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Inbreeding Depression and Male Survivorship in Drosophila: Implications for Senescence Theory

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ABSTRACT

The extent to which inbreeding depression affects longevity and patterns of survivorship is an important issue from several research perspectives, including evolutionary biology, conservation biology, and the genetic analysis of quantitative traits. However, few previous inbreeding depression studies have considered longevity as a focal life-history trait. We maintained laboratory populations of *Drosophila melanogaster* at census population sizes of 2 and 10 male-female pairs for up to 66 generations and performed repeated assays of male survivorship throughout this time period. On average, significant levels of inbreeding depression were observed for median life span and age-specific mortality. For age-specific mortality, the severity of inbreeding depression increased over the life span. We found that a baseline inbreeding load of 0.307 lethal equivalents per gamete affected age-specific mortality, and that this value increased at a rate of 0.046 per day of the life span. With respect to some survivorship parameters, the differentiation of lineages was nonlinear with respect to the inbreeding coefficient, which suggested that nonadditive genetic variation contributed to variation among lineages. These findings provide insights into the genetic basis of longevity as a quantitative trait and have implications regarding the mutation-accumulation evolutionary explanation of senescence.

INBREEDING depression is the loss of fitness that has often been observed within progeny produced by the mating of closely related individuals (Lynch and Walsh 1998). This phenomenon has been a continuing research interest in evolutionary biology, conservation biology, and the genetic analysis of quantitative traits (Lande and Schemske 1985; Charlesworth and Charlesworth 1987, 1990; Charlesworth *et al.* 1990; Frankham 1995; Pusey and Wolf 1996; Spielman *et al.* 2004). In the well-studied model system *Drosophila melanogaster*, inbreeding depression has most often been examined with respect to female reproductive traits or the viability of early life stages. A comparatively small number of studies, in contrast, have examined the inbreeding depression that may occur for adult longevity and patterns of survivorship (see Lynch and Walsh 1998, Chap. 10). The inbreeding depression that occurs for longevity, however, can be of special interest from several research perspectives. For example, highly inbred lineages are commonly used in experimental research on aging (*e.g.*, Giesel and Zettler 1980; Giesel *et al.* 1982; Nuzhidin *et al.* 1997; Khazaeli and Curtsinger 2000). The usefulness of such lineages has been questioned due to the potential for inbreeding depression (Rose and Charlesworth 1981; Johnson and Wood 1982; Rose 1984, 1991; Rose and Service 1985), which in some cases may have been the cause of experimental artifacts (*e.g.*, Wattiaux 1968; Mertz 1975; Tucic *et al.* 1996). The effects of inbreeding on survivorship are also relevant to the debate over evolutionary explanations of senescence (*i.e.*, mutation accumulation *vs.* antagonistic pleiotropy), since the alternative hypotheses make specific predictions regarding how inbreeding will affect survivorship patterns (Charlesworth and Hughes 1996). Finally, since the effects of inbreeding depend on the genetic architecture of phenotypic characters, studies of inbreeding effects on survivorship may provide insights into the genetic basis of longevity as a quantitative trait (Lande 1980; Lynch and Hill 1986; Falconer and Mackay 1996; Lynch and Walsh 1998).

Direct and indirect evidence that inbreeding reduces survivorship has been obtained in a number of studies of Drosophila (Gonzalez 1923; Pearl *et al.* 1923; Clarke and Maynard Smith 1955; Maynard Smith 1959; Gedda and Brenci 1969; Hughes 1995; Pletcher *et al.* 2000; Snoke and Promislow 2003; Vermeulen and Bijlsma 2004), the mouse (Russell 1966; Storer 1966), and humans (Epstein *et al.* 1966; Brown and Wisniewski 1983). The early Drosophila studies were among the first to demonstrate an association between genotype and longevity by showing that lineages fixed for recessive mutations had reduced life spans (Gonzalez 1923; Pearl *et al.* 1923; Gedda and Brenci 1969). Clarke and Maynard Smith (1955) and Maynard Smith (1959) later showed that crosses between inbred

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D. subobscura lines led to a doubling of the average life span, which suggested that inbreeding depression had reduced survivorship in the parental lineages. While these studies suggest that inbreeding depression for survivorship may occur, its magnitude may also be small compared to other life-history traits. In some experiments, for example, inbreeding has been found to have no significant effect on survivorship (*e.g.*, Gowen and Johnson 1946; Buzcuk and Husamoglu 1972; Lints *et al.* 1984). When clear evidence of inbreeding depression has been detected, the manner in which survivorship declined has varied in some cases. The rate of age-specific mortality, for example, is often characterized in terms of age-dependent and age-independent components (*e.g.*, the Gompertz model; see Pletcher *et al.* 2000). Hughes (1995) found that inbreeding primarily elevated the rate of age-dependent mortality (*i.e.*, the Gompertz slope), while Vermeulen and Bijlsma (2004) found that inbreeding primarily elevated the rate of age-independent mortality (*i.e.*, the Gompertz intercept).

The manner in which inbreeding increases age-specific mortality has important implications for evolutionary explanations of senescence. It is widely agreed upon that senescence is ultimately caused by the declining force or efficiency of natural selection throughout the life span (Hamilton 1966). This decline in the force of natural selection over the life span is thought to explain why genes with specific effects at late ages are more likely to be deleterious. It is unclear, however, whether such "aging genes" accumulate with or without the aid of natural selection. Under the mutation-accumulation theory of senescence, aging genes with deleterious effects limited to late ages accumulate without being driven by natural selection (Medawar 1952). Alternatively, under the antagonistic pleiotropy theory of senescence, deleterious aging genes may be selected for because of beneficial pleiotropic effects early in the life span (Williams 1957). These two hypotheses make different predictions regarding how inbreeding should affect age-specific mortality (Tanaka 1993; Charlesworth and Hughes 1996). Under the mutation-accumulation hypothesis, the inbreeding load is inversely related to the sensitivity of fitness to trait values (Charlesworth and Hughes 1996), such that inbreeding depression for survivorship and all other fitness characters is expected to become increasingly severe at advanced ages (when the sensitivity of fitness to trait values declines). Consequently, the mutation-accumulation theory of senescence predicts that inbreeding will entail an increase in the rate of age-dependent mortality (*e.g.*, Hughes 1995). Under the alternative antagonistic pleiotropy explanation of senescence, the inbreeding load is not related to the sensitivity of fitness to trait values (Charlesworth and Hughes 1996), and thus increases in the rate of mortality due to inbreeding are expected to be independent of age.

In this study, we investigated the inbreeding depression that affects male survivorship in *D. melanogaster*. We report results from an experimental study in which inbred lineages were extracted from a laboratory population and repeated assays of male longevity were performed over 66 generations. The first goal of the study was to examine the overall magnitude of inbreeding depression that potentially affects male survivorship. Given significant levels of inbreeding depression, the second goal of the study was to determine the extent to which such inbreeding depression was age dependent. To address this objective, we used a simple model based on the Gompertz parameters to partition the inbreeding load affecting age-specific mortality into age-dependent and age-independent components. Our results demonstrate the effects of inbreeding on male longevity and also test the mutation-accumulation theory of senescence prediction that the severity of inbreeding depression will increase over the life span.

MATERIALS AND METHODS

Experimental populations: Experimental populations used in this study were derived from a base population founded by 30 wild-inseminated females collected in Northwest Ohio between November 2000 and June 2001. In June 2001, this population was expanded to 720 individuals (360 pairs) and then maintained in six bottle cultures throughout the experiment with uniform mixing among bottles each generation. In August of 2001, two sets of 10 experimental lineages were derived from the base population. These two sets are referred to as the 2P and 10P lineages, respectively. The 2P lineages were initiated from 10 random samples of 4 flies (2 male-female pairs) obtained from the base population (generation −1) and were subsequently maintained at a constant population size ($N = 4$) for 44 generations using random samples of 2 pairs per generation as parents. Similarly, the 10P lineages were initiated from 10 random samples of 20 flies (10 male-female pairs) obtained from the base population and were subsequently maintained for 66 generations at a constant size ($N = 20$), using random samples of 10 pairs per generation as parents. All cultures were maintained in a dark incubator at 25° within single bottles containing standard cornmeal-molasses food medium supplemented with live yeast. Coefficients of inbreeding were calculated for lineages on the basis of effective population sizes (N_e) = 3.2 and 14 for the 2P and 10P populations, respectively. These effective population sizes correspond to N_e/N values that have been obtained previously for Drosophila lineages of similar census sizes maintained in single-bottle cultures (Kerr and Wright 1954; Wright and Kerr 1954; Crow and Morton 1955; Buri 1956; Ehiobu *et al.* 1989).

Survivorship assays: The survivorship of unmated male flies from each treatment and from the base population was assayed at several points throughout the experiment. In the 2P treatment, we performed survivorship assays at generations 1, 12, 24, and 44 ($F =$ 0.156, 0.870, 0.983, and 0.999, respectively). In the 10P treatment, we performed survivorship assays at generations 1, 12, 24, 41, 56, and 66 ($F =$ 0.036, 0.354, 0.582, 0.775, 0.870, and 0.909, respectively). In the base population, survivorship was assayed at generations 1, 12, 24, and 44 ($F \approx 0$ in each generation). In the 2P and 10P treatments, we assayed the survivorship of 3 samples of 10 unmated males per population (*i.e.*, 30 males per population), for a total of 300 individuals per treatment at each generation of measurement. In the base population, we assayed the survivorship of 30 samples of 10 unmated males (5 samples from each of the six bottle cultures), for a total of 300 individuals at each generation of measurement. During all survivorship assays, flies were maintained at 25° with dextrose food medium containing water, cornmeal, brewer's yeast, bacto agar, dextrose, 10% lexgard, and 1.5% benzyl benzoate. Dextrose was used in this food medium to reduce the growth of a lactobacillus that produces mucus on the surface of standard food medium, which may trap and kill flies (Ashburner 1989). In all assays, flies were maintained at an initial density of 10 individuals per dextrose vial, and surviving individuals were transferred to new vials weekly (without anesthesia) and counted daily until all had died. Survivorship assays were performed simultaneously on all lineages within a single generation of measurement.

Survivorship parameters: Survivorship parameters included median life span (T_{Med}), maximum life span (T_{Max}), and two model parameters that characterized the rate of age-specific mortality over the life span (α and β). We estimated mortality rate parameters on the basis of the Gompertz mortality model (Gompertz 1825), which allowed the inbreeding load to be partitioned into age-dependent and age-independent components. The Gompertz model assumes that the rate of age-specific mortality increases exponentially over the life span, such that the rate of age-specific mortality at age x [$\mu(x)$] is described by Equation 1:

$$\mu(x) = \alpha e^{\beta x}. \tag{1}$$

In Equation 1, the rate of age-dependent mortality (β) and the rate of age-independent mortality (α) are referred to as the Gompertz slope and intercept parameters, respectively. Both Gompertz parameters were estimated by maximum likelihood using the WinModest software package (Pletcher 1999). All survivorship parameters (T_{Med}, T_{Max}, α, and β) were estimated individually for each lineage at each generation of measurement (on the basis of 30 observations per lineage). With respect to the base population, the 300 observations obtained at generations 1, 12, 24, and 44 were randomly divided into 10 groups of 30 observations each, and individual parameter estimates were obtained for each group. This allowed 10 replicate estimates to be obtained, which provided an estimate of the sampling variance associated with survivorship parameters in the base population.

Haploid lethal equivalents: The inbreeding load was quantified by the estimated number of lethal equivalents per gamete. A lethal equivalent is a group of genes that would cause an average of one death if dispersed in different individuals (Morton *et al.* 1956). The inbreeding load represents the number of lethal equivalents that become expressed as a result of inbreeding and was estimated for both median life span ($L_{T_{\text{Med}}}$) and age-specific mortality [$L_{\mu(x)}$]. For age-specific mortality, the inbreeding load was partitioned into two separate components (L_α and L_β), as shown by Equation 2:

$$L_{\mu(x)} = L_\alpha + L_\beta x. \tag{2}$$

The values of L_α and L_β were estimated on the basis of the inbreeding depression observed for each of the Gompertz mortality parameters (α and β). The total inbreeding load affecting age-specific mortality at an inbreeding coefficient F can be expressed as the logarithm of the ratio of age-specific mortality observed in an inbred cohort [$\mu_{x(\text{I})}$] relative to that of an outbred cohort [$\mu_{x(\text{O})}$] (Lynch and Walsh 1998):

$$L_{\mu(x)} = \frac{1}{F}\ln\left(\frac{\mu_{x(\text{I})}}{\mu_{x(\text{O})}}\right). \tag{3}$$

By substituting $\alpha e^{\beta x}$ (see Equation 1) for $\mu_{x(\text{I})}$ and $\mu_{x(\text{O})}$, and denoting α_I (α_O) and β_I (β_O) as the mortality parameters of inbred and outbred individuals, respectively, the total age-specific mortality load can be expressed as a function of age-independent [$L_\alpha = \ln(\alpha_\text{I}/\alpha_\text{O})/F$] and age-dependent [$L_\beta = (\beta_\text{I} - \beta_\text{O})/F$] components, such that

$$L_{\mu(x)} = \frac{1}{F}\left[\ln\left(\frac{\alpha_\text{I}}{\alpha_\text{O}}\right) + (\beta_\text{I} - \beta_\text{O})x\right]. \tag{4}$$

An important aspect of Equation 4 is that the inbreeding load for age-specific mortality is constant over the life span if $\beta_\text{I} = \beta_\text{O}$, in which case $L_\beta = 0$. A significant estimate of L_β, therefore, provides evidence that the inbreeding depression affecting age-specific mortality increases over the life span.

The inbreeding load for median life span ($L_{T_{\text{Med}}}$) was estimated by regressing log-transformed parameter values onto the expected inbreeding coefficient of experimental populations (Morton *et al.* 1956; Lynch and Walsh 1998). Similarly, the inbreeding load affecting age-independent mortality (L_α) was estimated by the regression of $\ln(\alpha)$ onto the expected inbreeding coefficient. The slope of this regression is an estimator

of $\ln(\alpha_I)/F$, which is equal to L_α given that α_O is a constant with respect to F (see Equation 4). The inbreeding load affecting age-dependent mortality (L_β) was estimated by regressing β-estimates onto the expected inbreeding coefficient. The slope of this regression estimates β_I/F, which is equal to L_β given that β_O is a constant with respect to F (see Equation 4).

All inbreeding load estimates were obtained by weighted least-squares regression analysis (Neter *et al.* 1996, Chap. 10). The appropriate weights for each parameter were obtained directly from the data set by fitting a least-squares regression model of the observed increase in the among-population variance across levels of the inbreeding coefficient. Separate inbreeding load estimates were obtained with respect to the 2P treatment, the 10P treatment, and both experimental treatments pooled together (the same fitted regression model of the among-population variance was applied to generate weights in each case). A Durbin-Watson statistic was calculated for each weighted least-squares regression to test for significant autocorrelation of residuals over different levels of the inbreeding coefficient (Neter *et al.* 1996, p. 504). For cases in which the Durbin-Watson statistic was significant at a level of 0.05, we obtained parameter estimates using a generalized autoregressive conditional heteroscedasticity (GARCH) model (Bollerslev 1986), as implemented in the SAS AUTOREG procedure (SAS Institute 2004). This procedure obtains maximum-likelihood estimates of ordinary regression parameters, but also corrects for autocorrelation by simultaneously modeling changes in error over different levels of the predictor variable. Unlike weighted least-squares estimation, therefore, the GARCH model does not assume that residuals are independent across levels of the inbreeding coefficient.

To account for potential bias that may arise from adaptation to laboratory conditions, we also estimated the inbreeding load by treating the base population as a control. In this approach, we first obtained the mean values of survivorship parameters observed in the base population at generations 1, 12, 24, and 44. The parameter values of experimental populations in each generation were then adjusted by subtracting the mean values observed in the base population at corresponding generations. Since we did not have base population estimates for generations 56 and 66, we coupled generation 41 estimates of the 10P treatment with generation 44 estimates of the base population and omitted generation 56 and 66 estimates from the 10P treatment. Another potential bias that could influence inbreeding load estimates is the accumulation of spontaneous mutations that arise during the process of inbreeding. If the accumulation of spontaneously arising mutations was an important factor influencing inbreeding load estimates, it is expected that generation (or time) would have effects on parameter values independently of the inbreeding coefficient. To determine if this was the case, we examined whether changes in parameter values (adjusted for inbreeding) between measurements were related to the number of generations between measurements. For each experimental lineage, we calculated the difference in parameter values between successive measurements and standardized this value by the increase in inbreeding coefficient (*e.g.*, $\Delta \ln(T_{Med})/\Delta F$). This calculation was performed for all lineages in both experimental treatments and over all intervals between successive measurements (generations 0–1, 1–12, 12–24, and 24–44 in the 2P treatment and generations 0–1, 1–12, 12–24, 24–41, 41–56, and 56–66 in the 10P treatment). To determine whether generation number influenced parameter values independently of the inbreeding coefficient, we tested whether changes in parameter values per 1% increase in F between measurements were significantly correlated with the number of generations for each interval (which varied from 1 to 20).

RESULTS

The base population: Significant differences were found among the survivorship curves corresponding to the samples of 300 males obtained from the base population at generations 1, 12, 24, and 44 (log-rank test; $P < 0.001$). Figure 1A shows the observed survivorship curves corresponding to each sample, while Figure 1B shows age-specific mortality plots based on estimates of the Gompertz slope and intercept. The parameter estimates associated with Figure 1 are listed in Table 1. The general trend was a decline in survivorship with an increasing number of generations (Figure 1A). The regression of parameter values onto generation was significant for both median life span ($\beta = -0.094$; $P = 0.02$) and maximum life span ($\beta = -0.232$; $P < 0.001$). These regression coefficients indicate that median and maximum life span decreased by 1 day approximately every 10 and 5 generations, respectively. This trend was also reflected by an increase in the rate of age-dependent mortality in later generations ($\beta = -0.0434$; $P = 0.005$) (Figure 1B). In contrast, however, there was a marginally significant decrease in the rate of age-independent mortality over generations ($\beta = -0.0137$; $P = 0.077$) (Figure 1B).

Experimental populations: The survivorship curves observed for lineages in the 2P and 10P treatments at each generation of measurement are shown in Figure 2, while age-specific mortality plots based on Gompertz slope and intercept estimates are shown in Figure 3. The parameters and among-population variances associated with Figures 2 and 3 are listed in Table 1. On average, there was some indication of survivorship decline with respect to all parameters in each treatment (Table 1). A regression of parameter values onto F and F^2 provided no significant evidence that this decline was nonlinear with respect to the inbreeding coefficient (*e.g.*, for the 10P data: $\beta_{F^2} = 19.29$; $P = 0.1024$ for maximum life span; $P > 0.49$ for all other parameters). Among all populations, significant correlations were present among survivorship

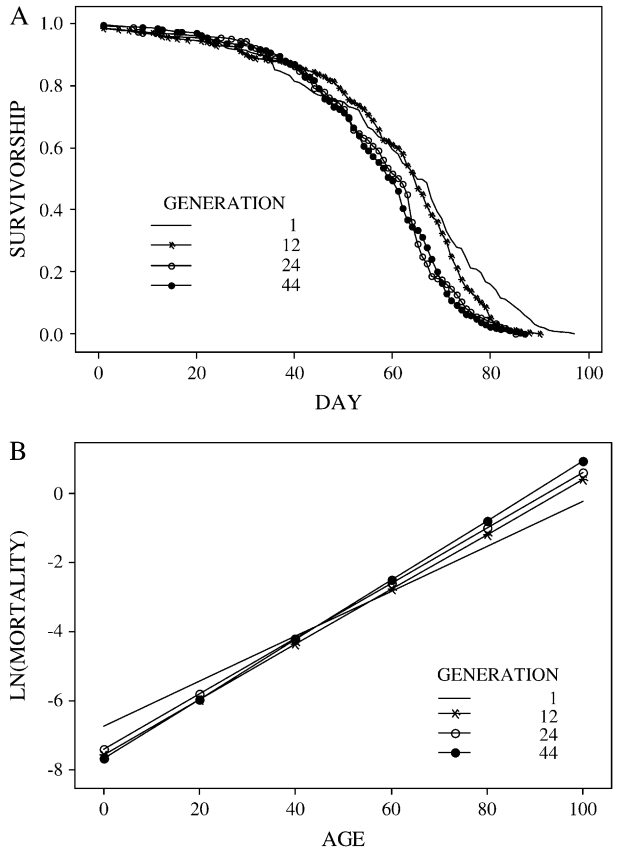

FIGURE 1.—Survivorship curves (A) and age-specific mortality plots (B) based on cohorts sampled from the base population at generations 1, 12, 24, and 44. Associated parameter estimates are listed in Table 1.

parameters (*e.g.*, T_{Med} and T_{Max}, $r = 0.807$; T_{Max} and β, $r = -0.652$; T_{Med} and α, $r = -0.547$; $P < 0.001$ for each).

At generation one, the variation among survival curves was significant in both experimental treatments (log-rank test; $P = 0.001$ and 0.02 for the 2P and 10P lineages, respectively). This variation in survivorship increased over time (see Figure 2), such that the variance of parameter estimates among lineages (V_B) was eventually much greater than that observed among replicate samples obtained from the base population (see Table 1). At generation 44, for example, the value of V_B in the 2P treatment ranged from 10 to 71 times the average sampling variance observed in the base population. As expected, estimates of V_B were slightly less substantial in the 10P treatment. The magnitude of V_B in the 10P treatment peaked at generation 56, when V_B estimates ranged from 7 to 31 times the average sampling variance of the base population. In the final survivorship assay carried out for each treatment, the range of median life span estimates among replicate lineages was considerable (21.5–67.5 days in the 2P treatment, 43–71 days in the 10P treatment).

For maximum life span and the Gompertz slope parameter, there was strong graphical and statistical evidence that the increase in variance among lineages over time was nonlinear with respect to the inbreeding coefficient. This trend was best examined with respect to the 10P treatment, since several (six) repeated measurements were performed on the 10P lineages throughout the experiment. Using data from the 10P treatment, when V_B was regressed onto F and F^2, the coefficient associated with the F^2 term was significant with respect to both maximum life span ($\beta_F = -113.3$; $\beta_{F^2} = 360.5$, $P = 0.016$) and the Gompertz slope parameter ($\beta_F = -61.10$; $\beta_{F^2} = 120.47$, $P = 0.046$).

Haploid lethal equivalents: Weighted least-squares estimates of the inbreeding load associated with median life span and components of age-specific mortality are listed in Table 2 for each treatment. In each case, the inbreeding load was significant with respect to median life span ($L_{T_{\text{Med}}}$) and the age-dependent component of age-specific mortality (L_β), but not with respect to the age-independent component (L_α). Significant evidence for inbreeding depression was therefore obtained for median life span and the age-dependent mortality rate, but not for the age-independent mortality rate. The lack of significance for the age-independent mortality component may have resulted from the relatively high variance associated with L_α estimates (see Table 2). Inbreeding load estimates changed only slightly with different methods of parameter estimation. For example, large Durbin-Watson (DW) statistics were calculated in the regressions used to estimate $L_{T_{\text{Med}}}$ (DW = 1.46–1.66; $P = 0.002$, 0.030, and 0.071 in the 2P, 10P, and pooled data, respectively). To account for autocorrelation of residuals, therefore, we fit the GARCH model (see MATERIALS AND METHODS), and similar results were obtained ($L_{T_{\text{Med}}} = 0.414$, 0.292, and 0.409 for the 2P, 10P, and pooled data, respectively, $P < 0.01$ for each). When the base population was treated as a control to account for potential adaptation to laboratory conditions (see MATERIALS AND METHODS), the inbreeding load affecting age-independent mortality became significant ($L_\alpha = 1.032$; $P < 0.01$ for pooled data), while the inbreeding load affecting median life span and age-dependent mortality remained significant ($L_{T_{\text{Med}}} = 0.776$, $L_\beta = 0.027$; $P < 0.01$ for the pooled data). For median life span and age-dependent mortality, there was no significant correlation between changes in parameter values per F and the number of generations between successive measurements ($r = -0.169$ and -0.079, respectively; $P > 0.10$ for both; 98 d.f.). With respect to age-independent mortality rates, there was a small but significant correlation between changes in $\ln(\alpha)$ per 1% increase in F and the number of generations between measurements ($r = 0.378$; $P < 0.01$; 98 d.f.).

In addition to the inbreeding load estimates from our experiment, we used Equation 4 to calculate the inbreeding load on the basis of data from three previous studies that have reported Gompertz slope and intercept parameters in inbred and outbred treatments (CURTSINGER *et al.* 1992; HUGHES 1995; VERMEULEN and BIJLSMA 2004). These estimates are listed in Table 3

TABLE 1

Mean survivorship parameters and among-population variances (V_B) associated with the base and experimental populations at each generation of measurement

	T_{Med}		T_{Max}		$\beta \times 10^2$		$\alpha \times 10^4$	
	Mean	V_B	Mean	V_B	Mean	V_B	Mean	V_B
Base Population								
Generation 1	64.3	37.6	91.0	13.8	6.49	3.53	1.18	1.80
Generation 12	64.6	9.04	82.9	14.5	7.96	2.10	0.52	0.23
Generation 24	60.9	7.10	82.4	9.6	8.03	1.93	0.60	0.18
Generation 44	59.1	20.2	79.6	29.8	8.61	1.05	0.46	0.08
2P Populations								
Generation 1	59.8	101.8	87.8	25.7	5.67	1.27	1.94	3.33
Generation 12	46.4	55.7	62.4	94.5	11.2	26.0	1.57	4.02
Generation 24	42.9	97.2	58.3	124.0	11.6	22.4	1.84	7.04
Generation 44	41.6	259.9	58.5	316.6	11.0	23.4	3.53	40.9
10P Populations								
Generation 1	68.9	31.8	92.4	15.8	6.47	1.02	0.66	0.15
Generation 12	59.3	55.3	81.2	32.8	7.21	1.13	0.94	0.35
Generation 24	56.3	70.4	80.1	31.4	7.85	4.30	1.14	1.30
Generation 41	48.6	74.7	71.9	120.3	9.07	15.0	1.61	4.57
Generation 56	47.1	124.3	71.3	161.3	9.09	26.9	2.35	17.7
Generation 66	56.8	56.3	81.1	219.0	9.07	60.7	0.81	0.76

Parameters listed include the median life span (T_{Med}), maximum life span (T_{Max}), Gompertz slope (β), and Gompertz intercept (α).

along with the pooled data estimates that have been obtained in our study. There is considerable variation among studies with respect to all parameters, but estimates were most variable with respect to L_α. Among all studies, the correlation was large between L_β and L_α ($r = -0.793$; $P = 0.109$), $L_{T_{Med}}$ and L_α ($r = 0.712$; $P = 0.178$), and $L_{T_{Med}}$ and L_β ($r = -0.666$; $P = 0.220$). The mean values of $L_{T_{Med}}$, L_α, and L_β across studies were 0.306, 0.552, and 0.033, respectively.

Direct estimates of the age-dependent portion of the mortality inbreeding load (L_β) were also obtained from studies that have reported sequential point estimates of the inbreeding load over the Drosophila life span (Charlesworth and Hughes 1996; Hughes *et al.* 2002). Charlesworth and Hughes's (1996) data suggest that the inbreeding load for male age-specific mortality increases at a mean rate of 0.011 per day ($L = 0.025$ at 14 days, 0.175 at 42 days, and 0.635 at 70 days), while the inbreeding load affecting male mating success increases at a mean rate of 0.042 per day ($L = 0.367$ at 3 days and 1.125 at 21 days). Similarly, Hughes *et al.*'s (2002) data suggest that the inbreeding load for male mating success increases at a mean rate of 0.055 per day ($L = 0.491$ at 7 days, 0.896 at 14 days, 0.945 at 21 days, 0.943 at 28 days, 1.414 at 35 days, and 2.885 at 42 days). These direct estimates compare well to those we obtained for age-specific mortality using Equation 4 (see Table 3).

DISCUSSION

The results from this study have yielded three main findings. First, our results showed that significant levels of inbreeding depression affect male survivorship in *D. melanogaster*. Second, we found significant levels of inbreeding depression for the age-dependent component of age-specific mortality (*i.e.*, the Gompertz slope), which indicated that the inbreeding load affecting age-specific mortality increased throughout the life span. Finally, with respect to maximum life span and the age-dependent rate of mortality, the differentiation of lineages over time showed significant nonlinearity with respect to the inbreeding coefficient. This suggested that nonadditive genetic variation contributed to the among-lineage variation associated with these parameters. We discuss these findings in relation to the quantitative genetic basis of longevity and the evolution of senescence.

The overall effects of inbreeding on survivorship are best summarized by the changes in median life span observed in experimental populations. We estimated that an inbreeding load of 0.39 lethal equivalents per haploid gamete affected median life span ($L_{T_{Med}}$), which is within the range of values calculated on the basis of data from previous studies (0.195–0.472; Table 3). Although our estimate of $L_{T_{Med}}$ was significantly greater than zero, its value also suggests that the inbreeding depression affecting median life span is less than that found to influence other life-history traits in Drosophila. A larger inbreeding load, for example, has been found to affect egg-to-adult viability (0.70), male mating ability (0.50–3.20), and competitive ability (1.80–3.50) (Simmons and Crow 1977; Latter and Sved 1994; Hughes 1995; Latter *et al.* 1995). The inbreeding depression that we observed may nonetheless be

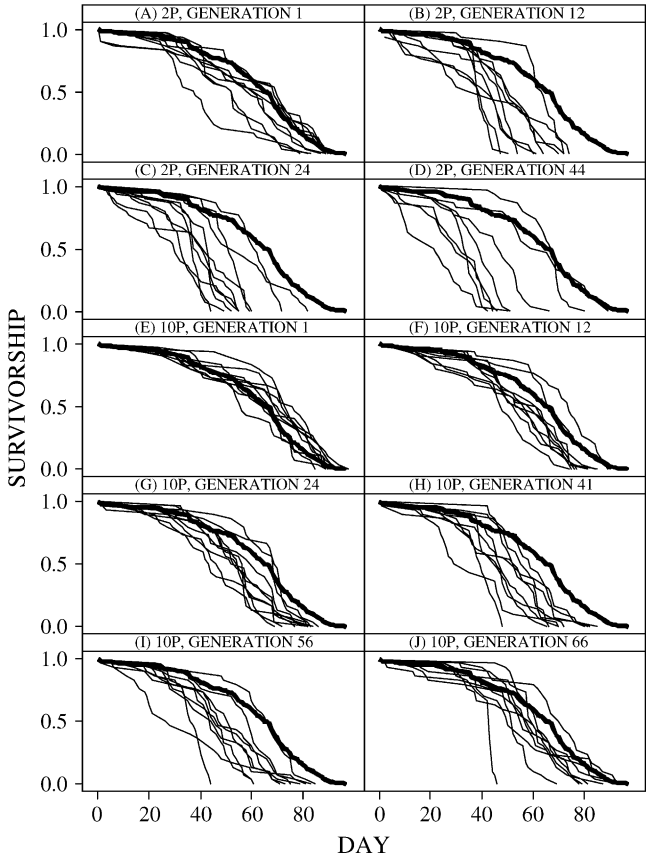

Figure 2.—Survivorship curves associated with lineages within the 2P treatment (A–D) and the 10P treatment (E–J). The survivorship curve associated with the 300 males sampled from the base population at generation 1 is represented by the thick line, and curves associated with replicate lineages are represented by thin lines. Associated parameter estimates are listed in Table 1.

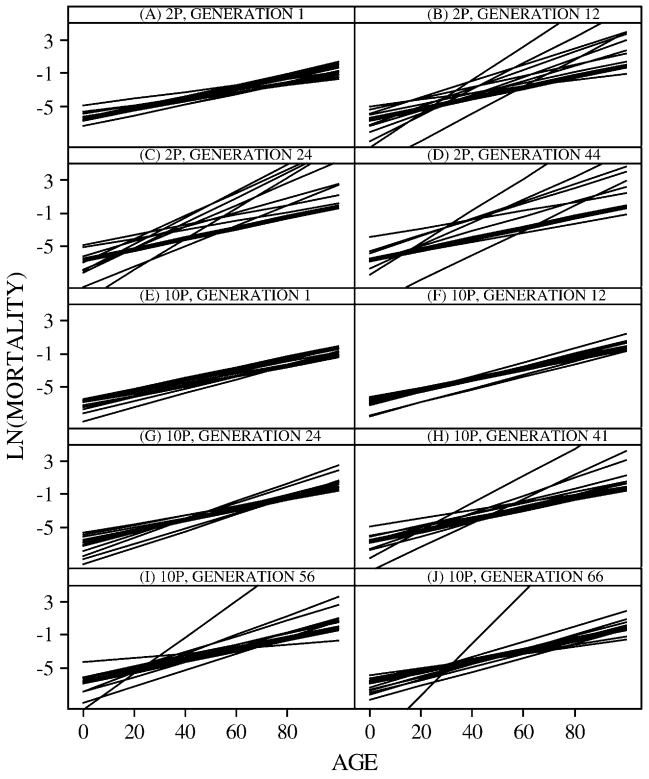

Figure 3.—Age-specific mortality plots associated with lineages within the 2P treatment (A–D) and the 10P treatment (E–J). The age-specific mortality rate associated with the 300 males sampled from the base population at generation 1 is represented by the thick line, and mortality rates associated with replicate lineages are represented by thin lines. Associated parameter estimates are listed in Table 1.

sufficiently large to impact survivorship within laboratory populations commonly used in experimental research on aging. Given an inbreeding load of 0.314 haploid lethal equivalents affecting median life span (*i.e.*, the mean estimate listed in Table 3), for example, a single generation of full-sib mating is expected to reduce life span by 8% on average. The concern that inbreeding depression in control or experimental populations has the potential to obscure treatment effects in longevity studies is therefore well founded (Rose and Charlesworth 1981; Johnson and Wood 1982; Rose 1984, 1991).

The effects of inbreeding on traits related to fitness have often been found to vary greatly among independent lineages (*e.g.*, Pray *et al.* 1994; Fowler and Whitlock 1999). Our results have shown similar variability of inbreeding effects with respect to the median life span. One lineage in the 10P treatment, for example, evidently became fixed for one or several deleterious recessive alleles that caused individuals to rapidly die shortly after the 40th day of the life span (see Figure 2). In generations 41, 56, and 66, this lineage had the lowest survivorship of all populations in the 10P treatment, with a maximum life span of only 46, 44, and 48 days in each generation, respectively. At the same time, however, the survivorship curves of other lineages were indistinguishable from that of the original base population throughout the entire experiment (Figure 2). For example, at generation 44, a 2P lineage had a median life span of 67.5 days, while at generation 66, a 10P lineage had a median life span of 71 days.

An important result obtained in this study was that the severity of inbreeding depression affecting age-specific mortality significantly increased over the life span. This finding provides evidence in support of the mutation-accumulation theory of aging (Charlesworth and Hughes 1996; Hughes *et al.* 2002; Snoke and Promislow 2003). We estimated that the baseline inbreeding load affecting age-specific mortality is 0.307 (L_α) and that this value increases at a rate of 0.046 (L_β) per day of the life span (Table 2). Both of these estimates are comparable to those calculated on the basis of data obtained from previous studies (Table 3). The mutation-accumulation theory of aging predicts that the magnitude of inbreeding depression and the ratio of dominance to additive genetic variation (V_D/V_A) will increase over the

TABLE 2

Inbreeding load estimates for median life span and age-specific mortality

	$L_{T_{Med}}$	L_α	L_β
2P treatment	0.428 (0.066)*	0.655 (0.552)	0.057 (0.011)*
10P treatment	0.334 (0.053)*	−0.129 (0.394)	0.033 (0.010)*
Pooled	0.389 (0.047)*	0.307 (0.359)	0.046 (0.008)*

For age-specific mortality, the inbreeding load is expressed as an age-independent component (L_α) and an age-dependent component (L_β) (see MATERIALS AND METHODS). All estimates were obtained by weighted least-squares regression. The standard errors associated with each estimate are given in parentheses. *Significant values, $P < 0.01$.

life span (CHARLESWORTH and HUGHES 1996). Of these two predictions, increases in inbreeding depression over the life span may provide the strongest evidence in support of the mutation-accumulation theory of senescence. Since estimating variances at different points in the life span entails several statistical challenges (PROMISLOW and TATAR 1998; SHAW *et al.* 1999; SNOKE and PROMISLOW 2003), experiments that have demonstrated increased V_A or V_D/V_A ratios over the life span have been difficult to interpret (*e.g.*, HUGHES and CHARLESWORTH 1994; CHARLESWORTH and HUGHES 1996; SNOKE and PROMISLOW 2003). While increases in inbreeding depression over the life span may also be subject to statistical challenges (PROMISLOW and TATAR 1998; PROMISLOW *et al.* 1999), most are effectively circumvented by the methods used in the present analysis. In previous studies, researchers have tested for an increasing trend in sequential point estimates of inbreeding depression obtained throughout the life span (TANAKA 1993; CHARLESWORTH and HUGHES 1996; HUGHES *et al.* 2002; SNOKE and PROMISLOW 2003). A problem with this discrete approach is that sampling variances differ among the point estimates. In the case of mortality and survival rates, the ability to detect inbreeding depression will increase over the life span, which may lead to an apparent increase in the inbreeding load even if it is constant (PROMISLOW and TATAR 1998; PLETCHER 1999). The simple model used in this experiment demonstrates that the inbreeding load increases over the life span if the Gompertz slope parameter differs significantly between inbred and outbred cohorts (Equation 4). This approach avoided the problems entailed in the discrete analysis of sequential point estimates of inbreeding depression.

TABLE 3

The inbreeding load affecting median life span and age-specific mortality estimated on the basis of the data from this and three other experimental studies

	$L_{T_{Med}}$	L_α	L_β
This study	0.389	0.307	0.046
CURTSINGER *et al.* (1992)[a]	0.261	−0.015	0.029
HUGHES (1995)[b]	0.195*	0.535*	0.045*
	0.211**	0.209**	0.040**
VERMEULEN and BIJLSMA (2004)[c]	0.472	1.722	0.003
Mean values	0.306	0.552	0.033

All estimates were obtained using *D. melanogaster* males. For age-specific mortality, the inbreeding load is expressed as an age-independent component (L_α) and as an age-dependent component (L_β) (see MATERIALS AND METHODS).

[a] Estimates are based on four inbred lineages that had undergone 30 generations of full-sib mating ($F \approx 0.99$).

[b] Estimates are based on third chromosome homozygotes. Calculations are based on either paired comparisons between homozygous and heterozygous chromosomes (*) or nonpaired comparisons averaged over blocks (**).

[c] Estimates are based on four inbred lineages that had undergone seven generations of full-sib mating ($F = 0.785$). Calculations are based on survivorship assays carried out at 25°.

Several points should be noted regarding our analysis of inbred and outbred mortality rates and our conclusions as they relate to senescence theory. First, the expected relationship between inbreeding load and age under the mutation-accumulation theory of senescence is based on the assumption that mutational effects at different ages are independent (CHARLESWORTH and HUGHES 1996). Due to a lack of empirical data, however, the degree to which this assumption generally holds is not yet clear. Second, while the increased Gompertz slope observed in inbred populations shows that the inbreeding load for age-specific mortality increases over the life span, it does not necessarily follow that the Gompertz slope is more important than the Gompertz intercept with respect to longevity differences between inbred and outbred individuals (see PLETCHER *et al.* 2000). Third, the linear pattern of increase in the inbreeding load over the life span is a consequence of our assumption that mortality rates follow the Gompertz model. If the increase in inbreeding load were in fact nonlinear with respect to age, our analysis would not have been able to detect it. Finally, there has been considerable debate with respect to whether independent factors act additively on the scale of mortality or survivorship (CHARLESWORTH and HUGHES 1996; PROMISLOW and TATAR 1998). Consequently, it is important to recognize the distinction between the inbreeding load affecting age-specific mortality and the inbreeding load affecting age-specific survivorship, since our inbreeding load estimates were obtained for the former and the same conclusions may not apply to the latter.

The pattern by which variation increased among inbred lineages over time provides insight into the genetic basis of longevity as a quantitative trait. With respect to maximum life span and the Gompertz slope parameter, we found that the increase in variation among lineages was significantly nonlinear with respect to the inbreeding coefficient. These nonlinear trends were clearly evident in plots of the among-population variance *vs.* the inbreeding coefficient (not shown). It was, in fact, surprising that the regression coefficient associated with

the F^2 term was significant in two cases, since the power of this test is relatively low. For neutral traits with an additive genetic basis, the among-population variance is expected to increase linearly with respect to the coefficient of inbreeding (Hill 1972; Lande 1980; Lynch and Hill 1986). However, as a life-history trait that is correlated with fitness to some extent, survivorship parameters may be associated with nonadditive components of genetic variation (Fox *et al.* 2004). The nonlinear increase in among-population variance that we observed, therefore, was most likely due to nonadditive gene action (Bryant *et al.* 1986; Goodnight 1987; Lynch 1988). This nonadditive gene action may indicate the presence of dominance or epistatic genetic variance (Lynch 1988). Since inbreeding depression was evident with respect to both Gompertz slope and maximum life span, it is likely that dominance genetic variation contributed to the nonlinear increase in among-population variance observed. It is also possible, however, that epistatic genetic variation contributed to the observed nonlinearity. Epistatic genetic variation affecting survivorship, for example, has been suggested by observations of F_2 hybrid breakdown of longevity in crosses between lineages of *D. pseudoobscura* (Vetukhiv 1956, 1957), by synergistic interactions between spontaneous mutations promoting longevity declines in parasitoid wasps (Rivero *et al.* 2003), and by strong background effects affecting QTL loci associated with longevity in gene-mapping studies using *D. melanogaster* and *Caenorhabditis elegans* (Shook and Johnson 1999; Spencer *et al.* 2003; Pasyukova *et al.* 2004).

We observed significant declines in survivorship in the base population used in this study over the course of 44 generations (Figure 1). This decline in survivorship was most likely the result of adaptation to laboratory conditions, given that the population had been recently established in the laboratory. Laboratory culture curtails the reproductive life span, such that natural selection may favor rapid development and early reproductive capacity (Promislow and Tatar 1998; Sgro and Partridge 2000). A decline in life span has often been observed as a correlated response to such selection for early reproduction in laboratory populations (Sokal 1970; Rose 1991; Reed and Bryant 2000; Partridge 2001; Partridge and Gems 2002). It is possible that laboratory adaptation may also have contributed to the survivorship declines we observed in experimental populations. While these effects might have upwardly biased our inbreeding load estimates, any such bias is likely to be small for several reasons. The effectiveness of natural selection (for laboratory adaptation) is expected to increase at larger population sizes (Frankham *et al.* 1968; Weber 1990, 1996). Consequently, adaptation to laboratory conditions would have been a less important factor in the experimental populations ($N = 4$ or 20) relative to that in the base population ($N = 720$). For the same reason, if laboratory adaptation did substantially contribute to the inbreeding depression that we observed, larger inbreeding depression estimates should have been obtained in the 10P treatment. This was not the case, however, as all inbreeding depression estimates were larger in the 2P treatment (see Table 2). A conservative approach to factoring out the effects of laboratory adaptation was employed by estimating the number of lethal equivalents while treating the base population as a control. When this analysis was performed, all of our inbreeding load estimates remained significant (or became significant in the case of L_α). This was conclusive evidence that survivorship declines in experimental populations were predominantly due to inbreeding depression rather than laboratory adaptation.

The curtailment of Drosophila life span in laboratory culture may allow spontaneous mutations with deleterious age-specific effects on survivorship to accumulate within experimental lineages during the course of inbreeding (*e.g.*, Shabalina *et al.* 1997), which was a second potential source of upward bias with respect to our inbreeding load estimates. With regard to median life span and the age-dependent portion of the age-specific mortality load, the effects of spontaneous mutation accumulation were most likely negligible. This is because observed changes in $\ln(T_{\text{Med}})$ and β per 1% increase in F between measurements showed no significant association with the number of generations between measurements. If spontaneous mutation accumulation had been an important factor, larger declines in $\ln(T_{\text{Med}})$ per F (and larger increases in β per F) should have been associated with measurement intervals covering a greater number of generations, which was not the case. With respect to the age-independent component of age-specific mortality, estimates of $\ln(\alpha)$ per F were greater when measurement intervals spanned a larger number of generations. This may have been due to spontaneous mutations arising during inbreeding, although since α represents the mortality rate at age zero, it would be surprising that such mutations were not eliminated by natural selection. In any case, if spontaneous mutation accumulation did upwardly bias our L_α estimate, this bias was probably small because our estimated value of 0.307 was nonsignificant and less than the mean value of 0.552 among other studies (see Table 3). For example, the largest L_α value listed in Table 3 (1.722) was obtained using data from the study of Vermeulen and Bijlsma (2004), in which spontaneous mutations would have been negligible because the inbreeding procedure used lasted only seven generations.

In conclusion, this study has used *D. melanogaster* as a model system to investigate the effects of inbreeding on male survivorship, a life-history trait that has rarely been the focus of experimental studies of inbreeding depression. In addition to observing significant inbreeding depression for parameters associated with longevity, our results have yielded several findings that underscore the uniqueness of longevity as a quantitative genetic

trait. We have found that the inbreeding depression affecting age-specific mortality increases throughout the life span, which provides support for the mutation-accumulation theory of senescence. In addition, the quantitative genetic basis of some survivorship parameters appeared to include nonadditive components of genetic variation, as evidenced by the temporal divergence pattern of parameter values among experimental lineages. These findings suggest that survivorship will continue to be a worthwhile focus in quantitative genetic studies aimed at understanding the genetic basis and evolution of complex phenotypic traits.

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