

## LETTER

## Effectiveness of sexual selection in removing mutations induced with ionizing radiation

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### Abstract

Because of the production of males, sexual populations are expected to incur a 50% cost in potential growth rate. However, theory predicts that sexual competition between males can compensate for this cost by decreasing the mutation load of sexual populations. To test this hypothesis, I induced mutations in male bulb mites with ionizing radiation and subjected their progeny ( $F_1$ ) to two selective regimes differing in opportunity for sexual selection. Mutations which were not removed by selection acting on the  $F_1$  decreased embryonic viability in the  $F_2$ . Viability was significantly higher in the treatment in which there was an opportunity for sexual selection than in the treatment in which sexual selection was experimentally eliminated. The results indicate that sexual selection can increase population fitness and, at least partly, compensate for the cost of sex.

### Keywords

Condition dependence, deleterious mutations, good genes, ionizing radiation, maintenance of sex.

*Ecology Letters* (2004) 7: 1149–1154

### INTRODUCTION

The evolutionary consequences of anisogamy have been extensively explored by evolutionary biologists for two main reasons. First, the reproductive success of the sex, which produces many small gametes, i.e. males, is expected to increase with the number of mating partners (Bateman 1948). This leads to the sexual competition underlying sexual selection (Darwin 1871; Andersson 1994). Secondly, population growth is limited by the reproductive rate of the sex producing fewer and larger gametes, i.e. females. Therefore, asexual lineages can in theory reproduce twice as fast as sexual ones (Maynard Smith 1978), raising a question about mechanisms compensating for the 'cost of producing males' (Maynard Smith 1978; West *et al.* 1999; Rice 2002). The areas of sexual selection and the evolution of sex have developed mostly in isolation, but recent theoretical advances have shown that sexual selection can have important consequences for the evolution of sex (Agrawal 2001; Siller 2001).

Sexual selection leads to evolution of traits that increase the male reproductive success, such as weapons used in contests over access to females, or ornaments increasing male attractiveness to females (Darwin 1871; Andersson 1994). The latter mechanism has been subject to much

debate, as it is not clear why females should show preferences for particular male phenotypes (reviewed in Andersson 1994; Kokko *et al.* 2003; Tomkins *et al.* 2004b). Zahavi (1975) proposed that such traits reveal male genetic quality, and therefore mate choice can provide choosy females with genetic benefits in terms of improved progeny fitness. The revealing function of sexual traits depends on their costs (Zahavi 1975; 1991; Grafen 1990). Costs of production and maintenance of sexual traits make their expression dependent on the phenotypic condition of individuals, and in consequence on genes that affect condition (Andersson 1982, 1986; Rowe & Houle 1996). As phenotypic condition is likely to be affected by many loci, condition-dependence of sexual traits may explain why these traits show considerable additive genetic variance ( $V_a$ ) (Pomiankowski & Møller 1995), despite the expectation that sexual selection should deplete  $V_a$ . Many loci imply that condition is a large target for mutations (Houle 1998), and therefore genetic variance in condition may be maintained by a continuous influx of deleterious mutations. Indeed, Houle & Kondrashov (2002) have shown that evolution of costly male displays and female preferences may proceed when genetic variance in condition is maintained solely by deleterious mutations. However, how male displays are influenced by mutation has been little investigated. Møller &

Mousseau (2003) found in the barn swallow that traits which are currently subject to sexual selection also decline more than average under radioactive contamination, which suggests that these traits may indeed be very sensitive to mutation.

Furthermore, Agrawal (2001) and Siller (2001) showed that by removing mutations from populations, sexual selection can compensate for the two-fold cost of sex. This cost arises because asexual populations, by sparing cost of producing males, can in theory grow twice as fast (Maynard Smith 1978). The models of Agrawal (2001) and Siller (2001) assume that effects of mutations are generally deleterious, such that mutations that decrease male sexual competitiveness also decrease female fecundity. In such a case the cost of producing males is counterbalanced by the increased fecundity of sexual females (Agrawal 2001; Siller 2001). However, the effectiveness of sexual selection in removing deleterious mutations from populations has been little studied.

Mutations with visible phenotypic effects that decrease female productivity also tend to decrease male mating success in *Drosophila melanogaster* (Whitlock & Bourguet 2000), which implies that sexual selection should purge these mutations from populations. Whether this would be the case for mutations with less dramatic phenotypic effects remains to be seen. The effects of mutations occurring spontaneously were studied by Radwan *et al.* (2004) in the bulb mite *Rhizoglyphus robini*. Natural selection was relaxed to allow accumulation of deleterious mutations and the effectiveness of sexual selection in prevention of the concomitant fitness decline was investigated. The decline in female fecundity observed after 12 generations of relaxed selection was similar in monogamous lines and in lines where reproductive competition between males was retained, suggesting that selection against deleterious mutations via sexual selection was of little importance. However, confidence intervals for the difference between monogamous lines and sexual selection lines were large and small to moderate effects of sexual selection could not be excluded (Radwan *et al.* 2004).

Here, I report on a more powerful design that investigates the effectiveness of sexual selection in removing mutations induced in the bulb mite with ionizing irradiation. Ionizing radiation causes a range of mutational effects, from base-pair substitution to chromosomal aberrations, with point mutations being predominant at low doses (reviewed by Evans & DeMarini 1999). Mutations were induced in males, whose progeny were subjected to treatments that differed in the opportunity for sexual selection. Thus, induced mutations could be selected against in heterozygotes, which mimics the natural situation where mutations are rare. The induced mutations could recombine in the F<sub>2</sub>, leading to lethal effects in some individuals. I, therefore, tested

whether sexual selection, through the removal of deleterious mutations, increased embryonic viability in the F<sub>2</sub>.

*Rhizoglyphus robini* (Acari: Astigmata: Acaridae) is a diploid, sexually reproducing pest of bulbs and tubers (Diaz *et al.* 2000). Sexual selection in this species takes place both before and after insemination (Radwan & Siva-Jothy 1996; Radwan *et al.* 2000). Although pre-copulatory female choice cannot be excluded, females are highly promiscuous (Radwan & Siva-Jothy 1996), making mate choice more likely to occur post-copulation. Indeed, Konior *et al.* (2001) have found that when post-copulatory sexual selection is prevented by enforcing monoandry, one of the major components of progeny fitness, daughter fecundity, decreases. Kozielska *et al.* (2004) further confirmed that this arises because of genetic rather than maternal effects. Thus, post-copulatory sexual selection may indeed act to improve fitness of the bulb mite populations. Another route of improvement may be via intrasexual selection. Male *R. robini* are dimorphic, with armoured 'fighter' males achieving on average higher reproductive success than unarmoured scramblers (Radwan & Klimas 2001). As morph is heritable and condition-dependent (Radwan 1995), population fitness might increase if selection for the fighter morph also selects for improved condition of their progeny, as is the case in another dimorphic acarid, *Sancassania berlesei* (Tomkins *et al.* 2004a). Here, I investigate the effects of sexual selection, both before and after insemination, on the rate of removal of induced mutations from population of the bulb mite.

## MATERIALS AND METHODS

The mites used in this study came from a stock culture derived from a colony of about 200 individuals found on onions in a garden near Cracow, Poland, in 1998 and kept in the lab as a large population (> 1000 individuals, subdivided into periodically mixed sub-populations) for about 150 generations before commencement of this research. They were maintained at 22–26 °C, > 90% humidity, and fed a 3 : 1 mixture of powdered yeast and wheat germ. Individually isolated mites, pairs and groups of 10 mites were kept in 0.8 cm diameter glass tubes (2 cm high) with plaster of Paris bases soaked with water, and were provided with food *ad libitum*.

In September 2003, 400 larvae were isolated in individual cells and reared to adulthood to obtain virgin females. Another group of 400 larvae were collected and group-reared to adulthood in a 2.5 cm diameter dish. After emergence of adults, group-reared males were irradiated with 3 krad of gamma rays from a Co<sup>60</sup> source except for 15 males which were left untreated. All males were irradiated at the same time in the same 2.5 cm diameter dish. Females were not irradiated to avoid maternal effects, which, conceivably, could be induced by irradiation. Both irradiated

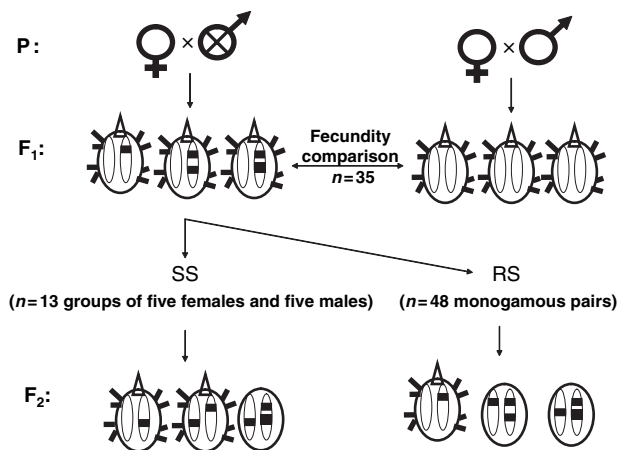
and untreated males were then mated to non-irradiated, virgin females. Ten eggs were collected from each pair and reared singly to obtain virgin females.

### Induced mutations and fecundity of F<sub>1</sub> females

This experiment was done to confirm that male irradiation had an effect on fitness of their progeny; fecundity was chosen for ease of measurement. A random sample of 22 female progeny of irradiated males was collected, each from a different family, and their fecundity was compared to that of the female progeny of non-irradiated males (Fig. 1). The F<sub>1</sub> daughters of both irradiated and non-irradiated males were paired in 0.8 cm diameter tubes with males obtained by isolation of larvae from the stock culture. The females remained paired with the males for 1 week, and then eggs laid by each female were counted (oviposition commences about 24 h after insemination, so females laid eggs for about 6 days).

### Opportunity for sexual selection and F<sub>2</sub> fitness

The main test designed to determine the effectiveness of sexual selection in removing mutations from populations was carried out on the progeny of irradiated males (Fig. 1). These mites carried induced mutations transmitted through their fathers' germ line and were thus heterozygous for these mutations. One male and one female were collected from



**Figure 1** Schematic representation of the design of the experiment. Irradiated males' (marked with cross) F<sub>1</sub> progeny, which inherited induced mutations (black spots on 'chromosomes'), was used in two procedures. The first procedure compared fecundity of the F<sub>1</sub> daughters of irradiated males to that of daughters of non-irradiated males. In the second procedure, F<sub>1</sub> progeny of irradiated males was divided between two treatments: sexual selection (SS) and relaxed selection (RS). Proportion of developing embryos was estimated in F<sub>2</sub> (see text for details).

each F<sub>1</sub> family fathered by irradiated males and assigned to one of the two treatments: relaxed selection (RS), where sexual selection were experimentally limited and sexual selection (SS) where males were allowed to compete for the access to females and their eggs. In the RS treatment each of the females was randomly assigned an unrelated male, whereas in the SS treatment, group of five females were placed in the same cell with five males. Thus, in the latter treatment competition between the males was allowed. Radwan *et al.* (2004) estimated the variance in male reproductive success in identical groups at 5.3 (i.e. about twice the number expected if differences in male reproductive success arose by chance). The assignment to treatments was random except that matings between brothers and sisters were avoided. Treatments lasted 6 days, after which the females were moved to new cells, where they laid eggs used for further procedures. Each female oviposited in a separate 0.8 cm diameter cell. Females laid eggs for 6 days and were then removed.

Following female removal, the proportion of hatched eggs was estimated in each cell. For 10 days, hatched larvae were counted and then removed, and on the day 10 the remaining eggs which failed to hatch were counted. In the RS treatment the embryonic viability was calculated for each female. In the SS, the proportion of hatched eggs was first estimated for each female separately. However, progeny of females kept in the same dish during the SS treatment could have been sired, in an extreme case, by one male. Therefore, to avoid the problem of non-independence, I used proportions of hatched eggs from individual females to calculate the mean embryonic viability for each group. Only such calculated averages were used in subsequent analyses. I set up 48 RS pairs and 13 SS groups. Three RS females did not lay any eggs, and a few other cells were lost because of random events, so the final sample sizes were 39 RS and 11 SS.

On the first day of oviposition, I collected 10 eggs from each female with the aim to rear them to adulthood and to assess a number of fitness components in the F<sub>2</sub>. This was precluded by a low proportion of hatched eggs and, consequently, an insufficient number of adults. Therefore, embryonic viability was the only fitness component I could analyse in F<sub>2</sub>.

### Statistics

The proportions of viable eggs were arcsine transformed. The transformed data did not depart from normality in any of the experimental group, but their variances were not homogenous. Therefore, I carried out the *t*-test with a separate estimate of variances which partly remedies this problem (Zar 1984). Furthermore, as in my data the larger variance was associated with the larger sample size,

inequality of variances could result in *t*-test inflating type II rather than type I errors, so the reported *P*-values are likely to be conservative (Zar 1984; Quinn & Keough 2002).

## RESULTS

In the  $F_1$ , 17 out of 22 females that were progeny of irradiated males laid eggs, whereas all females in the control group (sired by an untreated father,  $n = 13$ ) oviposited (difference n.s., Fisher exact  $P = 0.13$ ). Excluding females that failed to lay eggs, the fecundity of females sired by irradiated fathers (mean  $\pm$  SD =  $24.1 \pm 15.4$ ,  $n = 17$ ) was significantly lower than the fecundity of control females (mean  $\pm$  SD =  $39.5 \pm 22.6$ ,  $n = 13$ ,  $t_{28} = -2.22$ ,  $P = 0.034$ ). This shows that mutations induced in fathers had significant heterozygous effects in their offspring.

The proportion of eggs that hatched in the  $F_2$  in the SS treatment (36.7%, Fig. 2) was significantly higher than in the RS treatment (20%; *t*-test for separate variances,  $t_{46.2} = -3.89$ ,  $P < 0.001$ ).

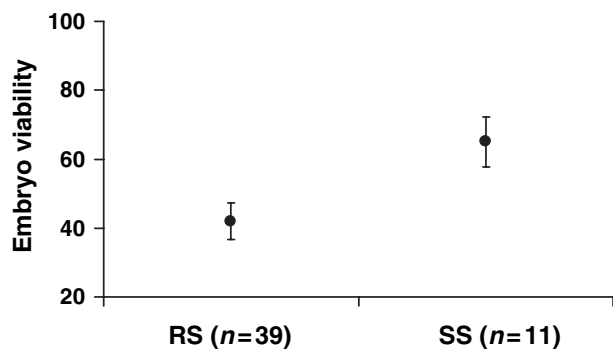
## DISCUSSION

The results of this study showed that sexual selection is effective in removing mutations that were induced with ionizing radiation. Sexual selection could remove these mutations when they occurred in the heterozygous state, a typical situation in natural populations where mutations are rare. When the induced mutations recombined in the next generation, the consequent embryonic viability was higher following the sexual selection treatment than after monogamous, relaxed selection treatment. Among acarid mites, unfertilised oocytes fail to develop (Heineman & Hughes 1970). Thus, if  $F_1$  males had decreased fertility, a failure to fertilize eggs would result in a smaller number of eggs laid rather than in decreased hatchability. Furthermore, in

untreated outbred bulb mites, on average over 98% eggs normally hatch (Radwan & Siva-Jothy 1996). Thus, the observed low embryonic viability in lineages founded by irradiated males could be attributed to the action of the induced mutations. Therefore, the results of the present study suggest that sexual selection helps to purge deleterious mutations from populations.

Radwan *et al.* (2004), on the other hand, did not find any effect of sexual selection when mutations accumulated spontaneously. Unlike in the present study, however, in the mutation-accumulation experiment of Radwan *et al.* (2004) the decline in fitness in relaxed selection lines and sexual selection lines could partly be because of inbreeding or evolution in control lines. Although Radwan *et al.* (2004) took care to reduce these additional effects, their joint action could nevertheless bias their results. Another possible reason of this disparity could be that ionizing radiation induces mutations that have effects different from those occurring naturally. However, ionizing radiation causes a wide spectrum of effects (Evans & DeMarini 1999), so that their distribution is not likely to be drastically different from that characterizing spontaneous mutations. Embryonic mortality, observed when induced mutations were allowed to recombine in the present study, was also recorded when mutations naturally occurring in bulb mite populations were made homozygous via inbreeding (Radwan 2003). Indeed, 13% of lines were lost this way during six generations of inbreeding (Radwan 2003). In other organisms a substantial proportion of spontaneous mutations also shows lethal homozygous effects (Houle *et al.* 1994; Wloch *et al.* 2001). Nevertheless, Wloch *et al.* (2001) recorded higher selection coefficients for mutations induced with EMS (ethyl methanesulfonate) than for spontaneous ones. However, the difference in distribution of mutational effects alone should not make a difference in the effectiveness of sexual selection in removing those mutations, unless the change in strength of selection against mutations is accompanied by changes in the magnitude of pleiotropic effects of these mutations. If spontaneous mutations show mostly trait-specific effects, whereas induced mutations have strong pleiotropic effects, thus creating positive covariances between male reproductive success and other components of fitness, then the results I report here for induced mutations may not be representative of the natural situation. This, however, seems unlikely, because spontaneous mutations are characterized by strong positive mutational correlations (Houle *et al.* 1994), which implies strong pleiotropic effects. Thus, using induced, rather than spontaneous mutations, should not substantially change the estimation of effectiveness of sexual selection in purging mutational load.

Finally, perhaps the most likely reason for the difference between conclusions reached by Radwan *et al.* (2004) and the results of the present study is that in order to accumulate



**Figure 2** Mean ( $\pm$  SE) proportions of hatched eggs (arcsine  $\times$  100) following treatments with sexual selection relaxed (RS) and sexual selection retained (SS). Viability was calculated as described in Materials and methods.

mutations in the former study, the selection was relaxed with respect to fecundity, but not with respect to embryonic viability. Indeed, Radwan *et al.* (2004) did not record any decrease in egg-to-adult survival after 12 generations of relaxed selection. Selection against embryonic mortality must have prevented detection of any effects of deleterious mutations with pleiotropic effects on viability and male reproductive success.

In conclusion, the results of the present study imply that deleterious mutation can be an important factor in sexual selection and the evolution of sexual reproduction. However, more experimental work in this neglected area is clearly needed.

## ACKNOWLEDGEMENTS

I thank D. Hosken, W. Babik, M. Puurtinen and anonymous referees for their comments on the previous versions of this manuscript, and M. Ciuk for improving the English. This project was supported by the State Committee for Scientific Research KBN 0408/P04/2001/21.

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Editor, Laurent Keller

Manuscript received 22 July 2004

First decision made 11 August 2004

Second decision made 31 August 2004

Manuscript accepted 13 September 2004