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PATTERNS OF DIVERGENCE IN THE EFFECTS OF MATING ON FEMALE REPRODUCTIVE PERFORMANCE IN FLOUR BEETLES

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Abstract.—Sexual selection can lead to rapid divergence in reproductive characters. Recent studies have indicated that postmating events, such as sperm precedence, may play a key role in speciation. Here, we stress that other components of postmating sexual selection may be involved in the evolution of reproductive isolation. One of these is the reproductive investment made by females after mating (i.e., differential allocation). We performed an experiment designed to assess genetic divergence in the effects of mating on female reproductive performance in flour beetles, *Tribolium castaneum*. Females were mated to males of three different wild-type genotypes at two different frequencies, in all possible reciprocal combinations. Male genotype affected all aspects of female reproduction, through its effects on female longevity, total offspring production, reproductive rate, mating rate, and fertility. Moreover, male and female genotype interacted in their effects on offspring production and reproductive rate. We use the pattern of these interactions to discuss the evolutionary process of divergence and suggest that the pattern is most consistent with that expected if divergence was driven by sexually antagonistic coevolution. In particular, the fact that females exhibited a relatively weak response to males with which they were coevolved suggests that females have evolved resistance to male gonadotropic signals/stimuli.

Key words.—Cost of reproduction, cryptic female choice, male accessory substances, sperm competition, Tenebrionidae.

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Sexual selection has been implicated as a key mechanism of speciation because it can generate rapid evolutionary change in reproductive characters (Lande 1981; West-Eberhard 1983; Andersson 1994; Coyne and Orr 1998). If such change occurs in allopatric populations, it will result in divergence, thus promoting the evolution of reproductive isolation. Most discussions of the role of sexual selection in generating speciation to date have focussed on premating sexual selection (e.g., Hoy et al. 1988; Barraclough et al. 1995; McMillan et al. 1997; Seehausen et al. 1997). This focus is reasonable for monandrous species, in which premating events such as male-male competition and/or overt female choice are the main sources of variance in reproductive success among males. In polyandrous species, however, variance in reproductive success can also arise through events taking place after mating, resulting in postmating sexual selection. For example, males can influence their reproductive success by outcompeting other males' sperm already present in the female's reproductive tract (Parker 1970; Smith 1984; Birkhead and Møller 1998). To do this, males may influence the uptake and/or transport of sperm to storage sites (Eberhard 1996; Chapman et al. 2000). Similarly, a male may increase the proportion of a female's offspring he fathers by inducing a refractory period during which the female is unsusceptible to further matings (Simmons and Gwynne 1991; Eberhard 1996) or by increasing female reproductive rate immediately following copulation (Chapman et al. 1995; Eberhard 1996). Despite the fact that the role of postmating sexual selection has only relatively recently received attention, postmating processes may commonly be at the heart of reproductive isolation (see Howard 1999).

Variance in reproductive success among individuals will be elevated if females invest differently in offspring production depending on which male she mates with. First proposed by Burley (1986, 1988; differential allocation), empirical studies of this phenomenon have focused mainly on precopulatory traits in birds and mammals (e.g., visual or vocal; Møller and Thornhill 1998; Cunningham and Russell 2000; Sheldon 2000). However, such differential allocation is also expected to be important in postcopulatory sexual selection, because various male signals perceived by females during mating may affect subsequent female reproductive effort (see Eberhard 1996; Wedell 1996). If male signal–female receptor systems diverge, they may thus contribute to the evolution of reproductive isolation. In addition to random processes, such as founder effects and/or genetic drift, at least two adaptive evolutionary scenarios can also lead to divergence in male signal–female receptor systems that affect female reproductive rate (cf. Colegrave 2001; Cunningham and Russel 2001; Gil and Graves 2001). First, they may evolve by sexually antagonistic coevolution (Rice 1996, 1998). Differences in the evolutionary interests of males and females are ubiquitous. Such sexual conflicts are known to influence many aspects of the reproductive biology of both sexes. Conflicts occur over, for example, mate choice, parental care, sperm competition, mating rate, and female reproductive rate (Parker 1979, 1984; Choe and Crespi 1997; Partridge and Hurst 1998). In polyandrous species, males are selected to increase their mates' immediate reproductive rate, because offspring produced by females later in life are likely to be sired by other males. For females, high investment in egg production at one point in life is likely to have negative pleiotropic effects such as reduced lifespan (see Roff 1992; Stearns 1992). Females can therefore be assumed to produce offspring at a rate that is in some sense optimal, resulting from the negative genetic correlation between reproductive rate and longevity. Consider the relative interests of a male and a female after mating. The female will have an optimal

reproductive rate, representing evolved trade-offs, her own condition, and the environmental conditions. This rate will in most cases be lower than the male optima, particularly in species with no paternal care, thus generating sexual conflict. This can lead to sexually antagonistic coevolution in which adaptations in males that increase female reproductive rate are detrimental for female fitness, thus selecting for counteradaptations in females to reduce male-induced costs, in turn selecting for further adaptations in males and so on (e.g., Rice 1996, 2000). The idea that sexual selection driven by sexual conflict can constitute an engine of divergence and/or speciation has recently received support as a result of novel theoretical, experimental, and comparative work (Rice 1996, 1998; Rice and Holland 1997; Howard et al. 1998; Parker and Partridge 1998; Arnqvist et al. 2000; Gavrilets 2000; Andrés and Arnqvist 2001; Gavrilets et al. 2001).

Second, male signal–female receptor systems that affect female reproductive rate may also coevolve as a result of indirect genetic benefits to females. In contrast to the scenario described above, a female may actually benefit by elevating her reproductive rate when mated to males with efficient signals/stimuli, if by doing so she secures genetic benefits for her offspring (e.g., good genes or sexy sons; Eberhard 1996; Wedell 1996; Sheldon 2000). As with any form of sexual selection, such indirect benefits scenarios may lead to allopatric divergence (West-Eberhard 1983).

A wide range of male traits may affect female reproductive rate including visual (Burley 1986, 1988), vocal (Balzer and Williams 1998; Martin-Vivaldi et al. 1999), olfactory (Okelo 1979; Schmidt and Albutz 1994; Schmidt and Othman 1994), and tactile (Ashworth and Wall 1994) stimuli. In addition, males can affect female reproduction by the transfer of seminal fluid substances during copulation that act on female receptors. In most organisms, such ejaculate compounds are produced in male accessory glands and are referred to as accessory proteins (ACPs). ACPs have been particularly well studied in insects, where they are known to influence female egg production rate, sperm uptake, and induction of unreceptivity to further matings (Chen et al. 1988; Herndon and Wolfner 1995; Chen 1996; Gillott 1996; Wolfner 1997; Heifetz et al. 2000). ACPs are known to evolve very rapidly in fruit flies (Aguade et al. 1992; Thomas and Singh 1992; Civetta and Singh 1995) and have been implicated in the evolution of reproductive isolation (Gregory and Howard 1994; Price 1997; Howard 1999; Gavrilets 2000).

The purpose of this study is twofold. First, we determine whether potentially differentiated populations of the red flour beetle (*Tribolium castaneum*) have diverged in the effect mating has on female reproductive performance and document any male × female interactions that might occur. Second, we assess the evolutionary mechanisms that might cause such divergence. We conducted a large-scaled lifetime mating experiment, where females were mated reciprocally to males of all strains at two different mating frequencies to vary the intensity of signals provided by males. The emerging pattern of genetic and treatment effects on female fecundity, fertility, and longevity are used to discriminate between the evolutionary processes at the within-population level that might cause divergence between populations (cf. Clark et al. 1999; Andrés and Arnqvist 2001). We focus on two different measures of female reproductive response to males: initial reproductive rate and lifetime offspring production.

Material and Methods

Experimental Organism

We studied three wild-type strains of the red flour beetle, *T. castaneum*. The stocks were provided by the *Tribolium* stock center at the U.S. Grain Marketing Research Laboratory in Manhattan, Kansas. The three strains were Georgia-1 (G), Tiw-6 (T), and CTC-485 (C). The G strain was collected in Georgia (USA) in 1980, the T strain was collected in India in 1989, and the C strain was collected in Australia in 1988; all have been maintained at large population sizes in the laboratory since then. The phylogeny of the different strains (hereafter genotypes) is not well established, but genetic sequence data have shown that the C and G genotypes are more closely related to each other than either are to the T genotype (Beeman et al. 1996). A phenotypic marker strain, Black, which is homozygous for a semidominant autosomal mutation causing black body coloration (Sokoloff et al. 1960) was also used in these experiments. All beetles were maintained in dark rearing chambers at 32°C and at 70% (±10%) relative humidity. The medium was a standard mixture of 95% whole wheat flour and 5% brewers' yeast (Sokoloff 1972).

Female Reproductive Rate

To ensure virginity, all beetles used in the experiment were sexed as pupae and males and females were kept separately during emergence. Males used in the experiment were kept in holding jars, one for each genotype, together with Black females to assure the nonvirginity of males (i.e., sexually experienced). These beetles were transferred to fresh jars approximately every three weeks, so that no eggs laid by the Black females in the holding jars had the chance of developing to adults.

We performed a full factorial experiment of the effects of genotype and mating frequency on female reproductive rate (offspring production). Females (all virgins and 4–7 days old at the start of the experiment) were mated to males of the three different genotypes in all possible reciprocal combinations and at two different mating frequencies, high (three times a week) and low (once every second week), generating 18 different treatment combinations ($3 \times 3 \times 2$). The number of replicates for each of the 18 treatment combinations ranged between nine and 25 (mean = 20.3 ± 0.9 SE). All females were kept individually in 9-cm oviposition vials (petri dishes) containing 12 g of standard medium (sifted to enable offspring counting) in the rearing chambers, except during exposure to males. The oviposition vials were replaced once a week and stored for two additional weeks in rearing chambers. All offspring produced (late instar larvae, pupae, and adults) were subsequently recorded. Females that failed to produce eggs during the first week of the experiment were discarded.

During exposure to males, each female and a portion of her standard medium was transferred to a mating vial of smaller diameter (3.5 cm) together with three males. The females were given the opportunity to mate for 3 h at room

TABLE 1. The results of a generalized linear model, using binomial errors and a logit link function, of variance in the probability of mating during the second week of the experiment. The full model was highly significant ($LLR = 87.1$, df = 13, $P < 0.001$). The contribution of each source was tested by analysis of deviance, by deletion of (1) each main factor from a model including all main factors only; and (2) each interaction from the full model.

Source	*LLR*	df	*P*
Female genotype	22.0	2	<0.001
Male genotype	39.0	2	<0.001
Mating frequency	0.3	1	0.584
Female genotype × male genotype	20.6	4	<0.001
Female genotype × mating frequency	8.1	2	0.017
Male genotype × mating frequency	0.7	2	0.705

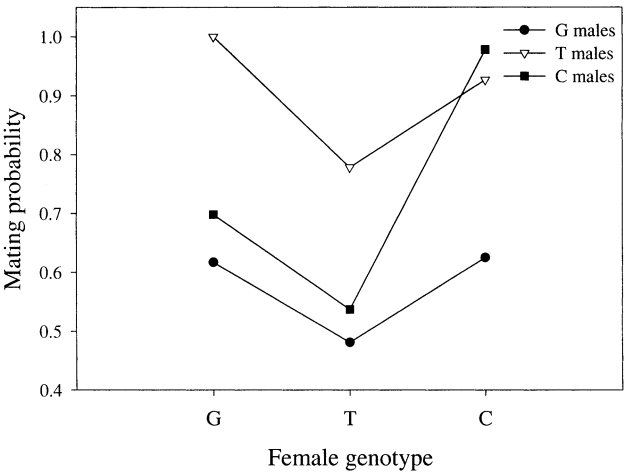

FIG. 1. Average mating probabilities during the second week of the experiment, for each male-female genotype combination.

temperature, after which the males were removed and the female (including medium) was reintroduced into the oviposition vial. All experimental females, regardless of their mating frequency, were taken out from the rearing chambers into room temperature during each mating occasion to avoid confounding effects of time spend outside the rearing chambers. To increase male persistence, all males were taken from the holding jars and placed individually in separate vials 20–24 h prior to matings. The mating frequency of individual females was assayed during the second week of the experiment by visually scoring females as mated (in copula for 35 sec or more) or not mated by continuous observation during the 3-h mating period.

The experiment continued for the entire female lifespan and the time of death was recorded at the mating events and/or during the weekly change of oviposition vials once a week. The body size of all females was subsequently measured using a digitizing tablet under a side-mounted camera lucida attached to a dissecting microscope.

Female Fertility

Virgin females and males were obtained by the methods described above. At 8 to 12 days after exclosure, five females were placed with five males in 9-cm vials containing ad libitum food, in all reciprocal genotype combinations. The number of replicates per genotype combination ranged between nine and 18 (mean = 12.1 ± 0.9 SE). Five days after the introduction, females and males were transferred to a 9-cm oviposition vial containing 12 g of finely sifted standard medium (mesh size 300 μm). These oviposition vials were replaced once a day for 3–6 days, incubated for another 2 days, after which the medium was sifted and the eggs were collected in 1.5-ml Eppendorf vials filled with 70% ethanol. The Eppendorf vials were vortexed and the contents examined under a dissecting microscope. Eggs were scored as developing normally or not.

Statistical Analysis

The effects of our experimental variables on female longevity, fecundity, and fertility were analyzed with appropriate general and generalized linear models. Response variables were transformed prior to analysis, if needed to stabilize variance and meet the assumptions of the models used. To enable analysis of fecundity functions, we also regressed weekly offspring production against time for each individual female using the sigmoidal model:

$$\text{Number of offspring} = A/\{1 + \exp[-(\text{week} + X_0)/B]\}. \quad (1)$$

These coefficients represent initial reproductive rate (A), the location of the function in time (X_0), and the rate of decline in offspring production over time (B) for each female. Least-squares estimates of A, X_0, and B for each female were then analyzed in a multivariate model.

RESULTS

Probability of Mating

A series of different factors affected the probability of females mating during the mating frequency assay the second week of our experiment (Table 1). The genotype of both sexes had marked effects on this probability. It is notable that whereas T males were most able to achieve matings, T females were least willing to mate. Further, the probability of mating was elevated when C males were paired with females of their own genotype, thus producing an interaction between male and female genotype (Fig. 1).

Female Lifespan

The analysis of female lifespan is presented in Table 2. The female genotypes differed considerably in their average lifespan (G = 126 ± 6.2 [SE], T = 197 ± 5.6, C = 194 ± 6.3 days). Male genotype and mating frequency also affected female lifespan when the effect of reproductive rate on female lifespan was controlled for. Females mated to C males lived shorter on average (165 ± 4.2 [SE] days) than did females mated to either T (179 ± 4.5 days) or G (179 ± 4.7 days) males. Females on high mating frequency had shorter lives than females with low mating frequency (169 ± 3.5 [SE] and 180 ± 3.8 days, respectively), when controlling for reproductive rate. Within each genotype, female body size was overall positively related to lifespan (univariate regressions, G: $P = 0.006$, T: $P = 0.003$, C: $P = 0.426$).

TABLE 2. The results of an analysis of covariance, using female lifetime offspring production and female size as a covariates, of the effects of our factorial variables on female lifespan. Residuals from this model did not differ significantly from normality (Kolmogorov-Smirnov one-sample test; $P = 0.110$).

Source	SS	df	F	P
Female genotype	14.612	2	76.836	<0.001
Male genotype	0.708	2	3.725	0.025
Mating frequency	0.628	1	6.607	0.011
Female genotype × male genotype	0.601	4	1.579	0.179
Female genotype × mating frequency	0.072	2	0.378	0.686
Male genotype × mating frequency	0.155	2	0.815	0.443
Female genotype × male genotype × mating frequency	0.209	4	0.549	0.700
Female offspring production	12.091	1	127.150	<0.001
Female body size	1.054	1	11.085	0.001
Error	32.616	343		

Lifetime Offspring Production

A series of factors affected female lifetime offspring production (Table 3). Most importantly, females of the various genotypes differed in their overall fecundity (average offspring production: G = 941 ± 34.7 [SE], T = 647 ± 31.1, and C = 913 ± 35.1). Independent of this effect, however, female fecundity also depended on which male genotype they were paired with (G = 843 ± 30.0 [SE], T = 730 ± 27.6, C = 929 ± 26.7) and was higher under the high-mating-frequency treatment (mean = 891 ± 22.4 [SE]) compared to the low treatment (mean = 777 ± 23.7). Female and male genotype also interacted in their effect on female reproductive output, but this interaction was complex and depended on the mating frequency of females (see three-way interaction in Table 3). Further analysis revealed that the interaction between male and female genotype was significant in the high- ($F_{4,173} = 2.804$, $P = 0.027$) but not in the low- ($F_{4,170} = 2.230$, $P = 0.068$) mating-frequency treatment (see Fig. 2). A closer examination of the high-mating-frequency treatment showed that male genotype significantly affected female lifetime offspring production in two of three female genotypes (Tukey's HSD multiple comparisons). In those genotypes (G and T), females responded least strong to males of their own genotype compared to males of the other two genotypes. In G females, however, no statistical difference could be detected between the response to G and T males ($P = 0.531$), whereas the response to G males differed significantly from that to C males ($P = 0.032$). In T females, the response to T males was significantly lower than either G or C males ($P = 0.006$, $P = 0.006$, respectively). From the male point of view, the offspring production of the mates of a given male genotype differed across female genotypes in two of three cases (T and C). T males paired with T females showed a lifetime offspring production that was lower than when T males were paired with either G or C females ($P < 0.001$, $P = 0.007$, respectively). C males paired with G females showed a lifetime offspring production that was higher than when C males were paired with either T or C females ($P = 0.030$, $P = 0.037$, respectively), but there was no significant difference between the response to T and C females ($P = 1.00$). As was the case for female lifespan, female body size was overall positively related to lifetime fecundity within genotypes (univariate regressions, T: $P = 0.327$, C: $P = 0.246$, G: $P = 0.017$).

Fecundity Functions

A multivariate analysis of covariance (MANCOVA) was performed to determine the simultaneous effects of male genotype, female genotype, mating frequency, and female body size on the shape of the relationship between offspring production and time (see Table 4). A series of factors affected this relationship, one of them being female genotype. T females had the highest initial reproductive rate and also the sharpest drop in reproductive rate, whereas G females kept a fairly low and constant reproductive rate before the drop. C females began at an intermediate reproductive rate and continued with a slow decline for the rest of their reproductive lifespan. Male genotype also affected the shape of the relationship between offspring production and time, in that male genotype affected initial reproductive rates of females. Interestingly, the genotype in which males had the lowest ability to elicit high initial reproductive rate in females (T) also

TABLE 3. The results of an analysis of covariance, using female size as a covariate, of the effects of our factorial variables on female lifetime offspring production. Residuals from this model did not differ significantly from normality (Kolmogorov-Smirnov one-sample test; $P = 0.355$).

Source	SS	df	F	P
Female genotype	5,366,121.546	2	29.659	<0.001
Male genotype	2,456,292.908	2	13.576	<0.001
Mating frequency	1,099,465.277	1	12.154	0.001
Female genotype × male genotype	440,819.660	4	1.218	0.303
Female genotype × mating frequency	1,362,624.820	2	7.531	0.001
Male genotype × mating frequency	94,002.509	2	0.520	0.595
Female genotype × male genotype × mating frequency	1,326,177.687	4	3.665	0.006
Female body size	386,313.310	1	4.270	0.040
Error	3.11192×10^7	344		

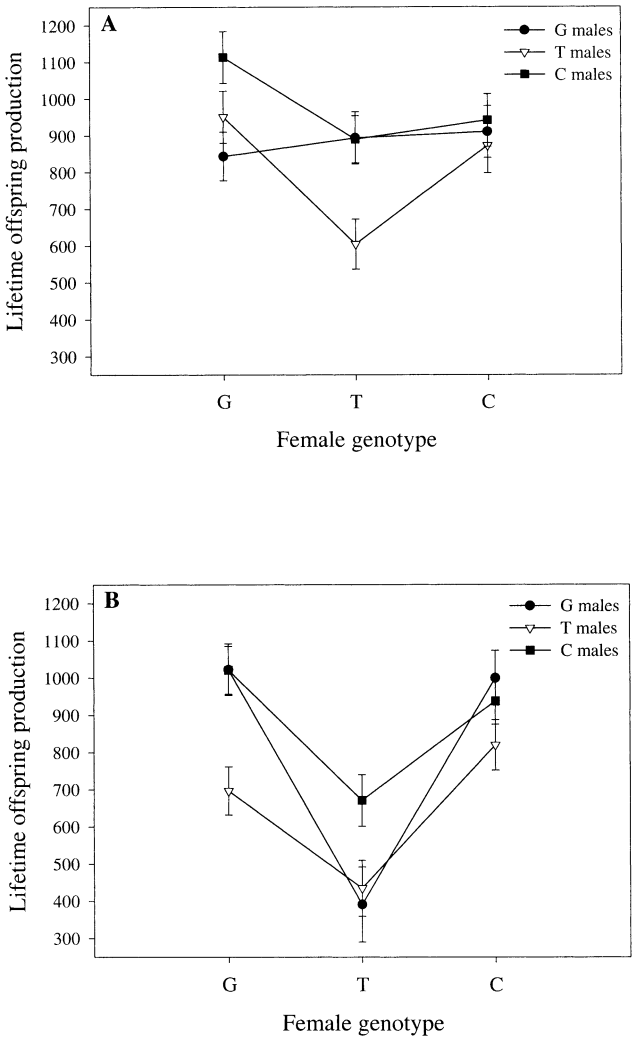

FIG. 2. Average female lifetime offspring production (±SE) under the (A) high- and (B) low-mating-frequency treatments.

have females with the lowest initial reproductive rate. Male and female genotype also interacted in their effect on initial reproductive rate (cf. A in Table 4, Fig. 3). Post hoc tests (Tukey's HSD multiple comparisons) showed that male genotype significantly affected female initial reproductive rate in two of three genotypes (G and T). In G females, the response to their own males was lower than the response to C males ($P = 0.003$), but there was no significant difference between the response to G and T males ($P = 0.431$). In T females, their own males elicited a response that was weaker than with either G males ($P = 0.05$) or C males ($P = 0.029$). From the male point of view, female genotype had no effect on males' ability to influence female initial reproductive rate in C and T males. However, G males mated to females of their own genotype showed a lower female initial reproductive rate than when mated to T ($P = 0.034$) but not to C ($P = 0.575$) females.

To assess how the response variables above relate to one another, we first standardized (zero mean and unit variance) initial reproductive rate, lifetime offspring production, and lifespan within each female genotype. For each female genotype, we then computed the average value when mated to each of the three different male genotypes and correlated these means ($n = 9$). This analysis evaluates the relationship between response variables, while controlling for the effects of female genotype. Initial reproductive rate was positively correlated with female lifetime offspring production ($r = 0.88$, $P = 0.002$), but not with female lifespan ($r = 0.29$, $P = 0.456$). Female lifespan, however, was not correlated with lifetime offspring production ($r = 0.28$, $P = 0.468$).

Male and Female Fertilities

We analyzed variation in female fertility in a generalized linear model of the number of normally developing eggs, using the total number of eggs in each replicate as a binomial denominator (Table 5). Although male genotype significantly affected female fertility, the fertility was very high for all males (G = 0.96 ± 0.005 [SE], T = 0.92 ± 0.01, C = 0.93 ± 0.008). Furthermore, and most importantly, male and female genotype did not interact in affecting fertility.

To ascertain whether difference in fertility between male genotypes might have affected the outcome of our analyses of female offspring production (see above), we repeated all analyses using offspring numbers adjusted for male fertility. However, in no case did these analyses differ from those reported above, in terms of our ability or inability to reject null hypotheses at $\alpha = 0.05$.

DISCUSSION

This study has documented differences between populations in the effects of mating on female reproductive performance. We found that male genotype affects female lifespan, female lifetime offspring production, and female reproductive rate. Male and female genotype also interacted in their effects on female offspring production and reproductive rate. We will now discuss the proximate and ultimate causes of these differences and some of the implications of our findings.

Male Signals in Flour Beetles

Females may respond reproductively to a range of different stimuli provided by males during mating (see introduction). In flour beetles, males produce pheromones (Qazi et al. 1998) that have been suggested to be of importance for mate choice and sperm precedence (Lewis and Austad 1994). Males also perform copulatory courtship during mating by tactile stimulation of female elytra, which is known to affect sperm precedence patterns (Edvardsson and Arnqvist 2000). Several facts also strongly suggest that male flour beetles transfer seminal fluid proteins to females during copulation. First, we found that females under high mating frequency had a higher reproductive rate but also a shorter lifespan compared to females under low mating frequency. These results are consistent with the documented dose-dependent effects of many male ACPs (Eberhard 1996). Second, it has been shown that males of many other Coleopterans produce proteins in their accessory glands that are transferred to the females, where they pass through the walls of the reproductive tracts to the

TABLE 4. Multivariate analysis of covariance of the simultaneous effects of male genotype, female genotype, mating frequency, and female body size on the shape of the relationship between offspring production and time. These parameters represent: *A*, initial reproductive rate; *B*, the rate of decline in reproductive rate; X_0, the location of the fecundity function along the abscissa.

Factor	Wilks' λ	F_1	df	*P*	F_2	df	*P*
Female genotype	0.844	9.606	6, 652	<0.001			
Univariate effect on *A*					5.276	2	0.006
Univariate effect on *B*					4.525	2	0.012
Univariate effect on X_0					23.381	2	<0.001
Male genotype	0.864	8.271	6, 652	<0.001			
Univariate effect on *A*					12.111	2	<0.001
Univariate effect on *B*					1.876	2	0.155
Univariate effect on X_0					0.919	2	0.400
Mating frequency	0.922	9.130	3, 326	<0.001			
Univariate effect on *A*					8.883	1	0.003
Univariate effect on *B*					1.326	1	0.250
Univariate effect on X_0					16.308	1	<0.001
Female genotype × male genotype	0.910	2.608	12, 862	0.004			
Univariate effect on *A*					3.226	4	0.013
Univariate effect on *B*					0.460	4	0.765
Univariate effect on X_0					0.398	4	0.810
Female genotype × mating frequency	0.875	7.513	6, 652	<0.001			
Univariate effect on *A*					9.428	2	<0.001
Univariate effect on *B*					1.816	2	0.164
Univariate effect on X_0					7.917	2	<0.001
Male genotype × mating frequency	0.964	1.984	6, 652	0.066			
Univariate effect on *A*					0.691	2	0.502
Univariate effect on *B*					0.375	2	0.687
Univariate effect on X_0					1.151	2	0.318
Female genotype × male genotype × mating frequency	0.917	2.400	12, 862	0.005			
Univariate effect on *A*					1.316	4	0.264
Univariate effect on *B*					2.564	4	0.038
Univariate effect on X_0					0.984	4	0.416
Female body size	0.988	1.311	3, 326	0.271			
Univariate effect on *A*					2.809	1	0.095
Univariate effect on *B*					0.016	1	0.900
Univariate effect on X_0					0.662	1	0.417

[1] Rao's *F*.
[2] Univariate *F*-test (ANOVA).

haemolymph and affect their reproduction (Das et al. 1980; Huignard 1983; Boucher and Huignard 1987; Rooney and Lewis 1999). In *Tenebrio molitor*, confamiliar to *Tribolium*, one such ACP has been shown to have high sequence similarity to *Drosophila* ACPs (Feng and Happ 1996). Third, *T. castaneum* males are indeed equipped with two pairs of large accessory glands (Sokoloff 1972), the only known function of which is to synthesize proteins.

Although the effects detected in this study could be mediated by any signal/stimulus provided by males, we suggest that differences in male ACPs and female receptivity to these across genotypes are responsible for the observed results. The occurrence of ACPs and their effects on female physiology has been well documented in a wide range of insect taxa, such as Lepidoptera, Orthoptera, Coleoptera, Hemiptera, and Diptera (Davey 1958; Pickford et al. 1969; Yamaoka and

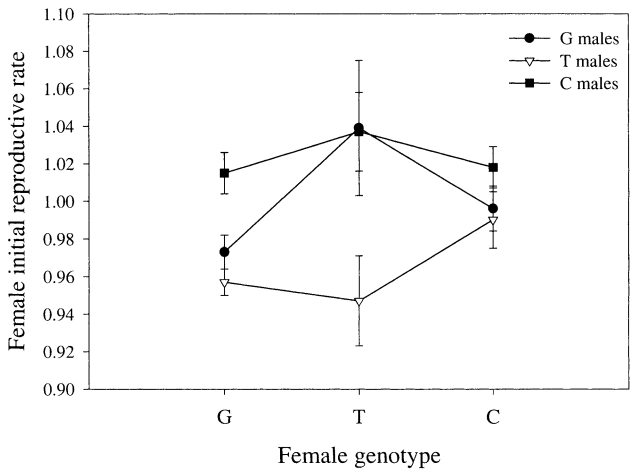

FIG. 3. Average female initial reproductive rate (log-transformed, ±SE), estimated from fecundity functions, for each male-female genotype combination.

TABLE 5. The results of a generalized linear model, using binomial errors and a logit link function, of variance in the proportion of developing eggs (i.e., female fertility). The full model was significant (*LLR* = 17.4, df = 8, *P* = 0.026). The contribution of each source was tested by analysis of deviance, by deletion of (1) each main factor from a model including both main factors only; and (2) the interaction from the full model.

Source	*LLR*[1]	df	*P*
Female genotype	0.56	2	0.756
Male genotype	14.85	2	0.001
Female genotype × male genotype	0.91	4	0.923

[1] To compensate for overdispersion (McCullagh and Nelder 1989) the method of Williams (1982) was implemented prior to statistical inference.

Hirao 1977; Chen 1984; Simmons and Gwynne 1991; Ramaswamy et al. 1997; Andersson et al. 2000). ACPs are well known to include substances that affect female egg production (Chen et al. 1988; Herndon and Wolfner 1995; Chen 1996; Gillott 1996; Wolfner 1997; Heifetz et al. 2000) and are known to evolve at a high rate (Aguade et al. 1992; Thomas and Singh 1992; Civetta and Singh 1995). We are unaware of support for the possibility that male pheromones affect female egg production in *Tribolium*.

Divergence in the Effects of Mating

Our results show that the male signal–female receptor system differs between the strains studied, and that this evolutionary divergence involves qualitative aspects of the signals and/or receptors involved rather than merely quantitative alterations. For example, the patterns observed do not fit a simple dose-dependency scenario (assuming that the amount of signals per mating is similar across male genotypes). Females mated to C males exhibited higher offspring production and shorter lifespan than did females mated to any other males, even though the former females did not mate more frequently and C males did not have higher fertility than other males. Similarly, T males exhibited the highest mating rates, but females mated with T males overall showed low egg production. Most importantly, however, the interactions between male and female genotype show that the effects are not only due to quantitative variation across males in, for example, the amount of signals provided, but that male signals and female receptivity have diverged in a more complex and qualitative manner (cf. Lewis and Austad 1990; Wilson et al. 1997; Clark et al. 1999; Andrés and Arnqvist 2001). The fact that there was no male × female interaction in our fertility experiment shows that our results are not simply due to inherent incompatibilities between genotypes.

Divergence between allopatric populations in the reproductive effects of mating can be generated by several processes. Random changes in the traits involved, by founder effects and/or genetic drift, can clearly cause such differences. However, this seems highly unlikely in our case for several reasons. The traits involved obviously mediate important fitness components in both sexes and should therefore be subject to strong selection. This is supported not only by observations of very rapid rates of evolution of reproductive proteins (e.g., Aguade et al. 1992; Civetta and Singh 1995) but also by direct demonstrations of selection on such traits (Tsaur and Wu 1997; Aguade 1999; Swanson et al. 2001). Differences in the reproductive effects of mating could also arise as pleiotropic side effects of adaptation unrelated to sexual selection. Again, this seems unlikely because most of the reproductive proteins thought to be responsible for such effects are produced in highly specialized reproductive glands (e.g., Gillott 1988, 1996). More importantly, however, the pattern of male × female interactions documented here is not consistent with either of these two possibilities (see below).

Alternatively, sexual selection via either sexually antagonistic coevolution (Rice 1996, 2000; Andrés and Arnqvist 2001; Gavrilets et al. 2001) or male-female coevolution driven by indirect benefits to females (Sheldon 2000; Colegrave 2001; Cunningham and Russel 2001; Gil and Graves 2001) can generate divergence in the effects of mating. Clark et al. (1999) and Andrés and Arnqvist (2001) both pointed out that the pattern of male × female genotypic interactions should differ under the various contending processes. In short, random processes as well as pleiotropy should both result in a pattern where females exhibit an average relative reproductive response to males from their own population and/or strain. In contrast, females should evolve resistance to males with which they are coevolved if divergence is driven by sexually antagonistic coevolution, due to fitness costs of male adaptations, thus responding weaker than average to their ''own'' males (see also Parker and Partridge 1998). Under the alternative hypothesis, divergence through indirect benefits, females should evolve preference for male signals, thereby responding stronger than average to males with which they are coevolved.

The male × female interactions detected here are complex and not entirely consistent. Furthermore, the limited number of populations used does not allow us to critically test the statistical associations mentioned above and thus does not permit firm conclusions about evolutionary processes. Nevertheless, a closer inspection of the male × female interaction in the effect of mating on lifetime offspring production (i.e., under high mating frequency) does show that females responded least strong to males of their own genotype in both cases in which male genotype had a significant effect on female lifetime offspring production (Fig. 2A). Similar male × female interactions were seen in the effects of mating on initial female reproductive rate. Male genotype significantly affected female initial reproductive rate in two of three genotypes. In one of these cases, female reproductive rate was significantly lower with males with which females had coevolved than with any other genotype. In the other case, the reproductive rate with coevolved males was, together with a second male genotype, significantly lower than with the third genotype. These results indicate that female reproductive response typically was lower than average when exposed to males with which females share a coevolutionary history. The lifetime offspring production elicited by males in the high-mating-frequency treatment differed significantly across female genotypes in two of three cases. In one of these cases, offspring production elicited by males was significantly lower with females with which they had coevolved than with any other genotype. In the other case, offspring production elicited by males was, together with a second female genotype, significantly lower with their own females than with the third genotype. This was true also in the only genotype in which the female initial reproductive rate elicited by males differ significantly across female genotypes. From the male point of view, thus, males tended to elicit a relatively weak response in females with which they had coevolved. In summary, our collective results are most consistent with the pattern predicted by sexually antagonistic coevolution. They are also in line with data presented in some recent studies of Diptera (Clark et al. 1999; Civetta and Clark 2000; Andrés and Arnqvist 2001) in indicating that females have evolved resistance to signals provided by males.

Two correlations across the sexes lend some further support to the suggestion that sexually antagonistic coevolution is a key process in flour beetles. First, the genotype with

males most able to achieve matings has females that are least willing to mate. Such correlation between male persistence and female resistance has also been documented across *Drosophila* populations (see Parker and Partridge 1998) and is predicted by sexually antagonistic coevolution theory (Parker 1979). Second, males least able to elicit high initial reproductive rate among females have females who respond strongest to males in terms of elevated initial reproductive rate. Again, this is predicted by sexually antagonistic coevolution theory (Gavrilets et al. 2001): Females can afford a low resistance to males in genotypes in which males are inefficient at manipulating female reproductive rate.

It might seem counterintuitive that females under high mating frequency did worse, in terms of lifetime offspring production, when mated to males of their own genotype given that they should have evolved adaptive resistance to these males (Gavrilets et al. 2001). Initial reproductive rate was also positively correlated with female lifetime offspring production within female genotypes. We suggest that these results are due to the fact that our assay was performed under conditions different from those under which females have adapted, in the following way. The optimal female reproductive rate in *Tribolium*, as in any other iteroparous species, will represent a trade-off between the costs and benefits of elevated current reproductive effort (Roff 1992; Stearns 1992). Thus, at this optimum, any increase in reproductive rate will be more than offset by a decrease in offspring quality and/or survival. Partial female resistance to male signals with gonadotropic effects is likely to reflect females striving toward their optimum in the face of male gonadotropic manipulation. However, when competition is relaxed, optimal female reproductive rate is elevated. In our assays, we provided superabundant food and competition was essentially absent. It is thus likely that an elevated reproductive rate translated into a higher lifetime offspring production in these assays, even though this might have been detrimental to female fitness in the crowded and relatively harsh conditions of their ''natural'' environment (i.e., culture containers). Thus, total lifetime offspring production is a very poor estimator of female fitness in our experiments. Our results illustrate that one should be cautious when extrapolating differences in reproductive responses into differences in net fitness. If the purpose of a study is to quantify net fitness, this should be done in an environment to which the experimental organisms are adapted.

Conclusions

We have documented evolutionary divergence in the effects mating has on female reproduction in flour beetles. Our results thus suggest that selection mediated by differences in female reproductive rate (i.e., differential allocation) can contribute to the evolution of reproductive isolation and that divergence in reproductive characters may result from this form of postmating sexual selection (see also Andrés and Arnqvist 2001). We have also identified a series of complex interactions between male and female genotypes. The pattern of these interactions was most consistent with that expected if divergence was driven by sexually antagonistic coevolution. Most studies of this phenomenon to date have focused on patterns of sperm precedence and the potential sexual conflicts that concern female sperm utilization (e.g., Robinson et al. 1994; Wade et al. 1994; Price 1997; Clark et al. 1999; Howard 1999; Civetta and Clark 2000). We stress that sexual conflict over female reproductive effort can also provide ample fuel for sexually antagonistic coevolution (see also Parker and Partridge 1998; Rice 2000), and male signal–female receptor systems involved in the regulation of reproductive rate may thus evolve rapidly. Our study is thus consistent with a line of recent studies indicating that postmating sexual conflict may be an important engine of speciation (Price 1997; Parker and Partridge 1998; Rice 1998; Clark et al. 1999; Gavrilets 2000; Gavrilets et al. 2001; Arnqvist et al. 2000).

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