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Adaptations to sexual selection and sexual conflict: insights from experimental evolution and artificial selection

Dominic A. Edward[†], **Claudia Fricke**[†] **and Tracey Chapman**[*]

School of Biological Sciences, University of East Anglia, Norwich, Norfolk NR4 7TJ, UK

Artificial selection and experimental evolution document natural selection under controlled conditions. Collectively, these techniques are continuing to provide fresh and important insights into the genetic basis of evolutionary change, and are now being employed to investigate mating behaviour. Here, we focus on how selection techniques can reveal the genetic basis of post-mating adaptations to sexual selection and sexual conflict. Alteration of the operational sex ratio of adult *Drosophila* over just a few tens of generations can lead to altered ejaculate allocation patterns and the evolution of resistance in females to the costly effects of elevated mating rates. We provide new data to show how male responses to the presence of rivals can evolve. For several traits, the way in which males responded to rivals was opposite in lines selected for male-biased, as opposed to female-biased, adult sex ratio. This shows that the manipulation of the relative intensity of intra- and inter-sexual selection can lead to replicable and repeatable effects on mating systems, and reveals the potential for significant contemporary evolutionary change. Such studies, with important safeguards, have potential utility for understanding sexual selection and sexual conflict across many taxa. We discuss how artificial selection studies combined with genomics will continue to deepen our knowledge of the evolutionary principles first laid down by Darwin 150 years ago.

Keywords: selection; sperm competition; ejaculate allocation; selection experiment; reproductive isolation

1. INTRODUCTION

(a) *Insights from selection experiments into evolutionary process*

Darwin was acutely aware that artificial selection and breeding experiments could give powerful insight into evolutionary process (Darwin 1868) and he derived from these sources much of the evidence to support the main themes of *The Origin of Species by Means of Natural Selection* (Darwin 1859). Since Darwin's time, the study of natural selection under controlled conditions by using artificial selection and experimental evolution has provided evidence for mechanisms of inheritance, mutation accumulation and quantitative genetics, especially in the fruitfly and the mouse (Falconer 1992; Falconer & Mackay 1996; Mackay 2001; Barton & Keightley 2002). There has also been an extremely fruitful tradition of experimental evolution research using microbes (Mortlock 1984; Dykhuizen 1990), which is now also providing novel insights into mechanisms underlying social evolution (West *et al.* 2007; Buckling *et al.* 2009).

Among the many classic artificial selection experiments conducted in *Drosophila melanogaster* are those that have targeted bristle numbers, flight ability, ethanol tolerance, body size and life history (Mackay *et al.* 2009). These experiments show that sustained and replicated responses to selection are possible (e.g. Yoo 1980). They also reveal the contribution of standing genetic variation, new mutations and effective population size to selection responses (e.g. Weber 1990; Barton & Keightley 2002; Snook *et al.* 2009). The use of artificial selection or experimental evolution (i.e. the creation of a specific set of conditions to which a suite of traits may evolve) has also been an important tool in microbial genetics. Classic studies have illuminated the spread of new mutations (e.g. Atwood *et al.* 1951), biochemical adaptation (Mortlock 1984), the evolution of resistance to phage (e.g. Lenski 1988) and the effects of inter-specific competition for limited resources (e.g. Gottschal *et al.* 1979). The value of these approaches looks set to increase in the future as their significance is realized across traditional disciplines. For example, recent reviews have emphasized the lack of knowledge of mechanisms of genetic adaptation to pervasive forces such as rising global temperatures (Gienapp *et al.* 2008).

(b) *Insights from selection experiments into post-copulatory sexual selection and sexual conflict*

There has been relatively less attention until recently on the application of artificial selection techniques to the study of behavioural ecology, sexual selection and

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[*] Author for correspondence (tracey.chapman@uea.ac.uk).
[†] These authors contributed equally to the study.

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sexual conflict. One exception is Manning's work on mating speed in *D. melanogaster* (Manning 1961, 1963). It was realized from the 1980s onwards that artificial selection and experimental evolution techniques could be used to investigate sexual selection and sexual conflict. Rice and colleagues pioneered the approach of exploring the consequences of the evolutionary manipulation of mating systems to alter the intensity of sexual selection and sexual conflict (e.g. Rice 1992, 1996; Holland & Rice 1999). Sexual conflict arises because of the different evolutionary interests of males and females (Parker 1979), and evolutionary approaches can reveal sexual conflict that occurs because of the expression of the same or different genes in males versus females (intra- versus interlocus sexual conflict, respectively, e.g. Rice 1992, 1996). For example, when males were allowed to evolve against a static female phenotype, alleles favouring an increase in fitness through the male lineage spread more easily because of the lack of counter selection in females (Rice 1996).

Selection experiments that have imposed different mating systems (e.g. monogamy versus polyandry) can reveal the effects on overall male and female fitness of the removal of sexual selection and conflict (Holland & Rice 1999; Hosken *et al.* 2001, 2009; Martin & Hosken 2003; Crudgington *et al.* 2005, 2009; Tilszer *et al.* 2006; Bacigalupe *et al.* 2007; Fricke & Arnqvist 2007; LaMunyon *et al.* 2007; Simmons & Garcia-Gonzalez 2008; Gay *et al.* 2009; Maklakov *et al.* 2009). For example, female *D. melanogaster* had longer lifespans following single matings to males from lines selected for 47 generations of monogamy, in comparison with females mated to polyandrous males (Holland & Rice 1999). These findings suggest that monogamous males evolve to be less harmful to females and that monogamous females can be more susceptible to mating costs. It is not yet clear whether costs that can arise from selection owing to sexual conflict can be offset via indirect benefits in subsequent generations (Kirkpatrick & Barton 1997; Cameron *et al.* 2003; Head *et al.* 2005; Priest *et al.* 2008) and, if possible, how often this occurs.

An attraction of applying regimes of monogamy and polyandry is that it can be done across many different taxa. For example, monogamous and polyandrous mating regimes have been applied in the fruitflies *D. melanogaster* and *Drosophila pseudoobscura* (Holland & Rice 1999; Crudgington *et al.* 2005, 2009; Bacigalupe *et al.* 2007), the seed beetle *Callosobruchus maculatus* (Fricke & Arnqvist 2007; Gay *et al.* 2009; Maklakov *et al.* 2009), the bulb mite *Rhizoglyphus robini* (Tilszer *et al.* 2006), the dung flies *Sepsis synipsea* and *Scathophaga stercoraria* (Hosken *et al.* 2001, 2009; Martin & Hosken 2003), the dung beetle *Onthophagus taurus* (Simmons & Garcia-Gonzalez 2008) and the round worm *Caenorhabditis elegans* (LaMunyon *et al.* 2007). Experiments which manipulate the degree of polyandry have also been performed (e.g. Wigby & Chapman 2004; Crudgington *et al.* 2005; Bacigalupe *et al.* 2007; Linklater *et al.* 2007; Reuter *et al.* 2008; Hosken *et al.* 2009). Together, these studies support the idea that experimental evolutionary manipulations of sexual selection and sexual conflict can lead to significant divergence in both pre- and post-mating traits.

The types of studies described above have also been useful in testing the important prediction that elevated sexual conflict can lead to antagonistic coevolution (e.g. Parker 1979; Holland & Rice 1998), which in turn can promote reproductive isolation leading to speciation (e.g. Parker & Partridge 1998; Rice 1998; Gavrilets 2000; Gavrilets *et al.* 2001). A distinctive prediction of sexual conflict is that reproductive isolation can evolve more quickly under large population sizes (Gavrilets 2000), in contrast to classical predictions (Lande 1981). There is not yet a consistent pattern of results, with some studies supporting a role for conflict in reproductive isolation (Martin & Hosken 2003; Hosken *et al.* 2009) and others not (Wigby & Chapman 2006; Bacigalupe *et al.* 2007; Gay *et al.* 2009). This may mean that conflict has a role to play in reproductive isolation only under some conditions, that such experiments have not yet run over a sufficiently long period of evolutionary time or that not all relevant traits have been studied (see below for discussion of further pitfalls).

There is also considerable interest in using selection techniques to study rates of adaptation. Theory states that sexual selection can speed up the rate of adaptation via the partitioning of genetic variation, with beneficial alleles being more effectively channelled through fewer breeding individuals because of mate choice (e.g. Lorch *et al.* 2003). Sexual selection can therefore reinforce natural selection and accelerate the rate of adaptation. This is an important prediction, but to date there are contradictory empirical results. Tests of population fitness in lines of *D. melanogaster* maintained with and without sexual selection revealed no, or negative, effects of sexual selection on the rate of adaptation (Holland 2002; Rundle *et al.* 2006). However, a study on adaptation to host shifts in seed beetles (*C. maculatus*) under polygamy and monogamy found that sexual selection increased the speed of adaptation, measured as fitness in the new environment (Fricke & Arnqvist 2007). In addition to the predicted beneficial effects of sexual selection, negative effects on population fitness can also arise owing to sexual conflict (Arnqvist & Rowe 2005). This may explain some of the discrepancies, as the extent of sexual conflict may counterbalance the beneficial effects of sexual selection.

An additional useful technique is to combine simultaneous evolutionary manipulations of mating systems and life histories. For example, Maklakov *et al.* (2009) combined selection for monogamy and polyandry with selection for early and late age reproduction in *C. maculatus*. The results showed that adaptation to life history had the most important effects on fitness, with the presence or absence of sexual selection having relatively little influence.

2. ARTIFICIAL SELECTION AND EXPERIMENTAL EVOLUTION IN *DROSOPHILA MELANOGASTER*

The breadth of organisms and traits targeted by experimental evolution and artificial selection is evident

Table 1. The effect of female-biased (FB) and male-biased (MB) selection regime and the presence or absence of rivals on variation in the latency to mate, mating duration, intermating egg production, intermating egg fertility and competitive reproductive success. Shown are the results of generalized linear models (for details, see electronic supplementary material).

source	latency to mate	mating duration	intermating egg production	intermating egg fertility	competitive reproductive success
FB/MB selection regime	$F_{1,4} = 1.615$, $p = 0.273$	$F_{1,4} = 2.418$, $p = 0.195$	$F_{1,4} = 1.866$, $p = 0.244$	$\chi^2_1 = 0.155$, $p = 0.693$	$\chi^2_1 = 3.003$, $p = 0.083$
± rivals	$F_{1,355} = 0.062$, $p = 0.804$	$F_{1,356} = 62.569$, $p < 0.001$	$F_{1,355} = 0.964$, $p = 0.327$	$\chi^2_1 = 16.712$, $p < 0.001$	$\chi^2_1 = 31.708$, $p < 0.001$
FB/MB regime × rivals interaction	$F_{1,355} = 5.538$, $p = 0.019$	$F_{1,355} = 1.663$, $p = 0.198$	$F_{1,355} = 3.826$, $p = 0.051$	$\chi^2_1 = 5.751$, $p = 0.016$	$\chi^2_1 = 0.002$, $p = 0.965$

from the research summarized above. We expand one example below, focusing on our recent studies of the consequences of manipulating the intensity of sexual selection and conflict in *D. melanogaster*. We then also describe a new investigation of the evolution of male responses to rivals. To manipulate sexual selection and sexual conflict, we created experimental evolution lines in which the adult sex ratio was male-biased (MB, one female : three males), equal sex (ES) or female-biased (FB, three females : one male). Previously, females from these lines were tested for evidence of female resistance to the costly effects of elevated matings with males (Wigby & Chapman 2004). Given that the number of matings per female was significantly higher in the MB lines, we predicted that MB females would be selected to evolve resistance to mating costs. The survival of females from the MB lines in the presence of wild-type males was significantly higher than for females from the ES and then FB lines (Wigby & Chapman 2004). There were no intrinsic differences in female survival in the absence of males, which argues that females had evolved a specific resistance mechanism to counter the costly effects of elevated matings (Wigby & Chapman 2004).

The males from the MB and FB lines showed ejaculate allocation patterns consistent with their evolutionary history of sexual selection. Given that the risk of usurpation by other males is very high under MB conditions, males are predicted to allocate more ejaculate to their earlier mates, rather than to be prudent, and they therefore run the risk of ejaculate exhaustion. To test this idea, males from the MB and FB lines were mated to five wild-type virgin females in succession. MB males did indeed lose fertility significantly faster than did males from the FB lines (Linklater *et al.* 2007). The loss of fertility over successive mates was associated more strongly with a reduction in the size of the male accessory glands than with the testes.

(a) *Evolutionary manipulations of male responses to rivals*

The way in which males can tailor matings and ejaculate transfer in response to rivals was first demonstrated in insects (Gage & Baker 1991) and has subsequently been studied across a range of vertebrate and invertebrate taxa (reviewed by Wedell *et al.* 2002; see also Neff *et al.* 2003; Siva-Jothy & Stutt 2003; Pound & Gage 2004; Friberg 2006; Carazo *et al.* 2007). There is good evidence that males that experience, or perceive, high levels of sperm competition can ejaculate more sperm (Parker *et al.* 1996, 1997; Wedell *et al.* 2002; Engqvist & Reinhold 2005), and can transfer more seminal fluid proteins (Wigby *et al.* 2009).

Male *D. melanogaster* respond to the presence of rival males both before and during mating. However, it is primarily exposure to rivals prior to mating that evokes adaptive male responses resulting in significantly higher competitive reproductive success under conditions where sperm competition is strong (Bretman *et al.* 2009*a*). The data are consistent with the idea that it is the length of exposure to rivals that is most important, rather than the number of males or male density, although the exact mechanisms are not yet known (Bretman *et al.* 2009*b*). What is clear is that the plastic responses exhibited by males are tightly calibrated to their socio-sexual environment and result in significantly increased competitive reproductive success.

(b) *Empirical study of male responses to rivals*

It is not yet known whether the magnitude, presence or sign of the plastic responses of males to their rivals can themselves evolve. These questions were investigated in new empirical work that we report here. We tested whether responses to rivals had evolved in males from the MB and FB lines described above (see the electronic supplementary material for detailed methods). We predicted that changes in the relative balance of intra- versus intersexual selection, and specifically the evolutionary history of elevated male–male competition in the MB lines, would lead to stronger selection on male responses to rivals in MB when compared with FB lines. We also tested the idea that these manipulations would select for differences in female responses to matings. We examined how females from the lines responded to single matings with males that did and did not transfer an ejaculate component (sex peptide, SP) that modulates egg production, female receptivity and that mediates sexual conflict (Wigby & Chapman 2005; Fricke *et al.* 2009).

Our evolutionary manipulation of adult sex ratio produced opposing responses in mating latency in MB versus FB males (table 1 and figure 1*a*). MB

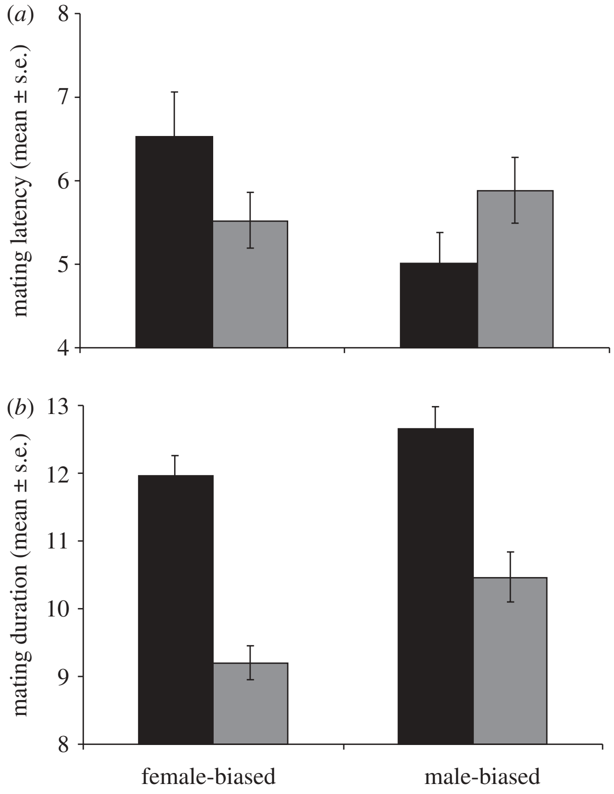

Figure 1. (*a*) Mating latency (mean minutes ± s.e.) for males from the male-biased (MB) and female-biased (FB) selection lines either exposed or not exposed to rivals for 5 days prior to mating. (*b*) Mating duration (mean minutes ± s.e.) for males from the MB and FB selection lines either exposed or not exposed to rivals for 5 days prior to mating. Black bars, +rivals; grey bars, −rivals.

males showed significantly decreased latency until mating and FB males significantly increased latency following exposure to rival males. Hence MB, but not FB, males retained the pattern found in wild-type males (Bretman *et al.* 2009*a*). Males from both lines showed significantly increased mating duration following exposure to rival males (table 1 and figure 1*b*). Hence, males from both regimes retained significant plasticity in mating duration, though there was a non-significant overall tendency for MB males to mate for longer. Egg production also showed a significant interaction (table 1). Females laid more eggs following matings to MB males that had been exposed to rivals and fewer eggs following matings to similarly exposed FB males (figure 2*a*). Hence, again MB males exhibited the wild-type pattern, whereas FB males showed the opposite. Further contrasts were evident in egg fertility, which was significantly lower in females mated to FB males without rivals, but not significantly different in mates of either group of MB males (table 1 and figure 2*b*). MB males fathered more, but not significantly more, progeny than FB males in a competitive context ($p = 0.08$; table 1 and figure 3), which may be linked to their longer mating duration (Bretman *et al.* 2009*a*). Both MB and FB males exposed to rivals prior to mating had significantly higher reproductive success, showing that plastic male responses to rivals were maintained in both regimes. Finally, the tests on females from the MB and FB lines showed, as expected based on the known phenotype of SP, significant differences in receptivity and intermating interval following receipt of SP. However, there were no consistent differences owing to selection regime (electronic supplementary material, table S1), and therefore no evidence that FB and MB females differed in receptivity and fecundity following single matings, or that they responded in a qualitatively different way to receipt of SP.

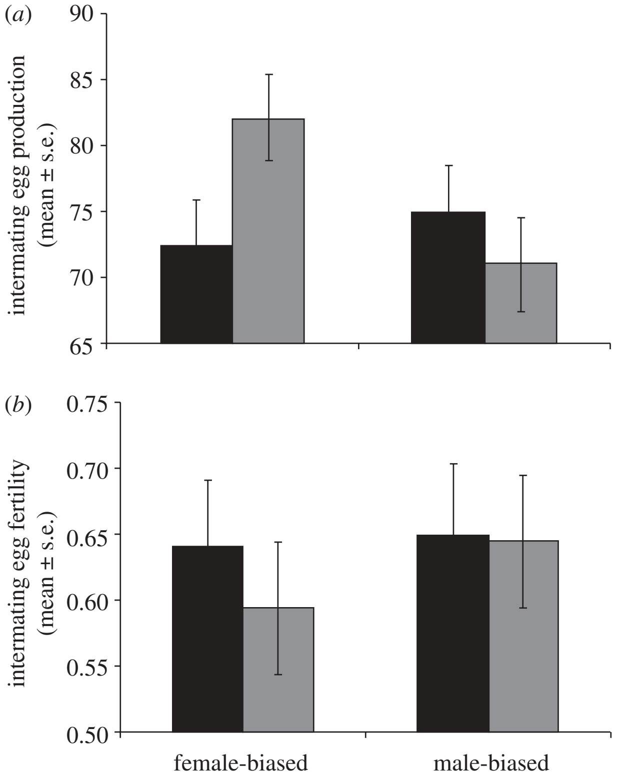

Figure 2. (*a*) Female fecundity in the 24 h intermating interval (mean number of eggs ± s.e.) for females mated to males from the MB and FB selection lines either exposed or not exposed to rivals for 5 days prior to mating. (*b*) Female fertility in the 24 h intermating interval (proportion of eggs fertilized ± s.e.) for females mated to males from the MB and FB selection lines either exposed or not exposed to rivals for 5 days prior to mating. Black bars, +rivals; grey bars, −rivals.

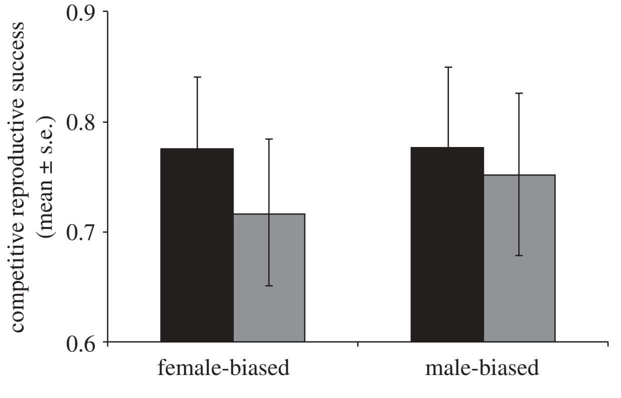

Figure 3. Competitive reproductive success (proportion of offspring sired ± s.e.) for males from the MB and FB selection lines either exposed or not exposed to rivals for 5 days prior to mating. Black bars, +rivals; grey bars, −rivals.

Overall, the study shows that experimental evolution under MB and FB regimes can lead to consistent and significant changes in the way that males respond to rivals, with males reared under MB conditions exhibiting responses similar to those of wild-type males. Differences were seen in both pre-mating and post-mating traits and there were significant interactions, where males from the FB and MB regimes (or the females to which they were mated) showed opposing responses. Consistent with these findings is the idea that the relaxation of male–male competition, and the potentially increased opportunity for female choice in the FB lines, led to the evolution of altered responses to rivals in FB males. An alternative explanation is the strong selection arising from the high frequency of male mating in the FB regimes to avoid sperm depletion, leading to constraints in how FB males responded to competitors.

Sexual selection can speed up the rate of adaptation because it can: (i) reinforce natural selection; (ii) speed up the fixation of advantageous alleles; and (iii) speed up the purging of deleterious alleles (Whitlock 2000; Lorch *et al.* 2003). However, these models are based on the assumption that both male–male competition and female choice are simultaneously elevated, or present/absent. The scenario employed in the creation of the MB and FB lines alters the *balance* of intra- to intersexual selection, and predictions for the speed of adaptation under these conditions have not yet been explored. Few significant effects owing to selection regime were found in our study, which is consistent with the idea that changes in the intensity of male–male competition relative to female choice (rather than elevated sexual selection *per se*) selected for altered responses to rivals.

Males exposed to rivals mate for longer and can transfer more seminal fluid proteins during those longer matings (Wigby *et al.* 2009). An artificial selection experiment on male accessory gland size suggested that the seminal fluid complement of a male can itself evolve (Wigby *et al.* 2009). Differences in the complement of male ejaculates could select for differences in how females respond to a single mating. However, there was no evidence that females from the FB, ES or MB lines differed in how they responded to single matings that either did or did not transfer the SP ejaculate component (electronic supplementary material). Collectively, these data show how relatively simple evolutionary manipulations to the way that adults interact can provide significant insight into the mechanisms underlying both pre- and post-mating sexual selection.

3. DISCUSSION

(**a**) *New avenues for artificial selection and experimental evolution in the study of sexual selection and sexual conflict*

The potential value of the selection experiment in investigating evolutionary responses to factors such as adult sex ratio and the relative balance of intra- versus inter-sexual selection is significant, as illustrated above. There is now an exciting possibility of combining such selection studies with genomic profiling to identify genes that underpin these important fitness traits (Toma *et al.* 2002). To date there are relatively few such tests. One exception is a study on mating speed in *D. melanogaster* (Mackay *et al.* 2005) in which replicated artificial selection for fast and slow mating speed was performed. Transcriptional profiling of the responses to selection in fast and slow mating speed lines revealed a large fraction of genes (approx. 20% of the genome) with potentially altered expression. As with other studies of mating-related genes (e.g. Lawniczak & Begun 2004; McGraw *et al.* 2004), changes were subtle and generally less than twofold. A useful feature of this kind of microarray study is that it can test for biases in the chromosomal locations of the differentially expressed genes. This is of interest in the context of sexual conflict because there are predicted ‘hot spots’ for genes involved in mediating sexual antagonism (Gibson *et al.* 2001). The Mackay study found that the X chromosome harboured fewer differentially expressed genes than expected (Mackay *et al.* 2005). X-linked genes are filtered more often by their passage through the selective environment of females rather than males, and the X chromosome contains an excess of genes that are female-biased in their expression (Parisi *et al.* 2003; Ranz *et al.* 2003; Sturgill *et al.* 2007). Hence, the X chromosome potentially harbours many more ‘female benefit’ genes than expected.

(**b**) *Avoiding the pitfalls*

We have so far emphasized the utility of selection experiments for studying mechanisms of adaptation in response to sexual selection and sexual conflict. However, it is also necessary to consider the potential pitfalls (Harshman & Hoffman 2000; Fuller *et al.* 2005). For example, there has been, to date, perhaps a rather uncritical tendency to view inconsistencies between different studies testing for the evidence of reproductive isolation driven by sexual conflict to be the result of the focal-selective forces under study, rather than because of inadvertent selection or other causes. Other potentially confounding factors that can contribute to differences include differential genetic drift, inbreeding, feeding regimes, differential strengths of selection, gene × environment interactions, constancy of selective environment and differential accumulation of age-specific mutations. All these potential pitfalls have been discussed previously (Harshman & Hoffman 2000; Fuller *et al.* 2005); however, they have not yet been fully incorporated into recent investigations of sexual conflict using selection techniques. To give an example, it has been assumed that elevated sexual conflict sometimes does and sometimes does not lead to measurable differences in reproductive isolation (Hosken *et al.* 2009). However, there are other possibilities, for example (i) sexual conflict may not actually be manipulated in all studies; (ii) inadvertent selection may play a role; (iii) there may be differential inbreeding or genetic drift owing to large differences in effective population sizes; (iv) the use of constant environments and abundant food may obscure differences; (v) there may be differential gene × environment interactions between regimes. So far, the only factor that has been considered in detail is the effective population size, N_e (Wigby & Chapman 2004; Rice & Holland 2005; Snook *et al.* 2009). A recent review suggests that N_e is not likely to have confounded the differences between studies (Snook *et al.* 2009), though it clearly can have a significant effect (Reuter *et al.* 2008). The use of replicate populations to guard against the mistaken attribution of a response to selection when it is in fact drift has proved to be very useful.

In the study reported here, we saw significant, consistent responses in the different replicate lines in male responses to rivals, suggesting that those responses may have a common genetic underpinning. However, responses in females from the same lines to the effects of a single mating were more variable, perhaps indicating divergent responses. Differential genetic drift is unlikely to be an explanation (Snook *et al.* 2009). Inbreeding effects can be problematic, but can in general be countered by crossing between different replicate lines (Hosken & Ward 2001; Hosken *et al.* 2001). However, this procedure can then obscure coevolved differences within lines or indeed any distinct evolutionary trajectories that may have occurred. Whenever lines are tested in a different environment to that for which they are adapted, there is also the potential for differential gene × environment interactions to be expressed. In different environments, fitness may also be favoured by different combinations of alleles, potentially limiting the general utility of the results. An over-abundance of food might also represent a problem if it reduces the likelihood of the expression of differences between regimes. Regimes that employ discrete generations over extended periods may be more likely to accumulate age-specific mutations than are populations that can be maintained under overlapping generations (Harshman & Hoffman 2000), but the latter methodology is not always possible within the context of the selection regimes that are applied.

In short, it is necessary to consider all these factors in concluding whether divergence between different lines is because of the selective regime applied and more caution in this respect is required. The next generation of experiments should seek to equalize N_e as far as possible, increase the numbers of replicates (to provide rigorous tests of the possibility of different evolutionary trajectories, while ruling out drift) and provide fluctuating selection pressures through variable food supply, humidity and/or temperature. Studies in which the results from selection experiments converge with the outcomes from phenotypic manipulations and comparative studies will also be informative. When these principles are rigorously adopted, we will gain increasingly valuable insights into mechanisms of adaptation and evolutionary contingency.

4. CONCLUSION

Unique and powerful inferences have been gained since the time of Darwin by using artificial selection and experimental evolution techniques. Recently, the utility of these approaches has been realized in the context of studying the evolution and mechanistic basis of selection arising from sexual selection and sexual conflict. We highlighted with new empirical data the potential utility of selection approaches for testing how the responses of males to their rivals evolve. We found that alterations of the balance between male–male competition and female choice altered in a predictable and repeatable manner the way that males responded to mating rivals. Finally, we discussed the potential pitfalls and the necessary and important safeguards required in order to investigate important questions such as whether sexual conflict can lead to independent evolutionary trajectories ultimately resulting in reproductive isolation.

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