Extended Longevity Lines of Drosophila melanogaster: Characterization of Oocyte Stages and Ovariole Numbers as a Function of Age and Diet

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Vitellogenic and previtellogenic oocyte stages, as well as ovariole numbers, were characterized in the selected (O) lines of D. melanogaster that have been selected for late-life reproduction and extended longevity. On day 4 post-eclosion, O females had more ovarioles than control (B) females, but this difference diminished at older ages. From days 4 to 24, O females showed a marked increase in average vitellogenic oocyte stage, whereas this parameter did not increase in B females as they aged. The previtellogenic gradient of oocyte maturation declined in both B and O females. Specifically, terminal previtellogenic stages were lost with advancing age, and this loss occurred relatively gradually in O females regardless of food regime. Among the reproductive traits assessed, differential persistence of the previtellogenic gradient of maturation is most strongly associated with selected versus control line differences in longevity.

Known from studies on a broad range of taxa, there is a general relationship between reproduction early in life and later-life reproduction as well as longevity. Relatively higher reproduction early in life is correlated with reduced reproduction later in life and also correlated with reduced longevity. The usual interpretation of this relationship is that there is a cost of present reproduction that is paid in terms of future reproduction and survival (1). Presumably, the cost of reproduction stems from constraints that exist between resources allocated to present reproduction versus future reproduction and survival (2–5). Although there is extensive empirical support for reproductive costs (6–26), there are studies that serve as counter examples, in which no cost of reproduction was observed (15,27–33). Certainly, there is motivation to investigate mechanisms underlying the cost of reproduction; the value of conducting such studies at multiple levels of biological organization is argued by Stearns (1).

Selection for late-life female reproduction in Drosophila melanogaster generally results in decreased early age fecundity and greater longevity (34–36). In relevant selection experiments, little is known about the state of female reproductive traits investigated throughout life. To generate the selected (O) and control (B) lines that are the focus of the present study, O females were selected for late-life reproduction, whereas control females (B) must reproduce quickly after eclosion (34). Young O females have very dry weights that are approximately one-third those of B females (34). There is a corresponding relative reduction in fecundity in young selected females. However, the fecundity difference between selected and control lines disappeared in later generations of this long-term selection experiment except when the females were assayed on a specific version of the food medium (23). Nevertheless, there is evidence for vitellogenic oocyte stage and ovariole number differences when young O and B females are compared (37). Ovarioles are the subdivisions of the ovary, down which developing egg chambers (oocytes and affiliated cells) proceed, and vitellogenesis is the process of provisioning developing oocytes with yolk protein. During the first 4 days post-eclosion, young O females mature vitellogenic oocytes relatively more slowly and have more ovarioles than B females (37). The delay in vitellogenic oocyte maturation in O females may underlie their reduced early-age fecundity. In terms of ovariole number differences, it was suggested that O females may begin adult life with more ovarioles to ameliorate the impact of any age-dependent loss of ovariole function and thus maintain late-life reproduction (37).

The goal of this study was to conduct an analysis of oocyte stages and ovariole numbers in O and B line females throughout most of their comparable lifetime. At defined intervals post-eclosion, until the time of approximately 50% mortality within line types, vitellogenic oocyte stages were tabulated and ovarioles counted in samples of females. In D. melanogaster, there is evidence for an age-dependent decline in oocyte maturation to the extent that some ovarioles have no previtellogenic egg chambers (38). Therefore, characterization of previtellogenic oocyte stages also was included in this study. The effect of supplemental food on lifetime fecundity and survival has previously been investigated in the O and B lines (22,23). Consequently, added yeast was included as a treatment to investigate the effect of supplemental food on oocyte stages and ovariole numbers throughout life.

Method

Lines and Culture Conditions

The B and O lines of Drosophila melanogaster were derived from a long-term laboratory population (34). Each derivative line is an independent interbreeding population. There are five B (control) lines and five O (selected) lines. The B lines are maintained on a 2-week generation cycle corresponding to the generation time of the source population. The O lines are maintained on a 10-week generation cycle and thus selected for late-life reproduction. Extended longevity and reduced early-age reproduction are correlated responses to selection.

For all treatments, flies were reared under constant illum-
nation at 25°C except for the period of time required to ship larvae from the University of California–Irvine to the University of Nebraska–Lincoln. Larval density was standardized at moderate numbers by controlling the number of eggs (approximately 60–80) transferred to each vial. Also for the purposes of standardization—in this case by conforming to the B line culture regime—all flies were reared for two generations on a 2-week cycle prior to characterization of female reproductive traits.

Experimental Design

As a source of adults for experimentation, rearing vials were cleared after the first flies eclosed. Two days later, females and males were separated (light ether used for immobilization), and a standard number of individuals were added to each holding vial. All vials contained 10 females and 10 males, held at 25°C under constant illumination. Every 48 hours, the adults in each vial were transferred to vials with fresh medium. Half of the holding vials contained the standard B and O line medium without added live yeast on the surface. In the remainder, live yeast (3.125 mg/vial) was added in a slurry that was allowed to dry overnight to ensure that introduced flies did not stick to the surface.

The B flies were assayed at specific intervals post-eclosion, but their age at each timepoint could vary by 2 days. Specifically, adult B females were assayed at days 2–4, 6–8, 14–16, 22–24, 30–32, and 38–40 post-eclosion, and the O flies were assayed on days 2–4, 6–8, 14–16, 22–24, 30–32, 38–40, 46–48, 54–56, and 62–64 post-eclosion. For the purposes of presentation clarity, the assay times in the figures are generally presented as post-eclosion days 4, 8, 16, 24, 32, 40, 48, 56, and 64. The 40-day (B lines) and 64-day (O lines) assay endpoints were defined by maintaining a cumulative record of mortality until approximately 50% of the initial number of flies had died. In spite of cumulative mortality, flies were maintained at a constant density and sex ratio (50:50) by addition of flies of the same age taken from extra holding vials. Ovariole numbers and oocyte stages were tabulated in the O lines up to the endpoint of 50% mortality to allow for inspection of any late-life differences between line types. However, the statistical analysis was based on a comparison of B and O line females during the first 40 days post-eclosion, when all lines were assayed.

Females of defined age were immobilized by light ether anesthesia, then counted into eppendorf tubes before storage at −20°C. A limited number of females, approximately 10, were frozen in each tube. Consequently, when a tube was thawed for dissections there was not an extended period of time between processing of the first and last females. All B and O lines were included in the present study, and an equal number of females were analyzed from each line at each timepoint. For each experimental cell, defined by combination of line, diet, and time post-eclosion, five females were dissected and analyzed. Reproductive characters were measured on individual flies, but lines were the units of statistical analysis. Ovaries were dissected into a Drosophila Ringers solution (130 mM NaCl, 5 mM KCl, and 1.4 mM CaCl₂), using fine stainless steel needles to tease apart the ovarioles. For every female analyzed, all of the ovarioles in each ovary were counted. Vitellogenic and previtellogenic oocyte stages were determined under a light microscope by comparison to King’s stages of oogenesis (39). In King’s system, the stages are numbered in order of developmental maturity ranging from the first (stage 1) to the last (stage 7) previtellogenic stage and first (stage 8) to last (stage 14) vitellogenic stage. All of the vitellogenic oocyte stages present in each female were recorded. The previtellogenic oocyte stages are normally found in a gradient of maturation such that a terminal previtellogenic stage is preceded sequentially by a continuous series of all of the less mature previtellogenic stages. Based on findings of Carlson and associates (37), when previtellogenic oocyte stage 7 is present, the next previtellogenic oocyte usually is stage 5 preceded sequentially by stages 4 through 1. In this case, recording the presence of stages 5 and 7 was sufficient to characterize the entire gradient of maturation. In other cases, one terminal previtellogenic stage was sufficient to characterize the entire previtellogenic oocyte gradient of maturation.

Statistical Analysis

A partially nested factorial analysis of variance was used for data analysis throughout the study. The grand average, for any of the characters assessed, is the sum of the means for all lines of a given type (B or O) divided by the number of lines of that type.

RESULTS

The B line (control) females were assayed until 40 days post-eclosion and the O line (selected) females were assayed until 64 days post-eclosion. At the last assay point, there was 55% mortality among B females held without added yeast, 57% mortality among B females held with added yeast, 63% mortality among O females held without added yeast, and 66% mortality among O females held with added yeast.

Previtellogenic oocyte stages in O and B females were characterized in the present study. In this case, the variate per female is the sum of all previtellogenic oocyte stages in a female divided by the number of previtellogenic oocytes. The per female variate was summed for all females in the same experimental cell and divided by the number of females (5 females) to generate a line average for each time point post-eclosion and diet treatment. Grand averages for the previtellogenic stages are presented in Figure 1 as a function of time post-eclosion and diet for the line types. The grand average for each line type is the sum of the replicate line means divided by the number of B lines or the number of O lines. For clarity, the standard deviations are not shown in Figure 1. The standard deviations corresponding to Figure 1 are presented in Table 1. For this data set (Table 1), and throughout the study, there is no strong correspondence between variance and age. With respect to the presumption of homoscedasticity, “the consequences of moderate heterogeneity of variances are not too serious for the overall test of significance” for multiple degree of freedom comparisons in ANOVA (40, p. 376). For the ANOVA on data from days 4 to 40, the factors were day post-eclosion, line type (B or O), and diet (with or without added yeast). Among the possible interactions among the factors, statistical support is indicated only for the interaction between diet and day post-eclosion (p = .0153). The inclusion of data from a timepoint (day 40) with high mortality in the B lines could have contributed to this interaction. The level of support for an effect of diet is p = .0005, and for day post-eclosion it is p = .0001. There also is support for a statistically significant effect of line type (p = .0021).
Figure 1. Grand average previtellogenic oocyte stage for B (control) or O (selected) lines with, or without, added yeast on the medium surface. The sum of all previtellogenic oocyte stages was divided by the total number of previtellogenic oocytes in each adult female to obtain a mean value. The grand average was derived from the sum of B or O line means divided by the number of replicate lines.

Table 1. Grand Average Previtellogenic Stages as a Function of Line Type, Diet, and Days Post-Eclosion

<table>
<thead>
<tr>
<th>Line Type</th>
<th>Yeast</th>
<th>Time (Days)</th>
<th>Mean</th>
<th>SD</th>
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<tr>
<td>Control (B)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>H</td>
<td>4</td>
<td>5.05</td>
<td>0.066</td>
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<tr>
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<td>H</td>
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<td>H</td>
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<td>H</td>
<td>40</td>
<td>4.53</td>
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<td></td>
</tr>
<tr>
<td>L</td>
<td>4</td>
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<td>0.037</td>
<td></td>
</tr>
<tr>
<td>L</td>
<td>8</td>
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<td>L</td>
<td>40</td>
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</tr>
<tr>
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<tr>
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<td>0.270</td>
<td></td>
</tr>
<tr>
<td>L</td>
<td>64</td>
<td>4.23</td>
<td>0.410</td>
<td></td>
</tr>
</tbody>
</table>

Note: H = high yeast, L = unmodified Drosophila medium.
Figure 2. Grand mean percentage of ovarioles missing two terminal previtellogenic oocyte stages (stages 5 and 6) for B (control) or O (selected) females with, or without, added yeast on the medium surface. All ovarioles were characterized in each adult female. The grand mean was derived from the sum of B or O line means divided by the number of replicate lines.

Figure 3. Grand average vitellogenic oocyte stage for B (control) or O (selected) lines with, or without, added yeast on the medium surface. The sum of all vitellogenic oocyte stages was divided by the total number of vitellogenic oocytes in each adult female. The grand average was derived from the sum of B or O line means divided by the number of replicate lines.
eclosion ($p = .0004$). The source of the interaction is indicated by post hoc comparisons of mean pairs. Specifically, on day 4, post-eclosion B females have a higher average vitellogenic stage (averaged across diets) than do the O females ($p = .0212$). The differences between line types are not statistically significant on day 16 post-eclosion, when the average vitellogenic stage in O females has increased (Figure 3). Among the main factors considered independently, the level of support suggests a statistically significant effect of diet ($p = .0275$).

As a second measure of vitellogenic activity, Figure 4 presents the grand average percentage of ovarioles with vitellogenic oocytes in B and O females as a function of age and diet. The ANOVA shows that among the main effects (line type, diet, and day post-eclosion), there is no support for a statistically significant effect of line type ($p = .5851$), but there is support for a statistically significant effect of diet ($p = .0001$). Specifically, a high-yeast diet is associated with an increased incidence of vitellogenic oocytes in ovarioles. Among the possible two factor interactions, there is support for a statistically significant interaction between line type and day post-eclosion ($p = .0001$). In terms of this interaction, it can be observed that O females show an increase in percentage of vitellogenic ovarioles as a function of age until approximately day 24 post-eclosion, whereas this parameter does not increase in B females as a function of age (Figure 4). Correspondingly, pairwise mean contrasts between O females on day 4 with O females on day 24 post-eclosion indicate support for differences on low yeast ($p = .0001$) and high yeast ($p = .0002$), whereas there is no support for a difference when B females are compared at days 4 and 24. A notable occurrence is that on day 4 post-eclosion, O females on the low-yeast diet have fewer vitellogenic ovarioles than O females on high-yeast ($p = .0001$), B females on low-yeast ($p = .0001$), or B females on high-yeast diets ($p = .0001$).

Figure 5 shows the grand average number of ovarioles per adult female at ages post-eclosion for the two dietary treatments. The low and high standard errors, as proportions of the grand average considered across all assay points post-eclosion, range from 9.17% to 18.81% (B lines–low yeast), 10.01% to 14.87% (B lines–high yeast), 9.87% to 15.03% (O lines–low yeast), and 10.99% to 14.74% (O lines–high yeast) of the mean value. As indicated by the ANOVA, there is statistical support for an effect of line type on ovariole number ($p = .0313$). Long-lived females (O) have more ovarioles than control (B) females. There was a greater difference between O and B females on day 4 post-eclosion than throughout most of the comparable life span of B and O females. For example, a pairwise mean contrast of O and B line ovariole numbers on day 4 post-eclosion revealed a statistically significant difference ($p = .0259$), but there was no support for a significant difference on day 8 post-eclosion ($p = .1728$). There was no support for statistically significant effects.
Figure 5. Grand average number of ovarioles for B (control) or O (selected) females with, or without, added yeast on the medium surface. All of the ovarioles in both ovaries were counted in each female. The grand average was derived from the sum of the B or O line means divided by the number of replicate lines.

Discussion

The relative delay in the decline of the previtellogenic gradient of oocyte maturation in long-lived O females shown in this study may be a salient observation (Figures 1 and 2). The long-lived lines were selected for late-life female reproduction, which appears to have resulted in persistence of female reproductive function in terms of maintaining the previtellogenic gradient of maturation. This may suggest that selection can act to increase the persistence or replicative potential of germ line stem cells. There was little evidence for line-type differences in late-life vitellogenic activity. Apparently, differential ability to maintain vitellogenesis does not underlie the difference in B and O life span. Early-age line-type differences in ovariole number in the present study act to confirm the results of an earlier study (37). Specifically, O females have more ovarioles than B females on day 4 post-eclosion. However, this study indicates that no statistically significant O versus B difference in ovariole number is evident at day 8 post-eclosion or at older ages. Consequently, there is little evidence for the hypothesis that long-lived O females possess more ovarioles in order to ameliorate fecundity decline in later life.

Ovariole number variation is thought to be associated with differential reproductive potential (41). For example, solitary locusts (Schistocerca gregaria) have approximately 40% more ovarioles and approximately 60% more maturing eggs than females of the gregarious form (42). Similarly, in D. melanogaster, ovariole number variation has been observed to be positively correlated with fecundity (43,44). However, in a study using homozygous chromosome substitution lines of D. melanogaster, segregating variance for ovariole number was correlated neither with variation in body size nor with competition-based measures of viability or fertility (45).

Carlson and associates (37) present evidence that females from lines selected for late-life reproduction (O lines) have significantly more ovarioles than control line females (B) on day 1 and day 4 post-eclosion. In the present study, O females were observed to have a greater number of ovarioles when first sampled on day 4 post-eclosion (Figure 5). During the first 4 days post-eclosion, O females have been observed to have relatively low fecundity, suggesting an inverse relationship between ovariole number and fecundity at early ages post-eclosion. Correspondingly, there is evidence for a relative delay in vitellogenic oocyte maturation in O females during the first 4 days post-eclosion (37). The question arises as to whether there is any relationship between ovariole number and increased late-life reproduction in long-lived females. Old D. melanogaster females exhibit reduced fecundity, and the suggestion was made that O females may have evolved an increased number of ovarioles as a correlated response to selection for late-life reproduction (37). Females could have evolved to counter the reproductive decline associated with age by an increase in the
number of oocyte maturing units (ovarioles) that can produce eggs. However, on day 8 post-eclosion, there was no statistically significant B versus O line difference in ovariole number. In fact, throughout most of the comparable life span of O and B females, there is only a modest difference in ovariole number (Figure 5). Ovariole number differences between line types are probably not sufficient to account for the increasing fecundity in O females as a function of age and the relative level of fecundity in older O females.

Several questions arise from the pattern of temporal changes in ovariole number observed in this study (Figure 5). The first is whether ovarirole numbers in B females increase after the first 4 days post-eclosion. Ovariole numbers are thought to be influenced primarily by the larval environment and determined by an early pupal stage (46). Nevertheless, it is possible that there is a delay in developing the total number of ovarioles in B females associated with the “control line” culturing regime. In this regime, there is selection for rapid egg production—perhaps at the expense of ovarirole formation, which may be delayed in part to the post-eclosion period. The second question is whether there is evidence for a relationship between ovarirole number and survival at older ages. We saw a suggestion of a decrease in ovarirole number at a time of high mortality in both B and O lines (Figure 5). For both B and O females, the terminal assay point corresponds to approximately 50% cumulative mortality and, irregardless of diet treatment, there appears to be a pronounced decline in ovarirole number at the last timepoint (Figure 5). Ovarioles could be lost with advancing age, or there might be a decrease in survival associated with greater ovarirole number that could reflect the direct or indirect cost of ovarirole formation.

This research characterized vitellogenic oocyte stages and determined the percentage of ovarioles with vitellogenic oocytes. Yolk protein, used to provision the egg during vitellogenesis, is one of the most abundant molecules produced for female reproduction. In a single day a female can synthesize an amount of yolk protein that is equivalent to 5%–10% of her weight (47). In part because vitellogenesis is so costly, variation in the process could underlie differential longevity.

In the present study, O females exhibited a lower average vitellogenic oocyte stage on day 4 post-eclosion regardless of diet (Figure 3). This observation reinforces the results from Carlson and colleagues (37), wherein it was determined that vitellogenic oocyte maturation was slower in O females from day 1 to day 4 post-eclosion. In general, a delay in oocyte maturation may underlie early age fecundity generally observed as a correlated response to selection for late-life female reproduction in various independent selection experiments (11,23,35,36). In this study, the B females exhibited no change, or a gradual decline, in average vitellogenic oocyte stage from days 4 to 16 post-eclosion, whereas O females showed an increase in this parameter (Figure 3). These trends were largely independent of diet. However, O females on an added yeast diet attained a relatively high average vitellogenic stage by day 8 post-eclosion, whereas O females without added yeast did not attain an approximately equivalent average vitellogenic stage until days 16 to 24 post-eclosion.

An attempt was made to relate the temporal pattern of average oocyte stage to survival and fecundity data from an earlier study on O and B females (22). Both studies were conducted using the same conditions of light, temperature, medium composition, and presence or absence of supplemental yeast. After approximately the first 5 days post-eclosion, the daily fecundity of O and B females declined steadily throughout the remainder of life on a high-yeast medium. On the diet without added yeast, daily fecundity remained relatively constant after eclosion, exhibiting a gradual decline in later life (22). In the present study, there was stasis or a slight decline in average vitellogenic oocyte stage throughout life in B females, whereas O females showed an increase in this parameter to a relatively high level throughout the remainder of the study, irrespective of the presence or absence of added yeast. In general, there is no obvious correspondence between the lifetime pattern of fecundity in the selected and control lines under two dietary regimes (22) and the temporal pattern of average vitellogenic stage as determined in the present study. Female survivorship curves are also available for O and B line females (22). From the longest average line life span, in decreasing order, the line-type rankings of survival were: O females on low-yeast medium, O females on high-yeast medium, B females on low-yeast medium, and B females on high-yeast medium. A comparison of rank average oocyte stage in the present study with rank longevity in the former study does not suggest a clear relationship between average vitellogenic stage and differential longevity.

The average percentage of ovarioles with vitellogenic oocytes is another measure of vitellogenic activity (Figure 4). On the low-yeast diet, there was a relatively low percentage of vitellogenic oocytes in O females on day 4 post-eclosion followed by a steady increase to day 24 post-eclosion. In general, this study suggests that selection for late-life reproduction results in a reduced level of vitellogenic activity early in life and a subsequent increase in vitellogenic activity in older O females. The addition of yeast increased the percentage of ovarioles with vitellogenic oocytes, perhaps corresponding to the high-yeast stimulation of fecundity and concomitant decrease in age-specific survival observed in Chippindale and colleagues (22).

The present study also included characterization of previtellogenic oocyte stages in aging O and B females on different diets (Figures 1, 2). It is known that D. melanogaster fecundity decreases with age (48). Moreover, a decrease in fecundity as a function of moderately advanced age has been documented in extended longevity (O) and control (B) females (22). In general, the underlying basis of declining fecundity as a function of age is not known. One possibility is that the rate of oocyte maturation declines as a function of age. In the present study, insight into the temporal decline of the previtellogenic gradient of oocyte maturation was obtained by examining the percentage of ovarioles that have lost two terminal previtellogenic stages (Figure 2). Independent of diet treatment, there is a marked loss of terminal stages in B females between days 8 and 16 post-eclosion. This loss corresponds to the decrease in average previtellogenic stage in B females between days 8 and 16 (Figure 1). In O females, the loss of terminal previtellogenic stages is temporally delayed and the decline is relatively gradual, especially in O females on low yeast. In these females (Figure 2), the decline starts approximately on day 16 post-eclosion and continues to day 40. In terms of longevity, the persistence of the O female previtellogenic gradient of matura-
REFERENCES

reviewers made valuable and appreciated comments on the manuscript. Nebraska-Lincoln to L.G. Harshman, and National Institute on Aging grant AG-08761. We thank Tony Zera for comments on the manuscript and the time.


Acknowledgments

This research was supported by a grant from the University of Nebraska–Lincoln to L.G. Harshman, and National Institute on Aging grant AG-08761.

We appreciate Alan Christensen's input, which accelerated the inception of this study. We thank Tony Zera for comments on the manuscript and the time devoted to graduate student instruction on the topic of life history studies. The reviewers made valuable and appreciated comments on the manuscript.

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This research was supported by a grant from the University of Nebraska–Lincoln to L.G. Harshman, and National Institute on Aging grant AG-08761.

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Received September 18, 1998
Accepted April 23, 1999

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