No reduction in the cost of mating for *Drosophila melanogaster* females mating with spermless males

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SUMMARY

Drosophila melanogaster females can incur a cost of mating, manifested as a decrease in longevity and lifetime reproductive success. We investigated whether the cost of mating was a cost of storing and/or receiving sperm by using two types of males that do not transfer sperm (*transformer* pseudomales and the male offspring of homozygous *tudor* mothers). Females that were intermittently exposed to males that did and did not transfer sperm did not differ in lifespan, in the absence of any differences in other costly aspects of reproduction, showing that there was no cost to receiving sperm. There was a cost of mating with spermless males; this suggested a potential cost of receiving accessory fluid. However, it was not possible to distinguish this possibility from other explanations, e.g. female injury at mating and the transfer of parasites. The reasons why females continuously exposed to males remated more than was in their reproductive interests is discussed.

1. INTRODUCTION

If life-history traits such as survival and fertility compete for a share of a finite resource pool, they cannot all be simultaneously maximized by natural selection; there will be constraints upon the set of life histories that organisms can achieve. An important type of constraint on life-history optimization is the 'cost of reproduction' (see reviews by Reznick 1985; Bell & Koufopanou 1986; Partridge & Harvey 1988), which occurs if reproductive activity adversely effects either survival or future fertility (Williams 1966). Although the existence of reproductive costs is generally accepted (Partridge & Harvey 1988), their physiological basis is poorly understood.

In *Drosophila melanogaster* females both elevated rates of egg production and increased exposure to males cause a drop in lifespan (Partridge *et al.* 1987), and part of the survival cost of exposure to males is some consequence of mating itself (Fowler & Partridge 1989). The nature of this cost of mating is unclear, and there seem to be at least three possibilities: mechanical injury at copulation (Lloyd & Park 1962; Ikeda 1974), the transfer of disease or parasites to the female, and some effect of sperm or accessory fluid.

The aim of this work was to test whether the cost of mating is in part a cost of receiving or storing sperm. Mating causes females to undergo behavioural and physiological changes, and sperm may be costly to receive or store for several reasons. Mating by *Drosophila melanogaster* females turns on their egg production (David 1963; Partridge *et al.* 1986; Partridge & Fowler 1990) and causes a decrease in female receptivity that can last 6–9 days (Manning 1962, 1967). The increase in egg production and decrease in female receptivity following mating appear to be due both to a short-lived effect of accessory gland components, e.g. the 'sex peptide' (Chen *et al.* 1988) and a longer-term effect of receiving sperm (Manning 1962; Gilbert *et al.* 1981*a*, *b*; Gromko *et al.* 1984*a*, *b*) and both effects may carry costs to females. In addition, the maintenance of sperm in storage organs could incur a physiological cost for females (Thornhill & Alcock 1983). It is also possible that accessory fluid molecules with costly effects on females might adhere to sperm and be transferred at mating.

One way to test for a cost of receiving sperm is to make use of males that are incapable of transferring it. XO males, which do not transfer motile sperm to the female at mating, were used in two previous experiments (Trevitt & Partridge 1991; Chapman 1992). Trevitt & Partridge (1991) interpreted the difference in lifespan between females exposed to males that did and did not transfer motile sperm as a cost of receiving sperm. However, mutants can have pleiotropic effects, and the non-mating costs of exposure to XO males that could not mate (because of ablation of their external genitalia) were lower than for exposure to otherwise equivalent XY males. The difference appeared to be attributable to differences in male behaviour; XO males were found to deliver significantly less courtship than wild-type males, and this reduction in courtship was associated with significantly lowered levels of female mobility (Chapman 1992). Exposure to XY

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males may therefore have resulted in extra energy expenditure or prevention of feeding in females. Therefore, in these two experiments, it proved impossible to test satisfactorily for costs of receiving sperm, because any such effects were confounded with differences in behaviour between XO and XY males (Trevitt & Partridge 1991; Chapman 1992).

The experiments described here used spermless males that were indistinguishable from control males in all the relevant aspects of their behaviour. Two experiments were done with two different types of males that could not transfer sperm, *transformer* (*tra*) pseudomales and the male offspring of homozygous *tudor* females. The *transformer* gene is part of the regulatory hierarchy controlling sex determination (see, for example, Belote 1989). XX *tra*/*tra* homozygotes are transformed into pseudomales with fully developed sex combs, a male coloured abdomen and normal male external and internal genitalia, although testis size is reduced. XX *tra*/*tra* males do not produce sperm and are therefore sterile, but they do transfer other ejaculate components. Dosage compensation is not affected by the *tra* mutation. XX *tra*/*tra* pseudomales are female in size and larger than XY males, presumably as a result of the effects of non-dosage-compensated size-determining genes on the X chromosome. The *transformer* gene is not essential in males; XY *tra*/*tra* flies are normal, fertile males (Lindsley & Zimm 1992); *tudor* is a maternal effect mutation that affects germ plasm assembly (Boswell & Mahowald 1985). The male offspring of homozygous *tudor* mothers have no germline and therefore do not make sperm; like *transformer* pseudomales, they transfer only accessory fluid at mating; *transformer* and *tudor* males were used in this study to test for decreased costs of reproduction in females that did not receive sperm at mating.

The aim of the two experiments was to investigate the cost of receiving sperm by comparing the survival of females exposed to males that transferred only accessory fluid (XX *tra*/*tra* males or *tudor* males) with that of females exposed to males that transferred both sperm and accessory fluid (XY *tra*/*tra* males or wild-type males) while controlling for female fecundity, fertility and non-mating costs of exposure to males.

2. MATERIALS AND METHODS

(*a*) *Fly stocks*

The Dahomey wild-type stock used in the experiments was random bred and maintained with overlapping generations in population cages at 25 °C on a 12 h:12 h light:dark lighting regimen. The stock was derived from individuals collected in Dahomey (now Benin), West Africa in 1970, and has been maintained according to the above regimen since collection.

A *transformer* stock, $B^{S}Y/yw$; *mwh tra*/*TM3*, *Sb Ser* was used to generate XX *tra*/*tra* pseudomales. The *Bar*, *yellow* and *white* mutations were removed from the stock by outcrossing to Dahomey wild type, and the resulting *mwh tra*/*TM3*, *Sb Ser* stock maintained by tossing onto new culture bottles every 2–3 d. Both XX *tra*/*tra* pseudomales and XY *tra*/*tra* males were therefore free of mutant markers. XY *tra*/*tra* males were a good control for the XX *tra*/*tra* pseudomales because they had identical genetic backgrounds and could be obtained from the same cultures as XX *tra*/*tra* flies. The males were sorted apart on the basis of their size: XX *tra*/*tra* homozygotes are larger than XY *tra*/*tra* males. The stocks were reared in uncrowded conditions and males for use in the experiment always collected from fresh cultures; this ensured that size differences between XX and XY *tra*/*tra* males remained constant over the whole experiment. The XX *tra*/*tra* pseudomales have smaller testes than normal which were easily scored by dissection. Samples of XX *tra*/*tra* ($n = 98$) and XY *tra*/*tra* males ($n = 101$) were later dissected to calculate the efficiency of sorting them by eye.

The *tudor* stock used was *bw sp* $tud^{1}/SM5$; spermless *tudor* males were obtained by collecting the male progeny of homozygous *tudor* virgin females of this stock crossed to Dahomey wild-type males. Wild-type males were used as controls for *tudor* males, as the two types of males exhibited comparable levels of courtship and were therefore expected to have similar non-mating effects on females.

(*b*) *Experimental methods*

To standardize female egg production and egg hatchability and to examine the effects of exposure to different males in normal mated females, all four groups of females in each experiment were exposed to intact wild-type males for 1 day in 3. On the other two days, one group of females in each experiment could receive both sperm and accessory fluid (by exposure to intact *tra*/*tra* XY males or wild-type males), one group in each experiment only accessory fluid (by exposure to intact *tra*/*tra* XX pseudomales or *tudor* males), and two groups in each experiment neither sperm nor accessory fluid (by exposure to XY *tra*/*tra*, XX *tra*/*tra*, wild-type or *tudor* males with ablated external genitalia). The two groups of females in each experiment exposed to males that could not mate were included to test for differences in non-mating costs of exposure to the different types of males.

Males were prevented from mating by ablation of their external genitalia using a microcautery procedure. A potential of approximately 80 V was applied to the external genitalia of 2-day-old males through two tungsten electrodes 40 μm apart, until the penis had been ablated. To check that the males could not mate, they were screened for mating ability 2 d after the microcautery. Effectively microcauterized males attempted to mount females but were unable to attach properly. The penis was the target for cauterization, but slight damage to the claspers surrounding the penis was sometimes evident. Males were put three per phial together with four 5-day-old virgin females; any males that mated within the next 3 h were discarded. In each screen for mating ability of the microcauterized males, control phials of intact 4-day-old wild-type males and 5-day-old virgin females were set up to control for any environmental effects that might have affected the willingness of flies to court and mate (e.g. barometric pressure (Ankney 1984)).

The effect of sperm on female lifespan could therefore be deduced, provided that the non-mating effects of the different males did not differ, and that female fecundity and fertility were unaffected by the experimental treatments.

(*c*) *The transformer experiment*

Experimental females were from the Dahomey stock and were reared at a density of 100 larvae per Sugar Yeast (SY) food vial (Trevitt & Partridge 1991). Adult virgin females (160) were collected at eclosion by using CO_2 anaesthesia, and were then aged in groups of 20 per phial for 3 d. Females

were then randomly assigned to four experimental groups, each of 40 females: (i) females kept with two wild-type males for 1 day in 3, and two XY *tra/tra* intact males for the other 2 days = *sperm+accessory fluid* (*tra XY*) group; (ii) females kept with two wild-type males for 1 day in 3 and two XX *tra/tra* intact males for the other 2 days = *no sperm* (*tra XX*) group; (iii) females kept with two wild-type males for 1 day in 3 and two XY *tra/tra* microcauterized males for the other 2 days = *no sperm or accessory fluid* (*tra XY*) group (control group for the non-mating effects of XY *tra/tra* males in group (i)); and (iv) females kept with two wild-type males for 1 day in 3 and two XX *tra/tra* microcauterized males for the other 2 days = *no sperm or accessory fluid* (*tra XX*) group (control group for the non-mating effects of XX *tra/tra* males in group (ii)).

Each female was placed with two males in a freshly yeasted SY food phial and was given new food every time that the males were changed. Males were initially 5 days old when introduced into the experiment, and were renewed from fresh cultures every 9 days. Males were stored 20 per phial when not kept with females. Female deaths were recorded daily. Eggs were counted in the phials laid up on the days when all females had wild-type males, and these phials were retained to count emerging adult progeny.

Remating frequency was recorded every 3 days on the days when all females were with wild-type males, by scanning phials every 20 min for a period of 3–5 h starting 1–3 h before lights-on, and counting the number of matings. Female behaviour was assayed every 3 days on one of the 2 days out of 3 when females were exposed to the four types of experimental males, to assess whether the males differed in their activity or courtship behaviour. Phials were scanned every 20 min for 3 h starting 1 h before lights-on, and the behaviour of the female recorded as moving or not moving and as courted or not courted.

(*d*) *The tudor experiment*

The methods used were exactly as for the *transformer* experiment except that the experimental females were obtained from uncrowded cultures and not reared at standardized density, and experimental food phials were supplied with live yeast granules only after day 22 of the experiment. The four experimental groups for this experiment, each of 40 females as previously, were: (i) females kept with two wild-type (wt) males for 1 day in 3, and another set of two wild-type intact males for the other 2 days = *sperm+accessory fluid* (*wt*) group; (ii) females kept with two wild-type males for 1 day in 3 and two *tudor* (*tud*) intact males for the other 2 days = *no sperm* (*tud*) group; (iii) females kept with two wild-type males for 1 day in 3 and two wild-type microcauterized males for the other 2 days = *no sperm or accessory fluid* (*wt*) group (control group for the non-mating effects of wild-type males in group (i)); and (iv) females kept with two wild-type males for 1 day in 3 and two *tudor* microcauterized males for the other 2 days = *no sperm or accessory fluid* (*tud*) group (control group for the non-mating effects of *tudor* males in group (ii)).

3. RESULTS

(*a*) *The transformer experiment*

The efficiency of sorting XY *tra/tra* males ($n = 101$) was 100% and was 75% for XX *tra/tra* males ($n = 98$). Therefore, the *no sperm* (*tra XX*) and *no sperm or accessory fluid* (*tra XX*) groups had some exposure to XY *tra/tra* males, and any differences between females exposed solely to XY as opposed to XX *transformer* males may have been slightly reduced. Female survival curves (figure 1) were analysed by using a distribution-free Mantel-Cox test (BMDP Software, W. J. Dixon 1988), which generalizes the Log Rank test (Miller 1981). There was no significant difference in longevity between the two groups of females intermittently exposed to microcauterized males (*no sperm or accessory fluid* (*tra XY*) median lifespan = 20 d, and *no sperm or accessory fluid* (*tra XX*) median lifespan = 19 d) or between the two groups of females exposed to intact males (*sperm+accessory fluid* (*tra XY*) median lifespan = 17 d, and *no sperm* (*tra XX*) median lifespan = 15 d).

Within a genotype, however, exposure to microcauterized males did have a significant effect on female lifespan: *no sperm or accessory fluid* (*tra XY*) females lived significantly longer than *sperm+accessory fluid* (*tra XY*) females (Mantel-Cox (MC) test statistic = 5.21, $p = 0.02$), and *no sperm or accessory fluid* (*tra XX*) females lived significantly longer than the *no sperm* (*tra XX*) group (MC = 5.87, $p = 0.01$).

Egg production (figure 2) was analysed by using multiple comparison Kruskal–Wallis tests (BMDP Software, W. J. Dixon 1988). Egg production did not differ significantly between the groups except in the initial sampling interval where the *sperm+accessory fluid*

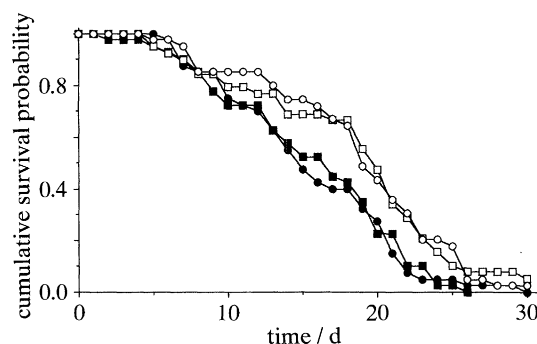

Figure 1. Cumulative probability of survival against time (days) for females exposed to wild-type males intermittent with XY *tra/tra* intact males (*sperm+accessory fluid* (*tra XY*); filled squares), XX *tra/tra* intact males (*no sperm* (*tra XX*); filled circles), XY *tra/tra* microcauterized males (*no sperm or accessory fluid* (*tra XY*); open squares), or XX *tra/tra* microcauterized males (*no sperm or accessory fluid* (*tra XX*); open circles).

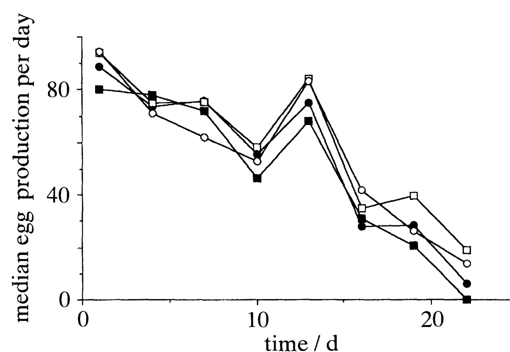

Figure 2. Median number of eggs produced against time (days) per 1 day sampling interval for the same groups of females as in figure 1; symbols are as figure 1.

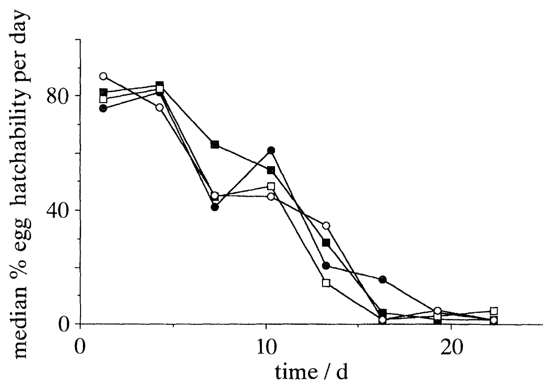

Figure 3. Median percentage egg hatchability against time (days) per 1 day sampling interval for the same groups of females as in figure 1; symbols are as figure 1.

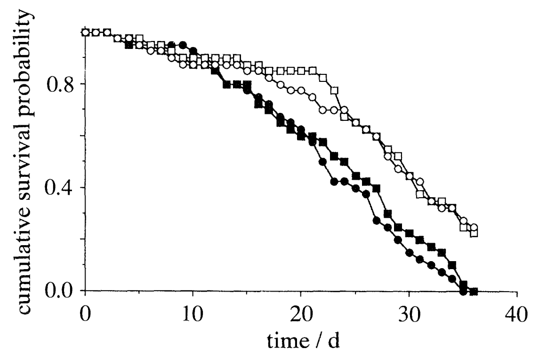

Figure 4. Cumulative probability of survival against time (days) for females exposed to wild-type males intermittent with wild-type intact males (*sperm + accessory fluid* (*wt*); filled squares), *tudor* intact males (no sperm (*tud*); filled circles), wild-type microcauterized males (*no sperm or accessory fluid* (*wt*); open squares), or *tudor* microcauterized males (*no sperm or accessory fluid* (*tud*); open circles).

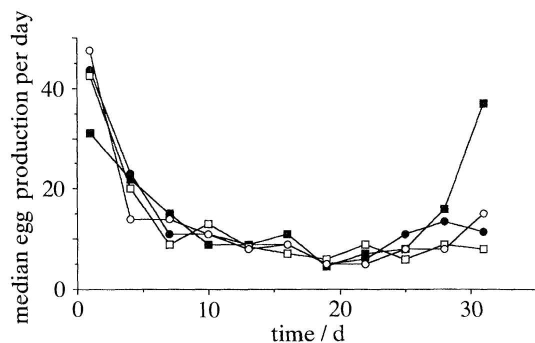

Figure 5. Median number of eggs produced against time (days) per 1 day sampling interval for the same groups of females as in figure 4; symbols are as figure 4.

(*tra XY*) group laid significantly fewer eggs than the *no sperm or accessory fluid* (*tra XY*) and *no sperm or accessory fluid* (*tra XX*) groups ($p < 0.05$). Median egg hatchability was calculated and plotted against female age (figure 3) and analysed by using Kruskal–Wallis tests; egg hatchability did not differ between the four groups except in the first sampling interval where the *no sperm or accessory fluid* (*tra XX*) group had significantly higher egg hatchability than the *no sperm* (*tra XX*) and *sperm + accessory fluid* (*tra XY*) groups ($p < 0.05$). Both of these results would be expected by chance with so many comparisons (i.e. 1 in 20 at the $p < 0.05$ level).

Mating frequency was recorded on the days when all females were kept with wild-type males. Each morning of mating observations was treated as one mating opportunity 'offered' for each female and, if a mating was scored, this was a mating opportunity that was 'taken'. The number of matings recorded in each sampling interval was small (maximum percentage of opportunities taken was 10%, minimum 0%), so the remating frequencies were summed throughout the experiment to give a larger sample size. The data for the four groups were compared by using a χ^2 test, and there were no significant differences in remating frequency, $\chi^2 = 3.39$, 3 d.f. Because the mating frequencies did not differ significantly on days when intact wild-type males were present, and because matings with XY *tra/tra* and XX *tra/tra* males on the other days were seen, those females continuously exposed to intact males must have had higher overall mating frequencies than females intermittently exposed to microcauterized males.

The data on behaviour were used to calculate, for each female in each sampling interval, the proportion of observations in which she was either courted or not courted and either moving or still. The data were then subjected to the angular transformation and one-way analysis of variance. The four groups of females did not differ significantly in the amount of courtship they received or the proportion of time that they spent moving around, except in the first two sampling intervals, where females exposed to XY *tra/tra* intact males were courted significantly more ($p < 0.05$) and moved around significantly more ($p < 0.001$) than females in the other groups. However, the differences in female lifespan were not consistent with the view that any initial and short-term difference in the non-mating costs of exposure to the different males had a qualitative effect on the experimental outcome.

(*b*) *The tudor experiment*

All data were analysed as described for the *transformer* experiment. Analysis of female survival curves (figure 4) showed that there was no significant difference in longevity between the two groups of females intermittently exposed to microcauterized males (*no sperm or accessory fluid* (*wt*) median lifespan = 30 d, and *no sperm or accessory fluid* (*tud*) median lifespan = 29 d), or between the two groups of females exposed to intact males (*sperm + accessory fluid* (*wt*) median lifespan = 24 d, and *no sperm* (*tud*) median lifespan = 22 d).

Within a genotype, however, exposure to microcauterized males did have a significant effect on female lifespan: *no sperm or accessory fluid* (*wt*) females lived significantly longer than *sperm + accessory fluid* (*wt*) females (Mantel-Cox (MC) test statistic = 10.28, $p = 0.0013$) and *no sperm or accessory fluid* (*tud*) females lived significantly longer than the *no sperm* (*tud*) group (MC = 12.94, $p = 0.0003$).

The median number of eggs produced by the four groups of females are shown in figure 5. There were no differences in egg production between groups except on the first and last sampling days ($p < 0.01$ and $p <$

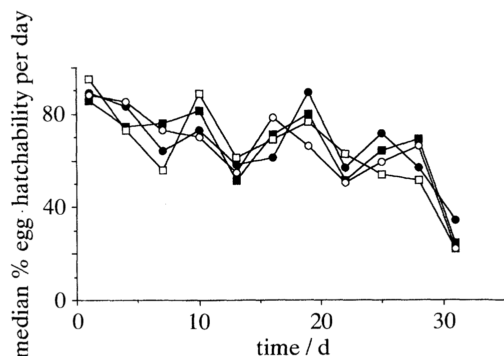

Figure 6. Median percentage egg hatchability against time (days) per 1 day sampling interval for the same groups of females as in figure 4; symbols are as figure 4.

0.05, respectively). Egg hatchability is shown in figure 6; there were no significant differences in the fertility of eggs laid by the four groups on any of the sampling days of the experiment.

The number of matings recorded in each sampling interval was slightly higher than in the *transformer* experiment (maximum percentage of mating opportunities taken was 25 %, minimum 2 %) and, as before, remating frequencies were summed throughout the experiment to give a larger sample size. There were no significant differences in remating frequency, $\chi^2 = 6.14$, 3 d.f. Matings with wild-type and *tudor* males were observed on the 2 days out of every 3 when experimental males were present and, therefore, because mating frequencies did not differ significantly on days when all females were exposed to intact wild-type males, those females continuously exposed to intact males had higher overall mating frequencies than females intermittently exposed to microcauterized males that could not mate.

Analysis of the amount of courtship received showed that there were mostly no significant differences in the proportion of time females were courted, except on the first two sampling days ($p = 0.012$ and $p = 0.033$, respectively). The proportion of time that females spent moving around was not significantly different except in the first sampling interval ($p = 0.001$). Unlike the *transformer* experiment, there was no consistent pattern to the differences between the groups in the initial sampling intervals. Any initial variance in non-mating effects could not explain the differences in female lifespan that were seen.

(c) *Lifetime reproductive success of females in both experiments*

The total number of progeny produced from egg samples over the lifetime of each female in each of the two experiments were calculated to give a measure of lifetime reproductive success (LRS). The skewness and kurtosis of all data were calculated and found not to differ significantly from normality, except for the kurtosis of the *no sperm* (*tud*) group in the *tudor* experiment ($0.05 > p > 0.01$). However, because in all other comparisons the data could be assumed normal, a three-way ANOVA procedure was used to compare female LRS between groups and experiments (SPSS™ data analysis system 1988). Male genotype, mating status (i.e. intact against microcauterized) and experiment (i.e. *transformer* against *tudor*) were the main effects. There were significant differences in lifetime progeny production due to mating status ($F = 5.69$, $p = 0.018$), experiment ($F = 71.78$, $p < 0.01$) but not to male genotype ($F = 0.025$, $p = 0.875$). Females exposed to intact males produced significantly fewer offspring in their lifetimes (mean LRS for *transformer* experiment = 186.8, *tudor* experiment = 122.6) than did females exposed to microcauterized males (mean LRS for *transformer* experiment = 204.7, *tudor* experiment = 140.7). Differences in female LRS between experiments would have been expected because females in the *tudor* experiment (mean LRS for all females = 131.7) were not supplied with yeast until day 22, and their lifetime reproductive successes were correspondingly lower than for females in the *transformer* experiment (mean LRS for all females = 195.5). There were no significant two- or three-way interactions between any of the main effects ($p \gg 0.05$).

4. DISCUSSION

The most important result from these two experiments was that there seemed to be no cost of receiving sperm. This was evident because the lifespans, egg production, egg hatchability and remating rates of females intermittently exposed to males that could (either XY *tra*/*tra* males or wild-type) or could not (either XX *tra*/*tra* males or *tudor*) transfer sperm were not significantly different.

There were no significant differences in the amount of courtship delivered by spermless (i.e. XX *tra*/*tra* or *tudor*) and fertile (i.e. XY *tra*/*tra* or wild-type) males, and no significant differences in the lifespans of females intermittently exposed to microcauterized spermless or microcauterized fertile males. This suggests that within each experiment there were no significant differences in the non-mating costs of exposure to the two types of males used. In the *transformer* experiment, however, the efficiency of sorting XX *tra*/*tra* males from XY *tra*/*tra* males was only 75 %, but females intermittently exposed to intact XX *tra*/*tra* males would still have received substantially lowered sperm levels overall because they were kept for 2 days out of 3 with males of which 75 % could not transfer sperm.

Exposure to intact males resulted in a significant decrease in female lifetime reproductive success over both experiments. The differences in lifetime reproductive success of females in the *transformer* experiment were small and could have been made greater if the courtship intensity and remating rates of the males had been increased (e.g. by keeping three males with each female), therefore exposing females to more reproductive costs, resulting in larger differences in lifespan between females exposed to intact and microcauterized males. The larger differences between the lifetime reproductive success of females exposed to intact and microcauterized males in the *tudor* experiment may have been due to the fact that remating frequencies were slightly higher than in the *transformer* experiment, and females may therefore have incurred a higher

mating cost (Fowler & Partridge 1989). The flies used in these experiments had a long history of laboratory culture and the mutant stocks were probably subjected to some inbreeding depression. However, the females used were wild type from an outbred stock, and mutant stocks too show costs of reproduction (see, for example, Bellen & Kiger 1987); controls for effects of the mutants used were also present in the experimental design.

It is not clear why females remated to such an extent that their reproductive success was impaired. It is possible that these experiments revealed a potential conflict to which females in population cages are not normally exposed. The Dahomey wild-type females used in these experiments came from cages with an evolutionary history of approximately 20 years. Females would have been expected to evolve remating rates in response to conditions prevailing in the cages, and it is possible that an ancestral conflict between the sexes, resolved for conditions within population cages, may have been revealed by altering the conditions under which males and females met. For example, females in cages may be in poor nutritional condition, and there is evidence that when food levels are so low as to reduce longevity, the cost of mating disappears, perhaps because remating rates are much lower when food is limiting (Chapman *et al.* 1993). There is no evidence for nutritional benefits accrued through multiple mating in food-starved females (Chapman *et al.* 1993). The males used in these experiments were also renewed from fresh cultures on a regular basis. Females may also have been subjected to more courtship and matings than in population cages, where, for example, evasion of males by flying away is probably less restricted.

New food is provided in the population cages used in these experiments once a week. Females that can capitalize on the new food resource and free oviposition space, i.e. those with a full sperm store and capable of high rates of egg laying, will be favoured. This strategy could also be adaptive under natural conditions where food resources may be both ephemeral and unpredictable. Males also congregate around the fresh food and females may come into contact with large numbers of courting males; remating by females might be adaptive in these circumstances to avoid harassment and to enable them to lay fertile eggs on the new food. Being amongst the first females to lay eggs on a new food medium may be advantageous for several reasons. The first larvae to hatch in the new food will have a competitive advantage, and therefore a higher proportion of the eggs laid by females that arrive first will be successful. As the severity of larval competition increases, the number of available oviposition sites on the food medium will also be reduced, as will feeding sites that are free from larval interference.

The results also confirmed the cost of mating with males that cannot transfer sperm (Trevitt & Partridge 1991; Chapman 1992). Females intermittently exposed to microcauterized males lived significantly longer than females intermittently exposed to spermless males; this is suggestive of an accessory fluid cost. The increase in egg production and decrease in receptivity of mated females are caused, at least in part, by components of the accessory fluid (see, for example, Kummer 1960; Garcia-Bellido 1964; Leahy 1966; Leahy & Lowe 1967; Merle 1968; Chen & Bühler 1970; Burnet *et al.* 1973; Chen *et al.* 1988). However, it is not possible, based on the design of these experiments, to distinguish whether the cost of mating with spermless males is due to an accessory fluid cost or to a cost of receiving other ejaculatory duct components, injury at mating, or the transfer of disease or parasites, and these possibilities merit further investigation.

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