SEXUAL SELECTION CAN REMOVE AN EXPERIMENTALLY INDUCED MUTATION LOAD

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Sexual selection is argued to be important for the removal of deleterious mutations, promoting population fitness, accelerating adaptation and compensating for the two-fold cost of sex. Here we induced mutations in the dung beetle *Onthophagus taurus* using ionizing radiation, and tested the efficacy of sexual selection in their removal. Mutations reduced male pre-copulatory (strength) and post-copulatory (testes mass) sexual traits. Two generations of sexual selection were sufficient to remove mutations that affected male strength, but not testes mass. Induced mutations did not affect female productivity, which was elevated by sexual selection. Our results provide empirical support for the hypothesis that condition dependent traits offer a large target for mutational variation, and that sexual selection can purge the genome of deleterious mutations and promote population fitness.

An enduring problem in evolutionary biology has been explaining the maintenance of genetic variation in the face of directional selection (Barton and Turelli 1989). This problem has been particularly prominent in sexual selection research. Females are proposed to choose as mates only those males who are able to provide indirect genetic benefits for offspring (Andersson 1994). In theory, sexual selection is expected to erode genetic variation in fitness thereby removing any benefits of mate choice, and yet mate choice persists; the so called Lek Paradox (Kirkpatrick and Ryan 1991). Theoretically, if sexual traits are condition dependent, and condition itself depends on a large number of loci distributed across the genome, then sexual traits may represent a large target for variation induced by spontaneous mutations. In general mutations tend to be detrimental to fitness and will contribute to the maintenance of fitness variation, offering a resolution to the Lek Paradox (Rowe and Houle 1996; Tomkins et al. 2004). Theoretical models suggest that costly female preferences can evolve rapidly where mutations supply a constant input to variation in genotypic quality (Houle and Kondrashov 2002). Indeed, sexual selection on males generally, through male contest competition or female choice, has been argued to be a potent mechanism by which the genome can be purged of deleterious mutations, elevating population fitness (Whitlock and Agrawal 2009), accelerating the rate of adaptive evolution (Lorch et al. 2003), and eliminating the cost of sex (Agrawal 2001; Siller 2001). However, the importance of sexual selection for these key evolutionary processes has received mixed empirical support (Candolin and Heuschele 2008).

The majority of studies that have sought to demonstrate the role of sexual selection in purging deleterious mutations have adopted experimental evolution approaches with flies in the genus *Drosophila*, and mostly *D. melanogaster*. Male mate searching effort in *D. melanogaster* is affected by a number of deleterious

mutations, against which sexual selection can act (MacLellan et al. 2009). In their studies of *D. melanogaster*, Sharp and Agrawal (2008) found that sexual selection acted against 5 of 8 deleterious alleles, while Clark et al. (2012) found that male mating success, but not competitive fertilization success, was negatively affected by 4 of 6 deleterious alleles. Mutation accumulation experiments have also revealed how naturally occurring mutations reduce male mating success and competitive fertilization success (Mallet et al. 2012), and have relatively stronger effects on male than female fitness (Mallet et al. 2011; Sharp and Agrawal 2013). The well documented effect of mutations on male fitness traits is expected to promote the removal of these mutations via sexual selection (Whitlock and Agrawal 2009). Using experimental evolution, Hollis *et al*. (2009) found that the frequency of the deleterious *alcohol dehydrogenase* null allele was rapidly reduced in populations of *D. melanogaster* that were subject to sexual selection compared with populations subject to enforced monogamy. However, sexual selection was found to be either ineffectual or to inhibit the purging of 6 different deleterious alleles from populations of *D. melanogaster* evolving with or without sexual selection (Arbuthnott and Rundle 2012). Similarly, sexual selection was unable to restore fitness among populations of *D. melanogaster* in which a mutational load had been induced by the chemical mutagen ethyl methanesulphonate, even after 60 generations of selection (Hollis and Houle 2011). Indeed, net reproductive output was actually higher among populations subject to enforced monogamy (Hollis and Houle 2011). *Drosophila melanogaster* may not be the best model to address this problem however, because the species is subject to intense sexual conflict which has been demonstrated to reduce population fitness (Holland and Rice 1999). In *D. bunnanda* sexual selection prevented the accumulation of mutations affecting variance in male mating success and

productivity, though the mean values of these traits remained unchanged (McGuigan et al. 2011). In the only non-*Drosophila* organism studied, Radwan (2004) showed that sexual selection was effective in purging deleterious mutations induced by ionizing radiation in bulb mites, *Rhizoglyphus robini*, and sexual selection was found to counteract extinction in small populations of this species (Jarzebowska and Radwan 2010). For a more general understanding of the role of mutations in maintaining fitness variation, we need more studies of a greater range of species, and in particular species in which sexual conflict is not a strong opposing selective force on population fitness.

We examined experimentally the contribution of mutations to both pre-copulatory (male strength) and post-copulatory (testes size) sexual traits in the dung beetle *Onthophagus taurus*, and the effectiveness of sexual selection in removing mutations. Sexual selection is a prominent feature of this species' mating system. Horned major males compete for access to females while hornless minor males compete for fertilizations under sperm competition. Male reproductive success depends strongly on their competitive ability (Hunt and Simmons 2001). Strength is an important pre-copulatory sexual trait as horned males engage in trials of strength over the possession of breeding tunnels (Moczek and Emlen 2000). Strength is also condition dependent, at least among horned males (Knell and Simmons 2010). Male condition exhibits considerable additive genetic variance and is genetically correlated with courtship rate, a trait on which females base their choice of mate (Kotiaho et al. 2001). Testes mass is also condition dependent and affects a male's post-copulatory sperm competitive success (Simmons and Kotiaho 2002; Simmons and García-González 2008). Thus, sexual selection acts both before and after mating in this species. Females acquire indirect genetic benefits from mate choice that affect the

viability of offspring at no apparent cost to female lifetime reproductive success (Simmons and Holley 2011). As such sexual conflict does not appear to be a prominent feature of the mating system (Simmons and García-González 2008). Here we ask whether mutational variance can contribute to the maintenance of good genes sexual selection in this species.

Methods

The source population of *O. taurus* consisted of ~500 beetles collected from a pasture in Serpentine, Western Australia. A sample of 250 females were placed in individual breeding chambers and allowed to build broods for one week (Simmons and García-González 2008). Broods were sieved and incubated at 28°C in 10-L plastic boxes. Newly emerged beetles (~1400) were sexed and maintained in single sex cultures with constant access to fresh dung for one week. Half of the males were anesthetized for five minutes under nitrogen and then exposed to a 2krad (20 Gray) dose of gamma radiation from a Cobalt 60 source. Low doses of radiation typically produce single base substitutions (point mutations), nucleotide deletions and reduced DNA repair capacity (Evans and DeMarini 1999; Sudprasert et al. 2006). Variation in the natural levels of ionizing radiation can be a source of mutational variation in natural populations of animals (Møller and Mousseau 2013). Irradiated males were combined with half of the females and left to mate freely for one week in a 30L bucket filled with damp sand and topped with fresh dung. The remaining males and females were combined and also left for one week to mate freely. All females were then established in individual breeding chambers and left for a further week to produce broods. The F1 offspring derived from these females formed two treatment groups, one in which

beetles were heterozygous for ionization induced mutations and a control group in which beetles were free of experimentally induced mutations.

Each treatment group was split between two experimental evolution regimes; beetles were subjected to either enforced monogamy or sexual selection following the procedures outlined in Simmons & Garcia-Gonzalez (2008). In brief, monogamy was enforced by placing each of 60 females with a single male in an individual box for one week. The sexual selection regime involved placing 10 females and 10 males into a 30-L bucket and allowing them to interact freely for one week. Six sub-populations were thus established to provide 60 multiply mated females. The intensity of sexual selection in these buckets was estimated from the standardized Bateman Gradient to be in the region of 0.8 (Simmons and García-González 2008). After the mating period, females were established in individual breeding chambers to produce F2 offspring. The number of broods each female produced was counted. Experimental evolution was conducted for a second generation, providing F3 offspring.

Male offspring were sampled for strength and testes mass. At one week of age a sample of males were weighed and their pronotum width measured. Some were dissected and their testes removed and weighed. Others were assessed for strength using the methods described in Knell & Simmons (2010). In brief, a length of cotton was attached to the elytra and the male allowed to enter an artificial tunnel between two pieces of glass, lined with fine sandpaper allowing the beetles to attain a firm grip on the substrate. A small polythene bag was attached to the end of the cotton, and the tunnel inclined to an angle of 60° from the horizontal. The cotton was pulled gently to encourage the beetle to grip onto the sandpaper. Water was then dripped into the bag at a constant rate until the beetle was unable to maintain its grip and was pulled from the tunnel. The weight of the bag plus water was determined and converted to

Newtons to give a measure of the beetle's strength. We assayed samples of males from the F1, F2 and F3 generations.

Data were log transformed where necessary and analyzed using general linear models. For male sexual traits, treatment, selection regime, generation, and male morph were entered as main effects; males were assigned to a morph based on their pronotum width (minors < 5.0mm ≥ majors Simmons et al. 1999). Body weight was also entered as a covariate. Non-significant interaction terms were removed from the models. All means are provided ±1SE.

Results

Induced mutations in their heterozygous state had only weak effects on male sexual traits (Table 1). Although male body size, strength and testes weight of F1 males were lower when their fathers had been exposed to ionizing radiation, the differences among treatment groups were not statistically significant (Tables S1 − S3 in the online supporting material). The number of broods produced by F1 female offspring did not differ between treatment groups or the mating regime to which they were subjected (Table 2 and Table S4). However, when induced mutations could occur in their homozygous state, in the F2 and F3 offspring, male sexual traits were affected.

Male body size among the F2 and F3 generations did not vary across selection regimes or treatments (Table S5). However, selection regime ($F_{1,90}$=5.82, P<0.001), treatment ($F_{1,90}$= 24.32, P<0.001) and generation ($F_{1, 90}$=32.64, P<0.001) all influenced beetle strength (Table S6). Importantly, the interaction between selection and treatment was significant ($F_{1,90}$=5.90, P=0.017). Thus male strength was reduced in populations subjected to ionizing radiation but increased in these populations when they were subject to sexual selection (Figure 1). There was also a significant effect of

morph ($F_{1,90}$=4.76, P=0.032) and an interaction between morph and treatment ($F_{1,90}$=4.60, P=0.035). Major male strength was reduced by the mutational load while minor male strength was not (Majors: with mutations 0.06±0.01 N; control 0.13±0.01 N; Minors: with mutations 0.05±0.01 N; control 0.07±0.01 N).

Testes mass was reduced in populations containing induced mutations ($F_{1,110}$=17.37, P<0.001) (Figure 2 and Table S7). Testes mass tended to be greater in populations subjected to sexual selection, but this effect was not statistically significant ($F_{1,110}$=3.75, P=0.056). Testes mass was lower in the F3 generation ($F_{1,110}$=40.56, P<0.001), and the difference in testes mass between generations tended to be greater, though not significantly so, among populations with induced mutations (interaction between treatment and generation, $F_{1,110}$=3.77, P=0.055).

Productivity of F2 females was significantly greater among populations subjected to sexual selection (broods produced: sexual selection, 18.0±0.7; enforced monogamy, 15.4±0.7; $F_{1,206}$=6.37, P=0.012) but induced mutations had no significant effect on brood production (irradiated, 16.4±0.7; control,17.0±0.7) (Table S8).

Discussion

Ionizing radiation induced mutations in male dung beetles, *Onthophagus taurus*, which, when potentially homozygous in the F2 and F3 generations, impacted male pre-copulatory (strength) and post-copulatory (testes size) sexual traits. Most mutations only decrease fitness by one or two percent when heterozygous (Houle et al. 1997; Lynch et al. 1998), so it is perhaps not surprising that we were unable to detect a significant impact of induced mutations in the F1 offspring. Likewise, Pekkala et al. (2009) found only very weak effects of ionization induced mutations on

the mating behavior of male *D. montana* when these mutations were in their heterozygous state.

In F2 and F3 offspring mutations can occur in their homozygous state. We found that ionization induced mutations affected the strength of major but not minor males. Major males control access to females in their breeding tunnels, and engage in physical trials of strength attempting to push rivals from breeding tunnels (Moczek and Emlen 2000). Minor males do not fight for access to females, but achieve matings by sneaking into breeding tunnels to encounter females unnoticed by guarding males. These different mating tactics are associated with differences in patterns of condition dependence whereby minor males do not invest in strength, and strength is only condition dependent in major males (Knell and Simmons 2010). Our data thereby offer strong evidence for the hypothesis that condition dependent sexual traits are particularly sensitive targets for genetic variation in fitness that is induced by new mutations (Rowe and Houle 1996). Induced mutations also affected testes mass, a condition dependent predictor of competitive fertilization success for both male morphs (Simmons and Kotiaho 2002; Simmons and García-González 2008). However, sexual selection was only effective in removing those mutations that impacted male strength.

Male strength increased in populations with induced mutations that were also subjected to sexual selection. The effect of sexual selection was greater in the F3 offspring. Indeed, following two generations of sexual selection, the strength of males in populations with induced mutations was almost restored to that of males from the control populations. In contrast, patterns of variation in testes mass were markedly different. While males from populations subjected to sexual selection tended to have larger testes, sexual selection had no significant impact on testes mass. Accordingly,

sexual selection was ineffective in removing deleterious mutations that reduced testes mass.

One potential explanation for the different findings for strength and testes mass could be that post-copulatory sexual selection in these populations was relatively weaker than pre-copulatory sexual selection. If true, we might have found a more marked effect of post-copulatory sexual selection on testes mass had we conducted a greater number of generations of selection. Indeed, more than six generations of selection were necessary to see divergence in testes mass in a previous experimental evolution study of this species (Simmons and García-González 2008). Testis mass varied greatly among generations, being lowest in the F1 and highest in the F2. Condition dependent traits such as testis mass are expected to be acutely sensitive to the quality of dung provided, which is difficult to control across generations. Indeed, large fluctuations in testes mass were also found among generations in Simmons & García-González's experimental evolution study (see Figure S1 in Simmons & García-González 2008). In this regard, it is interesting to note that populations with induced mutations tended to be more susceptible to the among-generational effects that reduced testis mass.

Populations subjected to sexual selection had greater F2 female productivity, consistent with previous studies showing that polyandry can promote female reproductive success in this species via paternal environmental effects (Kotiaho et al. 2003; Simmons and Holley 2011). However, we found no significant effect of induced mutations on the productivity of F2 females. This might at first seem surprising given its effects on male fitness traits. Females must dig tunnels, collect dung from the surface, transport it to the brood chamber and pack it into a brood ball before laying a single egg inside. Maternal provisioning is costly, reducing female

lifespan (Hunt et al. 2002). We might have expected mutations across the genome that reduce male strength to have reduced female productivity. Indeed, the argument that sexual selection can promote population fitness relies on the fact that sexual selection on males removes mutations that impact the non-sexual fitness of both sexes (Whitlock and Agrawal 2009). Because we only examined a single episode of brood production, and not lifetime brood productivity, we are unable to draw any firm conclusions from our data. However, there is little additive genetic variance among females in the number of broods they produce (Hunt and Simmons 2002). Thus, female productivity likely presents only a small mutational target, accounting for the lack of an effect of induced mutations on this trait. The same is true for body size, which is also determined primarily by the environment provided by females for offspring development (Hunt and Simmons 2000, 2002). Moreover, our findings are consistent with recent mutation accumulation studies of *D. melanogaster*, which suggest that new mutations may generally have a greater impact on male, than female fitness (Mallet et al. 2012; Sharp and Agrawal 2013).

In conclusion, our experimental manipulations offer empirical support for the theoretical argument that condition dependent traits present a large target for new mutations that can affect the expression of male sexual traits, and that sexual selection can be effective in removing deleterious mutations (Rowe and Houle 1996; Tomkins et al. 2004). Whether mutational variation can account for the maintenance of good-genes sexual selection in *O. taurus* will depend largely on the number of loci that contribute to condition and the natural rate of mutations at these loci (Houle and Kondrashov 2002).

ACKNOWLEDGEMENTS

LITERATURE CITED

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

Andersson, M. 1994. Sexual Selection. Princeton University Press, Princeton.

Arbuthnott, D., and H. D. Rundle. 2012. Sexual selection is ineffectual or inhibits the purging of deleterious mutations in *Drosophila melanogaster*. Evolution 66:2127-2137.

Barton, N. H., and M. Turelli. 1989. Evolutionary quantitative genetics: how little do we know? Annual Review of Genetics 23:337-370.

Candolin, U., and J. Heuschele. 2008. Is sexual selection beneficial during adaptation to environmental change? Trends Ecol. Evol. 23:446-452.

Clark, S. C. A., N. P. Sharp, L. Rowe, and A. F. Agrawal. 2012. Relative effectiveness of mating success and sperm competition at eliminating deleterious mutations in *Drosophila melanogaster*. Plos One 7:e37351.

Evans, H. H., and D. M. DeMarini. 1999. Ionizing radiation-induced mutagenesis: radiation studies in Neurospora predictive for results in mammalian cells. Mutation Research/Reviews in Mutation Research 437:135-150.

Holland, B., and W. R. Rice. 1999. Experimental removal of sexual selection reverses intersexual antagonistic coevolution and removes a reproductive load. Proc. Natl. Acad. Sci. USA 96:5083-5088.

Hollis, B., J. L. Fierst, and D. Houle. 2009. Sexual selection accelerates the elimination of a deleterious mutant in *Drosophila melanogaster*. Evolution 63:324-333.

Hollis, B., and D. Houle. 2011. Populations with elevated mutation load do not benefit from the operation of sexual selection. J. Evol. Biol. 24:1918-1926.

Houle, D., K. A. Hughes, S. Assimacopoulos, and B. Charlesworth. 1997. The effects of spontaneous mutation on quantitative traits. II. Dominance of mutations with effects on life-history traits. Genetics Research 70:27-34.

Houle, D., and A. S. Kondrashov. 2002. Coevolution of costly mate choice and condition-dependent display of good genes. Proceedings of the Royal Society of London. Series B: Biological Sciences 269:97-104.

Hunt, J., and L. W. Simmons. 2000. Maternal and paternal effects on offspring phenotype in the dung beetle *Onthophagus taurus*. Evolution 54:936-941.

Hunt, J., and L. W. Simmons. 2001. Status-dependent selection in the dimorphic beetle *Onthophagus taurus*. Proc. R. Soc. Lond. B 268:2409-2414.

Hunt, J., and L. W. Simmons. 2002. The genetics of maternal care: Direct and indirect genetic effects on phenotype in the dung beetle *Onthophagus taurus*. Proc. Natl. Acad. Sci. USA 99:6828-6832.

Hunt, J., L. W. Simmons, and J. S. Kotiaho. 2002. A cost of maternal care in the dung beetle *Onthophagus taurus*? J. Evol. Biol. 15:57-64.

Jarzebowska, M., and J. Radwan. 2010. Sexual selection counteracts extinction of small populations of the bulb mites. Evolution 64:1283-1289.

Kirkpatrick, M., and M. J. Ryan. 1991. The evolution of mating preferences and the paradox of the lek. Nature 350:33-38.

Knell, R. J., and L. W. Simmons. 2010. Mating tactics determine patterns of condition dependence in a dimorphic horned beetle. Proc. R. Soc. Lond. B 277:2347-2353.

Kotiaho, J. S., L. W. Simmons, J. Hunt, and J. L. Tomkins. 2003. Males influence maternal effects that promote sexual selection: A quantitative genetic experiment with dung beetles *Onthophagus taurus*. Am. Nat. 161:852-859.

Kotiaho, J. S., L. W. Simmons, and J. L. Tomkins. 2001. Towards a resolution of the lek paradox. Nature 410:684-686.

Lorch, P. D., S. R. Proulx, L. Rowe, and T. Day. 2003. Condition-dependent sexual selection can accelerate adaptation. Evolutionary Ecology Research 5:869-881.

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

MacLellan, K., M. C. Whitlock, and H. D. Rundle. 2009. Sexual selection against deleterious mutations via variable male search success. Biol. Let. 5:795-797.

Mallet, M., J. Bouchard, C. Kimber, and A. Chippindale. 2011. Experimental mutation-accumulation on the X chromosome of *Drosophila melanogaster* reveals stronger selection on males than females. BMC Evolutionary Biology 11:156.

Mallet, M. A., C. M. Kimber, and A. K. Chippindale. 2012. Susceptibility of the male fitness phenotype to spontaneous mutation. Biol. Let. 8:426-429.

McGuigan, K., D. Petfield, and M. W. Blows. 2011. Reducing mutation load through sexual selection on males. Evolution 65:2816-2829.

Moczek, A. P., and D. J. Emlen. 2000. Male horn dimorphism in the scarab beetle *Onthophagus taurus*: do alternative reproductive tactics favor alternative phenotypes? Anim. Behav. 59:459-466.

Møller, A. P., and T. A. Mousseau. 2013. The effects of natural variation in background radioactivity on humans, animals and other organisms. Biol. Rev. 88:226-254.

Pekkala, N., M. Puurtinen, and J. S. Kotiaho. 2009. Sexual selection for genetic quality: disentangling the roles of male and female behaviour. Anim. Behav. 78:1357-1363.

Radwan, J. 2004. Effectiveness of sexual selection in removing mutations induced with ionization. Ecol. Let. 7:1149-1154.

Rowe, L., and D. Houle. 1996. The lek paradox and the capture of genetic variance by condition dependent traits. Proc. R. Soc. Lond. B 263:1415-1421.

Sharp, N. P., and A. F. Agrawal. 2008. Mating density and the strength of sexual selection against deleterious alleles in *Drosophila melanogaster*. Evolution 62:857-867.

Sharp, N. P., and A. F. Agrawal. 2013. Male-biased fitness effects of spontaneous mutations in *Drosophila melanogaster*. Evolution 67:1189-1195.

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

Simmons, L. W., and F. García-González. 2008. Evolutionary reduction in testes size and competitive fertilization success in response to the experimental removal of sexual selection in dung beetles. Evolution 62:2580-2591.

Simmons, L. W., and R. Holley. 2011. Offspring viability benefits but no apparent costs of mating with high quality males. Biol. Let. 7:419-421.

Simmons, L. W., and J. S. Kotiaho. 2002. Evolution of ejaculates: patterns of phenotypic and genotypic variation and condition dependence in sperm competition traits. Evolution 56:1622-1631.

Simmons, L. W., J. L. Tomkins, and J. Hunt. 1999. Sperm competition games played by dimorphic male beetles. Proc. R. Soc. Lond. B 266:145-150.

Sudprasert, W., P. Navasumrit, and M. Ruchirawat. 2006. Effects of low-dose gamma radiation on DNA damage, chromosomal aberration and expression of repair genes in human blood cells. International Journal of Hygiene and Environmental Health 209:503-511.

Tomkins, J. L., J. Radwan, J. S. Kotiaho, and T. Tregenza. 2004. Genic capture and resolving the lek paradox. Trends. Ecol. Evol. 19:323-328.

Whitlock, M. C., and A. F. Agrawal. 2009. Purging the genome with sexual selection: reducing mutation load through selection on males. Evolution 63:569-582.

Table S1. Analysis of variance in pronotum width of male *Onthophagus taurus* sired by control males and males exposed to 2 krads of ionizing radiation.

Table S2. Analysis of variance in log pulling strength of male *Onthophagus taurus* sired by control males and males exposed to 2 krads of ionizing radiation.

Table S3. Analysis of variance in log testes mass of male *Onthophagus taurus* sired by control males and males exposed to 2 krads of ionizing radiation.

Table S4. Analysis of variance in productivity of female *Onthophagus taurus* sired by control males and males exposed to 2 krads of ionizing radiation.

Table S5. Analysis of variance in pronotum width of male *Onthophagus taurus* from populations with and without induced mutations, and subject to sexual selection or enforced monogamy for two generations.

Table S6. Analysis of variance in log pulling strength of male *Onthophagus taurus* from populations with and without induced mutations, and subject to sexual selection or enforced monogamy for two generations.

Table S7. Analysis of variance in log testes mass for male *Onthophagus taurus* from populations with and without induced mutations, and subject to sexual selection or enforced monogamy for two generations.

Table S8. Analysis of variance in the number of broods produced by female *Onthophagus taurus* from populations with and without induced mutations, and subject to sexual selection or enforced monogamy for one generation.

Figure legends

Figure 1. Pulling strength of male *Onthophagus taurus* from populations with (irradiated) and without (control) induced mutations, and subject to sexual selection or enforced monogamy for two generations. Untransformed means are displayed although statistical analyses were conducted on log pulling strength.

Figure 2. Least squares mean testes mass (controlling for body mass) of male *Onthophagus taurus* from populations with (irradiated) and without (control) induced mutations, and subject to sexual selection or enforced monogamy for two generations. Untransformed means are displayed but statistical analyses were conducted on log testes mass.

Table 1. Mean (±SE) body size, testes mass and pulling strength of F1 male offspring derived from control males or males exposed to 2 krads of ionizing radiation.

Trait	Irradiated	Controls
Pronotum width (mm)	4.83±0.05	4.89±0.05
Testes mass[1] (mg)	1.30±0.07	1.49±0.07
Strength (Newtons)	0.02±0.01	0.04±0.01

[1]Least Square means correcting for soma mass

Table 2. Mean (±SE) number of broods produced by F1 female offspring derived from control males or males exposed to 2 krads of ionizing radiation, and subject to enforced monogamy or sexual selection during matings.

Mating regime	Irradiated	Controls
Enforced monogamy	17.1±0.8	15.4±0.7
Sexual selection	16.6±0.7	16.0±0.8