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Patterns of male fitness conform to predictions of evolutionary models of late life

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Abstract

We studied lifetime male virility, a male fitness component, in five populations of *Drosophila melanogaster*. Virility was measured as the number of females, of eight total, that a male could fertilize in 24 h. Individual males were measured at weekly intervals until they died. Virility declined in an approximately linear fashion for the first 3 weeks of adult life. It then stayed low but relatively constant for another 3 weeks, exhibiting a clear plateau. These observations are consistent with the evolutionary theories of late life. The results were not consistent with a simple heterogeneity theory of late life. This is the first demonstration of a late-life plateau for a male fitness component. We also found that the virility of males that were within 7 days of death was significantly lower than that of similarly aged males that were not about to die. This rapid deterioration of virility prior to death, or death spiral, is similar to a decline in fecundity that we had previously documented.

Introduction

The life cycle of sexually reproducing organisms can be divided into three main periods: development, ageing and late life (reviewed in Rose *et al.*, 2002; Shahrestani *et al.*, 2009; Mueller *et al.*, 2011). The developmental period occurs at ages before the first age of reproduction and is expected to experience the strongest selection for high survival. After the first age of reproduction, the ageing phase begins and the strength of selection decays exponentially with age (Hamilton, 1966; Charlesworth, 1994; Partridge & Gems, 2002; Hughes & Reynolds, 2005). Late life characterizes advanced ages at which selection is so weak that the dynamics of alleles affecting traits at these ages is primarily determined by drift (Mueller *et al.*, 2011).

The observations of plateaus in mortality (Carey *et al.*, 1992; Curtsinger *et al.*, 1992) were at first viewed as somewhat anomalous but most likely due to lifelong heterogeneity in demographic rates (Vaupel *et al.*, 1979).

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A number of studies have produced evidence against the lifelong heterogeneity theories of mortality plateaus (Carey *et al.*, 1995; Khazaeli *et al.*, 1995; Drapeau *et al.*, 2000; Carey, 2003; Mueller *et al.*, 2003, 2011), although others have evidence that appears to be consistent with lifelong heterogeneity (Miyo & Charlesworth, 2004; Steinsaltz, 2005). Thus, there is still support for the lifelong heterogeneity theory (Curtsinger *et al.*, 2005).

Mueller & Rose (1996) suggested that this pattern of mortality is in fact expected from simple models of natural selection and drift. Theories based on different models of natural selection have produced similar results (Moorad & Promislow, 2011). An important corollary of this theory was that other fitness-related traits should show late-life plateaus also (Mueller *et al.*, 2011). Such predictions did not follow from heterogeneity theories. The evolutionary theory of late life can explain the existence of late-life plateaus in mortality and of late-life plateaus in fecundity (Rauser *et al.*, 2005; Mueller *et al.*, 2007; Reynolds *et al.*, 2007). A corollary of this theory is that all fitness components should show late-life plateaus. In particular, Mueller *et al.* (2011) show how female fecundity can evolve late-life plateaus. Those simulations could apply to males by simply recasting the

numerical scores for female fecundity as a measure of male fertility. As a test of that theory, the research described here will examine a male fitness component to see whether it also shows a late-life plateau.

The evolutionary predictions for age-specific male virility, V_t, can be simply modelled, as Rauser *et al.* (2006) have carried out for female fecundity, as a two-stage linear function:

$$V_t = \begin{cases} v - \beta t & \text{if } t \leq bd \\ v - \beta bd & \text{if } t > bd \end{cases} \quad (1)$$

At ages less than *bd*, virility decreases at a rate, β ($\beta > 0$), reflecting the declining force of natural selection (Hamilton, 1966; Charlesworth, 1994). The *y*-intercept, *v*, is proportional to virility at young ages. Late life begins at age *bd*, called the breakday. At this age, the forces of natural selection acting on age-specific virility are expected to be no stronger than genetic drift, giving rise to a plateau equal to v-βbd (Mueller & Rose, 1996; Mueller *et al.*, 2011).

It may be possible that male virility reaches a plateau in late life due to population heterogeneity in a manner similar to the mechanisms proposed for late-life mortality plateaus (Vaupel *et al.*, 1979). If males vary in virility and those with low virility when they are young also have low virility in late life but live longer than males with high virility, then a plateau may simply reflect the removal of these high-virility males from the population. Our experiments should also allow us to test this possibility.

We discovered a fourth life cycle phenomenon in a large-scale study of age-specific patterns of female fecundity in *Drosophila*, which we call the 'death spiral' (Rauser *et al.*, 2005; Mueller *et al.*, 2007). For a period of 6–15 days prior to death, the fecundity of females that are about to die drops at a much faster rate than the fecundity of similarly aged females that are not about to die. This result was found by comparing the slopes of the line describing fecundity vs. age as a function of the prospect of death for individual females. This decline in fecundity shortly before death was in turn incorporated into models that accurately describe the age-specific fecundity of *D. melanogaster* (Mueller *et al.*, 2007) and into models that accurately predict the death of individual female *D. melanogaster* using fecundity estimates (Mueller *et al.*, 2009). The death spiral has also been independently documented in *D. melanogaster* by other laboratories (Rogina *et al.*, 2007). The death spiral is detectable across all adult ages and may signal a very general decline in physiological health prior to death.

In this study, we followed lifetime male virility for five populations of *D. melanogaster*. We asked whether male virility reaches a plateau in late life, as is predicted from the evolutionary theory of late life. A second goal of this research is to determine whether male virility exhibits a death spiral phenomenon.

Materials and methods

Populations

Two hundred male *D. melanogaster* from each of five replicate populations were tested for daily mortality and weekly virility throughout their lifespan. The replicate experiments were staggered in time between January and December of 2010.

For the mating assays, we used virgin female *D. melanogaster* from the IV population (Ives, 1975). The IV population is a single population kept on a 2-week generation with a breeding population of about 1000–1500 adults. The IV has been maintained contemporaneously with the five B populations described next. The *D. melanogaster* males used in this study came from five replicate baseline populations ($B_{1\text{-}5}$) derived from the IV population (Rose, 1984). The IV and $B_{1\text{-}5}$ populations are replicates; they have been isolated from each other since 1980 (Rose, 1984), but all six populations are maintained under identical conditions on 14-day generation cycles, at densities of 60–80 flies per eight-dram vials with 5 mL of banana-molasses food per vial, at 25 °C and 24-h light.

Male mortality

At 14 days of age from egg, the B flies from each of the five replicate populations were separated into 200 numbered eight-dram glass vials; each vial contained one male and one female from the same replicate population. For each replicate population, 40 vials each containing 10 backup female partners and two males were also collected, and flies in these vials were transferred into new food every other day. Individual mortality data on the 200 males from each population were recorded daily; if the male's partner was dead, she was replaced with a backup female of the same age. Each male and his partner were transferred into a fresh vial with new banana food every day. Brief carbon dioxide exposure was used to anaesthetize the flies during the vial transfers to eliminate the possibility of flies escaping during transfers.

Virgin collections

We used young (5–8 days old as adults) virgins from the IV population in mating assays to eliminate the confounding effects of population, age and previous female mating on virility measurements. IV eggs were collected from the stock population once a week at densities of 60–80 eggs per eight-dram vial. Approximately 9 days after each egg collection, at 4-h intervals, all emerging adult IV females were removed from vials using carbon dioxide anaesthesia and isolated individually into clear plastic, $3^{7/8}$-inch straws with standard banana-molasses food at one side and capped at both ends with pipette tips.

Male virility assay

Virility assays were conducted once a week for 6 weeks. After 6 weeks, the sample sizes became too small to obtain reasonable estimates of virility in both groups of spiral and nonspiral males. Each male was placed in an eight-dram vial containing 5 mL of banana food with eight virgin IV females for 24 h. After the mating period, each B male was returned to a vial containing his female B partner. The eight IV mated females were isolated individually into clear plastic, $7^{3/4}$-inch straws containing banana food with a layer of yeast paste (added to stimulate oviposition) on one side and capped at both ends with pipette tips. The mated IV females were given 4 days to lay eggs in the straws, and on the fourth day, each straw was examined for the presence of larvae, which indicated that the female was successfully inseminated by the male. Male virility was defined as the number of females (of eight possible females) that the male had inseminated successfully in 24 h of mating. In the course of this experiment, 20 376 females were used in these virility assays.

Data analysis

In this experiment, individuals and populations are expected to vary. For individuals, this variation may be due to genetic or environmental differences. For populations, the major source of variation is due to random genetic drift. Individual males are also measured several times, and thus, they represent sampling units that may produce correlated virility values. These data are best analysed by nonlinear mixed effect models (Pinheiro & Bates, 2000). Although the basic model of virility (eqn 1) appears to be linear, fitting the two stages simultaneously requires nonlinear methods.

Let the index i indicate one of the five populations, j be one of the 200 individuals, k specify group membership (nonspiral ($k = 1$), spiral ($k = 2$)), and t be the male age from egg ($t = 16, 23, 30, 37, 44, 51$). Males that died within 7 days of a virility assay were placed in the spiral group; all other males were placed in the nonspiral group. Then, the virility of male j in population i, group k and with age t is V_{ijkt}. The basic nonlinear model is given by the following equation:

$$V_{ijkt} = f(\boldsymbol{B}_{ijk}, t) + \varepsilon_{ijkt} \tag{2}$$

where $\boldsymbol{B}_{ijk}$ is the vector of parameters (v_{ijk}, β_{ijk}, bd_{ijk}) from eqn 4 and ε_{ijkt} is the within-population variation.

We examined a two-stage linear model. For this model, the functional relationship is the following:

$$f(\boldsymbol{B}_{ijk}, t) = \begin{cases} v_{ijk} - \beta_{ijk} t & \text{if } t \le bd_{ijk} \\ v_{ijk} - \beta_{ijk} bd_{ijk} & \text{if } t > bd_{ijk} \end{cases} \tag{3}$$

In this model, there is a linear decline in virility until age bd_{ijk}, when the virility becomes constant. In line with similar models we have studied for mortality (Rose *et al.*, 2002) and fecundity (Rauser *et al.*, 2006), we call the age bd_{ijk} the virility breakday. We assume that there are both fixed effects and random effects on the values of the model parameters. The fixed effects can be varied to determine whether the membership in the death spiral group has a significant effect. The parameters are also assumed to vary randomly between populations due to founder/drift types of effects and between individuals who are measured multiple times prior to their death. These assumptions translate into the system of equations:

$$v_{ijk} = \alpha_1 + \gamma_1 \delta_k + b_i + c_{ij} \tag{4a}$$

$$\beta_{ijk} = \alpha_2 + \gamma_2 \delta_k \tag{4b}$$

$$bd_{ijk} = \alpha_3 + \gamma_3 \delta_k \tag{4c}$$

where b_i is variation due to population and c_{ij} is variation due to individuals that are nested within populations. The y-intercept (v), slope (β) and breakday (bd) have a main effect, α, and an effect due to membership in the death spiral group, γ. The parameter δ_k is an indicator random variable such that it equals one when $k = 2$ and is zero otherwise. This model allows random variation in only the v_{ijk} parameter. In fact, we tested different models with variation in either v_{ijk}, β_{ijk} or bd_{ijk}. The model with variation in v_{ijk} (eqn 4a) had the highest likelihood and lowest Akaike and Bayesian information indices. Models with variation in more than one parameter failed to converge.

The mean number of females fertilized decreases substantially with age in some of these populations, and thus, it is likely that the within-population variance, ε_{ijkt} (eqn 2), will also decrease. Accordingly, we assumed that the within-population variance was a function of mean virility. The general formulation for the within-population variance was the following: $\mathrm{Var}(\varepsilon_{ijkt}) = \sigma^2 |\widehat{\mu}_{ijkt}|^{\omega}$ where $\widehat{\mu}_{ijkt} = E(V_{ijkt}|b_i, c_{ij})$ (Pinheiro & Bates, 2000, pg. 210). The parameter ω is estimated from the data. The random factors b_i and c_{ij} are assumed to have a normal distribution with mean zero and variance σ_b^2 and σ_c^2, respectively. The estimated values of these variances are given in Table 1. The plateau height of the nonspiral males is α_1-$\alpha_2\alpha_3$, for example, let $\delta_2 = 0$ in eqn 4 and plug this into eqn 3. The variance of the plateau height can be approximated by a Taylor series expansion (Mood *et al.*, 1974, pg. 181), which yields, $\mathrm{var}(\alpha_1) + \alpha_3^2 \mathrm{var}(\alpha_2) + \alpha_2^2 \mathrm{var}(\alpha_3) + 2\alpha_3 \mathrm{cov}(\alpha_1\alpha_2) - 2\alpha_2 \mathrm{cov}(\alpha_1\alpha_3) + 2\alpha_2\alpha_3 \mathrm{cov}(\alpha_2\alpha_3)$. We will use this result to determine whether the plateau height is significantly greater than zero. The estimates of the parameters in eqn 4 were made with the *nlme* function in R version 2.13.1 (R Development Core Team 2010). The *nlme* function provides maximum likelihood parameter estimates and uses a variant of a Gauss–Newton algorithm to numerically find the maximum (Pinheiro & Bates, 2000, pg. 324–328).

Table 1. The results of the nonlinear mixed effects model and estimated parameters from eqn 4

Parameter	Value	SE	d.f.	*t*-value	*P*-value
Intercept (v)					
Nonspiral males (α_1)	11.5	0.285	1844	40.3	0.0000
Spiral male effect (γ_1)	−1.66	0.611	1844	−2.72	0.0066
Slope (β)					
Nonspiral males (α_2)	0.299	0.00942	1844	−31.8	0.0000
Spiral male effect (γ_2)	−0.0184	0.0226	1844	0.814	0.416
Breakday (bd)					
Nonspiral males (α_3)	35.4	0.455	1844	77.8	0.0000
Spiral male effect (γ_3)	−1.91	0.750	1844	−2.55	0.0109

$\sigma_b^2 = 0.143$, $\sigma_c^2 = 0.249$, $\sigma^2 = 1.46$, $\omega = 0.376$.

Results

At the time of each weekly virility assay, males were classified as either death spiral males, if they died in the next week, or nonspiral males, if they did not die. Males in the death spiral have lower intercepts (v) and earlier breakdays (bd) than males that are not in the death spiral (Table 1). Males in the death spiral at young ages fertilize roughly 1–2 fewer females than nonspiral males. The virility plateau is also reached at a significantly earlier age for death spiral males (Table 1). Before the breakday, virility declines at the same rate in both death spiral and nonspiral males (Table 1), and after the breakday, virility plateaus at lower values in death spiral males compared to nonspiral males (Fig. 1). The plateau in male virility is 3 weeks long and is at least as long as the ageing-phase decline in male virility. Although the plateau height for the nonspiral males is low, 0.88, it is significantly greater than zero (± 95 confidence interval, ± 0.36).

An alternative lifelong heterogeneity interpretation of the plateau in Fig. 1 can be constructed and tested with these data. Suppose that males showed a correlation between virility and longevity such that males with low, lifelong virility also happened to live longer. Then in the present experiment, as males age, the high-virility males are lost, eventually leaving a homogenous group of males with low virility giving rise to a plateau. If this theory is correct, then we would expect virility early in life to predict longevity or the chance of surviving to late life.

We tested this idea by splitting the initial sample of all males into two groups: those that survived to week 4 of adult life, the start of the virility plateau, and a second group of males that did not survive to the fourth week. We then estimated the mean virility in week 1 for each group and tested whether the group surviving to week 4 had significantly lower virility than the nonsurviving group. In fact, the males surviving to the fourth week had greater virility (6.9 vs. 6.5). The null hypothesis that the males surviving to the fourth week had the same or greater virility in week 1 than males dying before week 4 was not rejected (*t*-test, $P = 0.9991$).

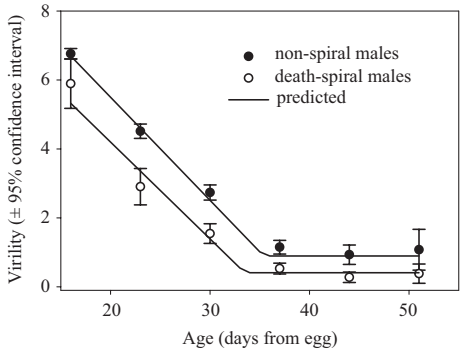

Fig. 1 Male Virility. The mean virility of males 1 week or less prior to death (death spiral males) and males more than 1 week from their death (nonspiral males). The lines are the predictions from eqn 3 and the parameter estimates in Table 1. The sample sizes at the six ages, 16, 23, 30, 37, 44 and 51 days, for the nonspiral males were 627, 538, 386, 207, 104 and 39, respectively. For the spiral males, the samples sizes were 56, 95, 145, 181, 101 and 68.

Discussion

Previous studies have shown that male virility declines with advancing age (Economos *et al.*, 1979; Kosuda, 1985; Borash *et al.*, 2007), but these studies were of insufficient size to determine whether there were virility plateaus. To measure virility, we used methods similar to those used by Borash *et al.* (2007) except that instead of using an 8-h mating period, we used a 24-h mating period. Although there are many ways to measure male reproduction in flies (e.g. Wu, 1983; Kosuda, 1985; Partridge *et al.*, 1985; Hughes, 1995; Borash *et al.*, 2007; Sepulveda *et al.*, 2008), the method that we used is appropriate for this study because it is related to successful reproduction, for example, the production of offspring, not merely mating ability, and thus fitness. Therefore, the evolutionary theory for age-specific male virility, which is based on age-specific fitness, should be relevant. Like previous work on male virility, we found that virility declines with age. However, our results show that at late adult ages, virility stops declining. The plateau in male virility at late ages is consistent with the evolutionary theories of late life. Together with the observations of reproductive plateaus in female *D. melanogaster* (e.g. Rauser *et al.*, 2003), they provide a strong and consistent corroboration of these evolutionary theories.

While our results do not directly contradict the lifelong heterogeneity theories of late-life mortality plateaus, they show how the evolutionary theory of late life provides a comprehensive understanding of patterns of late-life fitness components, which is not possible with heterogeneity theories. However, we have shown that these results are inconsistent with one possible heterogeneity theory of male virility. We should add that the two theories of late life are not mutually exclusive.

However, to date, it appears that lifelong heterogeneity can only explain a small part of the patterns of late-life mortality plateaus (Mueller *et al.*, 2011) and has been unsuccessful in explaining either fecundity plateaus (Rauser *et al.*, 2005) or virility plateaus.

In addition to showing reproductive plateaus, studies of female fecundity in *D. melanogaster* have also shown that fecundity declines at a faster rate for females that are within 6–15 days of death compared to the rest of the population (Rauser *et al.*, 2006). This fecundity decline prior to death has been called the 'fecundity death spiral' (Mueller *et al.*, 2009). The phenomenon has been replicated by an independent laboratory (Rogina *et al.*, 2007) where it was called the 'terminal phase'. Our results of male virility demonstrate that males also exhibit a reproductive death spiral; males that were within 7 days of death had significantly lower virility estimates at all ages compared to males that were not near death.

A study on *D. melanogaster* male virility by Economos *et al.* (1979) also showed signs of plateaus in virility at late ages as well as virility death spirals, but the sample sizes were too small to make strong inferences. Specifically, the percentage of males that were fertile and the number of viable offspring produced from individual males became minimal by 12–15 weeks of age, which was several weeks preceding the death of these males. Also, length of life was positively correlated with week of last mating and week of last fertile mating. Economos *et al.* (1979) suggested the possibility of using the correlation between measures of mating ability of individual male flies and their length of life as a predictor for time of death of these individuals.

We have already described methods for using measures of female fecundity to predict death of individual female flies (Mueller *et al.*, 2009). Such predictions allow us to dichotomize the population at any age into death spiral and nonspiral groups, and we can thus study physiological and other changes that happen during the dying process. Given the results of this study, it is likely that male virility can also be used as a predictor of death, allowing for the study of the dying process to extend to males as well.

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