DOI: 10.1534/genetics.103.024372

The Quantitative Genetic Basis of Male Mating Behavior in *Drosophila melanogaster*

Amanda J. Moehring[1] and Trudy F. C. Mackay

Department of Genetics, North Carolina State University, Raleigh, North Carolina 27695-7614

Manuscript received November 7, 2003
Accepted for publication April 5, 2004

ABSTRACT

Male mating behavior is an important component of fitness in Drosophila and displays segregating variation in natural popluations. However, we know very little about the genes affecting naturally occurring variation in mating behavior, their effects, or their interactions. Here, we have mapped quantitative trait loci (QTL) affecting courtship occurrence, courtship latency, copulation occurrence, and copulation latency that segregate between a *D. melanogaster* strain selected for reduced male mating propensity (2b) and a standard wild-type strain (Oregon-R). Mating behavior was assessed in a population of 98 recombinant inbred lines derived from these two strains and QTL affecting mating behavior were mapped using composite interval mapping. We found four QTL affecting male mating behavior at cytological locations 1A;3E, 57C;57F, 72A;85F, and 96F;99A. We used deficiency complementation mapping to map the autosomal QTL with much higher resolution to five QTL at 56F5;56F8, 56F9;57A3, 70E1;71F4, 78C5;79A1, and 96F1;97B1. Quantitative complementation tests performed for 45 positional candidate genes within these intervals revealed 7 genes that failed to complement the QTL: *eagle*, *18 wheeler*, *Enhancer of split*, *Polycomb*, *spermatocyte arrest*, *l(2)05510*, and *l(2)k02206*. None of these genes have been previously implicated in mating behavior, demonstrating that quantitative analysis of subtle variants can reveal novel pleiotropic effects of key developmental loci on behavior.

QUANTITATIVE traits demonstrate a continuous range of phenotypes in natural populations, resulting from the combined effects of multiple genes whose expression is influenced by the environment. A major challenge of modern geneticists is to identify the quantitative trait loci (QTL) and environmental factors causing variation in quantitative traits. Most aspects of morphology, physiology, and behavior are quantitative traits, as are many human diseases and disorders, including schizophrenia and susceptibility to addictive behaviors. These behaviors can be further understood through the quantitative genetic dissection of behavioral traits in a model organism, such as *Drosophila melanogaster*.

Drosophila mating behavior provides an excellent model system for determining the genetic architecture of behavioral traits. The courtship behavior of Drosophila is composed of sequential actions that exchange auditory, visual, and chemosensory signals between males and females, allowing for the individual components of the behavior to be separated (Hall 1994; Greenspan 1995). First the male aligns himself with the female. Then he taps the female with his foreleg, performs a "courtship song" by vibrating one wing, extends his proboscis to lick the female's genitalia, attempts to copulate, and (if the female accepts his advances) copulates. These actions vary within and among species in the duration of courtship (Greenspan and Ferveur 2000) and copulation (Markow 1996).

P-element insertional and chemical mutagenesis have been used to identify genes involved in Drosophila courtship behaviors (Hall *et al.* 1980; Yamamoto and Nakano 1998). Mutations in *courtless* (*col*; Yamamoto and Nakano 1998), *cuckold* (*cuc*; Castrillon *et al.* 1993), *he's not interested* (*hni*; Yamamoto *et al.* 1997), *pale* (*ple*; Buchner 1991; Neckameyer 1998), and *tapered* (*ta*; Bien-Willner and Doane 1997) exhibit a decrease in male courtship intensity, while mutations in *spinster* (*spin*; Suzuki *et al.* 1997), *chaste* (*cht*; Yamamoto *et al.* 1997), and *dissatisfaction* (*dsf*; Finley *et al.* 1997) display decreased female receptivity. Mutations in *period* (*per*; Kyriacou and Hall 1980) and *cacophony* (*cac*; von Schilcher 1976; Peixoto and Hall 1998) result in altered rhythmicity in courtship song, and disruptions in components of the sex-determination pathway genes *Sex-lethal* (*Sxl*), *transformer* (*tra*), *transformer-2* (*tra-2*), or *fruitless* (*fru*; Cline 1993; Barbash and Cline 1995; MacDougall *et al.* 1995; Finley *et al.* 1997; Schütt and Nöthiger 2000) result in altered sexual orientation.

In Drosophila, male mating ability is a critical component of reproductive fitness (Partridge *et al.* 1985) and exhibits significant inbreeding depression (Sharp

[1]*Corresponding author:* Department of Genetics, Box 7614, North Carolina State University, Raleigh, NC 27695.
E-mail: ajmoehri@unity.ncsu.edu

1984). However, there is a considerable amount of segregating variation for the individual components of mating behavior, as well as for mating preference (Manning 1961, 1963; Parsons 1964), with estimates of heritability (h^2) for male mating speed ranging from 0.3 to 0.6 (Manning 1961, 1963; Parsons 1964; Collins and Hewitt 1984; Casares *et al.* 1993). This variation is plausibly maintained by genotype-by-sex interactions (Casares *et al.* 1993; Nuzhdin *et al.* 1997; Vieira *et al.* 2000).

We know very little about the loci contributing to naturally occurring variation in behavior, their effects, and their interactions. Possibly, alleles at loci identified through analysis of mutations contribute to variation in mating behavior in natural populations. However, mutations generated in an isogenic background have not yet been screened for their quantitative effects on mating behavior. Such screens for subtle allelic effects of hypomorphic mutations are likely to reveal novel loci affecting behavior (Anholt *et al.* 1996; Lyman *et al.* 1996; Norga *et al.* 2003) since genes affecting behavioral traits are usually highly pleiotropic, with null mutations leading to homozygous lethality (Sokolowski 2001). Alternatively, we can directly address the question of which genes affect variation in mating behavior by mapping QTL by linkage to polymorphic molecular markers in populations that have been selected for reduced courtship or copulation latency. The major difference in using this technique to study behavioral traits, rather than morphological ones, is that the former are more sensitive to uncontrolled (or uncontrollable) environmental fluctuations, necessitating the measurement of larger numbers of animals to obtain accurate estimates of the genotypic value of each line.

Here, we have mapped four QTL affecting components of male mating behavior in a population of recombinant inbred lines derived from the wild-type strain Oregon-R (Ore) and a strain selected for low male mating activity, 2b (Kaidanov 1990). We used deficiency complementation mapping (Pasyukova *et al.* 2000; Fanara *et al.* 2002; De Luca *et al.* 2003) to fine map the autosomal QTL regions with higher resolution and quantitative complementation tests (Long *et al.* 1996; Mackay and Fry 1996; Fanara *et al.* 2002; De Luca *et al.* 2003) to mutations at positional candidate genes to identify seven novel candidate genes contributing to the difference in mating behavior between Ore and 2b.

MATERIALS AND METHODS

Genome scan for QTL affecting mating behavior: *Drosophila stocks:* An inbred *D. melanogaster* line, 2b, was selected over 550 generations for reduced male mating activity (Kaidanov 1990). We measured the proportion copulated and copulation latency (see below) for the 2b strain and for Ore, a standard wild-type and unrelated stock (Lindsley and Zimm 1992), during a 2.5-hr observation period. Only 1 2b male of 42 observed mated within this time period, whereas 24 of 42 Ore males mated. This difference is highly significant ($G = 35.47$, $P < 0.0001$). However, there was no significant difference between Ore and 2b females when paired with Ore males in proportion copulated ($G = 0.80$, $P > 0.05$) or copulation latency ($t_{22} = 0.57$, $P > 0.05$). Therefore, we focused our analyses on male mating behaviors.

Ore and 2b were crossed and the F_1 progeny were backcrossed to 2b and allowed to randomly mate for 4 generations. A total of 200 individual pairs were selected in the fifth generation and their offspring were inbred by full-sib mating for 25 generations to create 98 recombinant inbred (RI) lines, each with a unique combination of Ore and 2b genomes (Nuzhdin *et al.* 1997). The genetic composition of these RI lines has been determined using 80 *roo* transposable elements with polymorphic insertion sites between the parental lines and an average spacing of 3.2 cM, and a visible marker *sparkling* (*spa*) that is fixed within Ore on chromosome *4* but is absent in 2b (Nuzhdin *et al.* 1997; Vieira *et al.* 2000). The *roo* transposable elements are located cytologically at 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, and 100A. Chromosome *2* is divided into two linkage groups because the recombination between markers 50F and 57C was >0.5 cM (Nuzhdin *et al.* 1997).

Mating behavior assays: All flies were maintained at 25° under 12-hr light-dark cycles. Virgin flies were collected under brief CO_2 exposure, separated by sex, and aged 5–7 days in small groups (25 individuals or fewer per vial) to ensure reproductive maturity. Behavioral assays were performed for each line by aspirating (*i.e.*, not anesthetizing) three females and one male of an RI line into 8-dram food vials containing ~3 ml of standard cornmeal-agar-molasses medium. All experiments were performed at 25°, 75% humidity, within 3.5–5.5 hr of "lights on" to eliminate the known temperature, humidity, and circadian influences. We recorded courtship latency (time from the entry of the male into the vial to the initiation of courtship), copulation latency (time from male vial entry to copulation), and courtship duration (copulation latency − courtship latency) for a period of 60 min. Since only 40 test vials could be observed within each session, one vial for each of 40 lines was observed, the 40 lines being chosen at random each day, thus randomizing the environmental variation within RI lines. A total of 20 independent measurements were performed per RI line.

Quantitative genetic analysis: The latency and duration data were analyzed by single classification, random-effects analysis of variance (ANOVA) among the RI lines using the GLM and VARCOMP procedures in SAS (Version 8.2; SAS Institute 1988). Examination of residuals from these tests indicated that the assumptions of the ANOVA were satisfied without transformation. The categorical trait of occurrence was analyzed with a *G*-test as well as with ANOVA. Broad sense heritability (H^2) for the traits in this population of RI lines was computed as $\sigma^2_L/(\sigma^2_L + \sigma^2_E)$, where σ^2_L is the among-line and σ^2_E the within-line variance component. Pairwise genetic correlations (r_G) between traits were calculated as $r_G = \mathrm{cov}_{12}/(\sigma_1\sigma_2)$, where cov_{12} is the product moment covariance between line means for traits 1 and 2 and σ_1 and σ_2 are the square roots of the among-line variance components for each trait separately.

QTL affecting mating behavior: For each of these measurements, the mean value per line was calculated in seconds. Since the observation period lasted 3600 sec (1 hr), flies that did not mate within the time period were given a score of 3601. Line means were not transformed prior to analysis since

they approximated a normal distribution. Genome scans for QTL were performed for courtship latency, courtship occurrence, copulation latency, and copulation occurrence using composite interval mapping (CIM; Zeng 1994) as implemented by QTL Cartographer (version 1.13; ftp://esssjp.stat.ncsu.edu/pub/qtlcart/) software (Basten *et al.* 1994, 1999). CIM computes the likelihood-ratio (LR) test statistic, $-2\ln(L_0/L_1)$, where (L_0/L_1) is the ratio of the null hypothesis (there is no QTL within the test interval) to the alternative hypothesis (there is a QTL within the test interval), taking into account the segregation of unlinked QTL by multiple regression. Marker cofactors were chosen for each trait by forward-backward stepwise regression. LR test statistics were computed every 0.1 cM using a "window size" (*i.e.*, the distance from the test interval within which marker cofactors are included) of 10 cM. Empirical significance thresholds were determined by randomly permutating the data 1000 times and calculating the maximum LR across each marker interval for each permutation (Doerge *et al.* 1997). If the maximum LR statistic for the permutated data exceeded the original LR statistic <50 times, the marker interval was declared to be significant at $P \leq 0.05$ (Churchill and Doerge 1994; Doerge and Churchill 1996).

Test for epistasis among the random effects of markers: Pairwise epistatic interactions were tested by running ANOVA models including each marker closest to the significant QTL and one pairwise interaction between markers (Dilda and Mackay 2002). A Bonferroni correction was applied to account for multiple tests.

Quantitative complementation tests to deficiencies and mutations: *Deficiency complementation mapping:* We used deficiency complementation mapping (Pasyukova *et al.* 2000) to fine map the three autosomal QTL affecting mating behavior. The deficiencies tested and their cytological locations are given in Table 1. Deficiency complementation cannot be used to map traits expressed in males on the *X* chromosome; fine-scale recombination will be necessary to map this QTL. Female flies containing deficiencies (*Df/Bal*) uncovering autosomal QTL regions were crossed to Ore or 2b males. Virgin males of the four resulting genotypes (Ore/*Df*, 2b/*Df*, Ore/*Bal*, and 2b/*Bal*) were collected, where *Df* denotes the deficiency and *Bal* the balancer chromosome. Some stocks also contained a *white* mutation, which could modify behavior due to decreased visual acuity. For these stocks, deficiency males were first crossed to *Samarkand Cy/Pm* or *Samarkand H/Sb* females (depending on whether the deficiency was present on the second or third chromosome). Virgin females (*Df/Cy* or *Df/Sb*) were collected and crossed to Ore and 2b, and w^+ males from this cross were collected and assessed for mating behavior.

Mating behavior assays: Behavioral assays were performed as described above with the following exceptions: Three *Samarkand* females were combined with one test male; 20 assays were performed per deficiency per genotype (Ore/*Df*, 2b/*Df*, Ore/*Bal*, and 2b/*Bal*); and one male of each genotype was observed per deficiency in each observation period, the deficiencies to be tested per day chosen at random (40 vials maximum = 10 deficiency stocks/observation period). For example, one observation was made for each of the four genotypes used to evaluate *Df(2R)CX1* (Ore/*Df(2R)CX1*, 2b/*Df(2R)CX1*, Ore/*Bal*, and 2b/*Bal*) for each observation period in which *Df(2R)CX1* was randomly chosen.

Statistical analysis: The test for quantitative failure to complement is whether the difference between the mating behavior of the Ore/*Df* and 2b/*Df* flies is the same as the difference in mating behavior between Ore/*Bal* and 2b/*Bal*. In other words, quantitative complementation occurs when (Ore/*Df* − 2b/*Df*) = (Ore/*Bal* − 2b/*Bal*), and quantitative failure to complement occurs when (Ore/*Df* − 2b/*Df*) > (Ore/*Bal* − 2b/*Bal*). Quantitative failure to complement results in a significant line-by-genotype interaction term in a two-way ANOVA cross-classified design,

$$y = \mu + L + G + L \times G + E,$$

where μ is the overall mean, L is the main effect of the parental line (Ore or 2b), G is the main effect of genotype (*Df* or *Bal*), $L \times G$ is the interaction term, and E is the error variance within $L \times G$. If the variance of the differences ($L \times G$) is significant (ANOVA, $P \leq 0.05$), and the difference between Ore/*Df* − 2b/*Df* is greater than the difference between Ore/*Bal* − 2b/*Bal*, *i.e.*, consistent with an allelic interaction, then we conclude that the deficient region failed to complement Ore and 2b QTL (Pasyukova *et al.* 2000). Error variances for the categorical traits of courtship and copulation occurrence are not normally distributed and could violate the ANOVA assumption of normality, yet ANOVA has been shown to be robust in spite of departures from normality (Lush *et al.* 1948; Robertson and Lerner 1949; Dempster and Lerner 1950). Deficiency stocks that were found to be significant were retested. The ANOVAs and tests of significance for these deficiencies were repeated, pooling across replicates by adding the random effect of replicate nested within the $L \times G$ term. QTL locations were inferred using proximal and distal breakpoints of nonsignificant deficiencies overlapping significant deficiencies. Failure of deficiencies to complement QTL confirms the presence of QTL in the candidate region.

Complementation tests to candidate genes: Quantitative complementation tests to candidate genes in the QTL regions defined by deficiency mapping were used to further identify putative candidate genes corresponding to the QTL (Table 2). All 34 candidate genes within regions defined by deficiency complementation mapping that had healthy stocks available were assayed and analyzed using the procedure described above for deficiency complementation mapping. An additional 11 candidate genes generated by insertion of the *P{GT1}* element (Lukacsovich *et al.* 2001) in an isogenic derivative of Canton-S as part of the Berkeley Gene Disruption Project (http://www.fruitfly.org; Norga *et al.* 2003) were tested using a slightly altered paradigm. For the Baylor gene lines, single males were paired with one female and whether or not copulation occurred in 40 min was recorded since the previous assays indicated that this is a sufficient protocol for determining significance.

RESULTS

Mating behavior phenotypes and genetic variation in mating behavior in RI lines: The average times to the initiation of courtship behavior (1440 ± 39.3 sec = 24.0 min) and copulation (2645 ± 25.8 sec = 44.1 min), as well as the occurrence of courtship (0.781 ± 0.012) and copulation (0.495 ± 0.011), are similar for the population of RI lines and the Ore parental strain (1371 ± 238.1; 2637 ± 171.1; 0.950 ± 0.050; 0.524 ± 0.078, respectively). A comparison to the 2b parental strain is not possible since only a single 2b male courted and copulated within 2.5 hr—the maximum period for direct observation.

There was significant genetic variation between the 98 RI lines for courtship latency ($P \leq 0.0001$; Figure 1A), courtship occurrence ($P \leq 0.0001$; Figure 1B), copulation latency ($P \leq 0.0001$; Figure 1C), copulation occurrence ($P \leq 0.0001$; Figure 1D), and courtship du-

TABLE 1

Stocks used for deficiency complementation mapping

Chromosome *2*			Chromosome *3*		
QTL	Genotype	Cytogenetic location	QTL	Genotype	Cytogenetic location
57C–57F	*Df(2R)CX1*	49C1–4; 50C23–D2	72A–85F	*Df(3L)vin7*	68C8–11; 69B4–5
	Df(2R)L48	50F6–9; 51B3		*Df(3L)iro-2*	69B1–5; 69D1–6
	Df(2R)trix	51A1–2; 51B6		*In(3LR)C190*	69F3–4; 70C3–4
	Df(2R)03072	51A5; 51C1		*Df(3L)fz-CAL5*	70C2–6; 70E1
	Df(2R)Jp1	51D3–8; 52F5–9		*Df(3L)Brd6*	70E; 71F
	Df(2R)vg89e88	52B3–C1; 53E2–F2		*Df(3L)brm11*	71F1–4; 72D1–10
	Df(2R)Jp6	52E3–5; 52F		*Df(3L)st-F13*	72C1–D1; 73A3–4
	Df(2R)Jp8	52F5–9; 52F10–53A1		*Df(3L)81k19*	73A3; 74F
	Df(2R)Pcl7B	54E8–F1; 55B9–C1		*Df(3L)W10*	75A6–7; 75C1–2
	Df(2R)PC4	55A; 55F		*Df(3L)VW3*	76A3; 76B2
	Df(2R)P34	55E2–4; 56C1–11		*Df(3L)kto2*	76B1–2; 76D5
	Df(2R)017	56F5; 56F15		*Df(3L)XS-533*	76B4; 77B
	Df(2R)min	56F8–17; 56F8–17		*Df(3L)rdgC-co2*	77A1; 77D1
	Df(2R)AA21	56F9–17; 57D11–12		*Df(3L)ri-79c*	77B–C; 77F–78A
	Df(2R)D4	57A1–3; 57B13		*Df(3L)ME107*	77F3; 78C8–9
	Df(2R)exu1	57A2; 57B1		*Df(3L)Pc-2q*	78C5–6; 78E3–79A1
	Df(2R)Pu-D17	57B4; 58B		*Df(3L)Delta1AK*	79F; 80A
	Df(2R)X58-7	58B1–2; 58E4–10		*Df(3R)ME15*	81F3–6; 82F5–7
	Df(2R)X58-12	58D1–2; 59A		*Df(3R)3-4*	82F3–4; 82F10–11
	Df(2R)59AB	59A1–3; 59B1–2		*Df(3R)Tp110*	83C1–2; 84B1–2
	Df(2R)59AD	59A1–3; 59D1–4		*Df(3R)roe*	84A6–B1; 84D4–D9
	Df(2R)twi	59C3–4; 59D1–2		*Df(3R)dsx2*	84C1–3; 84E1
	Df(2R)bw-S46	59D8–11; 60A7		*Df(3R)p712*	84D4–6; 85B6
	Df(2R)or-BR6	59D5–10; 60B3–8		*Df(3R)p-XT103*	85A2; 85C1–2
	Df(2R)Chi[g230]	60A3–7; 60B4–7		*Df(3R)by10*	85D8–12; 85E7–F1
	Df(2R)Px1	60B8–10; 60D1–2		*Df(3R)by62*	85D11–14; 85F16
	Df(2R)Px2	60C5–6; 60D9–10		*Df(3R)M-Kx1*	86C1; 87B1–5
	Df(2R)M60E	60E2–3; 60E11–12		*Df(3R)T-32*	86E2–4; 87C6–7
	Df(2R)ES1	60E6–8; 60F1–2		*Df(3R)ry615*	87B11–13; 87E8–11
	Df(2R)Kr10	60F1; 60F5			
	Df(2R)Kr14	60F2; 60F5			
			96F–99A	*Df(3R)96B*	96A21; 96C2
				Df(3R)Espl3	96F1; 97B1
				Df(3R)Tl-P	97A; 98A1–2
				Df(3R)D605	97E3; 98A5
				Df(3R)3450	98E3; 99A6–8
				Df(3R)Dr-rv1	99A1–2; 99B6–11
				Df(3R)01215	99A6; 99C1
				Df(3R)L127	99B5–6; 99E4–F1
				Df(3R)B81	99C8; 100F5
				Df(3R)awd-KRB	100C;100D

ration ($P \leq 0.0001$; Figure 1E). Heritabilities were 0.28, 0.19, 0.25, 0.22, and 0.20, respectively, for courtship latency, courtship occurrence, copulation latency, copulation occurrence, and courtship duration.

There was a positive genetic correlation between courtship and copulation latency ($r_G = 0.70$; Figure 2A), as well as between courtship and copulation occurrence ($r_G = 0.72$; Figure 2B). However, there was a negative genetic correlation between courtship latency and courtship occurrence ($r_G = -0.89$; Figure 2C) as well as between copulation latency and copulation occurrence ($r_G = -0.94$; Figure 2D), demonstrating that the later that courtship or copulation is initiated, the less likely it is to be successful. Additionally, there was a stronger correlation, albeit negative, between latency and occurrence (for both courtship and copulation) than between courtship and copulation occurrence and courtship and copulation latency, demonstrating that the simpler measure of occurrence can be used as a correlate for latency.

Genome scan for QTL affecting mating behavior: There is significant variation among RI lines for all of the traits measured, making it possible to implement QTL mapping to identify candidate loci. We used CIM

TABLE 2

Genes used for quantitative complementation tests

Significant QTL	Gene tested	Cytological location
56F5; 57B4	*smooth*^{BG00756}	56D15–E1
	l(2)k08002^{k08002}	56F6–9
	18 wheeler$^{Delta7\text{-}35}$	56F8
	humpy^{1}	56F9; 58A1
	mus209^{k00704}	56F10–11
	CG11208;CG11209^{BG01288}	56F11
	l(2)s1866^{s1866}	56F11–12
	CG13871;CG8920^{BG02518}	56F16
	l(2)s4831^{s4831}	57A3–4
	l(2)k09920^{k09920}	57A3–6
	l(2)k16204^{k16204}	57A5–6
	l(2)05510^{05510}	57A5–6
	CG13432;CG13434^{BG02102}	57A5–6; 57A6
	bancal^{k07917}	57A6–7
	l(2)k02206^{k02206}	57A8–9
	BG01609	57A9
	l(2)k06409^{k06409}	57B1–3
	CG13438;CG13439^{BG02471}	57B1; 57B2–3
	inscuteable^{P49}	57B3
78C5; 79A1	*spermatocyte arrest*^{1}	78A2; 78C9
	l(3)04063^{04063}	78A3; 78D2
	l(3)neo29^{1}	78C
	l(3)ry3^{2}	78C; 78D
	CG5051;CG11308^{BG01493}	78C3–4; 78C7
	Polycomb^{1}	78C6–7
	Aef1^{BG01171}	78C8
	l(3)78Da^{2}	78C8–9
	SR3-7$^{XS\text{-}5R}$	78D; 79A
	Hr 78^{2}	78D1
	l(3)00534^{00534}	78D1–2
	M6^{BG00390}	78D4
	eagle^{1}	78E5–6
	CG7145^{BG02816}	78F1
	mushroom-body expressed^{BG01362}	78F4–79B1
	CG14560^{BG01919}	79A7
96F; 97A	*taxi*^{1}	96A20; 96F9
	l(3)rQ197^{rQ197}	96F1–2
	Enhancer of split^{R1}	96F8
	CG8354;CG8361^{BG02029}	96F8
	groucho^{C105}	96F8–9
	Prickly^{L}	96F11–14
	spindle-D^{2}	97A1–2
	goulash^{1}	97A1; 98A2
	Aldolase^{2}	97A6
	CG5467;CG5468^{BG01280}	97B6; 97B9

to localize QTL affecting components of male mating behavior (Zeng 1994). Four QTL affecting courtship occurrence and latency, as well as copulation occurrence and latency, were significant on the basis of permutation-derived significance thresholds: 1A;3E, 57C;57F, 72A;85F, and 96F;99A. A fifth QTL at 61A;65A affecting copulation latency had a LR (16.41) that was only slightly less than the threshold (16.75). These regions range from 2 to 21 cM and encompass 87–1467 candidate genes. While two of the regions contribute to all four mating behavior traits (Table 3, Figure 3), the remaining two QTL contribute to only copulation latency or courtship latency. We did not detect any QTL for courtship duration despite the significant genetic variation for this trait. The effects of this trait must be too small to detect given the limited number of measurements taken for courtship duration, which required both courtship latency and copulation latency to occur in many of the lines. It is possible that there are additional QTL for male mating behavior traits, but

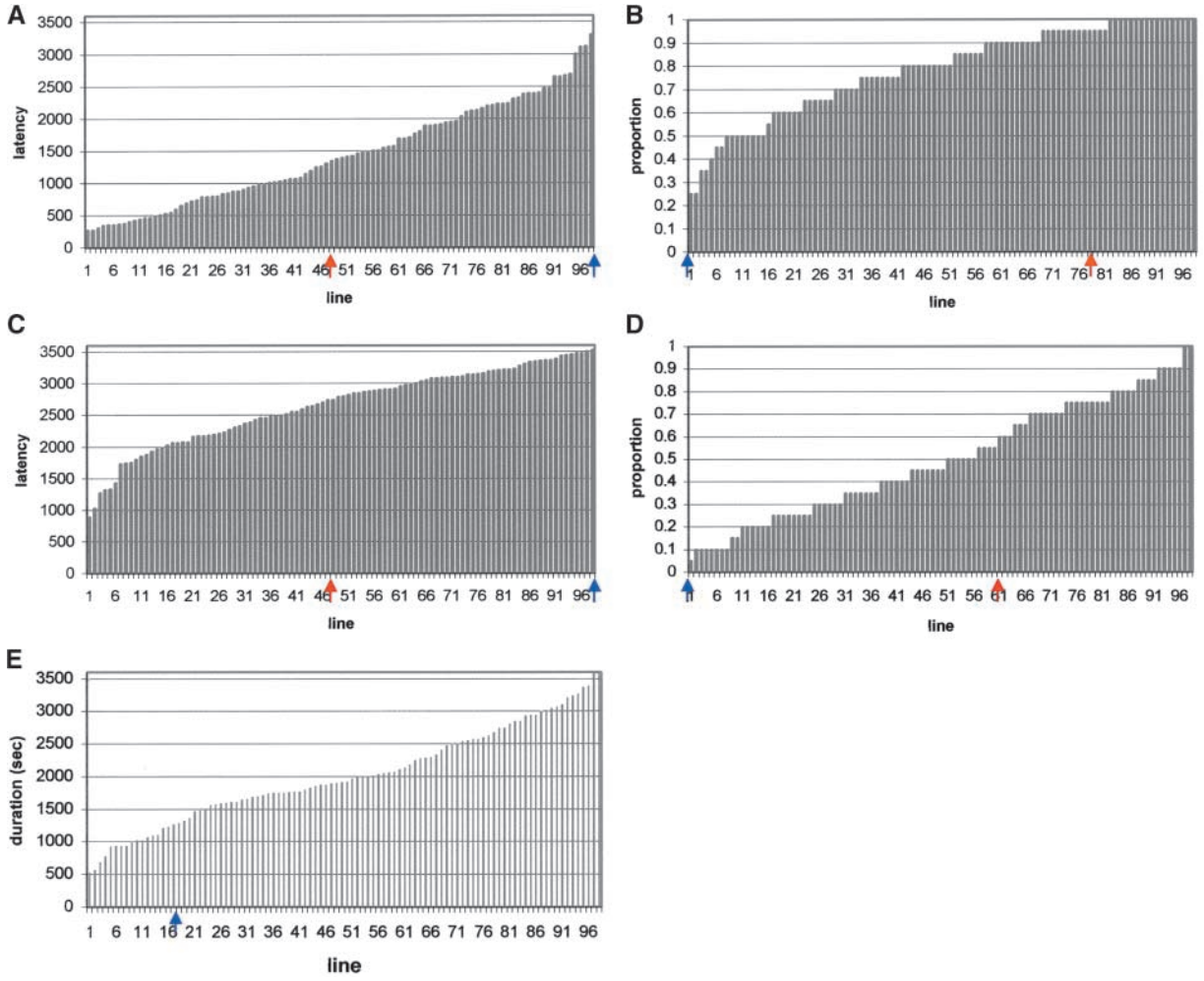


FIGURE 1.—Continuous distributions of means among the 98 RI lines for (A) courtship latency, (B) courtship occurrence, (C) copulation latency, (D) copulation occurrence, and (E) courtship duration, sorted from least to greatest. The arrows represent the mean of the Ore (red) and 2b (blue) parental lines.

their effects are too small to be detected given the sample size used. Even if this is true, it is still quite clear that of the genes that have the greatest effect there are some genetic factors that overlap in their contribution to mating behavior as a whole, while others are specific to individual components of mating behavior.

Epistasis between mating behavior QTL: We tested for epistatic interactions between the marker closest to each significant QTL (Table 3) and all of the other markers. After Bonferroni correction for multiple tests, none of the epistatic interactions were significant.

Deficiency complementation mapping: The sizes of the QTL intervals range from 2 to 21 cM (with an average of 8.8 cM) and include 87–1467 genes. We therefore utilized deficiency complementation mapping to reduce the size of each QTL interval (Table 4; supplementary Table 1 at http://www.genetics.org/supplemental/; Figures 4 and 5). The QTL from 57C;57F fractionated into two much smaller regions at 56F5;56F8 and at 56F9;57A3. The region from 72A;85F was reduced from ~13,000 kb to two smaller regions. One region at 70E1;71F1–4 was 25 kb, containing 22 genes for which no mutant stocks were available, and another region from 78C5–6;78E3–79A1 was 675 kb and contained 94 genes. The region from 96F;99A was reduced from ~2900 kb to a single smaller region at 96F1;97B1, with 580 kb and 98 genes. While each refined region could represent the effect of a single gene, it is quite possible that there are multiple closely linked genes within a region contributing to mating behavior. It should be noted that no candidate genes within these refined regions have been previously implicated as affecting components of male mating behavior. The inferences are subject to the usual caveat regarding deficiency complementation tests: Failure to complement might be attributable to epistatic interactions between deficiencies and Ore and 2b QTL not uncovered by the deficiencies (PASYUKOVA *et al* 2000).

Seven candidate genes are associated with variation in mating behavior: Of the mutations in 45 genes that were tested (Table 2), mutations in 7 genes were found to fail to complement Ore and 2b QTL alleles for com-

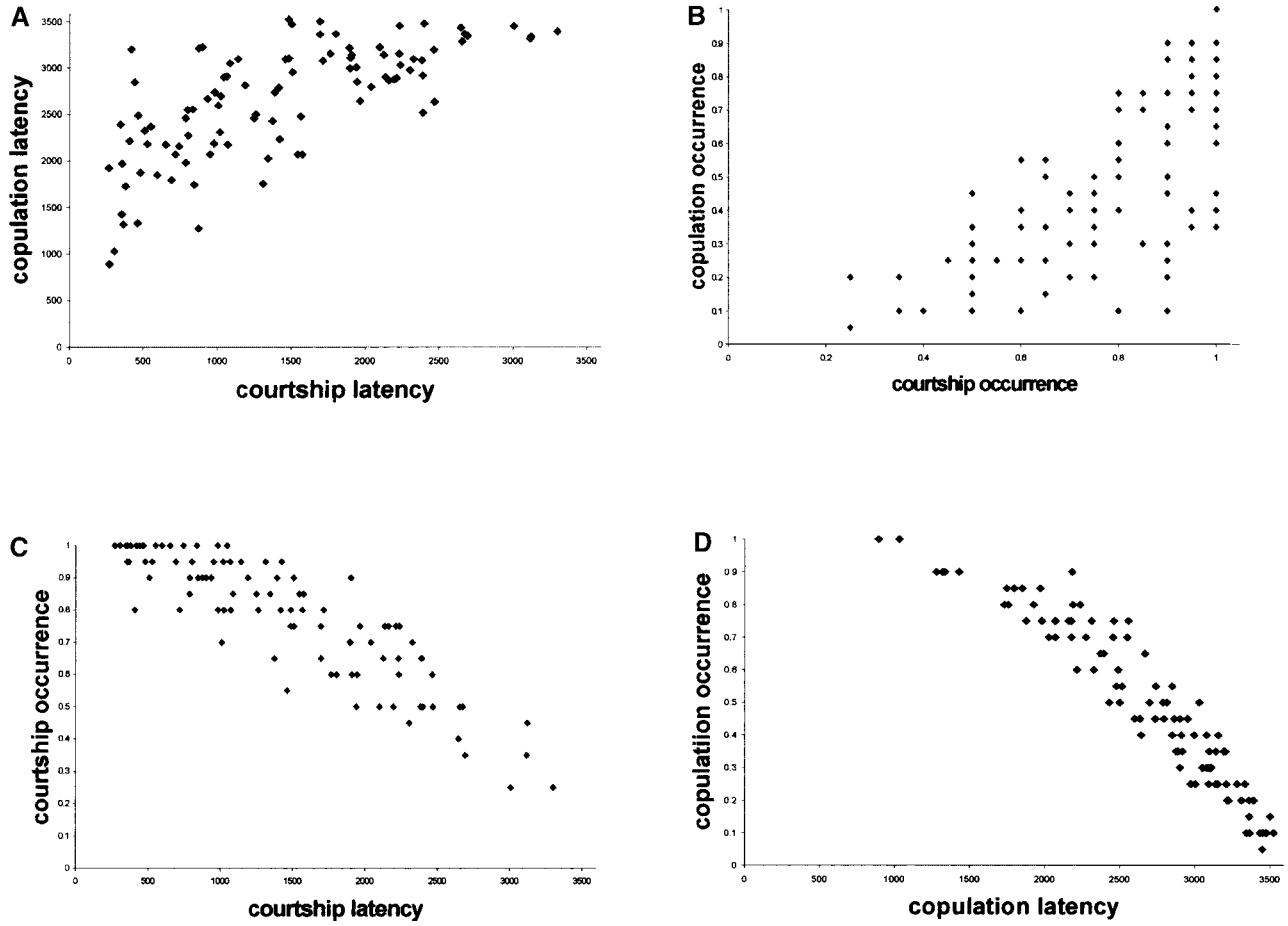


FIGURE 2.—Correlations among line means for (A) courtship and copulation latency, (B) courtship and copulation occurrence, (C) courtship occurrence and latency, and (D) copulation occurrence and latency.

ponents of male mating behavior: *l(2)k02206*, *l(2)05510*, *18 wheeler*, *spermatocyte arrest*, *eagle*, *Polycomb*, and *Enhancer of split* (Table 5; supplementary Table 1; Figure 6). None of these genes have previously been implicated to affect these traits.

DISCUSSION

QTL affecting mating behavior: We performed a genome scan for QTL affecting variation in components of male mating behavior between Ore, a standard wild-type strain, and 2b, a strain selected for reduced male mating activity. At least four QTL, one each on the *X* and second chromosomes, and two on chromosome *3*, contributed to the divergence between these strains in courtship occurrence and latency and copulation occurrence and latency. Quantitative complementation tests to deficiencies (PASYUKOVA *et al.* 2000) spanning the autosomal QTL enabled fine mapping of these QTL to five small regions, ranging in size from 25 to 675 kb and containing from 22 to 98 positional candidate genes.

We are confident that the QTL affecting male mating behavior are not simply a consequence of generally reduced fitness, since Ore and 2b do not differ genetically for fitness (WAYNE *et al.* 2001). Further, the QTL mapped in this study may be specific for mating behavior, since we and others have mapped QTL for additional traits in the same set of recombinant inbred lines, and they do not colocalize with the mating behavior QTL. Specifically, QTL affecting variation between Ore and 2b in sex comb tooth number (which are used to grip the female during copulation; NUZHDIN and REIWITCH 2000), courtship song (GLEASON *et al.* 2002), and locomotor activity (K. W. JORDAN, personal communication) do not overlap any of the QTL affecting male mating behavior. Either the variation in these lines for sex comb tooth number, courtship song, and locomotor activity does not contribute significantly to the initiation of courtship and copulation or variation in these modalities alone does not affect the overall success of courtship in these lines.

The same caveat applies to this study as to all genome scans for QTL using line-cross analysis: The number of QTL detected is always a lower bound to the number of QTL affecting naturally occurring variation in the trait (MACKAY 2001). More QTL, with smaller effects, contributing to the divergence in components of mating behavior between Ore and 2b could be detected by

TABLE 3

Summary of QTL mapping results

QTL	Marker[a]	95% CL	Trait	LR[a]	Effect[b]	kb[c]	cM[d]	No. loci[e]
1	1B	1A–3E	Courtship latency	20.84	0.38	3,689	5	419
2	57C	57C–57F	Courtship latency	37.93	0.55	768	2	87
			Courtship occurrence	23.16	0.46			
			Copulation latency	22.02	0.43			
			Copulation occurrence	32.28	0.47			
3	63A	61A–65A	Copulation latency	16.41	0.45	5,336	21	606
4	76A	72A–85F	Copulation latency	23.50	0.42	12,911	6	1,467
5	98A	96F–99A	Courtship latency	26.87	0.44	2,885	10	328
		97E–99A	Courtship occurrence	26.38	0.54			
			Copulation latency	20.10	0.45			
			Copulation occurrence	32.37	0.48			

CL, confidence limits.

[a] Peak LR.

[b] Proportion of the phenotypic variance accounted for by each QTL ($= a/\sigma_A$).

[c] Size of the QTL region given by the 95% CL (= 2-LOD support interval), in kilobase (kb) pairs (Sorsa 1988).

[d] Size of the QTL region given by the 95% CL, in centimorgans; (Lindsley and Zimm 1992).

[e] Average number of genes in the QTL region defined by the 95% CL, based on a total eukaryotic genome size of 120 Mbp and 13,600 genes and predicted genes; 1 gene on average = 8.8 kb (Adams *et al.* 2000).

increasing the number of recombinants as well as the number of individuals tested per recombinant genotype. Further, these strains represent only a subset of naturally occurring variation, and expanding the study to other strains is likely to uncover additional QTL. Nevertheless, it is interesting to compare these results with studies in which QTL affecting aspects of mating behavior have been mapped between "races" of *D. melanogaster* and between different Drosophila species.

The Zimbabwe subpopulation of *D. melanogaster* is partially reproductively isolated from other African populations as well as all continental *D. melanogaster* strains that have been tested: Zimbabwe females do not mate readily with non-Zimbabwe males (Wu *et al.* 1995). Each of the major chromosomes affects the ability of non-Zimbabwe males to mate with Zimbabwe females (Hollocher *et al.* 1997). Further recombination mapping of the third chromosome (Ting *et al.* 2001) showed that all of the third-chromosome QTL identified in this study potentially overlapped those contributing to partial reproductive isolation between the Zimbabwe subpopulation and cosmopolitan strains. High-resolution mapping is necessary to determine whether the QTL affecting variation in components of male mating behav-

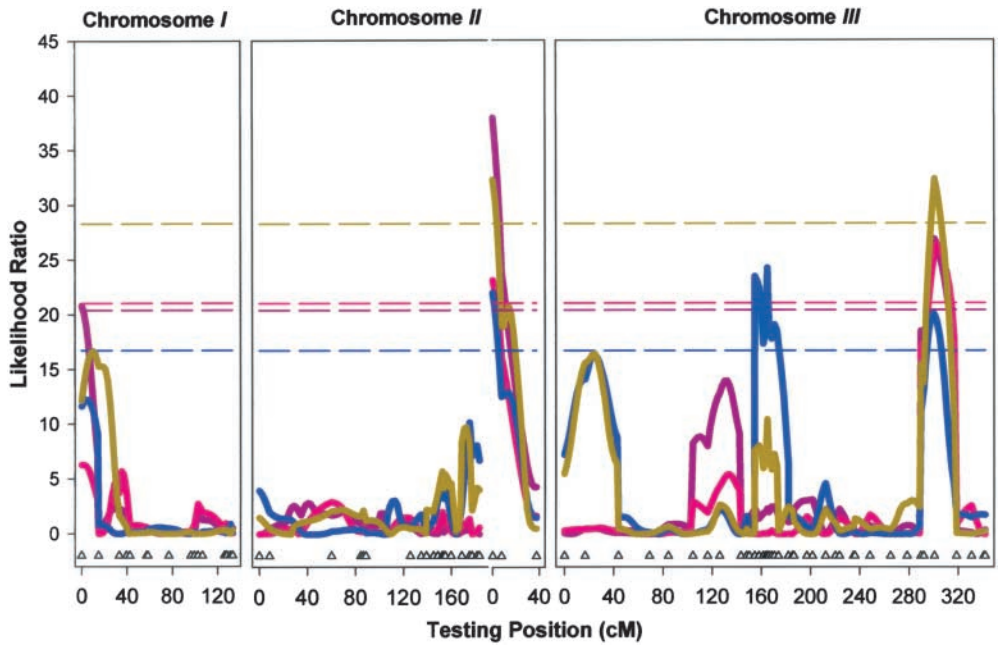


Figure 3.—LR scores and significance thresholds plotted against chromosome location from multiple-trait composite interval mapping for courtship latency (purple), courtship occurrence (pink), copulation latency (dark blue), and copulation occurrence (gold). Horizontal lines represent the significance thresholds for each trait and the triangles on the *x*-axis represent the locations of cytogenetic markers.

TABLE 4

Refined QTL regions as determined by deficiency complementation mapping with the *P*-values from ANOVA for deficiencies that showed failure to complement

Refined QTL	kb[a]	No. loci[a]	Genotype	Courtship latency	Courtship occurrence	Copulation latency	Copulation occurrence
56F5–56F8	160	25	*Df(2R)017*	0.0144	0.0071	0.0491	0.0119
56F9–57A3	225	53	*Df(2R)AA21*	0.0009	0.0002	0.0020	0.0002
			Df(2R)exu1	0.0146	0.0078	NS	0.0350
70E1–71F4	25	22	*Df(3L)Brd6*	0.0033	<0.0001	0.0133	<0.0001
78C6–79A1	675	94	*Df(3L)Pc-2q*	<0.0001	<0.0001	<0.0001	<0.0001
96F1–97B1	580	98	*Df(3R)Espl3*	<0.0001	<0.0001	<0.0001	<0.0001

NS, $P > 0.05$.

[a] Approximate size of the QTL region in kilobase (kb) pairs and number of genes in QTL region defined by FlyBase (FlyBase Consortium 2003).

ior identified in this study are the same as those that cause incipient reproductive isolation between the Zimbabwe and Cosmopolitan races of *D. melanogaster*.

To what extent do QTL affecting variation in mating behavior in *D. melanogaster* coincide with QTL affecting variation in mating behavior and related traits between the Drosophila species? Moehring *et al.* (2004, accompanying article, this issue) mapped QTL affecting the one-way sexual isolation between *D. mauritiana* and *D. simulans*. *D. mauritiana* females do not mate with *D. simulans* males in the laboratory, but the reciprocal cross occurs readily. At least seven QTL affect the preference of *D. mauritiana* females for conspecific males, two of which (52F;59C and 95C;97D) overlap the QTL mapped in this study. At least three QTL affect the male *D. simulans* traits against which *D. mauritiana* females discriminate, one of which (95D;100E) overlapped the third-chromosome QTL mapped here. The tip of chromosome *3R* also contained a QTL affecting courtship song differences between *D. pseudoobscura* and *D. persimilis*, at the cytological location equivalent to 93–98 in *D. melanogaster* (Williams *et al.* 2001), overlapping the QTL we found for courtship and copulatory behavior at 96F–97B. In contrast, Civetta and Cantor (2003) mapped only one QTL affecting variation in courtship latency and copulation latency between *D. simulans* and its sibling species *D. sechellia*, to 84A–86B on the third chromosome. This QTL does not overlap those detected in this study.

It is intriguing that some QTL affecting variation in mating behavior within *D. melanogaster* do colocalize to those affecting interspecific variation in mating behavior, but future high-resolution mapping studies are necessary to demonstrate whether or not the same genes are responsible. Lack of congruence between intra- and interspecific QTL affecting variation in mating behavior could be attributable to the restricted sample of genetic variation within *D. melanogaster* or because largely different loci contribute to segregating variation in mating behavior within species and to prezygotic sexual isolation between species. This issue can be resolved in the future only by high-resolution mapping of QTL within multiple species and by all possible interspecific comparisons among them, an endeavor for which Drosophila is especially well suited.

Positional candidate genes affecting mating behavior: Mapping QTL contributing to variation in components of mating behavior within and between species can reveal potentially novel and evolutionarily significant loci affecting adaptation and speciation, but only if the underlying genes within the QTL intervals can be identified. Defining the contributing quantitative trait genes is not trivial, since they have small and environmentally sensitive effects, and tens of thousands of recombinations must be screened to positionally clone each gene (Mackay 2001). It is not surprising that only a handful of QTL have been mapped to the level of genetic loci (Glazier *et al.* 2002) to date. In model organisms with excellent genetic resources, conducting quantitative complementation tests of mutations at positional candidate genes in the QTL intervals can define candidate quantitative trait genes for further study (Mackay 2001; Fanara *et al.* 2002; De Luca *et al.* 2003). Here, we have used this approach to identify seven novel candidate genes associated with variation in mating behavior between Oregon and 2b: *l(2)k02206*, *l(2)05510*, *18 wheeler*, *spermatocyte arrest*, *eagle*, *Polycomb*, and *Enhancer of split*.

The gene product of *l(2)05510* has not been characterized. *l(2)k02206* encodes a protein of unknown function that interacts with *pannier*. It is expressed in dorsocentral and scutellar bristles and in tormogen and trichogen cells, implicating a role in peripheral nervous system development. *18 wheeler* (*18w*), located cytologically at 56F8, encodes a transmembrane receptor localized to the plasma membrane. It is involved in morphogenesis during pattern formation and imaginal cell determination, and mutants often display morphological defects in their appendages. It is also a critical component of the humoral immune response (Eldon *et al.* 1994; Williams *et al.* 1997).

spermatocyte arrest (*sa*), located at 78A2–C9, encodes a product involved in spermatid development (Fuller 1998). It is required for male meiotic cell cycle progression and the initiation of postmeiotic differentiation (Lin *et al.* 1996). Mutants have multipolar spindles in male meiosis and irregular mitotic figures in the larval neuroblasts, which is the result of aberrant behavior of the mitotic spindle during embryonic cleavage (Wilson and Fuller 1991). The meiotic arrest phenotype is similar to that seen for meiosis I maturation arrest infertility in human males, suggesting that the pathway control is conserved from flies to humans (Lin *et al.* 1996).

Polycomb (*Pc*), located at 78C6–7, is named for the ectopic sex comb teeth on the second and third legs of mutants (Duncan and Kaufman 1975). It interacts with at least 59 other genes, including *Antennapedia* and *trithorax* (FlyBase Consortium 2003). *Polycomb* expression is localized to the nucleus where it encodes a transcriptional repressor (Gould *et al.* 1990; Roseman *et al.* 2001).

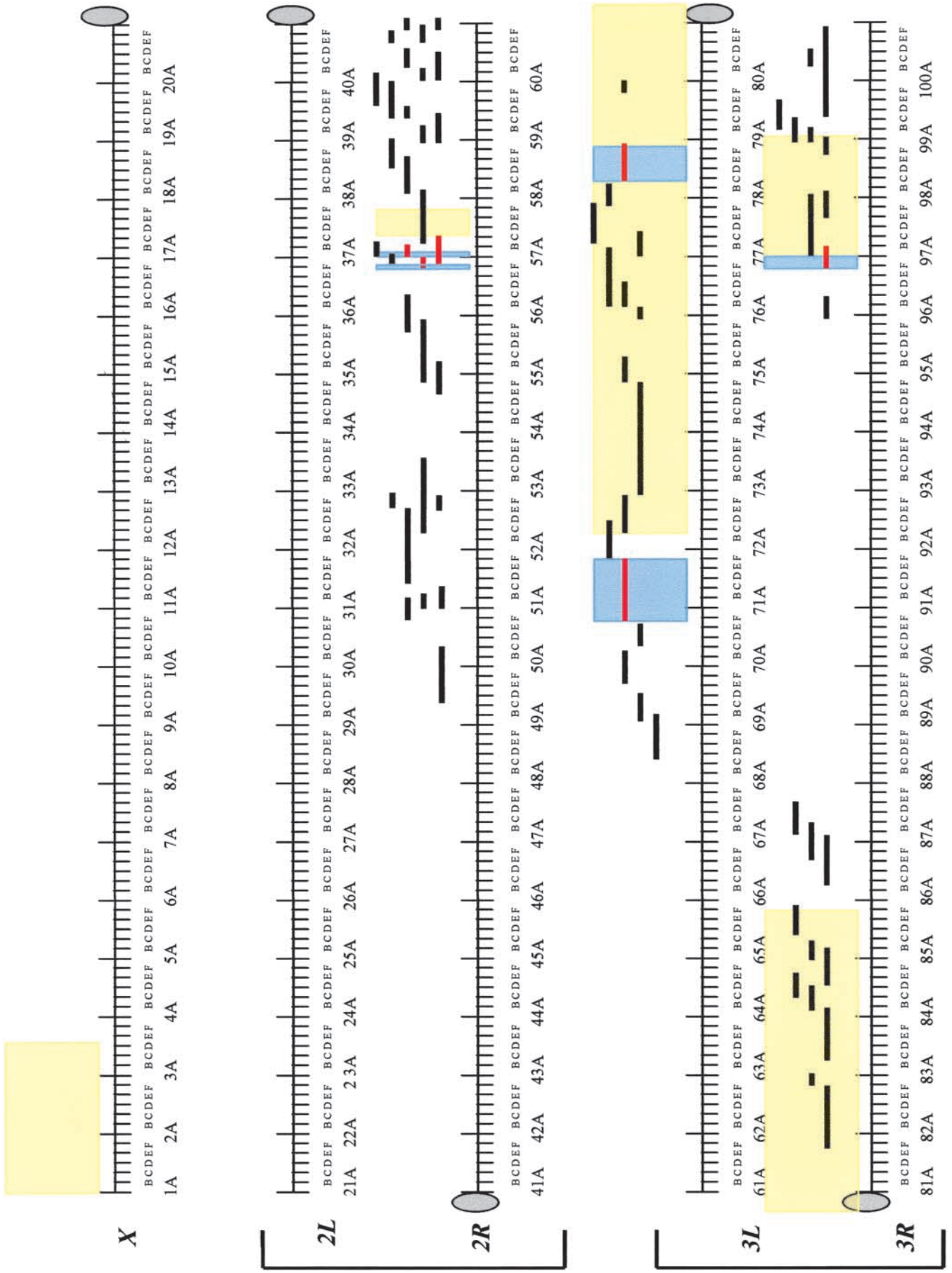
eagle (*eg*), located at 78F3, is aptly named for the spread wing phenotype of mutants (Duncan and Kaufman 1975). It encodes a nuclear transcription factor involved in fate determination of sister serotonin neurons in the central nervous system (Dittrich *et al.* 1997). Serotonergic cells are almost entirely lacking in loss-of-function mutants, while hypomorphic alleles result in a dramatic reduction in the number of serotonin-producing neurons (Lundell and Hirsh 1998). This gene is particularly interesting for three reasons. First, *Dopa decarboxylase* (Tempel *et al.* 1984) and *pale* (Buchner 1991), which are involved in the synthesis of dopamine and serotonin, have previously been shown to affect mating behavior. Second, the male-specific FRU^M protein produced by the *fruitless* gene is coexpressed in male-specific serotinergic neurons in abdominal ganglia that project toward regions of the abdomen involved in male reproduction, suggesting that *fruitless* may control formation of these cells or serotonin production in them (Lee and Hall 2001). Third, a polymorphism in 5-hydroxytryptamine 2 (5-HT2), which encodes a serotonin receptor, was exceptionally strongly associated with male *D. simulans* traits discriminated against by *D. mauritiana* females (Moehring *et al.* 2004). These observations strongly implicate the synthesis and regulation of biogenic amines in regulating mating behavior and causing variation in mating behavior within and between species.

Genes of the *Enhancer of split* [*E(spl)*] complex, located at 96F10, act as a functional unit composed of redundant genes that can partially substitute for each other. *E(spl)* encodes an RNA polymerase II transcription factor whose product is thought to function as a receptor rather than serving as a signal (Technau and Campos-Ortega 1987). It is involved in mesoderm development (Corbin *et al.* 1991) and differentiation of the neural ectoderm into epidermoblasts and neuroblasts (Knust *et al.* 1987). Increased levels of E(spl) product favor epidermal differentiation, whereas decreased levels favor neuronal differentiation. Mutants have no ventral cuticle and display hyperplasia of the central nervous system (Knust *et al.* 1987).

None of these genes have been implicated previously to affect mating behavior, most likely because their effects on behavior had not been (or could not be) tested. Null mutations in many genes have an embryonic, larval, or adult-lethal phenotype as homozygotes, precluding the characterization of their behavioral effects. However, assessing the effects of subtle, hypomorphic alleles at candidate genes, either directly or through quantitative complementation tests against QTL alleles, enables us to characterize specific effects on behavior of highly pleiotropic loci (Sokolowski 2001). One limitation of this method is that we were able to test only the 45 candidate genes within our mapped regions for which a mutant stock was available. There were an additional 247 positional candidate genes for which mutant stocks are not currently available, many of which might also contribute to variation in mating behavior. The future availability of mutations in all Drosophila genes (Spradling *et al.* 1999) will greatly facilitate identification of candidate genes corresponding to QTL, using quantitative complementation tests.

It is interesting to note that none of the genes that are known to affect aspects of mating behavior (*e.g.*, *fruitless*, *period*, and *transformer*) are located in QTL regions exhibiting significant variation for components of mating behavior between Ore and 2b. This highlights the complementary nature of mutational and quantitative genetic approaches to the dissection of the genetic architecture of any complex trait. Mutational analysis is crucial for identifying the factors required to produce normal mating behavior, but will miss mutations with specific effects on behavior unless hypomorphic mutations are induced in a controlled, homozygous genetic background and their effects are assessed quantitatively (Anholt *et al.* 1996; Lyman *et al.* 1996; Sokolowski 2001; Norga *et al.* 2003). However, only a subset of loci necessary to produce normal behavior will actually harbor alleles that affect variation in behavior between any two strains, or in a large natural population, for two reasons. First, as noted above, the two strains used for

Figure 4.—The significant regions from QTL mapping (yellow) and deficiency complementation mapping (blue) for the three major Drosophila chromosomes. Individual deficiencies that were tested are black lines, while those that showed failure to complement are in red. Centromeres are represented by a gray oval.


X
2L
2R
3L
3R

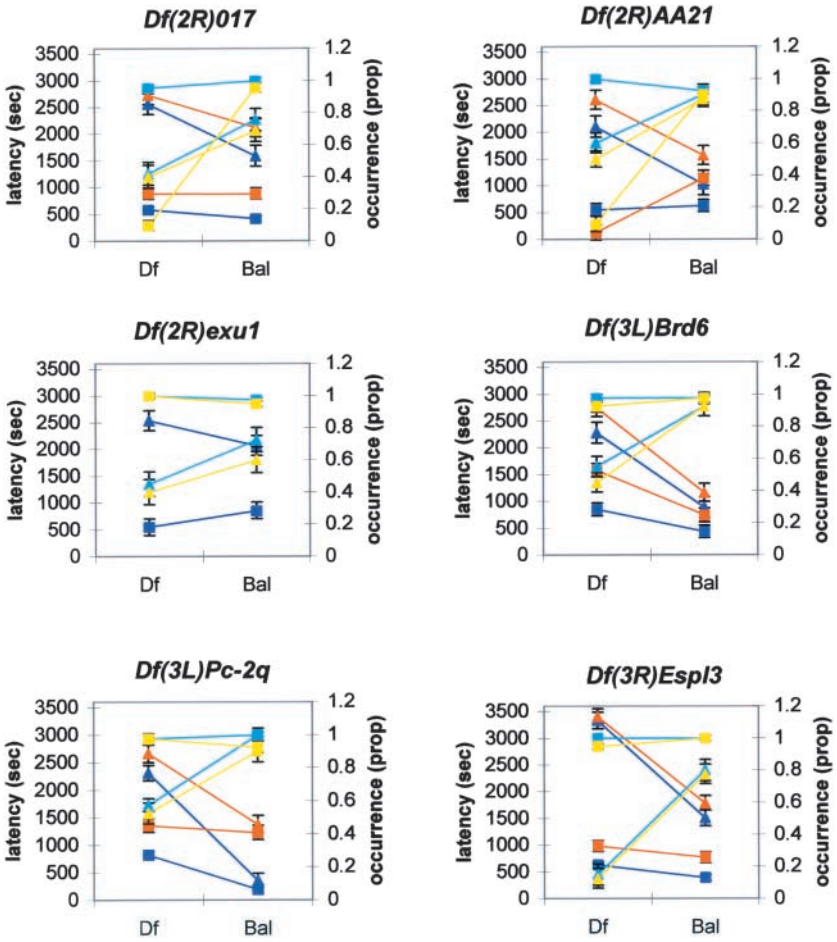


FIGURE 5.—Deficiency complementation tests and the standard error for the deficiencies and traits that showed failure to complement. Deficiencies (*Df*) and Balancers (*Bal*) crossed to Oregon (squares) and 2b (triangles) were measured for courtship latency (dark blue), courtship occurrence (light blue), copulation latency (orange), and copulation occurrence (yellow). Latency is measured in seconds; occurrence is measured as a proportion.

mapping QTL encompass only a small fraction of the total genetic variation. Second, it is possible that some loci required for producing normal behavior play such a critical role in the development of the proper sexual orientation and other pleiotropic functions that they do not vary in nature, due to selective constraint against any changes that would greatly decrease overall reproductive success. On the other hand, QTL mapping identifies gene regions haboring functional alleles affecting the trait in nature, but the actual genetic loci contributing to these QTL are most readily identified using complementation tests to existing mutations.

The seven genes that fail to complement Ore and 2b alleles for quantitative differences in male mating behavior are candidate quantitative trait genes in the absence of further proof that they are functionally associated with variation in behavior. Such studies will include analysis of gene expression and genetic interactions with other loci affecting mating behavior, molecular population genetic analysis of sequence variation within

TABLE 5

***P*-values from ANOVA for candidate genes that showed failure to complement**

Candidate gene	Courtship latency	Courtship occurrence	Copulation latency	Copulation occurrence
l(2)05510	0.0001	<0.0001	0.0018	NS
l(2)k02206	NS	0.0300	NS	0.0008
18w	0.0177	NS	NS	NS
sa	0.0002	0.0405	0.0116	NS
Pc	0.0008	NS	<0.0001	0.0074
eg	0.0202	<0.0001	0.0138	0.0017
E(spl)	0.0011	0.0003	NS	0.0205

NS, $P > 0.05$.

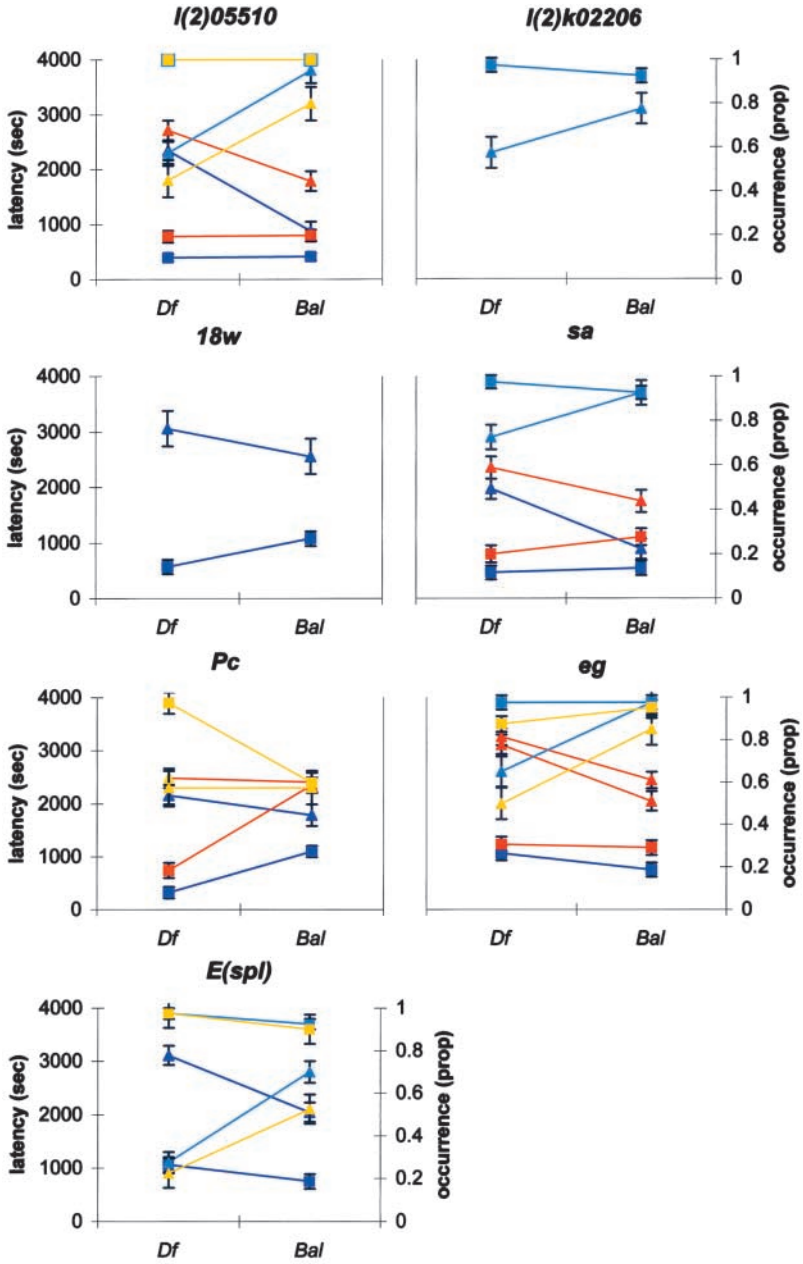


FIGURE 6.—Complementation tests and the standard error for the genes and traits that showed failure to complement. Deficiencies (*Df*) and Balancers (*Bal*) crossed to Oregon (squares) and 2b (triangles) were measured for courtship latency (dark blue), courtship occurrence (light blue), copulation latency (orange), and copulation occurrence (yellow). Latency is measured in seconds; occurrence is measured as a proportion.

and between species, and demonstration of a functional difference between the Ore and 2b alleles by transformation into a strain containing a null allele of the candidate gene.

Further, it is important to recognize that the genes affecting variation in mating behavior between Ore and 2b may not be responsible for naturally occurring variation in mating behavior. It is possible that inbreeding has fixed deleterious mutations affecting mating behavior in one or the other of these lines. If these polymorphisms are rare in nature, they will contribute little to variation. It is necessary to conduct linkage disequilibrium mapping studies to demonstrate that molecular polymorphisms in the candidate genes are associated with naturally occurring variation in behavior (MACKAY 2001). This approach has been used successfully to show that *Dopa decarboxylase* (*Ddc*), a candidate gene affecting variation in longevity between Oregon and 2b, is associated with naturally occurring variation in life span (DE LUCA *et al.* 2003). Since longevity is also affected by inbreeding depression, this study serves as proof of the concept that one can identify candidate genes by mapping QTL that segregate between inbred strains and then subsequently test whether they are responsible for variation in wild populations.

Mutagenesis studies have identified single genes affecting many aspects of mating behavior, providing the genetic framework by which the formation of sexual orientation, neural processing of external stimuli, and manifestation of response are built. The definition of

quantitative trait genes affecting mating behavior will further characterize the components responsible for the variation in the courtship ritual seen within and among species, enhancing the picture of how genes and environment interact to produce complex behavior. This is a critical step in understanding the genetic basis of adaptive evolution since it is only upon variation that selection acts to create shifts in gene frequencies, leading to the divergent evolution of two populations and, ultimately, speciation.

This work was funded by predoctoral fellowships from the North Carolina State University W. M. Keck Center for Behavioral Biology and the National Institutes of Health (NIH MH 85051) to A.J.M and NIH grant GM45344 to T.F.C.M. This is a publication of the W. M. Keck Center for Behavioral Biology.

LITERATURE CITED

Adams, M. D., S. E. Celniker, R. A. Holt, C. A. Evans, J. D. Gocayne *et al.*, 2000 The genome sequence of *Drosophila melanogaster*. Science **287:** 2185–2195.

Anholt, R. R. H., R. F. Lyman and T. F. C. Mackay, 1996 Effects of single *P*-element insertions on olfactory behavior in *Drosophila melanogaster*. Genetics **143:** 293–301.

Barbash, D. A., and T. W. Cline, 1995 Genetic and molecular analysis of the autosomal complement of the primary sex determination signal of *Drosophila melanogaster*. Genetics **141:** 1452–1471.

Basten, C. J., B. S. Weir and Z-B. Zeng, 1994 Zmap—a QTL cartographer, pp. 65–66 in *Proceedings of the 5th World Congress on Genetics Applied to Livestock Production: Computing Strategies and Software*, edited by C. Smith, J. S. Gavora, B. Benkel, J. Chesnias and W. Fairfull. Organizing Committee, 5th World Congress on Applied Genetics Applied to Livestock Production, Guelph, Ontario, Canada.

Basten, C. J., B. S. Weir and Z-B. Zeng, 1999 *QTL Cartographer*, Version 1.13. Department of Statistics, North Carolina State University, Raleigh, NC.

Bien-Willner, R. D., and W. W. Doane, 1997 *13th International Congress on Developmental Biology*, Abstract 291. Snowbird, UT.

Buchner, E., 1991 Genes expressed in the adult brain of *Drosophila* and effects of their mutations on behavior: a survey of transmitter- and second messenger-related genes. J. Neurogenet. **7:** 153–192.

Casares, P., M. C. Carracedo, E. San Miguel, R. Pineiro and L. Garcia-Florez, 1993 Male mating speed in *Drosophila melanogaster*: differences in genetic architecture in relative performance according to female genotype. Behav. Genet. **23:** 349–358.

Castrillon, D. H., P. Gönczy, S. Alexander, R. Rawson, C. G. Eberhart *et al.*, 1993 Toward a molecular genetic analysis of spermatogenesis in *Drosophila melanogaster*: characterization of male-sterile mutants generated by single *P* element mutagenesis. Genetics **135:** 489–505.

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

Civetta, C., and E. J. F. Cantor, 2003 The genetics of mating recognition between *Drosophila simulans* and *D. sechellia*. Genet. Res. **82:** 117–126.

Cline, T. W., 1993 The *Drosophila* sex determination signal: How do flies count to two? Trends Genet. **9:** 385–390.

Collins, M. F., and J. K. Hewitt, 1984 The genetic architecture of the male courtship sequence in *Drosophila melanogaster*. Heredity **53:** 321–337.

Corbin, V., A. M. Michelson, S. M. Abmayr, V. Neel, E. Alcamo *et al.*, 1991 A role for the *Drosophila* neurogenic genes in mesoderm differentiation. Cell **67:** 311–323.

De Luca, M., N. V. Roshina, G. L. Geiger-Thornsberry, R. F. Lyman, E. G. Pasyukova *et al.*, 2003 *Dopa decarboxylase* (*Ddc*) affects variation in *Drosophila* longevity. Nat. Genet. **34:** 429–433.

Dempster, E. R., and I. M. Lerner, 1950 Heritability of threshold characters. Genetics **35:** 212–236.

Dilda, C. L., and T. F. C. Mackay, 2002 The genetic architecture of Drosophila sensory bristle number. Genetics **162:** 1655–1674.

Dittrich, R., T. Bossing, A. P. Gould, G. M. Technau and J. Urban, 1997 The differentiation of the serotonergic neurons in the *Drosophila* ventral nerve cord depends on the combined function of the zinc finger proteins Eagle and Huckebein. Development **124:** 2515–2525.

Doerge, R. W., and G. A. Churchill, 1996 Permutation tests for multiple loci affecting a quantitative character. Genetics **142:** 285–294.

Doerge, R. W., Z-B. Zeng and B. S. Weir, 1997 Statistical issues in the search for genes affecting quantitative traits in experimental populations. Stat. Sci. **12:** 195–219.

Duncan, I. W., and T. C. Kaufman, 1975 Cytogenetic analysis of chromosome *3* in *Drosophila melanogaster*: mapping of the proximal portion of the right arm. Genetics **80:** 733–752.

Eldon, E., S. Kooyer, D. D'Evelyn, M. Dunman, P. Lawinger *et al.*, 1994 The *Drosophila 18 wheeler* is required for morphogenesis and has striking similarities to *Toll*. Development **120:** 885–899.

Fanara, J. J., K. O. Robinson, S. M. Rollmann, R. R. Anholt and T. F. C. Mackay, 2002 *Vanaso* is a candidate quantitative trait gene for Drosophila olfactory behavior. Genetics **162:** 1321–1328.

Finley, K. D., B. J. Taylor, M. Milstein and M. McKeown, 1997 *dissatisfaction*, a gene involved in sex-specific behavior and neural development of *Drosophila melanogaster*. Proc. Natl. Acad. Sci. USA **94:** 913–918.

FlyBase Consortium, 2003 The FlyBase database of the Drosophila genome projects and community literature. Nucleic Acids Res. **31:** 172–175 (http://flybase.org/).

Fuller, M. T., 1998 Genetic control of cell proliferation and differentiation in *Drosophila* spermatogenesis. Semin. Cell Dev. Biol. **9:** 433–444.

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

Gleason, J. M., S. V. Nuzhdin and M. G. Ritchie, 2002 Quantitative trait loci affecting a courtship signal in *Drosophila melanogaster*. Heredity **89:** 1–6.

Gould, A. P., R. Y. K. Lai, M. J. Green and R. A. White, 1990 Blocking cell division does not remove the requirement for *Polycomb* function in *Drosophila* embryogenesis. Development **110:** 1319–1325.

Greenspan, R. J., 1995 Understanding the genetic construction of behavior. Sci. Am. **272:** 72–78.

Greenspan, R. J., and J.-F. Ferveur, 2000 Courtship in *Drosophila*. Annu. Rev. Genet. **34:** 205–232.

Hall, J. C., 1994 The mating of a fly. Science **264:** 1702–1714.

Hall, J. C., R. W. Siegel, L. Tomkins and C. P. Kyriacou, 1980 Neurogenetics of courtship on *Drosophila*. Stadler Genet. Symp. **12:** 43–82.

Hollocher, H., C.-T. Ting, M.-L. Wu and C.-I Wu, 1997 Incipient speciation by sexual isolation in *Drosophila melanogaster*: the genetics of the Zimbabwe race. Genetics **147:** 1191–1201.

Kaidanov, L. Z., 1990 The rules of genetical alterations in *Drosophila melanogaster* inbred lines determined by selection. Arh. Biol. Nauka **42:** 131–148.

Knust, E., K. A. Bremer, H. Vassin, A. Ziemer, U. Tepass *et al.*, 1987 The *Enhancer of split* locus and neurogenesis in *Drosophila melanogaster*. Dev. Biol. **122:** 262–273.

Kyriacou, C. P., and J. C. Hall, 1980 Circadian rhythm mutations in *Drosophila* affect short-term fluctuations in the male's courtship song. Proc. Natl. Acad. Sci. USA **77:** 6929–6933.

Lee, G., and J. C. Hall, 2001 Abnormalities of male-specific FRU protein and serotonin expression in the CNS of *fruitless* mutants in *Drosophila*. J. Neurosci. **21:** 513–526.

Lin, T. Y., S. Viswanathan, C. Wood, P. G. Wilson, N. Wolf *et al.*, 1996 Coordinate developmental control of the meiotic cell cycle and spermatid differentiation in *Drosophila* males. Development **122:** 1331–1341.

Lindsley, D. L., and G. G. Zimm, 1992 *The Genome of Drosophila melanogaster*. Academic Press, San Diego.

Long, A. D., S. L. Mullaney, T. F. C. Mackay and C. H. Langley, 1996 Genetic interactions between naturally occurring alleles at quantitative trait loci and mutant alleles at candidate loci affecting bristle number in *Drosophila melanogaster*. Genetics **144:** 1497–1510.

Lukacsovich, T., Z. Asztalos, W. Awano, K. Baba, S. Kondo *et*

al., 2001 Dual-tagging gene trap of novel genes in *Drosophila melanogaster*. Genetics **157:** 727–742.

Lundell, M. J., and J. Hirsh, 1998 *eagle* is required for the specification of serotonin neurons and other neuroblast 7–3 progeny in the *Drosophila* CNS. Development **125:** 463–472.

Lush, J. L., W. F. Lamoreux and L. N. Hazel, 1948 The heritability of resistance to death in the fowl. Poult. Sci. **27:** 375–388.

Lyman, R. F., F. Lawrence, S. V. Nuzhdin and T. F. C. Mackay, 1996 Effects of single *P*-element insertions on bristle number and viability in *Drosophila melanogaster*. Genetics **143:** 277–292.

MacDougall, C., D. Harbison and M. Bownes, 1995 The developmental consequences of alternate splicing in sex determination and differentiation in *Drosophila*. Dev. Biol. **172:** 353–376.

Mackay, T. F. C., 2001 The genetic architecture of quantitative traits. Annu. Rev. Genet. **35:** 303–339.

Mackay, T. F. C., and J. D. Fry, 1996 Polygenic mutation in *Drosophila melanogaster*: genetic interactions between selection lines and candidate quantitative trait loci. Genetics **144:** 671–688.

Manning, A., 1961 The effects of artificial selection for mating speed in *Drosophila melanogaster*. Anim. Behav. **9:** 82–92.

Manning, A., 1963 Selection for mating speed in *Drosophila melanogaster* based on the behavior of one sex. Anim. Behav. **11:** 116–120.

Markow, T. A., 1996 Evolution of *Drosophila* mating systems. Evol. Biol. **29:** 73–106.

Moehring, A. J., J. Li, M. D. Schug, S. G. Smith, M. deAngelis *et al.*, 2004 Quantitative trait loci for sexual isolation between *Drosophila simulans* and *D. mauritiana*. Genetics **167:** 1265–1274.

Neckameyer, W. S., 1998 Dopamine modulates female sexual receptiveness in *Drosophila melanogaster*. J. Neurogenet. **12:** 101–114.

Norga, K. K., M. G. Gurganus, C. L. Dilda, A. Yamamoto, R. F. Lyman *et al.*, 2003 Quantitative analysis of bristle number in *Drosophila* mutants identifies genes involved in neural development. Curr. Biol. **13:** 1388–1397.

Nuzhdin, S. V., and S. G. Reiwitch, 2000 Are the same genes responsible for intra- and interspecific variability for sex comb tooth number in Drosophila? Heredity **84:** 97–102.

Nuzhdin, S. V., E. G. Pasyukova, C. L. Dilda, Z-B. Zeng and T. F. C. Mackay, 1997 Sex-specific quantitative trait loci affecting longevity in *Drosophila melanogaster*. Proc. Natl. Acad. Sci. USA **94:** 9734–9739.

Parsons, P. A., 1964 A diallel cross for mating speeds in *Drosophila melanogaster*. Genetica **35:** 141–151.

Partridge, L., T. F. C. Mackay and S. Aitken, 1985 Male mating success and fertility in *Drosophila melanogaster*. Genet. Res. **46:** 279–285.

Pasyukova, E. G., C. Vieira and T. F. C. Mackay, 2000 Deficiency mapping of quantitative trait loci affecting longevity in *Drosophila melanogaster*. Genetics **156:** 1129–1146.

Peixoto, A. A., and J. C. Hall, 1998 Analysis of temperature-sensitive mutants reveals new genes involved in the courtship song of Drosophila. Genetics **148:** 827–838.

Robertson, A., and I. M. Lerner, 1949 The heritability of all-or-none traits: viability of poultry. Genetics **34:** 395–411.

Roseman, R. R., K. Morgan, D. R. Mallin, R. Roberson, T. J. Parnell *et al.*, 2001 Long-range repression by multiple *Polycomb Group* (*PcG*) proteins targeted by fusion to a defined DNA-binding domain in Drosophila. Genetics **158:** 291–307.

SAS Institute, 1988 *SAS/SYSTAT User's Guide*, Ed. 4. SAS Institute, Cary, NC.

Schütt, C., and R. Nöthiger, 2000 Structure, function and evolution of sex-determining systems in Dipteran insects. Development **127:** 667–677.

Sharp, P. M., 1984 The effect of inbreeding on competitive male mating ability in *Drosophila melanogaster*. Genetics **106:** 601–612.

Sokolowski, M. B., 2001 *Drosophila*: genetics meets behaviour. Nat. Rev. Genet. **2:** 879–890.

Sorsa, V., 1988 *Chromosome Maps of Drosophila*, Vol. II. CRC Press, Boca Raton, FL.

Spradling, A. C., D. Stern, A. Beaton, E. J. Rhem, T. Laverty *et al.*, 1999 The Berkeley Drosophila Genome Project gene disruption project: single *P*-element insertions mutating 25% of vital Drosophila genes. Genetics **153:** 135–177.

Suzuki, K., N. Juni and D. Yamamoto, 1997 Enhanced mate refusal in female Drosophila induced by a mutation in a spinster locus. Appl. Entomol. Zool. **32:** 235–243.

Technau, G. M., and J. A. Campos-Ortega, 1987 Cell autonomy of expression of neurogenic genes of *Drosophila melanogaster*. Proc. Natl. Acad. Sci. USA **84:** 4500–4504.

Tempel, B. L., M. S. Livingstone and W. G. Quinn, 1984 Mutations in the *dopa decarboxylase* gene affect learning in *Drosophila*. Proc. Natl. Acad. Sci. USA **81:** 3577–3581.

Ting, C.-T., A. Takahashi and C.-I Wu, 2001 Incipient speciation by sexual isolation in *Drosophila*: concurrent evolution at multiple loci. Proc. Natl. Acad. Sci. USA **98:** 6709–6713.

Vieira, C., E. G. Pasyukova, Z-B. Zeng, J. B. Hackett, R. F. Lyman *et al.*, 2000 Genotype-environment interaction for quantitative trait loci affecting life span in *Drosophila melanogaster*. Genetics **154:** 213–227.

von Schilcher, F., 1976 The behavior of *cacophony*, a courtship song mutant in *Drosophila melanogaster*. Behav. Biol. **17:** 187–196.

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.

Williams, M. A., A. G. Blouin and M. F. Noor, 2001 Courtship songs of *Drosophila pseudoobscura* and *D. persimilis*. II. Genetics of species differences. Heredity **86:** 68–77.

Williams, M. J., A. Rodriguez, D. A. Kimbrell and E. D. Eldon, 1997 The *18-wheeler* mutation reveals complex antibacterial gene regulation in *Drosophila* host defense. EMBO J. **16:** 6120–6130.

Wilson, P., and M. Fuller, 1991 Genetic analysis of spindle structure and function. Ann. Dros. Res. Conf. **32:** 46.

Wu, C.-I, H. Hollocher, D. J. Begun, C. F. Aquadro and Y. Xu, 1995 Sexual isolation in *Drosophila melanogaster*: a possible case of incipient speciation. Proc. Natl. Acad. Sci. USA **92:** 2519–2523.

Yamamoto, D., and Y. Nakano, 1998 Genes for sexual behavior. Biochem. Biophys. Res. Commun. **246:** 1–6.

Yamamoto, D., J.-M. Jallon and A. Komatsu, 1997 Genetic dissection of sexual behavior in *Drosophila melanogaster*. Annu. Rev. Entomol. **42:** 551–585.

Zeng, Z-B., 1994 Precision mapping of quantitative trait loci. Genetics **136:** 1457–1468.

Communicating editor: M. A. F. Noor