

QUANTITATIVE TRAIT LOCUS ANALYSIS OF MALE MATING SUCCESS AND SPERM COMPETITION IN *DROSOPHILA MELANOGASTER*

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Abstract.—Much of sexual selection theory depends on assumptions about the genetic basis of variation in male mating success and sperm competitive ability. Despite intense interest in this topic, few genes have been identified that contribute to variation in these traits. Here we report the results of quantitative trait locus (QTL) analyses of mating success of male *Drosophila melanogaster* when exposed to virgin females, remating success of males with previously mated females, and both defense and offense components of sperm competition. We found two to four significant QTLs for remating success, but no QTLs for mating success, even though mating success was more genetically variable than remating success in the recombinant inbred lines used in this study. By combining these results with data from previous gene-expression experiments, we were able to identify three X-linked candidate genes for variation in remating ability. For two of these genes, QTL and expression data were completely concordant with respect to directionality of effects: high mating success was associated with high levels of gene expression and with beneficial QTL effects on the trait. We found equivocal evidence for genetic variation in sperm offense and defense in the recombinant inbred lines, and we did not find any significant QTLs for either sperm competition trait.

Key words.—Gene mapping, male fitness, mating ability, quantitative genetics, sexual selection.

Received December 22, 2005. Accepted April 23, 2006.

In species where females mate with multiple males and store sperm, the ability of a male to sire offspring can be affected by behavioral, morphological, or biochemical differences between males. These differences can result in consistent differences between males in the proportion of offspring they produce with previously or subsequently mated females. Reproductive success in this context is influenced by differences in copulation success or by differences in the ability to fertilize eggs after copulation. The former is typically referred to as “mating success” and the latter as “sperm competition,” “sperm precedence,” or “sperm displacement.”

If males differ in either mating success or sperm competitive ability, then these traits are likely to be under strong directional selection. However, several experiments provide compelling evidence that populations can be highly genetically variable for both traits. Within-population genetic variation for mating success has been demonstrated in both vertebrates (Brooks and Endler 2001) and invertebrates (Kosuda 1985; Partridge et al. 1985; Hughes 1995a,b; Wedell and Tregenza 1999).

Sperm competition is generally a more difficult trait to measure in quantitative genetic experiments. However, two studies of bulb mites (Radwan 1998; Konior et al. 2005) and four studies of *Drosophila melanogaster* (Clark et al. 1995; Hughes 1997; Fiumera et al. 2005; Friberg et al. 2005) have identified naturally occurring genetic variation for both sperm “offense” and sperm “defense.” Sperm offense is the fertilization success of a male that mates with a previously mated female; it reflects a male’s ability to compete for fertilizations after successful copulation. Sperm defense is the fertilization success of a male that has mated with a female, when that female subsequently mates with other males; this trait reflects a male’s ability to defend against postcopulatory competition from the subsequent males.

Based on an analysis of third-chromosome variation in

mating success and sperm competition in a population of *D. melanogaster*, Hughes (1997) suggested that patterns of variation for mating success were consistent with a mutation-selection balance model of the maintenance of variation, but the patterns for sperm competition were more consistent with a model of balancing selection. Clark and colleagues found nonadditive interactions between male genotypes (Clark et al. 2000) and between male and female genotypes (Clark and Begun 1998; Clark et al. 1999); such interactions could maintain genetic variation if the rank ordering of male fitness depends on the genotypes of females or of competitor males (Prout and Bundgaard 1977).

An analysis of hemiclinal variation in *D. melanogaster* found significant additive genetic variation for both mating success and sperm competition traits, and evidence of negative correlations between male and female fitness components (Friberg et al. 2005). Single-locus theory predicts that negative genetic correlation between male and female fitness can maintain genetic polymorphism via induced overdominance or if fitness differences between the sexes are large (Haldane 1962; Livingstone 1992). However, in a recent theoretical analysis, Turelli and Barton (2004) concluded that additive polygenic variation is unlikely to be maintained by this form of selection.

Despite intense interest in the genetic basis of male fitness variation, few individual genes have been identified that contribute to naturally occurring variation in mating success or sperm competition. Clark et al. (1995) found significant associations between sperm defense and polymorphism at four accessory-gland protein (ACP) genes in *D. melanogaster*. A second study of flies derived from a natural population found that single-nucleotide polymorphism (SNP) in six ACP genes was associated with variation in sperm competition or mating success (Fiumera et al. 2005). Using a different approach (microarray analysis), Drnevich et al. (2004) identified 27 candidate genes for male reproductive success in *D. melan-*

ogaster. None of the genes identified by microarrays were ACPs, and genes expressed in male accessory glands or testes were not overrepresented among genes implicated in fitness variation. This result is not necessarily contradictory to the association studies, since sequence differences at ACPs do not necessarily lead to differential mRNA abundance in these genes. However, this study does suggest that genes other than those expressed in male reproductive tissue can contribute to differential male fitness. QTL mapping studies (Moehring and Mackay 2004) and microarrays (Mackay et al. 2005a) have also been used to identify candidate genes for male mating behavior in this species, but the relationship between behavior variation and mating success has not been established.

To produce a whole-genome screen for polymorphisms affecting male reproductive fitness, we conducted a QTL mapping experiment to test for regions of chromosomes associated with variation in male mating success and sperm competitive ability. By concentrating on male traits in this analysis, we do not imply that variation in male fitness is independent of the biology and behavior of females. Several studies have shown that interactions between sexes can contribute to variation in mating success and sperm competitive ability (Clark and Begun 1998; Clark et al. 1999; Civetta and Clark 2000). However, female behavioral variation is subtle and more difficult to assay in the large sample sizes required for QTL analysis. The approach used here will identify genomic regions that are variable and that have effects on male fitness when males are exposed to a random sample of females, but not those that cause phenotypic variation only in the context of specific male genotype-by-female genotype interactions.

METHODS

Lines

The recombinant inbred lines (RILs) used in this study were obtained from Dr. T. Mackay at North Carolina State University, Raleigh, NC. The parental strains used to construct the RILs were Oregon-R (Ore) and 2b. The 2b strain is a derivative of two separate stocks, LA and b. The LA (low activity) stock originated from the natural population in Essenkuti, Russia, and was artificially selected for decreased male sexual activity for more than 600 generations (Pasyukova and Nuzhdin 1993; E. G. Pasyukova pers. comm.). The b strain was constructed by substituting an X chromosome from a Swedish strain into the LA background. In this new b strain, the second chromosome from LA acquired a number of new *copia*, *mdg1* and *mdg3* insertion sites due to transposition (Pasyukova et al. 1988). This altered second chromosome was then substituted back into the original LA stock (containing the X, third, and fourth LA chromosomes to produce the 2b stock (Belyaeva et al. 1989).

Construction of the RILs is detailed in Nuzhdin et al. (1997) and described briefly here. Construction of the lines was initiated by crossing the Ore and 2b strains. F₁ progeny were backcrossed to 2b, and subsequent offspring allowed to undergo random mating for four generations. Males and females were divided into 200 individual pairs in generation 5, and RILs were produced by 25 generations of brother-

sister mating. The 98 lines that survived inbreeding were genotyped for the presence/absence of *roo* element insertions and found to be largely homozygous (mean homozygosity over markers and lines was 0.956). Eighty variable insertion sites were identified at cytological positions: 1B, 3E, 4F, 5D, 6E, 7D, 7E, 9A, 10D, 11C, 11D, 12E, 14C, 15A, 16D, 17C, 19A, 21E, 22F, 27B, 29F, 30AB, 30D, 33E, 34EF, 35B, 38A, 38E, 43A, 43E, 46A, 46C, 48D, 49D, 50B, 50D, 50F, 57C, 57F, 60E, 61A, 63A, 65A, 65D, 67D, 68B, 68C, 69D, 70C, 71E, 72A, 73D, 76A, 76B, 77A, 77E, 78D, 82D, 85A, 85F, 87B, 87E, 87F, 88E, 89B, 91A, 91D, 92A, 93A, 93B, 94D, 96A, 96F, 97D, 97E, 98A, 99A, 99B, 99E, 100A (Nuzhdin et al. 1997; Leips and Mackay 2000). We maintained three replicate vials of each RIL on a standard molasses-corn-agar medium. Flies were housed at 25°C with a 12:12 photoperiod.

Mating Success and Sperm Competition Assays

To assay mating success and sperm competition, we mated virgin *ebony* (*ele*) females to two males in sequence: one of the males was from an RIL, the other was an *ele* "competitor" male. The recessive *ebony* mutation had been introgressed into a large, randomly mating population of flies (the Ives population), by repeated backcrossing. RIL males have wild-type body color, so the use of *ele* females and *ele* competitor males allows unambiguous determination of paternity.

To reduce maternal and density effects, we raised each RIL and the *ele* stock at constant parental density (five males and females per vial) for two generations prior to sperm precedence assays. Virgin males were collected from the second-generation constant-density vials of the RILs and the *ele* stock population; virgin females were collected from the *ele* stock. We confirmed virginity of females by inspecting vials for eggs or larvae. Any vials that had eggs or larvae were discarded; virgin females will lay eggs, but we discarded all vials with eggs to be conservative. At three days of age, RIL and *ele* males were lightly anesthetized with CO₂ and placed into fresh vials such that each vial contained either two males from an RI line or two *ele* males. Virgin *ele* females were lightly anesthetized when they were three days old and placed individually into fresh vials. Mating trials began when flies were four days old to allow 24 h for recovery from anesthesia.

Two experiments were conducted simultaneously. In experiment 1, individual four-day-old virgin females were transferred into a vial containing two males of a specific RIL, without using anesthesia. We removed the males after 2 h. Ninety-six hours later, we placed the once-mated females into a vial with two *ele* males, without using anesthesia. Because once-mated females are somewhat refractory to remating, these males were left in the female vial for 24 h. Females were transferred to a new vial after the males were removed, and then transferred to fresh vials every seven days for a total of five weeks after removal of the second male. All offspring emerging from these vials were scored for phenotype (wild type or *ebony*) and then discarded. The experiment was conducted in 10 replicate blocks, with each RIL assayed once per block. The number of adult offspring counted per female, including only females that produced offspring from their first mate, averaged 88.3 (SD = 43.5, *n* = 428, range = 4–214). We calculated mating success of each RIL

as the proportion of females that produced at least one *e/+* offspring during the five-week assay. We calculated sperm defense (P1) as the proportion of *e/+* offspring produced after the second mating, and during the first two weeks of the assay. A substantial number of female deaths during week 3 meant that data after that time point could be biased. In addition, for the calculation of P1, we included only females that had produced at least one offspring from each mate during the entire five-week assay.

Experiment 2 was identical to experiment 1, except that females were first mated to *e/e* males, and then to males from a specific RIL. This experiment was conducted in 11 replicate blocks, with each RIL assayed once per block. From experiment 2, we calculated remating success of each RIL as the proportion of females that produced at least one *e/+* offspring during the five-week assay. We calculated sperm offense (P2) as the proportion of *e/+* offspring produced after the second mating and during the first two weeks of the assay; we included only those females that produced at least one offspring from each mating during the entire five-week assay. In experiment 2, the number of adult offspring counted per female, counting only females that produced offspring from their first mate, averaged 79.0 (SD = 36.7, $n = 827$, range = 4–204).

We used a single female and two males per vial, rather than groups of females and males, to provide more scope for successful female rejection of male mating attempts. In vials with many males, females may be less refractory to mating than in vials with few males. It is possible that the two measures of mating success include some contribution from male sterility. If a male were completely sterile, he would be scored as “unsuccessful.” Also, if a male’s sperm were completely displaced by the competing male, he would be scored as “unsuccessful.” However, no RILs exhibited complete male sterility, and analysis of line means showed no significant correlation between mating success and sperm offense or defense (see Results). Also, previous studies of sperm competition show little evidence for complete sperm displacement in this species (Hughes 1997; Fiumera et al. 2005). We therefore deemed that our measures of mating success were not confounded with male sterility or with sperm competition measures.

Data Analysis

Because mating and remating success measures are binary traits, we used generalized linear models modified for random effects (SAS macro GLMMIX; SAS Institute Inc., Cary, NC), with a logit link function and line and block as categorical effects, to assess among-line variation. Line means on the logit scale were transformed back into probabilities for the mapping analysis. To assess among-line variation in P1 and P2, we first transformed these measures into competitive-index measures (Hughes 1997), since these typically have better statistical properties than proportions. The competitive index (CI) is the number of wild-type offspring divided by one plus the number of ebony offspring. For analysis, the cube root of CI was calculated, and residuals of the linear models met the assumptions of the analysis. We assessed among-line variation in CI by general linear models as implemented in SAS PROC MIXED (Ver. 9.1), with line and

block as random effects in the model. Least-square means were calculated from the same model, and these were used in the interval mapping procedure. Confidence intervals for variance components of all traits were calculated by generating 1000 bootstrap samples from the original data and calculating the among-line variance of the sample. We then determined empirical 95% confidence limits from the distribution of 1000 variance components.

We identified QTLs using composite interval mapping (CIM; Zeng 1994) in QTL Cartographer (ver. 1.16; Basten et al. 2002) as outlined in Leips and Mackay (2002). This mapping procedure tests the hypothesis that an interval between adjacent markers contains a QTL affecting the quantitative trait while controlling for the effects of linked QTLs outside of the test interval. Although interval mapping is typically applied to traits with normal distributions, this is a suitable method to map QTLs affecting the focal traits of this study as it is generally unbiased even for non-normal data (Zou et al. 2003). Markers on which the QTL analyses were conditioned were based on a forward-backward elimination stepwise regression analysis. Because the results of each analysis can be sensitive to the conditioning window used around each test interval, we tested a range of window sizes (5, 10, 15, and 20cM) to evaluate the effect of window size on the likelihood ratios for each QTL. In the end we used the 10cM window because QTLs identified with this window size were significant in all analyses, regardless of window size. Thus, this window size represents a conservative choice. The significance level for each QTL analysis was determined by randomly permuting the phenotypic data 1000 times and calculating the maximum likelihood ratio statistic across all test intervals for each permutation. LR statistics from the original data that were exceeded by the permutation maximum LR statistics less than 50 times were considered significant at $P < 0.05$ (Churchill and Doerge 1994; Doerge and Churchill 1996).

The proportion of the total variation explained by variation at the QTLs was calculated in QTL Cartographer (Basten et al. 2000). To estimate the allelic effects of each QTL, we first calculated the least-square mean phenotype of alternative homozygotes at the QTL position using ANOVA. Our model was $y = QTL + cofactors$; where y is the observed phenotype for a given RIL, QTL is the genotype of the particular RIL at the QTL position (*Ore/Ore* or *2b/2b*), and $cofactor(s)$ is the genotype of each RIL at the marker positions on which the QTL analysis was originally conditioned (as determined by QTL Cartographer). The difference between the least-square means of the alternative homozygotes was divided by two to give the allelic effect. Confidence intervals for the allelic effects were calculated in SAS using the standard formula for the variance of the difference between two means from the ANOVA above.

We tested for the effect of epistatic interactions between all possible markers using general linear models (PROC GLM in SAS ver. 9.1) first by looking for significant pairwise interactions between QTLs that had significant additive effects on the trait. For each interaction, the genotype of the marker closest to each QTL peak was used to evaluate the significance of marker interactions. Because epistasis may also occur between loci without main effects on the trait (Sen and

TABLE 1. Trait means (standard deviation) among-line variance (V_G) with confidence interval, P -value for likelihood ratio test of V_G , residual variance (V_E) with confidence interval, and V_G divided by V_E ($Rel V_G$).

Trait	Mean (SD)	N	V_G (95% CI)	P	V_E (95% CI)	$Rel V_G$
Mating success	0.72 (0.15)	98	0.77 (0.49, 1.16)	0.002	0.79 (0.72, 0.84)	0.97
Remating success	0.59 (0.17)	98	0.28 (0.13, 0.45)	0.05	0.95 (0.92, 0.97)	0.29
Sperm defense	0.10 (0.07)	98	0.02 (0.01, 0.02)	0.16	0.13 (0.01, 17)	0.15
Sperm offense	0.62 (0.13)	98	0.06 (0.03, 0.10)	0.13	0.58 (0.47, 0.70)	0.10

Churchill 2001; Mackay et al. 2005b) we performed a whole genome screen for pair-wise interactions between all possible pairs of markers using the model $y = \mu + M_i + M_j + M_i \times M_j + error$, where M is the genotype of each marker at positions i and j in each line. Using 80 markers, we tested 3160 possible two-way interactions. We therefore expected 158, 31.6, 3.2, and 0.32, significant interactions by chance alone at $P < 0.05$, $P < 0.01$, $P < 0.001$, and $P < 0.0001$, respectively.

Finally, we compared our QTL results to previous investigations of male traits that used the same mapping population and to two studies of differential gene expression in adult males. One of the expression studies (Gibson et al. 2004) compared mRNA expression in the parental lines used to create the RILs that we used for our mapping study; the other (Drnevich et al. 2004) reported variation in gene expression that was significantly associated with male reproductive success within a randomly mating population (the Ives population).

RESULTS

Mating success (first male success) showed highly significant among-line variation based on both the likelihood tests

and the bootstrap confidence interval. The among-line variation for remating success (second male success) was marginally nonsignificant by the likelihood test but the 95% bootstrap confidence interval did not overlap zero (Table 1). Neither sperm precedence trait exhibited significant among-line variation based on likelihood tests. However, the 95% confidence interval did not overlap zero for either trait, suggesting that the likelihood tests might be overly conservative. Nevertheless, the magnitude of the variance components for sperm competition is substantially smaller than those for mating success, both on the absolute scale and relative to the residual variance (Table 1, last column). We therefore conclude that the measures of mating success are more genetically variable in the RILs than are the measures of sperm competition.

Surprisingly, we found four regions of significant logarithmic odds (LOD) scores for remating success indicating significant QTLs (Fig. 1, Table 2), but no significant LOD scores for mating success, despite the more significant among-line variation for the latter trait. We also did not find significant QTL regions for the sperm precedence traits, despite the evidence for significant among-line variation. Pearson correlations among line means are shown in Table 3.

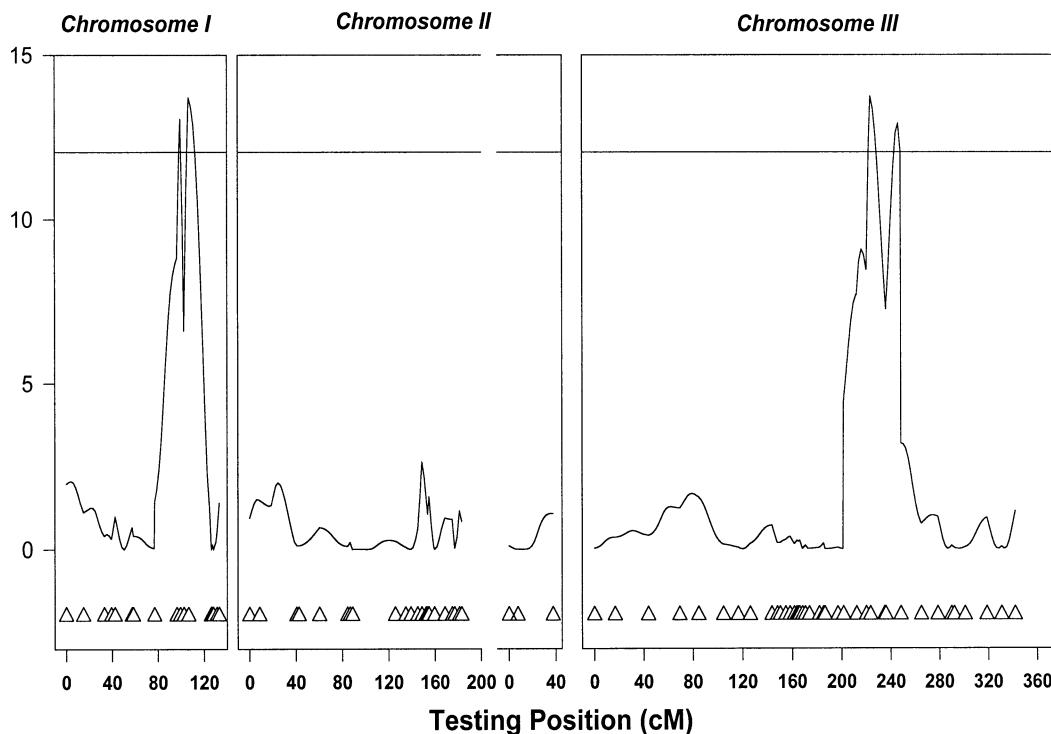


FIG. 1. QTL map for remating success showing position and logarithmic odds scores associated with each polymorphic marker (indicated by open triangles) used in the analysis.

TABLE 2. Significant QTLs for remating success. The allelic effect below is the change in the probability of remating success depending on the allele at that QTL. 3R, right arm of the third chromosome.

Chromosome	X	X	3R	3R
Location	11C	12E	92A	93B
95% CI	9A–14C	9A–15A	89B–96A	89B–96A
Effect of <i>Ore</i> allele	0.01	0.076	–0.065	–0.005
95% CI of effect	(–0.14, 0.18)	(0.01, 0.16)	(–0.01, –0.13)	(–0.09, 0.12)

Only the correlation between mating success and sperm defense is significant before correction for multiple tests. None of the correlations remain significant after sequential Bonferroni correction.

Of the four significant peaks for remating success, two are close together on the X chromosome, with overlapping 99% confidence intervals. The other two are on the right arm of the third chromosome, also with overlapping confidence intervals (Table 2). However, we assume that each peak is distinct because the likelihood scores between each pair of peaks dipped below the significance threshold. Whether a given peak represents one, two, or more QTLs is unknown and will need to be resolved by fine-scale mapping. For the X-linked QTLs, at position 11C the allele derived from the *Ore* parent increased remating success by 1%; at position 12E, the *Ore* allele increased remating success by 7.6%. For the QTLs on the third chromosome, the *Ore* allele at position 92A decreased remating success by 6.5%, while the *Ore* allele at position 93B decreased mating success by 0.5% (Table 2).

We found no significant epistatic interactions in the pairwise tests between the main effect QTLs; however, in the larger analysis of all markers we found 166 significant interactions at the $P < 0.05$ level, 23 of which had $P < 0.01$ and two of $P < 0.001$. At the $P < 0.05$ level, the number of significant interactions observed was higher than expected by chance and thus suggests that epistatic interactions among some subset of these markers contribute to the phenotypic variation in this trait.

Gene expression in adult males has been surveyed for the parental lines of the RILs used here (*Ore* and 2b), using microarrays (Gibson et al. 2004). Of the genes falling within the 99% confidence interval of the X-linked QTLs, 94 also had differential mRNA expression in that study. Two of these genes (CG1561 and CG15200) also had expression differences that were significantly associated with male reproductive success in a randomly mating population of *D. melanogaster* (Drnevich et al. 2004, see Table 4). Strikingly, the direction of the association with male fitness is the same for both genes in all three studies: higher mRNA abundance was associated with higher male fitness in both microarray stud-

ies, and the high QTL effect was due to alleles from the parental line with higher male fitness, *Ore* (Table 4).

One other gene in the X-linked QTL region (CG11699) was identified as a candidate gene for male fitness in the microarray study of Drnevich et al. (2004), and for this gene, high male fitness was associated with lower mRNA expression (Table 4). However, CG11699 did not exhibit significant expression differences in the study of the *Ore* and 2b lines. In addition, mRNA expression of both CG1561 and CG11699 exhibited a significant response to selection on male courtship behavior (Mackay et al. 2005a), although the direction of that response was not reported. Although expression differences may not be the causative factor underlying the QTL effects on this trait, these results suggest that the three genes listed in Table 4 warrant further investigation as candidate genes for variation in male reproductive fitness. Unfortunately, no functional annotation is currently available for any of these genes, although it is known that CG15200 is expressed in testes (Boutanaev et al. 2002) and CG11699 is expressed in the male accessory glands, which produce seminal fluid (Drnevich et al. 2004).

The QTL region on the third chromosome contains 315 genes that were differentially expressed between the parental lines (Gibson et al. 2004), of which 132 were also differentially expressed in the courtship-behavior selection experiment of Mackay et al. (2005a). However, none of these genes was significantly associated with male reproductive success in the Ives population (Drnevich et al. 2004).

A QTL study of male mating behavior used the same set of RILs to identify chromosomal regions affecting courtship occurrence and courtship latency, and the QTLs were refined using deficiency mapping (Moehring and Mackay 2004). None of the QTL regions we identified for remating success overlap with the candidate genes for mating behavior identified by Moehring and Mackay (2004). The remating success QTLs do overlap with QTLs affecting both virgin and mated male life span (Leips and Mackay 2002) but based on the most likely position of the life span QTLs, the loci affecting male life span are probably not the same as those affecting remating success.

DISCUSSION

Although first-male mating success demonstrated highly significant variation among RILs, no significant QTLs were identified. This pattern suggests that the variation for this trait is due to genes of small effect for which alternative alleles were fixed in the parental lines. Quantitative genetic analyses of a randomly mating population have supported similar conclusions: that genetic variation for first-male mating success is largely additive and that the amount of vari-

TABLE 3. Pearson correlation coefficients of line means for all traits. Asterisk indicates $P = 0.04$ before correction for multiple tests.

Trait	Mating success	Defense	Remating success	Offense
Mating success		0.20*	0.11	0.12
Defense			0.14	0.13
Remating success				0.02

TABLE 4. Genes within the QTL confidence intervals that are associated with male reproductive success in the Ives population. Two of these genes are also differentially expressed in adult males in parental lines of the RILs.

Gene	Position	Effect size ¹	Differential expression in Ore and 2b ²	Differential expression in selection lines ³	Male tissue-specific expression ⁴
CG1561	X: 10C9	0.17	yes, Ore >2b	yes	
CG15200	X: 10A10	0.20	yes, Ore >2b	no	T
CG11699	X: 10C7	-0.21	no	yes	M

¹ Mean difference in mRNA abundance between "high" and "low" male fitness genotypes on the log₂ scale, from Drnevich et al. 2004.

² From supplementary information of Gibson et al. (2004).

³ From table 5, Mackay et al. (2005).

⁴ Tissue-specific expression: M, male accessory gland (Drnevich et al. 2004); T, testis (Boutanaev et al. (2002).

ation is consistent with maintenance by mutation-selection balance (Hughes 1995a,b, 1997; Charlesworth and Hughes 2000). In the current study, one of the parental lines, 2b, was produced by both inbreeding and selecting for low male mating success when males were paired with virgin females (Kaidanov 1980). Males were categorized as having low mating ability if they failed to mate with a virgin female within 30 minutes of introduction. This line presumably fixed many alleles affecting first-male mating success. However, our analysis suggests that selection fixed few if any alleles of moderate or large effect. Large-effect alleles might have been eliminated because of the strong natural selection operating against them. Indeed, Kaidanov (1980) reports a very high failure rate of lines in his selection experiment due to low mating success and low fertility.

In contrast to the results for mating success, two to four significant QTLs were identified for second-male remating success, although the RILs were not as variable for this trait. This pattern is consistent with the hypothesis that differences between the lines are determined by a few loci with effects that are large relative to the total among-line variation in the trait. Our results indicate that the 2b line is fixed for at least one X-linked loci with deleterious effects on remating success, but also fixed for at least one third-chromosome locus with beneficial effects. In nature, and in normal laboratory culture, remating success is probably as important to male fitness as is first-male mating success because females mate multiply (Harshman and Clark 1998), and the last male to mate sires the highest proportion of offspring in this species (Clark et al. 1995; Hughes 1997; Fiumera et al. 2005; Friberg et al. 2005). However remating success per se was not under selection during the creation of line 2b.

The QTL regions for remating success that were identified on the third chromosome can be refined by deficiency mapping, as has been done for other complex traits (Pasyukova et al. 2000; Fanara et al. 2002; Harbison et al. 2004; De Luca et al. 2005). However, this method cannot be used to refine the X-linked regions because X-linked deficiencies are likely to be lethal or sublethal in male flies. For these regions, a different method of identifying candidate genes could be used, such as association mapping (e.g., Fiumera et al. 2005) or whole-genome expression profiling (e.g., Drnevich et al. 2004). By combining our QTL results with the results of expression profiling in the parental lines and in an independent population, we have identified three promising candidates on the X chromosome. Confirmation of these candidates

is now required, followed by functional characterization of any that are confirmed.

In contrast to previous studies, we found equivocal evidence for significant genetic variation in sperm competition traits. We suggest two potential causes of this discrepancy. The design of the mating trials in the current experiment provided substantial latitude for female rejection of mating attempts, and many females did not remate. This reduced the scope for variation among lines and reduced the power of the statistical tests for sperm competition relative to those for mating success. However, our assay was designed to reflect conditions in nature, where females can easily reject mating attempts. Indeed, other experiments have used similar protocols, but have detected significant genetic variation in sperm competition, suggesting an additional reason for failure to detect substantial variation in this study. The previous studies surveyed a large sample of genetic variation segregating within populations, but, as is the case in all QTLs mapping experiments, the variation present in the RILs represents only the variation present in the two parental lines. Also, variation among the RILs reflects only the homozygous effects of alleles. Neither homozygous effects nor variation in the two parental lines is necessarily representative of variation segregating within randomly mating populations (Mackay 2001, Hughes et al. 2006). If variation in sperm competition is maintained by strong balancing selection on a few loci, RILs generated from two inbred lines could fail to reflect variation that persists within randomly mating populations. Inbreeding, drift, and selection within lines will eliminate all but one allele at each locus, and different lines might fix functionally equivalent alleles, because selection will favor alleles with the highest homozygous fitness. However, QTL studies using inbred lines (including those used in this study) have successfully identified polymorphisms affecting morphological and fitness traits that appear to be maintained by balancing selection in natural populations (Long et al. 2000; De Luca et al. 2003; Carbone et al. 2006). Whether our failure to detect sperm competition QTL is due to unusual genetic architecture or to mating success variation obscuring the sperm competition variation present in the inbred lines remains to be determined. Given these possibilities, association studies and whole-genome expression profiling may be more effective than RIL mapping methods for identifying genes underlying natural variation in sperm competition.

ACKNOWLEDGMENTS

We thank C. Smith, R. Reynolds, S. Carlisle, and R. Sawby for help with the experiments; M. Reedy for help preparing the manuscript; and L. Rowe and two anonymous reviewers for insightful comments on a previous draft.

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