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Patterns of physiological decline due to age and selection in *Drosophila melanogaster*

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In outbred sexually reproducing populations, age-specific mortality rates reach a plateau in late life following the exponential increase in mortality rates that marks aging. Little is known about what happens to physiology when cohorts transition from aging to late life. We measured age-specific values for starvation resistance, desiccation resistance, time-in-motion, and geotaxis in ten *Drosophila melanogaster* populations: five populations selected for rapid development and five control populations. Adulthood was divided into two stages, the aging phase and the late-life phase according to demographic data. Consistent with previous studies, we found that populations selected for rapid development entered the late-life phase at an earlier age than the controls. Age-specific rates of change for all physiological phenotypes showed differences between the aging phase and the late-life phase. This result suggests that late life is physiologically distinct from aging. The ages of transitions in physiological characteristics from aging to late life statistically match the age at which the demographic transition from aging to late life occurs, in all cases but one. These experimental results support evolutionary theories of late life that depend on patterns of decline and stabilization in the forces of natural selection.

KEY WORDS: **Aging, age-specific physiology, *Drosophila*, experimental evolution, late life.**

Demographic aging is often interpreted in terms of Gompertz equations that predict an exponential increase in age-specific mortality with chronological age:

$$u(x) = Aexp(\alpha x) \quad (1)$$

where x is age, $u(x)$ is age-specific mortality rate, and the positive-valued parameters A and α are background mortality and aging rate, respectively. Equation (1) suggests that aging is an inevitable and unremitting process of deterioration, and that age-specific mortality rates should continue increasing until all members of a cohort die off (Finch et al. 1990; Rose 1991). But this assumption is undermined by laboratory experiments showing that mortality rates in medflies, fruit flies, wasps, nematodes, and yeast decelerate at advanced ages and in some cases plateau (Carey et al. 1992; Curtsinger et al. 1992; Fukui et al. 1993; Brooks et al. 1994; Vaupel et al. 1998; Carey 2003). Mortality rate deceleration at advanced ages has also been seen in humans (Greenwood and Irwin 1939; Gavrilov and Gavrilova 1991; Kulminski et al. 2007). The period of adult life in which mortality rates plateau is sometimes referred to as "late life" (Rose et al. 2005).

In addition to mortality plateaus, late life is also characterized by plateaus in other fitness characters, such as female fecundity (Rauser et al. 2005a; Rauser et al. 2006) and male virility (Shahrestani et al. 2012b). The existence of both phases, aging and late life, can be explained and predicted by evolutionary theories based on the age-specific intensity of natural selection. Hamilton (1966) derived the result that the force of natural selection acting on mortality is given by $s(x)/T$, where x is chronological age and T is a measure of generation length. The function s at age x is given by

$$\sum_{y=x+1}^{d} e^{-ry} l(y) m(y) \quad (2)$$

where r is the Malthusian parameter, or the growth rate of the population, associated with the specified $l(y)$ survivorship and

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m(y) fecundity functions. The *s(x)* function scales according to the impact on fitness of an individual's future reproduction, after age *x*. Before the first age of reproduction *s* is always equal to one, once reproduction has ended, *s* is equal to zero, and during the reproductive period *s(x)* progressively falls (reviewed in Rose et al. 2007). In evolutionary theories, equations such as equation (2) are expected to produce a progressive loss of adaptation with age (Rose 1991; Charlesworth 1994). But when the force of natural selection acting on mortality rates plateaus at or near zero late in adulthood, mortality rates should stabilize, albeit at high levels, because natural selection will no longer discriminate among genetic effects acting at these late ages (Mueller and Rose 1996; Mueller et al. 2011). At these advanced ages, the effective force of natural selection will be weaker than other, random, evolutionary forces, such as drift.

Predictions of the evolutionary theories of late life have been substantially corroborated in several studies (Rose et al. 2002; Rauser et al. 2005a; Reynolds et al. 2007). Even in organisms that reproduce at all ages, the force of natural selection is eventually overwhelmed by drift in late life, and when there are enough alleles that have age-independent beneficial effects, it is possible to have positive-valued average survival and fecundity during late life (Charlesworth 2001). This evolutionary explanation for mortality plateaus predicts that age-specific deterioration in individuals may slow down or stop at late ages. Most past studies of age-specific function did not extend into the late-life phase, and the few that did provided only slim and mixed results (Drapeau et al. 2000; Nghiem et al. 2000). Recently, we showed that aging and late life are different from each other with respect to the physiological characteristics of individuals from *Drosophila melanogaster* (Shahrestani et al. 2012a).

An important implication of "Hamiltonian" (Rose et al. 2007) evolutionary theories of late life is that late life is an evolutionarily distinct phase of life history, evolving according to strictures very different from those that mold aging. If the physiological transitions from aging to late life that we observed in Shahrestani et al. (2012a) evolutionarily depend on Hamiltonian patterns in the forces of natural selection, then we should be able to document earlier physiological transitions in populations with earlier plateaus in the forces of natural selection. We test this prediction in ten *D. melanogaster* populations that have evolved differences in their age of onset of the late life phase due to such shifts in the forces of natural selection (vid. Rose et al. 2002).

Methods

STUDY SYSTEM

Five "$CO_{1\text{-}5}$" populations derived from five ancestral "$O_{1\text{-}5}$" populations (Rose 1984) and maintained on discrete 28-day generation cycles (Rose et al. 1992; Phelan et al. 2003) were compared with five 5 "$ACO_{1\text{–}5}$" populations derived from the $CO_{1\text{-}5}$ populations and maintained on discrete 9–10 day generation cycles (Chippindale et al. 1997). Individual ACO_i and CO_i populations that were matched by a common index were tested at the same time. The common index indicates that the two populations have a common ancestral lineage deriving from a specific O_i population, with each ACO_i population derived from the corresponding CO_i population (Chippindale et al. 1997). Each population also has its own unique history of genetic change due to random genetic drift. All populations have been historically maintained with breeding population sizes of at least 1000 individuals, under 24-h illumination, at approximately 25°C, with banana-molasses medium at low larval densities (~60 – 80 eggs/8-dram vial). Prior to the start of each experiment, we reared the populations in identical conditions at controlled densities (60 ± 5 larvae per vial) for two generations to control for environmental and parental effects.

MORTALITY ASSAYS

For each population, ~13,000 individuals (half male and half female) were aged in 16 plexiglass population cages at densities of ~800 flies per cage. Four of the cages for each population were randomly marked as "mortality cages." The flies in these cages were used to estimate the age of onset of the mortality plateau. Dead flies were removed daily from these cages and the number and sex of dead flies was recorded. Flies in cages were fed daily with Petri dishes containing standard Rose-Lab banana-molasses fly food, without additional yeast supplement.

The remaining 12 cages per population were maintained in identical conditions to the mortality cages. From these cages, we sampled 48 flies per sex per population three times each week for the desiccation resistance, starvation resistance, time spent in motion, and negative geotaxis assays. The flies were removed from cages using light carbon dioxide anesthesia. For each day on which we tested for physiological characteristics, we removed flies from four cages at random, but each cage only received carbon dioxide exposure once each week, including the mortality cages.

DESICCATION RESISTANCE

Desiccation resistance assays were performed as described in Shahrestani et al. (2012a). In brief, flies were placed in 8-dram glass vials in groups of four males or four females (never mixed). A short foam-plastic plug was used to enclose the flies in the bottom of the vial and 3 g Drierite desiccant was added on top of the sponge. The vial was sealed with two layers of parafilm. Each vial was checked once every hour and the number of dead flies was recorded. Death was indicated by a fly's lack of movement upon mechanical provocation by tapping on the vial. We tested 24 flies per sex per population at each age.

TIME SPENT IN MOTION

Time spent in motion assays were performed as described in Shahrestani et al. (2012a). Flies were placed individually in 8-dram glass vials and confined to the bottom 1 cm of the vial using a plug. Flies were given 10 minutes to recover from anesthesia before a stopwatch was used to time the movement of the flies in a two-minute time interval. For each fly two recordings were made and averaged. We tested 24 flies per sex per population. The same flies were then used in the negative geotaxis assays.

NEGATIVE GEOTAXIS

Negative geotaxis assays were performed as described in Shahrestani et al. (2012a). In brief, for the vials used in the time spent in motion assays, the sponge was pulled back to 8-cm from the bottom of the vial, giving the fly more space. We recorded the percentage of flies that reached the top in a one-minute interval after having been tapped down to the bottom of the vial. All flies that made it to the top did so within the first $\sim$15 seconds. The flies used in the negative geotaxis assays were then used in the starvation resistance assay.

STARVATION RESISTANCE

Starvation resistance was measured as described in Shahrestani et al. (2012a). For each of the vials used in the negative geotaxis assays, an absorbent rayon ball and 5 ml distilled water were added on top of the plug and the vial was sealed with two layers of parafilm. Death from starvation was recorded every four hours. Death was inferred by a fly's inability to move upon mechanical provocation.

STATISTICAL METHODS

Mortality

Under the evolutionary theory of late-life (Mueller and Rose 1996; Mueller et al. 2011), mortality is expected to increase in an approximately exponential fashion through the majority of the adult life span and then level off at some advanced age. The age at which this leveling of mortality rates occurs is, under the evolutionary theory, the important landmark of late-life. Thus, our primary interest in the mortality data is not to arrive at the best-fitting model but rather to provide an estimate of the onset of late life in an objective fashion.

The simplest model that captures the essential features of the evolutionary model of late life is a two-stage Gompertz function. If we let $u(x)$ be the instantaneous mortality at age-x then the functional form of the two-stage Gompertz is,

$$u(x) = \begin{cases} A exp(\alpha x) \, if \; x \leq bd \\ \bar{A} \, if \; x > bd \end{cases}, \qquad (3)$$

The parameter *bd* of the two-stage Gompertz (eq. 3) is called the breakday and its value is used as an estimate of the boundary between the aging and late-life phases of life.

When age-specific mortality over a fixed period of time obeys a binomial distribution, our previous research has shown that the parameters of equation (3) are best estimated by a maximum likelihood technique (Mueller et al. 1995). It turns out that there are several different ways maximum likelihood may be implemented for the estimation of parameters in equation (3). A method we proposed (Mueller et al. 1995) and refer to here as the "deaths-per-census-period" method uses the total number of deaths that occur between each census period. Mueller et al. (1995) pointed out that since models like equation (3) are continuous time models, yet deaths can only be recorded over discrete intervals, there were some natural advantages to the deaths-per-census-period method. One of these is that the number of deaths is known exactly while the age-at-death is only known approximately. A second technique, called here the "age-at-death" method uses the age-at-death of each individual in the cohort to form the maximum likelihood function (Pletcher 1999). However, it has been shown recently that this method gives biased estimates relative to the deaths-per-census-period technique (Shahrestani et al. 2012a), and so it is not used in our analysis.

The deaths-per-census-period technique was used to estimate the breakdays for each population and each sex. Since our interest is ultimately not on any single replicate population we took the mean breakday for each sex and selection regime and used this to classify adult ages into an aging phase (ages $\leq bd$) and a late-life phase (ages $> bd$).

Alternative identification of late-life

We address the question, is there any value to breaking up the adult life-span into an aging phase and a late-life phase, and did we do it properly using the breakday as the boundary? One way to address this question is to use the physiological data only and determine if a linear description of physiology versus age can be improved by splitting the age range into two sections. Additionally, we would like to know if the location of that split corresponds to our estimate of breakday.

Suppose we have measurements for a physiological trait, y_{t_i}, at ages $t_0, t_1, \ldots, t_n$. We then created two regions, $R_1 = \{\text{all } t_i < t^*\}$ and $R_2 = \{\text{all } t_i \geq t^*\}$. We found a t^* that minimizes the equation, $\sum_{i:\, t_i \in R_1} (y_{t_i} - \hat{y}_{R_1})^2 + \sum_{i:\, t_i \in R_2} (y_{t_i} - \hat{y}_{R_2})^2$, where $\hat{y}_{R_j}$ is the fitted linear equation, $\hat{\beta}_0 + \hat{\beta}_1 t_i$, to the observations in R_j. To generate a confidence interval on the best cutpoint we used bootstrap resampling that replicated the total sample size and the sample size at each age. We implemented the bootstrap with the R package *boot*. 95% confidence intervals were generated from percentiles of the 1000 bootstrap replicates. Statistically this

problem is similar to creating a regression decision tree (Hastie et al. 2009, chapter 9) except that we are only doing one split of the data and using a linear regression rather than using the mean values of the traits in each group.

Physiology

We analyzed female and male physiological data (desiccation resistance, starvation resistance, time-in-motion) based on a regression-partition of aging and late life, using the linear-mixed effects package, *lme* in R (R Core Team 2013). For each physiological measurement, a vector of independent variables was recorded. These included the fly's age ($i = 2, 5, 7, \ldots$ *max.age*), sex ($j = 0$ (female) or 1 (male)), stage ($k = 0$ (late-life), 1 (aging)), selection regime ($l = 0$ (ACO), 1 (CO), population ($m =$ 1 (ACO1), 2 (ACO2),..., 10 (CO5)), vial ($n = 1, 2, \ldots,$ *max.vial*), and the *pth* individual. *max.age* and *max.vial* are integers that correspond to the maximum age and number of vials in an experimental population. With this notation, we modeled each physiological measurement at time t_i with the following linear model,

$$Y_{ijklmnp}(t_i) = \beta + \alpha t_i + \gamma\delta_j + \varphi\delta_k + \theta\delta_l + \Psi_j t_i\delta_j + \Psi_k t_i\delta_k + \Psi_l t_i\delta_l + \pi_{jk}\delta_j\delta_k \quad (4)$$

$$+\pi_{jl}\delta_j\delta_l + \pi_{kl}\delta_k\delta_l + \pi_{jkl}\delta_j\delta_k\delta_l + \Psi_{jk}t_i\delta_j\delta_k + \Psi_{jl}t_i\delta_j\delta_l + \Psi_{kl}t_i\delta_k\delta_l + \Psi_{jkl}t_i\delta_j\delta_k\delta_l + b_m + c_{mn} + \xi_{ijklmnp}, \quad (5)$$

In equation (5), the main effects of age, sex, stage, and selection are measured by α, γ, φ, θ, respectively. The effect of sex, stage, and selection on the slope is measured by Ψ_j, Ψ_k, and Ψ_l, respectively. The Ψ parameters with two or three subscripts measure the interaction between sex, stage, and selection with age. δ_x is the indicator random variable that equals 1 if $x = 1$ and 0 otherwise. There are three sources of random variation in equation (5): variation due to population is measured by b_m, vials within populations c_{mn}, and residual variation $\xi_{ijklmnp}$. These three sources of variation are assumed to be normally distributed independent random variables with a mean of zero, but different variances as estimated from the observations.

The parameters in equation (5) can be used to construct various tests of hypotheses about slopes. For instance, if we wanted to test whether the aging slope for ACO males was different than the late-life slope for ACO males we could test for whether the difference between these slopes was significantly different from 0. The aging slope for ACO males is $\alpha + \Psi_j$. The late-life slope for ACO males is $\alpha + \Psi_j + \Psi_k + \Psi_{jk}$. So the difference between the late-life slope and the aging slope is, $\Psi_k + \Psi_{jk}$. The statistical tests consists of constructing a confidence interval on this difference using the variance of the difference or, $Var(\Psi_k) + Var(\Psi_{jk}) + 2Cov(\Psi_k, \Psi_{jk})$. This methodology is followed in Tables 2 and 3 for two general types of tests: comparisons within a selection regime of the slopes between the aging versus the late-life ages and comparisons between selection regimes of like slopes, for example aging in ACO compared to aging in CO. The only difference between the analysis of starvation and desiccation was that there was no vial effect included in the starvation analysis. Since each fly was put in a separate vial to assay starvation resistance,

Table 1. The mean breakdays (in days) and 95% confidence intervals for each selection regime and sex.

Sex	ACO	CO
Male	33 (23, 49)	49 (42, 56)
Female	30 (17, 43)	44 (27, 61)

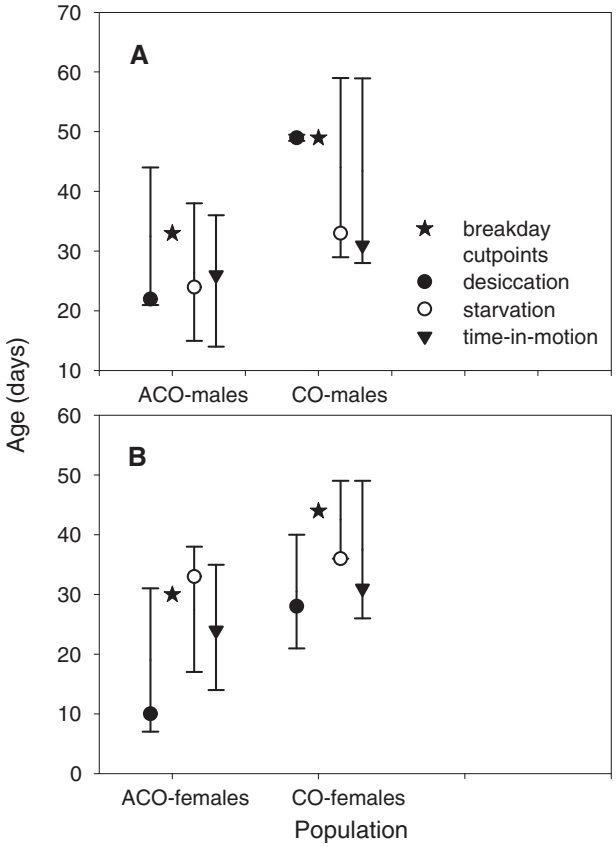

Figure 1. The breakdays (stars, Table 1) for the four population/sex combinations: (A) ACO-males, CO-males, and (B) ACO-females, CO-females. Next to each star are three 95% confidence intervals on cutpoints for the three physiological traits: desiccation resistance, starvation resistance, and time-in-motion. See the text for a description of how the confidence intervals were estimated. The points within each confidence interval are the estimated cutpoints. The breakdays are included in 11 of the 12 confidence intervals.

Table 2. Starvation resistance age-specific slopes.

Population	Sex	Stage	Slope	Difference	*P*-value
ACO	F	Aging	–1.57		
		Late-life	–0.12		
		Aging–late-life		–1.45	<0.0001
	M	Aging	–0.53		
		Late-life	0.045		
		Aging–late-life		–0.57	0.054
CO	F	Aging	–2.10		
		late-life	–0.55		
		Aging–late-life		–1.55	<0.0001
	M	Aging	–0.67		
		late-life	–0.016		
		Aging–late-life		–0.66	0.003
CO-ACO	F	Aging		0.52	<0.0001
		Late-life		–0.42	0.097
	M	Aging		–0.15	0.18
		Late-life		–0.06	0.86

The location of each slope is either before the breakday (aging stage) or after the breakday (late-life stage). Hypothesis tests are done within populations (aging–late-life) and between populations (CO-ACO).

the random effects of vials is confounded with the residual variation. Interestingly, the residual variance accounts for 98% of all the variation for starvation resistance but only 80% of the variation for desiccation resistance, consistent with the idea that there are more factors contributing to the residual variance of starvation resistance. Female flies continue to develop and mature after pupation, which leads to an increase in starvation resistance at early adult ages. This is because female flies continue to grow and increase their starvation resistance in early adulthood, which obscures the effect of aging (Service et al. 1985). To eliminate the effect of this continued development on our results, we looked at female starvation resistance by removing data prior to age 12, which was the period of time when female starvation resistance was increasing with age, as we have also done previously in our studies of late-life physiology (Shahrestani et al. 2012a).

Time-in-motion did not show a significant effect of sex nor was there a vial random component. Thus, the linear-mixed effects model was like equation (5) except all terms with a subscript *j* are removed and the random term, c_{mn}, is removed.

Geotaxis is measured as the fraction of 24 flies that reach the top of a vial in a specified time. Since the dependent variable in this analysis has a binomial distribution (e.g., number of flies reaching the top) the results were analyzed with logistic regression and a general linear model (GLM). The GLM model we are fitting looks like this,

$$Y_{ikln}(t_i) = \beta + \alpha t_i + \varphi\delta_k + \theta\delta_l + \Psi t_i\delta_k + \xi_{ikln}, \qquad (6)$$

where $Y_{ikln}(t_i)$ is the logit of stage-*k*, in selection regime-*l*, aged t_i weeks in the *nth* vial. The logit is equal to $\ln\{u(t_i)/[1\text{-}u(t_i)]\}$ if

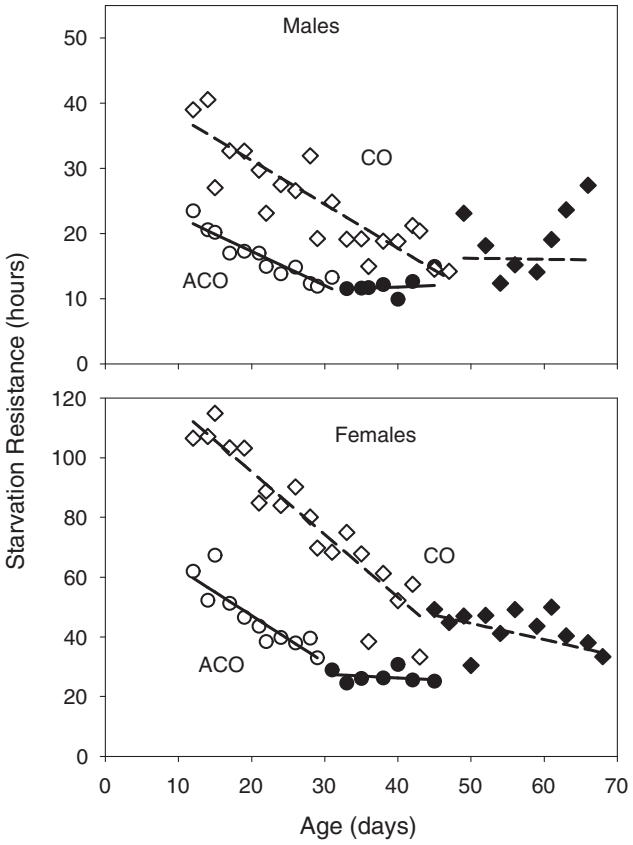

Figure 2. Starvation resistance in the ACO (circles) and CO (diamonds) populations. Open symbols are age-specific mean values prior to the mortality breakday and filled symbols are the means postbreakday. Lines (solid = ACO, dashed = CO) are predictions from the linear-mixed effects model.

Table 3. Desiccation resistance age-specific slopes.

Population	Sex	Stage	Slope	Difference	*P*-value
ACO	F	Aging	–0.15		
		Late-life	–0.072		
		Aging–late-life		–0.075	0.009
	M	Aging	–0.11		
		Late-life	–0.0090		
		Aging–late-life		–0.098	0.08
CO	F	Aging	–0.16		
		late-life	–0.075		
		Aging–late-life		–0.081	2×10^{-8}
	M	Aging	–0.10		
		Late-life	-0.076		
		Aging–late-life		–0.024	0.6
CO-ACO	F	Aging		–0.010	0.3
		Late-life		–0.0037	0.9
	M	Aging		0.0067	0.4
		Late-life		–0.067	0.09

The location of each slope is either before the breakday (aging stage) or after the breakday (late-life stage). Hypothesis tests are done within populations (aging–late-life) and between populations (CO-ACO).

$u(t_i)$ is the observed probability of reaching the top of the vial at aget_i. To convert the logit back to a probability the relationship, $u_{ijkn}(t_i) = \exp[Y_{ikln}(t_i)]/\{1+\exp[Y_{ikln}(t_i)]\}$ can be used.

To examine if the age-specific trends in late-life matter to a complete statistical description of the phenotypes desiccation resistance, starvation resistance, and time-in-motion, we compared two simple models to each other. The first model is a simple line with a constant rate of decline of each phenotype at all ages. The second model was a two-stage linear model in which we assume the linear decline in a phenotype stops at some age and levels off. The two-stage linear model is,

$$y = \begin{cases} a_0 + a_1 t, \ if \ t \leq a_2 \\ a_0 + a_1 a_2, \ if \ t > a_2 \end{cases}, \tag{7}$$

where y is the phenotypic value and t is the age of the individual. We cannot use equation (7) to model geotaxis since the age-dependent change for that phenotype accelerates in late-life relative to the aging phase of life. To assess the utility of each model we estimated the Akaike Information Criterion (*AIC*). Under the *AIC* the best model would have the smallest *AIC* value. The *AIC* is an increasing function of the residual sum of squares (*RSS*) and the number of model parameters (n_{par}). So while more complicated models may reduce the *RSS* it will increase the number of parameters. The *AIC* is calculated by,

$$Nln\left(\frac{RSS}{N}\right) + 2n_{par}, \tag{8}$$

where N is the total sample size. The regression results were carried out with the *lm* and *nls* R-functions.

Results

The average demographic breakdays are at younger ages in the ACO populations compared to the breakdays in the CO populations (Table 1). These observations are entirely consistent with earlier work on these populations (Rose et al. 2002).

We computed cut-points for the three physiological traits that were examined with standard linear regression methods: desiccation resistance, starvation resistance, and time-in-motion (Fig. 1). We see that 11 of the 12 cut-point confidence intervals (all except CO female desiccation resistance) include our breakday estimate (Fig. 1). In all cases, the residual sum of squares is improved by bisecting the observations at an age in the middle of the range. Thus, improvements in the statistical description of the age-specific declines in physiology are achieved by this division.

Females show significantly more shallow changes with age in starvation resistance (Table 2 and Fig. 2) and desiccation resistance (Table 3 and Fig.3) in late-life than they do during the aging phase of life. While the same qualitative effect is seen in males (Tables 2 and 3), it is only statistically significant in one out of four tests (CO male starvation resistance).

In contrast, significant differences are seen in only one out of eight comparisons of slopes between selection regimes (aging stage of male starvation resistance, Tables 2 and 3). So for starvation and desiccation resistance there tend to be similar declines with age for these physiological traits in both the CO and ACO populations during the aging phase, but there is a large difference in this pattern during the aging and late-life age classes. That is, aging appears to be parallel in its physiological effects, once the

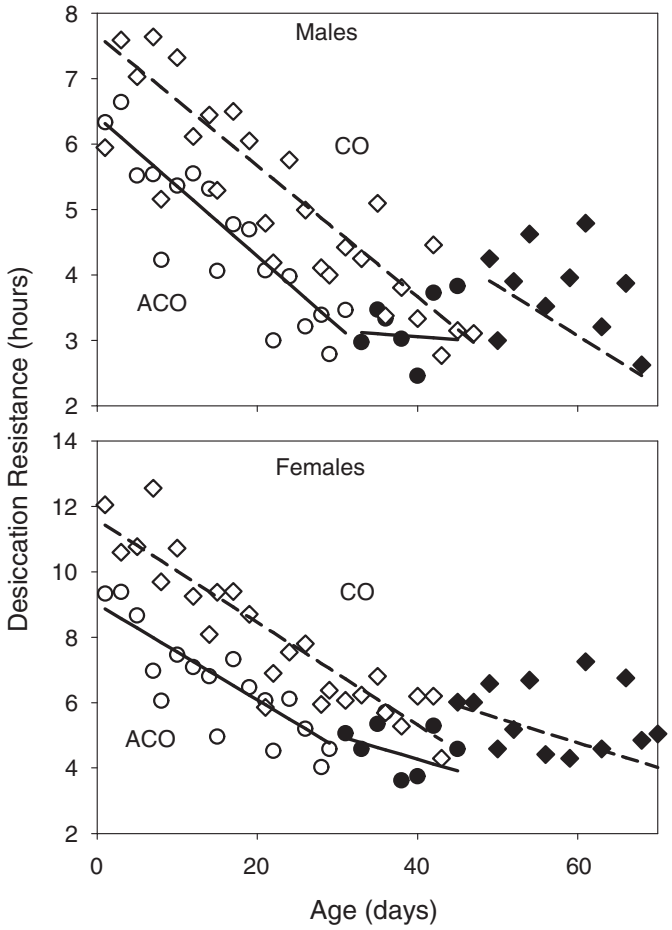

Figure 3. Desiccation resistance in the ACO (circles) and CO (diamonds) populations. Open symbols are age-specific mean values prior to the mortality breakday and filled symbols are the means postbreakday. Lines (solid = ACO, dashed = CO) are predictions from the linear-mixed effects model.

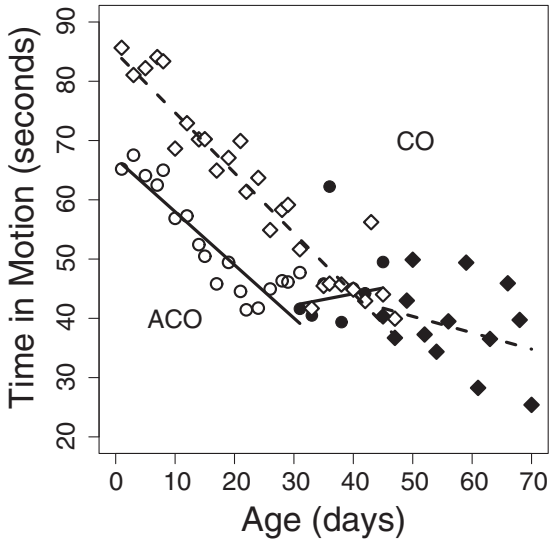

Figure 4. Time-in-motion in the ACO (circles) and CO (diamonds) populations. Open symbols are age-specific mean values prior to the mortality breakday and filled symbols are the means postbreakday. Lines (solid = ACO, dashed = CO) are predictions from the linear-mixed effects model. Male and female data are pooled. Since the breakdays for males and females are different some prebreakday points overlap postbreakday points.

aging demographic phase is correctly partitioned from the late-life demographic phase.

Similar results are obtained with time-in-motion (Table 4 and Fig. 4). The ACO and CO populations show significant differences with respect to the slope of the aging phase and the slope of the late-life phase (Table 4). But the age-specific declines in time-in-motion during aging are not different between the ACO populations and the CO populations (Table 4). Nor are they different during late life.

The analysis of geotaxis (Table 5, Fig. 5) shows significant effects on the GLM parameters due to both selection regime and stage. The large, positive value of φ indicates that flies in the aging phase have a higher probability of reaching the top of the vial than they do in late life, even after accounting for age. The large-positive values of θ indicates that CO flies have a higher probability of reaching the top of the vial than ACO flies, again after accounting for age. The negative value of ψ means that the

Table 4. Time in motion age-specific slopes.

Population	Stage	Slope	Difference	*P*-value
ACO	Aging	–0.90		
	late-life	0.19		
	Aging–late-life		–1.09	<0.0001
CO	Aging	–1.02		
	late-life	–0.28		
	Aging–late-life		–0.74	<0.0001
CO-ACO	Aging		–0.12	0.06
	Late-life		–0.47	0.08

The location of each slope is either before the breakday (aging stage) or after the breakday (late-life stage). Hypothesis tests are done within populations (aging–late-life) and between populations (CO-ACO).

Table 5. The GLM parameter estimates (eq. (6)), standard errors, and *P*-values for tests comparing them to 0.

Parameter	Value	Standard error	*P*-value
β	2.25	0.24	$<2 \times 10^{-16}$
α	–0.0579	0.0058	$<2 \times 10^{-16}$
φ	1.45	0.25	1.1×10^{-8}
θ	1.69	0.091	$<2 \times 10^{-16}$
Ψ	–0.0375	0.0059	2.2×10^{-10}

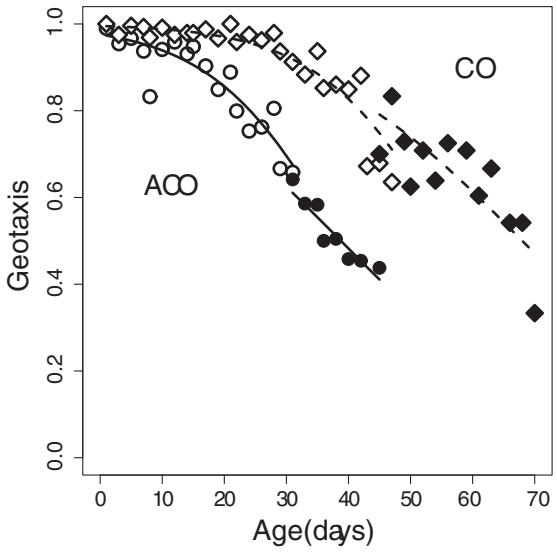

Figure 5. Geotaxis in the ACO (circles) and CO (diamonds) populations. Open symbols are age-specific mean values prior to the mortality breakday and filled symbols are the means postbreakday. Lines (solid = ACO, dashed = CO) are predictions from the general linear model. Male and female data are pooled. Since the breakdays for males and females are different some prebreakday points overlap postbreakday points.

probability of reaching the top of the vial declines at a faster rate in the aging phase than in late life. Geotaxis tends to remain high during early life, and it is apparent that this high plateau in geotaxis is extended in the CO populations. This is almost certainly a byproduct of the extended lifespan of the CO populations. There is also an effect of stage. We can express this effect by using the fitted model to predict at what age only 5% of the population would reach the top of the vial. This statistic clearly incorporates the changes that would occur in late life. For the ACO population, using only the aging data, the 5% age is predicted to occur at 70 days. However, if we predict this age using the late-life data from the ACO populations the age increases to 90 days. Thus, the observations from late-life suggest a slower decline in geotaxis than does the data from earlier ages. Likewise in the CO populations the aging data predict a 5% age of 87 days, while the late-life data predicts 119 days.

We were interested in assessing the value of taking into account the apparent differences in age-dependent changes of phenotypes in late life. One way to do this is determine if simply extending the linear age-dependent declines observed in early life can account adequately for the patterns in late life. Since desiccation and starvation resistance and time-in-motion all show a slowing of phenotypic decline in late life, we developed a simple alternative model in which the phenotype simply levels off at some advanced age. This two-stage linear model has a lower AIC for every phenotype and sex (Table S1). Thus the general pattern is one of decelerating physiological deterioration during late life. Evidently, this pattern is comparable to that of age-specific mortality (Rose et al. 2002), age-specific fecundity (e.g., Rauser et al. 2006), and virility (Shahrestani et al. 2012b), at least in these populations. Thus, in this one well-studied set of populations, we find a general tendency for aging-specific declines in functional characters to decelerate once populations achieve late life, with one notable exception, geotaxis in the study of Shahrestani et al. (2012a).

Discussion

In large sexually reproducing populations, age-specific mortality rates increase exponentially during early adulthood and stabilize in late adulthood (e.g., Carey et al. 1992). We call the periods of exponentially increasing mortality rates and stable mortality rates, the "aging" and "late life" phases of adulthood, respectively (Rose et al. 2005; Mueller et al. 2011). In *D. melanogaster*, late life is also marked by stabilization in the rates of decline in male virility (Shahrestani et al. 2012b) and female fecundity (Rauser et al. 2006). Evolutionary theories suggest that late-life mortality, fecundity, and virility plateaus arise from shifts in the balance between the actions of natural selection and drift affecting age-specific life-history characters (Mueller and Rose 1996; Charlesworth 2001; Mueller et al. 2011; Wachter et al. 2013).

Recently, we showed that the physiology of the aging phase is distinct from that of the late-life phase (Shahrestani et al. 2012a). In *D. melanogaster*, some physiological characters declined during aging and stabilized in late life, but other characters declined at a faster rate in late life (Shahrestani et al. 2012a). Thus, physiological transitions from aging to late life are complex, even paradoxical in some cases when functional characters decline at a faster rate during late life. In the present study, we tested the hypothesis that the timing of the physiological transitions from aging to late life depends on the evolutionary history of the population tested. We compared physiological transitions in *D. melanogaster* populations that differed in their age of onset of the mortality plateau and fecundity plateau (populations of Rose et al. 2002; Rauser et al. 2006). These populations, called $ACO_{1–5}$ and $CO_{1–5}$, were produced via manipulations of the force of selection on laboratory populations (Rose et al. 2002) and have since been widely studied (e.g., Rose et al. 2004; Burke et al. 2010).

In this study, we found a 14–16 day increase in the start of the late-life phase of CO populations relative to the ACO populations. However, when we compare the ACO and CO rates of decline specifically during the aging phase, for starvation resistance, desiccation resistance, and time-in-motion we find no differences except for male starvation resistance. A similar comparison of the late-life phase shows no differences for the same three traits. However, for all three of these characters, the age-specific patterns in aging are different from those in late life; the

rate of decline is faster during the aging phase compared to the late-life phase. While male and female results were similar for most traits, we consistently analyzed the sexes separately because of the large amounts of sexual dimorphism in flies (in body size, reproductive investment, etc.), which has the potential to affect physiology.

Geotaxis showed significant differences due to both selection and phase. Geotaxis declined at a faster rate in late life compared to aging in the ACO and CO populations, as we have found before (Shahrestani et al. 2012a), but the slopes during aging differed between the ACO and CO populations. The aging versus late-life results for geotaxis, along with the same comparison for desiccation resistance and time in motion, corroborate the results of Shahrestani et al. (2012a) for these three characters. Here our analysis of 10 different populations shows that the age of onset of the mortality plateau coincides with the age of onset of shifts in physiological characteristics from aging to late life, which supports Hamiltonian evolutionary theories of late life (vid. Mueller et al. 2011). Many *Drosophila* physiological characteristics, including the ones we have analyzed here, are tightly linked to life history and have been shown to correlate with life-history evolution (Rose et al. 2004). If fitness-related traits have pleiotropic effects on these physiological characteristics, then changing patterns of selection on fitness traits should impact patterns of age-specific physiological performance, as indeed we have demonstrated in our populations many times for the aging phase (Rose et al. 2004).

But our results were qualitatively different from those of Shahrestani et al. (2012a) for starvation resistance. While in our study, the decline in starvation resistance slowed down in late life compared to aging, in Shahrestani et al. (2012a) the slope of age-specific decline in starvation resistance was the same in aging and late life. This led us to pursue possible sources of artifact and inconsistency between the present study and that previous one. An important difference between these two studies from both evolutionary and physiological standpoints is that the present study was conducted in cages using populations that had long adapted to cage culture. By contrast, the previous study (Shahrestani et al. 2012a) was conducted in cages using "B" populations that had never adapted to laboratory cages. Thus the previous study was conducted using an evolutionarily novel environment.

Most previous work on the physiology of aging in our labs was done on flies that were aged in vials, as opposed to in cages. Vial and cage environments differ in many ways, which may influence social interactions and levels of activity and stress in flies. One obvious difference between the two environments is that flies kept in cages receive less food, by volume and surface area of food per fly, on average compared to flies kept in vials because of the experimental practicalities. One of the key nutrients in regular Rose-lab banana molasses fly food is yeast. As a first check on possible artifactual effects, we tested whether supplementing the diet with additional yeast in cages would affect the existence of mortality plateaus and whether the dietary supplement would affect longevity (see supporting files). For both males and females, longevity decreased when given supplemental yeast (Table S2). It is worth noting that the flies receiving regular diet are not necessarily under dietary restriction (supporting files), which may explain why the reduction in longevity seen in the flies given yeast supplement was smaller than what has been previously observed in dietary restriction studies (e.g., Chippindale et al. 1993; Partridge et al. 2005). Plateaus were present in both sexes under both nutrient levels, and in males these plateaus occurred at later ages when the flies were fed yeast supplement (Table S2). These results are in contrast to the results reported by Zajitschek et al. (2013) who suggested that the existence of plateaus was sensitive to the diets given flies. The methods used by Zajitschek et al. differ from ours—they varied yeast levels in cooked food while we varied the levels of live yeast on the food surface. Perhaps more importantly, our experiments differed substantially in population sizes. Zajitschek et al. used a total of 600 individuals per sex and food regime, while we used on average 1270. Statistically significant evidence for plateaus may not be found using small cohorts due to sample-size effects alone, and this may account for the absence of plateaus in some of Zajitschek et al.'s populations. The existence of fecundity plateaus in late life is also independent of dietary yeast supplement (Rauser et al. 2005b), which corroborates our results with mortality in cage cohorts given different levels of yeast. This result alleviated our concerns about artifacts arising from mere cage handling alone, where the study of age-specific physiology is concerned.

Then there remained the issue of the interaction of selection history with method of cohort handling, which potentially could have resulted in our qualitatively different results from Shahrestani et al. (2012a) for starvation resistance. Our previous study (Shahrestani et al. 2012a) featured a lack of correspondence between aging-cohort handling and long-standing culture regime; vial-adapted populations were handled in population cages. We devised experiments to similarly use a mismatch between test-cohort handling regime and evolutionary history, in order to test whether the interaction of selection history with method of cohort handling affects the physiological transition from aging to late life (supplemental files). We studied starvation resistance using cohorts handled in vials, where these cohorts were taken from CO populations that had adapted to cage handling for hundreds of generations. When the CO cohorts were handled in vials, there was still a significant difference in starvation resistance between aging and late life (Fig. S1). However, this difference was opposite to what we saw in cages for cohorts derived from the same CO populations, namely, the rate of decline in starvation resistance was steeper in late life compared to aging. This result suggests, perhaps unsurprisingly, that there can be an interaction of

environment with selection history for age-specific physiology, and also that the environment affects the physiological transition from aging to late life. Thus the qualitative nature of this physiological transition is affected by genotype-by-environment interactions that arise from evolutionary history.

Evolutionary theories of aging and late life based on Hamilton's forces of selection, suggest that any characteristic that is related to fitness may deteriorate during aging because of the declines in age-specific forces of natural selection that shape fitness. In late life, the forces of natural selection are consistently weak and overcome by drift, such that there is no longer a pattern of consistent changes in the forces of selection with increasing age (Mueller et al. 2011). Therefore, we would not expect the same age-specific declines of physiological aging to continue into late life. Because the forces of selection are negligible in late life, they may not affect age-specific changes in physiology and can therefore not be predictive about the direction of physiological change in late life. We have found that physiological characters vary in their trajectories during the transition from aging to late life, with their deterioration decelerating as a result of the transition to late life for some characters, but not all. We suggest that future research on the evolutionary physiology of aging and its cognate life-history characters should consider the transition from aging to late life, given the existence of considerable differences between these adult stages across the spectrum of functional characters.

While the evolutionary theory is the only theory that attempts to explain patterns of stabilization across all late-life life-history characters, there are alternative theories that have targeted late-life mortality. The lifelong heterogeneity theory (Vaupel et al. 1979) suggests that individual-level variation in demographic mortality patterns can alone produce mortality plateaus. Mueller et al. (2011) conclude that existing evidence does not support lifelong heterogeneity as the only cause of mortality plateaus (although see Chen et al. 2013). A variety of other theories have been proposed that typically possess only the ability to mimic patterns of mortality, featuring little other supporting evidence. These include Markov mortality models (Weitz and Fraser 2001), reliability theories (Gavrilov and Gavrilova 2001), evolutionary string models (Pletcher and Neuhauser 2000), optimality models (Abrams and Ludwig 1995), directionality theory (Demetrius 2001), and network theory (Vural et al. 2014). As pointed out by Steinsaltz and Evans (2004), the ability of such models to mimic mortality patterns cannot be considered strong support, because so many models possess this capability. In addition, those models do not naturally lead to predictions for fecundity and virility plateaus, unlike Hamiltonian evolutionary theories (cf. Rauser et al. 2006; Shahrestani et al. 2012b). However, we do not doubt the ability of proficient theorists to invent such model variations post hoc. Our point is instead that the Hamiltonian theory of late life proposed by Mueller and Rose (1996) both leads to and necessitates such general late-life stabilization among life-history characters, and is consistent with the observation that aging and late life are physiologically distinct.

Finally, we suggest that the present study provides support for the evolutionary approach to the study of aging, life history, and the life cycle generally, as opposed to the nonevolutionary approaches characteristic of much research on aging. Evolutionary theory can readily explain the existence of aging and late life (e.g., Hamilton 1966; Mueller and Rose 1996; Charlesworth 2001), experimental evolution can readily reshape both of them (e.g., Luckinbill et al. 1984; Rose et al. 2002; Rauser et al. 2006), and evolutionary physiology can parse their functional consequences (e.g., Shahrestani et al. 2012a; the present study). Once again, little about the transition from aging to late life makes sense except in the light of evolution (cf. Dobzhansky 1973).

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DATA ARCHIVING

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Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher's website:

Table S1. The Akaike Information Criterion (*AIC*, equation (7)) for two models: linear and two-stage linear (equation 6).
Table S2. The two-stage Gompertz parameters and longevity in yeasted (Y) and not yeasted (NY) food.
Figure S1. Starvation resistance measured in vials for CO females (circles) and CO males (diamonds).