

THE ROLES OF LIFE-HISTORY SELECTION AND SEXUAL SELECTION IN THE ADAPTIVE EVOLUTION OF MATING BEHAVIOR IN A BEETLE

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Although there is continuing debate about whether sexual selection promotes or impedes adaptation to novel environments, the role of mating behavior in such adaptation remains largely unexplored. We investigated the evolution of mating behavior (latency to mating, mating probability and duration) in replicate populations of seed beetles *Callosobruchus maculatus* subjected to selection on life-history ("Young" vs. "Old" reproduction) under contrasting regimes of sexual selection ("Monogamy" vs. "Polygamy"). Life-history selection is predicted to favor delayed mating in "Old" females, but sexual conflict under polygamy can potentially retard adaptive life-history evolution. We found that life-history selection yielded the predicted changes in mating behavior, but sexual selection regime had no net effect. In within-line crosses, populations selected for late reproduction showed equally reduced early-life mating probability regardless of mating system. In between-line crosses, however, the effect of life-history selection on early-life mating probability was stronger in polygamous lines than in monogamous ones. Thus, although mating system influenced male–female coevolution, removal of sexual selection did not affect the adaptive evolution of mating behavior. Importantly, our study shows that the interaction between sexual selection and life-history selection can result in either increased or decreased reproductive divergence depending on the ecological context.

KEY WORDS: Male–female coevolution, reproductive divergence, reproductive isolation, sexual conflict, speciation.

Divergence in mating preferences in sexually reproducing populations is a key component of models of speciation based on behavioral reproductive isolation (Kirkpatrick and Ryan 1991; Schluter 2000; Price 2008). The evolution of mating behavior can generate prezygotic reproductive isolation between populations and, in theory, can be affected by sexual selection and other forms of natural selection and the interaction between them (Lande 1981; Panhuis et al. 2001; Schluter 2001; Turelli et al.

2001; Blows 2002; Rundle et al. 2005; Schluter 2009). There are two distinct ways in which sexual selection can contribute to the evolution of mating behavior in allopatric populations (Schluter 2000; Rundle et al. 2005; Schluter 2009). First, sexual selection can affect the rate of adaptation of mating traits that are also subject to viability or fecundity selection, for example as a result of divergent selection on life-history traits (hereafter, "life-history selection") (Endler 1992; Rundle et al. 2005; Schluter 2009). Although theoretical models often consider the possibility that sexual selection will amplify the initial divergence by life-history selection (Lande 1982; Lande and Kirkpatrick 1988; Schluter and

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Price 1993), we note that this is not necessarily the case—the adaptive evolution of mating behavior can be constrained if life-history and sexual selection on a given trait proceed in opposite directions. For example, life-history selection may favor delayed mating and oviposition in females if resources are more abundant later in the season, while sexual selection on males can favor rapid mating and stimulation of early oviposition, because the benefits accrued in scramble and sperm competition outweigh the costs of suboptimal timing of oviposition.

The second general way in which sexual selection can contribute to the evolution of mating behavior is via the fixation of different alleles in different populations (i.e., by amplifying genetic drift) (Lande 1981; Rundle et al. 2005). Theory suggests that evolution of rapid reproductive isolation based on different mutations being fixed in different allopatric populations (mutation-order speciation sensu Schluter [2009]) is particularly likely under sexual conflict, which results from divergent reproductive strategies of males and females (Rice 1998; Gavrilets 2000; Gavrilets et al. 2001; Rice et al. 2005).

Strong support for the role of sexual selection in the evolution of reproductive barriers comes from comparative studies and from data on behavioral ecology and genetics of populations in hybrid zones (Schluter 2000; Coyne and Orr 2004; Price 2008). However, although there is substantial experimental evidence from laboratory evolution studies for the role of natural selection in the evolution of prezygotic reproductive isolation (Rice and Hostert 1993; Rundle et al. 2005), similar approaches toward addressing the role of sexual selection have been developed only recently, and the evidence is mixed (Martin and Hosken 2003; Wigby and Chapman 2006; Bacigalupe et al. 2007; Hosken et al. 2009). Moreover, while these experimental evolution studies focused on either life-history or sexual selection, these two evolutionary forces will proceed concurrently in nature, and may interact (Blows 2002). It is therefore crucial to conduct experiments in which both sexual selection and life-history selection are manipulated simultaneously to tease apart the relative contribution of these two processes to trait evolution. Recently, such an approach has been applied successfully to the study of life-history traits (Rundle et al. 2006; Fricke and Arnqvist 2007; Maklakov et al. 2009) and sexual dimorphism in a display trait (Chenoweth et al. 2008) during adaptation to novel environments. It is therefore a promising experimental approach for research on the evolution of mating behavior.

Despite the theoretical significance of interactions between sexual and life-history selection on mating behavior, we are aware of only two empirical studies that have used such an approach. The pioneering study by Blows (2002) assessed the importance of natural and sexual selection in the evolution of mate recognition in an artificially created hybrid population of *Drosophila serrata* and *D. birchii*. This population was created from a single cross

between a *D. serrata* female and *D. birchii* male, releasing a great deal of additive genetic variance in mating and life-history traits. The interaction between experimentally imposed natural and sexual selection had a very strong effect on the evolution of mate recognition based on cuticular hydrocarbons (Blows 2002). This *Drosophila* study only allowed comparisons between populations with or without natural selection and/or sexual selection, rather than comparing populations adapting to different sets of ecological conditions (divergent selection). Recently, Fricke (2006) tested for divergence of populations of *Callosobruchus maculatus* seed beetles reared on seeds from different host plants with and without sexual selection. She found no effect of the removal of sexual selection or sexual selection \times host plant interaction on the evolution of reproductive characters, but documented reduced divergence in reproductive characters in populations adapting to a new host compared to populations that remained on the ancestral host (Fricke 2006). One possible reason for the lack of an effect of sexual selection in this study is the small difference between treatments. Polygamous females encountered only two successive males over 24 h, which represents a rather benign level of sexual selection and sexual conflict compared to natural conditions in this species, such that, in practice, this study compared complete versus partial removal of sexual selection. Thus, to date, experimental evidence on concurrent effects of sexual selection and other forms of natural selection on the evolution of mating traits is very limited, and results are variable.

Here, we report the results of an experimental evolution study in which life-history and sexual selection were manipulated simultaneously in a full factorial design: populations of seed beetles were forced to adapt to novel reproductive schedules, while the opportunity for sexual selection was either completely removed or maintained at a level similar to the ancestral population.

We investigated the evolution of mating behavior in replicate populations of *C. maculatus* that were under selection for age at reproduction (hereafter, life-history regime)—the beetles were allowed to reproduce either early (“Young” lines) or late in life (“Old” lines). Sexual selection and sexual conflict were abolished in half of the lines in each life-history regime by enforcing random monogamy (“Monogamy”) whereas other lines were maintained as panmictic polygamous populations with ample opportunity for sexual selection and conflict (“Polygamy”). Our “Polygamy” treatment reflects the rearing conditions of the ancestral population as well as the recent evolutionary history of *C. maculatus* beetles, which have been associated with human grain stores for thousands of years (Tuda et al. 2006; Messina et al. 2007). We thus created a powerful 2×2 experimental evolution design that allowed us to examine the contribution of sexual selection to the short-term evolution of mating behavior under divergent life-history selection.

Because beetles in “Young” lines have very limited time for mating and egg-laying, we predicted that “Young” lines would evolve rapid mating behavior compared to “Old” lines. On the contrary, in “Old” lines, we predicted that delayed mating and delayed egg-laying (which is stimulated by mating in this species) would be beneficial, because eggs produced early in life would not contribute to the next generation. Selection is predicted to be particularly strong in this capital breeder (adults are facultatively aphagous, and typically kept without food in laboratory culture) that has a fixed amount of resources for lifetime egg production. Earlier studies confirmed genetic trade-offs between early- and late-life reproduction in another seed beetle with similar life-history *Acanthoscelides obtectus* (Tucic et al. 1996), and in this population of *C. maculatus* (Maklakov et al. 2009).

Whereas predictions with regard to life-history regime are relatively straight-forward, different sexual selection models predict different outcomes in relation to the potential role of sexual selection. “Good genes” models of mate choice evolution predict more rapid adaptive evolution of mating behavior in “Polygamy” lines. This should result in a mating system \times life-history regime interaction, with polygamous lines showing stronger adaptive responses than monogamous lines. In particular, “good-genes” models predict accelerated evolution of rapid mating in “Young Polygamy” lines, because rapid mating would be selected both via life-history selection in females and via sexual selection in males. In contrast, sexual conflict models predict increased rate of adaptation to novel environments in “Monogamy” populations freed from the “sexual selection load.” This should result in a different pattern of mating system \times life-history regime interaction, with monogamous populations showing stronger adaptive responses than polygamous populations. Moreover, sexual conflict theory predicts that sexual conflict over the timing of mating will be strongest in “Old Polygamy” lines (Fig. 1), where females will be selected for delayed mating and oviposition whereas males will be selected to mate sooner and induce earlier oviposition in their mates. In contrast, in other treatments, both sexes may be under similar selection (Fig. 1). Thus, the “Old Polygamy” lines may be expected to show a strong signature of sexual conflict, and this may be manifested in distinct patterns in sympatric versus allopatric crosses.

We also tested for divergence in reproductive traits by comparing mating behavior in allopatric (between-population) mating trials within each of the four combinations of selection regimes. First, it allowed us to test whether male–female coevolution proceeded similarly in monogamous and polygamous populations. Second, it allowed us to provide a further test of the “sexual conflict as engine of speciation” hypothesis (Gavrilets 2000; Martin and Hosken 2003; Wigby and Chapman 2006; Bacigalupe et al. 2007; Hosken et al. 2009). The general prediction is that prezygotic reproductive isolation, measured as proportion of failed

		Life history selection	
		Young reproduction	Old reproduction
Mating system	Polygamy	Females: early mating and oviposition Males: early mating and oviposition <u>Sexual conflict: weak</u>	Females: delayed mating and oviposition Males: early mating and oviposition <u>Sexual conflict: strong</u>
	Monogamy	Females: early mating and oviposition Males: early mating and oviposition <u>Sexual conflict: none</u>	Females: delayed mating and oviposition Males: delayed mating and oviposition <u>Sexual conflict: none</u>

Figure 1. Predicted selection pressures on each sex, and resulting intensity of sexual conflict over timing of mating and oviposition, under simultaneous regimes of divergent selection on the life-history schedule (“Young vs. “Old” reproduction) and manipulation of sexual selection intensity (“Monogamy” vs. “Polygamy”).

matings in allopatric versus sympatric crosses, will be more pronounced in polygamous populations that experience more sexual conflict than in monogamous populations (Gavrilets 2000; Martin and Hosken 2003; Wigby and Chapman 2006; Bacigalupe et al. 2007; Hosken et al. 2009). This prediction follows from Gavrilets’ (2000) model, which suggests that the degree of reproductive isolation between populations will increase with increased level of sexual conflict.

Materials and Methods

STUDY ANIMALS

The seed beetle *Callosobruchus maculatus* is a popular model organism for quantitative genetic studies of life-history traits (Messina 1993; Fox et al. 2004a,b, 2006; Bilde et al. 2008) and laboratory evolution (Messina and Karren 2003; Fricke and Arnqvist 2007; Maklakov et al. 2007a). *Callosobruchus maculatus* females glue their eggs to the surface of beans and hatching larvae bore a hole into the bean where they forage, pupate, and eclose as adults after 3–4 weeks. Adults can mate and oviposit straight after eclosion (Fox et al. 2003) and both sexes are polygamous. Males can usually be seen pursuing resistant females and attempting to mount them. The costs of reproduction and of male harassment for females are high (Rönn et al. 2006).

The beetles used in this experiment originated from an Australian population collected in Kingaroy in 2003 and maintained by the Department of Primary Industries and Fisheries (DPIF), Queensland. This population was started from a total of 357 beetles from infested mung beans (*Vigna radiata*) and was propagated with 250–300 beetles per generation. We reared the beetles in our laboratory from September 2006 for half a year, using ~500 beetles per generation on 200 g of organic mung beans kept at 30°C, 70% relative humidity (RH) and 14:10 light:dark (L:D) cycle before the start of the experiment (see Maklakov et al. 2009).

EXPERIMENTAL EVOLUTION

The details and the rationale for the selection protocol have been described elsewhere (Maklakov et al. 2009). Here, we briefly outline the most essential issues that are important for interpreting our results. The key idea behind our approach is to apply a novel life-history schedule in the form of selection for age at reproduction while simultaneously manipulating the mating system by removing sexual selection from half of our experimental populations (hereafter also called lines). Thus, we used a 2 × 2 design, which allowed us to test for the interaction between life-history and sexual selection in the evolution of mating behavior in experimental lines. Life-history selection regimes consisted of either “Young” (only allowed to reproduce for the first 24 h post-eclosion) or “Old” (only allowed to reproduce after 72 h post-eclosion) lines ($n = 8$ lines for each regime). “Young” lines underwent 18 generation of selection and “Old” lines—15 generations of selection. Both treatments were kept for one generation of relaxed selection prior to the mating behavior assays. Mating system was manipulated by keeping half of the lines in each life-history treatment either under polygamy (“Polygamy”) or random monogamy (“Monogamy”). Beetles in “Polygamy” lines (57 females and 57 males per line) were kept in panmictic populations, which reflect recent evolutionary history of these beetles in laboratory and, previously, grain storages. Beetles in “Monogamy” lines (50 males and 50 females per line) were collected as virgins and paired randomly for life. Once all beetles were dead, all of the beans within each line were mixed to ensure that beetles that were used to start a new generation came from randomly selected beans, allowing fecundity and larval viability selection to occur in the same way within all treatments. The sample sizes in mating system treatments (above) were adjusted to expected approximate differences in effective population size (Falconer and Mackay 1996) based on previously published data on variances in female fecundity and male reproductive success in this species (Eady 1994; see Fricke and Arnqvist 2007 for further details). We further note that the population sizes of our experimental lines make it unlikely that inbreeding levels will differ within the span of 15–18 generations (Rice and Holland 2005). Because nearly

all beetles survived the first 72 h (survival > 98% in preliminary trials), there was no need to adjust population size for different life-history regimes.

Potential differences in larval competition between selection regimes were avoided by providing beetles with a surplus of beans, such that only one egg per bean was typically laid. Inadvertent selection for early maturation was reduced by randomly collecting hatched adults 3 days after the start of hatching, rather than collecting beetles as soon as they emerged.

ASSAYS OF EARLY AGE MATING SUCCESS, AND COPULATION DURATION

We collected virgin beetles 24–48 h old from beans inoculated with a single egg and isolated in 48-well plates. We conducted sympatric (within-population) and allopatric (between-populations within the same experimental treatment) mating trials using the general design employed in three previous experimental evolution studies of reproductive isolation (Martin and Hosken 2003; Wigby and Chapman 2006; Bacigalupe et al. 2007). Note, however, that although the previous studies had only one level of experimental manipulation (mating system), our study has two (mating system and life-history schedule), allowing us to test for the interaction between them (see Bacigalupe et al. 2007 for the discussion of the statistical approach). Because we had four replicate populations within each of our treatment combinations, each population was represented in one sympatric ($n = 30$ within-population pairs) and three allopatric ($n = 10$ between-population pairs × 3 populations = 30 pairs) mating types. Thus, in total we conducted 16 populations × 60 pairs = 960 mating trials. During a mating trial, a male and a female were placed in a 60 mm diameter Petri dish and observed for 30 min. We recorded (1) whether mating occurred during the observation period (mating success); (2) time at the start of copulation (copulation latency), and (3) copulation duration.

STATISTICAL RATIONALE

We conducted three main types of analyses to answer the two main questions outlined in the Introduction: (1) what are the roles of life-history versus sexual selection in the adaptive evolution of mating behavior? and (2) how does male–female coevolution contribute to reproductive isolation? To answer the first question, we tested for the effects of life-history schedule (LH), mating system (MS), and the interaction between these two fixed factors on the evolution of mating behavior in our experimental populations using the data from sympatric matings. We used generalized linear models (*glm* function) to analyze mating success (proportion of successful matings per line) using quasi-binomial distribution and logit link function in R_{2.8.1} (R Development Core Team 2008). Changing the order of the independent variables in the models had no effect on qualitative significance. We also

note that the significance/nonsignificance of different terms was the same when the data were analyzed using arcsine-square-root transformation of the proportions and subsequent ANOVA in JMP (analyses not shown). Copulation latency and copulation duration were analyzed using the same fixed factors as above but in the general linear models in JMP, when individual values were averaged for each line (i.e., there were 16 datapoints in each model). Dependent variables from these latter models were tested for deviation from normality by conducting Shapiro-Wilks W test for the goodness-of-fit (all $P > 0.2$). To answer the second question, we first used the same set of models, with data from allopatric matings as response variables. Note that it would be statistically incorrect to use a single model with data from sympatric and allopatric mating trials and mating type as factor as discussed in Bacigalupe et al. (2007) and Hosken et al. (2009). We then conducted direct comparisons between allopatric and sympatric crosses for each population within each experimental treatment. This was done in two ways: first, we compared the proportion of allopatric versus sympatric crosses that resulted in copulation in monogamous and polygamous populations within each of the LH treatments (“Young” and “Old”) using log-likelihood ratio tests; second, following Hosken et al. (2009), we used the I index (Powell 1997), calculated as (Percentage of successful allopatric matings – Percentage of sympatric matings) / Percentage of sympatric matings.

Results

SYMPATRIC MATING TRIALS

There was a significant effect of life history on mating success during 30-min trials (Table 1A): “Young” lines had higher mating success than “Old” lines (Fig. 2A). There was no effect of mating system and no mating system \times life-history regime interaction for mating success (Table 1A, Fig. 2A). There was no significant effect of any of the fixed factors on latency to mating and copulation duration (Table 2A).

Table 1. The effects of selection for age at reproduction (LH; “Old” or “Young”) and mating system (MS; “Monogamy” or “Polygamy”) on proportion of successful matings between pairs of virgin beetles in (A) sympatric and (B) allopatric crosses.

Statistic effect	df	Deviance	Resid. df	Resid. Dev.	P
A)					
LH	1	23.191	14	52.974	0.015
MS	1	0.375	13	52.599	0.756
LH \times MS	1	2.522	12	50.077	0.421
B)					
LH	1	55.799	14	49.398	<0.001
MS	1	4.321	13	45.077	0.158
LH \times MS	1	16.150	12	28.927	0.006

Table 2. The effects of selection for age at reproduction (LH; “Old” or “Young”) and mating system (MS; “Monogamy” or “Polygamy”) on latency to mating and copulation duration in pairs of virgin beetles in (A) sympatric and (B) allopatric crosses.

Statistic effect	df	Sum of squares	F	P
A) Latency to mating				
LH	1, 12	9096.312	0.364	0.557
MS	1, 12	45807.955	1.834	0.201
LH \times MS	1, 12	1156.831	0.046	0.833
Copulation duration				
LH	1, 12	465.339	0.149	0.707
MS	1, 12	7468.191	2.385	0.148
LH \times MS	1, 12	251.054	0.080	0.782
B) Latency to mating				
LH	1, 12	49564.484	1.726	0.213
MS	1, 12	5354.151	0.186	0.673
LH \times MS	1, 12	30847.703	1.074	0.320
Copulation duration				
LH	1, 12	3225.625	2.835	0.118
MS	1, 12	3272.330	2.876	0.116
LH \times MS	1, 12	6580.538	5.783	0.033

ALLOPATRIC MATING TRIALS

There was a significant effect of life history on mating success during 30-min trials (Table 1B): similarly to the data on sympatric trials, “Young” lines had higher mating success than “Old” lines (Fig. 2B). However, although there was no net effect of mating system on mating success, there was significant mating system \times life-history regime interaction (Table 1B, Fig. 2B). There was no significant effect of any of the fixed factors on latency to mating (Table 2B), but there was significant mating system \times life-history regime interaction for copulation duration (Table 2B, Fig. 3).

DIRECT COMPARISON BETWEEN SYMPATRIC AND ALLOPATRIC TRIALS

The data from “Old” lines are roughly in line with the general prediction of relatively higher frequency of failure to mate in allopatric compared to sympatric crosses in “Polygamy” lines (likelihood ratio test for pooled data: $P = 0.052$) than in “Monogamy” lines (likelihood ratio test: $P = 0.133$) (compare also the parts of Figs. 2A,B that refer to “Old” lines). However, the “Young” lines show the opposite trend, with relatively lower frequency of failed matings in allopatric crosses compared to sympatric crosses in “Polygamy” lines (likelihood ratio test: $P = 0.042$) than in “Monogamy” lines (likelihood ratio: $P = 0.149$) (again, compare also Figs. 2A,B).

Analysis using the I index suggests that there are no significant effects of LH, MS, or their interaction on this measure of reproductive isolation (LH: $F = 0.06$, $df = 1, 12$, $P = .8037$;

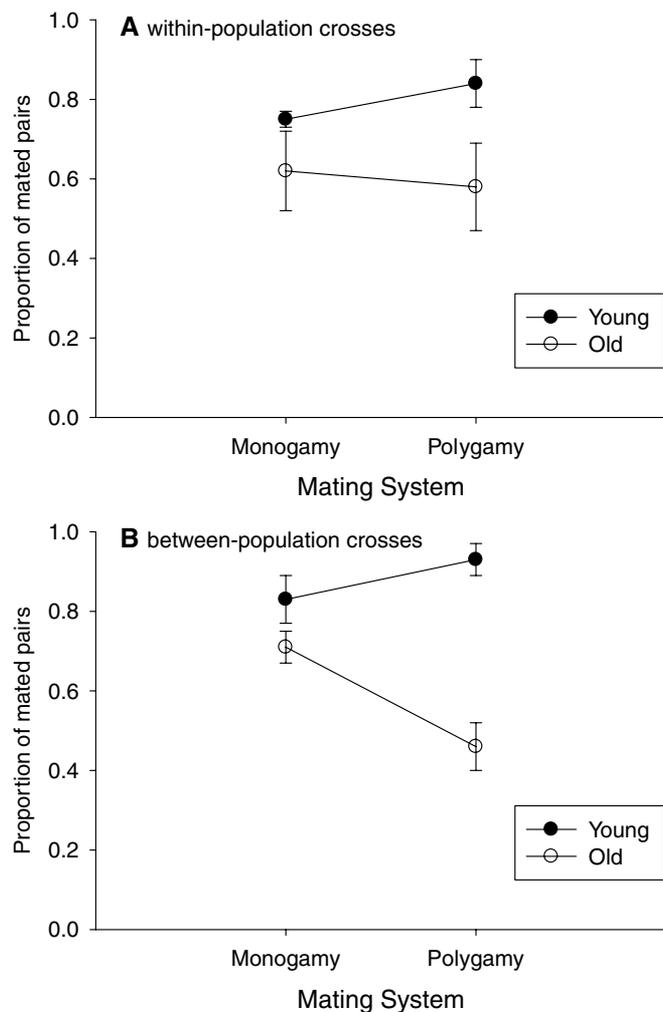


Figure 2. The effects of selection for age at reproduction (LH; “Old” or “Young”) and mating system (MS; “Monogamy” or “Polygamy”) on proportion of successful matings (mean across four populations per treatment \pm SE) between pairs of virgin beetles in (A) sympatric and (B) allopatric crosses.

MS: $F = 1.86$, $df = 1, 12$, $P = .1972$; LH \times MS: $F = 2.02$, $df = 1, 12$, $P = .1810$.

Discussion

We found that mating behavior evolved rapidly in our experimental populations and this evolution occurred in the predicted direction—beetles from populations that were selected for fast reproduction mated more frequently early in life than beetles from populations that were selected for late reproduction. However, sexual selection contributed little to this adaptive evolution, despite evidence that male–female coevolution proceeded differently in monogamous and polygamous populations. Below we discuss how this interesting result can help to explain differences in the outcomes of previous studies, and suggest directions for future research.

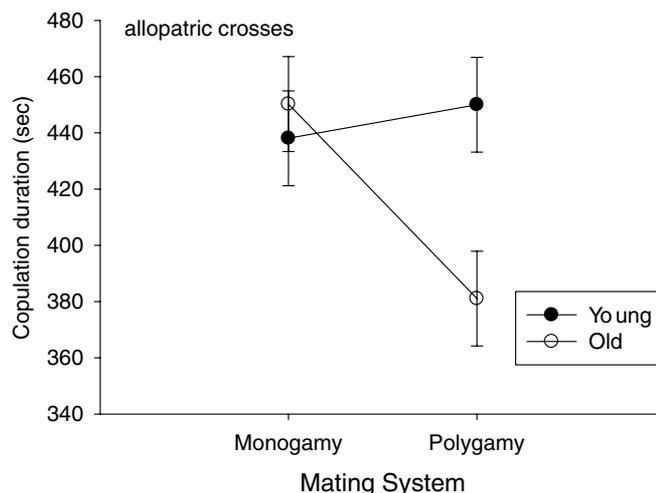


Figure 3. The effects of selection for age at reproduction (LH; “Old” or “Young”) and mating system (MS; “Monogamy” or “Polygamy”) on copulation duration in seconds (adjusted least squares mean across four populations per treatment \pm SE) between pairs of virgin beetles in allopatric crosses.

LIFE-HISTORY AND SEXUAL SELECTION ON THE EVOLUTION OF MATING BEHAVIOR

Mating induces rapid oviposition in seed beetles. We predicted, therefore, that life-history selection would favor rapid mating in “Young” lines (that could only reproduce within 24 h after pairing) and delayed mating in “Old” lines (that were selected to start reproducing 72 h after pairing), because females would benefit from accelerated reproduction in “Young” lines and from delayed reproduction in “Old” lines.

In theory, sexual selection could accelerate this predicted evolution of mating behavior in response to newly imposed life-history selection (Lande 1982; Schluter 2000; Blows 2002; Coyne and Orr 2004; Rundle et al. 2005), but such an effect was not observed in our study. This finding is in line with our previous investigation of the roles of life-history and sexual selection in local adaptation in these populations, where we documented rapid sex-specific evolution of life-history traits to life-history selection but little contribution from the imposed sexual selection treatment (Maklakov et al. 2009). The absence of sexual selection effects in our present study is intriguing because mating behavior is particularly likely to be affected by sexual selection. These results cannot be attributed to weak sexual selection for three reasons. First, the sexual selection treatment in this study mirrors conditions experienced by this species during its recent evolutionary history, i.e., high population density, where females are constantly and persistently harassed by males. Second, sexual selection treatment did have a significant effect on net female fitness: this effect showed that sexual conflict was occurring in the “Polygamy” treatment, but this conflict was eliminated in the “Monogamy” treatment

(Maklakov et al. 2009). Third, the pattern of allopatric matings suggests that male–female coevolution proceeded differently under “Monogamy” and “Polygamy” treatments (see below). We also note that mating behavior, as well as other traits, responded rapidly to selection for age at reproduction, suggesting ample standing genetic variation for these traits in our source population.

One possible reason for why we did not observe any positive effect of sexual selection on the rate of adaptation in mating behavior could be a counterbalancing effect of interlocus sexual conflict (Parker 1979; Rice 1996; Holland and Rice 1998; Holland 2002; Arnqvist and Rowe 2005). There is ample opportunity for sexual conflict in seed beetles: male *C. maculatus* have notoriously elaborate genitalia that damage the female reproductive tract (Crudginton and Siva-Jothy 2000; Rönn et al. 2007; Hotzy and Arnqvist 2009); male harassment is costly to females (Rönn et al. 2006); and male ejaculates of another seed beetle, *A. obtectus*, have been shown to contain toxic compounds (Das et al. 1980), perhaps akin to those in *D. melanogaster* (Chapman et al. 1995). Our previous study indicated that net fecundity in “Polygamy” lines was lower compared to “Monogamy” lines, suggesting that sexual conflict over reproduction might impose a female fecundity cost (Maklakov et al. 2009). Although females in “Old” lines benefit from delayed mating and oviposition, males in “Old Polygamy” lines can in theory benefit from mating early in life even if it reduces net female fitness (cf. Maklakov et al. 2007b). Such selection on males could counteract selection on delayed mating in “Old Polygamy” females resulting in a classic antagonistic coevolution. Our results lend some support to this hypothesis because the pattern of mating frequency in allopatric crosses was more in the direction of life-history selection than in sympatric crosses in “Polygamous” populations, even though all populations were selected to mate only in sympatry. In other words, “Polygamy” females would fare better by mating with allopatric rather than with sympatric males, which is indicative of antagonistic coevolution.

“Good-genes” sexual selection (Fisher 1930; Williams 1966; Trivers 1972; Zahavi 1975; Iwasa and Pomiankowski 1994; Houle and Kondrashov 2002) and sexual conflict can operate on different loci in a population, and the net result of these two processes for population fitness or trait evolution should be tested empirically. For example, a recent study demonstrated how “good-genes” sexual selection can benefit a population by helping to eliminate a deleterious allele in *D. melanogaster* (Hollis et al. 2009), a species in which sexual selection is harmful under a wide variety of experimental conditions (Holland and Rice 1999; Pitnick and Garcia-Gonzalez 2002; Wigby and Chapman 2005; Rice et al. 2006). On the other hand, “good-genes” sexual selection and sexual conflict can operate on the same loci and the net effect is likely to depend on the relative strength of each process and costs associated with mate choice (Cameron et al. 2003). *Callosobruchus*

maculatus males that are particularly successful in sperm competition can also be particularly damaging to female direct fitness (Hotzy and Arnqvist 2009), as well as produce low-fit daughters (Bilde et al. 2009), thereby reducing the strength of “good-genes” sexual selection.

Our findings are broadly in agreement with *Drosophila* studies by Holland (2002) and Rundle et al. (2006), as well as our previous analysis of life-history traits in these populations (Maklakov et al. 2009) in that sexual selection did not have a positive effect on adaptation to the novel environment (i.e., life-history selection regime). Interestingly, the only multigenerational study that found evidence in support of a positive net effect of sexual selection on adaptation (Fricke and Arnqvist 2007) was characterized by relatively weak sexual conflict. We thus agree with Hollis et al. (2009) that the detection of indirect benefits to populations through sexual selection would require experimental separation between “good-genes” sexual selection and interlocus sexual conflict. However, although such studies would provide valuable information on the operation of “good-genes” processes, and the evolution of particular loci in the genome (Whitlock and Agrawal 2009), we may also be interested in the effect of “good-genes” sexual selection on population fitness and trait evolution under natural conditions, that is, in the presence of natural levels of both inter- and intralocus sexual conflict.

Only a handful of studies have examined the correlated evolution of mating behavior in response to life-history selection (Pletcher et al. 1997; Sgro et al. 2000; Maklakov et al. 2006; Seslija et al. 2009) and, although sexual selection is often invoked as a potential explanation for observed patterns, the design of these studies precluded separate analysis of the effects of life-history and sexual selection. Although two studies using different sets of lines of *D. melanogaster* (Pletcher et al. 1997; Sgro et al. 2000) suggest that mating rate early in life is higher in “Young” lines, this was not corroborated in a seed beetle, *A. obtectus* (Maklakov et al. 2006; Seslija et al. 2009). Our results are in line with *Drosophila* studies and we would like to discuss why two species of seed beetles differed in their response to selection on age at reproduction. We suggest that the basic difference may lie in the fact that “Old” *A. obtectus* lines were selected without egg-laying substrate (beans) (Tucic et al. 1996) whereas our *C. maculatus* lines were selected in the presence of the egg-laying substrate. This is a crucial difference, which renders our experimental design more similar to the *Drosophila* study than to the *A. obtectus* study in this respect. In the absence of beans, seed beetle females lay fewer eggs and can use water or nutrients derived from male ejaculates for somatic maintenance. Therefore, *A. obtectus* females selected for late reproduction (10 days after pairing) could have used male ejaculates to survive until the beginning of egg-laying. This hypothesis is supported by increased life span of mated *A. obtectus* females maintained in the absence of beans (Tucic et al. 1996).

The net effect of sexual selection on adaptation can also differ depending on the timing of fitness assays (Lorch et al. 2003; Hollis et al. 2009; Whitlock and Agrawal 2009). Although Lorch et al. (2003) showed that “good-genes” sexual selection is likely to be important during early stages of adaptation to a novel environment (i.e., the first few generations), Whitlock and Agrawal (2009) suggested that short-term experiments may not always predict the long-term dynamics of sexual selection. It is important, however, that Whitlock and Agrawal’s analysis corresponds to long-term effects of sexual selection on population fitness rather than rate of adaptation to a novel environment. Our experiment replicates the latter scenario and the results do not provide support for more rapid adaptation under joint effects of life-history and sexual selection.

SEXUAL SELECTION AND SEXUAL CONFLICT AS AN “ENGINE OF SPECIATION”

Our data indicate that, although male–female coevolution within our experimental lines resulted in different mating patterns in sympatric versus allopatric crosses, the direction of this response with respect to mating system was roughly opposite in “Old” (lower mating frequency in allopatric versus sympatric matings under polygamy) and “Young” (higher mating frequency in allopatric versus sympatric matings under polygamy) lines. Hence, our results do not corroborate the hypothesis that sexual conflict may be a powerful engine of speciation, a prediction also tested in four previous studies (Martin and Hosken 2003; Wigby and Chapman 2006; Bacigalupe et al. 2007; Gay et al. 2009). This means that only two of five experimental evolution studies that conducted such a comparison supported this hypothesis. We note that Gay et al. (2009) experimental design differs from the other studies in that the authors manipulated the level of sexual conflict indirectly through increased population size, whereas only one of the studies that directly manipulated the opportunity for sexual conflict found the predicted effect (compare Martin and Hosken (2003) vs. Wigby and Chapman 2006; Bacigalupe et al. 2007; this study). Whether this difference in experimental design can explain the difference in observed patterns of reproductive isolation requires further research (see also Hosken et al. 2009). Currently, the evidence suggests that large population size per se, rather than any form of sexual selection, is likely to drive rapid reproductive isolation. Rate of adaptation is faster in large populations because they harbor more standing genetic variation, and more novel mutations contribute to additive variance per generation. Reproductive isolation observed in experiments that manipulated population size could therefore result from “ecological speciation,” because experimental populations had to adapt to novel laboratory conditions. Future studies in this field should aim to manipulate both mating system and population size to separate between these two factors.

Both life-history and sexual selection promote rapid mating in both sexes in “Young” lines, such that there is no conflict of interest between the sexes over timing of mating. Alternatively, in “Old” lines, life-history and sexual selection are in conflict because scramble competition and post-copulatory selection may favor the evolution of male traits that are detrimental to females. Post-copulatory sexual selection can result in the evolution of male traits that aid in sperm competition but damage females as a byproduct (Chapman et al. 1995; Hotzy and Arnqvist 2009) and such a scenario is possible here, because “Polygamy” populations had lower fitness than “Monogamy” populations (Maklakov et al. 2009). These considerations suggest that “Polygamy” treatment would impose selection on female resistance to early mating in “Old” but not in “Young” lines, which can potentially result in antagonistic coevolution in “Old” but not “Young” lines because of scramble competition among males. The pattern of early mating frequencies that we observe in our data fits this hypothesis: “Old Polygamy” females tend to mate less with allopatric males than with sympatric males.

Predicting the outcome of between-population crosses based on different modes of male–female coevolution is inherently difficult (Pizzari and Snook 2003; Rowe et al. 2003; Long et al. 2006; Rowe and Day 2006) and, at this stage, we would like to present the above considerations as a tentative hypothesis. However, one important outcome of this study is that mating system had opposite effects on the direction of male–female coevolution under different life-history regimes. In “Old” lines, the direction of the effect was seemingly indicative of reproductive isolation, where females were less likely to mate with allopatric males. In “Young” lines, the effect was in the opposite direction—females tended to mate more in allopatric crosses. These results suggest that the evolution of reproductive isolation by sexual conflict can be dependent on the environment, which may be one of the potential reasons for differences in outcomes of previous studies (reviewed in Hosken et al. 2009). One important implication is that experimental studies of reproductive isolation via any form of male–female coevolution should aim to manipulate not only the mating system but also the ecological context.

Conclusions

The main findings of the current study are that (1) mating behavior evolved rapidly in response to divergent life-history selection but (2) this evolution was neither assisted nor hindered by sexual selection. Moreover, there was no overall indication that sexual selection amplified genetic drift: this was not because sexual selection had no effect on male–female coevolution but because the direction of this effect on reproductive isolation was not consistent across the two life-history selection regimes. These data suggest that the interaction between sexual and life-history

selection can result in complex idiosyncrasies in the response of mating behavior to selection. Previous studies that manipulated both sexual and other forms of selection and assessed the effect of both processes on divergence of reproductive traits suggest that sexual selection tends to increase divergence whereas other forms of natural selection tend to decrease it (Fricke 2006; Chenoweth et al. 2008). Our study shows that the interaction between sexual selection and divergent life-history selection can result in either increased or decreased premating divergence depending on the ecological context. Despite the long-standing interest in and theoretical importance of the interaction between life-history and sexual selection in the evolution of mating behavior and reproductive isolation, we require more evidence from experiments manipulating both processes simultaneously (Blows 2002; Fricke 2006; Chenoweth et al. 2008, this study) to reach general conclusions. Nevertheless, our study suggests that sexual selection can play a less prominent role than life-history selection in the evolution of mating behavior during the initial stages of local adaptation.

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