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Quantitative trait loci for lifespan of mated *Drosophila melanogaster* affect both sexes

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Summary

The properties of alleles at quantitative trait loci (QTLs) contributing to variation in lifespan should be described to determine the mechanisms of evolution of life length and to predict its future changes. Previously, we and others conducted genome-wide screens for QTLs that segregate among one panel of recombinant inbred lines (RILs) using a dense molecular marker map. In non-stressful conditions, QTLs effecting the lifespans of virgin females and males were frequently sex specific. In an unrelated panel of RILs, the effects of QTLs in flies maintained in cages with mixed sexes were similar in both sexes. Here, we re-measured the lifespans of the former panel of RILs in cages with mixed sex cohorts. Lifespan declined owing to mating. The amount of decline correlated between sexes within lines. QTLs mapping to the intervals 15A–19C, 50B–57C, 63A–65A, and 96F–99B had similar effects on the lifespans of both males and females. These QTLs have previously been detected in virgin flies surveys and had sex- and/or environment-specific effects.

1. Introduction

Limited lifespan and senescence (the decline in survival and fertility with increasing age) are near-universal properties of organisms. We want to understand how we senesce or, in other words, what developmental or metabolic pathways are prone to increasing mortality with advancing age. These are identified as mutations with strong effects on duration of life, such as genes affecting the regulation of metabolism (Ewbank *et al.*, 1997), regulation of growth arrest and tumour suppression (Coates *et al.*, 1997), response to ultraviolet irradiation (Jazwinski, 1996), telomere shrinking (Bodnar *et al.*, 1998) and others (lifespan candidate pathways). It is also interesting why we senesce or, in other words, why organisms are made in such a way that the old are less fertile and more likely to die than the young. The evolutionary explanation is that senescence and limited duration of life might arise because weak natural selection late in life allows the accumulation of mutations with deleterious late-age effects that are either neutral (the mutation accumulation hypothesis) or beneficial (the antagonistic pleiotropy hypothesis) earlier in life (Charlesworth & Partridge, 1997).

It is thus interesting to understand the properties of alleles affecting lifespan. Both mutations accumulated in common isogenic background and alleles distinguishing independent wild-type stocks have been studied. In the former case, mutations were either induced (for instance, by transpositions of *P*-element derivatives (Stearns & Kaiser, 1996)) or spontaneous (Houle *et al.*, 1994; Pletcher *et al.*, 1998). Their effects were tested to infer cross-age correlations in mutational effects, patterns of pleiotropy and other important parameters required to verify evolutionary explanations of patterns of ageing (Charlesworth, 2001). In the latter case, QTL mapping experiments were undertaken both to map alleles affecting lifespan and to describe their properties. Using a dense molecular map, Nuzhdin *et al.* (1997) have conducted a genome-wide screen for QTLs segregating among a panel of recombinant inbred lines (RILs) and affecting the lifespan of individually grown virgin flies. Vieira *et al.* (2000) extended this study by measuring lifespan in five environments for mass-reared flies: standard culture conditions, high and low temperature, heat shock, and starvation stress. Leips & Mackay (2000) estimated the dominance of alleles affecting lifespan and alleles

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effecting sensitivity to larval crowding. There was highly significant genetic variance for lifespan between the RILs within each sex and environment. This variance was accounted for by a few QTLs. Interestingly, in non-stressful conditions, QTL effects were frequently sex specific. In a series of experiments with a different set of RILs, Curtsinger and colleagues observed a high degree of cross-sex correlations between QTL effects in mixed-sex cohorts (Curtsinger *et al*., 1998; Resler *et al*., 1998; Curtsinger & Khazaeli, 2002). Sex-limited effect of lifespan QTLs might thus be specific to the genotype of RILs or to the environments tested, including mating opportunities and the maintenance in cages. Here, we re-map QTLs affecting the lifespan of mated flies using the panel of RILs in which virgin lifespan QTLs were mapped in multiple environments. Although these RILs have undergone multiple generations between measurements, marker-based inferences should not be biased because newly accumulated mutations are not in linkage disequilibrium with the markers across RILs.

We expect to identify additional QTLs affecting the lifespan of mated flies. In insects, there is a marked cost associated with reproduction, typically seen as a strongly increased death rate (see Chapman *et al*., 1998). First, mating itself is energy demanding for both males and females (Fowler & Partridge, 1989; Partridge & Fowler, 1990). Second, the lifespan of females is decreased by egg production (Maynard Smith, 1956, 1958), affected by the act of mating itself, the amount and content of the transferred seminal fluid, the amount of protein in the diet, the availability of oviposition sites and so forth (Chapman *et al*., 1995, 1998). As an extreme point of view, one might envision the flies' life as a mixture of physiological modes with different demographic schedules of fertility and survival: a waiting mode in which both mortality and reproduction are low, and a reproductive mode in which mortality is very low at the onset of egg laying but accelerates as eggs are laid (Carey *et al*., 1998). It is thus interesting to compare the QTLs for lifespan of mated flies with those affecting the flies reproduction (Wayne *et al*., 2001).

2. Materials and methods

(i) Drosophila melanogaster *lines*

The origin of the homozygous line Canton S (Can) was described by Nuzhdin *et al*. (1998), and the origin of the RILs by Nuzhdin *et al*. (1997). Briefly, the F_1 progeny of the parental lines Oregon R (Lindsley & Zimm, 1992) and 2b (Pasyukova & Nuzhdin, 1993) were backcrossed to 2b, and the backcrossed progeny were randomly mated for four generations. At generation five, 200 individual pairs were selected and their progeny were inbred by full-sib mating for 25 generations. The 98 lines that survived inbreeding were maintained by mass matings of ~20 pairs for ~40 generations before the measurements were conducted. The genetic constitution of each RIL was previously determined by the analysis of 92 *roo* polymorphic cytological positions of TEs on polytene chromosomes and a visible marker spa^{pol} that are fixed within the parental lines but segregate between them.

(ii) *Lifespan measurements*

For each measurement of lifespan, all lines were multiplied for one generation at a density of ten pairs per vial and six vials per line to obtain a sufficient number of individuals for testing. In the following generation, ten pairs of flies were placed in each of ten vials per line for 3 days. Virgin males and females were collected at 8-h intervals over a 24-h period, 9 days after the parents were discarded. The following day, 25 males and females per RIL were placed in population cages made from 500 ml plastic dixie cups with attached glass vials containing 8 ml of cornmeal–agar–molasses medium plus yeast. Dead flies were removed by aspiration and deaths recorded at 2-day intervals. The food was changed every fourth day.

For each line and sex, we obtained two measurements of lifespan. For the first, homozygous RIL flies were mated within lines. A few flies escaped during the experiment, and 4775 deaths were recorded in total. For the second measurement, the lifespans of RIL males (or females) mated with Can females (or males) were scored. For this, Can flies were multiplied for a total of two sets of 300 vials, one transferred 6 days after the other (parents were discarded after three days) to ensure weekly replacement of mates for RIL males and females. Each sixth day, five Can flies were removed from each of the cages and replaced with five freshly collected (within the previous week) Can flies. Measurements are missing for males of four, and females of one, RIL. The deaths were recorded for 4149 flies.

(iii) *Statistical analysis*

The analysis of means and variances was performed using MEANS and GLM procedures (SAS Institute, 1988). QTLs for each of the traits were detected using composite interval mapping (Jansen & Stam, 1994; Zeng, 1994), as implemented by the QTL Cartographer 1.16 software (Basten *et al*., 1994, 1999, http://statgen.ncsu.edu/qtlcart/cartographer.html). We tested the hypothesis that an interval flanked by two adjacent markers contains a QTL affecting the trait, while simultaneously controlling for the effects of chromosomally linked QTLs. 76 cytological markers were used with the parameters 6 (model) and 30 (window size) for consistency with the analysis of Viera *et al*. (2000). The conditioning markers were automatically chosen by stepwise forward–backward regression. The likelihood-ratio test statistic (LR) is $-2\ln(L_0/L_1)$,

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Table 1. *Line means for different measurements of lifespan*

Measurement	Sex	Number of scored lines	Life span (standard error)	σ_p
Males and females caged individually[1]	Male	98	41·88 (1·88)	9·51
	Female	98	49·53 (2·08)	10·51
Males and females caged jointly	Male	98	26·53 (1·50)	7·59
	Female	97	21·83 (1·32)	6·62
RILs caged jointly with Can	Male	94	31·73 (1·32)	6·52
	Female	97	23·27 (1·18)	5·95

[1] Nuzhdin *et al*., 1997.

where L_0/L_1 is the ratio of the likelihood under the null hypothesis (there is no QTL in the interval) to the alternative hypothesis (there is a QTL in the interval). An empirical distribution under the null hypothesis of no association between any of the intervals and trait values was obtained by randomly permuting the trait data 1000 times and calculating the maximum *LR* statistics across all intervals for each permutation (Doerge *et al*., 1997). *LR* statistics from the original data that were exceeded fewer than 50 times by the maximum *LR* statistics from the permutation are significant at $p=0{\cdot}05$. Because the lifespans of males and females were correlated, we also performed multiple-trait composite interval mapping (Jiang & Zeng, 1995) that largely supported the single-trait analysis.

3. Results and discussion

(i) *Lifespan of mated flies*

The mean lifespan and its phenotypic variance in virgin homozygous flies caged individually (Nuzhdin *et al*., 1997) and mated homozygous flies maintained in small cages are presented in Table 1. As observed previously (Newport & Gromko, 1984), virgin females live significantly longer than virgin males: 49·53 days versus 41·88 days ($p<0{\cdot}0001$). When females are allowed to mate with congenic males, their lifespan is reduced by 56% ($p<0{\cdot}0001$). Similarly, the lifespan of males mated with congenic females is reduced by 37% ($p<0{\cdot}0001$). This reduction is significantly smaller than the reduction of female lifespan ($p<0{\cdot}0001$). This is consistent with the earlier reports for different strains of *D. melanogaster* (Newport & Gromko, 1984). The lifespan of both RIL males and females crossed to Can flies is lower than that of virgin flies (Table 1), although the reduction is somewhat smaller ($p<0{\cdot}0001$ for both males and females). Again, mated males live longer than mated females ($p<0{\cdot}0001$). When two measurements are treated as replicates, the effects of line ($F_{97,97}=3{\cdot}8$) and sex ($F_{1,97}=152{\cdot}8$) on the lifespan variation are significant ($p<0{\cdot}0001$), whereas sex-by-line interaction is not ($F_{97,190}=0{\cdot}72$, $p=0{\cdot}96$). Importantly, lifespans of congenic males and females are also significantly ($p<0{\cdot}002$) correlated (0·31) when males and females are crossed with the unrelated homozygous stock Can and caged separately. We thus predict a strong correlation of QTL effects on lifespan of both sexes.

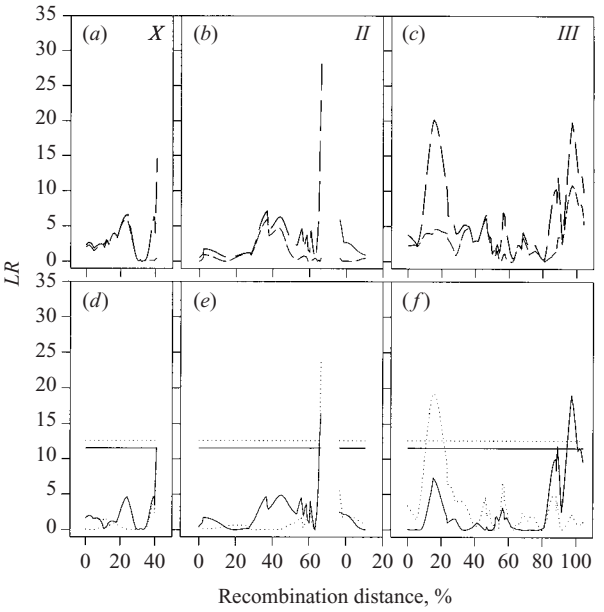

Fig. 1. Quantitative trait loci (QTLs) for lifespan on the three major *Drosophila* chromosomes (X, 2 and 3) for multiple-trait mapping (*a–c*), and for two sexes separately (*d–f*). Long-dashed lines depict mapping outcomes for two sexes simultaneously and short-dashed lines depict outcomes for sex-by-QTL interaction. Solid lines depict the likelihood profiles (log likelihood ratio, *LR*) for males and dashed lines show the same for females. Horizontal lines show statistical thresholds. There was no linkage disequilibrium between markers 50D and 57C, and so mapping in the interval between these markers is impossible and the second chromosome is treated as two separately segregating units.

(ii) *QTLs for lifespan of mated flies*

Multiple-trait analysis testing for the existence of a QTL with effects on both males and females is shown in Fig. 1 *a–c*, and Fig. 1 *d–f* shows analyses for each sex separately. QTL peaks for mated males and females show remarkable similarity in position and effects. The 2b allele of a QTL at 15A–19C decreases male lifespan

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Table 2. *Effects of lifespan QTLs across environments. Treatments: C, control; HD, high density*[1]*; HS, heat shock; HT, high temperature; LD, low density; LT, low temperature; S, starvation*[2]

Cytological (recombination) QTL position	Effects uncovered in previous studies
X, 15A–19C (0·40–end)	A female-specific QTL (*Ls6*) with antagonistic pleiotropic effects in C and HS environments[2]
II, 50B–57C (0·65–undefined)	Male-specific QTL (*3*) affecting increasing lifespan at high density[1]
III, 63A–65A (0·10–0·23)	Region (*Ls10*) affects lifespan at HT and has opposite effects in males and females
III, 96F–99B (0·86–0·90)	A male-specific QTL decreasing lifespan[3] A female-specific QTL (*Ls17*) with HS-specific effect[2]

[1] Leips & Mackay, 2000.
[2] Vieira *et al*., 2000.
[3] Nuzhdin *et al*., 1997.

by 3·4 days and female lifespan by 2·8 days. For QTLs at 50B–57C, 63A–65A and 96F–99B, the effects are −3·3 and −3·7, 1·5 and 2·7, and −4·3 and −2·1 days, respectively. It is interesting to note that QTLs in similar chromosomal regions were detected in previous mapping studies with these lines (Nuzhdin *et al*., 1997; Vieira *et al*., 2000; Leips & Mackay, 2000) but showed sex- and environment-specific effects (Table 2). Interestingly, Leips & Mackay (in press) measured the lifespan of mated flies of the same set of RILs maintained in vials. Sex-by-line interaction was highly significant, and four out of five detected QTLs had sex-limited effects. Three of these QTLs were within an interval 96F–99B identified in this study. The QTL at 63A–65A is located with the one (N14) partly accounting for selection response for extended lifespan (Resler *et al*., 1998).

Our analysis was robust to the model parameters except for the window size. With the 5 cM window, two more QTLs at 69D–76A and 85F–88E affected female lifespan by 2·9 and −3·4 days. These QTLs should be thought of as suggestive because few RILs have recombination breakpoints dissociating these closely positioned QTLs of similar magnitude but opposite sign. The complex picture of multiple closely linked QTLs was previously detected in these regions. Close to the region 69D–76A, Vieira *et al*. (2000, Table 4) detected three QTLs (*Ls12–14*) with female- and/or male-specific effects in different environments. Leips *et al*. (2000, Table 7) detected two QTLs (*4*, *5*) that increased male and female lifespan at low density but decreased female lifespan at high density; the followup precise scale deficiency mapping in this region revealed at least four QTLs (Pasyukova *et al*., 2000). In the region 85F–88E, both studies uncovered female-specific QTLs that decreased lifespan in control, heat-shock and low-density environments.

It appears that, by studying mated flies, we uncovered a subset of QTLs known to affect lifespan of virgin flies. This is a remarkable observation given the different fly life styles between treatments. We cannot currently exclude the possibility that this correspondence of QTL positions is by chance only, and that the QTLs detected here affect lifespan through reproduction-related mechanisms. Indeed, lifespan is strongly influenced by reproduction and associated stresses, for which genetic variation has been documented. Exposure of females to males (even without mating) makes the males court and hence waste energy, and causes the females stress. The lifespans of both sexes decline, the amount of decline varying with the males' genotype (Partridge & Fowler, 1990). Mating itself has associated costs on lifespan, and female remating speed in laboratory conditions varies significantly not only with female, but also with male, genotype (Van Vianen & Bijlsma, 1993). Seminal fluid transferred from males to females influences the egg-laying and remating frequency of the female, in turn affecting the lifespan. Genetic variance in both male seminal fluid and female reaction to it has been firmly established (Clark *et al*., 1995; Clark & Begun, 1999). Egg production shortens the life of *D. melanogaster* (Partridge *et al*., 1987). Abundant genetic variation for reproduction and its effects on lifespan are well established. It is interesting that two of the QTLs we have mapped are localized with the ovariole-number QTLs detected by Wayne *et al*. (2001).

Comparison of the data obtained here with those available from earlier studies hints at other interesting patterns. In the experiments of Mackay and colleagues, sex-limited effects were seen in flies maintained in standard environments, whereas sex-shared effects were seen in stressful environments. Out of five QTLs significant in at least one sex found by Nuzhdin *et al*. (1997), the effects in two sexes had the same sign in two, and different signs in three. Out of five QTLs significant in at least one sex in control environment in Vieira *et al*. (2000), three had the same sign and two different

signs across sexes. Overall, five QTLs had the same sign and five had different signs in two sexes, suggesting that their effects were uncorrelated between sexes. In stressful conditions, seven out of seven QTLs (with the main effect significant in at least one environment (Vieira *et al*., 2000)) had sex-shared effects (the sign test rejects sex-limited effect of these QTLs at $p<0{\cdot}01$). Unfortunately, Leips & Mackay (2000, in press) reported only effects that were significant after Bonferroni correction (i.e. mostly in one sex), and their data cannot be used to construct the sign test. QTLs mapped here have effects shared between sexes; the QTL-by-sex interaction term is only comparable with the main effect for the region 96F–99B (Fig. 1), and this term is not significant as evaluated by ANOVA (data not shown). Effects of four out of four QTLs (six out of six if we take into account QTLs at 69D–76A and 85F–88E) have the same sign in both sexes. Similarly, five out of five QTLs detected by Curtsinger & Khazaeli (2002) had the same sign in the two sexes. We conclude that, in cage-maintained mixed-sex cohorts of two genetically different sets of RILs, effects of QTLs affecting lifespan are shared between sexes (the sign test rejects sex-limited effect of these QTLs at $p<0{\cdot}01$ for combined data). Although the above patterns are interesting, they are suggestive at best because none of these proportions are significantly different from each other ($\chi^2=1{\cdot}63$ for the comparison of 5/10 and 9/9). Much more experimental data will be necessary to derive properties of QTLs as related to mating and/or stress.

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