

undifferentiated, but induction of differentiation results in massive cell death [6]. Fluorescence *in situ* hybridization (FISH) for *Xist* RNA reveals that a significant percentage of cells have inactivated both of their X chromosomes; about the same percentage as have inactivated only one X chromosome. These cells appear to display what Jeannie Lee calls 'chaotic choice'; each cell randomly inactivates zero, one or both of its X chromosomes, with only the cells inactivating a single chromosome surviving. The cells are no longer able to choose one and only one X chromosome to remain active.

To test whether the region containing the 5' portion of *Tsix* contains sequences necessary for counting, extra copies of this region and other nearby regions were introduced into male and female ES cells as multicopy transgenes [6]. All transgenes in a 15 kilobase region downstream of *Xist* which includes *Xite* and the 5' portion of *Tsix* result in a lack of *Xist* coating on both the transgene and the endogenous X chromosomes upon differentiation. This result is difficult to explain in a simple blocking factor-only model for X chromosome counting. If the 15 kilobase region contains the binding site for blocking factor, extra copies would be expected to titrate away blocking factor, inducing silencing of the single X chromosome in males and frequently resulting in two inactive X chromosomes in female cells.

Lee [6] suggests that the abolition of X inactivation upon introduction of *Tsix* or *Xite* transgenes implies instead that these sequences are titrating away something required for initiation of X inactivation—a competence factor. There are several problems with the competence factor/blocking factor model, though. First, if *Tsix* and *Xite* are binding sites for a competence factor, why does deleting these sequences lead to inactivation of the mutant chromosome in a heterozygous female [2,5,7]? Secondly, if competence factor is not present in male cells, how can the single X

in male cells carrying the 65 kb deletion be inactivated [7]?

It seems, then, that neither of the simplest models for X chromosome counting, blocking factor alone or blocking factor plus competence factor, is able to satisfactorily account for all of the data. The surprising effects of the homozygous *Tsix*^{ΔCpG} deletion in female cells suggest that making homozygous ES cells carrying other deletions in the *Xic*, particularly the 65 kb deletion, will be an important next step in trying to sort out what is happening in these cells. Alternatives to the blocking factor and competence factor models propose that differences between the two chromo somes prior to X-inactivation determine the choice of the active and inactive X chromosomes [13]. Perhaps a variation on one of these models will help to explain the X chromosome counting data. There are still pieces missing from the puzzle of how a cell counts its X chromosomes, but it looks like it will be an interesting picture in the end.

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University of California, San Francisco, Department of Biochemistry and Biophysics, 600 16th Street, Box 2200, San Francisco, California 94158, USA.

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Evolution: Do Bad Husbands Make Good Fathers?

Males sometimes harm their mates as they seek to maximise the number of offspring they sire. But are females really suffering or do the benefits of having sons that inherit their father's manipulative traits make up for the costs? Three recent studies provide the first hard data addressing this issue, but they differ in their conclusions.

David J. Hosken and Tom Tregenza

Mate choice, typically by females, and competition for matings and fertilisations, typically by males, are the two agents of sexual selection, and until relatively recently, the

predominant view was that sexual selection is either beneficial or neutral in relation to female fitness [1]. However, theory has long predicted that if there are direct costs of mate choice for females, it is unlikely that the indirect benefits that females might gain from having

genetically superior offspring could make up for these costs [2–4]. This debate has recently been reignited as it has become apparent that conflict between the sexes over reproductive decisions can generate direct costs for females. For instance, male flies poison their mates while attempting to manipulate their reproductive output [5]. The growing evidence for the prevalence of mating costs for females [5–10] raises the question of whether these are real fitness costs, which can be expected to select for females that avoid harmful mates, or whether costs are balanced because females mating to harmful males have sons that can manipulate the next generation of females.

There are three good reasons to be sceptical about the possibility that harmful males may be a good thing for females. Firstly, indirect effects through sons and daughters rely on the heritability of these traits which will never be perfect, and will often be slight. Secondly, in outbred populations, females share half their genes with their offspring, but only a quarter with their grandchildren, so for every child lost because of direct costs, the mother needs two grandchildren to fully compensate for it. Thirdly, the spread of harmful males will eventually create selection for females that resist mating with such males, so a stable equilibrium where harm is balanced by sexually selected benefits may not be possible [4]. Theory is all very well, but as critics have repeatedly pointed out [11,12] what we really need are good data. Three recent studies [13–15] have attempted to address the question of whether the sexually selected benefits of mating with harmful males can more than make up for direct mating costs; two of these studies [13,14] attempt to measure total fitness in a laboratory setting, whilst the third [15] uses an innovative new approach which sidesteps many of the problems associated with measuring fitness.

One way to investigate fitness is to use naturally occurring variation within populations and to evaluate the direct and indirect consequences of mating with more attractive or more successful males. Head *et al.* [13] used house crickets (*Acheta domesticus*) as their model, and divided males into two groups according to how rapidly they can induce a female to mate. They found that although females kept with attractive males have shorter lives, they have more attractive sons than females kept with unattractive males. By estimating the number of grandchildren produced they suggest that the net sexual selection benefits more than outweigh the costs, and hence mating with attractive males is ultimately beneficial.

Orteiza *et al.* [14] took a related approach, but made the opposite finding. Using *Drosophila melanogaster* they allowed females to mate once, then deprived half of them of further contact with males. This isolation deprived females of the opportunity to re-mate but freed them from harassment, and as a result they had substantially higher lifetime fecundity. The authors then showed that the sons sired by first or second mates in doubly mated females had very similar success in siring offspring in a competitive setting. Hence they conclude that there are clear direct costs of matings, but only limited genetic benefits.

So, these two studies produced opposite results: what are we to conclude? Perhaps, the main lesson is just how difficult it is to measure something as slippery as fitness. Head *et al.* [13] chose a single measurable parameter — how fast a male mates, as a measure of his ability to get matings — and examined a range of fitness estimators, one of which, intrinsic rate of increase, suggested compensation via sons. This leaves open the possibility that males that induce rapid mating are poor in other respects. It also ignores a range of possible costs to females,

including costs of discriminating between male types, something that could overwhelm the relatively small observed benefits. Additionally, the one fitness measure to show an association, intrinsic rate of increase, assumes all else is equal and that for example, growth rate does not trade-off against competitive ability or vulnerability to predators, which it does in other taxa [16].

Orteiza *et al.* [14] avoided the problems that arise from trying to identify exactly what it is that gives some males a mating advantage by simply putting groups of males together with females and counting how many offspring are sired by the sons of males that have mated to a virgin female (first mates) compared with the sons of males that managed to secure a mating with a non-virgin female (second mates). However, this approach ignores the possibility that females re-mate to set up sperm competition. So rather than females re-mating to trade-up — suggesting second males should be of higher sexual quality — they allow post-copulatory male-male competition to act as the agent of selection. A final issue that both these studies fail to address, is that although there may be indirect benefits of mating with seductive males there may also be indirect costs through having daughters who are vulnerable to harmful males. Neither study allows these costs to be expressed, so we do not have the whole picture.

A way to get around all these problems would be to examine the spread of a new gene that saves females from male harm, but also removes sexual selection on their mates. Stewart *et al.* [15] have done just such a study, based on the very neat idea of using *D. melanogaster* with one of two different eye colour alleles as markers and then physically moving females with one of these alleles (red eye) into a protected environment. This experimentally imposed a new ‘resistance-to-mating’ function on the red-eye allele. Initially, the ‘resistance allele’

was introduced at a low frequency and after controlling for pleiotropic effects, Stewart *et al.* [15] found that it increased in frequency over time. This shows that the indirect benefits of mating with persistent males for unprotected females — those not expressing the ‘resistance allele’ — do not compensate for the direct costs of mating, and represents the most compelling evidence that direct costs may not outweigh indirect benefits.

So, is this the end of the debate? Of course not. Stewart *et al.* [15] make it very clear that their aim is to examine what is going on in their particular laboratory system, designing their study so that it mimics the way they culture their fly populations. This means that the generality of their finding can partly be assessed by considering how similar their laboratory environment is to the real world. The population was established over 300 generations ago and has been maintained continuously at high population size to retain genetic diversity. The authors [15] claim that this approach means that the flies have had time to become adapted to the laboratory. Although this may be true in the sense that alleles present at low frequencies in the wild population that are beneficial in the lab can be expected to have raced to fixation, it cannot be true in the broader sense of gradual evolution through new mutations. A small glass tube in which males and females are forced into close proximity and walk around on a sea of food bears little resemblance to the varied natural environments where *D. melanogaster* are found, and it is safe to assume that there will be a continual stream of new mutations that prove beneficial on this laboratory island. Indeed, recent work [17] documents on-going evolution in fly populations maintained in the lab for over a 1000 generations. This constant adaptation to the lab may mean that naturally selected differences between individuals are much more important than

sexually selected differences, but whether this is the case in the wild is a point of contention. A second issue is that the lab is particularly unusual in relation to conflicts over mating because of the high-density housing conditions and females cannot escape from males since they are robbed of their major natural defence, which is to simply fly away (but see [18]).

The real benefit of Stewart *et al.*'s study [15] is probably that it answers critics who have worried that the stream of innovative studies coming out of the Rice lab may be ignoring a major component of selection on the mating system [11]. But we still have no clear idea what the relative magnitude of direct and indirect effects are generally. If we are really going to move this debate forward and out of the lab, incorporating the full gamut of costs and benefits of sexual selection, then systems are needed where trans-generational fitness can be measured in nature. Tellingly, in one of the few long-term studies of organisms in the wild [19], sexual selection is reported to have no fitness consequences, which suggests either the equilibrium situation Fisher [20] envisaged (where the benefits of choice are balanced by natural selection costs), or perhaps that the costs and benefits of sexual selection alone are balanced. If this is the case, and costs generated through sexual conflict are balanced by benefits through traditional sexual selection mechanisms, then we may not expect sexual selection to drive rapid evolutionary change. Determining how commonly males impose serious costs on females without compensatory benefits in nature is the next major challenge in the study of sexual conflict.

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Centre for Ecology and Conservation,
University of Exeter in Cornwall,
Tremough Campus, Penryn, Cornwall
TR10 9EZ, UK.