Reproductive Homeostasis and Senescence in *Drosophila melanogaster*

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**Abstract**

The homeostatic properties of reproduction in aging female *Drosophila melanogaster* are investigated. Classic studies based on cohort analysis suggest that homeostatic capacity declines gradually as daily oviposition rates decline in aging flies. Analysis at the level of individuals gives a very different picture: reproductive homeostasis remains relatively constant for most of adult life until a critical point when oviposition either ceases entirely or continues in dysregulated fashion. The collapse of homeostatic capacity is abrupt. Enhanced homeostasis is associated with increased lifetime fecundity and improved prospects for survival. The fractal concept of lacunarity can be used to parameterize the “roughness” of individual fecundity trajectories and is inversely related to homeostatic capacity.

**Keywords:** Fecundity trajectory, Lacunarity, Retirement, Oviposition, Life history

Homeostasis, classically defined as the ability of organisms to maintain stable function when challenged with internal or external environmental change, is a central concept in physiology and the biology of aging. It is also an evolving concept—historically there have been at least three lines of inquiry on the general theme of homeostasis that have been the subject of investigations employing the fruit fly *Drosophila melanogaster*.

One line of research has focused on genetic homeostasis, the tendency of homozygous genotypes to exhibit greater phenotypic variability than heterozygotes (1–3). The phenomenon is well documented, and is relevant to the repeatability of experimental results (4). The enhanced buffering ability of heterozygotes is generally viewed as a component of their superior fitness in comparison with homozygotes. A second and more recent line of research addresses the maintenance of physiological set points for individual organ systems and functional traits, including skeletal muscle, gut, heart, sleep, water balance, fat body, and glucose metabolism (5–12). A goal of this line of research is to identify conserved molecular mechanisms of homeostasis with potential application to human health. A third line of research broadens the definition of homeostasis beyond the classical notion of an unchanging physiological set-point. Davies (13) proposed the concept of adaptive homeostasis, defined as a transient expansion or contraction of the homeostatic range to cope with environmental stressors. Pomatto and colleagues (14) have argued that adaptive homeostasis declines with increasing age in a variety of experimental systems, and may be a causal agent of senescent decline of functional traits.

For a highly iteroparous organism such as *D melanogaster* abundant reproduction is the most meaningful measure of biological success. However, despite the widespread use of *Drosophila* in aging research and the centrality of reproduction, the homeostatic properties of the reproductive system have received little attention since 1970s. David and colleagues (15) studied longevity and fecundity in mated female *D melanogaster* and found that the between-fly variation in fecundity increased exponentially with increasing age. This observation was interpreted as an indication of progressive loss of resistance to environmental micro-variations in aging flies, leading to increasing between-fly variation. Giesel (16,17) confirmed that result and documented similar patterns in several species of small mammals. These foundational studies suggested that the age-related decline of reproductive homeostasis is a gradual process, proceeding hand-in-hand with the age-related loss of reproductive capacity (18).

Here, I examine reproductive homeostasis in *D melanogaster* using novel data and methods. The analysis differs from earlier studies in several ways. First, while previous studies used cohort-based observations, data analyzed here are individual-based, consisting...
of observations of survival and reproduction on several thousand individual female flies. The data include counts of more than four million eggs. Because the data include records of lifetime daily fecundity of individual flies they are well suited to investigating the relationships between age, reproductive senescence, and homeostasis. Second, the analysis incorporates the demographic concept of reproductive retirement (19,20) which provides an integrated treatment of survival, reproduction, and aging. Newly emerged flies begin adult life in the working stage, a period characterized by high levels of oviposition and steadily increasing age-specific mortality. Later, they transition to the retired stage, a terminal period typically lasting one-quarter of the adult life span, characterized by low or zero fecundity and relatively constant age-specific mortality. Third, I employ the fractal concept of lacunarity as a metric for homeostasis. Lacunarity, from the Latin for lacuna or gap, is a dimensionless measure of the “roughness” of spatial patterns. It was first developed in the context of fractal theory, and is now widely used in geology, ecology, dermatology, and radiology to quantify irregularities in one-, two-, and three-dimensional patterns (21). Lacunarity and other aspects of fractal analysis have proved useful for the analysis of a variety of age-related traits (22–26) but have not previously been employed in the analysis of homeostasis or reproductive senescence. The lacunarity metric adds a spatial dimension to the analysis of individual fecundity trajectories.

Results show that while cohort-level analysis supports the conventional view that reproduction and homeostatic capacity decline in coordination as flies age, a more discerning analysis at the level of individual flies gives a very different picture—homeostasis remains relatively constant for most of adult life until a critical point is reached. At the critical point, which coincides with the transition to retirement, individual flies follow one of two life history paths: they either cease oviposition entirely, or they continue to lay eggs in a highly dysregulated fashion.

Methods

Life history data on 3,923 individually housed, mated, fertile female D melanogaster are from four sources. Rauser and colleagues (27) studied lab-adapted, outbred populations derived from lines that were artificially selected for delayed reproduction (CO1-1, CO1-2, CO1-3). Raw data were obtained from Dr L. Mueller (University of California, Irvine). Le Bourg and Moreau (28) studied an outbred lab-adapted population (LB) previously selected for spontaneous locomotor activity. Data were obtained from Dr E. Le Bourg (Université Paul Sabatier). Klepsatel and colleagues (29,30) studied three populations that had been recently established with wild collections from Zambia, South Africa, and Austria (Zam, SA, and Aus, respectively). Khazaeli and CURTSINGER (31) studied two inbred lines, RI7 and SA9, derived from an artificial selection experiment for extended life span and its control, respectively.

Records of daily single-female egg counts commenced at emergence in six of the nine population. For the three CO1 populations, egg counts started 12 days after emergence. In all populations daily egg counts continued until death. Individual females were maintained with one or more males throughout adult life to ensure that sperm supply did not limit fecundity, except for the LB population, in which females were initially housed with one male that was not replaced if it died before the female.

Previous investigations employed the coefficient of variation (CV) as a measure of reproductive homeostasis (15–17). CV is defined as σ / X, where σ is the standard deviation of daily egg counts and X is the mean. Standardizing the variation by the mean corrects for the tendency of larger numbers to exhibit greater absolute variation. Here, I employ a related metric based on the statistical concept of lacunarity, formally defined as the ratio of the second moment to the one-dimensional lacunarity of individual fecundity records by equating mass with the number of eggs laid each day. Under that assumption lacunarity is estimated as CV2 (32).

Several measures of lacunarity are employed here. Ltot is defined as the lacunarity over the entire adult life span of individual flies, from emergence (except for CO1 populations, for which egg counts began on Day 12) until death. Lret is dimensionless, strictly positive, and sensitive to the degree of clustering of oviposition and to differences in numbers of eggs in each cluster. Greater values of Ltot indicate reduced homeostasis. Ltot is defined as CV2 over a window of t days. Ltot is computed on successive days to investigate trends in lacunarity over the life spans of individual flies, comparable to a moving average. Preliminary analysis with t = 5, 10, and 15 days gave qualitatively similar results; results for t = 5 days are presented here. Ltot is undefined for sequences of zeroes. Lwork is CV during the working stage of adult life, which commences at emergence and ends on the first day when no eggs are laid (19). Lret is lacunarity over the retired period.

Routine statistical analyses employed Systat version 10.2 (Systat Software, Inc., Richmond, CA).

Results

Representative examples of variation among individuals in the degree of reproductive homeostasis are shown in Figure 1. The top panel illustrates the most homeostatic pattern: daily fecundity rates peaked early in adult life and then declined gradually, with no large drops or gaps; in this case Ltot = 0.26. Figure 1b shows an intermediate case, with Ltot = 1.28; the decline in fecundity is more jagged, and there are gaps in the oviposition record after Day 30. The fecundity record in Figure 1c is the least homeostatic, with Ltot = 3.71. Fecundity dropped rapidly after the peak and was erratic after Day 15, with frequent gaps and no clear late-life temporal trend.

Average Ltot in the pooled data was 0.73. Averages for the nine experimental populations are shown in Table 1. While there are statistically significant differences between populations, those are minor. Almost all the variation was between individuals (analysis of variance with population as a factor nested within lab of origin, F lab = 8.1, df = 3/3899, p < .001; F pop(lab) = 5.3, df = 5/3899, p < .001; R2 = .01). There is slightly more variation between labs and populations in Lwork (F lab = 10.4, df = 3/3899, p < .001; F pop(lab) = 4.4, df = 5/3899, p < .001; R2 = .08) and Lret (F lab = 19.9, df = 3/1736, p < .001; F pop(lab) = 5.3, df = 5/1736, p < .001; R2 = .04).

Lacunarity describes the variability of reproductive rate over time. Given the generality of genetic homeostasis for life history characters, a reasonable a priori expectation is that Ltot for the inbred populations would be greater than Lret in the genetically heterogeneous populations. However, there is no obvious effect of inbred condition on Ltot evident in the population averages (Table 1). Analysis of variance of Ltot in the pooled data with inbred or outbred state as a factor is not statistically significant (F = 3.1, df = 1/3906, R2 = .001, p = .08). Inbreeding is also a nonsignificant factor for explaining variation in Ltot (F = 3.7, df = 1/1743, R2 = .002, p = .06). However,
inbreeding is statistically significant for $L_{work}$ ($F = 241, df = 1/3815, R^2 = .06, p < .001$). The least-squares mean of $L_{work}$ in outbred lines is 0.23, compared with 0.32 for the inbreds.

Low reproductive homeostasis is associated with reduced total fecundity. Product-moment correlation coefficients between $L_{tot}$ and total fecundity range from −0.32 for the LB population to −0.60 for Aus. In all nine populations, the correlation is negative and statistically significant at the $p = .001$ level (Table 1). Figure 2 shows results for pooled data, and Supplementary Figure S1 shows results for each population separately. Figure 2a includes data from six populations for which complete lifetime fecundity was measured. Figure 2b shows data from three populations in which egg counts started on Day 12. Smoothing employs the Lowess algorithm. In both cases, higher lacunarity is associated with reduced total fecundity (Figure 2a: $F_{Ltot} = 346, df = 1/1122, p < .001; F_{Ltot} = 737, df = 2/1122, p < .001$; $F_{pop(lab)} = 72, df = 3/1122, p < .001, R^2 = .66$). Figure 2b: $F_{Ltot} = 366, df = 1/2777. F_{pop} = 7.4, p = .001, R^2 = .12$. The magnitude of the effect of homeostasis on fecundity varies between populations, with $R^2$ ranging from 11% to 36%. Overall, flies with enhanced homeostasis ($L_{tot} < 1$) laid significantly more eggs than flies having $L_{tot} > 1$ (1267 vs 789, $t = 24, df = 3906, p < .001$).

$L_{tot}$, the measure of lacunarity over 5-day intervals, was computed on successive days to study changes in homeostatic capacity over the course of individual lifetimes. Figure 3a shows how $L_{i}$ changed on average in the pooled data. There is a slight peak immediately after emergence, corresponding to slight variation in the age at which oviposition commenced, followed by steady acceleration with increasing age. This observation confirms earlier reports by David and colleagues (15) and Giesel (16,17), who noted that CV increases exponentially with age, much like the temporal trend in age-specific mortality rate. At the oldest ages the pattern shown in Figure 3a is erratic, presumably because of small sample size. Figure 3b shows the average fecundity trajectory for the pooled data. The daily oviposition rate peaked early in adult life and then gradually declined. The temporal patterns illustrated in Figure 3a and b appear to be synchronized, suggesting that homeostatic capacity decays gradually and in coordination with reproductive decline.

Ontogenic changes in homeostatic capacity are associated with changes in prospects for future survival. For each fly $L_{i}$ and remaining life span were calculated on each day of life. As shown in Figure 4, remaining life declined substantially as $L_{i}$ increased from zero to one. Increases in $L_{i}$ greater than one appear to have little effect on survival.

The following analyses investigate the relationship between working and retired status of individual flies and the degree of reproductive homeostasis. Fifty-six percent of females in the pooled data (2,173/3,923) exhibited a simple fecundity trajectory: once oviposition declined to zero eggs over a 24-hour period no eggs were laid subsequently. This was the most common pattern in all lines, and was somewhat more frequent in wild stocks than in domesticated stocks.

Table 1. Average Life Span, Fecundity, and Measures of Reproductive Homeostasis in Nine Populations of Drosophila melanogaster

<table>
<thead>
<tr>
<th>Population</th>
<th>N</th>
<th>Life (Days) (SE)</th>
<th>Fecundity (SE)</th>
<th>$L_{tot}$ (SE)</th>
<th>$L_{work}$ (SE)</th>
<th>$L_{ret}$ (SE)</th>
</tr>
</thead>
<tbody>
<tr>
<td>S9</td>
<td>142</td>
<td>32.6 (0.8)</td>
<td>556 (16.8)</td>
<td>0.74 (0.04)</td>
<td>−0.54***</td>
<td>0.53 (0.03)</td>
</tr>
<tr>
<td>R17</td>
<td>191</td>
<td>49.5 (1.1)</td>
<td>706 (24.3)</td>
<td>0.90 (0.07)</td>
<td>−0.51***</td>
<td>0.51 (0.07)</td>
</tr>
<tr>
<td>CO1-1</td>
<td>1,085</td>
<td>40.7 (0.3)</td>
<td>1,128 (12.7)</td>
<td>0.67 (0.04)</td>
<td>−0.34***</td>
<td>0.20 (0.01)</td>
</tr>
<tr>
<td>CO1-2</td>
<td>1,103</td>
<td>44.2 (0.4)</td>
<td>1,111 (13.4)</td>
<td>0.72 (0.03)</td>
<td>−0.34***</td>
<td>0.23 (0.01)</td>
</tr>
<tr>
<td>CO1-3</td>
<td>605</td>
<td>41.5 (0.5)</td>
<td>1,190 (18.3)</td>
<td>0.65 (0.03)</td>
<td>−0.41***</td>
<td>0.22 (0.01)</td>
</tr>
<tr>
<td>Zam</td>
<td>167</td>
<td>35.4 (1.0)</td>
<td>1,342 (45.4)</td>
<td>1.12 (0.06)</td>
<td>−0.54***</td>
<td>0.34 (0.01)</td>
</tr>
<tr>
<td>SA</td>
<td>134</td>
<td>37.4 (0.9)</td>
<td>1,764 (55.3)</td>
<td>0.84 (0.04)</td>
<td>−0.59***</td>
<td>0.25 (0.02)</td>
</tr>
<tr>
<td>Aus</td>
<td>183</td>
<td>38.0 (0.7)</td>
<td>2,109 (42.5)</td>
<td>0.62 (0.03)</td>
<td>−0.60***</td>
<td>0.20 (0.01)</td>
</tr>
<tr>
<td>LB</td>
<td>313</td>
<td>33.4 (0.8)</td>
<td>1,189 (20.9)</td>
<td>0.90 (0.04)</td>
<td>−0.32***</td>
<td>0.37 (0.04)</td>
</tr>
<tr>
<td>Pooled</td>
<td>3,923</td>
<td>40.9 (0.2)</td>
<td>1,173 (8.4)</td>
<td>0.73 (0.02)</td>
<td>−0.31***</td>
<td>0.26 (0.01)</td>
</tr>
</tbody>
</table>

*Correlation between $L_{tot}$ and lifetime fecundity.

*** $p < .001$. 

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Figure 1. Complete daily fecundity records for three individual Drosophila melanogaster females. Cases were chosen to illustrate variation in the degree of reproductive homeostasis as measured by lacunarity $L_{tot}$. (a) SA population, fly #303, total fecundity = 2,609 eggs. $L_{tot} = 0.26$. (b) LB population, fly #200, total fecundity = 1,240 eggs. $L_{tot} = 1.28$. (c) LB population, fly #314, total fecundity = 1,154 eggs. $L_{tot} = 2.71$. High lacunarity is associated with reduced homeostasis, “jagged” changes in daily fecundity rate, and temporal gaps when no eggs were laid.

Figure 2a: $F_{Ltot} = 346, df = 1/1122, p < .001; F_{Ltot} = 737, df = 2/1122, p < .001$; $F_{pop(lab)} = 72, df = 3/1122, p < .001, R^2 = .66$. Figure 2b: $F_{Ltot} = 366, df = 1/2777. F_{pop} = 7.4, p = .001, R^2 = .12$. The magnitude of the effect of homeostasis on fecundity varies between populations, with $R^2$ ranging from 11% to 36%. Overall, flies with enhanced homeostasis ($L_{tot} < 1$) laid significantly more eggs than flies having $L_{tot} > 1$ (1267 vs 789, $t = 24, df = 3906, p < .001$).

$L_{tot}$, the measure of lacunarity over 5-day intervals, was computed on successive days to study changes in homeostatic capacity over the course of individual lifetimes. Figure 3a shows how $L_{i}$ changed on average in the pooled data. There is a slight peak immediately after emergence, corresponding to slight variation in the age at which oviposition commenced, followed by steady acceleration with increasing age. This observation confirms earlier reports by David and colleagues (15) and Giesel (16,17), who noted that CV increases exponentially with age, much like the temporal trend in age-specific mortality rate. At the oldest ages the pattern shown in Figure 3a is erratic, presumably because of small sample size. Figure 3b shows the average fecundity trajectory for the pooled data. The daily oviposition rate peaked early in adult life and then gradually declined. The temporal patterns illustrated in Figure 3a and b appear to be synchronized, suggesting that homeostatic capacity decays gradually and in coordination with reproductive decline.

Ontogenic changes in homeostatic capacity are associated with changes in prospects for future survival. For each fly $L_{i}$ and remaining life span were calculated on each day of life. As shown in Figure 4, remaining life declined substantially as $L_{i}$ increased from zero to one. Increases in $L_{i}$ greater than one appear to have little effect on survival.
In these cases, reproductive homeostasis can be assessed during the working stage but not in the retired stage, because CV is undefined for sequences of zeroes. Flies with this simple life history exhibited statistically significant change in reproductive homeostasis over time just before transition. \( L_5 \) was calculated for each fly at 5-day intervals preceding the day of transition, starting 1 day prior. Fecundity on the day of transition was treated as missing data. Analysis of variance of \( L_5 \) with time before transition, lab of origin, and population as factors is statistically significant (\( F_{\text{before}} = 1,390, df = 1/43,509, p < .001; \) \( F_{\text{lab}} = 36, df = 3/43,509, p < .001; \) \( F_{\text{pop(lab)}} = 4.3, df = 5/43,509, p < .001, R^2 = 0.15 \)). The statistically significant effect of time before the age of transition is due almost entirely to the increase in \( L_5 \) in the final 5-day period before transition. Least squares means of \( L_5 \) are <0.20 for most of the working period, and then double to 0.41 just before the transition. This analysis demonstrates that among flies exhibiting a simple fecundity trajectory there is an increase in lacunarity and corresponding loss of reproductive homeostasis in the period immediately preceding the age at which fecundity declines to zero.

Other females, 44% (1,745/3,923) of the total, exhibited a more complex fecundity trajectory: oviposition declined to zero eggs per day and then resumed on subsequent days. For these individuals, lacunarity can be estimated in both working and retired stages. Figure 5 shows reproductive homeostasis measured by \( L_5 \) over time, where time is measured in days before and after the age of transition to retirement for each fly. Observations of zero fecundity on the day of transition to retirement are treated as missing data. There is a striking pattern of abrupt increase in \( L_5 \) at the age of transition, indicating a collapse of reproductive homeostasis. The abrupt change at the age of transition is also evident in populations treated separately (Supplementary Figure S2). Average values of lacunarity during working and retired stages in each population are shown in Table 1. In all cases \( L_{\text{ret}} \) is substantially larger than \( L_{\text{work}} \), ranging from five- to thirty-fold higher. Ninety-nine percent \( (N = 1,728) \) of the females with this more complex life history exhibited higher lacunarity in the retired stage than in the working stage. Thus, while lacunarity estimated at the cohort level appears to increase gradually with age, at the individual level there is evidence for an abrupt dysregulation of reproduction at the age of the retirement transition.

**Discussion**

The conventional view of reproductive homeostasis in \( D \) \( m \) \( e \) \( l \) \( a \) \( n \) \( o \) \( g \) \( a \) \( s \) \( t \) \( e \) is that it declines in coordinated fashion with fecundity.
as the female reproductive system senesces. Results reported here suggest a different picture: homeostatic capacity is largely separable from senescent decline, and exhibits abrupt late-life collapse rather than gradual change. Over most of adult life average oviposition rates fall, but during that same period homeostatic capacity changes little; there is orderly decline. Dramatic change in homeostasis comes in the final stage of adult life, the retired stage. At that point individual flies follow one of two paths: about half the flies cease oviposition entirely, while the remainder continue to lay eggs. In the latter group, oviposition is sporadic and homeostatic capacity is minimal. It is important to note that these phenotypes are stage-dependent rather than age-dependent; the age of transition to the retired stage and then resumed on subsequent days. In these cases, there was an abrupt increase in lacunarity and loss of reproductive homeostasis after the transition.

At the individual level, reproductive homeostasis is a positive trait: its enhancement is associated with increased lifetime fecundity and increased survival prospects, while reduced homeostasis is associated with reduced fecundity and reduced survival. The relationship holds across all experimental populations studied here, including inbred, outbred, lab-adapted, and wild stocks. This observation argues against the idea that female D melanogaster sometimes hold back oviposition to recuperate and regain fecundity later in life. Indeed, the data suggest the opposite—the first gap in the fecundity record of an individual fly is a warning sign. Once the daily fecundity rate drops to zero remaining life is limited, remaining fecundity is low, and homeostatic capacity is minimal.

Among the populations studied here genetically heterogeneous strains exhibited superior reproductive homeostasis compared with inbred lines, but only in the working stage, not the retired stage. One possible explanation is differences between labs in the details of fly culture. The data were collected in four different laboratories, only one of which employed inbred strains. As a result strain type is confounded with differences in lab culture. An alternative and more interesting possibility is that genetic homeostasis, the superior ability of heterozygous genotypes to buffer against random environmental variations, is evident only when the trait is relevant to fitness. Productivity during the working stage, when the bulk of oviposition occurs, is a central component of Darwinian fitness. The retired stage, like the post-reproductive period it encompasses, is probably a non-Darwinian “add-on” (29). Reproductive homeostasis occurs in the earlier part of individual life history when it matters to fitness and, conversely, dysregulation occurs late in life when productivity is essentially irrelevant to fitness. Further support for the view that late-life fecundity is irrelevant to fitness is provided by the observation that egg hatchability declines with age, typically approaching zero several days before oviposition ceases (29).

Klepsatel and colleagues (29) fit a variety of parametric models to individual fecundity data, including some of the data analyzed here, and found that the best fit involved four adult life history stages: an initial period of reproductive maturation to peak fecundity, followed by linear and then exponential decline, and, finally, a post-ovipository period. The present analyses add to that result by demonstrating that the final stage of oviposition is, in almost half the flies, markedly erratic. The dysregulated nature of end-of-life fecundity might help to explain observations that seem to be at odds with the four-stage model: individual fecundity trajectories that sometimes remain steady or even increase in the days preceding death (28,33). Given that late-life oviposition can be erratic, it is not surprising that a variety of functional forms fit the data. Perhaps the salient conclusion is not that end-of-life fecundity fits one particular parametric model, but that it is dysregulated.

As far as I am aware, the analysis of fecundity data in terms of lacunarity is novel. As a practical matter this differs little from previous analyses—David and colleagues (15) and Geisel (16,17) used the CV to estimate the degree of homeostasis, while the lacunarity estimator used here is the squared coefficient of variation. Nevertheless, the novel statistical context is noteworthy, because it opens the possibility of applying other aspects of fractal analysis to reproductive senescence in experimental systems. As noted by Zaia and colleagues (34), complex systems, including senescent phenotypes, are highly dependent on initial conditions. As a result, small differences early in life can lead to major phenotypic differences later. Further application of fractal analysis might offer fruitful methods to analyze such ontogenic complexity.

**Supplementary Material**

Supplementary data are available at *The Journals of Gerontology, Series A: Biological Sciences and Medical Sciences* online.

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**Conflict of Interest**

The author has no conflicts of interest to declare.

**References**


