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Demographic heterogeneity explains age-specific patterns of genetic variance in mortality rates

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Abstract

The genetic variance of mortality rates in *Drosophila melanogaster* increases with age early in life, but declines at intermediate ages. A simulation study was done in order to evaluate two competing explanations for this age-specific pattern: (1) demographic heterogeneity, and (2) binomial sampling effects. The pattern can be explained by demographic heterogeneity among and within genotypes. In contrast, binomial sampling variance is not sufficient to explain this age-specific pattern of genetic variance in mortality rates. A previous publication that rejected the 'heterogeneity explanation' in favor of binomial sampling is shown to be mistaken.

Keywords: Mortality rate variance; Gompertz function; Heterogeneity theory; *Drosophila melanogaster*; Genetic variance; Binomial sampling; Ageing

1. Introduction

Mueller et al. (2003) would have us believe that 'heterogeneity theory' is dead and buried as an explanation for mortality patterns. They argue that my earlier findings (Service, 2000a) "are largely a consequence of sampling variation in estimated mortality rates or the effects of artificially truncated distributions on the estimates of variance" (Mueller et al., 2003, p. 379). Hence, they conclude that my results "have little to do with the heterogeneity model" (Mueller et al., 2003, p. 375). My simulations (Service, 2000a, Fig. 3b) were undertaken to demonstrate that a decline in genetic variance of $log(\mu(x))$ at 'intermediate' ages in *Drosophila melanogaster* (Promislow et al., 1996; Pletcher et al., 1998) could be explained as a result of within-genotype demographic heterogeneity (and the effects of using a logarithmic scale for mortality rates). My findings were corroborated by a more general mathematical analysis (Pletcher and Curtsinger, 2000).

In this short paper, (1) I will show that Mueller et al. are mistaken in their conclusion that binomial (or mixed binomial) sampling can explain age-specific patterns of among-genotype ('genetic') variation in mortality rates, given the cohort sizes and parameter values that they considered; (2) I will reproduce the probable analysis from which Mueller et al.'s mistaken conclusions are drawn and show how their analysis is wrong; and (3) I will present the results of a simulation that Mueller et al. should have done in order to properly test my earlier conclusions (Service, 2000a).

2. Methods

Both my earlier analysis (Service, 2000a) and its 'refutation' by Mueller et al. are based on simulations, and I will use the same simulation procedure here. The simulation model was designed to mimic an experiment which used 25 different genotypes (second-chromosome lines) of *D. melanogaster*. Each line consisted of a large number of genetically identical (for the second chromosome) individuals (Promislow et al., 1996). In my earlier paper (Service, 2000a), I referred to the among-line variation in mortality rates as genetic variation. That usage will be continued here, except that it is also understood that the among-line variation in mortality rates contains a component due to the stochasticity of mortality within lines. This latter source of variation is binomial (or mixed binomial) sampling (Mueller et al., 2003).

All simulations are based on Gompertz mortality functions, and each individual ages according to its own

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Gompertz function. If all individuals in a given line have the same Gompertz intercept and slope parameters, A and b, then there is no within-line ('environmental') demographic heterogeneity. If all lines have the same mean values of the Gompertz parameters, then there is no among-line (genetic) demographic heterogeneity. Four situations are simulated: (1) no demographic heterogeneity either within or among lines, (2) only within-line heterogeneity, (3) both within- and among-line heterogeneity, and (4) only among-line heterogeneity. Situations 1 and 2 are intended to be equivalent to the analyses presented by Mueller et al. in their Figs. 5a and d, respectively. Fig. 5f of Mueller et al. purportedly shows the results of modeling situation 3. Situation 3 is also the one described by Fig. 3b of Service, 2000a except that here the results will be extended to older ages. Situation 4 demonstrates the effect of removing within-genotype demographic heterogeneity when there is among-genotype heterogeneity. Mueller et al. did not do the latter simulation although it is critical to their argument.

Following the earlier papers (Service, 2000a; Mueller et al., 2003), each simulation included 24 cohorts (lines), and each cohort comprised 1250 individuals. There were 10 replicate simulations for each analysis. For the Gompertz intercept parameter, the overall mean of $\ln(A)$ was -8.57, and the overall mean of the slope parameter, b, was 0.193 in all simulations. In simulations with demographic heterogeneity, the variance of $\ln(A)$ was 0.766, and the variance of b was 0.0021. These values are based on published estimates of the means and genetic variances of Gompertz parameters in *D. melanogaster* (Promislow et al., 1996). The same variances were used to model both within-line (environmental) and among-line (genetic) heterogeneity. It is important to remember that in simulations with among-line heterogeneity, the mean values of $\ln(A)$ and b for any given line were not the overall means noted above. A was lognormally distributed and b was gamma distributed. For each of the 24 cohorts in a simulation, $\ln(\mu(x))$ was estimated at each age, x, and the variance of $\ln(\mu(x))$ was calculated across the cohorts. Then, the average values of the variance of $\ln(\mu(x))$ at each age were calculated across the 10 replicate simulations. The units of age are days. The remaining details of the simulation procedure can be found in Service, 2000a.

Mueller et al. argued that my earlier results depended in an important way on my decisions (1) to estimate mortality only at ages when >10 individuals remained alive in a cohort, and (2) to calculate among-cohort (genetic) variance in mortality rates only through ages for which there were survivors in all 24 cohorts of a simulation. In order to address that criticism, the results presented here are not truncated at all in the simulations: they are truncated in just two cases in the figures for the benefit of plotting all results on the same x-axis scale. For direct comparison of the present simulations with those of Mueller et al., all results are shown in terms of $\ln(\mu(x))$ rather than $\log_{10}(\mu(x))$ (Service, 2000a).

3. Results and discussion

3.1. Patterns of among-line variance in $\ln(\mu(x))$

All simulations resulted in superficially similar age-specific patterns of among-line variation in $ln(\mu(x))$ (Fig. 1). The patterns are roughly bimodal with a younger-age peak at 8–28 days of age, and a more variable and less well-defined older-age 'peak' or plateau. In the following discussion, I will refer to the ages between the two modes as intermediate ages. In simulations with no demographic heterogeneity (Fig. 1(a)), or only within-line heterogeneity (Fig. 1(b)), the among-line variation in $\ln(\mu(x))$ is due to binomial sampling effects (Mueller et al., 2003). Among-line (genetic) demographic heterogeneity (Figs. 1(c, e)) has large effects compared to simulations with only within-line (environmental) heterogeneity. In particular, the variance of $\ln(\mu(x))$ is increased at all ages. For example, the younger-age peaks are 3.4–6 times higher when there is among-line demographic heterogeneity than when there is not. This seems reasonable—the addition of an among-line parametric component of variance to the stochastic component represented by binomial sampling is expected to increase the overall variance.

3.1.1. Lack of agreement between the present results and those of Mueller et al.

Regarding the comparisons between simulations with and without among-line demographic heterogeneity, these results *do not* agree with those of Mueller et al. Their simulations showed no increase in the variance of $\ln(\mu(x))$ among lines due to the presence of among-line demographic heterogeneity. Taken at face value, Mueller et al.'s Fig. 5 appears to show that the combination of within-line and among-line demographic heterogeneity (their Fig. 5f) results in essentially the same, or less, among-line variance in $\ln(\mu(x))$ than when there is only within-line heterogeneity (their Fig. 5d).

This comparison between simulations that incorporate among-line demographic heterogeneity and those that do not is crucial. Mueller et al.'s conclusion that my earlier results (Service, 2000a) could be explained by sampling variation is based upon their mistaken belief that the age-specific pattern of variance shown their Fig. 5f is the same as the pattern shown in Fig. 3b of my earlier paper, and its equivalent in this paper, Fig. 1(c). ("In Fig. 5f we have reproduced the simulations of Service by sampling 24 computer-generated genetically variable lines. The results prior to day 40 closely resemble Service's results" (Mueller et al., 2003, p. 379)). In fact, the younger-age peak in Fig. 1(c) is about four times higher than the corresponding peak in Mueller at al.'s Fig. 5f.

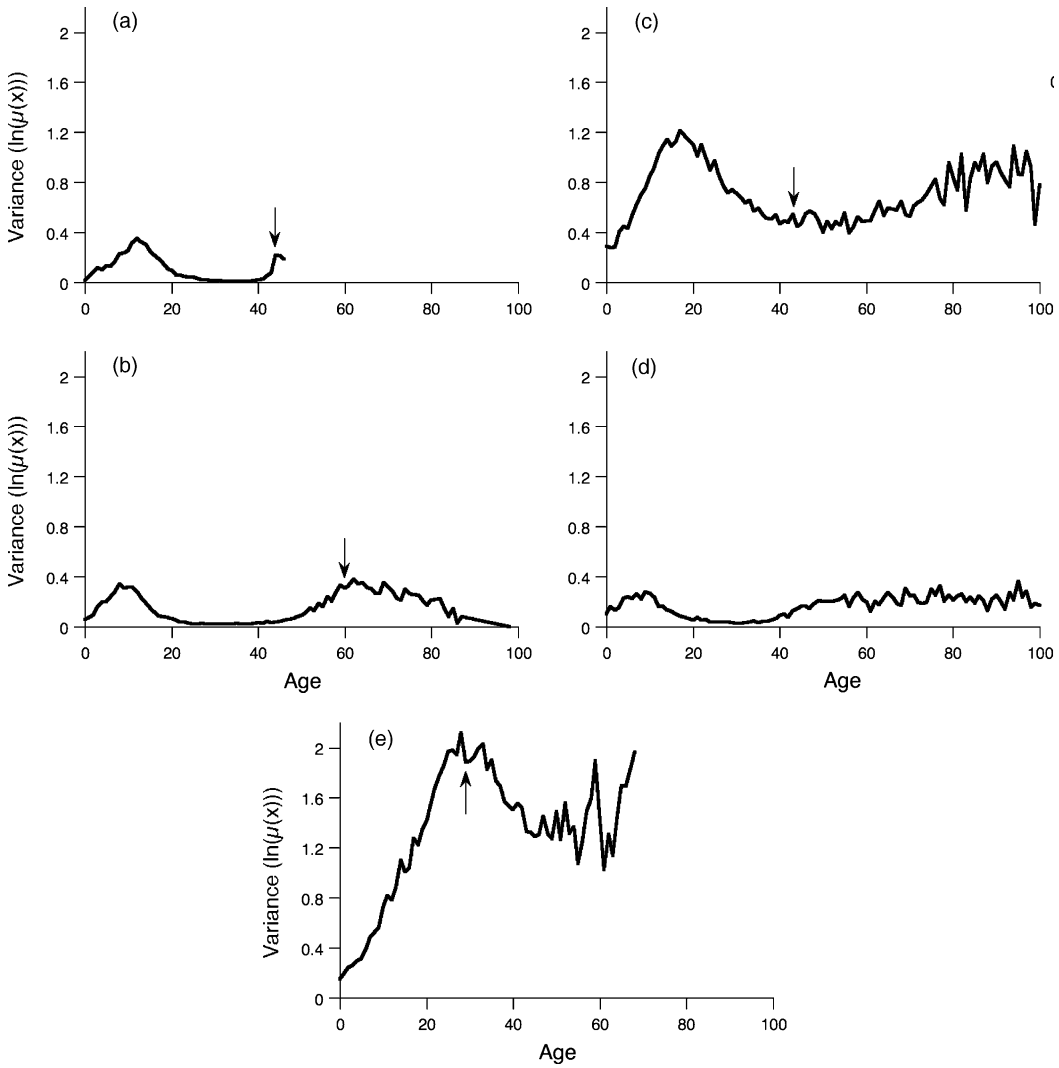

Fig. 1. Age-specific patterns of among-line variance in $\ln(\mu(x))$ under different combinations of within-line ('environmental') and among-line ('genetic') heterogeneity in Gompertz parameters: (a) no heterogeneity; (b) only within-line heterogeneity; (c) within- and among-line heterogeneity; (d) only within-line heterogeneity, but averaged over simulations with different Gompertz parameters; (e) only among-line heterogeneity. Arrows indicate the average oldest age, over 10 replicate simulations, for which all 24 cohorts in a simulation could be used for calculation of the variance of $\ln(\mu(x))$. Data are truncated in (c) and (d) for the benefit of plotting all graphs on a common x-axis.

3.1.2. The probable mistake in Mueller et al.'s simulations with among-line demographic heterogeneity

Contrary to what they seem to claim, I believe that Mueller et al.'s Fig. 5f is not a representation of among-line (genetic) variance in $\ln(\mu(x))$ when there is demographic heterogeneity both within and among lines ('genotypes') in the same simulation. To support my argument, I will reconstruct the methods by which Mueller et al. probably obtained the results shown in their Fig. 5f. Imagine that a single pair of Gompertz intercept and slope parameters is randomly chosen from the distributions described in the Methods section. Then simulate 10 heterogeneous cohorts of size 1250 using that pair of parameters. That is, all 10 cohorts are simulated with the same mean values of $\ln(A)$ and b. Finally, calculate the variance of $\ln(\mu(x))$ among the 10 cohorts. The resulting curve will look like one of the curves in Mueller et al.'s Fig. 5e (and like the curve shown in Fig. 1(b)). Note that this simulation of 10 cohorts (genotypes) does not include among-line heterogeneity (genetic variation) in Gompertz parameters. Repeat the process just described 23 more times, randomly sampling a new pair of Gompertz parameters each time. The result will be 24 curves similar to the three in Muller et al.'s Fig. 5e and arrayed along the x-axis. Finally, find the average value of the variance of $\ln(\mu(x))$ across the 24 curves at each value of x. Note that nowhere in this procedure have we calculated the variance of $\ln(\mu(x))$ among lines (genotypes) that have different Gompertz parameters. That is, our variance calculations do not include any contribution due to genetic variation of mortality parameters. The results of this procedure are shown in Fig. 1(d). There is good agreement between these results and those shown in Fig. 5f of Mueller

et al., at least up until about age 60. The height of the younger-age peak is about 0.25–0.28, and it occurs between about ages 10–15. The variance of $\ln(\mu(x))$ is low at intermediate ages (20–40), then begins rising again, reaching a peak (or plateau) by the late 50's.

In summary, what Mueller et al. appear to have done in their Fig. 5f is to estimate the 'average' contribution of binomial sampling effects to the variance of $\ln(\mu(x))$ in a genetically heterogeneous 'population'. This was done by calculating $\ln(\mu(x))$ across a set of lines that had the same Gompertz parameters, and then averaging over sets (genotypes) with other parameter values. This is not the variance of $\ln(\mu(x))$ among genotypes with different Gompertz parameters, which is what is modeled in Fig. 1(c). This interpretation of Mueller et al.'s procedure is, in fact, supported by their caption for Fig. 5. Fig. 5f, which due to a typographical error in the caption is referred to as 5e, shows the "average variation over 24 genetically different lines…". Note their use of 'average', and the inconsistency with the claim in the text of their paper to have 'reproduced' my earlier simulations. If I have correctly reconstructed the essentials of the procedure used to generate Mueller et al.'s Fig. 5f, then their claim that their results "prior to day 40 closely resemble Service's results" (Mueller et al., 2003, p. 379) is equivalent to claiming that Fig. 1(d) in this paper closely resembles Fig. 1(c). In fact, Mueller et al. never reproduced my original simulation (Service, 2000a, Fig. 3b).

3.2. Within-line (environmental) demographic heterogeneity is necessary for the decline in among-line (genetic) variance of $\ln(\mu(x))$ *at intermediate ages*

Although their Fig. 5f does not 'closely resemble' my earlier results, Mueller et al. might still argue that they were correct in their criticism of my earlier conclusion that environmental demographic heterogeneity causes the intermediate-age decline in genetic variance of $\ln(\mu(x))$ (Service, 2000a). In their argument, as I imagine it, they would attribute the age-specific pattern of changes in variance of $\ln(\mu(x))$ to binomial sampling, and leave no roles for demographic heterogeneity, other than to alter the heights and locations of the peaks. There are compelling reasons to reject such an argument. To understand why the younger-age peak in variance of $\ln(\mu(x))$ and its subsequent decline through intermediate ages (Fig. 1(c)) cannot be due solely or even mainly to changes in sampling variation, I will present a slightly simplified example. Consider the case in which genotypes have different mean values of the Gompertz slope parameter, b, but the same value for the intercept parameter, A. At relatively young ages, before the effects of within-genotype (environmental) heterogeneity become important, the mortality dynamics of each genotype will be essentially Gompertzian. On a logarithmic scale, the mortality trajectories of the several genotypes will be diverging straight lines with positive slopes, and the variance of $\ln(\mu(x))$ must increase with age. This contribution to the variance of $\ln(\mu(x))$ due to genetic variation in b is above and beyond any contribution due to sampling. As genotypes continue to age, however, within-genotype demographic heterogeneity begins to exert it effects, the mortality dynamics become non-Gompertzian, and the divergence of mortality rates among genotypes will be slowed and may be halted or reversed (Service, 2000a). At that point, even if mortality rates continue to increase with age and even if there is no actual convergence of mortality rates (when expressed without logarithmic transformation), it seems likely that the variance of $\ln(\mu(x))$ will decrease with further ageing (Pletcher and Curtsinger, 2000).

The importance of the logarithmic transformation has not been sufficiently appreciated, and it is worth considering briefly. Using the same parameter values as in these simulations, the among-line variance of $\mu(x)$ did not decline at intermediate ages (Service, 2000a, Fig. 3c). In other words, at least with these parameter values, both within-line (environmental) heterogeneity and the log transformation are required in order to produce a decline in among-line (genetic) variance of mortality rates at intermediate ages. It does not seem likely that the log transformation would be required for all parameter sets in order to produce the intermediate-age decline (see Fig. 1c of Service, 2000a, for example). A full consideration of the issue would probably require an analysis similar to that of Pletcher and Curtsinger (2000), but for $\mu(x)$ rather than $\ln(\mu(x))$, and is beyond the scope of this paper. However, a simple example may serve to illustrate the effect of the log transformation on among-line variances. Imagine a series of parallel lines described by the equation $y_i = a_i + bx$ ($b > 0$). The variance of y will be a constant that does not depend on the value of x. However, the variance of $\log(y)$ will decline with increasing x. If the slope parameter is also allowed to vary, so that the variance of y increases with x, it is still mathematically possible that the variance of $\log(y)$ will decrease with increasing x. This is essentially the reason why a logarithmic transformation is often used in statistical analyses when means and variances are positively correlated. Clearly, the common practice of using a logarithmic transformation for $\mu(x)$ can have important consequences on the observed patterns of variance in mortality rates.

The main point that I wish to make, however, is that it is the within-genotype (environmental) demographic heterogeneity that eventually causes the initially Gompertzian mortality trajectories of the genotypes to stop diverging (or to diverge more slowly). This fact is the root cause of the decline in genetic variance of mortality rates after the initial maximum, although the decline may be apparent only when mortality rates are transformed to logarithms. To contradict Mueller et al., I conclude that in fact the younger-age peak shown in Fig. 1(c) (and in Fig. 3b of Service, 2000a) has little, if anything, to do with binomial sampling variance.

3.2.1. The 'missing' simulation

The argument of the preceding section can be supported by simulating the situation in which there is only among-line demographic heterogeneity. Mueller et al. did not perform this simulation, even though it would seem to be necessary to test my conclusion that within-line (environmental) demographic heterogeneity causes the decline in among-line (genetic) variance of $\ln(\mu(x))$ at intermediate ages (Service, 2000a). When there is only among-line heterogeneity, the variance of $\ln(\mu(x))$ continues to increase to a considerably later age, and reaches a maximum value that is almost twice as great as when there is within-line demographic heterogeneity as well (cf. Figs. 1(c) and (e)). Thereafter, it declines to about age 50. The decline cannot be due to within-line heterogeneity, however, because that is not included in the simulation. Rather, the decline is attributable to extinction of the more rapidly ageing cohorts: that is, the surviving lines (of the original 24 in a simulation) are becoming more similar. Averaged across the 10 simulations, cohort extinction began at age 28.9 days, coinciding almost exactly with the younger-age peak in variance of $\ln(\mu(x))$ (Fig. 1(e)). Non-random cohort extinction cannot explain the decline in variance seen at intermediate ages in Fig. 1(c). In that simulation, the average age at which cohort extinction began was 43 days, which was after the decline in variance was almost complete.

3.3. What happened to the long-lived individuals?

It is odd that the simulations of Mueller et al. appear not to have produced long-lived individuals. Variation in the Gompertz slope parameter should result in some individuals having low values of that parameter, and these individuals should live a relatively long time. The effect is clearly demonstrated in the present simulations. An illustration can be seen in the comparison between Fig. 1(a) and (b)—within-line heterogeneity approximately doubles maximum life span from about 50 to almost 100 days. In contrast, the similar comparison in Mueller et al. shows a difference of only about 10–12 days in maximum life span (their Figs. 5a and d). Other comparisons of Mueller et al.'s figures give similarly puzzling results. For example, contrary to expectation, simulations that have within-line variation in the Gompertz slope parameter (their Fig. 5c) result in the same maximum life span as simulations with no variation in mortality parameters (their Fig. 5a). The comparison of Figs. 5e and f of Mueller et al. also suggests that long-lived individuals are missing. One of the simulations shown in Fig. 5e had individuals living to about age 80. Similar simulations, or simulations with even lower values of the Gompertz parameters, were presumably included among the simulations that comprise the results of Fig. 5f. Yet the maximum age shown in Fig. 5f is only about 60 days.

3.4. Points of agreement

I do agree with Mueller et al. that the older-age peaks seen in all simulations are a consequence of sampling variation when there are few surviving individuals. The fact that it is indeed a sampling artifact is reason enough to truncate analyses when only a few individuals remain alive (Service, 2000a)—it tells us nothing biologically interesting about the contribution of demographic heterogeneity to patterns of genetic variance in mortality rates. I also agree with Mueller et al. that when there is no among-line demographic heterogeneity, the younger-age peak in variance is due to sampling effects at ages when relatively few deaths are occurring (Fig. 1(a) and (b)).

3.5. Conclusions

The term 'heterogeneity theory' as used in this paper refers to a set of arguments that attribute observations about cohort mortality rates to among-individual variation in rates of ageing. In addition to explaining age-specific patterns of genetic variance in mortality rates, as in this paper, heterogeneity has been suggested as a general explanation for mortality rate plateaus and for the absence of catastrophic old-age mortality in most organisms (Service, 2000a). Heterogeneity theory does not seek to explain why organisms age. Furthermore, its success in explaining demographic observations does not preclude other explanations for those same observations. For example, it is possible that the decline in genetic variance of $\ln(\mu(x))$ at intermediate ages does reflect some intrinsic biological factor or aspect of natural selection that results in different genotypes having similar mortality rates. Mueller et al. (2003) advocate this latter position, and have attempted to advance it by attacking heterogeneity theory. However, their analysis is flawed.

Rather than refuting heterogeneity theory, what Mueller et al. have actually done is to estimate the contribution of sampling variance to among-cohort variation in mortality rates. In fact, with the cohort sizes and parameter values used, the overall contribution is small (perhaps unimportant) at young and intermediate ages in those cases where there is also genetic variation in mortality parameters among cohorts. In short, sampling effects cannot explain observed age-specific patterns of genetic variance in mortality rates of flies. However, sampling effects might be more important with very small cohorts and in cases where there is little or no among-genotype demographic heterogeneity.

Mueller et al., 2003, p. 382 contend that "Unlike evolutionary theories of demography, the lifelong heterogeneity theories do not rest upon well-established principles of biology." On the contrary, heterogeneity

theories as a class rest upon perhaps the most inescapable of all biological 'facts'—phenotypic variation. Phenotypic variation in rates of ageing does appear to offer a simple, powerful, biologically plausible explanation for age-specific patterns of genetic variance in mortality rates, and for mortality plateaus (Pletcher and Curtsinger, 2000; Service, 2000a). With apologies to Mark Twain, reports of the death of heterogeneity theory are greatly exaggerated, once again (Service, 2000b; de Grey, 2003).

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