Enhanced cost of mating in female sterile mutants of *Drosophila melanogaster*

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In *Drosophila* females, mating is known to cause a reduction in life span, which is referred to as 'the cost of mating'. Since mating enhances oogenesis and oviposition, the cost of mating may be regarded as a trade-off between reproduction and longevity. We examined whether the cost of mating exists in mutant females that are unable to produce eggs. Three different mutant alleles of *ovarian tumors* (*otu*) and an allele of *dunce* (*dnc<sup>M11</sup>*) of *Drosophila melanogaster* were used to sterilize females. For all the female sterile mutants tested, mating dramatically decreased the life span of homozygous sterile females. Even more extreme shortening of life spans were observed when the sex peptide gene (*Acp70A*) was expressed in homozygous *otu* females, though they were virgin, indicating that the shortening in life span is due to seminal factors. These results indicate that the cost of mating is greater in females defective in oogenesis than that in normally fertile females.

**Key words:** *Drosophila*, female sterile mutant, cost of mating, life span

**INTRODUCTION**

Reproduction of an organism is accompanied with costs. Typically, an increased reproductive activity results in a decreased probability of survival (for a review, see Bell and Koufopanou, 1986). The cost of reproduction was often regarded to occur as a trade-off between reproduction and life-history traits (Bell and Koufopanou, 1986 and literatures therein). Consistent with this hypothesis, *Drosophila subobscura* females, sterilized by exposure to high temperatures or X-ray irradiation, were shown to have extended life spans (Maynard Smith, 1958; Lamb, 1964). In *D. melanogaster* females, a manipulation of the egg-production rate by changing the availability of nutrition or oviposition sites has shown that increased rates of reproduction result in decreased survival (Partridge et al., 1987). Furthermore, artificial selection experiments in *D. melanogaster* for early or late reproduction have shown that increased reproduction early in life leads to a reduction in the females' life span (Rose and Charlesworth, 1980, 1981; Rose, 1984; Luckinbill et al., 1984).

It is also well-known in *Drosophila* females that mating decreases their longevity. This is referred to as 'the cost of mating' (Fowler and Partridge, 1989; Chapman, 1992; Chapman et al., 1993, 1995, 1996, 1998). During mating, a number of proteins contained in seminal fluid are transferred, along with sperm, from males into females. Some of the seminal fluid substances enhance the reproductive activity of the mated females, such as the stimulation of yolk protein synthesis and the accumulation in oocytes (Soller et al., 1997), and/or induction of ovulation and oviposition (Chen 1996, Heifetz et al., 2000). Therefore, the cost of mating may be regarded as a trade-off between increased reproduction and life span (for review, see Rose, 1991). However, studies with *D. melanogaster*, in which mating was experimentally manipulated, have demonstrated that mating itself reduces the life span of females, without affecting lifetime egg productivity, and therefore egg production alone cannot account for the cost of mating (Partridge et al., 1986, 1987). Further experiments have revealed that the decrease in female's life span is due to seminal substances produced in the main cell of male accessory glands (Chapman et al., 1993, 1995). Indeed, *Acp62F*, an accessory gland protein, was found to be toxic when expressed ectopically (Lung et al., 2002). Based on these results, the cost of mating is suggested to be a side-effect of evolutionary conflict between the sexes (Chapman et al., 1995).

Since seminal substances have diverse physiological effects on the mated female, it is difficult to determine to what extent the reduction of longevity in mated females is due to mating itself or to enhanced reproduction (Chap-
man et al., 1998). We reasoned, as did earlier studies (Maynard Smith, 1958; Lamb, 1964), that if enhanced egg production were largely responsible for the reduction in longevity, we might expect an extension in life span in those females which are unable to produce eggs. To test this, we examined the effect of mating on the longevity of *D. melanogaster* females whose oogenesis was genetically deprived. To sterilize the females, three different alleles of a female sterile mutant, *ovarian tumor* (otu), were used. The oogenesis of homozygous otu females arrests at various stages, depending on the alleles (King et al., 1986; Storto and King, 1988). In addition, we tested another female sterile mutant, *dunce* (dnc), of which the effect of mating on female longevity has previously been reported (Bellen and Kiger, 1987; Chapman et al., 1996).

In this paper we will show that, contrary to the above supposition, the longevity of genetically sterilized females decreases dramatically upon mating, which is in sharp contrast to the previous findings with *D. subobscura* (Maynard Smith, 1958; Lamb, 1964). We will also show that such effect of mating can be mimicked by ectopically expressing a seminal peptide gene, *Acp70A* (the sex peptide gene), in virgin sterile females.

**MATERIALS AND METHODS**

**Fly Strains.** Three strains each carrying different otu (ovarian tumor) mutant alleles, *et* otu1v otu2v /FM3, *y* ev /FM3 and otu6v v f /FM3 and otu7v /FM7c, were obtained from the Bloomington Stock Center. Females homozygous for these otu alleles rarely produce mature ova because of defective oogenesis. A dnc (dunce) strain, y dncM11 /FM3, was supplied by R. Hardy, University of California at San Diego. Females homozygous for dncM11 are unable to lay eggs due to a somatic cell defect (Bellen and Kiger 1987).

A sex peptide transgenic strain (G10) was described by Aigaki et al. (1991). It carries on the second chromosome a transgene consisting of an enhancer of the *Yolk protein 1* (*Yp1*) gene, the sex peptide gene (*Acp70A*) and the promoter of heat shock protein 70 gene (*hsp70Bb*). Hereafter, this transgene is abbreviated to *SP*. Females carrying this transgene are known to express constitutively the sex peptide gene (Aigaki et al. 1991). Another transgenic strain, abbreviated to the SP-GFP strain, carries on the second chromosome a transgene consisting of the promoter of the sex peptide gene and the green fluorescence protein (GFP) gene. Seminal fluid of this strain transferred into females is visible under a fluorescence microscope (Payre, 2001).

Males from a wild-type strain, Canton-S, were used for mating with test females.Balancer chromosomes and other markers were described by Lindsley and Zimm (1992).

Flies were grown on a cornmeal-yeast-glucose medium and maintained at 25°C under continuous lighting. Adult flies were collected within 8 h after eclosion and sexed under light etherization. For other manipulations of flies, a mouth aspirator was used. Sexed flies were kept in an incubator at 25°C under a 12:12 h light-dark cycle, and flies aged 4–5 days after eclosion were used for the experiments.

**Longevity test.** About 30 test females and 50 wild type males were introduced into a vial (3 × 11 cm) containing food, and kept for 24 hours. During this period, most of the females were likely to mate only once. Mated females were transferred to a fresh food vial. Every 2 or 3 days, flies were transferred to a new vial and the number of dead flies were counted until all the females died. More than 5 replicate vials were tested for each group and data were pooled for statistical analyses. Because of variation in fly's age at the onset of a longevity assay, the life span was defined in this study as a survival period from the start of an assay until death.

**Fecundity test.** About 30 test females and 50 wild type males were introduced into a food vial and mated pairs were aspirated out. After the copulation was completed, females were singly transferred to a new food vial. Every 2 days, until all the flies died, females were transferred to new vials and the numbers of eggs laid were counted.

**Examination of GFP-labeled seminal fluid.** Females were allowed to mate for 1 h with the SP-GFP males. The mated females were dissected at various periods after mating and the intensity of GFP fluorescence in the uterus was examined under blue light irradiation (515 nm) on a binocular microscope (LEICA MZFLIII) equipped with a filter (GFP2 filter). In most cases, the presence or absence of the GFP label in the uterus could unambiguously be determined, and in cases where a faint signal was discernible, they were classified as negative.

**Repeated mating.** Virgin females, 4–5 days after eclosion, were confined with males in a vial, and copulated females were aspirated out. After seven days, these females were given a chance to mate again. Remated females were separated from the males and their life span after the second mating, together with those of the unmated and the mated-once flies, were examined.

**Statistical analyses.** For the statistical analyses of the survival curves, a non-parametric test was carried out using the LIFETEST procedure of the SAS software package (SAS Institute Inc.).
Cost of mating in *Drosophila* females

Figure 1. Survival curves for mated and unmated females that were homozygous or heterozygous for three ovarian tumor alleles, *otu*\(^6\) (a), *otu*\(^1\) (b), and *otu*\(^7\) (c). Open and filled circles, respectively, indicate homozygotes and heterozygotes, and solid and broken lines, respectively, indicate mated and unmated females.

Figure 1. Survival curves for mated and unmated females that were homozygous or heterozygous for three ovarian tumor alleles, *otu*\(^6\) (a), *otu*\(^1\) (b), and *otu*\(^7\) (c). Open and filled circles, respectively, indicate homozygotes and heterozygotes, and solid and broken lines, respectively, indicate mated and unmated females.
RESULTS

Effect of mating on the longevity of \textit{otu} females.

Figure 1 shows the survival curves for females homozygous and heterozygous for mutant \textit{otu} alleles, and the mean life span is summarized in Table 1. For all the \textit{otu} alleles tested, the differences in the survival rates among the four groups of females were highly significant (Log-Rank test; $\chi^2$ (d.f. = 3) = 894.6, $p < 0.0001$ for \textit{otu}$^6$; $\chi^2$ (3) = 930.6, $p < 0.0001$ for \textit{otu}$^1$; $\chi^2$ (3) = 540.1, $p < 0.0001$ for \textit{otu}$^7$). A large part of the observed heterogeneity among the groups is apparently due to the shorter life spans of \textit{otu} homozygotes as compared with those of the heterozygotes. With respect to the effect of mating, the life span of the mated sterile females is significantly shorter than that of the unmated sterile females for all of the \textit{otu} mutant alleles tested (Log-Rank test; $\chi^2$ (1) = 33.7, $p < 0.0001$ for \textit{otu}$^6$; $\chi^2$ (1) = 261.1, $p < 0.0001$ for \textit{otu}$^1$; $\chi^2$ (1) = 32.5, $p < 0.0001$ for \textit{otu}$^7$). In contrast, with fertile heterozygous females, the mated females’ life span is significantly longer than or equal to that of the unmated females ($\chi^2$ (1) = 63.0, $p < 0.0001$ for \textit{otu}$^6$; $\chi^2$ (1) = 210.9, $p < 0.0001$ for \textit{otu}$^7$).

Fecundity of females heterozygous for \textit{otu} An unexpected result was the lack of a life-shortening effect of mating in females heterozygous for \textit{otu} mutants. To test the possibility that this is due to differential fecundity between mated and unmated females, lifetime egg production was examined with \textit{otu}$^6$ heterozygotes. The total number of eggs produced ± SE (N) were 1,161 ± 152 (17) for the mated females and 1,257 ± 165 (22) for the unmated females. There was no significant difference in the lifetime egg production between the mated and unmated heterozygous females (t-test; $p = 0.502$).

Fate of seminal fluid in mated females In fertile females, mating induces a quick ovulation response, followed by oviposition. Therefore, seminal fluid remaining in the uterus may be purged away together with eggs from the female body. If the sterile females could not expel the fluid because of an absence of eggs, they might be exposed to a toxic effect, if any, of seminal substances for a longer duration, leading to an early death of the sterile females. To explore the possibility of this effect, the fate of GFP labeled seminal fluid was traced in the uterus of the sterile and fertile females. Figure 2 shows changes after mating in the proportion of females in

<table>
<thead>
<tr>
<th>Females</th>
<th>\textit{Mean life span (days) ± SE (number tested)}</th>
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<tr>
<td>\textit{otu}^6 (QUI)</td>
<td>\textit{otu}' (ONC)</td>
</tr>
<tr>
<td>Mated \textit{otu}/\textit{otu}</td>
<td>27.9 ± 1.3 (142)</td>
</tr>
<tr>
<td>Unmated \textit{otu}/\textit{otu}</td>
<td>39.4 ± 0.5 (302)</td>
</tr>
<tr>
<td>Mated \textit{otu}/ +</td>
<td>63.8 ± 0.6 (200)</td>
</tr>
<tr>
<td>Unmated \textit{otu}/ +</td>
<td>58.3 ± 0.6 (209)</td>
</tr>
</tbody>
</table>

QUI, quiescent allele; ONC, oncogenic allele; DIF, differentiating allele

Figure 2. Proportion of females in which GFP labeled seminal fluid was detected in the uterus at respective times elapsed after copulation. Open and filled bars, respectively, indicate homozygous and heterozygous females for \textit{otu}'. For each class, 23 to 57 females were examined. Error bars show the 95% confidence limits.
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The half-time of the GFP signal persistence was 2.6 h and 2.1 h for homozygous and heterozygous females, respectively. At any time after mating, no significant difference was found between the fertile and sterile flies in the proportion of females with a positive GFP signal (p > 0.05 by Fisher’s exact probability method). It is unlikely that the observed decrease with time in the proportion of flies having a positive signal reflects the decay of GFP fluorescence because GFP-labeled sex peptide that is translocated from the uterus to the sperm storage organs is clearly visible at more than four days after mating (Peyre and Aigaki, unpublished).

**Effect of repeated mating on longevity**

Figure 3 shows the survival curves for virgin, once- and twice-mated females that are homozygous for *otu*<sup>6</sup> and *dnc<sup>M11</sup>*. The mean life span is summarized in Table 2. The life spans of unmated and once-mated females are compared.

<table>
<thead>
<tr>
<th>Mating status</th>
<th>Mean life span&lt;sup&gt;a&lt;/sup&gt; (days) ± SE (number tested)</th>
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<tr>
<td></td>
<td><em>otu</em>&lt;sup&gt;6&lt;/sup&gt;</td>
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<tr>
<td>Virgin</td>
<td>32.0 ± 0.6 (147)</td>
</tr>
<tr>
<td>Mated once</td>
<td>20.6 ± 1.0 (154)</td>
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<tr>
<td>Mated twice</td>
<td>16.7 ± 0.8 (154)</td>
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</table>

<sup>a</sup> survival time after the second mating.
were shorter than those observed in the experiments mentioned above (Table 1) because the assessment of life span started 8 days later in this experiment. Females that mated twice had a significantly shorter life span than did females who mated once (Log-Rank test: $\chi^2(1) = 10.7$, $p = 0.0011$ for $otu^6$; $\chi^2(1) = 20.8$, $p < 0.0001$ for $dnc^{M11}$) and the life span of the latter was significantly shorter than that of virgin females ($\chi^2(1) = 31.6$, $p < 0.0001$ for $otu^6$; $\chi^2(1) = 74.2$, $p < 0.0001$ for $dnc^{M11}$).

The present study clearly demonstrated that the cost of mating is much higher in females that are unable to produce mature eggs because of the $otu$ mutation. The phenotype of the $otu$ mutants are known to vary depending on different alleles. According to the affected stages in oogenesis, they are classified into three groups; quiescent alleles (QUI) in which germ cells are lacking, oncogenic alleles (ONC) which are defective in the proliferation of germ cells, and differentiating alleles (DIF) which are defective in nurse cell/oocyte differentiation (King et al., 1986; Storto and King, 1988). The three $otu$ alleles, $otu^6$, $otu^1$ and $otu^7$, used in the present study are one of QUI, ONC and DIF alleles, respectively. Despite such heterogeneity in the stages at which oogenesis was arrested, the life-shortening effect of mating was similar across the $otu$ alleles, indicating that the stages of oogenesis attained have little effect on the cost of mating. That the decreased life span of mated sterile females is due to copulation is supported by the finding that females homozygous for $otu^6$ that had mated twice lived shorter lives than those that mated once.

In another female sterile mutant of $D. melanogaster$, $dunce$ ($dnc$), inseminated homozygous females have been shown to live shorter lives than virgins (Bellen and Kiger 1987). This indicates that the increased cost of mating in sterile females is not unique to the $otu$ mutant. Chapman et al. (1996) suggested that an elevated rate of remating in sterile $dnc$ females was responsible for their short life span. They showed that there were no significant differences in life span between virgin females and females who had mated once. In contrast, differences

**DISCUSSION**

The effect of ectopic expression of a sex peptide transgene

Figure 4 shows the survival curves for virgin females that carry the $otu^6$ allele and express ectopically the sex peptide gene. As shown in Table 3, sterile females ($otu^6$ homozygotes) expressing the sex peptide gene had an extremely short life span and the difference between homozygous females with and without the $SP$ transgene was highly significant ($\chi^2(1) = 567.0$, $p < 0.0001$). In contrast, expression of the $SP$ transgene in $otu$ heterozygotes significantly extended their life span ($\chi^2(1) = 89.6$, $p < 0.0001$).

<table>
<thead>
<tr>
<th>Genotypes</th>
<th>Mean life span (days) ± SE (number tested)</th>
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<tbody>
<tr>
<td>$otu^6$/+$SP$/+$</td>
<td>11.4 ± 0.4 (300)</td>
</tr>
<tr>
<td>$otu^6$/+$+/+$</td>
<td>39.4 ± 0.5 (302)</td>
</tr>
<tr>
<td>$otu^6$/FM3$/SP$/+$</td>
<td>50.3 ± 0.9 (300)</td>
</tr>
<tr>
<td>$otu^6$/FM3$/+/+$</td>
<td>45.3 ± 0.8 (307)</td>
</tr>
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</table>
between virgin females and females who had mated twice were significant. In the present study, we also examined the effect of mating on the life span of dnc<sup>M11</sup> females. In agreement with the results obtained with <i>otu</i><sup>6</sup>, females' life spans were found to decrease with increasing numbers of mating. The two studies yielded somewhat different results, presumably because different <i>dnc</i> alleles were used, or the method of assessing life span was different. However, our results obtained with <i>otu</i> females indicate that the increased cost of mating in <i>dnc</i> females is due to their inability to produce mature eggs rather than their increased frequency of mating.

It has been reported in <i>Drosophila subobscura</i> that females sterilized by exposure to high temperatures or by ionizing radiation survived longer than fertile females, which is just the opposite of our results. The difference in longevity between fertile and sterile females was suggested to be due to their different fecundity (Maynard Smith 1958; Lamb 1964). Other studies, however, have shown that the fecundity of females might not be an important determinant of the longevity of females (Aigaki and Ohba 1984; Partridge et al. 1986, 1987). Our results also suggest that the life span of females may not be affected by fecundity, because in females heterozygous for <i>otu</i><sup>6</sup>, the life span of the mated females was significantly longer than that of unmated ones, though there were no significant differences in the fecundity between them.

It is well-known in wild-type <i>Drosophila</i> females that mating decreases their life span (e.g. Fowler and Partridge, 1989). In the present studies, however, a reduction in longevity in mated females heterozygous for <i>otu</i> was not observed, though they were as fecund as the wild-type females. Conversely, with two of the three <i>otu</i> alleles, the mated heterozygous females were found to live longer than the unmated females. In previous studies, females were allowed to mate repetitively, while in this study they were given a chance to mate only once. Such a brief exposure to males might be insufficient to reveal the cost of mating, although the reason why the mated females lived longer compared with virgin females remains unanswered.

The cost of mating in male <i>Drosophila</i> is known to arise from substances, including the sex peptide, that are produced in the male accessory glands and transferred to the female during copulation (Chapman et al., 1995). The extremely short life span found with the sterile females constitutively expressing the sex peptide transgene indicates that the life shortening effect of mating in sterile females is caused by substances contained in the seminal fluid. This does not necessarily mean that the sex peptide itself has an adverse effect on the survival of females. Indeed, no decrease in life span was observed with fertile females expressing the sex peptide transgene as compared with females lacking the transgene (data not shown). The sex peptide and some other peptides in the seminal fluid are known to stimulate the reproductive activity of mated females, such as the activation of juvenile hormone biosynthesis and the promotion of oogenesis, ovulation and oviposition (Moshitzky et al. 1996; Soller et al. 1997; Chen et al. 1988; Aigaki et al. 1991). Such physiological responses to seminal peptides may be disturbed in sterile females because of defective oogenesis. This may cause some metabolic imbalance resulting in early death. Whatever the reason, the exaggerated cost of mating revealed in sterile females seems to provide us with a useful system for elucidating the physiological basis for the cost of mating.

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