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The effect of sex on the mean and variance of fitness in facultatively sexual rotifers

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Abstract

The evolution of sex is a classic problem in evolutionary biology. While this topic has been the focus of much theoretical work, there is a serious dearth of empirical data. A simple yet fundamental question is how sex affects the mean and variance in fitness. Despite its importance to the theory, this type of data is available for only a handful of taxa. Here, we report two experiments in which we measure the effect of sex on the mean and variance in fitness in the monogonont rotifer, *Brachionus calyciflorus*. Compared to asexually derived offspring, we find that sexual offspring have lower mean fitness and less genetic variance in fitness. These results indicate that, at least in the laboratory, there are both short- and long-term disadvantages associated with sexual reproduction. We briefly review the other available data and highlight the need for future work.

Introduction

Despite the well-known costs of sex, this mode of reproduction is pervasive in plants and animals. The costs of sex are believed to be outweighed by the benefits of shuffling genes (Otto & Lenormand, 2002; Agrawal, 2006b), and such benefits may be realized in either of two (nonexclusive) ways. First, sexual offspring may have higher mean fitness than asexual offspring. This has been described as a short-term advantage to sex (Barton, 1995; Lenormand & Otto, 2000; Agrawal, 2009b). Second, sexual offspring may have higher variance in fitness than asexual offspring, thus allowing the sexually produced subpopulation to respond to selection faster than the asexually produced subpopulation. This is known as a long-term advantage to sex.

Numerous theories exist for the evolution of sexual reproduction (Kondrashov, 1993; Otto, 2009). In a landmark paper, Barton (1995) outlined the contributions of short- and long-term effects to the evolution of sex in a general theoretical framework. Since then, it has become clear that the various hypotheses for sex can be studied under this common conceptual framework. Numerous authors have applied this approach in order to understand the population genetic mechanisms by which sex is favoured under different hypotheses (Peters & Lively, 1999, 2007; Lenormand & Otto, 2000; Barton & Otto, 2005; Roze & Lenormand, 2005; Agrawal, 2006b, 2009b; Roze & Michod, 2010). Some hypotheses for the evolution of sex predict the benefit of sex arises primarily from a short-term advantage, whereas other hypotheses predict that long-term advantages are most important.

Sex alters how alleles are distributed among individuals by breaking down statistical associations that may exist between alleles within or among loci (i.e. linkage disequilibria, Hardy–Weinberg disequilibria). In principle, the short- and long-term effects of sex can be understood by knowing the patterns of the genetic associations and the fitness effects of gene interaction. If good alleles tend to be found with other good alleles and unfavourable alleles are found with other unfavourable alleles (i.e. if genetic associations are positive), then genetic variance is high (Agrawal, 2006a). Sexual reproduction mixes these good alleles with unfavourable ones, reducing the genetic variation in fitness (a long-term disadvantage to sex). The reverse is true if genetic associations are initially negative.

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Whenever there are nonadditive gene interactions, either dominance or epistasis, then breaking down genetic associations will not only alter the variance in fitness but also the mean, i.e. there will be a short-term effect of sex (Lynch & Gabriel, 1983; Lynch & Deng, 1994; Deng & Lynch, 1996). By definition, the existence of nonadditive gene interactions means that fitness depends on which alleles are combined with one another. Thus, rearranging how alleles are distributed will affect the mean fitness. Prior to reproduction (perhaps as a result of prior selection), alleles may be combined such that particularly deleterious combinations of alleles are rare or absent among parents. This may be manifest as an excess of heterozygotes if deleterious alleles are recessive or a deficit of the *ab* haplotype if *a* and *b* are deleterious alleles that have negative synergistic effects on fitness. In such cases, rearranging the existing combinations via sex will result in the production of poor gene combinations (e.g. deleterious homozygotes or the reconstitution of the unfit *ab* haplotype). This results in a negative short-term effect of sex (i.e. sexually produced offspring have lower mean fitness than asexually produced offspring), which has also been described as 'genetic slippage' (Lynch & Deng, 1994). In general, the short-term effect will be negative whenever genetic associations prior to reproduction already 'match' the relevant component of nonadditive selection. For example, if there is an excess of heterozygotes among parents when heterozygotes are more fit than the average of the homozygotes (i.e. deleterious alleles are recessive), then the short-term effect of sex will be negative because sex produces the unfit homozygotes. Conversely, the short-term effect is positive whenever there is a 'mismatch' between the genetic association among the parents and the relevant component of nonadditive selection (see Agrawal (2006a) for a more detailed discussion of this topic).

All hypotheses for sex are based upon some mechanism for generating genetic associations, e.g. epistatic selection (Kondrashov, 1984; Charlesworth, 1990; Barton, 1995), Hill-Robertson effects (Felsenstein, 1974; Barton & Otto, 2005), migration (Lenormand & Otto, 2000; Agrawal, 2009b). Most hypotheses make additional assumptions about the nature of gene interactions that determine the immediate consequences of allele shuffling. Although it is difficult to directly assess the sign of genetic associations or the nature of gene interactions, we can infer something about these properties from real populations by measuring how sex affects the mean and variance in fitness. For example, as discussed earlier, if sex results in a reduction in genetic variance, we can infer that disequilibria were positive. If sexually produced offspring also have lower fitness, then we can infer that the nonadditive components of selection tended to be of the same sign as the corresponding disequilibria.

Arguably, there is no empirical question in this field that is as fundamentally important as how sex affects the mean and variance in fitness. Answering this question will not uniquely identify one hypothesis as an explanation for sex but some answers can eliminate numerous contenders. For example, theories based on Hill-Roberston effects (Felsenstein, 1974; Felsenstein & Yokoyama, 1976; Barton & Otto, 2005; Keightley & Otto, 2006) as well as theories based on negative (synergistic) epistasis (Kondrashov, 1984; Charlesworth, 1990; Barton, 1995) both predict that sex will increase the variance in fitness. Finding the opposite result would directly oppose a key prediction of these theories.

Despite its importance, data of this type exist for only a handful of taxa (see Discussion). In this study, we measure the impact of sexual reproduction on mean and variance of fitness (life time per capita number of offspring produced) by comparing fitness of sexually and asexually produced offspring of the cyclic parthenogenetic rotifer *Brachionus*. Monogonont rotifers are common diploid zooplankter, which often have an extended phase with dominantly parthenogenetic reproduction and short sexual phases (Arndt, 1993; Armengol *et al.*, 2001; Wallace *et al.*, 2006). Sexual reproduction in monogonont rotifers is linked to production of dormant stages (resting eggs) that allow for spatial and temporal dispersal (Ellner *et al.*, 1999) and can be collected from the sediment of lakes and ponds. This study is not intended to test the adaptive value of how sex occurs naturally in these rotifers. Rather, we use the rotifer system to test for the fitness effects of changes in genetic associations because of sex.

In this experiment, we used a *Brachionus* population derived from resting eggs newly collected from the field to capture natural levels of genetic variation. In this approach, the genetic associations were built by forces in the field yet we are evaluating the consequences of sex by assessing fitness in the laboratory. This is especially problematic if epistatic selection in the field differs from that in the laboratory (Agrawal, 2006a). Therefore, we performed a similar experiment with populations that have adapted to the laboratory (approximately 100 generations after establishing in the laboratory), allowing us to evaluate the consequences of breaking down genetic associations in the same context in which they were developed.

Materials and methods

We use cyclic parthenogenetically reproducing rotifers to explore short- and long-term effects of sex on fitness. Monogonont rotifers of the genus *Brachionus* possess the ability to switch between reproducing asexually by ameiotic parthenogenesis or sexually if stimulated by an environmental cue (Gilbert, 2002, 2003; Stelzer & Snell, 2006). Asexual reproduction by amictic females (diploid, asexual females) continues often for extended periods of time, interrupted by sexual phases. High densities of conspecifics induce mixis (sexual reproduction) in the

genus *Brachionus*. A mixis-inducing protein (MIP) is produced and released by the rotifers themselves (Snell *et al.*, 2006). When the MIP level reaches a threshold, amictic females start to parthenogenetically produce micitc females. These sexual females meiotically produce haploid eggs from which dwarf (haploid) males hatch, or resting eggs (diploid) if sexual females are inseminated as young. Resting eggs can survive harsh conditions in the sediment till amictic females hatch when conditions are favourable (Schroeder, 2005). In the laboratory, resting eggs will hatch spontaneously after a few days if maintained under good conditions.

Rotifer isolation

A rotifer population of *Brachionus calyciflorus* (Pallas 1799) was established from resting eggs hatched in June 2009. Sediment containing the resting eggs was collected in May 2009 from the saddle region (17 m depth) of Lake Onondaga (NY, USA; 17 m depth). The sediment, containing several generations of resting eggs from several years in different sediment layers, was mixed and kept cool and dark until egg hatching. Hatching took place in the laboratory under constant light (24 h) and temperature conditions in shallow plastic bins with a mixture of filtered lake and aged tap water added to the bins (approximately 2 cm above sediment surface). Hatching of rotifers was detected daily by siphoning the water. Isolated and identified *Brachionus calyciflorus* were transferred to low-density cultures with the green algae *Monoraphidium minutum* (SAG 278-3, Algae Culture Collection University of Göttingen) as food. Two hundred and thirty-five rotifers hatched over the course of 2 weeks, and because resting eggs are the result of sexual reproduction, each hatched rotifer represents a unique genotype. Rotifers were kept at low densities at 25 °C with a 12 : 12 h L/D cycle (total laboratory stock consisted of 10 flasks with frequent exchange among flasks). Potential biases with respect to genetic variation in our stock culture because of selection during collection and hatching of the resting eggs are discussed below.

Production of sexually and asexually derived genotypes and fitness assay

Ideally, one would isolate the sexually and asexually derived offspring from the same individuals (Kelley *et al.*, 1988; Lynch & Deng, 1994; Pfrender & Lynch, 2000). This is not possible with rotifers. Instead, we took large random samples of our laboratory population and each sample was assigned to either a sexual or an asexual treatment. A similar approach has been used in other systems examining this problem (Colegrave *et al.*, 2002; Kaltz & Bell, 2002). In studies of this nature, it is possible that genetic differences arise between the sexual and asexual samples as a result of selection during the induction of sex. We took precautions to minimize this possibility but it is important to be aware of it. We consider its potential effects on this and other studies in the Discussion.

From the laboratory population, we created 10 random samples, each with an initial size of approximately 650 females (no resting eggs were present in the random samples). Five of these samples were assigned to the 'sex' treatment (hereafter sex replicates) and the other five to the 'asex' treatment (hereafter asex replicates). Rotifers in all replicates were allowed to grow for 4 days at high concentrations of their algal food, *M. minutum*. The five asex replicates were kept at high volumes (low densities) to prevent mixis induction and promote asexual reproduction. The five sex replicates were maintained in a smaller volume, thus increasing density, to induce sexual reproduction. The density used here (32 ± 5.4 females per mL at the end of the 4 day growth period) was well above the density required to induce sex in most genotypes (Becks & Agrawal, 2010) so it is unlikely that a heavily biased subset of individuals contributed to the sexual offspring (about 53% of the produced eggs were resting eggs). After the 4th day, 13 resting eggs were isolated from each replicate of the sex treatment and individually transferred to a single well of a 24-well plate for hatching. The same number of parthenogenetically produced eggs was transferred from each replicate of the asex treatment.

All rotifers hatched within 3 days from the isolated eggs. To avoid differences that could occur because of sexually derived genotypes developing from resting eggs and asexually derived genotypes developing from amictic eggs and other maternal effects, we maintained each genotype by amictic reproduction for three generations prior to measuring fitness. Specifically, the first five offspring from the second (asexual) generation after isolation were used to measure lifetime reproduction (5 individuals/genotype × 13 genotypes/replicate sample × 5 replicate samples/treatment × 2 treatments = 650 fitness measures). Each individual was placed in an individual well. Each day, the number of offspring was recorded and the female was transferred to a new well with fresh medium until she died. The lifetime per capita number of offspring was used as a measure for fitness.

All cultures and individual females of the fitness assays were fed algae (*M. minutum*) well above the incipient limiting concentration (Halbach & Halbach-Keup, 1974). Algae were taken from a continuous chemostat culture to ensure constant food quality.

Effect of sex on laboratory-adapted populations

The experiment described earlier was performed almost immediately after the laboratory population had been established from field-collected resting eggs (20 days from the first rotifer isolation and 7 days after the last rotifer isolation). This means that the genetic associations in this population were likely shaped by evolutionary

forces in the field. This is potentially problematic if patterns of selection in the laboratory differ dramatically from those in the field.

Ideally, we want to examine the consequences of breaking down genetic associations under the conditions in which those associations were developed. Of course, it would be best to do so in the field but this is impossible in most systems. We employed the alternative approach of studying the effects of sex in the laboratory using a population that had the opportunity to adapt to laboratory conditions.

Our second experiment followed the same design as the first except that it was performed after our laboratory population had been established in the laboratory for approximately 100 generations. During this period, there was a low rate of sex (males and resting eggs were occasionally observed in the stock populations during maintenance procedure). The laboratory population was kept at low densities as three subpopulations with weekly exchange between the three subpopulations (25 °C with a 12 : 12 h L/D cycle).

Because the genetic associations in the first experiment were likely formed by forces in the field, we refer to this as the 'field' experiment whereas we refer to the second experiment as the 'laboratory' experiment. We emphasize that all fitness assays were performed in the laboratory, and these labels only refer to where genetic associations developed.

Data analysis

Fitness data (W) were analysed using generalized mixed effect models (GLMM) with treatment (sexual or asexual reproduction) as a fixed effect and replicated sample and clone nested within replicated sample as random effects for the comparison of the fitness means [W = treatment + clone (replicate) + individual (clone (replicate)); with treatment either sex or asex]. Analyses were performed using the *lmer* function in *R* (Bates & Maechler, 2009, R Development Core Team, 2009) with quasi-poisson error structure.

The experimental design allowed us to calculate the variance among clones as a measure of genetic variation. The total variation in fitness (phenotypic variation) is the sum of genetic and environmental variation. Here, the latter is the within-clone variance of each replicated clone (Lynch & Deng, 1994; Deng & Lynch, 1996). Significance of the among-clone variation was assessed by comparing treatment-specific models with and without the clone term [W = replicate + clone (replicate) + individual (clone (replicate))] using maximum likelihood ratio tests. Technically, the among-clone variance includes both genetic variance and variance arising from maternal effects (Lynch & Walsh, 1998). However, as the among-clone variance includes maternal effect variance for both sexual and asexual populations, the difference between treatments (described below) should reflect the difference in genetic variance. In principle, it is possible that sexuals and asexuals are affected to different extents by maternal effects and we cannot exclude this possibility. This seems unlikely in the context of our experiment in which both the sexual and asexual offspring were derived from individuals following three generations of clonal reproduction.

To judge whether the differences in genetic variation were significantly different between the asexual and sexual treatments (at the $P < 0.05$ level), we calculated 95% confidence intervals (CI) using a bootstrap procedure. This was accomplished by resampling with replacement at the 'clone' level (i.e. all replicates within a clone were included), maintaining the original structure of the data. For each re-sampled dataset, we calculated the difference in among-clone variances between sexual and asexual treatments. From 5000 re-sampled data sets, we established approximate 95% confidence intervals for the difference in genetic variance between sexually and asexually produced offspring.

Result

Both experiments exerted distinct effects of sexual reproduction on mean and variance of fitness in comparison with asexual reproduction (Fig. 1, Table 1). Mean fitness of sexually produced offspring was approximately three times lower in the first experiment and approximately two times lower in the second experiment (field: GLMM $\chi^2 = 103.76$, df = 1, $P < 0.001$, lab: GLMM $\chi^2 = 123.17$, df = 1, $P < 0.001$, see Material and methods). Thus, the genetic slippage was reasonably consistent in both experiments and independent from the previous clonal selection, in the field in the first experiment and laboratory in the second experiment.

Sexual reproduction also led to a decrease in genetic variation (Table 1). Again, the first (field genetic associations) and second experiments (laboratory genetic associations) showed the same direction of change. There was significant variance in fitness among clones in the asexual treatment in both experiments (field: GLMM $\chi^2 = 92.67$, df = 1, $P < 0.001$, lab: GLMM $\chi^2 = 92.87$, df = 1, $P < 0.001$). In the sexual treatment, point estimates of genetic variance were much lower and the amount of genetic variance was statistically significant only in the first experiment (field: GLMM $\chi^2 = 32.67$, df = 1, $P < 0.001$, lab: GLMM $\chi^2 = 0.51$, df = 1, $P > 0.05$). Direct comparison between sexual and asexual treatments confirmed that there was significantly more genetic variance in the asexual treatment than the sexual treatment in both experiments ($P < 0.05$ for both).

Discussion

Both the long- and short-term consequences of sex are important for our understanding of sexual reproduction because nearly all theory on the evolution of sex predicts

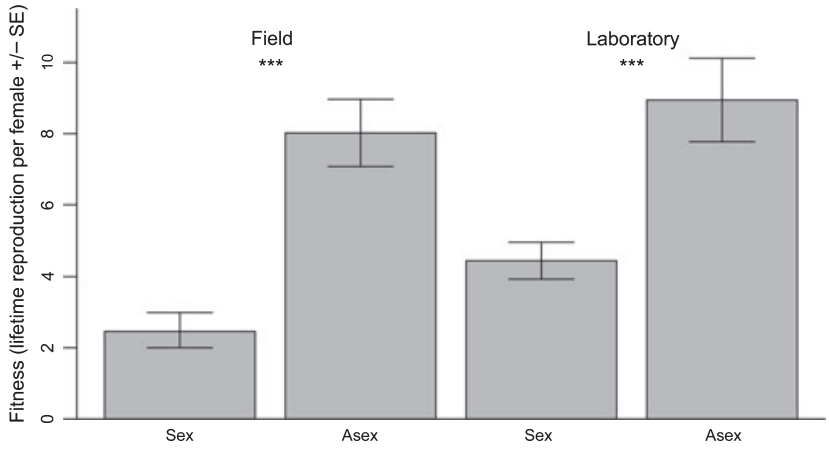

Fig. 1 Fitness (lifetime reproduction per female ± 1 SE) of third generation offspring of sexual (SEX) and asexually (ASEX) reproduced females of *Brachionus calyciflorus* measured shortly after isolation in the FIELD (left) and 4 month (approximately 100 generations) after establishing in the LABORATORY (right). Number of offspring of five identical clones of each third generation females was recorded daily till the females died (n = 5 replicates per treatment, 13 clones per replicate). ***P < 0.001.

an increase in the mean fitness or variance of fitness. This has been made explicit in models of the gradual evolution of sex (i.e. modifier models; Peters & Lively, 1999, 2007; Roze & Michod, 2010; Agrawal, 2009b; Agrawal & Otto, 2006; Roze & Lenormand, 2005; Lenormand & Otto, 2000; Barton & Otto, 2005; Agrawal, 2009a).

Despite the emphasis in the theoretical literature on these short- and long-term effects, there is little empirical data in how sex changes the mean and variance of fitness (Table 2). These studies are limited to only four organismal groups: *Anthoxanthum*, *Chlamydomonas*, *Daphnia*, and *Drosophila*. (Note that the *Drosophila* study is somewhat different in that it compares recombinant versus nonrecombinant offspring rather than sexual versus asexual offspring). We have added a fifth taxa to this list. For rotifers, we found that sexual reproduction yields offspring that have lower average fitness and are less variable in fitness than offspring produced by asexual reproduction.

Some caveats are necessary in interpreting our results as well as the results from the studies in Table 2. Ideally, studies of this type should measure the effect of the genetic shuffling of sex and nothing else. In principle, sex should only change genetic associations and not allele frequencies. However, different procedures are applied to obtain sexual and asexual offspring. An inadvertent by-product of these procedures is that sexual and asexual offspring may come from slightly different gene pools if differential selection occurs incidentally in the creation of sexual and asexual individuals. As in most experiments of this type, there were several opportunities for selection during the production of sexual offspring in our experiment. Below we consider different opportunities for selection and their potential to occur in our study as well as previous ones.

Induction of sex in most facultatively sexual organisms requires special conditions that may also cause selection. In our experiment, rotifers were held at high density and differential survival of genotypes might have occurred during this time period. However, we have no reason to believe there was much, if any, scope for this type of selection; the increased density was applied for only a short period, and there was no obvious increase in mortality (L. Becks, pers. obs). Nonetheless, we cannot formally exclude some selection. Nitrogen stress is used to induce sex in *Chlamydomonas* but the experimental evidence indicates this is not a source of selection in studies using this taxa (Colegrave *et al.*, 2002; Kaltz & Bell, 2002). In the studies that used *Daphnia* and *Anthoxanum*, sexual and asexual offspring were obtained from the same mothers so there was no opportunity for this source of selection.

When sex is induced, it is possible only a biased subset of individuals is induced into sexual reproduction, resulting in another form of selection. In our study, this is unlikely as we used stronger density cues than are necessary to induce sex in the vast majority of clones. This form of selection may have occurred in the *Chlamydomonas* studies if not all clones are induced into the sexual state by nitrogen stress. In the *Daphnia* studies, females carrying sexually produced eggs were collected from the field and asexual offspring were obtained from these same mothers. Thus, there was no opportunity for

Table 1 Variance in fitness for sexually (SEX) and asexually (ASEX) derived offspring in *Brachionus calyciflorus* measured shortly after isolation from the field (field) and after approximately 100 generations of culturing under laboratory conditions (lab). Ninety-five per cent confidence intervals (CI) were estimated by a bootstrapping procedure. Significance of genetic variance was determined by generalized mixed models and for the difference in genetic variance between asexual and sexual derived offspring (Δ = Variance$_{ASEX}$ − Variance$_{SEX}$) by judging whether 95% CI differs from zero.

	Variance	95% CI	P-value	Δ	95% CI for Δ	P-value for Δ
Field$_{ASEX}$	1.28	0.81–1.79	<0.001	1.02	0.44–1.53	<0.05
Field$_{SEX}$	0.26	0.09–0.50	<0.001			
Lab$_{ASEX}$	1.59	0.91–2.40	<0.001	1.56	0.71–2.23	<0.05
Lab$_{SEX}$	0.12	0.07–0.33	n.s.			

Table 2 Summary of published studies examining short- and long-term effects of sexual reproduction on fitness.

Study	Organism	Origin (place where genetic associations were created)	Fitness assay (place where genetic associations were broken down)	Direction of change in mean fitness after sex	Direction of change in variance of fitness after sex
Kelley *et al.*, 1988	*Anthoxanthum odoratum*	Field	Field	△	Not reported
Charlesworth & Charlesworth, 1975	*Drosophila melanogaster (recombinant vs. nonrecombinant)*	Field	Laboratory	▽	△
DaSilva & Bell, 1996	*Chlamydomonas rheinhardtii*	Mixture of lines that evolved for 500–600 generations (laboratory)	Laboratory	No change	△
Colegrave *et al.*, 2002	*Chlamydomonas rheinhardtii*	Mixture of lines (laboratory)	Laboratory	▽	△
Kaltz & Bell, 2002	*Chlamydomonas rheinhardtii*	Mixture of lines (laboratory)	Laboratory	▽	△
Lynch & Deng, 1994	*Daphnia pulex*	Field	Laboratory	▽*	▽*
Pfrender & Lynch, 2000	*Daphnia pulex*	Field	Laboratory	No change*	No change*
Allen & Lynch, 2008	*Daphnia pulicaria*	Field	Laboratory	▽[1]*	△[1]*▽[2]
Present study	*Brachionus calyciflorus*	(a) field (b) laboratory (see Materials and method)	Laboratory	(a) ▽ (b) ▽	(a) ▽ (b) ▽

*These studies measured several traits. We only include here 'clutch size' as a measure of fitness for easier comparison with the other studies.
[1]For low and medium–low rates of sex in the population.
[2]For medium–high rates of sex in the population.

selection of this form to cause differences between the sexual and asexual pools. However, it is possible that the entire pool of *Daphnia* genotypes is a biased subset of the whole population because only genotypes in the sexual state are selected for study. Presumably, this is less of a concern in the *Anthoxanum* study because there is less potential for bias in the selection of parents as all individuals are obligately sexual (asexual offspring are created 'artificially'). Even here, some bias is possible as not all individuals flower each year, possibly reflecting genetic variation for a growth-reproduction trade-off or variation in genetic quality.

It is also possible that selection occurs after sex but prior to the measurement of fitness, thus upwardly biasing the assessment of sexual fitness. It is easy to imagine scenarios in which new genotypes created by sexual reproduction do not survive to adulthood and so are not included in fitness assays. However, the scope for this form of selection was limited in our experiment as sexual offspring were isolated as resting eggs and almost all such lines survived to contribute to our fitness assay. It is difficult to assess the potential for this type of selection in previous studies of *Anthoxanum, Chlamydomonas* and *Daphnia*.

Finally, whenever sex occurs, there is the opportunity for sexual selection unless special precautions are taken (e.g. enforced monogamy). None of the studies to date, including our own, can exclude the possibility of sexual selection. In the rotifers, males are haploid so there is additional potential for sexual selection to eliminate recessive alleles. Because a large fraction of the haploid genome is expressed in pollen (Becker *et al.*, 2003), this is also true in *Anthoxanum*. Unlike the other forms of selection discussed earlier, sexual selection can be considered almost an inherent part of sex and some theories of sex have emphasized its importance (Agrawal, 2001; Siller, 2001; Hadany & Beker, 2007). Nonetheless, when trying to isolate the effects of genetic mixing caused by sex per se it would be preferable to avoid this form of selection.

If one or more of the forms of selection discussed earlier occurs during the process of producing sexual offspring, one might expect that this selection would eliminate deleterious alleles so that the resulting offspring would be more fit than expected. Thus, we expect that inadvertent selection, if it happens at all, would tend to cause an upward bias in the average fitness of sexual offspring. In our experiments, we observed a substantial reduction in the fitness of sexual offspring relative to asexual offspring. This result, in addition to the methodological reasons outlined earlier, suggests that the effects of inadvertent selection were likely small.

In our first experiment, we used a population shortly after it had been collected from the field. In principle, this population contains genetic associations as shaped by evolutionary forces in nature. However, our laboratory stock was initiated from field-collected resting eggs and this raises two issues. First, resting eggs obtained from sediment samples can represent offspring from multiple years. Thus, temporal admixture could potentially contribute to genetic associations in our laboratory population, although this will only be important force to the

extent that allele frequencies in nature change between years (i.e. if allele frequencies are reasonably stable, the effects of temporal admixture will be of little concern).

Second, all of the initial genotypes come from resting eggs and, thus, are the products of sex. Consequently, genetic associations in our initial laboratory population will not be as strong as if it was initiated from field-sampled asexual clones. A single episode of sex with random mating will completely eliminate all intralocus associations (e.g. excess homozygosity or heterozygosity) although it will only partly reduce interlocus associations (i.e. linkage disequilibrium). Thus, the initial laboratory populations should have contained interlocus genetic associations reflective of field conditions, though in a weakened form, but essentially no intralocus associations.

By the time we performed the second experiment, the laboratory population had been adapting to laboratory conditions for over 100 generations. At this point, we might reasonably expect that forces reflective of laboratory conditions have shaped associations. Because the rate of sex within the base laboratory population was reasonably low (L. Becks, pers. obs.), there was plenty of opportunity for intralocus associations to develop in addition to interlocus associations.

Although we primarily view our two experiments as differing with respect to where associations were generated (field vs. laboratory), it is important to recognize other differences between the treatments. First, as indicated earlier, the population in the first experiment was more recently derived from a fully sexual generation (i.e. the founding laboratory population) than the population used in the second experiment. Thus, intralocus associations are expected to play a smaller role in the first experiment than the second experiment. Second, the population used in the later experiment had existed under laboratory conditions for a longer period than the population in earlier experiment and thus might be expected to be more adapted to laboratory conditions. Surprisingly, the data in Fig. 1 provide at best very weak support for this idea. Nonetheless, these additional differences between experiments should be kept in mind when interpreting our results.

Qualitatively, both experiments yielded the same result. Sexual offspring were less fit on average and less variable in fitness than asexual offspring. The lower variance in fitness of sexually produced offspring implies the existence of positive genetic associations among parents (i.e. good alleles tend to be found with other good alleles). The reduction in mean fitness implies relatively strong nonadditive gene action, either dominance or epistasis (Agrawal, 2006a). The effects of dominance can only be detected when intralocus associations are present. As discussed earlier, intralocus associations are expected to be largely absent from the first experiment unless such associations developed during the small number of asexual generations during the initial establishment of the population from resting eggs. For this reason, epistasis may be the more likely cause of the reduction in mean fitness. The reduction in mean fitness was somewhat less in the second experiment than in the first. This could be because some of the most severely deleterious alleles were eliminated in the intervening time. Alternatively, it may indicate that beneficial effects with respect to intralocus associations and dominance partly mitigate the negative effects of sex with respect to interlocus associations and epistasis. However, these interpretations are highly speculative.

Most of the studies listed in Table 2 used populations that were newly isolated from the field to capture natural genetic variation (discussed below). But evaluation of the effect of sex on fitness took place under laboratory conditions (Charlesworth & Charlesworth, 1975; Deng & Lynch, 1996; Lynch *et al.*, 1998; Pfrender & Lynch, 2000; Allen & Lynch, 2008). This approach creates potential problems because consequences of breaking down associations were tested in a different context than where associations were developed (Agrawal, 2006a). Our first experiment also suffers from this potential problem, whereas our second experiment does not. The results are qualitatively similar between our two experiments. However, there is no reason to believe that this would apply in other systems; it is easy to imagine that patterns of nonlinear selection may be similar between laboratory and field in some organisms but not in others.

In addition to our second experiment, we are aware of only two other studies performed in the same environment in which they had evolved. The first used a mixture of *Chlamydomonas* lines that grew and evolved under laboratory conditions for 500–600 generations prior to the experiment (DaSilva & Bell, 1996). The fitness was then assayed under the same laboratory conditions, but no change in the mean fitness and little in the variance of fitness were observed. The second study evaluated the consequences of sex in the same field environment where building genetic associations took place (Kelley *et al.*, 1988). This study showed that the mean fitness of the sweet vernal grass *Anthoxanthum odoratum* is higher in sexually derived offspring.

The study of Kelley *et al.* (1988) is worthy of special comment as it is unique in being the only study to measure the effects of sex on the field. It is also the only study to find an increase in mean fitness, whereas many of the laboratory studies find the opposite. Some results from the sweet vernal grass study suggest that the increase in fitness is the result of selection by parasites (Kelley *et al.*, 1988). The Red Queen hypothesis is a major theory for the evolution of sex (Hamilton, 1980; Lively, 2010) and by excluding parasites in a laboratory setting (intentionally or unintentionally) we might miss the benefits of sex because of selection by parasites.

In our study, we observed both a short- and long-term disadvantage to sex (i.e. reductions in both mean and

variance in fitness of sexually derived offspring). These genetic disadvantages to sex would add to classic intrinsic costs of sex such as the cost of meiosis (Maynard Smith, 1971; Wiliams, 1975). Thus, we would expect the evolution of reduced sex. In other experiments, we have observed such a decline in sex when rotifers are maintained under constant conditions (Fussmann *et al*., 2003; Becks & Agrawal, 2010). Of course, sex is maintained in nature, suggesting important selective differences between the simple laboratory conditions used here and natural environments.

Standard laboratory experiments may be sufficient to detect benefits predicted from models based on deleterious mutations but inadequate to test theories that require special features of the natural ecology that are typically lacking in the laboratory (including parasites and environmental heterogeneity). If other field studies also show a different pattern from those in the laboratory, it will be a strong indicator that laboratory-based studies are lacking in key selective agents.

Additional studies from more taxa will give us more insights into the consequences of sex on fitness and the potential effect of the above outlined caveats. Comparison of sex-induced changes in the mean and variance of fitness across taxa and populations will provide the best way to disentangle the long- and short-term contributions of sex to fitness. Only a larger number of studies will put us in a better position when we are trying to understand one of the biggest challenges in evolutionary biology, the evolution of sex. Studies from more taxa are desperately needed, especially those where fitness can be assayed in the field.

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References

Agrawal, A.F. 2001. Sexual selection and the maintenance of sexual reproduction. *Nature* **411**: 692–695.

Agrawal, A.F. 2006a. Evolution of sex: why do organisms shuffle their genotypes? *Curr. Biol.* **16**: R696–R704.

Agrawal, A.F. 2006b. Similarity selection and the evolution of sex: revisiting the Red Queen. *PLoS Biol.* **4**: 1364–1371.

Agrawal, A.F. 2009a. Differences between selection on sex versus recombination in Red Queen models with diploid hosts. *Evolution* **63**: 2131–2141.

Agrawal, A.F. 2009b. Spatial heterogeneity and the evolution of sex in diploids. *Am. Nat.* **174**: S54–S70.

Agrawal, A.F. & Otto, S.P. 2006. Host-parasite coevolution and selection on sex through the effects of segregation. *Am. Nat.* **168**: 617–629.

Allen, D.E. & Lynch, M. 2008. Both costs and benefits of sex correlate with relative frequency of asexual reproduction in cyclically parthenogenic *Daphnia pulicaria* populations. *Genetics* **179**: 1497–1502.

Armengol, X., Boronat, L., Camacho, A. & Wurtsbaugh, W.A. 2001. Grazing by a dominant rotifer *Conochilus unicornis* Rousselet in a mountain lake: *in situ* measurements with synthetic microspheres. *Hydrobiologia* **446**: 107–114.

Arndt, H. 1993. Rotifers as predators on components of the microbial web (bacteria, heterotrophic flagellates, ciliates) – a review. *Hydrobiologia* **255**: 231–246.

Barton, N.H. 1995. A general-model for the evolution of recombination. *Genet. Res.* **65**: 123–144.

Barton, N.H. & Otto, S.P. 2005. Evolution of recombination due to random dirift. *Genetics* **169**: 2353–2370.

Bates, D. & Maechler, M. 2009. lme4: linear mixed-effects models using S4 classes. R package version 0.999375-31. http://CRAN.R-project.org/package=lme4.

Becker, J.D., Boavida, L.C., Carneiro, J., Haury, M. & Feijo, J.A. 2003. Transcriptional profiling of *Arabidopsis* tissues reveals the unique characteristics of the pollen transcriptome. *Plant Physiol.* **133**: 713–725.

Becks, L. & Agrawal, A.F. 2010. Higher rates of sex evolve in spatially heterogeneous environments. *Nature* **468**: 89–92.

Charlesworth, B. 1990. Mutation-selection balance and the evolutionary advantage of sex and recombination. *Genet. Res.* **55**: 199–221.

Charlesworth, B. & Charlesworth, D. 1975. Experiment on recombination load in *Drosophila-melanogaster*. *Genet. Res.* **25**: 267–274.

Colegrave, N., Kaltz, O. & Bell, G. 2002. The ecology and genetics of fitness in *Chlamydomonas*. VIII. The dynamics of adaptation to novel environments after a single episode of sex. *Evolution* **56**: 14–21.

DaSilva, J. & Bell, G. 1996. The ecology and genetics of fitness in *Chlamydomonas*. 7 The effect of sex on the variance in fitness and mean fitness. *Evolution* **50**: 1705–1713.

Deng, H.W. & Lynch, M. 1996. Change of genetic architecture in response to sex. *Genetics* **143**: 203–212.

Ellner, S.P., Hairston, N.G. Jr, Kearns, C.M. & Babai, D. 1999. The roles of fluctuating selection and long-term diapause in microevolution of diapause timing in a freshwater copepod. *Evolution* **53**: 111–122.

Felsenstein, J. 1974. Evolutionary advantage of recombination. *Genetics* **78**: 737–756.

Felsenstein, J. & Yokoyama, S. 1976. Evolutionary advantage of recombination. 2. Individual selection for recombination. *Genetics* **83**: 845–859.

Fussmann, G.F., Ellner, S.P. & Hairston, N.G. Jr 2003. Evolution as a critical component of plankton dynamics. *Proc. R. Soc. Lond. B* **270**: 1015–1022.

Gilbert, J.J. 2002. Endogenous regulation of environmentally induced sexuality in a rotifer: a multigenerational parental effect induced by fertilisation. *Freshw. Biol.* **47**: 1633–1641.

Gilbert, J.J. 2003. Specificity of crowding response that induces sexuality in the rotifer Brachionus. *Limnol. Oceanogr.* **48**: 1297–1303.

Hadany, L. & Beker, T. 2007. Sexual selection and the evolution of obligatory sex. *BMC Evol. Biol.* **7**: 7.

Halbach, U. & Halbach-Keup, G. 1974. Quantitative relations between phytoplankotn and population-dynamics of rotifer *Brachionus calyciflorus* Pallas – Results of laborartory experiments and field studies. *Arch. Hydrobiol.* **73**: 273–309.

Hamilton, W.D. 1980. Sex versus non-sex versus parasite. *Oikos* **35**: 282–290.

Kaltz, O. & Bell, G. 2002. The ecology and genetics of fitness in *Chlamydomonas*. XII. Repeated sexual episodes increase rates of adaptation to novel environments. *Evolution* **56**: 1743–1753.

Keightley, P.D. & Otto, S.P. 2006. Interference among deleterious mutations favours sex and recombination in finite populations. *Nature* **443**: 89–92.

Kelley, S.E., Antonovics, J. & Schmitt, J. 1988. A test of the short-term advantage of sexual reproduction. *Nature* **331**: 714–716.

Kondrashov, A.S. 1984. Deleterious mutations as an evolutionary factor. 1. The advantage of recombination. *Genet. Res.* **44**: 199–217.

Kondrashov, A.S. 1993. Classification of hypotheses on the advantage of amphimixis. *J. Hered.* **84**: 372–387.

Lenormand, T. & Otto, S.P. 2000. The evolution of recombination in a heterogeneous environment. *Genetics* **156**: 423–438.

Lively, C.M. 2010. A review of Red Queen models for the persistence of obligate sexual reproduction. *J. Hered.* **101**: S13–S20.

Lynch, M. & Deng, H.W. 1994. Genetic slippage in response to sex. *Am. Nat.* **144**: 242–261.

Lynch, M. & Gabriel, W. 1983. Phenotypic evolution and parthenogenesis. *Am. Nat.* **122**: 745–764.

Lynch, M. & Walsh, B. 1998. *Genetics and Analysis of Quantitative Traits*. Sinauer, Sunderland, MA.

Lynch, M., Latta, L., Hicks, J. & Giorgianni, M. 1998. Mutation, selection, and the maintenance of life-history variation in a natural population. *Evolution* **52**: 727–733.

Maynard Smith, J. 1971. What use is sex? *J. Theor. Biol.* **30**: 319–335.

Otto, S.P. 2009. The Evolutionary Enigma of Sex. *Am. Nat.* **174**: S1–S14.

Otto, S.P. & Lenormand, T. 2002. Resolving the paradox of sex and recombination. *Nat. Rev. Genet.* **3**: 252–261.

Peters, A.D. & Lively, C.M. 1999. The Red Queen and fluctuating epistasis: a population genetic analysis of antagonistic coevolution. *Am. Nat.* **154**: 393–405.

Peters, A.D. & Lively, C.M. 2007. Short- and long-term benefits and detriments to recombination under antagonistic coevolution. *J. Evol. Biol.* **20**: 1206–1217.

Pfrender, M.E. & Lynch, M. 2000. Quantitative genetic variation in Daphnia: temporal changes in genetic architecture. *Evolution* **54**: 1502–1509.

R Development Core Team. 2009. R: A language and environment for statitical computing. pp. R Foundation for Statistical Computing Vienna, Austria. ISBN 3-900051-07-0, URL http://www.R-project.org.

Roze, D. & Lenormand, T. 2005. Self-fertilization and the evolution of recombination. *Genetics* **170**: 841–857.

Roze, D. & Michod, R.D. 2010. Deleterious mutations and selection for sex in finite diploid populations. *Genetics* **184**: 1095–1112.

Schroeder, T. 2005. Diapause in monogonont rotifers. *Hydrobiologia* **546**: 291–306.

Siller, S. 2001. Sexual selection and the maintenance of sex. *Nature* **411**: 689–692.

Snell, T.W., Kubanek, J., Carter, W., Payne, A.B., Kim, J., Hicks, M.K. *et al.* 2006. A protein signal triggers sexual reproduction in *Brachionus plicatilis* (Rotifera). *Mar. Biol.* **149**: 763–773.

Stelzer, C.P. & Snell, T.W. 2006. Specificity of the crowding response in the Brachionus plicatilis species complex. *Limnol. Oceanogr.* **51**: 125–130.

Wallace, R.L., Snell, T.W., Ricci, C. & Nogrady, T. 2006. *Rotifera 1: Biology, Ecology and Systematics*. Backhuys Publisher, Leiden.

Wiliams, G.C. 1975. *Sex and Evolution*. Princeton University Press, Princeton, NJ.

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