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Populations with elevated mutation load do not benefit from the operation of sexual selection

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sexually antagonistic coevolution.

Abstract

Theory predicts that if most mutations are deleterious to both overall fitness and condition-dependent traits affecting mating success, sexual selection will purge mutation load and increase nonsexual fitness. We explored this possibility with populations of mutagenized *Drosophila melanogaster* exhibiting elevated levels of deleterious variation and evolving in the presence or absence of male–male competition and female choice. After 60 generations of experimental evolution, monogamous populations exhibited higher total reproductive output than polygamous populations. Parental environment also affected fitness measures – flies that evolved in the presence of sexual conflict showed reduced nonsexual fitness when their parents experienced a polygamous environment, indicating trans-generational effects of male harassment and highlighting the importance of a common garden design. This cost of parental promiscuity was nearly absent in monogamous lines, providing evidence for the evolution of reduced sexual antagonism. There was no overall difference in egg-to-adult viability between selection regimes. If mutation load was reduced by the action of sexual selection in this experiment, the resultant gain in fitness was not sufficient to overcome the costs of sexual antagonism.

Introduction

The relationship between nonsexual and sexual fitness remains a fundamental question in evolutionary biology. One possibility, suggested first by Darwin (1859), is that sexual selection will 'give its aid to ordinary selection'. This idea that sexual selection will favour well-adapted males developed into modern good genes theory, which explains how females can bear the cost of choosiness and suggests population-level benefits to the operation of sexual selection, including accelerated adaptation (Proulx, 1999; Lorch *et al.*, 2003) and purification of the genome (reviewed in Whitlock & Agrawal, 2009). Alternatively, the conflict of interests between the sexes may lead to antagonistic coevolution and depress nonsexual fitness. There is a growing body of evidence that there are direct costs to female fitness traded off for direct benefits to male fitness in many systems (Arnqvist & Rowe, 2005).

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A particularly powerful approach to measuring fitness effects has been manipulating the presence or absence of sexual selection in laboratory populations for one or several generations. This approach has given mixed results. Female choice leads to increased viability in some studies (Partridge, 1980; Promislow *et al.*, 1998), and the presence of sexual selection can help populations adapt to novel environments (Fricke & Arnqvist, 2007) and purge deleterious variation (Hollis *et al.*, 2009). However, the majority of recent work based on these multi-generation mating system manipulations has reported no benefit to populations experiencing sexual selection (Holland & Rice, 1999; Holland, 2002; Radwan *et al.*, 2004; Rundle *et al.*, 2006; Maklakov *et al.*, 2009).

The fact that both increases and decreases in nonsexual fitness have been observed makes it clear that the real unknown is the balance of costs and benefits accrued through sexual selection. This is an empirical question, and quantifying the relative magnitudes of the opposing forces will clarify whether there is a net advantage to populations experiencing sexual selection. An important caveat is that this balance is likely to differ across taxa with different mating systems; costs or benefits of sexual

selection determined for a population of promiscuous insects may not apply to, for example, a monogamous bird with parental care. Still, uncovering the pattern of antagonistic adaptations and indicators of genetic quality in any one system will go a long way towards strengthening our predictions across taxa.

The difficulty in establishing a clear picture is surprising, as we should expect the effects of sexual selection to be far-reaching. Sexual selection operates on more than just showy characters – indeed, whenever sexually selected traits are costly to produce, they should evolve to be an honest indicator of quality through the process of 'genic capture' (Rowe & Houle, 1996). As genetic variation for sexually selected traits is exhausted, theory predicts new variation will be recruited into building those traits. Through this process, much of the genome may become involved in the expression of sexually selected traits.

If sexual selection targets loci throughout the genome, it should amplify the effects of nonsexual selection and help to reduce genetic load. Few studies have directly tested whether this happens in populations. Radwan *et al.* (2004) relaxed nonsexual selection in bulb mite populations and found that the resultant decline in fecundity as deleterious mutations accumulated was not influenced by the presence of sexual selection. However, Radwan (2004) later found in a single-generation experiment that populations of irradiated bulb mites in which sexual selection operated showed elevated viability relative to populations where sexual selection was removed.

We addressed the question of whether or not sexual selection provides a benefit to populations with long-term experimental evolution of *Drosophila melanogaster* exhibiting increased levels of deleterious genetic variation. We did this by exposing flies to a mutagen and then manipulating the presence or absence of male–male competition and female choice. We then measured fitness components of flies from both mating treatments after 60 generations of experimental evolution, allowing us to observe any differences between treatments that evolved.

Materials and methods

Fly stocks and rearing

The experiments were carried out with a long-term laboratory population (the IV population) that was initiated from about 200 wild *D. melanogaster* of each sex collected in Massachusetts in 1975 (Charlesworth & Charlesworth, 1985). The IV population is maintained at 25 °C in ten bottles on a 14-day schedule and a 12L : 12D cycle with mixing between bottles every generation. The population is extremely crowded, increasing mortality at all phases of the life cycle. Selection on development time is particularly strong, as there is only time for one generation in each cycle. Previous work has characterized selection in these populations (Houle & Rowe, 2003) and shown only a narrow window of time (approximately 2 days) in which any laid eggs will reach adulthood before the next transfer. A particular advantage of the IV population is that it has adapted to this same environment for over 750 generations, and so what constitutes fitness is well understood.

Fitness assays involving a competitor used a second laboratory population derived from the IV population that carries a recessive *ebony* mutation (IVe). The IVe population was established in 1992 after a spontaneous *ebony* mutation was repeatedly backcrossed into the larger IV population. IVe has been maintained in the laboratory for more than 350 generations and, because of their darker coloration, provide a competitive standard easily distinguished from flies in our experimental populations.

Mutagenesis

Approximately 1000 male and 1000 female virgins were collected from the base IV population. These flies were starved for a 12-h period and then placed for 12 h in vials with filter paper soaked in a solution of 2.5 mM ethyl methanesulphonate (EMS) in 1% sucrose. EMS is a potent mutagen that produces primarily single base pair changes (Ashburner, 1989), and past work has shown that lethal mutation rate elevated from seven-fold (Huang & Baker, 1976) to > 50-fold (Ohnishi, 1977) at this dose. Fitness effects at this dose are also well studied; Keightley & Ohnishi (1998) showed significant decreases in viability, fecundity, hatchability, developmental time, longevity and mating speed.

In order to directly estimate the amount of induced mutation in our experiment, a standard test for detecting induced lethal mutations was performed (Fig. 1). First, immediately after mutagenesis, 250 EMS-treated males were mated with 250 FM7 females. FM7 is a first

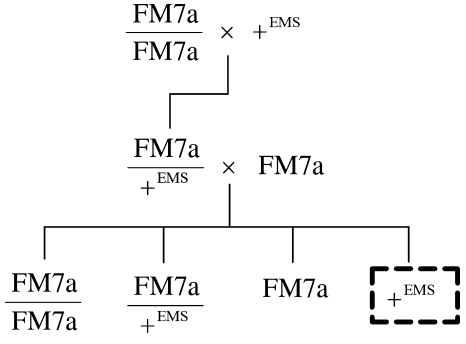

Fig. 1 Crossing scheme to detect recessive lethal mutations on chromosome I (the sex chromosome). The absence of wild-type males (indicated by the dashed box) implies a novel lethal mutation induced by mutagenesis.

chromosome (the sex chromosome) balancer that carries markers for yellow body coloration and white eyes and also prevents recombination, so the female progeny of these crosses received an FM7 chromosome from their mothers and an X chromosome from their mutagen-treated fathers. To determine whether that X chromosome is lethal in homozygous state, the female offspring of this cross were crossed again to FM7 males. All male offspring from this cross were then collected and scored for phenotypically yellow body and white eyes. If the X chromosome treated with EMS carried a lethal mutation, all males had a yellow body and white eyes and no wild-type males emerged from the cross. As a control, 80 untreated males were crossed in the same manner.

Measuring fitness deficits of mutagenized flies

In order to assess the initial fitness deficits of the mutagenized flies from which our experimental populations would be drawn, three different assays were performed one generation after mutagenesis. Competitive egg-to-adult viability was measured by placing groups of 50 eggs from either the mutagenized population or our IV control population into bottles in which 50 IVe females had been laying eggs for 12 h. IVe females were then returned to these bottles and continued laying eggs for the next 2 days, creating intense competition between IVe larvae and the larvae of interest. All eclosing flies were collected from day 9–14, and the number of the 50 transferred eggs reaching adulthood was scored.

Fecundity was measured by placing groups of 3-day-old virgin males and females into vials (three males, three females) and counting the number of eggs laid during a 12-h light period. These groups were transferred to holding vials overnight and in the morning placed into fresh vials for another 12 h of egg-laying. This was repeated once more, so that each group of flies' eggs was counted across three consecutive broods, and the total number of eggs laid was used as a measure of fecundity.

Male mating success of control and mutagenized flies was measured by placing five virgin males of interest with five IVe virgin females and five IVe virgin male competitors. After 2 days, all of the flies from these vials were discarded. Offspring emerging from days 9–14 were collected, and the proportion of each brood that was phenotypically wild type (as opposed to *ebony*-bodied) was used as a measure of male mating success.

Beginning the experiment

After one generation of mass breeding, the mutagenized IV population was subdivided into six replicate populations, each initiated from 100 virgin males and 100 virgin females. Three of these replicates experienced sexual selection (the polygamous mating system or S+) for the remainder of the experiment and three did not (the monogamous mating system or S−). Replicate numbers 1, 2 and 3 were arbitrarily assigned within each treatment.

Manipulation of sexual selection

In order to enforce monogamy in S− populations, each generation virgin females were randomly paired with one virgin male apiece and allowed to spend 2 days mating in these interaction vials. In contrast, in S+ populations, groups of five virgin females were combined with groups of five virgin males in vials and also allowed to spend 2 days mating. In both S− and S+ treatments, several extra interaction vials were made each generation to make up for any mortality.

After 2 days of opportunity for sexual selection, males from both treatments were discarded as were the interaction vials. Females from each replicate were placed into two bottles, 50 females per bottle. The mated females spent the next 3 days laying eggs in these bottles before also being discarded. These bottles were the source of the next generation's flies. This transfer of females to bottles for egg-laying (in the absence of males) helps to confine our manipulation to pre- and postcopulatory sexual selection while equalizing nonsexual selection across treatments. Nine or ten days after the initial set-up of these bottles, virgin collections began and continued until enough flies were collected to begin the next generation. These virgins were then passed back through the experimental treatment.

Measuring net reproductive rate

Net reproductive rate of S− and S+ populations was measured after 60 generations of experimental evolution. Prior to measurement, all experimental populations experienced two generations of a monogamous mating environment in order to remove any effects of antagonism between the sexes that persisted across generations.

Groups of three virgin females and three virgin males (all 3 days old) from each replicate population were placed in vials (n = 30 vials per replicate population). These flies were allowed to mate and deposit eggs continuously for 3 days before being discarded. The total number of flies produced by these vials represented net reproductive output.

Measuring fecundity and egg-to-adult viability

Fecundity and egg-to-adult viability of S− and S+ populations were also measured after 60 generations of experimental evolution. Preliminary measures during the course of experimental evolution had suggested a fecundity deficit in polygamous populations. The measured flies in these early estimates were collected directly from the population bottles, leaving open the possibility that trans-generational effects of polygamous and monogamous mating environments influenced our

measures. In order to control for this, all experimental populations were simultaneously reared under both mating treatments for one generation before components of fitness were measured.

Groups of three virgin females and three virgin males (all 3 days old) from each replicate population were placed in vials for three consecutive 12-h light cycles as in the original fecundity assay of the mutagenized population ($\overline{n}$ = 41 groups in the 12 replicate/parental mating environment combinations). Fecundity was measured as the number of eggs laid by each group of flies across these three broods. Egg-to-adult viability was determined by the proportion of these eggs that reached adulthood.

Statistical analysis

The fitness effects of mutagenesis were evaluated using a linear model with treatment (mutagenized or control) as a fixed effect. For the experimental evolution results, a generalized linear mixed model was fit using PROC GLIMMIX in SAS© version 9.1 (SAS Institute, 2003). Because parental environment was controlled (two generations of monogamy), the analysis of net reproductive rate included only the fixed effect of mating treatment and random replicate effects.

Measures of fecundity and egg-to-adult viability were each modelled with mating treatment and parental mating system as fixed effects and replicate as a random effect. For the egg-to-adult viability data, where the measure obtained was a proportion, egg number was also included as a covariate because density is an important determinant of the proportion of flies reaching adulthood in a vial.

Results

Mutagenesis

Our mutagenized flies experienced a lethal mutation rate of 0.152 (29 of 191 successful crosses resulted in no male offspring carrying the mutagenized first chromosome). There were no lethals detected in control crosses. Estimates of the spontaneous lethal mutation rate for the first chromosome vary between 0.001 and 0.003. Woodruff *et al.* (1983), summarizing the results of many studies, give a best estimate of 0.0016. At this spontaneous lethal rate, our mutagenesis treatment was roughly equivalent to 95 generations of spontaneous mutation accumulation.

Estimates of the fitness deficits for mutagenized flies are shown in Fig. 2. Egg-to-adult viability in the presence of a standardized competitor (IVe) was significantly higher in control populations (37.50 ± 1.06 flies) than in the mutagenized population (31.58 ± 1.33 flies) (Fig. 2a, F = 12.16, d.f. = 1, 22, P < 0.01). Fecundity, however, was not different between populations, with

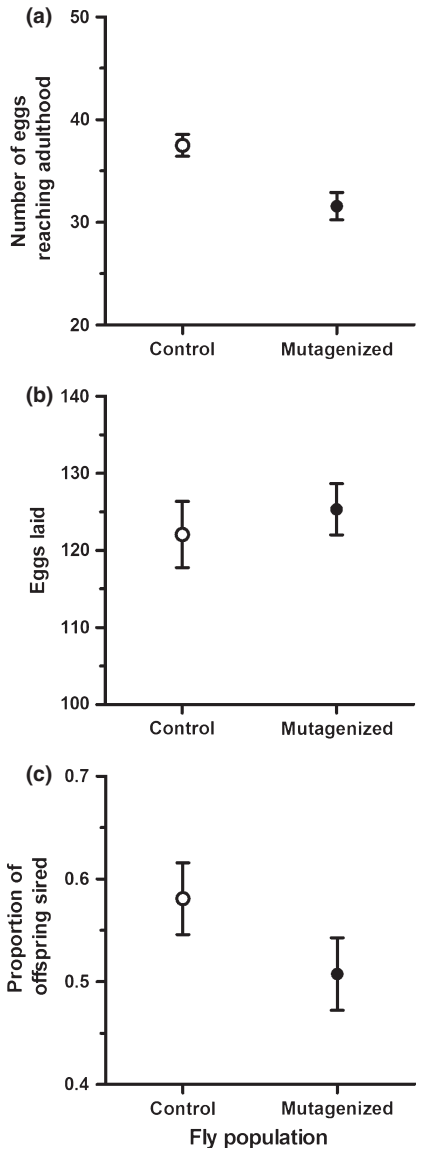

Fig. 2 Fitness estimates for mutagenized and control populations. Mutagenized flies exhibited reduced competitive egg-to-adult viability (a) but were not significantly different than the control population in measures of (b) fecundity and (c) competitive mating success.

control flies laying 122.08 ± 4.29 eggs and mutagenized flies laying 125.36 ± 3.32 eggs (Fig. 2b, F = 0.37, d.f. = 1, 98, P = 0.55).

Male mating success appeared reduced in the mutagenized population relative to the control, with mutagenized flies securing approximately half of an IVe female's brood (0.51 ± 0.04) whereas control flies sired a larger proportion in our trials (0.58 ± 0.03), but this difference was not significant (Fig. 2c, F = 2.20, d.f. = 1, 83, P = 0.12).

Experimental evolution

We measured fitness after 60 generations of mating system manipulation and two generations of monogamy (net reproductive rate) or one generation of rearing all populations in both mating environments (fecundity and egg-to-adult viability). Results of the statistical analysis of fitness measures are summarized in Table 1.

Net reproductive output after two generations of monogamous rearing was significantly higher in the S− treatment (126.59 ± 2.16 flies) than in the S+ treatment (117.60 ± 2.17 flies) (Fig. 3). This productivity assay is similar to the rearing protocol over the course of experimental evolution, with competition between adult flies extending several days and a high density of eggs and developing larvae.

Total fecundity – the sum of all eggs laid across three consecutive broods – was not significantly different between selection treatments. The mating environment of parents of measured flies did matter, however (Fig. 4a). This effect of parental rearing environment also differed between mating treatments (marginally significant, Table 1), with S+ flies showing a marked decline in the number of eggs laid if their parents experienced a polygamous mating environment (125.49 ± 6.86) vs. a monogamous parental mating environment (140.23 ± 7.23). S− flies showed the opposite pattern (125.80 ± 7.04 with monogamous parents and 129.76 ± 6.64 with polygamous parents), indicating reduced costs of sexual interactions in monogamous populations. There was also a significant brood effect, with all lines laying more eggs in the first 12-h window than in either of the later two laying periods (Fig. 4b). Lines from the S+ and S− treatments showed a different pattern of decline in the experiment (mating treatment × brood interaction in Table 1) with S+ fecundity dropping more quickly (48% decline from first to second brood if parents were monogamous, 46% decline if parents were polygamous) than S− fecundity (29% decline with monogamous parents, 34% decline if parents were polygamous).

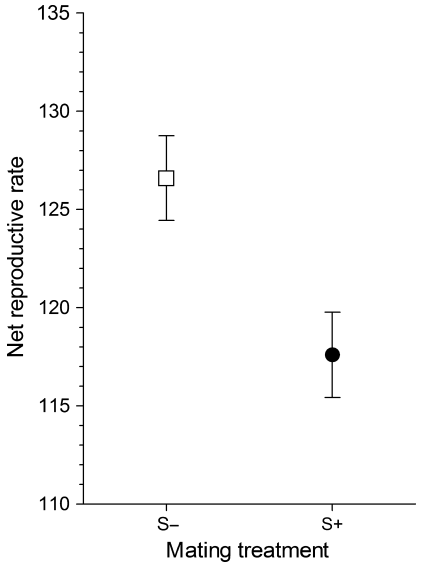

Fig. 3 Net reproductive rate after 60 generations of experimental evolution and two consecutive generations of monogamous rearing.

Table 1 Results of mixed model analysis testing the effects of sexual selection on experimental populations' (a) net reproductive rate, (b) fecundity and (c) egg-to-adult viability.

Source	d.f.	*F*	*P*
(a) Net reproductive rate			
Mating system (S+ vs. S−)	1, 4	8.62	0.043
(b) Fecundity			
Mating system (S− vs. S+)	1, 4	0.12	0.751
Parental environment (S− vs. S+)	1, 4	8.38	0.044
Mating system × parental environment	1, 4	6.74	0.060
Brood	2, 8	214.15	< 0.001
Mating system × brood	2, 8	14.06	0.002
(c) Egg-to-adult viability			
Mating system (S+ vs. S−)	1, 4	0.50	0.520
Parental environment (S− vs. S+)	1, 4	0.75	0.435
Mating system × parental environment	1, 4	3.13	0.151
Brood	2, 8	5.26	0.035
Mating system × brood	2, 8	7.12	0.017
Eggs	1, 1424	14.20	< 0.001

Egg-to-adult viability followed a generally similar pattern to the fecundity results. There was no significant difference between S− and S+ treatments in overall egg-to-adult viability, and there was no significant effect of the parental mating environment (Fig. 5a). As with the fecundity measures, egg-to-adult viability decreased in the S+ treatment when parents of assay flies were reared in a polygamous environment (76.4 ± 2.6% of eggs reaching adulthood) vs. parents reared in a monogamous environment (79.7 ± 2.4% of eggs reaching adulthood) but stayed relatively constant in the S− treatment (80.4 ± 2.3% vs. 80.1 ± 2.4%). Unlike for the fecundity measures, however, this mating treatment × parental environment interaction was not significant. In addition to an effect of brood number and egg density on egg-to-adult viability, there was a mating treatment × brood interaction (Table 1) where the S− treatment showed increased egg-to-adult viability in later broods whereas S+ egg-to-adult viability remained relatively constant (Fig. 5b).

Discussion

The multitude of predictions about sexual selection's potential benefits to populations – increased average nonsexual fitness, accelerated adaptation, purified genomes and an alleviated cost of sexual reproduction – are

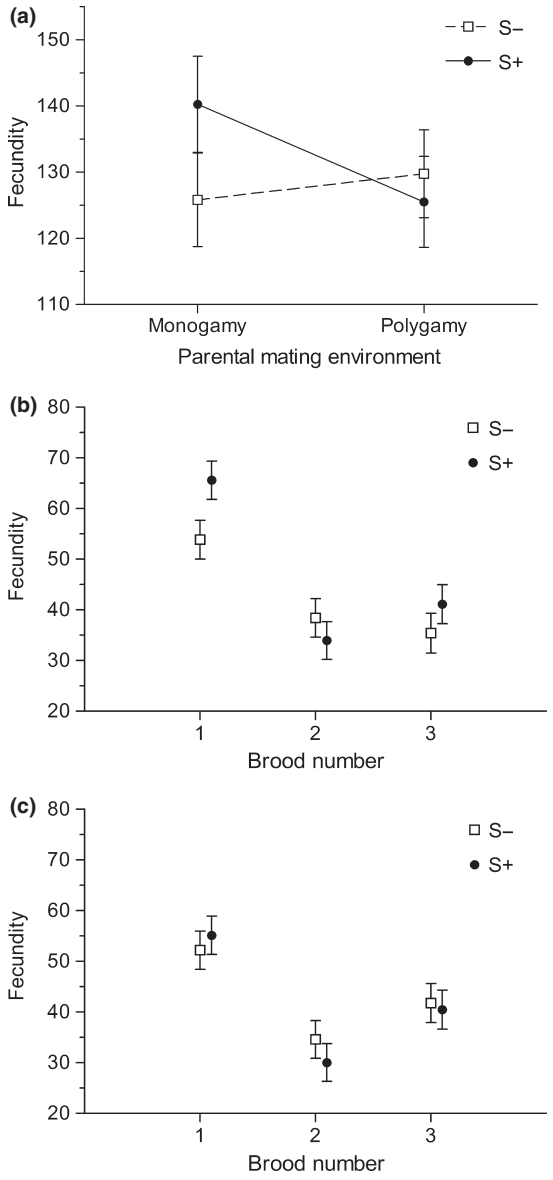

Fig. 4 Fecundity of S+ and S− lines after 60 generations of experimental evolution when the parents of assay flies were reared in either monogamy or polygamy. (a) Total fecundity over three sequential broods. (b) Fecundity of evolved lines when parents were monogamous, by brood. (c) Fecundity of evolved lines when parents were polygamous, by brood.

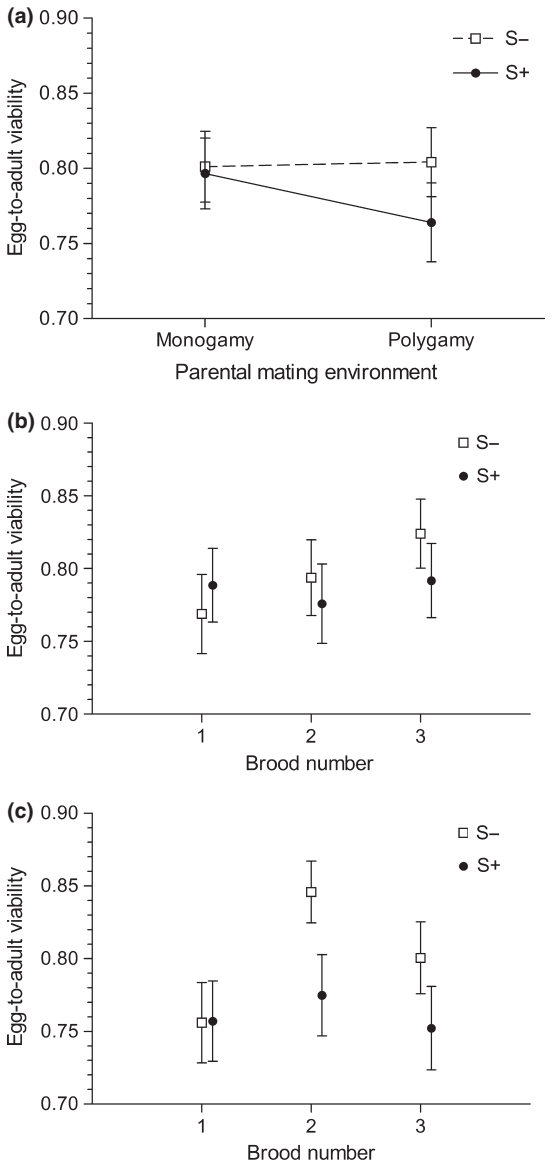

Fig. 5 Egg-to-adult viability of S+ and S− lines after 60 generations of experimental evolution when the parents of assay flies were reared in either monogamy or polygamy. (a) Total egg-to-adult viability over three sequential broods. (b) Egg-to-adult viability of evolved lines when parents were monogamous, by brood. (c) Egg-to-adult viability of evolved lines when parents were polygamous, by brood.

intuitively appealing because they offer an explanation for why females might bear the costs of being choosy. Also, because of the strong theoretical foundation behind 'good genes' thinking and evidence for the benefits of sexual selection in manipulated populations (Fricke & Arnqvist, 2007; Hollis *et al.*, 2009), these predictions are not easily dismissed. The main result of the experiment reported here is clear: even when substantial deleterious variation is present in a laboratory population of *D. melanogaster*, any benefits conferred by the operation of sexual selection are outweighed by its costs.

In our measures of components of fitness, there was no overall effect of mating treatment on either fecundity or egg-to-adult viability. However, there was a significant decline in fecundity for S+ lines if parents were polygamous, and this decline was absent in S− lines.

Egg-to-adult viability showed a similar but nonsignificant trend. This indicates trans-generational effects of the mating environment of assayed flies' parents on measures of fitness, a phenomenon that has been reported in other invertebrates (Bernasconi & Keller, 2001; Tregenza *et al.*, 2003; Kozielska *et al.*, 2004). Making any comparison between treatments without controlling for these parental effects is a major problem that renders the findings of several studies (e.g. Rundle *et al.*, 2006 and Fricke & Arnqvist, 2007) difficult to interpret.

In addition to the trans-generational costs of sexual antagonism to components of fitness, there is also evidence for within-generation costs. Fecundity of S+ flies declines more sharply in later broods than does the fecundity of S− flies. There is also a significant difference between the treatments in the change in egg-to-adult viability across broods. Viability increases in later broods of S− flies while staying relatively constant in S+ flies. These results are consistent with the marked difference between treatments in our net reproductive rate assay, as this harsher environment compounds effects of antagonism between the sexes. The detectable difference between treatments in net reproductive rate after 60 generations of experimental evolution but not individual components of fitness highlights the context-sensitivity of fitness measures and also the inherent difficulties of estimating absolute fitness from individual components.

The likely explanation for increased reproductive output in monogamous populations is the evolution of decreased manipulation of females by males. This is because the relaxation of competition for mating opportunities in the monogamy treatment and the ample 2-day window for mating relax selection on male competitive ability, whereas any costs of male persistence to female reproductive success must necessarily reduce male reproductive success.

The differing effects of parental polygamy (costly for evolved S+ lines but not for evolved S− lines), along with the greater reduction in measures of fitness in S+ lines across broods as they are housed with S+ males, all provide evidence for the evolution of reduced male harm in monogamous populations. This change in males either could be precopulatory, for example through less vigorous courtship and harassment of females, or could be occurring in a postcopulatory fashion, for example by reduced transfer of harmful male accessory gland proteins (ACPs). There is evidence that both pre- and post-copulatory events can increase male representation in future generations while simultaneously harming female reproductive success (Chapman *et al.*, 1995; Linder & Rice, 2005; Stewart *et al.*, 2005). Our results are consistent with postcopulatory male manipulation, as the role of many ACPs aligns with our data (e.g. increased egg-laying for only 1 day after mating, Herndon & Wolfner, 1995; a cost to mating for females, Chapman *et al.*, 1995; Wigby & Chapman, 2005), and past work has shown the evolution of enlarged accessory glands in populations with elevated promiscuity (Crudgington *et al.*, 2009).

Future research into the proximal mechanisms of changes in components of fitness after relaxation of sexual selection is likely to be informative, particularly in light of a similar pattern of reduced female fecundity detected in *Drosophila pseudoobscura* (Crudgington *et al.*, 2010). One way to approach this question is with functional studies of ACPs (e.g. Mueller *et al.*, 2008) or other sexually antagonistic traits coupled with genome-wide sequence and expression analysis of experimentally evolved monogamous and polygamous populations.

Although our work makes it clear that in laboratory populations of *D. melanogaster* sexual selection depresses nonsexual fitness by imposing a sexual conflict load, a role for sexual selection in facilitating the purging of deleterious mutations remains plausible. It is possible that in polygamous populations sexual selection is cleansing the genome while males and females concurrently experience an ongoing sexual arms race. This may be difficult to detect in experimental evolution studies lasting only tens of generations. The experiment reported here has 'stacked the deck' in favour of picking up this adaptive signal in several ways. First, females have the opportunity to choose between males and remate, but their period of confinement with males is limited to 2 days. Also, unlike in similar studies with heavily male-skewed sex ratios, females in the polygamous treatments in this experiment experience an equal sex ratio during the mating phase. Finally, substantial deleterious variation was present in these populations at the outset. These factors taken together should amplify the potential for sexual selection to accelerate adaptation while reducing the effects of conflict, and yet no advantage to sexual selection is seen. It is worth noting, however, that in the one situation in our experiment in which the negative effects of the sexual arms race are reduced (the first brood of flies, before there has been extended opportunity for harm to females, and when parents were reared monogamously and trans-generational effects removed), S+ flies demonstrate dramatically higher fecundity than S− flies.

The potential benefits of sexual selection are expected to be persistent due to an unremitting supply of slightly deleterious mutations. It is possible that the mutations induced by mutagenesis in this study were highly deleterious and therefore purged completely by populations from both selection regimes before measurements of fitness were conducted at generation 60. This is unlikely, however, as a large body of work on EMS mutagenesis indicates that changes in trait values are caused by many mutations of small effect (Keightley & Ohnishi, 1998; Yang *et al.*, 2001). For example, at the dose used in this study, the average homozygous effect of a single mutation on traits related to fitness is in the order of a few per cent (Keightley & Ohnishi, 1998). This suggests that the elevated mutation load in our

EMS-treated fly populations was caused by many new mutations of small average effect, each present primarily in heterozygous state, and persisted beyond the completion of the experiment.

The opposite scenario, in which fitness was measured before the benefits of sexual selection could be detected, is more likely. As suggested by Whitlock & Agrawal (2009), populations experiencing sexual selection may benefit on a timescale ignored in all experimental evolution studies, including this one. In order to pick up any signal of purged mutation load, future work will need to either include ambitious long-term experiments in a system amenable to this (e.g. the yeast mating-type system, Rogers & Greig, 2009) or specifically target known deleterious variation.

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