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Distinct Biological Epochs in the Reproductive Life of Female *Drosophila melanogaster*

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Abstract

Mating alters the physiology and behavior of female *Drosophila melanogaster* resulting in a surge of egg laying, a decrease in receptivity to other males, and a decrease in life span. Here, we show striking differences in patterns of *Drosophila* egg laying and mortality rate dependent upon mating history. Our data reveal previously unreported epochs in the reproductive life of females: optimal, vulnerable and declining-terminal. During the optimal period, mating induces females to respond with a surge in egg laying and has a reversible effect on mortality rate. In contrast, during the vulnerable period, mating does not induce females to respond with a surge in egg laying and causes an irreversible increase in mortality rate. The terminal period was always observed several days before death, irrespective of the chronological age, and is marked by sharp reductions in egg laying. The presence of these distinctive biological epochs may reflect increased female sensitivity to mating due to age-related decline.

Keywords

Aging; *Drosophila melanogaster*; Reproduction; Egg laying; Mating

1. Introduction

Reproduction significantly influences longevity, yet age also has a profound effect on the rate at which animals reproduce (Finch, 1990). In a variety of animals including humans, female fecundity declines with advancing age and results in menopause (Wood, 1989). *Drosophila* females also undergo age-related declines in fertility and have allowed detailed analysis of the effects of mating and reproduction on aging (Partridge, *et al.*, 1986; Partridge *et al.* 1987; Wolfner, 1997; Partridge *et al.* 2005). Mating markedly changes the physiology of female *Drosophila melanogaster*, resulting in a shortened life span compared to virgins (Fowler & Partridge, 1989; Wolfner, 1997). This has been attributed to a “cost of reproduction” that

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includes the allocation of physiological resources for courtship, mating, egg production, egg laying and a direct toxic effect of mating (Fowler & Partridge, 1989; Partridge *et al.* 2005). The post-mating changes in *Drosophila* females are caused by male accessory gland proteins (Acps) that are transmitted in the male seminal fluid during mating (Chapman & Davies, 2004). For instance, Acp62F causes the toxic effect of seminal fluid and thus is the causative agent in shortening the life span of females and may also influence sperm storage (Chapman, *et al.* 1995; Lung *et al.* 2002; Wigby & Chapman, 2005). In addition, mating-induced increases in egg production are caused by sex-peptides Acp70A and Acp26Aa (Chapman *et al.* 2001; Chapman *et al.* 2003; Liu & Kubli, 2003; Peng *et al.* 2005).

Age-associated changes in mating strategy have been observed in some species. For example, virgin female cockroaches became less selective in choosing their partners with increasing age (Moore & Moore 2001). Middle aged and old female ladybird beetles preferentially mate with the middle aged and older males (Pervez *et al.* 2004). Virgin female European grapevine moths display decreased fecundity and fertility if mated later in life but have increased survivorship (Torres-Vila *et al.* 2002). Mediterranean flies if kept on a sugar diet do not produce eggs and are in a type of “waiting mode” (Carey *et al.*, 1998a). When protein is added to their food after a prolonged period on sugar, they start laying eggs and live much longer than flies continuously fed only sugar or sugar and protein. The goal of our study is to determine the effect of age on reproduction, since only the effect of reproduction on survival has been carefully studied in *Drosophila* female. How does the age of the female affect her response to mating, particularly her ability to increase egg laying? A related unresolved issue is whether mating has a reversible effect on mortality. We addressed such questions by mating virgin female fruit flies of variable age to 3-day old males as life-long mates or for a single 10-day period. Analysis of the observed changes in egg laying patterns and mortality identified three distinct epochs in the reproductive life of females. The progression of *Drosophila* females through these three biological epochs may reflect age-related shifts in the physiology of female flies.

2. Materials and methods

2.1. Fly Strains

Two different inbred laboratory fly strains were used in the studies: The *PwgCyO* line, with an enhancer trap construct inserted into the *wingless* (*wg*) gene of a *CyO* balancer chromosome, was obtained from J. Kassis (Kassis *et al.* 1991). The standard wild type *Canton-S* (*CS*) line was obtained from the Bloomington Stock Center (Stock number 1). Both lines were used in experiments to determine the effect of mating at different ages on female egg production and mortality. *CS* flies were used in experiments to determine the optimal and critical time for mating, or is the ages when mating causes the smallest and biggest effects on female mortality. Both lines were kept on standard corn media in a temperature controlled incubator at 25° with a 12: 12 light: dark cycle.

2.2. Fertility studies

For the first fertility study 480 virgin *wg* females were collected on CO₂ and placed in individual plastic vials containing standard cornmeal agar medium with several grains of yeast at 25° in a humidified incubator. At age 0 (MD0) (n=98), 20 (MD20) (n=97), 40 (MD40) (n=95) or 60 (MD60) (n=96), subgroups of females were separated and each of the females was mated to a single 3-day old *wg* male. Dead males were replaced with sibling males of the same age. The flies were passed to new vials daily and the number of eggs and age of death for each individual female was recorded. A total of 501,558 eggs were counted in 5 different experimental trials. In the second study *wg* females were continuously mated to males from day 0 but *wg* males were replaced with 3-day old males on day 20, 40 and 60 (n=104). The control group was *wg* virgins (n=110). In the third fertility study, 120 *CS* females were collected as virgins and

divided into the following experimental groups: virgins, continuously mated (MD0) and groups in which males were present only during a 10-day period (0-10 (MD0-10), 10-20 (MD10-20), 20-30 (MD20-30) and 30-40 (MD30-40)). On the days previously noted, a single 3 day old CS male was added to a single female and kept together for a 10-day period. The number of eggs was recorded as described above.

2.3. Determination of the cost of mating at different ages

C5 females flies were collected as virgins and divided into the following experimental groups: virgins, continuously mated or males present during 10 day period (0-10, 10-20, 20-30, 30-40, 40-50, 50-60 and days 60-end). Each vial contained 40 females. On the days previously noted, twenty 3 day old CS males were added to twenty females and kept together for a 10-day period. After the males were removed 40 females were gathered and kept together. The flies were kept in a plastic vial containing a standard cornmeal agar medium with several grains of yeast at 25° in humidified incubator. The flies were passed to a new vial every Monday, Wednesday and Friday when the number of dead females was recorded. Each group contained between 163-521 females.

2.4. Statistical analysis

Survival analysis employed Kaplan-Meier survival functions, the log-rank test to assess statistical differences in median longevity, and Cox hazard regression. The latter was used to define average risk ratios for the entire life span (risk ratio 1) and for remaining lifetime after mating was initiated (risk ratio 2). Smoothed hazard rates were obtained for five cohorts (MD0, MD20, MD40, MD60 & virgins, using bandwidths 40, 35, 25, 35 and 30, respectively) by means of the transformation method described in (Müller *et al.* 1997). The period hazard rate ratio of all mated groups vs. virgin was defined as ratio of the number of deaths during a 20-day period and the number of subjects at risk at the mid-point of the period.

A Poisson regression model was applied to investigate differences in the average egg production of each group compared to virgins and how average egg production changes within the same group as the female age. Considering that the egg-laying pattern of mated groups over time may change differently from the virgin group, the interaction effects between different periods and groups are also included in the model. The virgin group in the period from day 0 to 19 is treated as baseline (See Supplemental Table 1b for details).

3. Results

3.1. The age of the females determines the effect of mating on egg-laying

To determine the effect of age on egg laying and mortality rate, we placed 480 virgin *wg* females in individual vials. At ages 0 (MD0), 20 (MD20), 40 (MD40) or 60 (MD60), equal subgroups of individual females were separated and each female was mated to a single 3-day old *wg* male. Numbers of eggs laid and the survival of each group were monitored daily and compared to control virgin females. We found that the average lifetime number of eggs in all experimental groups was similar, with the exception of flies in the MD40 group that produced the lowest life long number of eggs (MD40=843.1 eggs/female compared to virgin=1027.3, $p<0.025$, Table 1). These results confirm the findings of a previous report that also found similar fecundity in virgins and females mated throughout the life (Partridge *et al.*, 1986). Although females that were mated continuously or at a later point in life laid about the same average number of eggs as virgins, the dynamics of egg production depends on the presence of males and age of the females. While mating early in life leads to a short-term increase in eggs laid compared to age-matched virgins, this is compensated by a long-term reduction. Females that were mated from day 0 (MD0) laid the highest number of eggs between 5-10 days of age. They laid 109.7% more eggs compared to virgin flies during the first 20 days of life (MD0:

average=793.3, standard error (SE)=40.1; Virgin: average=378.2, SE=21.6, $p<0.0001$) (Supplemental Table 1A and B). After day 30, MD0 females laid fewer eggs relative to virgins (Fig. 1 and 2A). Between days 40-59, they laid 53.2% fewer eggs (MD0: average= 126.9, SE= 19.1; Virgin: average= 271.2, SE= 20.4, $p<0.0001$) and 48.3% fewer during the 60-end period (MD0: average=14.7; Virgins: average=28.5; $p<0.0001$) (Fig. 1 and 2A, Supplemental Table 1A). Females that began to mate on day 20, MD20, responded to males with a burst in egg production, laying 32.5% more eggs compared to virgins during 20-39 days of age (MD20; average=519.4, SE=29.4; Virgin: average=391.8, SE=22.5, $p<0.0001$, Fig. 1 and 2B, Supplemental Table 1A). This period is followed by a decrease in eggs laid compared to virgins after day 40. MD20 laid 37.5% fewer eggs than virgins between 40-59 days, and 52.7% fewer in the 60-end period (Fig. 1 and 2B, Supplemental Table 1A). Egg production of virgin, MD40 and MD60 females was not statistically different before males were added suggesting that their reproductive potential is very similar. Females that began to mate on day 40 did not respond with a surge in egg laying, instead, 56.1% fewer eggs were laid by MD40 compared to virgins between 40-59 days (MD40: average= 119.01; SE=19.0; Virgins: average= 271.24, SE=20.4, $p<0.0001$) and 77.1% fewer eggs during the 60-the end of life period (MD40: average= 6.5, SE=2.9; Virgins: average= 28.5, $p<0.0001$, Fig. 1 and 2C). When males were added to 60-day old virgins they laid 17.7% fewer eggs compared to the females that remained virgins (MD60: average=23.5, SE=4.2; Virgins: average=28.5, SE=5.0, $p<0.0001$, Fig. 1 and 2D). Interestingly, we observed that MD60 females were able to have viable offspring even though mating put them at high risk of their own mortality (Figure 4 and data not shown). These results indicate that there is a major shift in fertility during the female life span. The optimal time for egg laying seems to start immediately after eclosion and extends to at least 20 days of age and stops before age 40. This is illustrated by an initial increase in eggs laid by females mated from day 0 and on day 20. Furthermore, females mated at age 0, 20, or 40 laid fewer eggs after 35-40 days of age compared to the virgin females. Our data suggest that around age 40 females appear to enter a less optimal period for egg laying, since they may be more vulnerable to the costs of mating, particularly to the toxic effects of males (Chapman, *et al.* 1995; Lung *et al.* 2002).

In order to determine if the age-related pattern of egg laying is specific for the *wg* genotype or is a more general phenomenon, we determined life-long egg laying of wild type *Canton-S* (CS) females which were kept as virgins, mated continuously or mated during a 10 day period that began at different ages. The use of a 10 day period allowed more precise timing of the age-related changes in female mating responses. Continuously mated females (MD0) and females mated during the first 10 days (MD0-10) laid about 10 times more eggs during the first 10 days compared to virgin females (MD0; average=237.1, SE=13.4; MD0-10: average=241.1 SE=12.6; Virgins; average=24.3 SE=3.3, $p<0.0001$) and about 100% more eggs during the 10-19 day period (MD0; average=218.62, SE=8.3; MD0-10: average=261.4, SE=8.1; Virgins: average=106.69, SE=5.3, $p<0.0001$) (Fig. 3, Supplemental Table 1D and E). Both groups laid fewer eggs compared to virgins starting from 20 day of age (Fig. 3, Supplemental Table 1D and E). Females mated first at age 10 (MD10-20) and 20 (MD20-30) laid significantly more eggs compared to virgin females during the 10 days of mating (Supplemental Table 1D and E). Both groups laid fewer eggs compared to virgins after age 30. MD30-40 laid similar numbers of eggs compared to virgin females after addition of males. These results indicate that similar to *wg* females, wild type CS females respond with a surge in egg laying only when mated early in life. No surge in egg laying was observed if females were mated for the first time at age 30. In addition, all mated CS females laid fewer eggs compared to virgin females after age 25 (MD0), 30 (MD0-10 and MD10-20) or after age 40 (MD20-30 and MD30-40) (Supplemental Table 1D).

3.2. The effect of mating on life span depends on the age of the *wg* female

In order to determine how mating at different ages affects *wg* female life span, we measured survivorship for each experimental condition. While addition of males dramatically changes survivorship, the extent of this mating effect depends on the age of mating (Fig. 4). The highest cumulative impact of mating is seen in continuously mated females that were exposed to males starting at day zero and thus have a 30.3% shorter mean life span of 48 days compared to 68.9 days for virgin females ($X^2=40.5$ $p<0.0001$) (Table 2). With the exception of MD40 (mean=55.9 days), the females display a life span inversely proportional to the day of male addition (Table 2). Mean life span was the shortest for the continuously mated females, but the 90th quantile of life span was not significantly different from that of other mated females (Table 2). This may be a result of the fact that males in the continuously mated group were aging together with females and their effect on females was decreased as compared to the three days old males added to the MD20, MD40 and MD60 groups. The other explanation comes from the heterogeneity hypothesis. This hypothesis suggests that only certain robust females, characterized by lower mortality, survive to older age (Vaupel *et al.* 1979). To address this issue we conducted a new set of experiments with *wg* females, which strongly support the first explanation. The male present with a continuously mated female was replaced on days 20, 40, and 60 with a fresh 3-day old male. The mean and 90th quantile of the female's life span is reduced 35.3% and 25% compared to life span of virgins, respectively (data not shown).

3.3. Effect of mating on the hazard risk to life depends on age of the female

The immediate risk to life (i.e. death within 20 days of initial mating) caused by mating is mainly due to the transfer of Acps, which increase egg production and have the direct toxic effect (Chapman *et al.* 1995). It is possible to separate these two risks by measuring egg production. The highest immediate impact of mating, seen as an increased hazard risk of 330% and 309%, occurs in the MD40 and MD0 cohorts respectively. However, the number of eggs laid is quite different for these two groups (Supplemental Table 1A). Mating initiated at age 0 causes a surge in eggs laid, but mating initiated at age 40 reduces egg laying compared to age-matched virgins (Fig. 1 and 2C). The increased immediate hazard risk is likely to be only caused by the direct toxic effects of mating for the latter but not for the former group.

3.4. *Drosophila* females are most at risk from mating during the vulnerable epoch

Our findings suggest that at around age 40, females might be at their most vulnerable to the effects of mating. We refined our studies to more clearly determine optimal and unfavorable times for mating. In previous survivorship studies, once females started to mate, they were mated continuously until death. Thus, some females experienced larger cumulative effects of mating than others. In a new set of experiments, wild type *CS* females were mated for a 10 day window at different ages. Females mated during 0-10 and 10-20 days of age experience a reversible influence of mating on survivorship (Fig. 5A and B). The high initial death rate of females, when mated immediately, decreased to a level lower than that of virgins once males were removed. Thus, survivorship rate became almost identical to virgins at about age 60, (Fig. 5A and B) resulting in 90th quantiles of life span similar to that of virgins. For MD0-10 and MD10-20 females mean life span was shorter by 20% and 14% respectively compared to virgin flies, but 90th quantile was shorter by only 8.7% and 3.7% respectively (Supplemental Table 2). Next, we determined how late in life the effect of mating on mortality rate could be reversed. The mortality rates for females mated at age 20 or later never return to virgin levels after the removal of males, and there was little or no decline in the mortality rate when the males were removed (Fig. 5C). In fact, the highest immediate and long term impact of mating is seen in females mated between 20-30 days, illustrated by a decrease of 34.3% in the mean (38.8 days) and 32.8 % in the 90th quantile of life span (54.3 days) compared to virgin females (Virgins: mean=59.4, 90th quantile=80.8, $p<0.0001$, Supplemental Table 2). A similar difference in

mean and 90th quantile life span relative to virgins was observed in females that were mated over their entire life span (decrease in mean=33.8% and 90th quantile= 24.8% life span) (Supplemental Table 2). Interestingly, MD20-30 females have the highest risk ratios for the entire life span (5.08), even when compared to MD0 (3.85) (Supplemental Table 2). Furthermore, the highest increase in the number of dead females among all 10-day mating periods is between 25-30 days of life for females first mated on day 0, 10 and 20 (Fig. 6). A smaller increase in the number of dead females at period 60-70 was observed in a group that was mated for the first time at 60 days, analogous to our data from fertility experiments. High mortality at age 60 comes as a result of both advanced age of the female and the high mating rate of 3-day old virgin males. Our data suggest that differences in the mean and 90th quantile of life span of *Drosophila* females mated at different times in life result from an age-dependent response to mating. The egg-laying pattern in CS females in response to 10 day matings show that mating up to age 20 days leads to a short-term increase in egg-laying, followed by a decrease in eggs laid later in life (Fig. 3). In conclusion, mating early in life has a reversible effect on female 90th quantile of survivorship. During that time females increase egg laying after mating. This optimal epoch is followed by a vulnerable epoch during which the effect of mating on survivorship is irreversible. During this period all mated females laid fewer eggs compared to virgins.

3.5. Female flies have a terminal phase prior to death

Examination of the reproductive histories of all females revealed a post-fertile period during which females rapidly decrease egg laying, as illustrated by averages of 0.2 and no eggs laid on the penultimate and final days of life respectively, (Fig. 7A and B). In order to further correlate the decrease in eggs laid and death we plotted average eggs laid by females on the days prior their death (Fig. 7C and D). Early increases in eggs laid are followed by fecundity plateaus at mid-life, when viewed from the time of death rather than time of birth (Fig. 7C and D). At about 40 days prior to death, all females show a linear decline in eggs laid. This declining period ends with a terminal period that is present in all females regardless of duration of life or mating schedule (Fig. 7A-D). The presence of a declining phase in *Drosophila* females implies that, under laboratory conditions, death occurs after a period of physiological decline characterized by decreases in the number of laid eggs.

4. Discussion

Drosophila melanogaster has been a powerful model system for examining factors that affect aging and survival. In particular, the effects of reproduction on survival have been studied in some detail. Given this rich background, it was of interest to examine age-related changes in reproductive physiology. It has been shown that mating induced alterations in physiology and behavior of female *Drosophila melanogaster* result in increased egg production, decreased receptivity to further mating and shorter life span. One aspect of the aging-reproduction interaction that has not been explored previously in *Drosophila* females is how age affects female reproductive physiology, as measured by egg laying and mortality rate. Here, we showed that the age at which a female first mates affects egg laying and life span. Based on age-specific changes in the effect of mating on egg laying and mortality, our data suggest that the reproductive life history of female *Drosophila* can be divided into the three distinct biological epochs: optimal, vulnerable and declining.

4.1. Mating costs in *Drosophila* females can be reversed during optimal epoch

The optimal epoch occurs early in life, at 0-30 days of age for *wg* and 0-20 for CS flies. During this period females respond to mating with a surge in egg laying (Fig. 1, 2A and B). The observed higher immediate risk to life during this period is due to a high mating rate, the cost of increased egg laying, and a direct toxic effect of mating (Fig. 1, Table 2 and Supplemental

Table 1). Surprisingly, the negative influence of mating on mortality rate can be reversed during this time with the removal of males, resulting in a minimal accrued risk of mating to the 90th quantile of life span. (Fig. 5A and B).

4.2. A vulnerable epoch is defined by the highest immediate and long-term impact of mating on survivorship despite decreased fecundity

The epoch of optimal reproductive potential is followed by the vulnerable epoch, characterized by an irreversible negative effect of mating on female survivorship and an inability of females to increase egg laying after mating (Fig. 1-5). The negative effect is demonstrated in females mated at age 20 days or later by an increase in mortality rate that is not reversed by male removal (Fig. 5B and C). During this epoch females are most vulnerable to mating illustrated by the highest immediate impact of mating and an increased hazard risk of 330% on life span observed in MD40 females compared to virgins (Table 2). The impact comes mostly as the result of a direct toxic effect of males since the females laid fewer eggs after mating, and therefore, there is no increased risk due to cost of egg production (Fig. 1-3). Declining egg production during the vulnerable epoch might contribute to the leveling off the mortality rates of females *Drosophila* at older ages (Curtsinger *et al.* 1992).

4.3. Females in all experimental groups have a third declining-terminal epoch, irrespective of the duration of their life span

There is a linear decrease in egg laying starting over the last 40 days of female life. The fact that females decrease egg laying as they approach their death and stop laying eggs one to two days prior to their demise, suggests that physiological changes prior to death preclude egg laying. The supine behavior of male Mediterranean fruit flies also appears to “predict” the time of death (Papadopoulos *et al.* 2002). In mated *Drosophila* females, Rauser described accelerated declines of egg laying in females prior to death (Rauser *et al.* 2005). We found that the terminal period is present in all females on five different reproductive schedules and regardless of the length of life. The observation of a “terminal phase” of life in the fly irrespective of age implies that under controlled laboratory conditions, the death of these animals is not a sudden accidental event but the culmination of a process that includes physiological changes as reflected by declining egg laying. It also indicates that, despite the stochastic nature of death when viewed as a population phenomenon, life span follows a similar progression at the level of the individual. The presence of this terminal phase of life in a genetically powerful model system will allow detailed analysis of the “death” trigger and the physiological changes which define this terminal phase of life, providing a biopredictor for death in laboratory strains of *Drosophila*.

4.4. Distinct biological epochs in *Drosophila* females suggest physiological adjustments to age-associated increase in frailty

The underlying mechanism for the presence of the biological epochs might be physiological changes to cope with mating stress, particularly with the toxic effects of mating. Many species are able to adjust to challenging environmental conditions by changing their physiological modes. These changes include the dauer stage of *C. elegans*, diapause in insects and the stationary phase in yeast (Tatar & Yin, 2001). Furthermore, most organisms curtail reproduction when food is scarce in order to maintain their ability to reproduce under more favorable conditions (Finch 1990, Tatar & Yin, 2001, Carey *et al.* 1998a). In fact, Mediterranean fruit flies enter a physiological state of low mortality and reproduction in response to a diet lacking protein and revert to normal mortality and reproduction when protein is restored to the diet (Carey *et al.* 1998a). Similarly, when exposed to different nutrient levels females modulate their egg laying, so that under reduced nutrient conditions egg laying decreases and resources are mainly used for body maintenance (Partridge *et al.* 1987, Bross

et al. 2005). Allocation of physiological resources for courtship, egg production and egg-laying reduces the remaining resources available for DNA repair and growth (Müller *et al.* 2001). As females age, more resources may need to be allocated for the repair of damage caused by the direct toxic effect of males and damage accumulated during aging. Such a change in resources allocation could explain our observation that females do not increase egg laying in response to mating at midlife, and moreover laid fewer eggs compared to virgins. Our findings demonstrate an age-dependent increase in the vulnerability of female flies to external stresses, such as mating. The differential cost of mating, viewed as mortality, in relation to age at first mating is a consequence of increasing frailty and diminishing resources. In addition, our findings provide evidence for the presence of mechanisms to cope with increasing age-associated frailty and other age-associated changes in female *Drosophila*. The presence of these distinctive biological epochs may reflect physiological changes of the females that allow them to maximize fecundity early in life but focus resources on body maintenance during midlife and later in life, allowing them to cope with mating stress and increasing age-associated frailty. The fact that females with two different genetic backgrounds have similar age-related responses to mating with regard to egg laying and mortality suggests that the presence of three epochs is most likely a general phenomenon for laboratory strains of *Drosophila*.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1. Mating status determines female life-long egg laying patterns

The average number of eggs per 20 day periods laid by *wg* female virgins (Virgins), here compared with females that started to mate at day 0 (MD0), day 20 (MD20), day 40 (MD40) or day 60 (MD60). Continuously mated females (MD0) show 109.7% ($p<0.0001$) increase in egg laying during the first 0-19 days compared to virgin females. There is an increase of 32.5% in eggs laid for MD20 females during days 20-39 after mating compared to virgin females ($p<0.0001$). During the 40-59 day period all mated females laid fewer eggs compared to virgin females (MD0: 53.2%, $p<0.0001$; MD20: 37.5%, $p<0.0001$ and MD40: 56.1%, $p<0.0001$). Similarly, during the end of the life span (60 – end) all mated females laid significantly fewer eggs compared to the virgins (MD0: 48.3%, MD20: 52.8%, MD40: 77.1%, and MD60: 17.8, $p<0.0001$ for all). Insert is enlarged 60-end period.

Figure 2. Average difference in daily egg laying between virgin females and females mated at day 0 or later in life

A) High egg laying observed in continuously mated *wg* females early in life is followed with a decrease below the level of virgins after day 30. B) There is an increase in eggs laid in MD20 females during the first 20 days after mating. After age 40, these females laid fewer eggs relative to virgins. There is a significant decrease egg laying after adding males to the MD40 (C) and MD60 (D) group on day 40 and 60, respectively. Arrows indicate time when males were added.

Figure 3. Mating increases egg laying in early life but reduces it in midlife

The average egg-intensity in 10-day periods laid by CS female virgins, females that were mated continuously (MD0), or were mated for a 10-day period from 0-10 days (MD0-10), 10-20 (MD10-20), 20-30 (MD20-30) or 30-40 (MD30-40). MD0 and MD0-10 females laid more eggs during the first 0-19 days compared to virgin females but fewer eggs after day 20. Similarly, MD10-20 females laid more eggs during 10-29 period but fewer compared to virgin females after age 30. MD30-40 do not increase their egg laying after first mated at age 30. During the 40-59 day period all mated females laid fewer eggs compared to virgin females.

Figure 4. Effects of mating depends on the age of the female

Survivorship (A) and smoothed hazard function rate function (B) of the *wg* female flies that were mated the whole life (MD0) or started to mate at day 20 (MD20), 40 (MD40) and 60 (MD60) compared to virgin flies. B) Hazard function of MD0 and MD40 increases more than the other cohorts after initial mating. Different bandwidths was used to smooth the hazard rate: 40, 35, 25, 35 and 30 for MD0, MD20, MD40, MD60 and Virgins, respectively. Number of female flies included in experiments: MD0=97, MD20=97, MD40=95, MD60=96 and virgins=95.

Figure 5. Effect of mating on female survivorship is reversible early in life

A) Survivorship of the CS females mated only during a 10-day period that started at different times in life, for instance MD0-10 means that females were mated during 0-10 days of life. Female flies that were mated from 0-10 and 10-20 days have shorter mean life span, 47.6 days and 50.9 days respectively, compared to the virgin females (59.4 days) but 90th quantile of life span is similar to the virgin flies (Supplemental table 2). Females that were mated only during the age of 20-30 days have the shortest mean (38.8) and 90th quantile (58.7) of life span compared to females mated throughout the life span or any other experimental group (Supplemental Table 2). B) Age of the female determines changes in mortality rates after mating. Females mated during 0-10 days display an initial increase in mortality and an observed relative decline in mortality that became almost the same as the virgin females at age 40. C) An irreversible effect of mating is observed in females mated during 20-30 and 30-40 days; their mortality stays higher than virgin females after the removal of males. Number of female flies used in experiments: MD0=182, MD0-10=343, MD10-20=336, MD20-30=337, MD30-40=322 and Virgins=521.

Figure 6. *Drosophila* wild type females are most at risk to the adverse effects of mating during the vulnerable epoch

This figure shows the period hazard rate ratio of all the mated groups vs. virgin CS females. The highest immediate impact of mating on survivorship is observed at around age 25-30 for females that started mating between 0 and 20 day, suggesting that might be a particular window of vulnerability to mating. The period hazard ration is defined as ratio between death number during the 20-day period and number of subjects at risk until mid-point of the period.

Figure 7. Females flies decrease number of egg laid as they come close to their death

Daily numbers of eggs laid by individual continuously mated MD0 (A) and virgin (B) *wg* females is color-coded: Black= 0, green 1-10, yellow 11-30, 31-50, blue 51-more eggs/day. Each horizontal line represents the daily life-long egg production for one fly. All female flies were ordered according to their estimated survival probability (y-axis) (Carey *et al.*, 1998b). C) Regardless of their mating schedules, all females decrease egg laying as they approach death and lay no eggs the day prior to death. A vertical line indicates the beginning of this declining period. Most of the females display fecundity plateaus prior the declining period. D) Average eggs laid by females from different mating schedules before death on the days listed.

Table 1
Average life-long number of eggs /fly in wg females on different mating schedules.

Group	N	Average life-long number of eggs/fly	SE egg/fly	Average eggs/day	SE average eggs/day
MD0	97	1220.1	76.2	25.3	1.32
MD20	97	1058.1	55.7	17.8	0.84
MD40	95	843.1	54.5	14.5	0.90
MD60	96	1065.6	61.9	16.3	0.89
Virgins	95	1027.3	59.5	14.8	0.83

wg female flies produce about the same number of eggs during their life with exception of females mated at day 40 (MD40). Females were mated from day 0 (MD0), day 20 (MD20), day 40 (MD40), day 60 (MD60) or kept as virgins. MD40 produce significantly fewer eggs compared to MD0 ($p<0.0002$) and virgins ($p<0.025$). Total production for each group: virgin 97,593, MD0 118990, MD20 102629, MD40 80045 and MD60 102301. N, Number of flies in each experiment. SE is standard error.

Table 2

Effect of mating on the survivorship and remaining life span

	Mean Life Span (% change)	90 th Quantile of Life Span	Log-Rank Test (χ^2)	Risk ratio 1 (95% C.I.)	Risk Ratio 2 (95% C.I.)	Percent Increase Hazard Risk After Male Addition
MD0	48 (30.3)	75	40.5	2.50 (1.86, 3.36)	2.50 (1.86, 3.36)	309%
MD20	58.2 (15.5)	75	31.3	2.30 (1.70, 3.10)	2.44 (1.80, 3.31)	140%
MD40	55.9 (18.8)	69	55.2	3.41 (2.44, 4.79)	3.83 (2.69, 5.47)	330%
MD60	62.2 (9.7)	76	23.4	2.17 (1.58, 2.96)	2.67 (1.86, 3.82)	246%
Virgins	68.9	89				

The mean and 90th quantile of life span of wg females started to mate at day 0 (MD0), 20 (MD20), 40 (MD40), 60 (MD60) and virgin females. Parentheses indicate the % change in comparison to the virgin female flies. Log-Rank tests are mated group vs. virgin group and are all highly significant. P<0.0001 for each experiment. Risk ratios are obtained from fitting a Cox regression model, with risk ratio 1 for entire lifetime and risk ratio 2 for remaining life span only, where remaining life span is the life time after males are added. 90th Quantile of life span is the age at which 10% of the cohort live longer, and 90% have died. Hazard risk is the ration between number of death within 20 days of initial mating in certain cohort and number of risk set at the midpoint of the 20-day period.