DOI: 10.1534/genetics.105.054098

High-Resolution Quantitative Trait Locus Mapping Reveals Sign Epistasis Controlling Ovariole Number Between Two Drosophila Species

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Manuscript received November 30, 2005
Accepted for publication February 11, 2006

ABSTRACT

Identifying the genes underlying genetically complex traits is of fundamental importance for medicine, agriculture, and evolutionary biology. However, the level of resolution offered by traditional quantitative trait locus (QTL) mapping is usually coarse. We analyze here a trait closely related to fitness, ovariole number. Our initial interspecific mapping between *Drosophila sechellia* (8 ovarioles/ovary) and *D. simulans* (15 ovarioles/ovary) identified a major QTL on chromosome 3 and a minor QTL on chromosome 2. To refine the position of the major QTL, we selected 1038 additional recombinants in the region of interest using flanking morphological markers (selective phenotyping). This effort generated approximately one recombination event per gene and increased the mapping resolution by approximately seven times. Our study thus shows that using visible markers to select for recombinants can efficiently increase the resolution of QTL mapping. We resolved the major QTL into two epistatic QTL, QTL3a and QTL3b. QTL3a shows sign epistasis: it has opposite effects in two different genetic backgrounds, the presence *vs.* the absence of the QTL3b *D. sechellia* allele. This property of QTL3a allows us to reconstruct the probable order of fixation of the QTL alleles during evolution.

MOST phenotypic variation within and between species appears to be controlled by multiple genes. Such genes with quantitative effects are more difficult to identify than genes underlying Mendelian traits because of their low penetrance, their environmental sensitivity, and epistasis with other loci (Glazier *et al.* 2002). Interval mapping (Lander and Botstein 1989) and its variants [collectively named quantitative trait loci (QTL) mapping] (reviewed in Manly and Olson 1999; Broman 2001) have been used extensively to map QTL. In such experiments, inbred strains differing in traits of interest are crossed to generate a mapping population. QTL are identified and localized to chromosomal regions by correlating segregating genetic markers with trait values. This approach typically identifies large genomic regions that do not allow identification of the genes involved. The major problem is that an increasingly large number of recombination events is required to map the QTL to an increasingly small interval. Several strategies to increase the number of recombination events in the mapping population, either by using multiple generations of crossing or by selecting for recombination events at targeted loci, have been proposed (reviewed in Darvasi 1998, 2005). Here we exploit selective phenotyping, in which individuals that are recombinant in a region known to contain a QTL are selected for phenotyping (Darvasi 1998, 2005; Ronin *et al.* 2003; see also Jin *et al.* 2004; Xu *et al.* 2005). Such selective phenotyping requires only two generations of crossing to produce the mapping population, but a large number of progeny individuals must be screened. This strategy is commonly employed in mice to narrow QTL regions (see for example Purcell *et al.* 2001). Flies of the genus Drosophila are ideally suited to this approach because they can be reared *en masse* and morphological markers can be used to facilitate the screening for recombinants. This technique, also known as meiotic recombination mapping, is extensively used in *D. melanogaster* to identify genes underlying Mendelian traits. However, to our knowledge, selective phenotyping has not yet been used for QTL mapping in Drosophila. Our analysis highlights some of the advantages and pitfalls of this approach for mapping QTL.

Ovarioles represent the functional units of insect ovaries; they are the tubes in which eggs undergo maturation (Mahowald and Kambysellis 1980). Ovariole number is determined during the early pupal stage (King *et al.* 1968; Hodin and Riddiford 2000). Ovariole number is a fertility trait that is closely related to fitness; the more ovarioles, the more eggs females can potentially lay. Similar latitudinal clines in ovariole number have been found on four continents (David and Bocquet 1975a,b; Lemeunier *et al.* 1986; Capy *et al.* 1993; Azevedo *et al.* 1996; Gibert *et al.* 2004), suggesting that ovariole number is under contemporary natural

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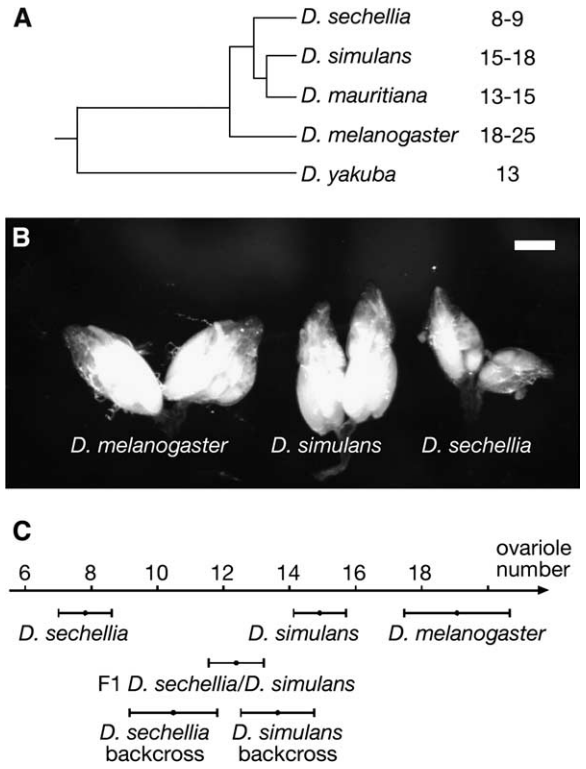

Figure 1.—Variation in ovariole number in the *D. melanogaster* subgroup. (A) Phylogeny (Powell 1997; Harr *et al.* 1998; Ting *et al.* 2000) showing the range in number of ovarioles per ovary for each species (David and Bocquet 1975b; Louis and David 1986; Coyne *et al.* 1991; Hodin and Riddiford 2000). (B) Ovarian morphology in *D. melanogaster*, *D. simulans*, and *D. sechellia*. Bar, 200 μm. (C) Mean ovariole number and standard deviation for *D. melanogaster* (Oregon-R, $n = 44$ flies), *D. simulans f;nt,pm;st,e* ($n = 29$), *D. sechellia* ($n = 48$), F_1 hybrids *D. sechellia*/*D. simulans* ($n = 47$), progeny from the *D. sechellia* ($n = 226$) and *D. simulans* backcrosses ($n = 383$).

selection. Variation in ovariole number is controlled by several loci, both within *D. melanogaster* (at least five QTL, Coffman *et al.* 2003; Wayne *et al.* 2001; Wayne and McIntyre 2002) and between species of the *melanogaster* species subgroup (at least three QTL, Coyne *et al.* 1991). Ovariole number is sensitive to environmental conditions such as temperature (David and Clavel 1967; Delpuech *et al.* 1995; Moreteau *et al.* 1997; Morin *et al.* 1997; Hodin and Riddiford 2000), rearing density (Robertson 1957), and larval nutrition (Hodin and Riddiford 2000), which complicates QTL analysis by introducing nongenetic variation.

D. sechellia has approximately half as many ovarioles as its closest relatives (Figure 1A), suggesting that ovariole number has decreased in the evolutionary lineage leading to *D. sechellia*. Such a dramatic decline in potential fecundity is possibly adaptive. *D. sechellia* is endemic to the Seychelles Islands where it feeds exclusively on the freshly dropped fruits of *Morinda citrifolia*. These fruits are highly toxic to other Drosophila species (Rkha *et al.* 1991), although the level of toxins declines as the fruits rot. Ecological specializations in Hawaiian drosophilids and Tephritid flies in the genus Dacus have previously been associated with reductions in ovariole number (Kambysellis and Heed 1971; Fitt 1990; Kambysellis *et al.* 1995). The possible advantages of such reductions in ovariole number are not known but may be related to larger egg sizes (Kambysellis and Heed 1971; Montague *et al.* 1981; Fitt 1990). Thus, *D. sechellia* specialization on a brief temporal niche during fruit rotting might have favored a dramatic reduction in its ovariole number.

Using four genetic markers, Coyne *et al.* showed that both chromosomes 2 and 3 harbored QTL controlling the ovariole number difference between *D. sechellia* and *D. simulans* (Coyne *et al.* 1991). The X chromosome had no effect. We performed an initial QTL mapping experiment to map more precisely the main regions responsible for the decrease in ovariole number in *D. sechellia*. We then used selective phenotyping to increase the resolution in the major QTL region.

MATERIALS AND METHODS

Strains and crosses: Female *f;nt,pm;st,e D. simulans* flies (kindly provided by C. Jones) were crossed to male *D. sechellia* flies (Tucson Drosophila Species Stock Center strain 14021-0248.07) and the female progeny were backcrossed to *D. simulans* males (*D. simulans* backcross) or *D. sechellia* males (*D. sechellia* backcross). Flies were raised on standard media at 25°. Since ovariole number is sensitive to nutrient conditions (Hodin and Riddiford 2000), flies were reared in uncrowded conditions.

Ovariole number: Ovaries were removed from 2- to 4-day-old females. Ovarioles were dissected with tungsten needles in phosphate-buffered saline + 0.1% Tween and counted under a Nikon SMZ1500 stereoscopic microscope. For each fly, the mean ovariole number of the left and right gonad was calculated. Only flies for which both ovaries were scored were included in the analysis.

Marker scoring: Following dissection, flies were frozen at −80°. DNA was isolated from frozen individuals (Gloor and Engels 1992). Molecular markers (supplemental Table 1 at http://www.genetics.org/supplemental/) were PCR amplified and separated on 2% agarose or 4.5% agarose SFR (AMRESCO). We scored natural variation in sequence length or differences in restriction enzyme sites.

Genetic marker map: We used the genetic map previously determined from *D. simulans*/*D. sechellia* hybrids (Macdonald and Goldstein 1999) (supplemental Table 1 at http://www.genetics.org/supplemental/). For markers not included in the original map, we estimated their genetic map position on the basis of their physical map position in *D. melanogaster* relative to flanking markers. The large inversion on chromosome 3 relative to *D. melanogaster* (84F1-93F6-7) that is present in both species (Mahowald and Kambysellis 1980) was taken into account.

QTL mapping: The distribution of markers and our sample size allowed us to identify every QTL responsible for a minimum of a one-ovariole effect (and thus a two-ovariole difference between parents if the QTL is additive) (calculation not shown, adapted from Soller *et al.* 1976; Soller and Genizi 1978; Darvasi and Soller 1992; Lynch and Walsh 1998). This detection limit is in the worst case of a QTL located

midway between two markers. In most genomic locations, QTL with lower effects were potentially detectable. To avoid any bias related to selective genotyping (Lander and Botstein 1989), data from the nonextreme progeny were included and their molecular marker genotypes were entered as missing. Composite interval mapping was performed using R/qtl (Broman *et al.* 2003) with the multiple imputation method of Sen and Churchill (2001). Background markers were chosen at the location of the maximum LOD score calculated by simple interval mapping. Background markers were included only if located on a different chromosome than the test position (see supplemental material at http://www.genetics.org/supplemental/). Statistical significance was determined by permutation tests (Churchill and Doerge 1994). Two- and three-dimensional scans for epistatic QTL were performed using the scantwo and scanqtl functions of R/qtl, respectively (Broman *et al.* 2003). To test for additional QTL, the maximum LOD score was compared between a model containing an additional epistatic QTL and a model without any additional QTL. An LOD difference <2 was considered as not significant. To test for epistasis between QTL, the model allowing for an interaction between the QTL was compared to the additive QTL model. With the present data, evidence for interaction between QTL3a and QTL3b was so clear that the formal calculation of a *P*-value was deemed unnecessary.

RESULTS

The mapping strain of *D. simulans* has a mean ovariole number of 14.9 ± 0.1 (SE) and *D. sechellia* has a value of 7.8 ± 0.1 ovarioles (Figure 1, A–C). F_1 hybrids have a mean of 12.4 ± 0.1 ovarioles (Figure 1C). This value is not significantly different from the mean of the *D. simulans* and *D. sechellia* values, as previously observed with other strains of *D. sechellia* and *D. simulans* (Coyne *et al.* 1991). This suggests that the ovariole difference involves alleles with additive effects or several alleles with opposite dominant effects in each species.

To survey the genomic regions responsible for the difference in ovariole number between *D. sechellia* and *D. simulans*, we performed composite interval mapping on separate *D. sechellia* and *D. simulans* backcross populations. As we were interested primarily in identifying the major QTL, we chose to selectively genotype the progeny flies exhibiting extreme phenotypes, *i.e.*, with lowest and highest ovariole numbers (selective genotyping, Lander and Botstein 1989). We genotyped ~42% of 226 progeny for the *D. sechellia* backcross and 25% of 383 for the *D. simulans* backcross. Selective genotyping reduces cost and time and efficiently detects major QTL (Lander and Botstein 1989; Darvasi and Soller 1992). However, the estimates of QTL position and effect are usually less precise than total genotyping and the identification of epistasis can be more difficult. We estimate here the effect of a QTL as the effect of substituting one *D. simulans* allele with a *D. sechellia* allele. Since *D. sechellia* contains fewer ovarioles than *D. simulans*, QTL are expected to have negative effects on ovariole number if they follow the general direction of evolution.

In both backcrosses the marker on the fourth chromosome was not significantly associated with the trait

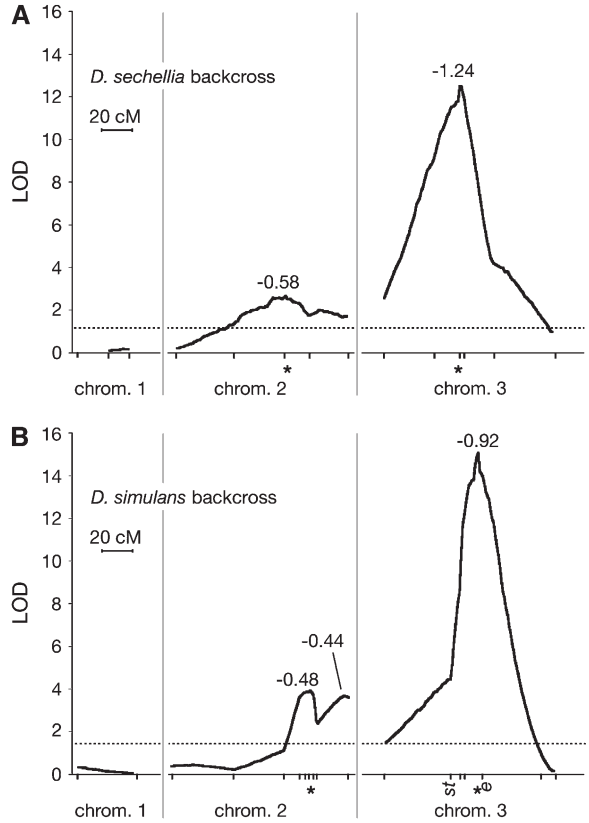

Figure 2.—Composite interval mapping of mean ovariole number between *D. sechellia* and *D. simulans* for the *D. sechellia* backcross (A) and the *D. simulans* backcross (B). Marker positions are indicated along the *x*-axis and LOD score on the *y*-axis. The asterisks indicate the positions of the background parameters (see supplemental material at http://www.genetics.org/supplemental/). The estimated effect of QTL is shown at the top of each peak and is expressed in ovariole number. The LOD threshold for a 5% significance threshold, estimated by a permutation test, is represented as a dotted line.

and results from this chromosome, which comprises only ~1% of the genome, are not shown.

***D. sechellia* backcross:** Results for the *D. sechellia* backcross are shown in Figure 2A. One region of chromosome 3 has the largest effect (a difference of −1.24 ovarioles between a *D. simulans/D. sechellia* heterozygote and a *D. sechellia* homozygote). Another QTL is detected on chromosome 2 with an effect of −0.58 ovarioles (LOD = 2.74, permutation-based LOD threshold = 1.15). No QTL is detected on chromosome 1. Tests for additional QTL on chromosome 2 or 3 are not significant (LOD = 0.66 for chromosome 2 and LOD = 0.87 for chromosome 3). Estimates of QTL positions and effects are summarized in Table 1. No significant epistatic interaction is detected between QTL (LOD = 0.67) and no further QTL exhibiting solely epistatic effects were identified (not shown).

***D. simulans* backcross:** Initial analysis of the *D. simulans* backcross using the five visible markers suggested that a major QTL resides between *scarlet* (*st*) and *ebony* (*e*) on chromosome 3 (not shown). Therefore, to

TABLE 1

Estimates of QTL positions, effects, and interactions for both backcross experiments

Cross	Chromosome	QTL name	Position	2-LOD support interval[a]	LOD	Estimated effect[c]	% of parental difference[d]	Candidate genes[e]
D. sechellia backcross	2	—	47C	25F–60F 94 cM, 5050 genes	2.7	−0.53 (0.19)	7 (3)	—
	3	—	78C	70E–83E 17 cM, 1400 genes	12.5	−1.28 (0.16)	18 (2)	*gig*, *gnu*, *l(3)80Fi*, *M(3)82B-C*, *polo*, *Rheb*, *rpr*
D. simulans backcross	2	—	59C	52C–60F 31.5 cM, 1600 genes	8.5	−0.36 (0.06)	5 (1)	*amz*, *Ark*, *Cdk4*, *Egfr*, *gbb*, *M(2)53*, *M(2)58F*
	3	3a	82C	75F5–83A2 5 cM, 650 genes	8.2[b]	0.15 (0.10)	−2 (1)	*gig*, *l(3)80Fi*, *M(3)82B-C*, *polo*
	3	3b	93D	93F1–90D 10.8 cM, 400 genes	29.2[b]	−0.36 (0.09)	5 (1)	*cdc2c*, *E2f*, *InR*, *PI3K*, *sqz*
	3	3a + 3b	—	—	5.5	−0.88 (0.07)	12 (1)	—

[a] Cytological intervals are given in *D. melanogaster* cytological units. Note that the *D. simulans*/*D. sechellia* hybrid genetic map is larger than the *D. melanogaster* map. The 2-LOD support interval of QTL3a and QTL3b is based on two-dimensional scans in a model including the QTL on chromosome 2 (Figure 4).

[b] The LOD of QTL3a and QTL3b include the interaction term.

[c] The effect of substituting one *D. simulans* allele with a *D. sechellia* allele is given in number of ovarioles with SE in parentheses.

[d] SE is given in parentheses.

[e] Candidate genes involved in cell proliferation or cell death were found with the FlyBase Genes Query Tool.

increase the resolution of this QTL, 70 additional *st–e* recombinant flies from the original backcross were scored (see supplemental material at http://www.genetics.org/supplemental/). Composite interval mapping with all markers revealed a major QTL on chromosome 3 with an effect of −0.92 ovarioles (Figure 2B, LOD = 15.06, permutation-based LOD threshold = 1.49). Two minor peaks were also detected on chromosome 2 with effects of −0.48 and −0.44 ovarioles, respectively (Figure 2B). However, a test for two linked QTL on chromosome 2 is not significant (LOD = 0.67). We cannot, therefore, conclude that there are two QTL on chromosome 2. No significant epistatic interaction was detected between QTL (LOD = 0.34) and no further QTL exhibiting solely epistatic effects were identified (not shown).

High-resolution QTL mapping of the main region on chromosome 3: To increase the resolution of the major QTL region on chromosome 3, we screened a large number of progeny flies for those that were recombinant between the morphological markers *st* and *e* in the *D. simulans* backcross population. The conspicuous phenotypes of the *st* (bright red eyes) and *e* (dark brown body) markers facilitated the screen. We estimated the recombination rate between *st* and *e* from our first backcross as 14.5%. We selected 1038 recombinants and thus screened ~7158 flies. Because the resolution of the QTL position is theoretically proportional to the number of analyzed progeny flies (Darvasi *et al.* 1993; Dupuis and Siegmund 1999; Visscher and Goddard 2004) the resolution is expected to increase by a factor of seven within the *st–e* region. We then selectively genotyped the progeny with extreme phenotypes (48% of 1038 flies).

Composite interval mapping using only the recombinants from this second *D. simulans* backcross indicates that the QTL responsible for the decrease in ovariole number in *D. sechellia* is located near *e* (Figure 3A). In the second backcross, the LOD score peak over *st* with precisely the opposite effect is an artifact resulting from the fact that every fly that is heterozygous for *st* is homozygous for *e*, and vice versa. Composite interval mapping on the pooled data from the first and second backcrosses reduces the artifactual peak near *st* and improves the resolution of the peak (Figure 3A). The new estimated effect of the main QTL on chromosome 3 (−0.73 ovarioles) is lower than the estimate obtained from the first backcross experiment (−0.92), as might be expected with the use of a larger mapping population (Broman 2001).

The selected *st–e* recombinants were also genotyped for several markers on chromosome 2. Two peaks are observed on the right arm of chromosome 2 (Figure 3B) but a test for two QTL on chromosome 2 is not significant (LOD = 1.08). We cannot, therefore, conclude that there are two QTL on chromosome 2. The estimated position and effect of the QTL on chromosome 2 is given in Table 1.

The major QTL splits into two QTL with epistatic interactions: From the pooled data of both *D. simulans*

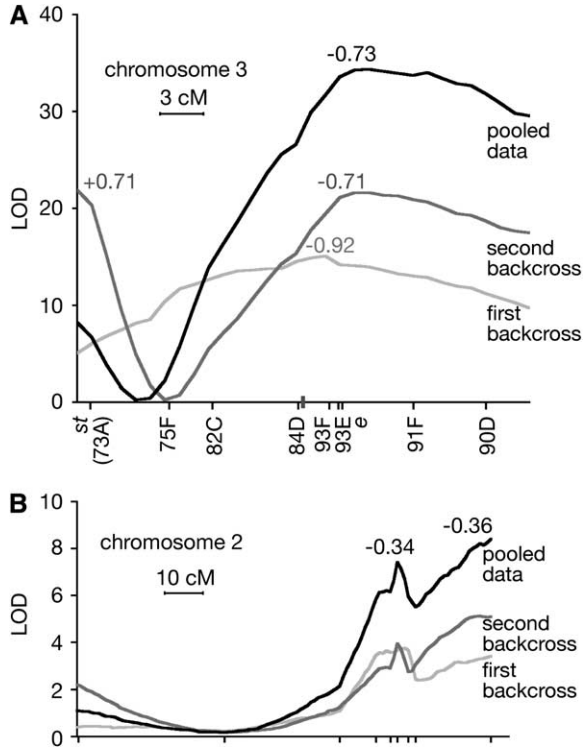

FIGURE 3.—High-resolution QTL mapping of the *D. simulans* backcross. (A) Section of chromosome 3. (B) Entire chromosome 2. Representation is as in Figure 2. Cytological locations of markers are shown along the *x*-axis. The shaded bar indicates the position of the inversion breakpoint relative to *D. melanogaster*. Results from composite interval mapping for the first backcross (light shaded line), the second backcross (dark shaded line), and the total analysis of both first and second backcrosses (solid line) are presented.

backcrosses, a model with two epistatic QTL on chromosome 3 (and one QTL on chromosome 2) gives a maximum LOD score of 40.9, whereas a model with a single QTL on chromosome 3 (and one QTL on chromosome 2) gives a maximum LOD score of 33.7. The LOD difference of 7.2 between these models provides strong evidence that there are actually two linked QTL on chromosome 3. To determine the positions and effects of the linked QTL, we performed a two-dimensional scan for QTL on chromosome 3, while simultaneously controlling for the effects of the QTL on chromosome 2 (Figure 4A). The highest LOD score is found at the intersection of 82C and 93E (see cross in Figure 4A) and the 2-LOD support interval delimits a region around 82C that we named QTL3a and a region around 93D named QTL3b (Figure 4A). A test for epistasis between the linked QTL is highly significant (LOD = 5.5, Figure 4B), but no epistasis is detected between the QTL on chromosome 2 and QTL on chromosome 3 (LOD = 0.16 with QTL3a and LOD = 0.41 with QTL3b). The *D. sechellia* allele of QTL3b decreases ovariole number in the absence of the QTL3a *D. sechellia* allele (Figure 4D), with an effect of −0.36 ovarioles at the highest LOD score position. Surprisingly, QTL3a acts in the opposite direction in the absence of the QTL3b *D. sechellia* allele, with an effect of +0.15 ovarioles at the highest LOD score position (Figure 4C). When both QTL are combined, they have a total effect of −0.88 ovarioles at the highest LOD score position (Figure 4E). The estimated effects and positions of QTL are summarized in Table 1. A test for a third QTL on chromosome 3 is not significant (LOD = 0.80).

DISCUSSION

QTL controlling ovariole number: Our study illustrates how finely one can map interspecific QTL of large effect for a quantitative trait that is susceptible to considerable environmental variation, on the basis of a backcross mapping population. We identified a minimal number of three QTL (if the same QTL are detected with both backcrosses) that account for ~42% of the total species difference (Table 1). The remaining 58% is due either to additional loci of smaller effect or to epistatic interactions that are not detectable in backcross conditions between the identified QTL.

Our results agree with previous lower-resolution mapping experiments between *D. simulans* and *D. sechellia* that identified a major effect on ovariole number near *ebony* (93C-D) and a smaller effect linked to *cinnabar* (43E) in a *D. simulans* backcross (COYNE *et al.* 1991). The previous study used different fly lines than our experiment, suggesting that the QTL that we have identified control the species difference in ovariole number and do not reflect simply strain differences. Our interspecific QTL for ovariole number are largely distinct from the intraspecific QTL for ovariole number that have been identified in *D. melanogaster*, with the possible exception of the 65A–87F region (WAYNE *et al.* 2001; WAYNE and MCINTYRE 2002; COFFMAN *et al.* 2003).

It has been suggested that evolutionary changes at the *ovo/shaven-baby* locus might have contributed to the evolutionary decrease in ovariole number in *D. sechellia* (JONES 2005). This hypothesis was motivated by the observation that evolution of the *ovo/shaven-baby* gene caused the loss of larval trichomes in the *D. sechellia* lineage (SUCENA and STERN 2000). Since *ovo/shaven-baby* is required for oogenesis in *D. melanogaster* (MEVEL-NINIO *et al.* 1995), Jones hypothesized that evolutionary changes at this locus might have pleiotropic effects on larval trichome patterning as well as on ovariole number. We can now reject this hypothesis for intermediate or large effects of the *ovo/shaven-baby* gene on ovariole number since we found no QTL for ovariole number on chromosome 1, the location of the *ovo/shaven-baby* locus.

Selective phenotyping: Traditional QTL mapping, which utilizes every individual from a mating design, usually provides limited resolution of QTL locations. This is due largely to the limited number of recombination events that are scored. Selective phenotyping, in

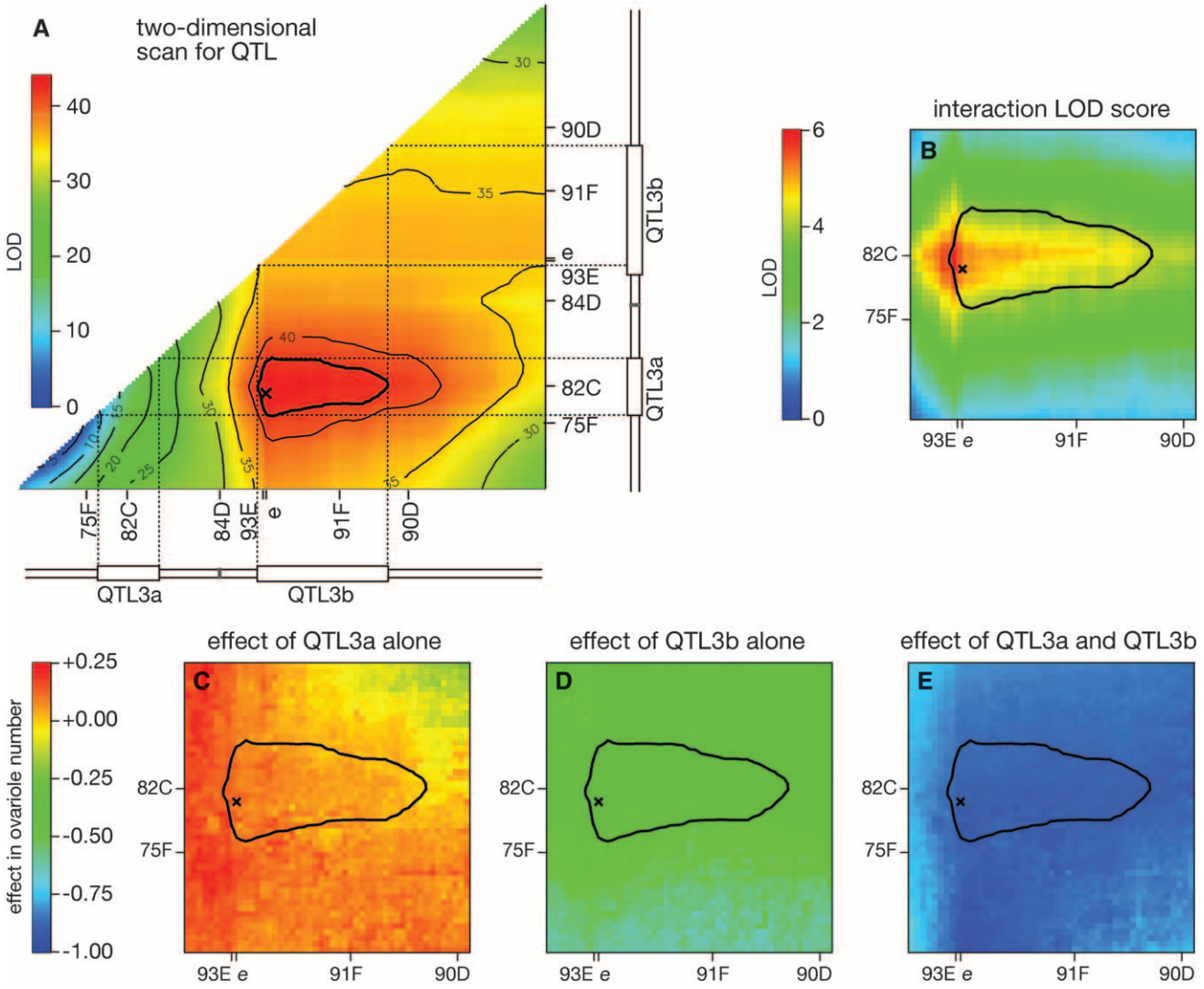

FIGURE 4.—Two-dimensional scan for linked QTL on chromosome 3. Values are given as a function of the two QTL positions tested by the model. Cytological locations are indicated for each marker. (A) The LOD score (relative to a model with no QTL) for the three-QTL model with a QTL in fixed position on chromosome 2 and two interacting QTL in varying positions on chromosome 3. (B) The interaction LOD score, comparing the model with two interacting QTL on chromosome 3 to that with additive QTL on chromosome 3. (C) The estimated effect of substituting one *D. simulans* allele by a *D. sechellia* allele at QTL3a (in a *D. simulans* background at QTL3a and QTL3b positions). (D) The estimated effect of substituting a *D. simulans* allele by a *D. sechellia* allele at QTL3b (in a *D. simulans* background at QTL3a and QTL3b positions). (E) The estimated effect of substituting a *D. simulans* allele by a *D. sechellia* allele at both QTL3a and QTL3b (in a *D. simulans* background for QTL3a and QTL3b). In A–E, the location of the QTL on chromosome 2 is kept fixed. The estimated locations of the two QTL are indicated by a cross and the 2-LOD support region by a thick solid line. Iso-LOD lines are shown in A with thin solid lines.

which only individuals carrying a recombination event in a region of interest are selected for phenotyping, has been predicted to greatly improve the resolution of QTL mapping (DARVASI 1998). We used selective phenotyping to better resolve the major QTL in the *st–e* region.

We phenotyped 1038 *st–e* recombinant flies. There are ~1150 genes between *st* and *e*, so we generated approximately one recombination event per gene in this region. Theoretically (DARVASI *et al.* 1993; DUPUIS and SIEGMUND 1999; VISSCHER and GODDARD 2004) this should refine the confidence interval for the QTL position by a factor of seven. We indeed observed a significant increase in the QTL mapping resolution. The initial QTL on chromosome 3, estimated to be responsible for 13% of the parental difference, was first mapped to a 2-LOD support interval of 20 cM (not shown). Then, using selective phenotyping, the QTL3a region was mapped to a region four times smaller (5 cM) although it accounts for only 2% of the parental difference in the absence of the QTL3b *D. sechellia* allele (Table 1). Selective phenotyping is therefore an efficient method to increase resolution and power to detect QTL of small effect.

Unfortunately, our *st–e* recombinants do not help to resolve the QTL3b region that extends to the right of *e*.

Correspondingly, the 2-LOD support interval for the position of QTL3b is large on the right of *e* and relatively small on the left of *e* (Figure 4A), with only 21 genes on the left of the highest LOD score peak. This suggests that a greater resolution would have been obtained if a visible marker located to the right of *e* had been used instead of *e*. This observation illustrates an inherent tradeoff in selective phenotyping. Markers must be close enough to allow the exclusion of many uninformative individuals, but they should also be far enough apart so that the flanked region covers the potential QTL. In our study, we mistakenly assumed that the QTL would lie within the *st–e* region because the initial highest LOD score peak was actually caused by two closely linked QTL. One solution to this problem is to use distant markers in a first step to refine the region and then closer markers in a second step.

Our selective phenotyping strategy can be applied to other genomic regions (there are ~50 available visible markers across the *D. simulans* genome at the Tucson Drosophila Species Stock Center) and to other organisms that can be raised *en masse* in the lab. For species with a limited number of existing visible mutations, transgenes carrying visible reporter constructs could be used as an alternative.

Identifying the genes underlying QTL: The identification of the genes and mutations underlying QTL, which appears to be extremely difficult, is nevertheless essential to improve our understanding of the genetic basis of complex traits. The reduced ovariole number in *D. sechellia* is associated with a slower rate of cell proliferation in the ovaries during the third instar larval stage (Hodin and Riddiford 2000). We observed no difference in the number of embryonic ovary cells between *D. sechellia* and *D. melanogaster* (V. Orgogozo and D. L. Stern, unpublished data). This suggests that the genes involved in the decrease in ovariole number in *D. sechellia* are likely to regulate cell proliferation or cell death. Each QTL region that we identified contains several such genes (Table 1). The *insulin receptor* gene, which regulates cell size and cell number in individual organs (Brogiolo *et al.* 2001), is an attractive candidate gene for the QTL3b region. It falls directly beneath the QTL3b peak and ovariole number is reduced in *D. melanogaster* mutants of the insulin pathway (Tu and Tatar 2003; Richard *et al.* 2005; V. Orgogozo and D. L. Stern, unpublished data).

Several approaches can be undertaken to identify the genes underlying the QTL for ovariole number (reviewed in Flint and Mott 2001). For example, candidate genes can be tested via interspecific transgenesis. Unfortunately Drosophila transgenesis is currently not feasible for genes that are longer than 40–50 kb (Venken and Bellen 2005), such as the *insulin receptor* gene. Alternatively, classical meiotic recombination mapping could be pushed further to identify the genes underlying QTL. First, a QTL should be isolated from the effects of other QTL by introgressing only the region of interest. Then, using markers that closely flank the introgression, a large number of recombination events could be identified. Measuring a large number of individuals for each recombination event (progeny testing) should allow identification of the causal genes and possibly nucleotides. An unresolved problem for genes with small effects and for traits that are sensitive to environmental variation such as ovariole number is that large sample sizes are required to estimate the effect of a genomic region. A second unresolved problem is that such an introgression approach is unlikely to identify epistatic QTL such as QTL3a, which causes a small increase in ovariole number in the absence of other QTL. Thus, a combination of approaches that search for QTL at multiple hierarchical levels of resolution is required to fully elucidate the genetic basis of quantitative traits.

Epistasis and evolution: There is increasing evidence that epistatic interactions play an important role in the expression of complex traits (see, for example, Nagel 2005). Detection of epistasis generally requires high-resolution mapping studies (see for example Gadau *et al.* 2002; Brem *et al.* 2005; Brem and Kruglyak 2005). Selective phenotyping enabled us to detect two linked epistatic QTL. The QTL3a *D. sechellia* allele increases ovariole number in the absence of the QTL3b *D. sechellia* allele, but decreases ovariole number in the presence of the QTL3b *D. sechellia* allele. This type of epistasis, in which an allele has opposite effects on two different genotypic backgrounds, has been named sign epistasis (Weinreich *et al.* 2005). This is in contrast with synergistic epistasis (the effect of two loci is higher than the sum of their individual effects) and antagonistic epistasis (the effect of two loci is lower than the sum of their individual effects, but still in the same direction).

Because sign epistasis introduces limitations on the selectively accessible mutational trajectories across a fitness landscape (Weinreich *et al.* 2005), it is possible to order past evolutionary events. If we assume that the ancestor of *D. simulans* and *D. sechellia* was *D. simulans*-like regarding ovariole number, and that alleles that increase ovariole number decreased fitness during *D. sechellia* evolution, it is unlikely that the *D. sechellia* QTL3a allele (which slightly increases ovariole number in the absence of QTL3b) appeared before QTL3b. It is more likely that the *D. sechellia* QTL3b allele appeared and was fixed first and then the QTL3a allele arose (Figure 5). It is also possible that both *D. sechellia* alleles segregated in the ancestral population at the same time and that the generation of a QTL3a + QTL3b superallele by recombination allowed rapid fixation of the pair of QTL. In any case, our results suggest that the *D. sechellia* QTL3a allele could not have been selectively fixed in the absence of the *D. sechellia* QTL3b.

Our results also have implications for use of the Orr Sign Test (Orr 1998) for detecting positive selection

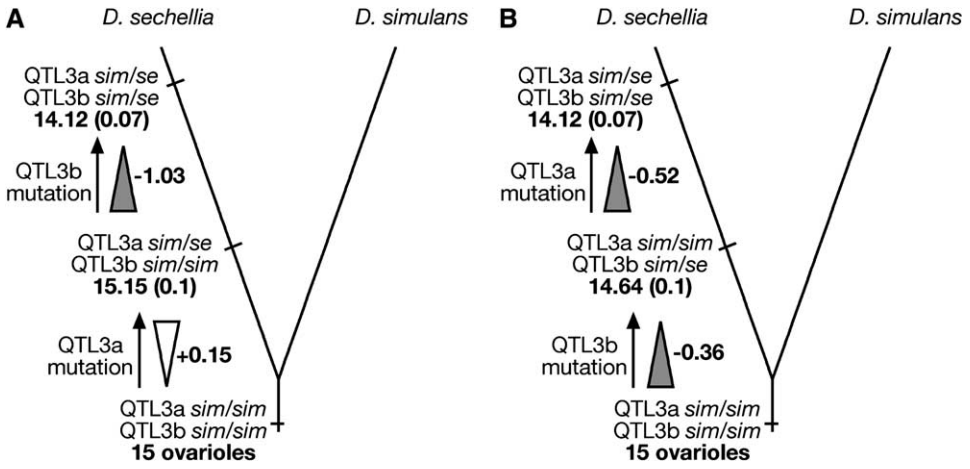

FIGURE 5.—Two evolutionary scenarios for the evolution of ovariole number. Only QTL3a and QTL3b are considered here. We assume that the ancestor of *D. simulans* and *D. sechellia* was *D. simulans*-like, with genotype QTL3a *sim/sim*, QTL3b *sim/sim*, and an initial phenotype of 15 ovarioles. In A, the QTL3a mutation appears first and is followed by the QTL3b mutation. B represents the alternative scenario. The estimated number of ovarioles and standard error are indicated at each evolutionary step (based on Table 1). Although it is possible that one QTL was fixed before the origin of the second, the *se/se* allelic conditions are not shown because we do not have ovariole number estimates for the *se/se* genotypes. If we assume that mutations that increase ovariole number (open inverted triangle) decreased fitness during *D. sechellia* evolution, then A is unlikely and B, which involves successive mutations that decrease ovariole number (shaded triangle), is more likely. *sim*, *simulans*; *se*, *sechellia*.

using QTL mapping data. This test determines whether the number of QTL with effects in one direction is greater than expected under a neutral model. An excess of QTL with effects in the direction expected, given the parental difference, suggests that directional selection operated on the trait. The possibility that sign epistasis may exist between QTL will complicate interpretation of the Orr Sign Test. In particular, sign epistasis may bias the test toward suggesting that directional selection did not act when in fact it did. This is because during evolution a QTL showing sign epistasis might have had an effect opposite that of its calculated effect if it happened to be in a different genetic background. It is not yet clear how often QTL underlying complex traits display sign epistasis (KROYMANN and MITCHELL-OLDS 2005; WEINREICH *et al.* 2005). Investigating sign epistasis between closely linked loci will require QTL mapping studies with higher resolution than is usually obtained.

We thank C. Jones and the Tucson Drosophila Species Stock Center for flies, T. Doak for the multichannel pipette, and D. Storton for the use of the high-throughput liquid handling Beckman Coulter Biomek FX and MJ Research Tetrad thermocyclers. We thank L. Kruglyak and M. Rockman for providing useful comments on this manuscript. V.O. is a Damon Runyon Fellow supported by the Damon Runyon Cancer Research Foundation (DRG-1789-03). K.W.B. acknowledges the National Institutes of Health (grant GM-074244) and D.L.S. acknowledges the National Institutes of Health (grant GM-6362201), the David and Lucile Packard Foundation, and Princeton University for financial support.

LITERATURE CITED

AZEVEDO, R. B. R., V. FRENCH and L. PARTRIDGE, 1996 Thermal evolution of egg size in Drosophila melanogaster. Evolution **50:** 2338–2345.

BREM, R. B., and L. KRUGLYAK, 2005 The landscape of genetic complexity across 5,700 gene expression traits in yeast. Proc. Natl. Acad. Sci. USA **102:** 1572–1577.

BREM, R. B., J. D. STOREY, J. WHITTLE and L. KRUGLYAK, 2005 Genetic interactions between polymorphisms that affect gene expression in yeast. Nature **436:** 701–703.

BROGIOLO, W., H. STOCKER, T. IKEYA, F. RINTELEN, R. FERNANDEZ *et al.*, 2001 An evolutionarily conserved function of the Drosophila insulin receptor and insulin-like peptides in growth control. Curr. Biol. **11:** 213–221.

BROMAN, K. W., 2001 Review of statistical methods for QTL mapping in experimental crosses. Lab. Anim. **30:** 44–52.

BROMAN, K. W., H. WU, S. SEN and G. A. CHURCHILL, 2003 R/qtl: QTL mapping in experimental crosses. Bioinformatics **19:** 889–890.

CAPY, P., E. PLA and J. R. DAVID, 1993 Phenotypic and genetic variability of morphometrical traits in natural populations of *Drosophila melanogaster* and *D. simulans*. I. Geographic variations. Genet. Sel. Evol. **25:** 517–536.

CHURCHILL, G. A., and R. W. DOERGE, 1994 Empirical threshold values for quantitative trait mapping. Genetics **138:** 963–971.

COFFMAN, C. J., R. W. DOERGE, M. L. WAYNE and L. M. MCINTYRE, 2003 Intersection tests for single marker QTL analysis can be more powerful than two marker QTL analysis. BMC Genet. **4:** 10.

COYNE, J. A., J. RUX and J. R. DAVID, 1991 Genetics of morphological differences and hybrid sterility between Drosophila sechellia and its relatives. Genet. Res. **57:** 113–122.

DARVASI, A., 1998 Experimental strategies for the genetic dissection of complex traits in animal models. Nat. Genet. **18:** 19–24.

DARVASI, A., 2005 Dissecting complex traits: the geneticists "around the world in 80 days." Trends Genet. **21:** 373–376.

DARVASI, A., and M. SOLLER, 1992 Selective genotyping for determination of linkage between a marker locus and a quantitative trait locus. Theor. Appl. Genet. **85:** 353–359.

DARVASI, A., A. WEINREB, V. MINKE, J. I. WELLER and M. SOLLER, 1993 Detecting marker-QTL linkage and estimating QTL gene effect and map location using a saturated genetic map. Genetics **134:** 943–951.

DAVID, J., and M. F. CLAVEL, 1967 Influence de la tempèrature subie au cours du développement sur divers caractères biométriques des adultes de Drosophila melanogaster meigen. J. Insect Physiol. **13:** 717.

DAVID, J. R., and C. BOCQUET, 1975a Evolution in a cosmopolitan species: genetic latitudinal clines in Drosophila melanogaster wild populations. Experientia **31:** 164–166.

DAVID, J. R., and C. BOCQUET, 1975b Similarities and differences in latitudinal adaptation of two Drosophila sibling species. Nature **257:** 588–590.

DELPUECH, J. M., B. MORETEAU, J. CHICHE, E. PLA, J. VOUIDIBIO *et al.*, 1995 Phenotypic plasticity and reaction norms in temperate and tropical populations of Drosophila melanogaster: ovarian size and developmental temperature. Evolution **49:** 670–675.

DUPUIS, J., and D. SIEGMUND, 1999 Statistical methods for mapping quantitative trait loci from a dense set of markers. Genetics **151:** 373–386.

Fitt, G. P., 1990 Variation in ovariole number and egg size of species of Dacus (Diptera, Tephritidae) and their relation to host specialization. Ecol. Entomol. **15:** 255–264.

Flint, J., and R. Mott, 2001 Finding the molecular basis of quantitative traits: successes and pitfalls. Nat. Rev. Genet. **2:** 437–445.

Gadau, J., R. E. Page and J. H. Werren, 2002 The genetic basis of the interspecific differences in wing size in Nasonia (Hymenoptera; Pteromalidae): major quantitative trait loci and epistasis. Genetics **161:** 673–684.

Gibert, P., P. Capy, A. Imasheva, B. Moreteau, J. P. Morin *et al.*, 2004 Comparative analysis of morphological traits among Drosophila melanogaster and D. simulans: genetic variability, clines and phenotypic plasticity. Genetica **120:** 165–179.

Glazier, A. M., J. H. Nadeau and T. J. Aitman, 2002 Finding genes that underlie complex traits. Science **298:** 2345–2349.

Gloor, G. B., and W. R. Engels, 1992 Single fly DNA preps for PCR. Dros. Inf. Serv. **71:** 148–149.

Harr, B., S. Weiss, J. R. David, G. Brem and C. Schlotterer, 1998 A microsatellite-based multilocus phylogeny of the Drosophila melanogaster species complex. Curr. Biol. **8:** 1183–1186.

Hodin, J., and L. M. Riddiford, 2000 Different mechanisms underlie phenotypic plasticity and interspecific variation for a reproductive character in drosophilids (Insecta: Diptera). Evolution **54:** 1638–1653.

Jin, C., H. Lan, A. D. Attie, G. A. Churchill, D. Bulutuglo *et al.*, 2004 Selective phenotyping for increased efficiency in genetic mapping studies. Genetics **168:** 2285–2293.

Jones, C. D., 2005 The genetics of adaptation in Drosophila sechellia. Genetica **123:** 137–145.

Kambysellis, M. P., and W. B. Heed, 1971 Studies of oogenesis in natural populations of drosophilidae.1. relation of ovarian development and ecological habitats of Hawaiian species. Am. Nat. **105:** 31–49.

Kambysellis, M. P., K. F. Ho, E. M. Craddock, F. Piano, M. Parisi *et al.*, 1995 Pattern of ecological shifts in the diversification of Hawaiian Drosophila inferred from a molecular phylogeny. Curr. Biol. **5:** 1129–1139.

King, R. C., S. K. Aggarwal and U. Aggarwal, 1968 The development of the female Drosophila reproductive system. J. Morphol. **124:** 143–166.

Kroymann, J., and T. Mitchell-Olds, 2005 Epistasis and balanced polymorphism influencing complex trait variation. Nature **435:** 95–98.

Lander, E. S., and D. Botstein, 1989 Mapping Mendelian factors underlying quantitative traits using RFLP linkage maps. Genetics **121:** 185–199.

Lemeunier, F., J. R. David, L. Tsacas and M. Ashburner, 1986 The *melanogaster* species group, pp. 147–256 in *The Genetics and Biology of Drosophila*, edited by M. Ashburner. Academic Press, London.

Louis, J., and J. R. David, 1986 Ecological specialization in the Drosophila melanogaster species subgroup: a case-study of Drosophila sechellia. Acta Oecol. Oecol. Generalis **7:** 215–229.

Lynch, M., and B. Walsh, 1998 *Genetics and Analysis of Quantitative Traits.* Sinauer, Sunderland, MA.

Macdonald, S. J., and D. B. Goldstein, 1999 A quantitative genetic analysis of male sexual traits distinguishing the sibling species *Drosophila simulans* and *D. sechellia.* Genetics **153:** 1683–1699.

Mahowald, A. P., and M. P. Kambysellis, 1980 Oogenesis, pp. 141–224 in *The Genetics and Biology of Drosophila*, edited by M. Ashburner and T. R. F. Wright. Academic Press, London.

Manly, K. F., and J. M. Olson, 1999 Overview of QTL mapping software and introduction to map manager QT. Mamm. Genome **10:** 327–334.

Mevel-Ninio, M., R. Terracol, C. Salles, A. Vincent and F. Payre, 1995 ovo, a Drosophila gene required for ovarian development, is specifically expressed in the germline and shares most of its coding sequences with shavenbaby, a gene involved in embryo patterning. Mech. Dev. **49:** 83–95.

Montague, J. R., R. L. Mangan and W. T. Starmer, 1981 Reproductive allocation in the Hawaiian Drosophilidae: egg size and number. Am. Nat. **118:** 865–871.

Moreteau, B., J. P. Morin, P. Gibert, G. Petavy, E. Pla *et al.*, 1997 Evolutionary changes of nonlinear reaction norms according to thermal adaptation: a comparison of two Drosophila species. C. R. Acad. Sci. III Sci. Vie **320:** 833–841.

Morin, J. P., B. Moreteau, G. Petavy, R. Parkash and J. R. David, 1997 Reaction norms of morphological traits in Drosophila: Adaptive shape changes in a stenotherm circumtropical species? Evolution **51:** 1140–1148.

Nagel, R. L., 2005 Epistasis and the genetics of human diseases. C. R. Biol. **328:** 606–615.

Orr, H. A., 1998 Testing natural selection vs. genetic drift in phenotypic evolution using quantitative trait locus data. Genetics **149:** 2099–2104.

Powell, J. R., 1997 *Progress and Prospects in Evolutionary Biology: The Drosophila Model.* Oxford University Press, London.

Purcell, M. K., J. L. Mu, D. C. Higgins, R. Elango, H. Whitmore *et al.*, 2001 Fine mapping of Ath6, a quantitative trait locus for atherosclerosis in mice. Mamm. Genome **12:** 495–500.

Richard, D. S., R. Rybczynski, T. G. Wilson, Y. Wang, M. L. Wayne *et al.*, 2005 Insulin signaling is necessary for vitellogenesis in Drosophila melanogaster independent of the roles of juvenile hormone and ecdysteroids: female sterility of the chico(1) insulin signaling mutation is autonomous to the ovary. J. Insect Physiol. **51:** 455–464.

Rkha, S., P. Capy and J. R. David, 1991 Host plant specialization in the Drosophila-melanogaster species complex - a physiological, behavioral, and genetic-analysis. Proc. Natl. Acad. Sci. USA **88:** 1835–1839.

Robertson, F. W., 1957 Studies in quantitative inheritance. X. Genetic variation of ovary size in Drosophila. J. Genet. **55:** 410–427.

Ronin, Y., A. Korol, M. Shtemberg, E. Nevo and M. Soller, 2003 High-resolution mapping of quantitative trait loci by selective recombinant genotyping. Genetics **164:** 1657–1666.

Sen, S., and G. A. Churchill, 2001 A statistical framework for quantitative trait mapping. Genetics **159:** 371–387.

Soller, M., and A. Genizi, 1978 Efficiency of experimental-designs for detection of linkage between a marker locus and a locus affecting a quantitative trait in segregating populations. Biometrics **34:** 47–55.

Soller, M., T. Brody and A. Genizi, 1976 Power of experimental designs for detection of linkage between marker loci and quantitative loci in crosses between inbred lines. Theor. and Appl. Genet. **47:** 35–39.

Sucena, E., and D. L. Stern, 2000 Divergence of larval morphology between Drosophila sechellia and its sibling species caused by cis-regulatory evolution of ovo/shaven-baby. Proc. Natl. Acad. Sci. USA **97:** 4530–4534.

Ting, C. T., S. C. Tsaur and C.-I Wu, 2000 The phylogeny of closely related species as revealed by the genealogy of a speciation gene, Odysseus. Proc. Natl. Acad. Sci. USA **97:** 5313–5316.

Tu, M. P., and M. Tatar, 2003 Juvenile diet restriction and the aging and reproduction of adult Drosophila melanogaster. Aging Cell **2:** 327–333.

Venken, K. J., and H. J. Bellen, 2005 Emerging technologies for gene manipulation in Drosophila melanogaster. Nat. Rev. Genet. **6:** 167–178.

Visscher, P. M., and M. E. Goddard, 2004 Prediction of the confidence interval of quantitative trait Loci location. Behav. Genet. **34:** 477–482.

Wayne, M. L., and L. M. McIntyre, 2002 Combining mapping and arraying: An approach to candidate gene identification. Proc. Natl. Acad. Sci. USA **99:** 14903–14906.

Wayne, M. L., J. B. Hackett, C. L. Dilda, S. V. Nuzhdin, E. G. Pasyukova *et al.*, 2001 Quantitative trait locus mapping of fitness-related traits in Drosophila melanogaster. Genet. Res. **77:** 107–116.

Weinreich, D. M., R. A. Watson and L. Chao, 2005 Perspective: sign epistasis and genetic constraint on evolutionary trajectories. Evolution **59:** 1165–1174.

Xu, Z., F. Zou and T. J. Vision, 2005 Improving quantitative trait loci mapping resolution in experimental crosses by the use of genotypically selected samples. Genetics **170:** 401–408.

Communicating editor: G. Gibson