Original Article

The Retired Fly: Detecting Life History Transition in Individual *Drosophila melanogaster* Females

James W. Curtsinger


Address correspondence to James W. Curtsinger, 1987 Upper Buford Circle, St. Paul, MN 55108. Email: jwcurt@umn.edu

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Abstract

Life history observations at the level of individual model organisms are relatively scarce, but highly informative. Here I analyze published data on the survival and lifetime fecundity of 3,971 individually housed, mated *Drosophila melanogaster* females from nine experimental populations. Data were collected from four laboratories and include counts of over 4.6 million eggs. Individual fecundity records are dominated by zero-egg-days (ZEDs). I show that the timing of ZEDs is informative about the survival and reproduction of individual flies. The first postmaturation ZED divides adult life into two functional stages: working and retired. The working stage is characterized by relatively high levels of oviposition and survival, while the retired stage is characterized by low levels of oviposition and reduced survival. The retired stage typically lasts one quarter of the total adult life span. The age of transition varies between flies; consequently age-synchronized cohorts will generally contain a mixture of working and retired flies, possibly influencing responses to experimental treatments. ZED can be used as a nonintrusive, real-time biomarker to distinguish live flies in the prime of life from those in a terminal state.

Key Words: Fecundity—Survival—*Drosophila*—Life history

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*Drosophila melanogaster* has been a model system for basic research on aging for almost a century (1). Many investigations have focused on variation in survival and reproduction (2–16). While survival data are routinely collected on individual flies, fecundity observations have mostly been made at the level of entire cohorts, obscuring individual variation. Since the 1990s, when biodemographic methods began to exert significant influence on aging research, there has been heightened appreciation of the importance of heterogeneity within cohorts and a corresponding emphasis on individual variation (17–21). In a landmark experimental study, Carey and his colleagues (22) reported complete survival and life-long daily fecundity of 1,000 individually housed female Med flies (*Ceratitis capitata*), and noted that the investigation of individual life histories in model systems has the potential to deepen our understanding of the relation between reproduction, longevity, and senescence.

Here I analyze published data on 3,971 individually housed, mated *D. melanogaster* females. Data were collected from four laboratories (13,15,23,24) and include 180,000 fly-day observations and counts of over 4.6 million eggs. I show that fecundity records of individual flies contain a previously unappreciated indicator of transition in life history. After maturation adult flies experience relatively high levels of fecundity and survival, a period that I term the *working* stage. Later in life they transition to a terminal stage characterized by limited fecundity and reduced survival, the *retired* stage. The age of transition varies substantially between flies, and can be detected nondestructively and in real time as life history data are collected.

Methods

Published data on the survival and daily fecundity of individually housed, mated female *D. melanogaster* are from four sources.
Rauser and his colleagues (23) studied three lab-adapted, outbred populations derived from lines subjected to artificial selection for delayed reproduction (CO1-1, CO1-2, and CO1-3). Raw data were obtained from Dr. L. Mueller (University of California, Irvine). Le Bourg and Moreau (24) studied a population that had been selected for spontaneous locomotor activity; this population, referred to as LB, is also lab-adapted and outbred. Data were obtained from Dr. E. Le Bourg (Université Paul Sabatier). Klepsatel and his colleagues (15) studied three populations that had recently been collected from Zambia, South America, and Austria (Zam, SA, and Aus respectively). Data are archived by Klepsatel and his colleagues (25). Khazaeli and Curtsinger (13) studied two inbred lines, RI7 and 1S9, that were derived from an artificial selection experiment for extended life span and its control, respectively. Raw data are archived in the supplement (13). With the exception of the LB population, individual females were maintained with one or more males throughout adult life to ensure that sperm supply did not limit fecundity. In the LB population individual females were initially housed with one male, but the male was not replaced if it died before the female (26). Statistical analyses employed Systat version 10.2 (Systat Software, Richmond, CA).

Results

Summary statistics on survival and fecundity of female *D. melanogaster* from nine experimental populations are shown in Table 1. For the following analyses sterile and low-fecundity flies (<100 eggs lifetime) and censored data due to escaped flies were trimmed.

Lifet ime daily fecundity records of individual flies are dominated by zero-egg-days (henceforth ZED; Figure 1), which constituted 20% of all observations. Following maturation during the first few days of adult life, the population frequency of ZEDs increased with age in almost linear fashion, eventually reaching 100% (Figure 1, inset). The age trajectory of ZED is distinct from that of other low egg counts (Figure 1, inset). The high frequency of ZEDs and the unique age-dependent trend are also seen in populations treated individually (Supplementary Figures S1 and S2).

The age specificity of ZED in individual flies can be visualized by constructing an event history diagram (22). In this graphical method each individual is represented by a bar with length proportional to life span. When the bars are sorted and stacked horizontally they produce an outline of cohort survivorship. Each bar can be shaded to indicate timing of life history events. Figure 2 shows an event history diagram for the LB population with shading indicating ZED. Note that ZEDs are concentrated in the first few days after emergence at and the end of life. All nine populations show a similar pattern (Supplementary Figure S3).

The clustering pattern suggests that the first postmaturation ZED marks the beginning of a terminal stage of low fecundity in individual flies. That is, whether life is long or short, there is generally a lack of ZED in mid-life and a cluster of ZEDs at the end of life. I propose that the first postmaturation ZED is a biomarker of life history transition. In the following I show that prior to the start of the terminal cluster of ZEDs, survivorship and fecundity rates are relatively high, but once ZEDs start occurring the flies transition to a state of relatively limited fecundity and reduced survival. For convenience, the first postmaturation ZED is referred to as ZED1. The period of adult life from emergence until ZED1 is the working stage; the subsequent period is the retired stage. The retired stage includes periods of postreproductive survival, if they occur. There were no observations of daily fecundity for the first 11 days after emergence

Figure 1. Distribution of eggs laid per day by individual *D. melanogaster* females. Data are from nine experimental populations, all ages pooled. Sterile and low-fecundity flies (<100 eggs lifetime) are trimmed. The top 0.1% of observations (>120 eggs per day) are not shown. The x-axis is offset slightly to reveal a peak at zero eggs per day, which constituted 20% of all daily observations. Inset: Following a peak immediately after emergence, the frequency of ZEDs increased approximately linearly with increasing age. The frequency of counts in the range 1–5 eggs per day did not show a comparable age trend.

Table 1. Summary statistics for nine populations of *Drosophila melanogaster*

<table>
<thead>
<tr>
<th>Population</th>
<th>Total Females</th>
<th>Low Fecundity</th>
<th>Life Span (days) (SD)</th>
<th>Retirement (days) (SD)</th>
<th>Total Eggs</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aus</td>
<td>183</td>
<td>0</td>
<td>38.0 (8.9)</td>
<td>7.8 (5.4)</td>
<td>385,873</td>
<td>15</td>
</tr>
<tr>
<td>CO1-1</td>
<td>1,111</td>
<td>50</td>
<td>40.1 (11.6)</td>
<td>7.7 (8.4)</td>
<td>1,223,965</td>
<td>23</td>
</tr>
<tr>
<td>CO1-2</td>
<td>1,111</td>
<td>31</td>
<td>44.0 (12.7)</td>
<td>11.5 (11.7)</td>
<td>1,225,115</td>
<td>23</td>
</tr>
<tr>
<td>CO1-3</td>
<td>606</td>
<td>12</td>
<td>41.4 (11.6)</td>
<td>8.3 (9.3)</td>
<td>720,021</td>
<td>23</td>
</tr>
<tr>
<td>LB</td>
<td>322</td>
<td>3</td>
<td>32.8 (13.7)</td>
<td>9.6 (9.4)</td>
<td>375,571</td>
<td>24</td>
</tr>
<tr>
<td>RI7</td>
<td>192</td>
<td>5</td>
<td>49.3 (15.0)</td>
<td>15.7 (13.3)</td>
<td>134,928</td>
<td>13</td>
</tr>
<tr>
<td>1S9</td>
<td>143</td>
<td>2</td>
<td>32.5 (9.5)</td>
<td>6.6 (7.9)</td>
<td>79,149</td>
<td>13</td>
</tr>
<tr>
<td>SA</td>
<td>134</td>
<td>0</td>
<td>37.4 (10.2)</td>
<td>9.0 (6.7)</td>
<td>236,325</td>
<td>15</td>
</tr>
<tr>
<td>Zam</td>
<td>169</td>
<td>0</td>
<td>35.0 (12.7)</td>
<td>11.6 (9.6)</td>
<td>224,486</td>
<td>15</td>
</tr>
<tr>
<td>All pooled</td>
<td>3,971</td>
<td>103</td>
<td>40.5 (12.8)</td>
<td>9.6 (10.0)</td>
<td>4,605,433</td>
<td></td>
</tr>
</tbody>
</table>

Note: *Lifetime fecundity <100 eggs.*
in CO1-1, CO1-2, or CO1-3 populations, the largest studies examined here. Consequently, ZED1 is operationally defined to occur on Day 12 or later, and variation in the maturation period is ignored. These simplifications make it possible to analyze all the data using uniform methods.

Observed ages of ZED1 varied between flies, ranging from 12 to 71 days. The mean was 36.1 days (SD = 11.3) and the distribution was approximately normal aside from the truncation at Day 12 (Supplementary Figure S4). Variation between populations in the age of ZED1 is statistically significant, though minor in magnitude (analysis of variance, \(R^2 = 0.05, F = 23.0, df = 8/3477, p < .001\)). In the CO1-1, CO1-2, and CO1-3 populations there were 242 flies that exhibited no postmaturation ZED. Ten flies with similar characteristics were observed in other populations. I interpret these cases as accidental deaths during the working stage and exclude them from further analyses. All other flies, 94% of the total, exhibited at least one postmaturation ZED. Increasing the stringency of the biomarker to two consecutive postmaturation ZEDs reduces the general applicability of the diagnostic criterion to 55% of fertile females.

In the pooled data, adult life spans averaged 40.5 days, with 30.9 days spent in the working stage and 9.6 days, or 24% of adult life, in the retired stage, with some variation between populations (Table 1; Supplementary Figure S5). Postreproductive survival averaged 5.0 days.

To quantify the relationship between adult stage and rate of oviposition, daily fecundity records were subjected to analysis of covariance, with age as covariate and population and stage as factors. Days when ZED1 occurred, which are diagnostic of stage, were excluded from this analysis, as were fecundity observations prior to Day 12. The effects of stage are statistically significant (\(R^2 = 0.50, F = 1,842, df = 1/89942, p < .001\)). Adjusted least squares means and SEs for working and retired flies are 36.5 (0.10) and 21.2 (0.34) eggs per day respectively, a difference of 72%. Analysis of covariance assumes a linear relationship between covariate and dependent variable that might not be valid here. Therefore additional analyses were performed in which the effects of age were accounted for by quadratic regression. Residuals were subjected to the analysis of variance, with population and stage as factors. Again the effect of stage was statistically significant (\(R^2 = 0.23, F = 1,560, df = 1/89,943, p < .001\)). Residuals were also analyzed by nonparametric methods. Both Kruskal–Wallis and Mann–Whitney U tests indicate a statistically significant effect of stage on age-corrected fecundity (both \(p < .001\)).

In the pooled data, flies laid an average of 1,132 eggs in the working stage and 77 eggs in the retired stage (Supplementary Figure S6). The figure underestimates fecundity in the working stage, because of the absence of data before Day 12 in CO1-1, CO1-2, and CO1-3 populations. The general pattern is also reflected in populations treated individually (Supplementary Figures S7 and S8). In spite of slightly different treatment described in the Methods section, the fecundity pattern of LB females did not differ noticeably from that of other populations.

Daily fecundity rates were corrected for age by quadratic regression, as described above, and then age-corrected residuals were examined on the days preceding ZED1. As shown in Figure 3A, age-corrected fecundity declined in the days before ZED1 at a rate significantly different from zero (\(t = 36.3, p < .001; 95\% CI −2.21\) to −2.46 eggs per day). Populations treated individually also exhibited fecundity declines prior to ZED1 (Supplementary Figure S9). These observations are consistent with reports by Mueller and his colleagues (27,28) and Shahrestani his colleagues (29,30), who noted that flies enter a “death spiral” phase near the end of life characterized by rapid decline in fecundity. After ZED1, average daily fecundity rates remained at low levels, with a general declining trend (Figure 3B, Supplementary Figure S10).
ZED1 predicts survival. In the pooled data, flies were classified as working or retired at each chronological age, and then remaining life expectancies were calculated for the two groups. At younger ages the life expectancy of retired flies was 10 days less than that of working flies, while at older ages the difference shrank to five days (Supplementary Figure S11). Differences between groups are statistically significant for each day from Day 15 to 60 (t test, Bonferroni correction, all \( p < .001 \)). Examples of the survival differences are shown in Figure 4, where stage-dependent conditional survivorship is shown for ages 15, 25, and 35 days. In each case, retired flies had significantly lower subsequent survival than working flies of the same age (log-rank test, \( p < .001 \) in all cases). To determine biomarker efficacy, I calculated odds ratios from 2 \times 2 contingency tables. Each fly in the pooled data was classified as working or retired at ages 15, 25, 35, and 45 days. The odds of subsequent survival for 5, 10, 20, 30, and 40 days were then calculated, subject to the constraint of limited sample size after 65 days. The odds ratio measures association between a diagnostic trait and future outcome for individual flies. Here diagnosis refers to working or retired status at a particular age, and outcome refers to being alive or dead a certain number of days after diagnosis. As shown in Supplementary Figure S12A, for example, at the age of 15 days retired flies were 19 times more likely to die by Day 20 than working flies of the same age. Working/retired status is an excellent predictor of 5- and 10-day survival at all ages (Fisher’s exact test, all \( p < .001 \) after Bonferroni correction). At younger ages ZED1 is also predictive of 20- and 30-day survival (Supplementary Figure S12).

Discussion

The retired stage as defined here includes postreproductive survival, if it occurs, plus other days at the end of adult life that are generally characterized by low rates of oviposition. The most obvious way to partition the adult life span of iteroparous organisms such as Drosophila is to distinguish two stages after maturation: reproductive and postreproductive (15,31–36). While conceptually sound, life stages defined in this way are difficult to study experimentally, for two reasons. First, the postreproductive period is often brief. In the pooled data studied here, 40% of flies exhibited postreproductive survival of 2 days or less. Second, because individual oviposition events in late life tend to be sporadic, the postreproductive state can be known with certainty only after death. In contrast, detecting the retired stage is unambiguous: observation of one postmaturational event is sufficient to identify individuals that have transitioned to a terminal state that typically lasts one quarter of the total adult life span. The classification is simple, nonintrusive, and predictive of subsequent survival and reproduction.

Why is ZED1 informative? One possible explanation involves the assembly-line nature of Drosophila oogenesis. The process starts with the generation of oocytes within the ovarioles, and is followed by release of the oocyte into the oviducts, movement down the oviduct, fertilization in the uterus, and finally, oviposition (37). Retirement might reflect the age at which individual flies no longer have adequate resources to keep the production line running. The observation of declining fecundity in the days before ZED1 is consistent with this interpretation. An apt mechanical analogy might be the slowdown and sputtering of an internal combustion engine as it runs out of gas. It is also possible that between-individual variation in age of ZED1 reflects differences in ovariole numbers, which are in at least some cases correlated with fecundity (15,38,39).

There are several limitations to the conclusions presented here. Because of the scarcity of appropriate data, male life history has not been considered (but see 29). The analysis does not take into account hatchability. Klepsatel and his colleagues (15) observed low hatchability of eggs laid by old females and suggested that including hatchability might significantly lengthen estimates of the true postreproductive period. On the other hand, Rauser and his colleagues (40) observed that eggs laid by old females are viable. The explanation for differing conclusions is not clear. It is not known whether the results extend to environmental conditions involving limited food or mate availability. The collection of lifelong fecundity data in individual flies is very labor intensive, which may limit future applications.

While the demonstration that ZEDs have predictive value is novel, aspects of the analyses presented here have precedent. ZEDs have been reported in D. melanogaster, though not recognized as informative (41–43). Carey (19) noted that in fertile Med flies ZEDs were clustered at maturation and at the end of life, with some ZEDs occasionally observed in young flies. The latter were interpreted as periods of “recovery” following bouts of heavy egg laying (p. 68). Müller and his colleagues (44) showed that reproductive potential in Med flies

![Figure 4](https://academic.oup.com/biomedi/article-abstract/70/12/1455/2605278)
predicts remaining life span. Rogina and his colleagues (43) studied unmated D. melanogaster females and found that adult stages, including a terminal stage, could be defined in terms of behavioral responses to mating. Arking and his colleagues (8) discussed health, transition, and senescent periods of adult life span as defined by differences in gene expression. Mueller and his colleagues (28,29) classified flies into “death spiral” and “nonsenp” categories on the basis of individual fecundity records. The classification involved 3 days of fecundity observations and assumptions about the duration of fecundity decline and Gompertzian mortality dynamics. Rera and his colleagues (45,46) showed that intestinal barrier dysfunction, which can be detected non-destructively by the observation of blue dye in the haemolymph after feeding, is a harbinger of death. Defining the healthspan of model systems has been recognized as a central problem in aging research (47); many perspectives on that problem are presented in this journal’s Special Issue: Biology of Aging Summit (February 2009).

The analyses presented here may be relevant to the design and interpretation of experimental results on life extension. Repeatability of experimental outcomes is sometimes problematic (48–52). Because flies retire at different ages, experimental cohorts will generally contain a mixture of working and retired flies, even when all individuals are the same chronological age. For example, at age of the average life span in the pooled data, 54% of flies were working and 46% were retired (Supplementary Figure S13). Given that working and retired flies exhibit distinct fecundity and survival characteristics, it is likely that they will differ in response to treatments such as dietary restriction, heat shock, and nutritional supplementation. Classifying individuals by the functional criterion proposed here might eliminate a significant source of uncontrolled experimental variation.

Supplementary Material
Supplementary material can be found at: http://biomedgerontology.oxfordjournals.org/

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References


