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Quantitative Trait Loci With Age-Specific Effects on Fecundity in *Drosophila melanogaster*

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ABSTRACT

Life-history theory and evolutionary theories of aging assume the existence of alleles with age-specific effects on fitness. While various studies have documented age-related changes in the genetic contribution to variation in fitness components, we know very little about the underlying genetic architecture of such changes. We used a set of recombinant inbred lines to map and characterize the effects of quantitative trait loci (QTL) affecting fecundity of *Drosophila melanogaster* females at 1 and 4 weeks of age. We identified one QTL on the second chromosome and one or two QTL affecting fecundity on the third chromosome, but these QTL affected fecundity only at 1 week of age. There was more genetic variation for fecundity at 4 weeks of age than at 1 week of age and there was no genetic correlation between early and late-age fecundity. These results suggest that different loci contribute to the variation in fecundity as the organism ages. Our data provide support for the mutation accumulation theory of aging as applied to reproductive senescence. Comparing the results from this study with our previous work on life-span QTL, we also find evidence that antagonistic pleiotropy may contribute to the genetic basis of senescence in these lines as well.

A major challenge in evolutionary genetics is to characterize the genetic architecture of natural variation in life-history traits, those components of fitness that directly influence age-specific survival and reproductive success. Life-history theory is founded on the idea that natural selection favors a particular strategy of age-specific allocation of energy to the competing demands of growth, development, reproduction, storage, maintenance, and repair in a given ecological setting (Stearns 1992). This suggests that understanding the genetic basis of life-history variation will require that we not only identify the genes that affect these traits but also characterize the age-specific effects of alleles at these loci. Knowledge of the genetic basis of life-history variation at the molecular genetic level not only would contribute to our understanding of the genetic architecture and evolution of quantitative traits in general but also would provide insight into mechanisms that maintain variation in fitness (Barton and Turelli 1989; Barton and Keightley 2002; Turelli and Barton 2004).

While numerous studies have documented the existence of genetically based variation in life-history traits in natural populations (Mousseau and Roff 1987; Hard *et al.* 1993; Shaw *et al.* 1995; Kruuk *et al.* 2000; Leips *et al.* 2000; Sommer and Pearman 2003; Drnevich *et al.* 2004; Fox *et al.* 2004; Windig *et al.* 2004), we know very little about the genes that underlie this variation. Further, although several studies have verified that mutations can have age-specific effects on fitness components (Houle *et al.* 1994; Hughes and Charlesworth 1994; Hughes 1995; Charlesworth and Hughes 1996; Promislow *et al.* 1996; Tatar *et al.* 1996; Pletcher *et al.* 1998, 1999; Mack *et al.* 2000; Yampolsky *et al.* 2000; Hughes *et al.* 2002), only a few have attempted to identify the actual loci that influence these age-related changes in natural populations (Curtsinger and Khazaeli 2002; Jackson *et al.* 2003; Khazaeli *et al.* 2005; Nuzhdin *et al.* 2005).

One of the more important life-history traits is fecundity, that is, the number of offspring, propagules, or eggs produced. The fecundity of an individual sets the baseline number of offspring that can be contributed to the next generation and so represents the maximum possible fitness of an organism. Further, the age-specific allocation of energy to reproduction is a more important determinant of fitness than life-time fecundity in populations with age structure (Charlesworth 1994). The age-specific allocation of energy to reproduction can also affect fitness by producing trade-offs with other traits such as adult survival and future reproduction (Rose 1984; Gustafsson *et al.* 1994; Tatar and Promislow 1997; Jouventin and Dobson 2002; Oksanen *et al.* 2002; Fedorka *et al.* 2004). Thus, loci that control the age-specific allocation of energy to

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reproduction should have pleiotropic effects on other fitness components and so may act as genetic constraints on the independent evolution of other fitness components.

Fecundity exhibits an age-related decline in many species (Aigaki and Ohba 1984; Rose 1991; Partridge and Barton 1993; Rauser *et al.* 2003; Ricklefs *et al.* 2003; Broekmans *et al.* 2004; Novoseltsev *et al.* 2004; Ottinger *et al.* 2004; Skoracka and Kuczynski 2004; Nichols *et al.* 2005) and so understanding the genetic basis of reproductive senescence should also be useful for understanding the genetics of aging. A large number of studies have identified genes through mutational analysis or genetic manipulation that influences rates of senescence as reflected in age-specific mortality rates (Kenyon *et al.* 1993; Lin *et al.* 1998; Hsin and Kenyon 1999; Tatar *et al.* 2001; Sun *et al.* 2002; Tu *et al.* 2002; Marden *et al.* 2003; Picard *et al.* 2004; Rogina and Helfand 2004; Halaschek-Wiener *et al.* 2005; Kaeberlein *et al.* 2005). However, genes that influence age-specific mortality may be different from those causing senescence in other traits, such as reproduction. Further, the above studies used artificially induced mutations to identify candidate genes for aging that may or may not contribute to natural variation in senescence. Thus, to understand how the genetic architecture of life-history traits influences senescence and the maintenance of variation in senescence it is important to characterize the influence of natural allelic variation (Knight *et al.* 2001).

Quantitative trait locus (QTL) mapping procedures provide a useful avenue to search for loci that contribute to variation in fitness components. Many QTL studies have identified chromosomal regions that affect some aspect of fitness (Nuzhdin *et al.* 1997, 2005; Fry *et al.* 1998; Shook and Johnson 1999; Lin 2000; Wayne *et al.* 2001; Slate *et al.* 2002; Steinmetz *et al.* 2002; Wayne and McIntyre 2002; Ayyadevara *et al.* 2003; Ungerer and Rieseberg 2003; Weinig *et al.* 2003; Moehring and Mackay 2004; Peripato *et al.* 2004; Valenzuela *et al.* 2004; Verhoeven *et al.* 2004; Colosimo *et al.* 2005; Fiumera *et al.* 2005; Khazaeli *et al.* 2005; Zhong *et al.* 2005). In *Drosophila melanogaster* fine mapping of these QTL has allowed refinement of the position of candidate genes within QTL that affect a number of traits, including life span (Pasyukova *et al.* 2000, 2004), male mating behavior (Moehring and Mackay 2004), ovariole number (Wayne and McIntyre 2002), starvation resistance (Harbison *et al.* 2004), and olfactory behavior (Fanara *et al.* 2002). Verification of the effects of natural genetic variation in candidate genes within QTL regions has also been initiated using association mapping (De Luca *et al.* 2003). While the "gold standard" for final confirmation of the effect of sequence variation on phenotypic variation is to functionally characterize the effects of alleles on phenotypes (Mackay 2001; Rong *et al.* 2002; Sun *et al.* 2004), the mapping process has proven to be a promising technique for identifying genes on which to focus our functional genetic efforts (Long *et al.* 1995; Gurganus *et al.* 1999; Geiger-Thornsberry and Mackay 2004; Moehring and Mackay 2004; Nguyen *et al.* 2004; Palsson and Gibson 2004; Pasyukova *et al.* 2004).

In this study we use a population of recombinant inbred lines (RIL) of *D. melanogaster* to address three issues related to the genetic architecture of age-specific fecundity. First, we map the location of QTL that produce variation in fecundity at two ages (1 week and 4 weeks) and assess their relative influences on fecundity at each age. Second, because the effects of allelic variation at QTL can be influenced by the genetic background (Mackay *et al.* 2005) we tested for epistatic effects on fecundity in a genome-wide analysis. Finally, because age-specific fecundity is often (Rose 1984, 1991) but not always (Harshman and Hoffmann 2000) negatively correlated with life span, we compare the fecundity results from this study to our previous study on life span to identify putative pleiotropic loci affecting these two traits. We discuss these results in light of the two leading evolutionary theories of aging, antagonistic pleiotropy (AP) (Williams 1957) and mutation accumulation (MA) (Medawar 1952). To explore the potential influence of AP, we compare the locations and age-specific allelic effects of fecundity QTL with the locations and allelic effects of QTL affecting mated life span, which were previously mapped using the same set of RIL under similar environmental conditions (Leips and Mackay 2002). Under the AP model, QTL affecting fecundity and life span should colocalize and the alleles at these loci should exhibit antagonistic effects on these traits. To examine our data for evidence in support of MA, we use data on the genetic components of variation in fecundity among lines at each age. A unique prediction of this theory applied to our mapping population of RIL (a population of homozygous lines) is that the genetic component of variation in fecundity should increase with age (Hughes and Charlesworth 1994; Hughes and Reynolds 2004).

MATERIALS AND METHODS

Fly stocks: Our mapping population consisted of a set of 92 RIL (Nuzhdin *et al.* 1997) derived from a cross between two isogenic strains, the *Oregon-R* (*Ore*) (Lindsley and Zimm 1992) and the Russian *2b* strain, an isogenic line derived from a population of flies selected for decreased male sexual activity (Pasyukova and Nuzhdin 1993). The procedure used to construct the RIL has been previously described (Nuzhdin *et al.* 1997) and is summarized here. F_1 offspring of the cross between the *Ore* and *2b* strains were backcrossed to the *2b* strain and then randomly mated for four generations. After the last generation of random mating, 200 male-female pairs were used to create sublines from this population by carrying out brother-sister matings for 25 generations. Ninety-eight RIL were subsequently created from these sublines. Of the original 98 RIL, 92 were extant at the time this experiment was carried out.

Early and late-age fecundity assay: The total number of eggs laid by single females over a 2-day period was used as an estimate of fecundity at 1 and 4 weeks of age. Two replicate sets of flies of each genotype were set up simultaneously so that early and late-age fecundity could be measured on different females from each RIL. We measured early and late-age fecundity on different females to minimize the effects of handling at early age on late-age fecundity and to decrease the influence of nongenetic phenotypic correlations between early and late-age fecundity. One limitation of this design, however, is that because fecundity was measured on different females at early and late age we could not calculate the phenotypic correlation between early and late-age fecundity.

To produce females for each fecundity estimate, 15–20 pairs of flies from the *Ore* and *2b* strains and each of the 92 RIL were allowed to lay eggs over a 4-day period in egg-collecting chambers (described below). From these chambers, 50 first or second instar larvae of each genotype were collected and placed in vials containing 5 ml of standard cornmeal/agar/molasses medium to standardize larval density. This procedure was repeated with five replicate vials per line. Larval density was controlled in this manner to reduce the influence of variation in larval density on female size at eclosion, a trait that is positively correlated with fecundity (Robertson 1957; Tantawy and Rahka 1964; Partridge *et al.* 1986; Nunney 1996; Zwaan *et al.* 1995). From each replicate vial, three virgin females that emerged on the same day were collected and placed in a vial containing standard fly food. To provide mates, six young males (<1 week old) of the *Samarkand* (*Sam*) strain were added to each vial (two males:one female per vial). *Sam* (an unrelated isogenic laboratory stock) males were used as mates to standardize the potential effects of male genotype on female fecundity.

Age-specific fecundity of individual females was measured at young (4–5 days) and old (28 days) age in a fashion similar to that of Houle *et al.* (1994) and Service (2000). These dates were chosen because reproductive output typically peaks in *D. melanogaster* at ~6–12 days posteclosion and declines beyond that point (Houle *et al.* 1994; Tatar *et al.* 1996; Gasser *et al.* 2000). At each age, single females were removed from each vial and placed in an egg-laying chamber with a single *Sam* male. Egg-laying chambers consisted of standard 10-ml fly vials, containing 1 ml of hardened 2% agar to provide a source of moisture and 1 ml of fly food to provide a site for oviposition. Food was placed on the flat side of a hardened foam plug (which was also used to cap the vial). The vial was then inverted so that the plug of food was on the bottom of the vial. Females were allowed to lay eggs on food plugs for 24 hr, after which each plug was removed to count eggs. A fresh food-containing plug was replaced in the vials and the above process was repeated for a second 24-hr period. After this second day of egg laying, all females were discarded.

At each age, fecundity estimates were made on 15 females/line (five replicate vials containing 3 females/vial/line) for a total of 2760 observations (1380 young flies and 1380 old flies).

Statistical analyses: We tested for differences in age-specific fecundity between the inbred parental strains (*Ore* and *2b*) and the RIL in separate analyses. Differences in fecundity between the parental strains were tested using a mixed-model ANOVA according to the model $y = \mu + A + S + (A \times S) + R(A \times S) + error$, where μ is the overall mean, A is the fixed effect of age (1 or 4 weeks), S is the fixed effect of parental strain (*Ore* or *2b*), and R is the random effect of the replicate nested within age and strain. Flies in a particular replicate were those that had shared a vial until the age that we measured fecundity.

To assess genetically based differences in fecundity among the RIL, we used three separate analyses. The first two analyses used a random-effects ANOVA to test for genetic differences among lines at 1 and 4 weeks of age and provided estimates for the among-line variance components for fecundity at each age. The model partitioned the random effects of line (L) and replicate (R) within line and residual error according to the model $y = \mu + L + R(L) + error$ for flies in each age group. In the third model we used a mixed-model ANOVA on the entire data set to examine the potential for a line-by-age interaction that would in essence tell us if the effect of age on fecundity was similar among lines. For this analysis we used the model $y = \mu + A + L + (A \times L) + R(A \times L) + error$, where A is the fixed effect of age on fecundity and all other effects are random.

All statistical analyses were carried out using SAS V.9.1. The PROC GLM and VARCOMP procedures were used for the analyses of variance on RIL within each age and for estimating variance components within each age. The PROC MIXED procedure was used for both of the mixed-model analyses and the significance of random effects was determined using likelihood-ratio tests (Littell *et al.* 2002). Phenotypic data were ln-transformed to meet the assumptions of ANOVA.

Variance components from the random-effects analyses above were used to calculate the proportion of the total phenotypic variation in fecundity explained by genetic differences among lines at each age (also separately estimated as the coefficient of genetic variation) and the genetic correlation of fecundity at young and old ages. The coefficient of genetic variation (CV_G) was calculated at each age as $CV_G = 100(V_L)^{1/2}/\bar{x}$, where V_L is the among-line variance component and $\bar{x}$ is the overall mean fecundity (Houle 1992). The genetic correlation across ages (r_{GA}) was computed as $\mathrm{cov}_{12}/(\sigma_{L1}\sigma_{L2})$ (Robertson 1959), where cov_{12} is the covariance among-line means at 1 and 4 weeks of age and σ_{L1} and σ_{L2} are the square roots of the among-line variance components of fecundity at 1 and 4 weeks of age from the reduced model analyses.

QTL mapping: Molecular markers used to determine the genotype of the RIL were the cytological insertion sites of the *roo* transposable element (Nuzhdin *et al.* 1997). Eighty-one informative markers were used (Nuzhdin *et al.* 1997; Leips and Mackay 2000) with an average spacing between markers of 7.9 cM. Spacing between markers was estimated from the observed recombination (r) frequencies between pairs of markers using the Kosambi map function $100d_M = 0.25 \ln[(1 + 2r)/(1 - 2r)]$. The distance between markers in this study is slightly greater than that of previous studies using these RIL (Nuzhdin *et al.* 1997; Wayne *et al.* 2001; Leips and Mackay 2002) because the loss of 6 of the original 98 lines reduced the number of observed recombination events in the mapping population.

QTL mapping was done using composite interval mapping (Zeng 1994) in QTL Cartographer (Version 1.14) and as outlined in Leips and Mackay (2002). This mapping procedure tests the hypothesis that an interval between adjacent markers contains a QTL affecting the quantitative trait, while controlling for the effects of linked QTL outside of the test interval. Markers on which the QTL analyses were conditioned were based on a forward-backward elimination stepwise regression analysis. Because the results of each analysis can be sensitive to the conditioning window used around each test interval, we tested a range of window sizes (5, 10, 15, and 20 cM) to evaluate the effect of window size on the likelihood ratios for each QTL. On the basis of the results from this set of analyses we used a window size of 10 cM because QTL identified with this window size were also determined to be significant in all analyses regardless of window size and so represent a conservative choice. The significance level for each QTL analysis was determined by randomly permuting the fecundity data 1000 times and calculating the maximum-likelihood ratio

TABLE 1

Summary statistics for the fecundity data from the set of recombinant inbred lines

Age	Fecundity	$(V_L/V_L + V_R)^a$	$CV_G{}^b$	$P(GAI)^c$	$r_{GA}{}^d$ $(L_1, L_2)^e$
1 wk	14.8 ± 0.3	0.08	1.91	****	0.05 (−0.11, 0.30)
4 wk	15.2 ± 0.1	0.13	2.46		

[a] Proportion of the total phenotypic variance explained by variation among RIL where V_L is the variance component from ANOVA for testing differences among lines and V_R is the variance component of the vial replicate plus the residual variance from ANOVA.
[b] $CV_G = 100\ (V_L)^{1/2}/\bar{x}$ where $\bar{x}$ is the average life span among the RIL.
[c] *P(GAI)* is from the test of significance for the line by age interaction term, ****$P < 0.0001$.
[d] r_{GA} is the genetic correlation of fecundity of the lines between each age.
[e] Lower and upper confidence limits of r_{GA}.

statistic across all test intervals for each permutation. LR statistics from the original data that were exceeded by the permutation maximum LR statistics <50 times were considered significant at $\propto = 0.05$ (Churchill and Doerge 1994; Doerge and Churchill 1996).

We used ANOVA (PROC GLM in SAS V.9.1) to test for epistasis first by looking for significant pairwise interactions between QTL that had significant additive effects on fecundity on the basis of our mapping analysis. For each interaction, the genotype of each marker (homozygous for either the *Ore* or the *2b* allele) closest to each significant QTL peak was used to evaluate the significance of marker interactions on fecundity. Because epistasis may also occur between QTL without main effects on the trait (Mackay *et al.* 2005), we performed a whole-genome screen for pairwise interactions between all possible pairs of markers using a two-way ANOVA where $y = \mu + M_i + M_j + (M_i \times M_j) + error$, where M is the genotype of each marker at positions i and j in each line. Using 81 markers means that we tested 3240 possible interactions. As such, we expect 162, 32.4, 3.24, and 0.324 significant interactions by chance alone at $P < 0.05$, < 0.01, < 0.001, and < 0.0001, respectively.

RESULTS

Genetic variation in age-specific fecundity (parental strains): The average fecundity of the *Ore* strain (±1 standard error) at 1 and 4 weeks of age was 19.5 ± 3.0 and 10.06 ± 2.7 eggs, respectively. The average fecundity of the *2b* strain at 1 and 4 weeks was 10.87 ± 2.6 and 8.53 ± 1.4 eggs, respectively. Despite these differences there was no significant difference in fecundity between the parental strains when averaged across both ages ($F_{1,16} = 2.51$, $P = 0.13$). There was also no significant effect of age ($F_{1,16} = 3.36$ $P = 0.08$) nor was there a significant age by parental strain interaction ($F_{1,16} = 1.23$, $P = 0.28$).

Genetic variation in age-specific fecundity (RIL): Fecundity at 1 and 4 weeks averaged over all RIL was 14.8 and 15.2, respectively (Table 1) and the range of fecundity among the RIL at 1 and 4 weeks of age was similar (week 1: 5–23 eggs/female; week 4: 3–27 eggs/female). Thus, fecundity changed very little between weeks 1 and 4 when averaged over all lines.

On the basis of the ANOVA at each age, however, we found significant differences in fecundity among the RIL at both 1 ($F_{91,368} = 2.03$, $P < 0.0001$) and 4 ($F_{91,368} = 2.44$, $P < 0.0001$) weeks of age. Notably, the genetic component of the total variation in fecundity at 4 weeks was almost twice what it was at 1 week (Table 1). This is not because there was less phenotypic variation in older aged individuals. In fact, the amount of residual variance in fecundity was 30% higher in the analysis of 4-week-old females compared to that of 1-week-old females. This increase in the environmental component of variance with age is consistent with other studies of fecundity (Rose and Charlesworth 1981) and longevity (Charlesworth and Hughes 1996) in Drosophila and suggests that older individuals may be more sensitive to environmental variation than younger flies (Charlesworth and Hughes 1996). Another possible explanation is that because older flies have experienced a greater range of environments the cumulative effect of this variation results in greater phenotypic variation among older aged individuals.

Although the RIL differed in fecundity at each age, the effect of age on fecundity differed dramatically among lines (Figure 1). The line-by-age interaction term was significant ($\chi^2_{(1)} = 23.4$, $P < 0.0001$) and the genetic correlation of fecundity at 1 and 4 weeks of age was not significantly different from zero (Table 1).

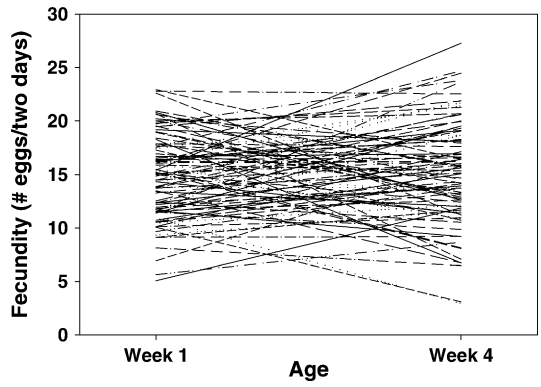

Figure 1.—The average fecundity of each line at weeks 1 and 4. Fecundity was measured as the number of eggs laid by a single female over a 2-day period. Fecundity counts within lines were measured for different females at each age.

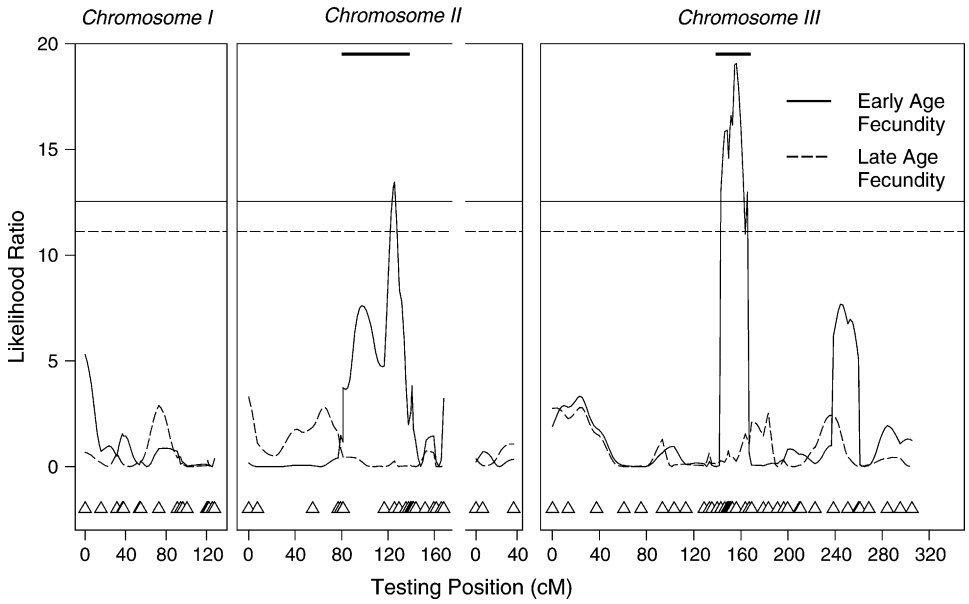

FIGURE 2.—Composite interval mapping results indicating the positions of QTL affecting fecundity at each age. The threshold value for significance at each age is given by the horizontal lines. Positions of informative markers are denoted by triangles on the *x*-axes. Lines above peaks indicate 2-LOD support intervals for the location of the QTL.

Together these results suggest that genes contributing to the variation in early age fecundity are distinct from those producing variation in fecundity at 4 weeks and/or that the allelic effects of loci that contribute to genetic variation in fecundity vary with age.

Age-specific QTL for fecundity: We identified two or three QTL affecting fecundity at 1 week of age, one on the second chromosome and another one or two on the third chromosome (Figure 2). Given the proximity of the two QTL on the third chromosome and the confidence intervals around each, it is not clear if these are distinct QTL. The most likely position of the QTL on the second chromosome is at cytological position 34E with the 2-LOD support interval (LYNCH and WALSH 1998) extending from cytological positions 30D to 38A. Variation at this QTL explained 10% of the variation in fecundity among lines at 1 week of age. The additive effect of the *Ore* allele at this locus increased the fecundity of females by 0.78 eggs/day compared to the effects of the *2b* allele. One of the QTL on the third chromosome has the highest likelihood of being at position at 85F (2-LOD support interval 73D–87E). Variation at this locus explained 14% of the genetic variation in fecundity, and the *Ore* allele at this locus also increased the fecundity by 0.74 eggs/day compared to the effect of the *2b* allele. The third QTL appears at position 87B (2-LOD support interval also ranges from 73D to 87E) and explains an additional 10% of the genetic variation. At this locus, the *Ore* allele increases fecundity by 0.67 eggs/day relative to the *2b* allele. Interestingly, the direction of effects of the *2b* alleles is consistent at both sites and may reflect the fact that this line is derived from a population selected for decreased male mating activity. However, the *2b* and *Ore* parental strains do not differ genetically for fitness (WAYNE *et al.* 2001) so it is unlikely that the effects seen here are due to a general effect of *2b* alleles in reducing overall fitness.

Unexpectedly, we found no QTL that influenced fecundity at 4 weeks of age, despite the fact that the ANOVA indicated a higher degree of genetically based variation in late-age fecundity than in early age fecundity. This suggests that genetic variation in fecundity among lines at the older age is due to alleles with small late-age specific effects. Our observation that there was no genetic correlation of fecundity between young and old ages is thus explained by the diminution of the effects of QTL affecting early age fecundity.

Effects of epistasis among marker loci on fecundity: None of the pairwise tests for epistasis among the QTL with main effects on fecundity were significant. In the global test for pairwise interactions among loci at early age, a total of 97 markers exhibited significant epistatic effects on fecundity ($P < 0.05$). Of these, 83 interactions had *P*-values between 0.01 and ≤0.05, 13 had *P*-values between 0.001 and <0.01, and only one interaction had a *P*-value of <0.001. In a comparable test using late-age fecundity a total of 98 markers exhibited significant epistatic effects on fecundity ($P < 0.05$). Of these, 92 interactions had *P*-values between 0.01 and ≤0.05, and 6 had *P*-values between 0.001 and <0.01. Given 3240 possible pairwise interactions, the number of significant interactions that we found were well within the numbers of interactions expected by chance alone (expected: 162 $P \leq 0.05$, 32.4 $P < 0.01$, and 3.2 $P < 0.001$).

DISCUSSION

Age-specific effects of QTL: Our results show that the relative influence of genes regulating the age schedule of reproduction changes with age. This interpretation is supported by the high degree of variation among

lines in the effect of age on fecundity and the lack of a genetic correlation between early and late-age fecundity in the RIL. Combining the QTL results with those from the analyses of genetic components of variation leads to the interpretation that variation in fecundity at the younger age was determined at least in part by a few genes of moderate effect but fecundity at old age was determined by many more loci of smaller effect. While it is unclear how many actual genes within the QTL regions contribute to the variation in fecundity at the early age, it is clear that their relative effect on fecundity diminishes greatly with age.

Genes with age-specific effects on other fitness components have been implicated in many other studies of *D. melanogaster* (Kosuda 1985; Engstrom *et al.* 1989; Hughes and Charlesworth 1994; Hughes 1995; Charlesworth and Hughes 1996; Promislow *et al.* 1996; Tatar *et al.* 1996; Pletcher *et al.* 1998, 1999; Mack *et al.* 2000; Yampolsky *et al.* 2000; Curtsinger and Khazaeli 2002; Hughes *et al.* 2002; Snoke and Promislow 2003) and a few studies have begun to map the QTL underlying these age-specific effects on mortality rates (Curtsinger and Khazaeli 2002; Nuzhdin *et al.* 2005) and metabolic rates (Khazaeli *et al.* 2005). Our findings of the age-specific effects of QTL are similar to those seen by Khazaeli *et al.* (2005). In their study, metabolic rates at days 16 and 29 posteclosion and life span appeared to be affected by the same QTL, but these QTL did not affect metabolic rates at the youngest and oldest ages examined in their study. The results from Nuzhdin *et al.* (2005) are also consistent with ours in that no QTL identified in their study had a significant influence on mortality rate at all ages studied. Even more intriguing, their results suggested that at two of the QTL, the allelic effects on mortality rate were negatively correlated across different ages, with the same allele having either a positive or a negative effect on mortality, depending on age.

Given the transient nature of the effects of QTL on senescent phenotypes, an important goal for future studies will be to identify the factors that give rise to age-specific genetic effects. One possibility is that age-specific mutational effects result from genotype-by-environment interactions. Under this scenario, genetic influences on the phenotype depend on the internal physiological conditions; age-related changes in the physiology of the organism modulate the effects of these loci such that they have a notable phenotypic effect only within a certain range of conditions. In addition, age-specific changes in gene expression may give rise to age-specific effects. Numerous studies have demonstrated age-dependent changes in gene expression (Rogina *et al.* 1998; Jin *et al.* 2001; Weindruch *et al.* 2001; Pletcher *et al.* 2002; Seroude *et al.* 2002; McCarroll *et al.* 2004; Kim *et al.* 2005) and it may be that the age at which the effects of allelic variation are notable coincides with the ages of peak expression of these loci. Along these same lines, a growing body of evidence implicates age-related changes in chromatin structure, which directly regulates gene expression, as a mechanism for regulating aging (Chang and Min 2002; Rogina *et al.* 2002; Tissenbaum and Guarente 2002; Issa 2003; Jaenisch and Bird 2003). It is possible that genetic variation in the enzymes controlling age-related changes in chromatin remodeling produces the age-specific effects of particular loci on senescence.

Gene action: We found little compelling evidence for the influence of epistasis on fecundity among our RIL at either age. Admittedly, the method used to correct for the number of expected false positives is conservative and so does not preclude the potential importance of epistasis on fecundity in our mapping population. A more sophisticated statistical approach that has greater power to detect epistasis might have been more useful in this regard (Kao *et al.* 1999) but such methods require much larger sample sizes than we had in this study. If our interpretation about epistasis is correct—that there are few if any significant epistatic interactions among loci that affect fecundity—then this strengthens our interpretation of the QTL results. This is because the composite interval mapping method used produces biased estimates of the position and marginal effects of QTL, given any amount of epistasis and linkage between epistatic QTL (Kao and Zeng 2002).

The lack of epistatic interactions affecting fecundity is in stark contrast with previous work on these lines, which found extensive epistasis among QTL affecting virgin life span (Leips and Mackay 2000; Mackay *et al.* 2005). Interestingly, when these lines were measured for mated longevity, only a single pair of markers appeared to interact epistatically. Thus, differences in the mating status appeared to influence the degree to which epistasis affected longevity. More work is necessary to evaluate the extent to which epistasis is dependent on the environmental/physiological condition and whether different traits are more or less affected by epistatic interactions.

Implications for evolutionary theories of aging: Many studies on the genetic basis of senescence have used age-specific variance components to test the predictions of the MA and AP theories of aging. These studies typically use breeding designs that allow estimation of the additive and dominance genetic components of variation in a trait with age that can then be evaluated in light of the predictions of each theory. While increases in the additive component of variation (V_A) with age can be expected from either model of aging (Charlesworth and Hughes 1996), a unique prediction of the MA theory is that the genetic component of variation among homozygous lines will increase with age. Using this metric, the results of our study lend support for MA producing variation among lines in reproductive senescence. Within the age span covered in this experiment (ages 1 to 4 weeks) our results

are in agreement with most studies of age-specific genetic effects on a number of traits, including fecundity (Engstrom *et al.* 1989; Tatar *et al.* 1996), age-specific mortality (Hughes and Charlesworth 1994; Charlesworth and Hughes 1996; Promislow *et al.* 1996; Tatar *et al.* 1996; Hughes *et al.* 2002; Snoke and Promislow 2003), and aspects of male mating success (Kosuda 1985; Hughes 1995; Charlesworth and Hughes 1996; Hughes *et al.* 2002; Snoke and Promislow 2003). The lack of a significant genetic correlation between early and late-age fecundity adds additional support for the MA theory because this model assumes that the effects of alleles on fitness early in life are uncorrelated with allelic effects on fitness later in life (Partridge and Barton 1993). Under the AP model we would expect to see a negative genetic correlation between early and late-age fecundity. On the basis of these summary statistics we can conclude that MA contributes to the variation in age-specific fecundity in our mapping population.

To examine evidence supporting the AP theory, we compared the results of this study with our earlier study mapping QTL affecting the life span of mated males and females in the same population of RIL (Leips and Mackay 2002). The earlier experiment differed from the current one in that the life-span measurements were made on the offspring of the cross between each RIL and the inbred parental strains, *Ore* and *2b*. Thus, the allelic effects of QTL on life span were estimated in different genetic backgrounds. However, there was only one instance in which life-span QTL were shown to interact epistatically in that earlier study and neither of the two QTL involved were those identified as fecundity QTL. Therefore, comparison of the additive allelic effects of fecundity QTL on life span using these two studies is appropriate in looking for evidence supporting the AP theory. While the antagonistic relationships assumed by AP could presumably exist between any number of traits that influence fitness at early age and that are negatively correlated with longevity (Leroi *et al.* 2005), one of the most commonly observed trade-offs that support the AP theory is the trade-off between early age fecundity and life span (Rose and Charlesworth 1981; Rose 1991; Marden *et al.* 2003; Leroi *et al.* 2005). Application of this theory to our QTL mapping studies would predict a negative correlation between early age fecundity and life span among the RIL. At the QTL level we would expect that QTL affecting life span and early age fecundity should colocalize and that the allelic effects at these loci should have opposite effects on these two traits. To investigate these possibilities, we first calculated the correlation between the average mated life span of males and females from our earlier study with early and late-age fecundity. As predicted by AP, we did find a significant negative correlation ($r = -0.43$, $P < 0.0001$) but interestingly this correlation was between early female fecundity and the life span of *males* from the RIL × *Ore* cross. At the QTL level, both of the early age fecundity QTL colocalize with QTL affecting life span and alleles at these loci do indeed have antagonistic effects. Oddly enough and in accordance with the correlation analysis described above, it is the life span of *males* and not females that exhibits antagonistic allelic effects with female fecundity at early age. At the QTL on the second chromosome the *Ore* allele increases fecundity by 0.78 eggs/day compared to the *2b* allele but decreases male life span by 4 days. The allelic effects at the QTL on the third chromosome are similar to those at the QTL on the second; the *Ore* allele increases early age fecundity by 0.74 eggs/day but decreases male life span by 5 days. Thus, it appears that the allelic effects of these QTL may exhibit sexual antagonism with alleles having an advantageous effect on one sex but a deleterious effect on the other. Sexual antagonism in QTL studies is not unusual (Nuzhdin *et al.* 1997; Leips and Mackay 2000; Vieira *et al.* 2000; Wayne *et al.* 2001) although neither is it universal (Curtsinger and Khazaeli 2002). Another possible explanation is that male longevity is genetically correlated with male fecundity (which was not measured) and so would indicate a positive correlation between male and female fitness. It should be noted here that the allelic effects at these QTL potentially represent the combined effects of many genes within the QTL region. Therefore, the actual genes that contribute to the variation in fecundity may be in the same region as, but distinct from, those affecting male life span. Only by identifying the actual loci underlying the variation in these traits can this issue be resolved. Also, genotype-by-environment interactions can influence the sign and magnitude of the allelic effects of QTL (Leips and Mackay 2000; Vieira *et al.* 2000) and it may well be that under different environmental conditions a trade-off between fecundity and female life span would be evident. Marden *et al.* (2003) found just such a situation in studying the combined influence of the *Indy* mutation on life span and fecundity. A trade-off between these traits was evident only when flies were reared on a calorically restricted diet. Confirmation of the degree to which particular alleles contribute to trade-offs among traits will require that we not only identify the loci that affect these traits, but also observe the allelic effects on all traits affected in a range of ecologically relevant environments.

From QTL to gene: Identification of QTL with age-specific effects on life-history traits represents the first of many steps toward understanding the complexities of the genetic basis of variation in these traits. Identification of the actual genes that contribute to the variation in fecundity identified in this study will require fine-scaled mapping of the QTL regions using crosses to deficiency strains and complementation tests to candidate genes within refined QTL regions (*e.g.*, Pasyukova *et al.* 2000). Once candidates are identified, testing for the effects of naturally segregating variation on

fecundity can be accomplished by association mapping studies (*e.g.*, De Luca *et al.* 2003). The QTL regions identified in this study contain many candidate genes that are involved in some aspect of reproduction/oogenesis and might contribute to the variation in this study. These genes include *daughterless*, *rho-6*, *zucchini*, *kekkon-1*, *vasa*, *cactus*, and *kelch* on the second chromosome and *maelstrom*, *jim*, *rpk*, *bicoid*, *poached*, and *squid* on the third chromosome. As there are hundreds of genes within each of these QTL regions, most of unknown function, resolving the actual loci contributing to variation in fecundity will require additional mapping efforts.

One limitation of this study, which is indeed a limitation of QTL studies in general, is that only a limited sample of genetic diversity is represented in our lines. Also, the mapping population used was derived from two inbred laboratory strains and not isolates from a natural population. As such, the generality of our results needs to be tested by repeating this study with independent lines ideally constructed from a natural population. The fact that the parental strains did not differ in fecundity but we were still able to map QTL in the RIL derived from them is not unusual (*e.g.*, Leips and Mackay 2000; Vieira *et al.* 2000). These results suggest that QTL with positive and negative effects on fecundity that were fixed for each parental strain were revealed when they appeared in different combinations in the RIL.

Assuming that we can make use of the rapidly developing technological tools to identify and characterize the genetic architecture of life-history traits at the molecular genetic level, many questions will remain after we have the loci in hand. For example, given genetic variation in the age-specific expression of a trait, does variation result from the action of a different subset of genes acting on the trait at different ages or does variation arise from differences in the influence of particular alleles at the same genes with age? Do physiological changes with age modulate allelic effects on traits (in a fashion similar to genotype-by-environment interactions)? And if so, what are the relevant physiological changes that alter these allelic effects? Such questions represent a few of the many challenges that remain in understanding the genetic basis of life-history variation.

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