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Quantitative trait locus mapping of fitness-related traits in *Drosophila melanogaster*

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Summary

We examined the genetic architecture of four fitness-related traits (reproductive success, ovariole number, body size and early fecundity) in a panel of 98 *Oregon-R* × *2b3* recombinant inbred lines (RILs). Highly significant genetic variation was observed in this population for female, but not male, reproductive success. The cross-sex genetic correlation for reproductive success was 0·20, which is not significantly different from zero. There was significant genetic variation segregating in this cross for ovariole number, but not for body size or early fecundity. The RILs were genotyped for cytological insertion sites of *roo* transposable elements, yielding 76 informative markers with an average spacing of 3·2 cM. Quantitative trait loci (QTL) affecting female reproductive success and ovariole number were mapped using a composite interval mapping procedure. QTL for female reproductive success were located at the tip of the *X* chromosome between markers at cytological locations 1B and 3E; and on the left arm of chromosome *2* in the 30D–38A cytological region. Ovariole number QTL mapped to cytological intervals 62D–69D and 98A–98E, both on the third chromosome. The regions harbouring QTL for female reproductive success and ovariole number were also identified as QTL for longevity in previous studies with these lines.

1. Introduction

Evolutionary biologists are often surprised at the amount of genetic variation present in natural populations. Fisher's Fundamental Theorem states that the response to natural selection for fitness is equal to the additive genetic variance of fitness. In an equilibrium population in a stable environment, therefore, little genetic variation for fitness is expected and it will be primarily non-additive (Fisher, 1958). However, genetic variation for quantitative traits, including those closely related to fitness (Houle *et al.*, 1996), is widespread, and it is important to determine what evolutionary forces act to maintain it.

There are several, non-mutually exclusive mechanisms that can act to maintain genetic variation for fitness in natural populations. Some fraction of the variation we see must be attributable to a balance between the introduction of new, deleterious alleles in each generation and their subsequent elimination by natural selection (Simmons & Crow, 1977). In addition, variation for fitness may be maintained by one of several balancing selection mechanisms: antagonistic pleiotropy, genotype by environment interaction, and genotype by sex interaction. Long-term selection on an index of fitness components is expected to generate negative correlations between the components, or antagonistic pleiotropy (Robertson, 1955; Rose & Charlesworth, 1981; Rose, 1984). Genotype by environment interaction for alleles affecting fitness can maintain genetic variation in spatially or temporally heterogeneous environments (Levene, 1953; Haldane & Jayakar, 1963; Gillespie & Turelli, 1989). One environment which is heterogeneous by definition is the environment of sex. Autosomes find themselves in the female environment about half the time; and *X* chromosomes spend two-thirds of their time in the female environment. Therefore alleles which are differentially sensitive to their sex environment can

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never be at equilibrium (Rice, 1992, 1996; Mackay *et al*., 1996; Wayne *et al*., 1997).

Efforts in the past to evaluate models of the maintenance of genetic variation have focused on estimating variances and evaluating genetic correlations, to determine whether there are negative genetic correlations between traits (antagonistic pleiotropy) or environments (trade-offs) (Harshman & Hoffmann, 2000). For example, work by Houle *et al*. (1996) comparing mutational and genetic variances suggests that mutation–selection balance may explain most if not all genetic variation (but see Wayne & Mackay, 1998). However, these phenotypic descriptions summarize the net effects of all loci affecting quantitative traits, and conceal any heterogeneity of allelic effects between loci. For example, if the absolute value of the genetic correlation between two traits, $|r_G| = 1$, then we may assume that the same genes are responsible for variation in both traits. However if, as is more common, $|r_G| < 1$, one cannot infer to what extent different genes affect the two traits, and to what extent some loci have opposite effects on the two traits. Further, we have no reason to expect that variation at all of the loci contributing to variance for a trait will be maintained by the same forces.

A quantitative trait locus (QTL) level of analysis is necessary to disentangle the causes of imperfect genetic correlations. Identifying QTL contributing to genetic variation in fitness is a necessary first step towards the identification of the actual genetic loci responsible. Only when we have attained this level of resolution will it be possible to infer the nature of the evolutionary forces maintaining variation for fitness on a locus-by-locus basis. Here we mapped QTL for fitness-related traits in a panel of recombinant inbred lines (RILs). The RIL design is useful for studying traits related to fitness, in which we expect the environmental component to be a large determinant of the phenotype, because of our ability to replicate genotypes and hence to obtain an accurate estimate of the genotypic value of each line within and across environments. We chose two morphological traits: ovariole number (David, 1970; Boulétreau-Merle *et al*., 1982) and body size (Partridge & Farquhar, 1983); and two complex fitness characters: early fecundity and reproductive success. As the same panel of lines has been used previously to map QTL affecting longevity (Nuzhdin *et al*., 1997; Vieira *et al*., 2000), we were able to compute genetic correlations for this suite of traits, as well as to infer whether the same QTL contributed to variation in multiple characters.

2. Materials and methods

(i) *Fly lines*

The mapping stocks used were a set of 98 RILs (Nuzhdin *et al*., 1997) derived from the cross of two isogenic lines, *Oregon-R* (Lindsley & Zimm, 1992) and *2b* (Pasyukova & Nuzhdin, 1993). *Oregon-R* is a standard wild-type strain, and *2b* is an isogenic derivative of a line that was selected for low male mating activity (Kaidanov, 1990). The F1 animals were backcrossed to the *2b* line and were randomly mated for four generations prior to dividing the population into sublines and subjecting them to 25 generations of brother–sister mating. They were maintained subsequently as small mass mating populations of 20 males and 20 females. Ovariole number and body size were measured on the lines approximately 20 generations after the cessation of inbreeding. Reproductive success was measured on the lines approximately 5 generations later; early fecundity was measured approximately 30 generations later.

The competitor strain used in the reproductive success experiment was *ebony 39·36* (Houle *et al*., 1997), an effectively isogenic line derived from the Ives population. The line was subjected to full-sib inbreeding for 40 generations, and was cryopreserved 2 generations after the cessation of inbreeding. It was revived about 10 generations before use in the reproductive success experiment.

All cultures were maintained in 10 ml cornmeal–agar–molasses medium in shell vials at 25 °C.

(ii) *Reproductive success*

Reproductive success (RS) was estimated by a competition assay between each of the 98 RILs and *ebony* (*e*) flies (see above). As *e* is recessive, heterozygous progeny are wild-type, while homozygous *e* progeny are dark-bodied. RS was measured by competing males and females separately.

Flies used for the RS assays were reared from parent vials of 5 inseminated females at 25 °C, which were allowed to lay eggs for 5 days. Multiple parent vials were set up for each line. Virgin males and females were stored on fresh medium for 1–5 days before use. Virgins of the same genotype reared in different vials were combined prior to setting up the competition vials. Flies were anaesthetized with CO_2 during virgin collection and assignment to vials.

Estimates of RS for both sexes were obtained by competing 12 males and 6 females per vial. Pilot experiments indicated that these parameters would yield approximately 50 % $e/+$ and 50 % e/e progeny. For the male RS assay, 6 *e* males competed against 6 recombinant inbred males for matings with 6 *e* females. Female RS was estimated by placing 12 *e* males with 3 recombinant inbred females and 3 *e* females. Flies remained in the competition vials for 5 days. The progeny were frozen 12 and 14 days after establishment of the cultures and were stored in Eppendorf tubes at −70 °C until scoring their *e* phenotype and sex. On average, each vial produced 145 flies.

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The experiment was repeated in two blocks, run 3 weeks apart. Each block consisted of 5 replicate vials for both the male and female RS assays for each of the 98 RILs, for a total of 980 competition vials per block. A grand total of 290598 flies were scored for the experiment.

(iii) *Ovariole number*

Flies from the 98 RILs to be measured for ovariole number (ON) emerged from cultures set up with 5 mated females per vial, which laid eggs for 5 days. Progeny were collected and mated females were aged 5–7 days prior to dissection. Both ovaries were removed and fixed in a saturated solution of potassium dichromate for approximately 5 min before dissection in Ringer's solution (Coyne *et al*., 1991) before being dissected and counted. The number of ovarioles in the left (dorsal view) and right ovaries were recorded separately. Ovariole number was recorded from 4 flies in each of three replicates per RIL, for a total of 1176 flies scored.

(iv) *Body size*

Flies for measurement were reared at 25 °C from parent vials with a density of 5 mated females per vial, as described above. Flies were frozen and stored at −70 °C prior to measurement. Body size was quantified as thorax length (mm), using an ocular micrometer. For each of the 98 lines, 10 males and 10 females were measured from each of two replicates, for a total of 3920 flies.

(v) *Early fecundity*

Early fecundity was assayed by the number of eggs laid over a 24 h period by females aged 3–4 days. Parent vials were reared at a common density as above. Virgin female progeny were collected for 24–48 h and then aged for 48 h in vials with fresh medium supplemented with live dry yeast. The flies were transferred to laying cages the following day and given mates of their respective genotypes. Virgin collection and fly transfer were performed using CO_2 anaesthesia. The cages consisted of 7 shell vials, whose bottoms had been sawed off, glued together in a circle with the seventh vial in the middle. Nylon net, which was too small for the flies to escape through but sufficiently large enough for them to lay eggs through, was placed over the open ends of the vials and secured to the vials by glue (Leech flexible cement) and rubber bands. Three female flies were placed in each of the six exterior vials with three virgin male flies and the other open end closed with rayon balls. The netted end of the cage was placed in a 100×15 mm Petri dish containing approximately 30 ml medium, coloured dark green. The laying cage was attached to the Petri dish by parafilm and placed in a 25 °C Percival incubator on a 12 light/12 dark cycle for 24 h. Cages were then removed and the Petri dishes covered and placed in a −20 °C freezer until they were counted. The experiment was performed with two cage replicates per genotype, for a total of 12 estimates per genotype.

(vi) *Statistical analysis*

All statistical analyses were performed by SAS version 6.10 for the Macintosh or version 6.12 for UNIX, except for QTL mapping (see below). ANOVAs were performed using PROC GLM, and variance components calculated by PROC VARCOMP. Pearson's product-moment correlation was used for correlation analysis (PROC CORR).

Standard errors were calculated from line means rather than from individuals in order to communicate the amount of variation relevant to QTL mapping. Likewise, phenotypic standard deviations were calculated based on variance among line means, because this quantity relates most directly to the average effects of a QTL as estimated here (see below).

(vii) *Quantitative trait locus* (*QTL*) *mapping*

The RILs were genotyped for insertion sites of *roo* transposable elements (Nuzhdin *et al*., 1997). There were a total of 76 informative markers, with an average spacing of 3·2 cM on the standard *Drosophila* map. The informative markers and their estimated map positions, cytological (recombinational) were: 1B (1–0·00), 3E (1–14·85), 4F (1–33·16), 5D (1–39·36), 6E (1–42·43), 7D (1–57·28), 7E (1–58·30), 9A (1–76·81), 10D (1–96·29), 11C (1–99·36), 11D (1–102·43), 12E (1–106·52), 14C (1–126·01), 15A (1–127·03), 17C (1–131·16), 19A (1–134·27), 21E (2–0·00), 22F (2–8·59), 27B (2–61·19), 29F (2–85·53), 30AB (2–87·65), 30D (2–89·77), 33E (2–126·77), 34EF (2–135·75), 35B (2–140·15), 38A (2–146·00), 38E (2–148·49), 43A (2–152·16), 43E (2–153·38), 46C (2–154·52), 48D (2–163·49), 49D (2–169·92), 50B (2–172·04), 50D (2–175·15), 50F (2–178·47), 57C (3–0·00), 57F (3–7·65), 60E (3–39·23), 61A (4–0·00), 63A (4–16·72), 65A (4–43·80), 65D (4–67·70), 67D (4–83·06), 68B (4–103·00), 68C (4–114·92), 69D (4–124·71), 70C (4–142·04), 71E (4–146·59), 72A (4–148·85), 73D (4–153·26), 76A (4–156·75), 76B (4–160·18), 77A (4–162·34), 82D (4–164·65), 85F (4–169·26), 87B (4–177·28), 87E (4–180·67), 87F (4–181·81), 88E (4–192·05), 89B (4–196·56), 91A (4–207·31), 91D (4–215·33), 92A (4–218·65), 93A (4–230·09), 93B (4–231·15), 94D (4–243·34), 96A (4–260·05), 96F

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(4–273·56), 97D (4–284·86), 97E (4–287·06), 98A (4–295·83), 99A (4–313·98), 99B (4–326·17), 99E (4–337·22), 100A (4–338·28), and spa (5–0·00).

QTL mapping was performed using composite interval mapping (Jansen & Stam, 1994; Zeng, 1994). The analyses were performed using window sizes ranging from 5 to 20 cM, and the Kosambi map function. The number of background markers used as covariates in the analyses was determined by forward–backward stepwise regression. Experiment-wise significance thresholds ($\alpha = 0{\cdot}05$) were determined by permutation (1000 shuffled datasets). The estimates of average QTL effects were scaled by the phenotypic standard deviations (σ_P).

3. Results

(i) *Reproductive success*

The analysis variable was the percentage of *ebony* (*e*) flies emerging from each competition vial. We also recorded the sex of the progeny. Initial analyses showed that neither progeny sex nor its interactions with other sources of variation was significant, so this term was consequently dropped from the analyses.

Averaged over blocks, replicate vials and RILs, the proportion of *e* flies (±standard error) was 0·41 (±0·01) from the male RS assays and 0·35 (±0·01) from the female RS assays. Variance in RS among RILs was partitioned into the main effects of Line (*L*, random), Sex (*S*, fixed), and Block (*B*, random), and their two- and three-way interactions by a mixed model, cross-classified ANOVA: $Y = L + S + B + L \times S + L \times B + S \times B + L \times S \times B + Error$. ANOVA was also performed for each sex separately ($Y = L + B + L \times B + Error$).

There was no significant variation among lines, between sexes, or between blocks in the ANOVA pooled across sexes (Table 1). However, the Line × Sex interaction was highly significant, indicating that there is genetic variation for the difference in reproductive success in males and females. In the analyses of sexes separately, there was highly significant variation among lines for female RS, but not for male RS (Table 2). The genetic correlation in RS across sexes, r_{GS}, was calculated using among-line variance components from the analyses of sexes pooled and separately:

$$r_{GS} = \frac{\sigma^2_{Line_{M,F}}}{\sqrt{\sigma^2_{Line_M}\sigma^2_{Line_F}}}$$ (Robertson, 1959).

The estimate of r_{GS} was low: 0·197. The lower and upper confidence limits, −0·001 and 0·381, were computed using Fisher's *z* transformation (Sokal & Rohlf, 1981). The confidence limits include zero; therefore, different genes affect RS in males and females in these lines.

In both the pooled sex and separate sex analyses, all interactions of Line and Sex with Block were highly significant (Tables 1, 2). Similar to the genetic variation for the difference in RS in males and females, there is genetic variation for the difference in RS in different environments, although the nature of this environmental difference is unknown. An estimate of the magnitude of genotype × environment interaction is r_{GE}, where *E* in this case is the uncontrollable and unknown differences between blocks. We performed a one-way ANOVA of RS separately for males and females within each block (data not shown). The among-line variance in female RS was highly significant in each block, as well as across blocks. The estimates of variance components were $1{\cdot}63 \times 10^{-2}$ and $1{\cdot}35 \times 10^{-2}$ for blocks 1 and 2, respectively. For female RS, the estimate of r_{GE} computed as above from variance components was 0·577, with lower and upper confidence limits of 0·428 and 0·696. As the cross-environment genetic correlation is significantly different from both zero and one, some genes for female RS are expressed in both block environments, while others are environment-specific. The extent to

Table 1. *ANOVA and variance components for reproductive success*

Source	d.f.	Type III MS	Variance component $\times 10^{-3}$	% variance
Line	97	0·4107	1·06	1·8
Sex	1	4·5838	Fixed	—
Block	1	1·3882	0·00	
Line × Sex	97	0·2695**	4·80	8·2
Line × Block	97	0·2736**	5·10	8·8
Sex × Block	1	4·6734***	4·64	8·0
Line × Sex × Block	96	0·1691***	11·03	19·0
Rep(Line × Sex × Block)	1564	0·0588***	27·44	47·2
Error	1955	0·0040	4·02	7·0

**$P < 0{\cdot}01$; ***$P < 0{\cdot}001$.

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Table 2. *ANOVA and variance components for reproductive success, sexes separately*

	d.f.	Type III MS	Variance component $\times 10^{-3}$	% variance	d.f.	Type III MS	Variance component $\times 10^{-3}$	% variance
Source	Males				Females			
Line	97	0·4025	3·08	4·0	97	0·2720***	8·57	23·8
Block	1	5·5009***	5·27	6·9	1	0·3926*	0·30	0·8
Line × Block	97	0·3409***	25·99	33·9	96	0·1012***	6·45	17·9
Rep(Line × Block)	784	0·0810***	38·75	50·7	780	0·0367***	16·07	44·8
Error	980	0·0035	3·48	4·5	975	0·0045	4·56	12·7

*$P < 0{\cdot}05$; ***$P < 0{\cdot}001$.

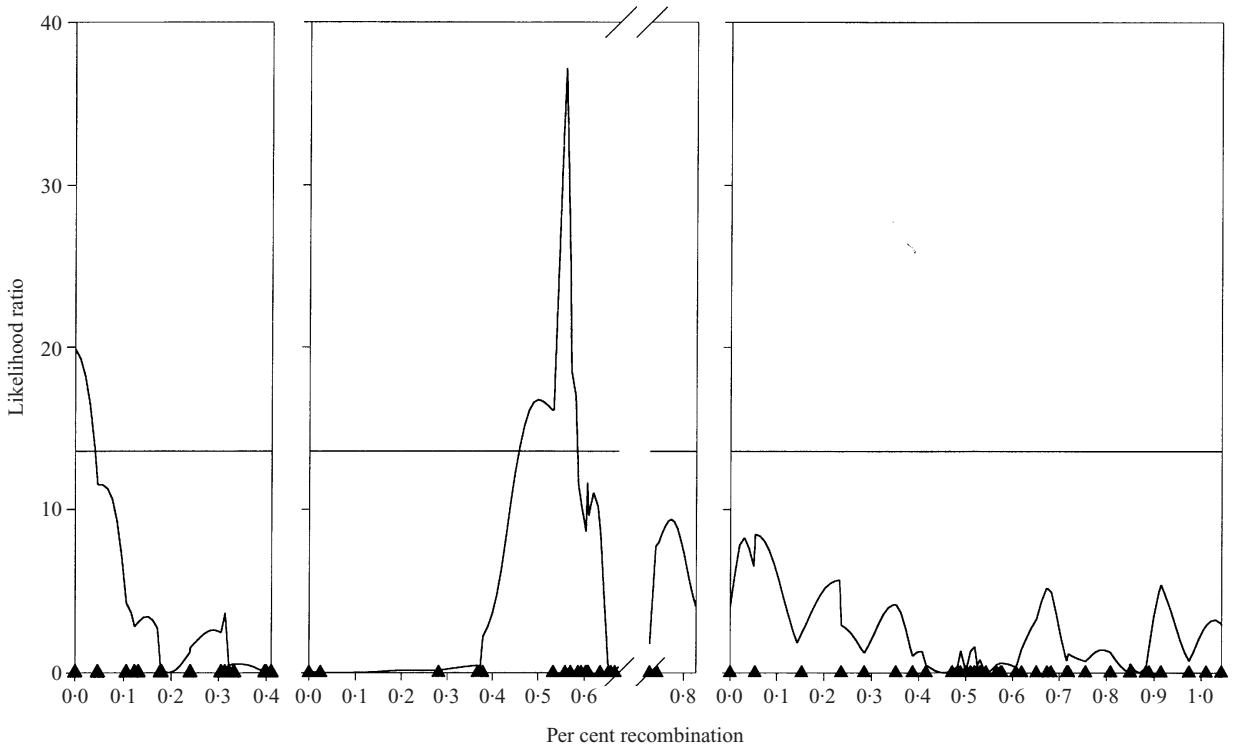

Fig. 1. QTL for female reproductive success. Each panel represents a *Drosophila* chromosome (*X*, *2* and *3*). The triangles represent the cytological markers (listed in Section 2). The axis break in the middle panel depicts a break in the linkage map for chromosome 2. The horizontal line is the significance threshold, determined by permutation as explained in Section 2.

which the phenotypic differences between RILs are attributable to genetic differences is indicated by the ratio $V_G:(V_G + V_R)$, where V_G is the among-line variance component plus half the Line × Block variance component, and V_R is the sum of the variance components between and within replicates. For female reproductive success, this ratio is 0·33.

In contrast, all genetic variation for RS in males is confined to the interactions with Block. The among-line variance in male RS was highly significant within each block. The estimates of among-line variance components were $2{\cdot}65 \times 10^{-2}$ and $3{\cdot}15 \times 10^{-2}$ in blocks 1 and 2, respectively. The estimate of r_{GE} for male RS, again computed from variance components, was 0·106 with lower and upper confidence limits of −0·095 and 0·299. Thus, different constellations of genes affect male RS in different block environments.

This differential sensitivity to the environment of genes affecting female and male RS is reflected in the highly significant Line × Sex × Block interaction term in the ANOVA for RS pooled over sexes and blocks (Table 1).

Although there was significant genetic variation for male RS within each block, we did not map QTL for male RS within blocks since we are only interested in QTL that represent experimentally replicable genetic variation. We mapped QTL for female RS for line means across blocks using composite interval mapping

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Table 3. *ANOVA and variance components for ovariole number*

Source	d.f.	Type III MS	Variance component	% variance
Line	97	93·22***	2·29	15·1
Side	1	301·43***	Fixed	—
Line × Side	97	7·96	0·00	0·0
Rep(Line)	196	39·35***	3·78	25·0
Error	1960	9·07	9·07	59·9

***$P < 0{\cdot}001$.

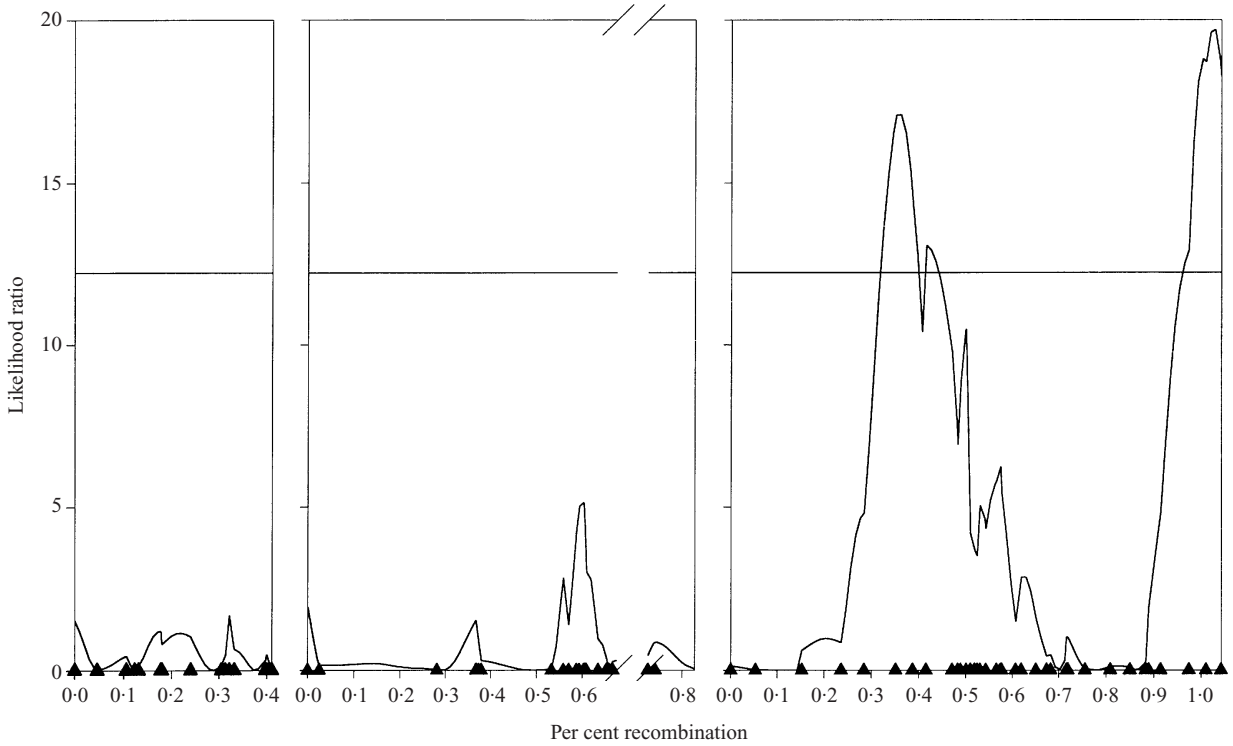

Fig. 2. QTL for ovariole number. As in Fig. 1, each panel represents a *Drosophila* chromosome (*X*, *2* and *3*). The triangles represent the cytological markers (listed in Section 2). The axis break in the middle panel depicts a break in the linkage map for chromosome 2. The horizontal line is the significance threshold, determined by permutation as explained in Section 2.

(Zeng, 1994). The exact results of composite interval mapping can vary according to the size of the window within which marker cofactors were excluded. We explored the stability of the mapping results using window sizes of 5, 10, 15 and 20 cM. The same QTL were found in all analyses, although the size of the chromosomal regions to which the QTL mapped varied. We present the results from the analysis using a 15 cM window, because this is the most conservative analysis (i.e. the intervals containing the QTL are the widest for this parameter value). The experiment-wise significance threshold for this window size is the likelihood ratio (LR) value 13·58. Two QTL exceeded this value: one at the tip of the *X* chromosome, in the interval between the telomere and 3E; and the second on the right arm of chromosome 2 between cytological markers 30D and 38A (Fig. 1). The highest LR statistic (19·88) for the X chromosome QTL was associated with the most distal marker, 1B. The effect of this QTL was $-0{\cdot}008$ phenotypic standard deviations. The peak LR statistic for the second chromosome QTL, 37·18, was associated with the marker at 34EF. This QTL had an effect of $-0{\cdot}016$ phenotypic standard deviations.

(ii) *Ovariole number*

The mean ovariole number (ON) for the RILs was $19{\cdot}69 \pm 0{\cdot}08$. Variation for ON was partitioned using a two-way factorial mixed model ANOVA: $Y = L + D + L \times D + R(L) + Error$, where L (random) and D (fixed) are the cross-classified main effects of Line and Side; and $R(L)$ is Replicate vial nested within Line. The line term was highly significant (Table 3). The

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Table 4. *ANOVA and variance components for body size*

Source	d.f.	Type III MS	Variance component $\times 10^{-4}$	% total variance
Line	97	0·0156	0·91	3·4
Sex	1	14·77***	Fixed	—
Line × Sex	97	0·0024	0·00	0·0
Rep(Line)	97	0·0129***	4·78	17·2
Sex × Rep(Line)	98	0·0034***	1·39	5·2
Error	3528	0·0020	19·70	73·6

***$P < 0{\cdot}001$.

Table 5. *ANOVA and variance components for fecundity*

Source	d.f.	Type III MS	Variance component	% variance
Line	95	2844·1	29·6	4·6
Cage(Line)	87	2527·6***	392·7	61·5
Error	896	215·9	215·9	33·9

***$P < 0{\cdot}001$.

extent to which the phenotypic differences between RILs are attributable to genetic differences is indicated by the ratio $V_G:(V_G + V_R)$, where V_G is the among-line variance component and V_R is the sum of the variance components between and within replicates. This ratio is 0·15 for ON. There was also highly significant variation for ON between the right and left ovaries. The significant D term indicates the presence of directional asymmetry. However, there is no genetic variation for asymmetry, as indicated by the non-significant $L \times D$ term.

QTL were mapped for ON under a range of parameter values as described above, and again, the most conservative window size was 15 cM. The experiment-wise significance threshold was an LR value of 12·23, as determined by permutation. Two QTL for ON exceeded the significance threshold, both on chromosome 3 (Fig. 2). The first ON QTL was on the left arm of the chromosome between cytological positions 62D and 69D, with a peak LR of 17·10 between 68B and 68C. The effect of this QTL was −2·71 phenotypic standard deviations. The second ON QTL was at the tip of the right arm between cytological positions 98A and 98E. The peak LR statistic of 19·70 occurred in the 99B to 99E interval; the effect of this QTL was −2·98 phenotypic standard deviations.

The discovery of QTL on the third chromosome for ovariole number is consistent with our previous demonstration of significant segregating variation in natural populations for these traits on the third chromosome (Wayne *et al*., 1997). Earlier work also localized genes contributing to variation in ovariole number to the third chromosome (Thomas-Orillard, 1975).

(iii) *Body size*

The mean body size for males was 0·846 mm ± 0·001 and for females was 0·969 mm ± 0·001. Variance for body size among RILs was partitioned using a two-way factorial mixed model ANOVA: $Y = L + S + L \times S + R(L) + Error$, where L (random) and S (fixed) are the cross-classified main effects of Line and Sex; and $R(L)$ is Replicate vial nested within Line. The only significant term in this analysis was the main effect of sex (Table 4); no significant genetic variation for body size was observed.

(iv) *Early fecundity*

The mean early fecundity across RILs was 45·3 eggs ± 1·86. Variance in female fecundity was analysed by a random-effects, nested ANOVA according to the model $Y = L + C(L) + Error$, where L is Line and Cage (C) is nested within Line. No significant genetic variation for early female fecundity was observed under these conditions (Table 5).

Discussion

(i) *Genetic architecture for fitness traits in RILs*

We observed significant variation among this panel of RILs for female reproductive success and for ovariole number, but not for male reproductive success, body size (either sex) or early female fecundity. It is unlikely that the genetic variation in female RS observed could be due to differences in egg-laying capacity and hence systematic differences in rearing density among the RILs: there was no detectable genetic variance in

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fecundity among these lines, nor was there significant genetic variance detected for body size, which would be expected to be affected by density differences.

The differences in genetic architecture for male and female RS are perhaps not surprising. While both assays measured viability and larval competitive ability, there were also traits specific to each assay. The estimate of male RS includes male mating success and characters such as sperm precedence, and the estimate of female RS includes early fecundity and female components of sperm precedence. Lack of genetic correlation between female and male RS could thus be attributable either to genetic variation in female RS-specific traits or to female-specific alleles in the common traits. Sex-specific genetic variation has been observed for other life history traits (e.g. viability: Wayne and Mackay 1998; and longevity: Maynard-Smith, 1959; Nuzhdin *et al*., 1997, Leips & Mackay, 2000; Vieira *et al*., 2000); therefore the latter explanation cannot be excluded. It was, however, surprising that no genetic variation was observed for male RS, given that the *2b* parental line was derived from a strain selected for low male mating activity. This suggests the possibility of a trade-off between mating activity and another male fitness component, which bears further investigation.

Likewise, the genetic architecture of female reproductive success was distinct from that for ovariole number. The product-moment correlation of line means for female RS and ON was $-0{\cdot}110$, with lower and upper confidence limits of $-0{\cdot}302$ and $0{\cdot}091$, suggesting that different genes affect variation for these traits in this set of lines. This inference is supported by lack of concordance of map positions of QTL affecting these traits.

As a number of traits have been measured on this set of RILs, we had an opportunity to estimate genetic correlations (r_G) between traits and to compare the map positions of QTL identified in different composite interval mapping analyses. There is a highly significant negative correlation between ON and male longevity, using the data of Nuzhdin *et al*. (1997) ($r_G = -0{\cdot}225$, $P = 0{\cdot}001$). Consistent with this observation, the map positions bracketed by ovariole number QTL (68B–68C and 99B–99E) are both identified in Nuzhdin *et al*. (1997) as QTL affecting male longevity. The coincidence in map position of ovariole number QTL and QTL for longevity is not likely to have occurred by chance. Nuzhdin *et al*. (1997) detected four male-specific QTL, each with a peak LR associated with one of the 76 markers. Thus, the probability of detecting any single QTL affecting another trait at the same location as one of the male longevity QTL is 4/76, or 0·053. The probability of both ovariole number QTL coinciding with two male-specific longevity QTL is therefore 0·0028. The negative correlation between male longevity and ovariole number is supported by the observation of a significant negative correlation between dry weight of 'young' ovaries and longevity across sexes (Rose *et al*., 1984). We have found suggestive negative associations between ovariole number and male fitness before (Wayne *et al*., 1997). Negative genetic correlations between the sexes at the level of the QTL for longevity (Vieira *et al*., 1999) are also consistent with these observations. The relationship between these two traits bears further investigation in other laboratory and natural populations.

At the level of correlations among line means, there are positive but not formally significant correlations between ON and female longevity using the data of Vieira *et al*. (2000) ($r_G = 0{\cdot}122$, $P = 0{\cdot}075$) and between female reproductive success and female ($r_G = 0{\cdot}131$, $P = 0{\cdot}056$) and male longevity ($r_G = 0{\cdot}130$, $P = 0{\cdot}057$) using the data of Nuzhdin *et al*. (1997). However, non-significant correlations at the level of line means do not preclude significant associations at the level of QTL. For example, the ON QTL between 68B and 68C corresponds to a female longevity QTL in the same region identified by Vieira *et al*. (2000). Nuzhdin *et al*. (1997) identified two closely linked, sex-specific chromosome 2 QTL for longevity that are both in the region spanned by our female RS QTL. Interestingly, marker 1B, the other female RS QTL, was identified as a female-specific longevity QTL by Vieira *et al*. (2000), although there is no significant correlation of line means between female RS and female longevity using these data.

(ii) *Genotype × environment interaction for fitness traits*

One of the most striking results of this experiment was the exceptional sensitivity of genetic variation affecting RS to slight, random differences in the environment between nearly contemporaneous assays, conducted under highly standardized laboratory conditions. In these RILs, male RS was more sensitive to small, stochastic environmental fluctuations than female RS: the genetic correlation of female RS across blocks was 0·577, while the genetic correlation of male RS across blocks was 0·106 (not significantly different from zero). Vieira *et al*. (2000) examined the cross-sex and cross-environmental correlations of adult longevity in these RILs in five environments: standard culture conditions, high and low temperature, and heat shock and starvation stress. The genetic correlation of longevity across sexes and environments was not significantly different from zero, and all QTL detected were sex- and/or environment-specific. Taken together, these data may indicate that allelic sensitivity may be a hallmark of genetic variation for life history traits, although this hypothesis needs to be evaluated for other traits and other lines. It is possible that the

homozygosity of the RILs may exacerbate this environmental sensitivity. However, Leips & Mackay (2000) observed similar environment- and sex-specific effects for longevity in a largely heterozygous background. If generally true, variable allelic effects in the face of environmental heterogeneity, such as encountered in nature, could contribute to maintenance of genetic variation for life history traits.

(iii) *Relevance of RILs to naturally occurring variation for fitness*

Fitness is arguably the most complex of all quantitative traits, since mutations at every gene are potentially capable of affecting fitness. If we wish to study genetic variation for fitness then we must begin with the simplest possible scenario: two segregating genomes. In nature, we expect the gene frequencies of alleles affecting variation in fitness to be extreme. Thus, the contribution of any one locus to the total genetic variation in fitness will be small. The use of two genomes in a recombinant inbred design maximizes genetic variation attributable to any one locus, because gene frequencies are intermediate (Falconer & Mackay, 1996). However, this increase in power to detect segregating variation for fitness comes at the expense of generality: only a small subset of the total segregating variation in natural populations is sampled. Further, we can make no inferences about gene frequencies in natural populations. And even in this simplified genetic scenario and with our ability to obtain multiple measurements of each RIL genotype, very large sample sizes were necessary to map QTL for reproductive success: nearly 300000 individuals were scored.

The ultimate goal of QTL mapping is to identify genetic loci affecting the quantitative trait of interest. In *Drosophila*, this effort is facilitated by the availability of deletion and *P*-element stocks, as well the complete genome sequence (Adams *et al*., 2000). Until actual genetic loci affecting life history variation are identified, it will not be possible to make inferences about gene frequencies or allelic effects in other genetic backgrounds and in other environments. However, given the large sample size necessary to detect segregation using RILs, a quantitative genetic study of individual fitness loci in natural populations is not feasible, given entire segregating genomes, extreme allele frequencies at loci affecting variation in fitness and uncontrolled environmental variation. Fortunately, once we have identified the genetic loci, such an experiment is largely unnecessary: we can make inferences about the relevance of the gene to fitness in natural populations by using the tools of molecular population genetics and statistical tests of neutrality (Kreitman, 1991; Wayne & Simonsen, 1998).

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