

RAPID ADAPTATION TO A NOVEL HOST IN A SEED BEETLE (*CALLOSOBRUCHUS MACULATUS*): THE ROLE OF SEXUAL SELECTION

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Received June 7, 2006

Accepted October 19, 2006

Rapid diversification is common among herbivorous insects and is often the result of host shifts, leading to the exploitation of novel food sources. This, in turn, is associated with adaptive evolution of female oviposition behavior and larval feeding biology. Although natural selection is the typical driver of such adaptation, the role of sexual selection is less clear. In theory, sexual selection can either accelerate or impede adaptation. To assess the independent effects of natural and sexual selection on the rate of adaptation, we performed a laboratory natural selection experiment in a herbivorous bruchid beetle (*Callosobruchus maculatus*). We established replicated selection lines where we varied natural (food type) and sexual (mating system) selection in a 2×2 orthogonal design, and propagated our lines for 35 generations. In half of the lines, we induced a host shift whereas the other half was kept on the ancestral host. We experimentally enforced monogamy in half of the lines, whereas the other half remained polygamous. The beetles rapidly adapted to the novel host, which primarily involved increased host acceptance by females and an accelerated rate of larval development. We also found that our mating system treatment affected the rate of adaptation, but that this effect was contingent upon food type. As beetles adapted to the novel host, sexual selection reinforced natural selection whereas populations residing close to their adaptive peak (i.e., those using their ancestral host) exhibited higher fitness in the absence of sexual selection. We discuss our findings in light of current sexual selection theory and suggest that the net evolutionary effect of reproductive competition may critically depend on natural selection. Sexual selection may commonly accelerate adaptation under directional natural selection whereas sexual selection, and the associated load brought by sexual conflict, may tend to depress population fitness under stabilizing natural selection.

KEY WORDS: Artificial selection, beneficial alleles, female oviposition behavior, host acceptance, monogamy.

Herbivorous insects are a classic example of an adaptive radiation (Schluter 2000) and such insects are known to coevolve with the host plants they utilize as food. Many cases of insect–plant coevolution have been described, often involving comparisons

of phylogenetic congruence (Farrell 1998; Farrell and Sequeira 2004; see examples in Coyne and Orr 2004). Diversification, in this case, begins with an insect population colonizing a new food source. In insect–plant associations, a key question concerns the

kind of adaptations that result from host shifts and whether these can ultimately result in reproductive isolation. Ehrlich and Raven (1964) pointed to an interaction between plant secondary chemical substances and physiological adaptations in insects to such compounds as the engine driving coevolution between butterflies and their food plants. Ehrlich and Raven (1964) placed special emphasis on larval food choice for the insect–food plant relationship, as inappropriate food can lead to the death of the larvae. Larval food choice, however, is just of importance if larvae disperse. If not, then female choice of oviposition site is key. Thus, to understand the initial phases of host shifts, we need to study female oviposition behavior and larval adaptations to novel hosts.

Adaptive radiation is characterized by rapid evolution (Schluter 2000). Natural selection generally generates ecological adaptation to novel environments (Schluter 2000; Nosil et al. 2002; Rundle and Nosil 2005). In contrast, the role of sexual selection is more controversial (Arnqvist and Rowe 2005). Some models of sexual selection suggest that the rate of fixation of beneficial alleles increases (Whitlock 2000; Lorch et al. 2003), or that deleterious alleles are purged more effectively (Agrawal 2001; Siller 2001), under sexual selection and that adaptation is accelerated as a consequence. Empirical evidence that relates to this question is very limited. Sexually reproducing populations adapt faster than nonsexuals to new environments (e.g., Colegrave 2002) and recombination is known to enhance the strength of natural selection (Rice and Chippindale 2001a; Goddard et al. 2005), but these empirical findings only show that there is an advantage of sexual reproduction. Other sexual selection models, assuming no net benefit of female choice, show that sexual selection can instead impose a load on populations that may constrain populations from reaching their fitness optimum (Lande 1980; Kirkpatrick 1982) potentially causing extinction (Tanaka 1996; Houle and Kondrashov 2002; Kokko and Brooks 2003). There is some comparative support for the idea that sexual selection can increase the probability of extinction (McLain et al. 1995, 1999; Sorci et al. 1998; Doherty et al. 2003; Morrow and Pitcher 2003). Additionally, Kokko and Brooks (2003) pointed out that sexual conflict is likely to cause extinction to a higher extent, because costs and benefits are not carried by the same individual. Theory predicts that sexual selection that results from sexual conflict (Gavrillets et al. 2001) can result in a reproductive load as females are harmed or are forced to expend on the evolution of resistance (Arnqvist and Rowe 2005) and there is some empirical support for this (Holland and Rice 1999). Furthermore, intralocus sexual conflict can depress population level fitness in theory (Chippindale et al. 2001; Rice and Chippindale 2001b; Arnqvist and Rowe 2005) and provides one potential way in which sexual selection can impede the rate of adaptation. Thus, sexual selection can both accelerate and impede the rate of adaptation to novel environments (Holland 2002). There is, however, little consensus with regards to the net

effect of sexual selection and whether natural and sexual selection generally reinforce one another or, conversely, act in opposite directions in adaptive evolution.

Herbivorous beetles are very species rich and their radiation is intimately linked with the diversification of the angiosperms (Farrell 1998). Within one of the most diverse groups, the Chrysomeloidea, the radiation of bruchid beetles is well investigated over a broad geographic range (Jermy and Szentesi 2003; Kergoat et al. 2004, 2005a, b). Phylogenetic analyses of host plant–bruchid beetle associations have revealed that seed beetles often exhibit specializations and that the members of one beetle group predominantly use closely related plant taxa belonging to a single host tribe (Jermy and Szentesi 2003; Kergoat et al. 2004, 2005a, b; Morse and Farrell 2005). Diversification patterns in seed beetle genera reflect the topology of the phylogeny of their host plants (Kergoat et al. 2004, 2005b) and host–plant secondary compounds seem to be a strong selective agent (Kergoat et al. 2005b). However, there is ample evidence for the occurrence of several major host shifts in the evolutionary history of bruchid beetles (Kergoat et al. 2005a, 2005b; Morse and Farrell 2005; Tuda et al. 2006). Host shifts are hypothesized to occur due to changes in the female’s chemosensory system, causing changes in host selection behavior resulting in the occupation of a novel host (Jermy and Szentesi 2003). Relatively few genes are believed to underlie variation in oviposition behavior in the seed beetle *Callosobruchus maculatus* and although there are population differences in the genetic architecture of oviposition behavior, the genetic basis seems to depend on the environment in which these genes are expressed (see Fox et al. 2004). Messina (2004a) showed that preference for a new host can evolve in only 40 generations in this model species, that oviposition preference is rather labile but that a new host is readily accepted after this time. Furthermore, even after many years of laboratory culturing, there is still considerable plasticity in traits related to host use in *C. maculatus* (Guedes et al. 2003). However, a host shift exposes larvae to a completely new environment causing changes in larval feeding behavior (Guedes et al. 2003), larval competitive behavior (Tuda and Iwasa 1998; Messina 2004b), and adult life-history traits (Messina 2004b). Thus, host shifts lead to adaptations to new food sources both in the laboratory and in the wild in this group of insects (Tuda et al. 2006). This is thus a system that is suitable for studies of the rate of adaptation to a novel host.

Bruchid beetles of the genus *Callosobruchus* are also well studied in aspects of their reproductive biology other than host-plant associations. Although mating and multiple mating can be advantageous for females, enhancing life-span and lifetime fecundity (Fox 1993a), male genitalia also injure females during mating (Crudgington and Siva-Jothy 2000). The interaction between the positive and negative effects of mating in this species seems quite complex (Arnqvist et al. 2004) and, furthermore, the fitness effects

of mating on females vary across species in the genus *Callosobruchus* (Rönn et al. 2006). Even though both sexual conflict and direct benefits seem to contribute to the interaction between the sexes in these beetles, there are reasons to believe that sexual conflict predominates and is important in causing reproductive divergence (Fricke and Arnqvist 2004).

The primary goal of this study was to assess the net effect of sexual selection on the rate of adaptive evolution. This was done in an artificial selection experiment using *C. maculatus*, where we measured the rate of adaptation in replicated selection lines. Half of these lines were subjected to a host shift whereas half were kept on their natural laboratory host. Further, sexual selection (and sexual conflict) was removed in half of the lines by enforcing strict genetic monogamy but allowed in the other half by permitting polygamy.

Material and Methods

STUDY ORGANISM

The seed beetle *C. maculatus* is a worldwide pest on leguminose seeds in human bean storages. Mated females cement their eggs on the host bean, and newly hatched larvae then burrow into the seed. The larvae complete their development and pupate inside a single host seed. Adult beetles live on average for 10 days when kept without food and water. The entire life cycle from egg to egg is completed in about 21–24 days at 30°C. These biological features facilitate rearing of seed beetles in the laboratory, making them suitable model organisms for artificial selection experiments. We received three beetle populations from Dr. Peter Credland (University of London) in 2002, collected in large numbers from three adjacent locations in Nigeria (Oyo, Zaira, and Lossa), Africa. Populations had been kept for two years (approx. 24–30 generations) in the laboratory prior to their transfer to our laboratory at Uppsala University. Beetles were reared on black-eyed beans (*Vigna unguiculata*), with 250–350 randomly chosen adult beetles transferred to 120–140 g of host medium every new generation. Colonies were held in incubators under constant conditions at 30°C ± 0.5° and 45% RH ± 10% with a 12–12 h light-dark cycle.

SELECTION LINES

A base population for the selection lines was established from the three Nigerian populations (see above) by mixing 50 males and 50 females each from each of the three populations. Fusing the three related populations reduces the risk that a lack of additive genetic variation would obstruct response to selection, whereas restricting the elevation of nonadditive genetic variation that may result from mixing diverged and unrelated populations (Lynch and Walsh 1998). The base population was reared for five generations before establishing 16 distinct selection lines. In order to test for

the effect of sexual selection on adaptation to a novel environment, we employed a laboratory natural selection experiment (sensu Fuller et al. 2005), imposing a host bean and a mating system treatment on our selection lines.

Half of the selection lines were reared on chick peas (*Cicer arietinum*), a novel host to these beetles (hereafter referred to as CP lines), whereas the other eight lines were continued on their natural host, the black-eyed bean (*Vigna unguiculata*; hereafter BE lines). The two hosts belong to different host tribes (Cicereae and Phaseoleae), each representing one of two large sister clades within the subfamily Papilionoideae (Kajita et al. 2001; Doyle and Luckow 2003; Wink and Mohamed 2003). The two hosts differ in the composition and concentration of chemical defense substances; for example, Phaseoleae species lack a toxic secondary compound (L-canavanine) observed in Cicereae (e.g., Bisby et al. 1994) as well as the size of the bean and the texture and hardness of the seed coat. Thus, the host shift imposed in CP lines should lead to new conditions for larval development, competition, and female oviposition. In addition to the host treatment, we enforced monogamy (M lines) on half of the selection lines whereas the other eight lines were kept under polygamy (P lines; see below). These two selection treatments were applied in a full factorial orthogonal design with four replicates for each of the four treatment combinations.

In each generation, we established the P lines with 57 and the M lines with 50 pairs each (see below). To start the selection lines, we first collected beans infested with larvae ready to hatch from the source population and placed them in virgin chambers. Here, beans are kept individually in square petri dishes subdivided into 25 small compartments to avoid matings between males and females. Virgin chambers were checked several times a day for emergence of adult beetles. Virgin females and males were then kept individually until enough beetles were collected to establish the selection lines as described below. In the first generation, beetles were randomly paired and randomly assigned to a selection treatment. Two replicates for each treatment combination were first established (eight selection lines). In the following generation, two additional replicates for each treatment combination were established from the base population resulting in the establishment of all 16 selection lines. In each subsequent generation, approximately 100 beans from each selection line were separated in virgin chambers where virgin beetles were collected. Females were kept individually, whereas males were kept together, prior to mating. Mating was conducted in the following way. A randomly chosen male was introduced to a single virgin female and they were allowed to mate. All matings occurred at ambient room-temperature and natural lighting. After 3 h, mates were rotated once within the P lines by moving each male from his first female to another female within his line, thus introducing a new but non-virgin male to each female. This rotation was not conducted in the

M lines, where each female was only exposed to a single male. Three hours after initiating matings, we also added one bean of the respective host-type to each beetle pair in the polygamous as well as the monogamous treatment, to elevate the remating rate (Eady et al. 2004). Thus, sexual selection in the P lines was potentially comprised of overt female choice, cryptic female choice, and sperm competition, whereas direct premating competitive interactions between males were not allowed. In contrast, variance in male mating success in the M lines could only occur by some males failing to mate completely and females could not gain from being reluctant to mate with the male randomly allocated to them. Twenty-four hours after introducing the mates to each other, males were removed and discarded while all females from a given selection line were introduced together into 1-L glass vials with abundant oviposition medium (120 g of the appropriate host-type). The selection lines were then maintained in an incubator under constant conditions at $30^{\circ}\text{C} \pm 0.5^{\circ}\text{C}$, and $45\% \text{ RH} \pm 10\%$ with a 12–12 h light-dark cycle until the next generation hatched and the above selection protocol was repeated. Our lines were maintained under this selection protocol for more than 35 generations.

We used unequal absolute population sizes in the two mating system treatments (100 and 114, respectively, see above) to produce comparable effective population sizes. The observed variances in female fecundity and male mating/sperm competition success (Eady 1994) correspond to variances in family size for a mean family size of 2.0 of $V_{kf} = 3.0$ for females and $V_{km} = 4.4$ for males in P lines. This, in turn, yields the following equality between the effective population sizes in M (left expression) and P (right expression) lines (Falconer and Mackay 1996):

$$N_e \approx \frac{4 \times 100}{V_{kf} + 2} = \frac{8 \times 114}{V_{kf} + V_{km} + 4} = 80.$$

REPEATED FITNESS ASSAYS

To track evolutionary responses in our selection lines, standardized fitness assays were performed in generation 6, 20, and 35. In each assay, we tested all 16 selection lines and established 10 replicates per line, with measures for each replicate representing an average over 20 individuals. Thus, a given point estimate for a line was based on 100 males and 100 females. At all three occasions the following protocol was implemented. Individuals for the fitness assay were collected in parallel with the normal collection necessary to establish the next generation as described above. For the fitness assay, 10 virgin males and females belonging to the same selection line were introduced into a petri dish (ϕ 9 cm) containing 12 g of respective host-type. They were then allowed to mate and oviposit there until their death. Fifteen days into each assay, we counted all eggs laid (1) on beans and (2) on the bottom of the petri dish in each replicate. At peak emergence, on day 28, we removed and counted all adult offspring (at 30°C adult eclosion

starts after 21 days). Fifty randomly chosen adult offspring were preserved in 98% ethanol and stored in a freezer at -20°C . Forty days into the fitness assay, the remaining adult offspring were counted. Thus, assay conditions were constant over the course of our experiment although they differed from both selection regimes (e.g., allowed for male–male competition and lifelong cohabitation between the sexes). Further, assay conditions were identical for M and P lines but different for C and BE lines.

For each replicate from each fitness assay, we separated preserved individuals by sex and dried them for two days at 50°C , after which dry weight per beetle was measured using a microbalance. These fitness assays thus yielded measures of (1) male and female body size (dry weight), (2) total fecundity (number of eggs laid), (3) total offspring production, (4) larval survival (number of adults produced divided by the number of eggs laid on beans), (5) development rate (number of adults hatched at day 28 divided by the total number of adults produced), (6) host acceptance (proportion of all eggs laid on beans), and (7) the intrinsic rate of population increase (see below).

MATING RATE ASSAY

In parallel with the fitness assays described above, we also recorded female mating rate. Over a period of 24 h, we continuously observed 10 females from each selection line with spot checks once every fifth minute and recorded the number of matings performed during this period. Following the selection protocol, we rotated males in the polygamous lines and added a single bean to each pair 3 h after the onset of the assay.

DEVELOPMENT RATE ASSAY

To measure development rate for all lines, the following assay was conducted (in generation 37 for four selection lines and generation 38 for the remaining lines). One hundred twenty virgin males and a similar number of virgin females were collected from each selection line at the same time as founders for the next generation were collected. These males and females were kept together in groups of 20 pairs and allowed to mate for 45 min in an incubator at standard conditions. After this period, males were discarded. The 120 females from each selection line were randomly split into two groups: 60 were allowed to oviposit on 144 g of the host they were adapted to (hence, familiar host: BE lines on black-eyed beans and CP lines on chick peas), whereas 60 females were presented with 144 g of the alternative host (hence, unfamiliar host: BE lines on chick peas and CP lines on black-eyed beans). After 6 h of oviposition, all females were removed and the vials were incubated at standard conditions. Nineteen days after the onset of this experiment, we started to monitor the vials for emerging offspring. During the peak emergence time, we recorded the number of emerging offspring three times a day and reduced the

frequency of spotchecks to once or twice daily before and after peak emergence time until we reached day 32.

POPULATION FITNESS UNDER NONCROWDED CONDITIONS

The fitness assays described above were performed under conditions with considerable larval competition. We also repeated these assays under more benign conditions. We used the same experimental setup as described above, apart from the following two differences. First, the 20 individuals from each replicate were introduced onto 120 g of oviposition medium instead of 12 g and, second, we additionally tested each line on their unfamiliar host. In generation 36/37, we performed five replicates of each line introducing beetles to their familiar host and five replicates on their unfamiliar host. In generation 39, we performed five additional replicates for each of the two host types. Together, we measured the fecundity of 100 beetles subdivided into 10 replicates for each host type, under such noncrowded larval conditions. After 25 days, we counted the number of hatched offspring a first time and the remaining offspring after 35 days. After the onset of adult emergence, we collected 10 males and females from each replicate that were kept on the familiar host and preserved them in alcohol. The beetles were used as described in the repeated fitness assay part above, to obtain measures of body dry mass.

STATISTICAL RATIONALE

We analyzed the results of the experiments described above using two complementary approaches. (1) To assess effects on rates of evolution, we used repeated measures analyses of variance on the mean values (across all replicates) for all lines. Here, selection treatments were regarded as between-subject factors and time (i.e., generation) as a within-subject factor. This analysis is appropriate for estimating rates of evolution (i.e., effects of time) and for assessing interactions between the rate of evolution and selection treatments. We base our main conclusions with regard to response to selection on the within-subjects parts of these models, because these describe and directly test for evolutionary change over time and are thus free from any direct effects of assay condition (which was standardized over time). Note that the between-subject effects of "host type" parameterizes the direct effects of food type during our assays and are thus of no relevance here. In contrast, a between-subject effect of "mating system" would indicate a main effect of selection because assay conditions were identical in M and P lines. (2) Repeated measure analyses of variance yield rather conservative estimates of the net effects of selection and do not allow the use of covariates unique for each fitness assay and line (e.g., body size). To better evaluate the net effects of selection, we thus also employed mixed model analyses of variance/covariance on all observations for each of the fitness assays separately, using the orthogonally crossed selection treatments as fixed factors, line

as a random factor nested within selection treatments and, in some cases, used appropriate variables as covariates. The random effects of line within treatments are, however, not reported below for brevity. Mixed models were fitted using a restricted maximum likelihood approach, as implemented in JMP version 6.0.0 (SAS Institute 2005).

For both analytical approaches, transformations appropriate for stabilizing variances and error distributions of our response variables were applied prior to model estimation and inference. This involved arcsine transformation variants of all ratios. Because some assumptions made in repeated measures analyses of variance cannot be tested (i.e., sphericity; see Looney and Stanely 1989), we used Huynh-Feldt corrected *P*-values for all within-subjects effects and also evaluated all repeated measures models by resampling tests, involving bootstrapping (2000 replicates) the residuals of the original models (see ter Braak 1992; Manly 1997). We note that the more conservative tests provided by this bootstrap procedure were in close agreement with conventional tests based on the *F*-distribution. Means are presented below with their associated SE.

Our analyses include multiple inferential tests, which increase the risk of committing type I statistical errors. We chose not to compensate for this fact by lowering our α level (Holm 1979; Storey 2003) because of the increased within-study type II error rate and deflation of statistical power that inevitably follows from such procedures (Cohen 1988). Instead, we minimize the impact of type I errors by basing our interpretation of the results on the overall pattern of phenotypic responses to selection. We note that none of our main conclusions rests upon a single significant test.

The data from the development rate assay were used to statistically describe adult emergence from the seeds over time. We regressed the proportion of adults emerged against time using the following nonlinear (sigmoidal) regression model:

$$\text{Proportion of adults emerged} = A / \{1 + \exp[-(\text{time} - X_0)/B]\}.$$

These coefficients represent the level at which the proportion of adults emerged levels out (*A*), the time at which 50% emergence is reached (*X*₀), and the rate of increase in adult emergence over time (*B*). Estimates of the three parameters for each selection line and food type were then jointly considered response variables in a multivariate analysis of variance of the effects of selection and food type on development rate (see Nilsson et al. 2002).

Results

We first present our main results from the repeated measures analyses of variance to characterize phenotypic responses to our selection treatments over time. We then give the complimentary results of the mixed models for data from the individual fitness assays,

and finally describe the effects of our selection regimes on development rate and population fitness under noncrowded conditions.

REPEATED FITNESS ASSAYS

The statistical evaluation of the pattern of phenotypic response to selection over time is summarized in Table 1. Unsurprisingly, host type had a strong main effect on almost all variables measured, with few exceptions. Further, many of the variables measured responded to selection over time as shown by significant effects of time. The total number of eggs laid in the fitness assays tended to increase over time during the course of the selection experiment and was higher among BE lines (757 ± 49) compared to CP lines (649 ± 41), but the mating system treatment had no apparent main effects on fecundity. Host type also had a main effect on the proportion of eggs laid on beans, a measure of host acceptance, such that a lower proportion was laid on beans among beetles kept on the novel host (i.e., the CP lines). More importantly, the rate of host acceptance increased over time among beetles kept on the novel host. Interestingly enough, although host acceptance increased more rapidly under polygamy in lines adapting to the novel host, it also increased in monogamous lines kept on the old host (see Fig. 1 and Table 1).

Juvenile survival, measured as the proportion of eggs laid on beans that successfully hatched into adult beetles, was lower among beetles reared on black-eyed beans (0.62 ± 0.04) compared to chick peas (0.72 ± 0.05) and tended to decrease over time (0.70 ± 0.06 , 0.69 ± 0.05 , and 0.63 ± 0.05 for the three generations, respectively). We note, however, that this measure of juvenile survival confounds genetic adaptation to the novel host with treatment induced differences in the degree of density-dependent larval competition. We suggest that the pattern found is the combined result of lower rates of host acceptance, and thus lower larval density, on chick peas and of the adaptive increase in host acceptance over time.

Total offspring production per generation, a rate-independent measure of population fitness, was higher overall in lines reared on black-eyed beans (see Fig. 2). Adaptation was evident in beetles reared on the novel host, where offspring production increased markedly over time, but not in lines reared on the old host. Moreover, the effect of mating system on population fitness interacted with the effect of host type, such that polygamous lines showed higher offspring production when adapting to a novel host whereas, if anything, monogamous selection lines showed a higher offspring production when reared on the familiar host.

Female mating rate during our behavioral assays did not change significantly over time, but was higher in lines reared on black-eyed beans compared to chick peas (1.50 ± 0.10 vs. 1.25 ± 0.08 ; see also below) and in polygamous compared to monog-

amous lines (1.43 ± 0.09 versus 1.31 ± 0.08). There were no between-subject effects of selection on the body size of either sex. Female body size did, however, tend to increase during the selection experiment and this trend was more pronounced when beetles were reared on black-eyed beans.

Development rate was not significantly affected by any of our explanatory variables in this repeated measures analysis of variance, although mating system was involved in marginally non-significant interaction effects. We note, however, that this model is conservative and does not allow the use of egg density as a covariate (see below).

INDIVIDUAL FITNESS ASSAYS

As the results of our mixed model analyses of variance were very similar across the three fitness assays, we will only present a detailed account of the results for the last assay (performed in generation 35) here. Overall, the host treatment had a strong and significant effect on nearly all variables measured (see Table 2). In general, the CP lines had a lower lifetime fecundity than the BE lines in generation 35 (total eggs: CP: 682.09 ± 9.09 ; BE: 783.01 ± 10.92 ; total offspring: CP: 296.68 ± 5.31 ; BE: 408.41 ± 4.47). This pattern persisted even though acceptance of chick peas as a suitable oviposition site clearly increased over time (proportion eggs laid on beans: gen. 35: CP: 0.66 ± 0.01 ; BE: 0.89 ± 0.01 ; gen. 6: CP: 0.46 ± 0.01 ; BE: 0.88 ± 0.01) and, furthermore, juvenile survival was still higher on the novel than the ancestral host (CP: 0.67 ± 0.01 ; BE: 0.60 ± 0.01) (see above). We also, calculated a rate-sensitive measure of population fitness in generation 35 by dividing the total number of offspring produced with mean generation time (from the development rate assays) for each line. The selection lines kept on the ancestral host had a significantly higher intrinsic rate of increase than the lines on the novel host (CP: 0.52 ± 0.01 ; BE: 0.74 ± 0.01).

Interestingly enough, the trend observed in the repeated measures analyses of variance of development rate, was confirmed in the mixed models when using egg density as a covariate (see Table 2). Although there was no significant effect of our mating system treatment on development rate in generation 6 ($F_{1,12.36} = 0.647$), there was a significant interaction between mating system and host treatment in the two later assays (see Table 2). Under these conditions, polygamous lines developed slower than monogamous lines when evolving on the ancestral host, whereas the opposite was true among lines evolving on the novel host (see Fig. 3).

MATING RATE

Female mating rate was analyzed separately for all three fitness assays, in generalized linear models with Poisson errors and a log-link function. After six generations of selection, females held on the ancestral host mated significantly more often than females held

Table 1. Results of repeated measures analyses of variance of phenotypic responses to selection over time during the selection experiment. Given are Huynh-Feldt corrected *P*-values for within subjects effects and (within brackets) the results of bootstrap tests of all effects.

Source	Total egg production			Proportion of eggs laid on beans		
	df	<i>F</i>	<i>P</i>	df	<i>F</i>	<i>P</i>
Between subjects						
Mating system	1	0.000	0.987 (0.994)	1	0.276	0.609 (0.597)
Host type	1	38.749	<0.001 (0.002)	1	210.04	<0.001 (<0.001)
Mating system×Host type	1	0.507	0.490 (0.478)	1	2.528	0.138 (0.191)
Error	12			12		
Within subjects						
Time	2	12.029	<0.001 (0.006)	2	18.71	<0.001 (0.001)
Time×Mating system	2	1.466	0.251 (0.266)	2	6.213	0.007 (0.015)
Time×Host type	2	0.080	0.924 (0.914)	2	4.579	0.021 (0.029)
Time×Host type ×Mating system	2	0.358	0.703 (0.692)	2	4.215	0.027 (0.049)
Error	24			24		
Source	Juvenile survival			Development rate		
	df	<i>F</i>	<i>P</i>	df	<i>F</i>	<i>P</i>
Between subjects						
Mating system	1	0.060	0.811 (0.803)	1	0.892	0.364 (0.359)
Host type	1	31.272	<0.001 (0.002)	1	0.069	0.798 (0.788)
Mating system ×Host type	1	0.212	0.653 (0.632)	1	3.541	0.084 (0.083)
Error	12			12		
Within subjects						
Time	2	17.922	<0.001 (0.001)	2	0.784	0.468 (0.440)
Time×Mating system	2	1.405	0.265 (0.253)	2	2.967	0.071 (0.087)
Time×Host type	2	4.576	0.021 (0.034)	2	2.593	0.096 (0.118)
Time×Host type ×Mating system	2	0.710	0.502 (0.482)	2	1.517	0.240 (0.232)
Error	24			24		
Source	Total offspring production			Female mating rate		
	df	<i>F</i>	<i>P</i>	df	<i>F</i>	<i>P</i>
Between subjects						
Mating system	1	3.231	0.097 (0.101)	1	4.988	0.045 (0.052)
Host type	1	710.533	<0.001 (<0.001)	1	17.074	0.001 (0.006)
Mating system ×Host type	1	4.875	0.047 (0.047)	1	1.94	0.189 (0.198)
Error	12			12		
Within subjects						
Time	2	8.201	0.002 (0.003)	2	0.173	0.842 (0.800)
Time×Mating system	2	0.730	0.492 (0.471)	2	0.625	0.544 (0.512)
Time×Host type	2	12.157	<0.001 (0.002)	2	0.628	0.542 (0.524)
Time×Host type ×Mating system	2	0.108	0.898 (0.885)	2	0.266	0.768 (0.707)
Error	24			24		

continued

Table 1. continued

Source	Female body size			Male body size		
	df	<i>F</i>	<i>P</i>	df	<i>F</i>	<i>P</i>
Between subjects						
Mating system	1	0.008	0.928 (0.924)	1	0.868	0.370 (0.455)
Host type	1	1.779	0.207 (0.183)	1	0.712	0.415 (0.467)
Mating system						
×Host type	1	0.034	0.856 (0.820)	1	0.991	0.339 (0.430)
Error	12			12		
Within subjects						
Time	2	24.481	<0.001 (0.001)	2	2.098	0.166 (0.392)
Time×Mating system	2	0.202	0.818 (0.797)	2	0.973	0.362 (0.437)
Time×Host type	2	4.839	0.017 (0.026)	2	0.779	0.422 (0.466)
Time×Host type						
×Mating system	2	0.622	0.545 (0.472)	2	1.041	0.344 (0.429)
Error	24			24		

on the novel host (CP: 1.30 ± 0.06 ; BE: 1.53 ± 0.10) ($\chi^2 = 4.10$, $df = 1$, $P = 0.043$). This effect of host treatment was also present after 20 generations of selection (CP: 1.2 ± 0.07 ; BE: 1.56 ± 0.09) ($\chi^2 = 9.57$, $df = 1$, $P = 0.002$) but was not significant in generation 35. Neither mating system, nor the interactions between the two selection factors, significantly affected mating rate in these models (but see Table 1).

DEVELOPMENT RATE ASSAY

There was a strong effect of the host selection treatment, as well as whether beetles were reared on their familiar host, on the three parameters characterizing the cumulative adult emergence function

(see Table 3). The CP lines developed significantly faster than the BE lines (CP: $549.12 \text{ h} \pm 5.74$; BE: $579.48 \text{ h} \pm 7.29$) and beetles in general developed faster on their familiar host compared to the host they did not evolve upon (familiar: $561.62 \text{ h} \pm 2.70$; unfamiliar: $566.97 \text{ h} \pm 10.42$). This difference in development rate was, however, not very large due to the contrasting behavior of the CP and BE lines as revealed by the significant interaction term (see Table 3). The CP lines developed faster on their unfamiliar host ($527.52 \text{ h} \pm 2.42$) than on their familiar host ($570.72 \text{ h} \pm 1.37$). In contrast, the BE lines had a much slower development on their unfamiliar ($606.43 \text{ h} \pm 3.83$) compared to their familiar host ($552.52 \text{ h} \pm 2.37$) (see Fig. 4).

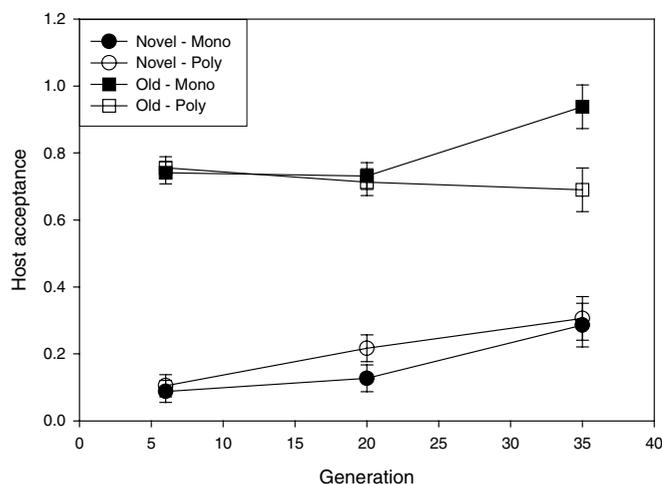


Figure 1. Evolution of host acceptance in the four selection treatment combinations, with chick peas being the novel and black-eyed beans the old (i.e., ancestral) host, respectively. Acceptance rate was measured as the proportion of eggs laid on beans over the total number of eggs laid (arcsine transformed).

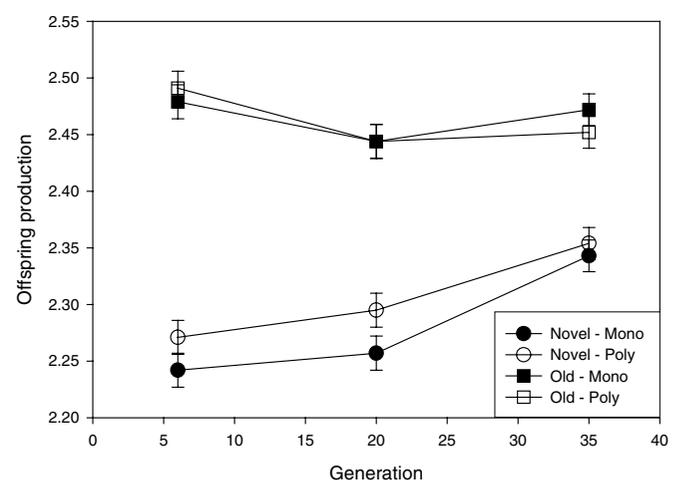


Figure 2. Evolution of total offspring production in the four selection treatment combinations, with chick peas being the novel and black-eyed beans the old (i.e., ancestral) host, respectively. Transformed values of offspring production, a rate-insensitive measure of fitness, are shown.

Table 2. The results of nested mixed model analyses of variance of the selection treatment effects, measured in generation 35. Selection lines are treated as a random factor nested within the two selection treatments. Only effects of fixed factors are given below.

Source	Total egg production		Proportion of eggs on beans		Juvenile survival		Total offspring production	
	df ^a	F	df ^a	F	df ^a	F	df ^a	F
Mating system	1,11.89	0.323	1,12.07	2.555	1,11.92	0.415	1,11.93	0.253
Host type	1,11.89	11.634**	1,12.07	58.048***	1,11.92	6.166*	1,11.93	62.985***
Mating system								
×Host type	1,11.89	0.391	1,12.07	4.533	1,11.92	0.073	1,11.93	1.181
Source	Female body size		Male body size		Development rate		Intrinsic rate of increase	
	df ^a	F	df ^a	F	df ^a	F	df ^a	F
Mating system	1,11.92	0.095	1,12.02	0.117	1,11.76	0.224	1,11.9	0.122
Host type	1,11.92	0.170	1,12.02	2.562	1,22.19	0.044	1,11.9	68.916***
Mating system								
×Host type	1,11.92	0.651	1,12.02	0.061	1,11.64	4.769*	1,11.9	0.819
Egg density					1,148.2	8.564**		

P* < 0.05; *P* < 0.01; ****P* < 0.001.

^aKHKR adjusted denominator degrees of freedom (SAS Institute 2005).

POPULATION FITNESS UNDER NONCROWDED CONDITIONS

The rate-insensitive measure of population fitness (total offspring production) was significantly influenced by the evolutionary history of the selection lines as well as of the actual food source they encountered (see Table 4). Beetles had clearly adapted to their respective host, such that lines adapted to a given host had

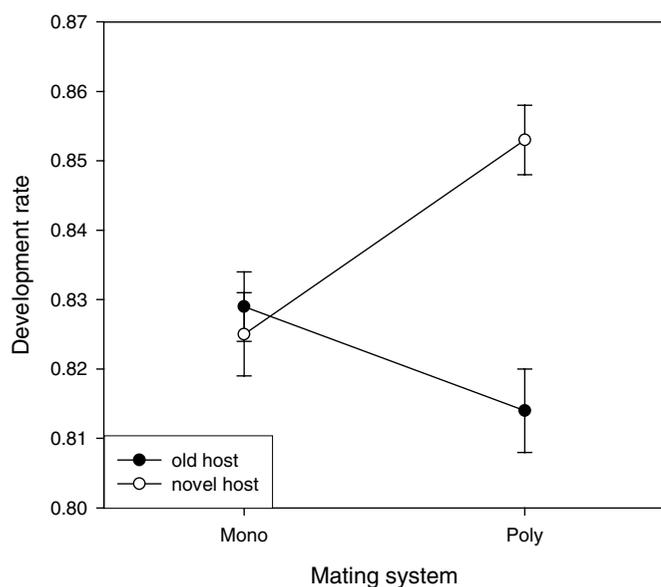


Figure 3. Development rate after 35 generations of selection in selection lines held on chick peas (novel host) or black-eyed bean (old, i.e., ancestral, host) and experiencing polygamy or monogamy.

highest fitness on that host (see Fig. 5). Notably, CP lines showed lower fitness when reared on black-eyed beans compared to BE lines. There was a strong decrease in fecundity among BE lines when forced to use chick peas. The CP lines, on the contrary, had higher lifetime fecundity on their ancestral compared to their novel host, even though the difference in host performance was not as marked as in BE lines. The results for the rate-sensitive intrinsic rate of increase were the same as those for total offspring production described above. Under these noncrowded conditions,

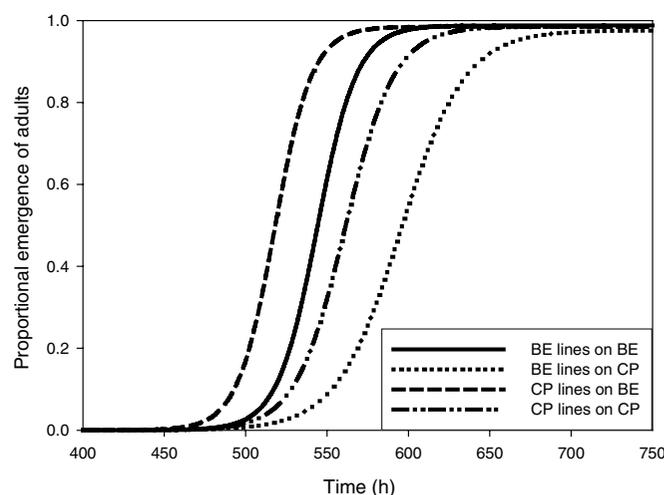


Figure 4. Nonlinear regression functions, fitting proportional adult emergence against time in lines selected for reproduction on either black-eyed beans or chick peas when reared either on the host they were evolving upon or on the alternate host.

Table 3. The results of a multivariate analysis of variance of the effect of selection treatments (mating system and host type) and actual food source (familiar/unfamiliar) on development rate (see text). Host type describes the host seed type the selection lines evolved upon over the course of the selection experiment. Familiar/unfamiliar host denotes whether the seeds used in this assay were of the same kind that lines evolved upon or not.

Factor	Wilk's λ	Rao's F	df	P
Mating system	0.866	1.134	3, 22	0.357
Host type	0.052	133.413	3, 22	<0.001
Familiar/unfamiliar host	0.564	5.674	3, 22	0.005
Mating system \times Host type	0.911	0.713	3, 22	0.555
Mating system \times Familiar/unfamiliar host	0.978	0.169	3, 22	0.916
Host type \times Familiar/unfamiliar host	0.066	103.258	3, 22	<0.001
Mating system \times Host type \times Familiar/unfamiliar host	0.977	0.169	3, 22	0.916

beetles of both sexes were larger when emerging from black-eyed beans compared to chick peas. Development rate was affected by a significant interaction between the host type and the familiarity of the food source. CP lines developed faster overall compared to BE lines, but the BE lines were very slow when developing in the unfamiliar host (Fig. 6). Despite the strong influence of host use and host use history on development rate, we found a significant effect of our mating system treatment. In general, offspring development rate was higher in the polygamous lines compared to the monogamous ones (polygamous: 0.54 ± 0.02 ; monogamous: 0.49 ± 0.02).

Discussion

Two main results emerged from our experiments. First, we observed rapid adaptation to the novel host. Second, the rate of adaptation interacted with our mating system treatment. We will discuss the implications of each of these points below.

The novel host clearly posed a challenge to *C. maculatus* in terms of population fitness. Wasserman (1986) found that chick peas were consistently chosen as the least acceptable host in an assay of oviposition preference of 22 strains of this seed beetle. However, chick peas were obviously not a hostile environment to juvenile beetles, as larval mortality was fairly low in our

Table 4. The results of nested mixed model analyses of variance of the effect of selection treatments (mating system and host type) and actual food source (familiar/unfamiliar) on fitness under benign conditions, with lines as a random factor nested within the two selection treatments. Host type describes the host seed type the selection lines evolved upon over the course of the selection experiment. Familiar/unfamiliar host denotes whether the seeds used in this assay were of the same kind that lines evolved upon or not.

Source	Total offspring production		Development rate		Intrinsic rate of increase	
	df ^a	F	df ^a	F	df ^a	F
Mating system	1,12.01	0.165	1,12.01	7.814*	1,12.01	0.072
Host type	1,12.01	6.070*	1,12.01	239.465***	1,12.01	13.225**
Familiar/unfamiliar host	1,300	385.373***	1,300	170.790***	1,300	295.124***
Mating system \times Host type	1,12.01	0.0001	1,12.01	1.035	1,12.01	0.011
Host type \times Familiar/unfamiliar host	1,300	890.557***	1,300	1946.857***	1,300	1268.359***
Mating system \times Familiar/unfamiliar host	1,300	0.335	1,300	0.083	1,300	0.333
Source	Female body size		Male body size			
	df ^a	F	df ^a	F		
Mating system	1,11.84	0.410	1,11.86	0.025		
Host type	1,11.84	10.951**	1,11.86	25.234***		
Mating system \times Host type	1,11.84	0.005	1,11.86	0.105		

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

^aKHRR adjusted denominator degrees of freedom (SAS Institute 2005).

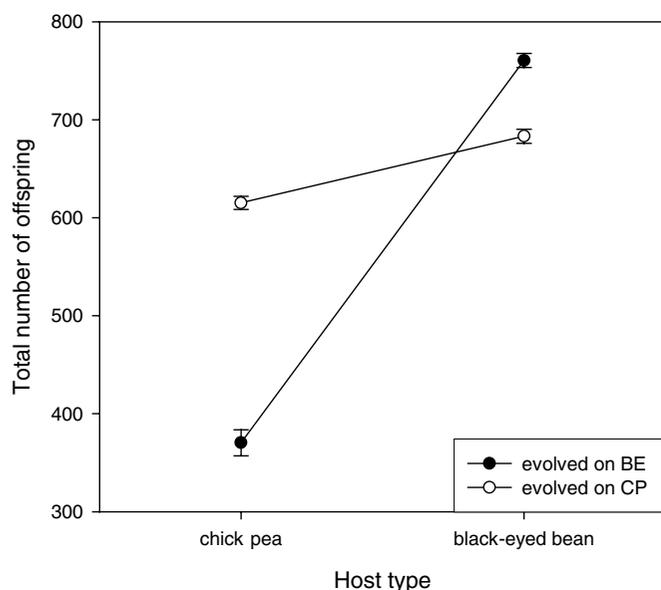


Figure 5. Total offspring production when beetles were allowed to utilize the host they were evolving upon or the alternate host in the fitness assay performed under noncrowded conditions. Host type along the abscissa represents the host encountered during the assay.

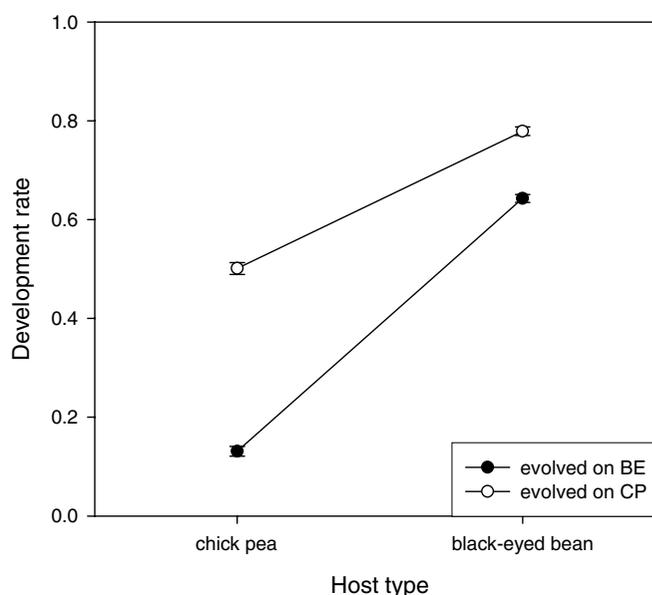


Figure 6. Development rate when beetles were allowed to utilize the host they were evolving upon or the alternate host in the fitness assay performed under noncrowded conditions. Host type along the abscissa represents the host encountered during the assay.

experiments. Instead, the main hindrance was that seeds were not readily accepted as oviposition sites by females. Yet, as a result of selection, female oviposition behavior evolved to accept the novel host at an increasing rate in 35 generations. Although this translated into an increase in larval competition during the course of the experiment, resulting in decreased juvenile survival rate, the net effect was a steady increase in population fitness. Thus, the rate of adaptation to this novel host due to natural selection was clearly quite rapid.

In herbivorous insects, the incorporation of a new host in the diet is thought to necessitate changes in female oviposition behavior as well as physiological changes in larval ability to use the novel food source (Jaenike 1990). Theory predicts that females should preferentially oviposit on hosts beneficial for larval performance and the two traits should thus become genetically correlated. However, empirical results in this field are somewhat divergent (see Futuyma and Peterson 1985 and Jaenike 1990 for reviews). The two traits often seem to be under distinct genetical control and thus may potentially evolve independent from each other (Wasserman and Futuyma 1981), although there are also examples of a positive genetic correlation between these traits (Via 1986). There is compelling evidence that the genetic correlation between female oviposition preference and larval performance is low in *C. maculatus* (Wasserman and Futuyma 1981; Fox 1993b), at least across closely related hosts. Female oviposition behavior is known to show heritable genetic variation (Futuyma and Peterson 1985; Messina 2004a) and egg-laying preferences

in *C. maculatus* seems to be quite evolutionary labile in general (Messina 2004a,b). Although Fox et al. (2004) suggested that relatively few genes underlie oviposition behavior, the work of Messina et al. (1987) suggests that many genes with small effect may influence this trait because several types of sensory input causes female host discrimination. Divergence in female host acceptance has been documented in two previous artificial selection experiments with *C. maculatus*, (Wasserman and Futuyma 1981; Messina 2004a; but see Kawecki and Mery 2003). In this study, we found rapid evolution of female oviposition behavior with an increase in novel host acceptance, in agreement with Wasserman and Futuyma's (1981) as well as Messina and Karren's (2003) findings. In contrast to Wasserman and Futuyma (1981), however, we also found associated changes in larval performance during the process of adaptation. Most importantly, we observed acceleration in larval development rate and, presumably as a result, a slight decrease in body size. Thus, evolution of several polygenic traits to the novel host occurred in concert during adaptation, collectively elevating population fitness on chick peas. Accelerated development rate (i.e., a shorter time spent as juvenile) on a novel host is predicted by theory, to the extent that it represents an adaptation to a less favorable environment. If juvenile mortality rate is accelerated and/or growth rate decelerated on chick peas, the optimal trade-off between growth and mortality will shift towards earlier emergence at a smaller adult size.

Our data also revealed a relatively weak but significant effect of sexual selection on the rate of adaptation. However, the impact

of sexual selection was distinct under the two natural selection regimes (novel versus old host), as revealed by interactions between natural and sexual selection in terms of their effects on fitness. When populations were challenged with adapting to a novel host, the adaptation seen was more rapid under a polygamous mating system compared to a monogamous one. This was perhaps most obvious in terms of the rate of change on host acceptance: female acceptance of the novel host for oviposition evolved more rapidly under polygamy. When maintained on the ancestral host, in contrast, the monogamous lines exhibited a slight increase in host acceptance not seen in the polyandrous lines (see below). The same basic pattern was seen also for population fitness and development rate. After 35 generations of selection, the total number of offspring produced on the novel host was highest among polygamous lines whereas offspring production on the ancestral host was highest among monogamous lines. Thus, contrasting effects of sexual selection dominated under the two natural selection regimes.

Theory suggests that sexual selection may be a “double-edged sword.” Although sexual selection may permit increased rates of fixation of beneficial mutations/alleles, leading to accelerated adaptation (Whitlock 2000; Lorch et al. 2003), it also imposes a selection load and sexual conflict may further depress fitness (Brooks and Jennions 1999; Holland and Rice 1999; Arnqvist and Rowe 2005). Empirical data directly addressing this question are rare and somewhat equivocal. Although some studies have found positive effects of sexual selection on components of adaptation (Promislow et al. 1998; Crudgington et al. 2005), others have found no clear net effect (Holland 2002; Martin and Hosken 2003) and yet others have documented negative effects (Holland and Rice 1999; Martin and Hosken 2004; Martin et al. 2004). Holland and Rice (1999) pointed to the fact that different environmental conditions should change the relative costs and benefits to populations from sexual selection. Our study suggests that the prevailing natural selection regime may indeed critically affect the outcome of such studies. When populations are under strong directional natural selection, such as when adapting to a novel environment, sexual selection may promote the rapid accumulation of novel beneficial alleles leading to a positive net effect. In contrast, for populations residing close to their adaptive peak, the liberation from a sexual selection load and/or sexually antagonistic adaptations that result from genetic monogamy (Rice 2000) may lead to increased fitness. We suggest that our results are in broad agreement with this general interpretation. Selection lines maintained on chick peas were, evidently, under very strong directional selection to adapt to this novel host. Assuming a heritability of host acceptance of $h^2 = 0.3$ (Fox 1993b), the response to selection seen during our experiment corresponds to an average standardized linear selection gradient for host acceptance in CP lines of $s' = 0.28$ between generations 6 and 20 and $s' = 0.27$

between generations 20 and 35. However, a caveat to the general conclusion above is warranted. We note that the two natural selection regimes also differed in another aspect: the realized female mating rate was slightly, but significantly, higher in lines kept on the ancestral host. This suggests that the intensity of sexually antagonistic selection (Wigby and Chapman 2004) might have been different in the two natural selection regimes, such that our experimental removal of sexual conflict may have had a larger net effect in lines kept on the ancestral host.

Brooks and Jennions (1999) suggested that “the costs of sexual selection can be laid at the door of postcopulatory processes.” The results of our study do not quite corroborate this suggestion. In our polygamous lines, the main form of sexual selection should have occurred at the postcopulatory level. Yet, the net effect of sexual selection was apparently positive when lines were adapting to a novel environment. It is possible that males that were better adapted to chick peas also gained higher post-mating fertilization success, given that such success among males may be condition dependent (Tomkins et al. 2004). If host choice and larval performance on hosts is at all genetically correlated, postmating sexual selection could then generate the adaptational edge seen among polyandrous lines. In this sense, postmating sexual selection may not be essentially different from premating sexual selection.

There are several candidates for costs of sexual conflict that may have been reduced as a result of our experimental removal of sexual antagonism by enforcing monogamy (Rice 2000). For example, sperm competition is thought to be responsible for the maintenance of male genitalia that harm females (Edvardsson and Tregenza 2005) and substances in the ejaculate that are disfavorable to females (Arnqvist et al. 2004). Removal of sperm competition may have promoted the evolution of more benign males. Additionally, ridding lines from intralocus genetic conflicts (Chippindale et al. 2001; Rice and Chippindale 2001b) may have allowed females to evolve a better adapted phenotype, which could at least partly be responsible for the increase in host acceptance seen in the monogamous lines kept on the ancestral host.

We also note that the sexual selection treatment in our experiment was relatively weak. In the polygamous lines, males and females were given the opportunity to interact only for 24 h and no premating male-male competition was allowed. This is in contrast to the standard laboratory settings, with frequent encounters between the sexes and ample sexual harassment. Thus, our polyandry treatment should have resulted in a marked relaxation of sexual conflict compared to the ancestral conditions. This fact may even have contributed to the positive net effect of sexual selection on the rate of adaptation in a novel environment. In a similar study on fruit flies, Holland (2002) failed to find such an effect and suggested that male-harm induced costs to females might have offset any benefits accrued due to sexual selection. It is, however, hard to judge the underlying causes for the different

results in these two studies, as inconsistent outcomes of selection experiments are not rare and can have a multitude of causes (Harshman and Hoffman 2000). Although natural laboratory selection experiments are powerful tools to study microevolutionary changes (Harshman and Hoffmann 2000) and reveal whether a putative selective agent is capable of generating long-term adaptive changes (Fuller et al. 2005), we need to exhibit caution in drawing conclusions from the outcome of this type of experiments and in extrapolating our results into nature (Harshman and Hoffmann 2000; Fuller et al. 2005).

In general, our findings show that the central conditions necessary for a successful host shift are met in *C. maculatus*: evolutionary changes in female oviposition behavior and physiological traits in larvae occurred over the course of only 35 generations. This observation has implications for speciation in this group (Tuda et al. 2006). However, speciation requires that reproductive isolation evolves in addition to ecological divergence (Coyne and Orr 2004). For two distinct host races to evolve, two changes are required. First, populations should show host preference for the novel host and discriminate against their ancestral host. Although we did not perform host choice tests, preliminary observations indicate that the lines held on chickpeas still showed a high preference for black-eyed beans at the end of our selection experiment, despite adaptation to the novel host. This result would be in line with the findings of two other studies of this species (Wasserman 1986; Kawecki and Mery 2003), showing that host preference hierarchies are conservative whereas the threshold for host acceptance evolves more rapidly. Second, to facilitate ecological distinctness, adaptation to a new host should result in impaired performance on the ancestral host (Jaenike 1990). Host-associated fitness trade-offs can then act as postzygotic barriers to reduce gene flow (Feder 1998). Chick pea is a nonpreferred host that clearly posed a challenge to the beetles in our experiment. This can be due to toxic substances and/or the hardness of the seed coat (Janzen 1977; Janzen et al. 1977; Bisby et al. 1994; Wink and Mohamed 2003). Despite this challenge, the beetles were able to adapt to the novel host. There was, however, no clear evidence in our data for a reduced performance of lines adapted to chick peas when reared on their ancestral host. The CP lines instead produced more offspring when encountering their ancestral host. Notably, the acceleration of larval development as a response to the novel host translated into a more rapid development on black-eyed beans, even surpassing that of the BE lines. Although this indicates that accelerated development was part of the adaptation seen, it is in line with other studies also showing no apparent trade-off in performance on a novel compared to an ancestral host in a laboratory setting (Futuyma and Philippi 1987; James et al. 1988; Karowe 1990; Messina 2004b; but see Gould 1979).

To conclude, we found rapid evolution of female oviposition behavior and larval performance to a novel host in the seed beetle

C. maculatus, as a result of strong directional natural selection. Sexual selection had a slight but significant influence on the rate of adaptation. The role of sexual selection was, however, more complex. For populations residing close to their adaptive peak, monogamy resulted in higher fitness. In lines kept in a novel environment, polygamy increased the rate of adaptation. Thus, it seems that although sexual selection can accelerate adaptation to novel environments, it can also be associated with a reproductive load that becomes apparent under weak natural selection. We conclude that natural selection was effective in fuelling adaptation to a new environment, but that sexual selection modified the effects of natural selection on the rate of adaptation. In systems with intense sexual conflict, the resulting reproductive load may effectively reduce the rate of adaptation.

ACKNOWLEDGMENTS

We thank Peter Credland for providing us with the beetle colonies used in this experiment. Furthermore, Mari Katvala and Alexei A. Maklakov occasionally took care of the lines and we are extremely grateful for their help. Noelia Amaro, David Canale, Katia Chavez-Abiega, Damian Dowling, Urban Friberg, Natacha Kremer, Josefine Larsson, Alexei A. Maklakov, Johanna Rönn, Candice Slevinski, and Agnetha Säfsten kindly assisted in the laboratory at times. Richard G. Harrison and his group (Cornell University) were kind enough to play host to CF during the summer of 2004. We thank Damian Dowling, Urban Friberg, Mari Katvala, Oliver Martin, Alexei A. Maklakov, Johanna Rönn, Merrill Peterson, Frank Messina, and an anonymous reviewer for comments on earlier drafts of this manuscript. This study was made possible by financial support from the Swedish Research Council (GA), Stiftelsen för Zoologisk Forskning at Uppsala University (CF), and a fellowship from the Deutscher Akademischer Austausch Dienst, Doktorandenstipendium im Rahmen des gemeinsamen Hochschulsonderprogramms III von Bund und Ländern (CF).

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Associate Editor: M. Peterson