

# Quantitative Trait Loci for Aggressive Behavior in *Drosophila melanogaster*

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## ABSTRACT

Aggressive behavior is observed across animal taxa and is likely to be evolutionarily conserved. Although potentially advantageous, aggression can have social and health consequences in humans, and is a component of a number of psychiatric disorders. As a complex genetic trait, it is modulated by numerous quantitative trait loci (QTL) with allelic effects that can vary in direction and magnitude and that are sensitive to environmental perturbations. Assays to quantify aggressive behavior in *Drosophila melanogaster* have been developed, making this an ideal model system in which to dissect the genomic architecture underlying manifestation of and variation in aggressive behavior. Here, we map QTL affecting variation in aggression between two wild-type *Drosophila* strains. We identified a minimum of five QTL in a genome-wide scan: two on chromosome 2 and three on chromosome 3. At least three and possibly all five of these QTL interact epistatically. We used deficiency complementation mapping to subdivide two linked, epistatically interacting QTL of large effect on chromosome 3 into at least six QTL, and complementation tests to mutations identified four candidate quantitative trait genes. Extensive epistasis poses a serious challenge for understanding the genetic basis of complex traits.

**A**GGRESSIVE behavior is observed throughout the animal kingdom and can be essential for survival. Aggression is used to gain access to territory, food, or mates; in defense against predators; to protect offspring; and to gain social status in a dominance hierarchy. On the other hand, aggression can be costly, since violent interactions with others can cause injury, and aggressive displays are energetically expensive and interfere with other essential behaviors, such as feeding and mating. The persistence of high levels of genetic variation for aggression within populations (DOW and VON SCHILCHER 1975; HOFFMANN 1988, 1989; HOFFMANN and CACOYIANNI 1989) suggests that a balance of evolutionary forces acts to maintain the variation. Theoretical modeling has suggested that frequency-dependent selection could be generating genetic variation in aggression (MAYNARD SMITH and HARPER 1988). The observation that many behavioral phenotypes vary widely in a population is often attributed to life history trade-offs (CABRAL *et al.* 2008; MANEY 2008); recent work also suggests that animal “personalities” within a population might be adaptive (WOLF *et al.* 2007). Another potential explanation is that the pleiotropic nature of many genes affecting aggressive behavior subjects them to opposing

or multidirectional selective pressures, maintaining variation.

In humans, aggression is often associated with impulsive behavior, and can reach pathological levels. It is a component of a number of behavioral or psychiatric disorders, such as alcoholism, borderline personality disorder, conduct disorder, and Alzheimer’s disease. It can also constitute a behavioral pathology in and of itself (*e.g.*, intermittent explosive disorder). Therefore, an understanding of the genetic basis of variation in aggressive behavior is important from the dual perspectives of evolutionary genetics and human health.

Considerable work on the neurobiology and genetics of aggressive behavior in mammals and invertebrates has revealed key molecules required to mediate and modulate aggression, in particular biogenic amines. Normal levels of serotonin (5-hydroxytryptophan, 5-HT) inhibit aggressive behavior in vertebrates, and decreased levels of 5-HT and 5-hydroxyindoleacetic acid (5-HIAA), a metabolite of 5-HT, are associated with increased levels of impulsivity and aggression (MANUCK *et al.* 1999; NELSON and CHIAVEGATTO 2001). In invertebrates, increased 5-HT results in increased aggressive behavior (EDWARDS and KRAVITZ 1997; HUBER *et al.* 1997; BAIER *et al.* 2002; DIERICK and GREENSPAN 2007). Monoamine oxidase A (MAOA), which catabolizes 5-HT as well as dopamine and norepinephrine, has also been associated with aggressive behavior in humans; males lacking a functional copy of MAOA (which is X-linked) are highly aggressive (BRUNNER *et al.* 1993), and expression levels of brain MAOA are inversely correlated with aggression (ALIA-KLEIN *et al.* 2008). The

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noradrenergic (DE ALMEIDA *et al.* 2005) and dopaminergic (HALLER *et al.* 1998) systems also modulate aggressive behavior. The *Drosophila* homolog of noradrenaline, octopamine, also appears to modulate aggression, since males lacking octopamine display hardly any aggressive behaviors (BAIER *et al.* 2002; HOYER *et al.* 2008). Polymorphisms in catecholamine-O-methyltransferase, which degrades dopamine and norepinephrine, are also associated with variation in aggression in schizophrenic men (HAN *et al.* 2004).

The neurotransmitters nitric oxide (NO) and  $\gamma$ -aminobutyric acid (GABA) (MICZEK *et al.* 2003) also modulate aggressive behavior. In humans, haplotypes in the gene encoding nitric oxide synthase (NOS) are associated with increased aggression and/or suicidal behavior (RUJESCU *et al.* 2008). In mice, knocking out expression of neuronal NOS is associated with increased aggression (NELSON *et al.* 1995), although this may be attributable to the effect of the knockout on 5-HT (CHIAVEGATTO *et al.* 2001). Neuropeptide Y and its invertebrate homolog, Neuropeptide F, affect aggression in mammals (KARL *et al.* 2004; GAMMIE *et al.* 2007; KARL and HERZOG 2007) and *Drosophila* (DIERICK and GREENSPAN 2007). In *Drosophila*, correct expression of the male-specific transcript of *fruitless*, a gene in the sex-determination pathway, is required for executing male aggressive behaviors (LEE and HALL 2000; VRONTOU *et al.* 2006; CERTEL *et al.* 2007; CHAN and KRAVITZ 2007).

As a quantitative trait, we expect natural variation in levels of aggression to be influenced by segregating alleles at multiple interacting loci with varying effects that depend on the social and physical environment. Recent studies documenting correlated responses of the transcriptome to artificial selection for divergent levels of aggressive behavior from wild-derived (EDWARDS *et al.* 2006) and laboratory (DIERICK and GREENSPAN 2006) strains of *Drosophila* hint that the genetic basis of natural variation in this behavior may be very complex. However, these studies are unable to discriminate which of the correlated transcriptional responses are caused by polymorphic alleles at loci directly affecting aggressive behavior and which are transregulated by the causal polymorphisms, or hitchhike along with the selected causal polymorphisms. Therefore, we need to use QTL mapping to identify causal loci affecting variation in aggressive behavior between wild-type lines.

Here, we map QTL affecting the difference in aggressive behavior between two laboratory stocks: *Oregon-R* (*Ore*), a standard laboratory wild-type strain (LINDSLEY and ZIMM 1992), and *2b*, a Russian strain selected for low male mating ability (KAIDANOV 1990). Recombinant inbred lines (RILs) derived from these strains have been used previously to map QTL affecting a wide variety of quantitative traits: life span (NUZHGIN *et al.* 1997; LEIPS and MACKAY 2000; PASYUKOVA *et al.* 2000; VIEIRA *et al.* 2000; LEIPS and MACKAY 2002; REIWITCH and NUZHGIN 2002; LAI *et al.* 2007); starvation (VIEIRA *et al.* 2000;

HARBISON *et al.* 2004); heat and cold stress resistance (MORGAN and MACKAY 2006); reproductive success (FRY *et al.* 1998; WAYNE *et al.* 2001); numbers of sensory bristles (GURGANUS *et al.* 1998); sex comb teeth (NUZHGIN and REIWITCH 2000) and ovarioles (WAYNE *et al.* 2001); flight velocity and metabolic traits (MONTTOOTH *et al.* 2003); courtship song (GLEASON *et al.* 2002); and olfactory (FANARA *et al.* 2002), male mating (MOEHRING and MACKAY 2004), and locomotor (JORDAN *et al.* 2006) behavior. We used a population of introgression lines derived from the recombinant inbred lines to map QTL affecting aggressive behavior. We inferred that there are a minimum of five QTL affecting aggressive behavior in these strains and that at least three of these QTL interact epistatically. Two of the three epistatic QTL were localized to a small region on the left arm of the third chromosome. We used complementation tests to deficiencies and their co-isogenic wild-type strains to map the epistatic QTL in this region with higher resolution. Surprisingly, the region fractionated into at least six QTL and complementation tests to mutations implicated four novel candidate genes affecting variation in aggression between *Ore* and *2b*. The expression and function of these genes informs our understanding of the genetic influences on aggression and lays the foundation for future research.

## MATERIALS AND METHODS

**Drosophila stocks:** We derived 20 introgression lines from the population of 98 *Ore*  $\times$  *2b* RILs (NUZHGIN *et al.* 1997). We screened the RIL genotypes, one chromosome at a time, for partially overlapping segments of the *2b* genome in an otherwise *Ore* background, and chose 20 segmental introgressions that together constitute the entire *2b* genome. We then substituted each of the chromosomes containing a *2b* introgressed fragment into the *Ore* background, by standard crosses using balancer chromosomes that had themselves previously been substituted into *Ore*. The cytological limits of the introgressed portion of the *2b* genome, as assessed by insertion sites of *roo* transposable element markers, are given in supporting information, Table S1.

We obtained *DrosDel* (RYDER *et al.* 2007) and *Exelixis* (PARKS *et al.* 2004) deficiency (*Df*) stocks with defined molecular breakpoints that had been generated in a *w<sup>1118</sup>* isogenic background and stocks with mutations in positional candidate genes from the Bloomington *Drosophila* Stock Center, Bloomington, Indiana.

**Assay to quantify aggressive behavior:** Eight male flies of the same genotype, aged 3–7 days, were transferred without anesthesia to an empty vial and deprived of food for 90 minutes. They were then exposed to a droplet of standard food and allowed to acclimate for 2 minutes, after which they were observed for 2 minutes. The number of aggressive encounters observed during this period was recorded as the aggression score for the replicate. The average of these scores is the mean aggression score (MAS). All assays were performed between 8 and 11 AM, at 25° and 75% humidity.

**QTL mapping using introgression lines:** We scored 20 replicate samples of each introgression line for aggressive behavior. We partitioned variance in aggressive behavior between (*L*) and within (*E*) introgression lines using the

one-way random effects ANOVA model  $Y = \mu + L + E$ . We estimated the broad sense heritability ( $H^2$ ) of aggressive behavior as  $H^2 = \sigma_L^2 / (\sigma_L^2 + \sigma_E^2)$ , where  $\sigma_L^2$  and  $\sigma_E^2$  are the among- and within-line variance components, respectively. We then used *t*-tests to assess whether the mean level of aggression in each introgression line was significantly different from *Ore* and/or *2b*. Introgression lines with significantly different MAS scores from *Ore* contain a *2b* QTL allele affecting aggressive behavior.

**Deficiency complementation mapping:** The DrosDel and Exelixis deficiency stocks have isogenic genomic background control lines ( $w^{1118}$ ). We crossed *Ore* and *3-88Я* to each deficiency stock and to the control, and assessed aggressive behavior for 10 replicates of each of four genotypes: *Df/Ore*, *Df/3-88Я*,  $w^{1118}/Ore$ , and  $w^{1118}/3-88Я$ . The statistical model used to analyze the behavioral data is a two-way ANOVA:  $Y = \mu + L + G + L \times G + E$ , where  $\mu$  is the overall mean,  $L$  is the main effect of line (*Ore* or *3-88Я*),  $G$  is the main effect of genotype (*Df* or  $w^{1118}$ ), and  $E$  is the environmental variance. A significant  $L \times G$  term indicates quantitative failure to complement. As for all complementation tests, failure to complement could arise from different effects of *Ore* and *2b* alleles at one locus in the region uncovered by the deficiency, or nonallelic interactions. Note that failure to complement due to epistasis is minimized in these tests, because the *Df* strains have no additional mutations and a co-isogenic control strain rather than a balancer chromosome is used as the wild-type allele. Also, the introgression design limits epistasis to other QTL in the introgressed region.

**Mutant complementation tests:** The test for quantitative failure to complement using mutants is similar to that for deficiencies. However, since no genomic control line is available, we instead assessed aggressive behavior for 10 replicates of each of the following genotypes: *mutant/Ore*, *mutant/3-88Я*, *Bal/Ore*, and *Bal/3-88Я*, where *Bal* is the balancer chromosome over which the mutation is maintained. The same ANOVA model was used, and the same criteria were applied to assess quantitative complementation. We excluded cases of failure to complement where the difference between *Bal/Ore* and *Bal/3-88Я* means was not significantly different from that between *mutant/Ore* and *mutant/3-88Я* means. Mutants meeting these criteria were retested using 20 replicates per genotype. Results from both blocks of testing were pooled and tested together using the model

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

where  $L$  is the line term,  $G$  is the genotype term, and  $B$  is the block term.

All statistical analyses were conducted using Statistical Analysis Software Version 8.2 or JMP Version 7.0 (SAS; Cary, NC).

## RESULTS AND DISCUSSION

**QTL mapping using introgression lines:** A mapping population of RILs derived from *Ore* and *2b* has been used to map QTL affecting a large number of complex traits. We assessed whether these strains also differed in aggressive behavior and found that the MAS of *Ore* males ( $20.82 \pm 2.25$ ) was greater than twice that of *2b* males ( $8.55 \pm 1.17$ ) ( $t = 2.09$ ,  $P < 0.0001$ ). We hypothesized that we could improve the power to map QTL while reducing the number of lines to be scored if we constructed a population of segmental introgression

lines, each of which contains a fragment of the *2b* genome in an otherwise *Ore* background, but which together comprise the entire *2b* genome (Figure 1, Table S1).

There was significant variation in mean aggression scores among the 20 introgression lines ( $F_{19, 359} = 10.72$ ,  $P < 0.0001$ ; Figure 1). The estimate of broad sense heritability of aggression levels in the introgression lines is  $H^2 = 0.33$ . All but two lines (*2-16* and *3-34Я*) exhibited significantly ( $P < 0.05$ ) higher levels of aggression than *2b*. Five lines differed from *Ore*: *2-16*, *3-17*, *3-31Я*, and *3-34Я* were less aggressive and line *3-88Я* was more aggressive.

We used these data to infer the locations of QTL affecting the difference in aggressive behavior between *Ore* and *2b*. None of the  $X$  chromosome introgressions were significantly different from *Ore*, and all were different from *2b*. The most parsimonious inference is that there are no  $X$ -linked QTL affecting the difference in aggressive behavior between these strains (Figure 1).

The MAS of chromosome 2 introgression line *2-16* is significantly different from *Ore*, but not *2b*, and therefore contains at least one QTL affecting aggressive behavior. However, the introgressions in lines *2-18* and *2-81Я* together include the entire *2b* fragment in *2-16*, which is not consistent with a single QTL model. There are three possible two-QTL models that explain this observation (Figure 1). (1) There is a QTL with a negative effect on aggressive behavior between 29F;30D and another with an equal positive effect in the region of 27B. Line *2-16* contains only the negative effect QTL, while lines *2-78Я* and *2-18* contain both QTL. (2) There is a QTL with a negative effect on aggressive behavior between 34EF;38E and another with an equal positive effect between 39A;50B. Line *2-16* contains only the negative effect QTL, while line *2-81Я* contains both QTL. (3) Line *2-16* contains two QTL between 29F;30D and 34EF;38E that interact epistatically to reduce the MAS; neither QTL on its own has a detectable effect on aggressive behavior. These models are indistinguishable on the basis of the available data.

There are at least three QTL on chromosome 3. Lines *3-34Я*, *3-31Я*, *3-17*, and *3-27* do not have statistically different MAS from each other (data not shown); *3-34Я*, *3-31Я*, and *3-17* have significantly lower MAS than *Ore*, and *3-31Я*, *3-17*, and *3-27* have significantly higher MAS than *2b*. Taken together, these results are consistent with a QTL affecting reduced aggressive behavior in the *2b* region common to all these introgressions, from 97D;100F. Surprisingly, the MAS of line *3-88Я*, which includes only the 65D;69D region of the *2b* genome, is significantly different from both *Ore* and *2b*, and is nearly twice as aggressive as *Ore*. Therefore, the effect of one or more QTL in this interval must be suppressed by another QTL elsewhere on the *2b* chromosome 3. Further, this introgression is completely overlapped by the introgressions in *3-15* and *3-5*, indicating that there

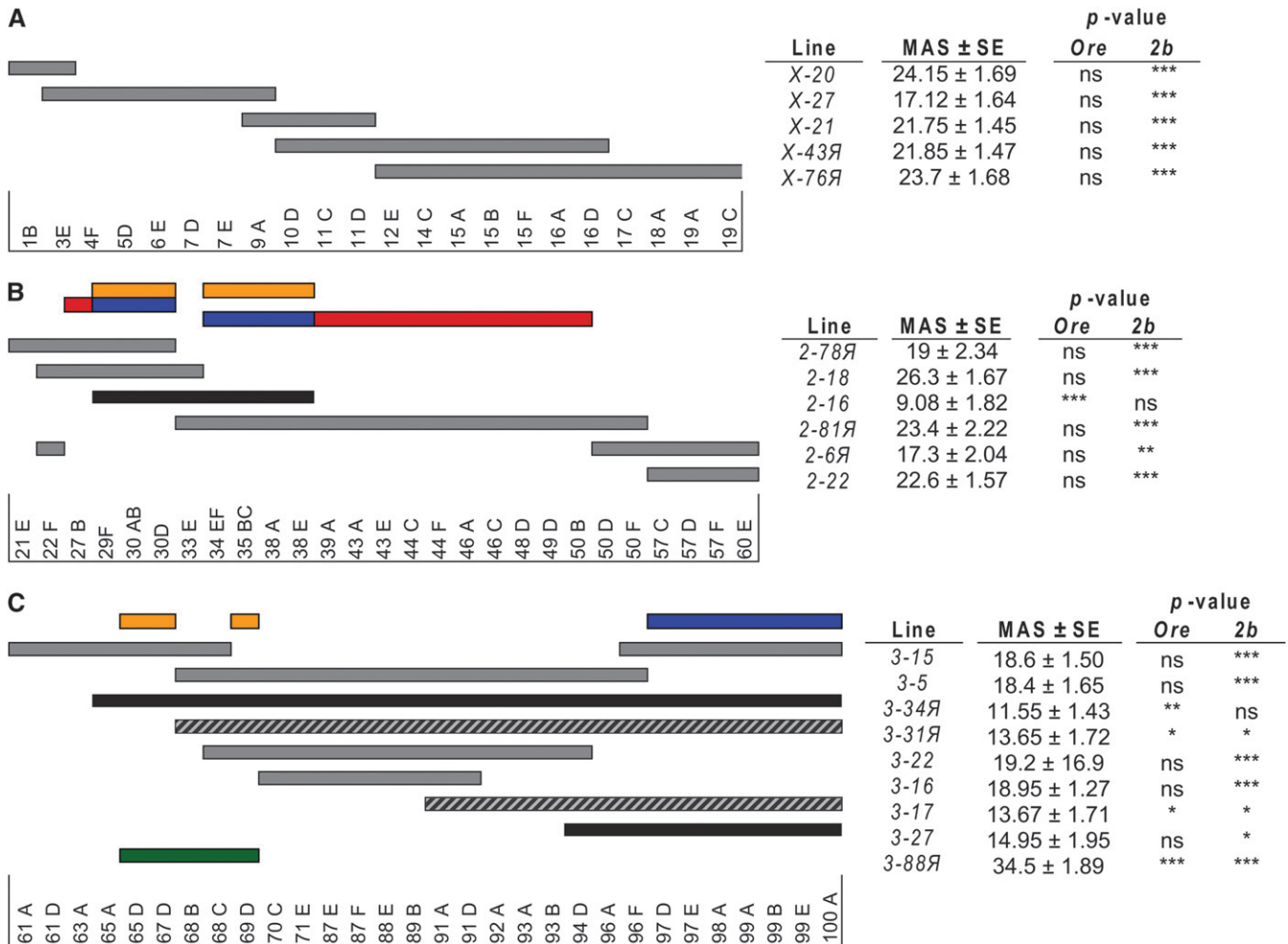


FIGURE 1.—QTL mapping using introgression lines. Gray bars represent the region of the genome derived from the *2b* parental line for each introgression line on (A) the X chromosome, (B) chromosome 2, and (C) chromosome 3. The cytological locations of *roo* transposable element markers are depicted below each set of lines. Gray bars denote introgressions with a mean aggression score (MAS) that is not significantly different from *Ore* but is different from *2b*; black bars denote introgressions with MAS that are significantly different from *Ore* but not significantly different from *2b*, and gray/black bars denote introgressions with MAS that are intermediate between *Ore* and *2b*, and significantly different from both parental lines. The green bar denotes an introgression with a MAS that is significantly greater than either parent line. The QTL locations are inferred using this physical map in conjunction with the MAS of each introgression line and statistical comparisons of the MAS between the introgression line and the parental lines. Orange bars represent QTL with epistatic effects; red bars indicate QTL with positive additive effects; and blue bars represent QTL with negative additive effects. Three possible models explain the QTL on chromosome 2, shown here in different rows (see text for explanation). Significance levels were determined using Student's *t*-tests. \* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$ .

must be a least two epistatic QTL in the 65D;69D region, one between 65D;67D and another in the region of 69D (the region of nonoverlap of 3-15 and 3-5). We must further postulate that neither epistatic QTL has an effect on its own, but that the effect of both are suppressed by the QTL at 97D;100F, since line 3-34A contains all three QTL, but is not significantly different from lines 3-17 and 3-27, which only contain the 97D;100F QTL (Figure 1).

Therefore, of the five QTL affecting aggressive behavior, at least three and possibly all five interact epistatically. We previously inferred that epistatic interactions were prevalent among loci affecting natural variation in aggressive behavior, since the estimate of

additive genetic variance from response to artificial selection (EDWARDS *et al.* 2006) was much less than the estimate of the total genetic variance among inbred lines derived from the same base population as the selection lines (A. C. EDWARDS, J. F. AYROLES, E. A. STONE, M. A. CARBONE, R. F. LYMAN and T. F. C. MACKAY, unpublished data). In the light of extensive epistasis shaping the genetic architecture of aggressive behavior, we cannot be certain that there are no QTL for aggressive behavior on the X chromosome, only that we cannot detect them with the available genotypes. Furthermore, this experimental design enables us to detect QTL affecting aggressive behavior and infer the presence of interactions among them, but we do not

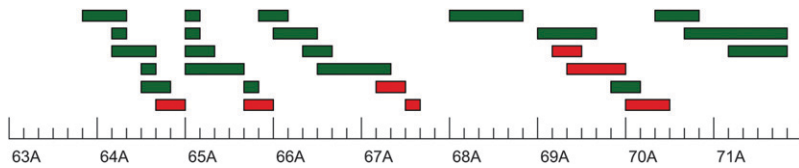


FIGURE 2.—Deficiency complementation mapping. The bars depict the approximate cytological locations of deficiencies used for complementation mapping. Red bars indicate deficiencies that fail to complement QTL affecting aggressive behavior; green bars represent deficiencies that complement QTL affecting aggression.

have enough information to estimate all additive and epistatic effects.

**Deficiency complementation mapping:** There are at least two epistatically interacting QTL with a large effect on aggressive behavior in the 65D;69D region. Therefore, we used complementation to deficiencies (PASYUKOVA *et al.* 2000) to map these QTL with higher resolution. Deficiency mapping has been used to successfully fine-map QTL affecting variation between *Ore* and *2b* for many quantitative traits (PASYUKOVA *et al.* 2000; FANARA *et al.* 2002; DE LUCA *et al.* 2003; HARBISON *et al.* 2004; MOEHRING and MACKAY 2004; JORDAN *et al.* 2006). These analyses typically reveal that single QTL fractionate into multiple linked QTL.

The precise breakpoints of this QTL are somewhere between 65A;65D and 69D;70C; therefore we used deficiencies spanning the 65A;70C cytological interval. We introduced two improvements over previous deficiency complementation tests. First, introgression line *3-88Y* contains this region of the *2b* genome in an otherwise *Ore* genetic background. Therefore we used *3-88Y* and *Ore* as the parent lines for the complementation tests. This limits the potential for noncomplementation due to epistatic interactions to interactions between QTL in this region only. Second, we used deficiency stocks from the DrosDel and Exelixis (PARKS *et al.* 2004; RYDER *et al.* 2007) collections. These deficiencies have been constructed in isogenic backgrounds and do not contain any additional mutations, which reduces the likelihood of identifying a region that fails to complement due to epistatic interactions between mutations carried on the deficiency chromosome, and not the deficiency itself. Furthermore, the breakpoints of the deficiencies are molecularly defined, enabling more precise mapping.

We crossed *Ore* and *3-88Y* to 27 deficiency stocks (*Df/Bal*, where *Bal* is a balancer chromosome) with overlapping breakpoints spanning the QTL region (Figure 2), and to a *w<sup>1118</sup>* control line, which had the appropriate isogenic background in which the deficiency stocks were constructed. We scored the aggressive behavior of F<sub>1</sub> males of four genotypes: *Df/Ore*, *Df/3-88Y*, *w<sup>1118</sup>/Ore*, and *w<sup>1118</sup>/3-88Y-3*. We analyzed the data for each deficiency by factorial ANOVA (Table 1), which partitions variation among the genotypes into the cross-classified main effects of line (*Ore*, *3-88Y*), genotype (*Df*, *w<sup>1118</sup>*) and the line  $\times$  genotype interaction. We inferred complementation if the line  $\times$  genotype interaction term was not significant, as this indicates that the difference in phenotype between *Ore* and *3-88Y* was

the same in the *Df* and *w<sup>1118</sup>* chromosome backgrounds. We inferred failure to complement if the line  $\times$  genotype interaction was significant, which indicates the difference in aggressive behavior between *Ore* and *3-88Y* varied between the *Df* and *w<sup>1118</sup>* chromosome backgrounds. We then delineated the QTL locations by the region of nonoverlap of deficiencies complementing the trait phenotype with those that fail to complement the trait phenotype.

Note that the interpretation of failure to complement in these tests is complicated by the epistatic gene action of the QTL in the 65A;65D region from *2b*, when introgressed in the *Ore* background. The QTL in the 65D;67D region do not have a main effect on aggressive behavior; neither do the QTL in 69D. The hyperaggressive phenotype only occurs when both QTL alleles from the *2b* strain co-occur. Thus, if we observe that a deficiency fails to complement the aggressive phenotype, it must simultaneously both uncover one of the QTL in the introgressed region, and interact epistatically with another.

Six of the deficiency stocks exhibited significant ( $P < 0.05$ ) quantitative failure to complement (22% of those tested). Four of these tests were significant following a Bonferroni correction for multiple tests (Table 1). Thus, the QTL region was fractionated into multiple smaller QTL. Four of the QTL were in the first of the epistatic QTL inferred from the analyses of the introgression lines (64E5;64F5, 65E8;65F4, 67B10;67C5, and 67D1;67D2), while the other two QTL were in the region of the second epistatic QTL (69B5;69C4 and 69C4;69F6). The effects of all six of these QTL were in the same direction, with the *Df/3-88Y* genotype, which elucidates the effect of the *2b* allele in that region, exhibiting higher aggression than the *Df/Ore* genotype. Each of these significant deficiencies must uncover one of the QTL alleles and interact epistatically with another QTL allele. We cannot map the QTL with which the *Df*s interact; all could interact with a single QTL not uncovered by any of the deficiencies, or genes in the regions uncovered by the deficiencies could interact with each other.

An additional QTL in the region of the second epistatic QTL, 70A3;70C10, approached formal significance ( $P = 0.0554$ ). We included candidate genes from this region in complementation tests to mutants, although the direction of effect of this QTL was opposite to the others, with the *Df/Ore* allele exhibiting higher levels of aggression.

The 65A;70C cytological interval encompasses nearly 1000 genes mapped to the sequence. Deficiency complementation mapping narrowed the QTL intervals to  $\sim 300$

**TABLE 1**  
**Deficiency complementation tests**

Deficiency genotype	Deficiency breakpoints	Mean aggression score (SE)		ANOVA <i>P</i> -values		
		<i>Df/88</i> MAS	<i>Df/Ore</i> MAS	Line ( <i>L</i> )	Genotype ( <i>G</i> )	<i>L</i> × <i>G</i>
<i>w</i> <sup>1118</sup> ; <i>Df(3L)ED4341/TM6C, Sb</i> <sup>1</sup>	63F6;64B11	12.1 (1.12)	12.0 (1.21)	0.9820	0.0082	0.9224
<i>w</i> <sup>1118</sup> ; <i>Df(3L)ED4342/TM6C, Sb</i> <sup>1</sup>	64B1;64B13	15.6 (1.54)	16.1 (1.89)	0.8175	0.8947	0.9054
<i>w</i> <sup>1118</sup> ; <i>Df(3L)ED210/TM6C, Sb</i> <sup>1</sup>	64B11;64D1	22.0 (1.75)	18.3 (1.86)	0.2237	0.0026	0.1850
<i>w</i> <sup>1118</sup> ; <i>Df(3L)Exel6105/TM6B, Tb</i> <sup>1</sup>	64D1;64D6	16.9 (0.98)	19.9 (0.66)	0.0499	0.0103	0.4879
<i>w</i> <sup>1118</sup> ; <i>Df(3L)Exel6106/TM6B, Tb</i> <sup>1</sup>	64D6;64E2	22.8 (1.04)	22.5 (1.83)	0.6360	<0.0001	0.4709
<b><i>w</i><sup>1118</sup>; <i>Df(3L)Exel6107/TM6B, Tb</i><sup>1</sup></b>	<b>64E5;64F5</b>	<b>30.3 (2.30)</b>	<b>21.8 (1.87)</b>	<b>0.0089</b>	<b>&lt;0.0001</b>	<b>0.0003*</b>
<i>w</i> <sup>1118</sup> ; <i>Df(3L)Exel7210/TM6B, Tb</i> <sup>1</sup>	65A1;65A5	17.5 (0.98)	20.2 (2.23)	0.0997	0.0080	0.6151
<i>w</i> <sup>1118</sup> ; <i>Df(3L)Exel8101/TM6B, Tb</i> <sup>1</sup>	65A3;65A9	21.1 (1.84)	18.5 (1.46)	0.6404	0.0007	0.1048
<i>w</i> <sup>1118</sup> ; <i>Df(3L)ED211/TM6C, Sb</i> <sup>1</sup>	65A9;65B4	11.9 (1.57)	11.3 (0.94)	0.8709	0.0036	0.7789
<i>w</i> <sup>1118</sup> ; <i>Df(3L)ED212/TM6C, Sb</i> <sup>1</sup>	65A9;65D5	21.4 (2.77)	18.8 (1.05)	0.4203	0.0043	0.3622
<i>w</i> <sup>1118</sup> ; <i>Df(3L)Exel6110/TM6B, Tb</i> <sup>1</sup>	65E4;65E8	16.5 (1.15)	17.0 (1.11)	0.4007	0.2697	0.6828
<b><i>w</i><sup>1118</sup>; <i>Df(3L)Exel6111/TM6B, Tb</i><sup>1</sup></b>	<b>65E7;65F4</b>	<b>24.3 (1.24)</b>	<b>16.9 (1.02)</b>	<b>0.0114</b>	<b>&lt;0.0001</b>	<b>0.0002*</b>
<i>w</i> <sup>1118</sup> ; <i>Df(3L)Exel8104/TM6B, Tb</i> <sup>1</sup>	65F7;66A4	19.3 (1.63)	15.7 (1.16)	0.4032	0.1167	0.0519
<i>w</i> <sup>1118</sup> ; <i>Df(3L)ED4408/TM6C, Sb</i> <sup>1</sup>	66A22;66C5	12.8 (1.32)	10.2 (1.44)	0.3737	0.0032	0.3146
<i>w</i> <sup>1118</sup> ; <i>Df(3R)Exel7317/TM6B, Tb</i> <sup>1</sup>	66C5;66D3	17.8 (0.88)	16.2 (1.12)	0.9453	0.1820	0.1838
<i>w</i> <sup>1118</sup> ; <i>Df(3L)ED4421/TM6C, Sb</i> <sup>1</sup>	66D14;67B1	11.9 (1.89)	8.8 (1.89)	0.3173	0.0005	0.2679
<b><i>w</i><sup>1118</sup>; <i>Df(3L)Exel6114/TM6B, Tb</i><sup>1</sup></b>	<b>67B10;67C5</b>	<b>18.5 (1.21)</b>	<b>15.3 (0.87)</b>	<b>0.4438</b>	<b>0.2143</b>	<b>0.0450</b>
<b><i>w</i><sup>1118</sup>; <i>Df(3L)Exel9048/TM6B, Tb</i><sup>1</sup></b>	<b>67D1;67D2</b>	<b>32.2 (1.33)</b>	<b>18.1 (1.27)</b>	<b>&lt;0.0001</b>	<b>&lt;0.0001</b>	<b>&lt;0.0001*</b>
<i>w</i> <sup>1118</sup> ; <i>Df(3L)ED4470/TM6C, Sb</i> <sup>1</sup>	68A6;68E1	14.0 (1.86)	11.6 (2.20)	0.4551	0.0590	0.3935
<i>w</i> <sup>1118</sup> ; <i>Df(3L)ED4483/TM6C, Sb</i> <sup>1</sup>	69A4;69D3	17.7 (1.17)	17.0 (2.03)	0.8489	0.2353	0.7615
<b><i>w</i><sup>1118</sup>; <i>Df(3L)ED215/TM6C, Sb</i><sup>1</sup></b>	<b>69B5;69C4</b>	<b>16.9 (1.93)</b>	<b>10.9 (0.72)</b>	<b>0.0372</b>	<b>0.2946</b>	<b>0.0282</b>
<b><i>w</i><sup>1118</sup>; <i>Df(3L)ED4486/TM6C, Sb</i><sup>1</sup></b>	<b>69C4;69F6</b>	<b>23.9 (2.16)</b>	<b>14.3 (1.59)</b>	<b>0.0018</b>	<b>0.0212</b>	<b>0.0013*</b>
<i>w</i> <sup>1118</sup> ; <i>Df(3L)Exel6261/TM6B, Tb</i> <sup>1</sup>	69F6;70A3	18.5 (1.44)	15.8 (1.09)	0.5931	0.1556	0.0805
<i>w</i> <sup>1118</sup> ; <i>Df(3L)ED4502/TM6C, Sb</i> <sup>1</sup>	<i>70A3;70C10</i>	<i>14.9 (1.08)</i>	<i>20.3 (1.45)</i>	<i>0.0424</i>	<i>0.1539</i>	<i>0.0554</i>
<i>w</i> <sup>1118</sup> ; <i>Df(3L)ED4543/TM6C, Sb</i> <sup>1</sup>	70C6;70F4	14.6 (2.34)	11.9 (1.18)	0.3929	0.1078	0.3366
<i>w</i> <sup>1118</sup> ; <i>Df(3L)ED217/TM6C, Sb</i> <sup>1</sup>	70F4;71E1	20.0 (1.75)	17.8 (1.43)	0.4725	0.0267	0.4068
<i>w</i> <sup>1118</sup> ; <i>Df(3L)ED218/TM6C, Sb</i> <sup>1</sup>	71B1;71E1	17.6 (0.90)	15.6 (0.91)	0.4800	0.4708	0.4072
<i>w</i> <sup>1118</sup> (Exel control)	N/A	14.8 (0.67)	16.2 (0.96)	N/A	N/A	N/A
<i>w</i> <sup>1118</sup> (ED control)	N/A	15.6 (0.80)	15.7 (1.16)	N/A	N/A	N/A

Deficiency stocks used in quantitative complementation tests. ED denotes DrosDel deficiencies and Exel indicates Exelixis deficiencies. The isogenic background stocks for both sets of deficiencies are listed as controls. Boldface type indicates deficiencies that failed to complement the QTL for aggressive behavior (*P*-values of the *L* × *G* term from ANOVA < 0.05). \*Significant following a Bonferroni correction for multiple tests. *Df(3L)ED4502* (results in italics) was marginally significant and was therefore included in complementation tests to mutations.

genes: 43 in 64E5;F5, 17 in 65E8;F4, 49 in 67B10;C5, 14 in 67D1;D2, 22 in 69B5;C4, 86 in 69C4;F6, and 70 in 70A3;70C10. Many of the genes in these regions affect metabolism and development.

**Mutant complementation tests:** We used quantitative complementation tests to mutations in positional candidate genes in QTL intervals defined by deficiency complementation tests to identify candidate genes corresponding to QTL affecting the difference in aggressive behavior between *Ore* and *2b*. The logic of quantitative complementation tests to mutations is the same as that for deficiency complementation tests. However, the mutations are maintained over balancer chromosomes with visible markers, and they are not all available in a common isogenic background. Therefore, we crossed each mutant stock to both *3-88Y* and *Ore* and measured the aggressive behavior of F<sub>1</sub> males of the four genotypes: *mutant/3-88Y*, *mutant/Ore*, *Bal/3-88Y*, and *Bal/Ore*, where *Bal* is the balancer chromosome.

We initially screened 58 mutants in positional candidate genes from all seven QTL defined by the deficiency complementation tests (Table S2). Where possible, we used *P*-element insertional mutations that were generated in the same background. We also chose stocks with few additional mutations, and included mutations in computationally predicted genes. The interpretation of the complementation tests to mutations is not as clear-cut as that from the complementation tests to deficiencies, because the genetic background of the mutant and control (balancer) genotypes are not co-isogenic. If the difference between *88Y* and *Ore* were due to alleles with strictly additive effects, this would not matter. However, if epistasis is extensive, the failure to complement could be attributable to differences between the mutant and balancer genotypes that lie outside the *88Y* introgression region, but which interact with QTL in this region. Therefore, we imposed the additional filter that the pattern of failure to complement in these tests must be

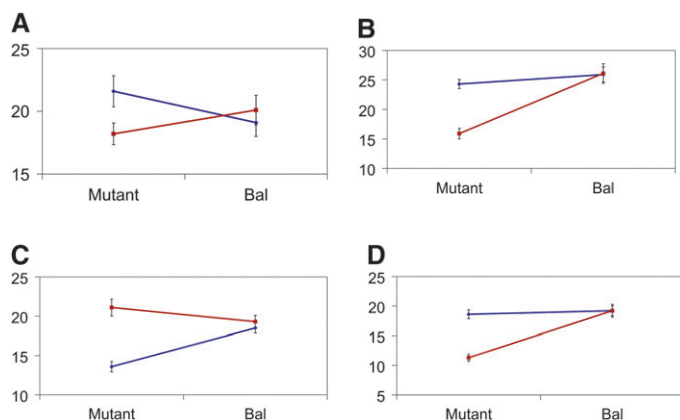


FIGURE 3.—Mutant complementation tests. Means and standard errors of aggression scores (y-axis) are depicted for the four genes that exhibit quantitative failure to complement *Ore* and *3-88Y* alleles. The blue lines represent the *3-88Y* line, and the red lines represent the *Ore* line. Genotype is depicted on the x-axis, such that four genotypes are shown: *Ore/Bal*, *3-88Y/Bal*, *Ore/mutant*, and *3-88Y/mutant*. (A) *CG10754*<sup>103161</sup>; (B) *CG11006*<sup>EY12079</sup>; (C) *mus312*<sup>D1</sup>; and (D) *Rgl*<sup>GT-000359</sup>.

consistent with an interaction with the mutant chromosome and not the balancer chromosome.

Of the 58 mutants tested, 12 (~21%) exhibited quantitative failure to complement, inferred by a significant ( $P < 0.05$ ) line  $\times$  genotype interaction term. We excluded five of these mutations from further analyses because the difference between *Bal/Ore* and *Bal/3-88Y* means was not significantly different from that between *mutant/Ore* and *mutant/3-88Y* means. We retested the remaining seven mutants, using twice the number of replicates as in the initial screen, and confirmed four mutations that exhibited quantitative failure to complement the QTL alleles affecting the difference in aggressive behavior between *Ore* and *3-88Y*: *CG11006*, *CG10754*, *mutagen-sensitive 312*, and *Ral guanine nucleotide exchange factor 2* (Figure 3). None of these loci have been previously associated with aggressive behavior. The *3-88Y/mutant* genotype exhibited higher levels of aggression than the *Ore/mutant* genotype for *CG10754*, *CG11006*, and *Rgl*; this observation was reversed for the *mus312* mutant. That flies hemizygous for the *88Y-3* allele at all loci are not the more aggressive underscores the complex nature of QTL affecting aggression. The cumulative effects and potential epistatic interactions at these and additional, unidentified, loci must be teased apart in great detail, as they are neither necessarily predictable nor intuitive.

We did not identify candidate genes in the 64E5;64F5, 67B10;67C5, 67D1;67D2, and 69B5;69C4 QTL, and it is possible there are additional candidate genes in the QTL in which we did identify a candidate gene. Ideally, all positional candidate genes should be screened, but there were many of the 300 genes for which no mutation was available, and we did not test mutations in candidate genes when the stock contained multiple additional mutations.

**Candidate quantitative trait genes for aggressive behavior:** *CG11006* has four predicted transcripts and is annotated as being involved in “cellular component organization and biogenesis” (LYNE *et al.* 2007). *CG11006* is highly expressed in the brain, ovary, and male accessory glands (CHINTAPALLI *et al.* 2007). The *CG11006* protein interacts with 10 other gene products, many of which are also computationally predicted

(GIOT *et al.* 2003). However, those that have been described function in central nervous system development, bristle morphogenesis, and calcium ion binding. It is orthologous to the human DMD gene, which is implicated in both Becker and Duchenne muscular dystrophy (WOOD *et al.* 1987; BOYCE *et al.* 1991).

*CG10754* is adjacent to and partially overlapping with *CG11006*. *CG10754* has one predicted transcript, is annotated to function in RNA splicing, metabolism, and zinc binding (LYNE *et al.* 2007), and is expressed in the ovary and male accessory glands (CHINTAPALLI *et al.* 2007). No gene ontology information is available for the two predicted gene products with which *CG10754* interacts (GIOT *et al.* 2003). Variation in *CG10754* transcript abundance is associated with chill coma recovery time (AYROLES *et al.* 2009) and sleep behavior (HARBISON *et al.* 2009) in a reference panel of inbred lines derived from a natural population.

*mutagen-sensitive 312* (*mus312*) has three transcripts, and functions in DNA damage recognition and repair, protein binding, and metabolism (LYNE *et al.* 2007). *mus312* is expressed in the ovary and testes, as well as other tissues (CHINTAPALLI *et al.* 2007). The MUS312 protein interacts with *CG5410* and *CG8942* gene products, which are involved in mitochondrial transport and Wnt receptor signaling, respectively (GIOT *et al.* 2003).

*Ral guanine nucleotide exchange factor 2* (*Rgl*) encodes four transcripts and is involved in G-protein coupled receptor and tyrosine kinase signaling (LYNE *et al.* 2007). It is highly expressed in the brain, crop, ovary, and male accessory glands (CHINTAPALLI *et al.* 2007). *RGL* interacts with *CG2091*, encoded by a computationally predicted gene with no known function (GIOT *et al.* 2003). *Rgl* is orthologous to the human gene *SOS1*, which is implicated in the dysmorphic Noonan syndrome (ROBERTS *et al.* 2007; TARTAGLIA *et al.* 2007).

The four candidate quantitative trait genes affecting the difference in aggressive behavior between *Ore* and *3-88Y* have little in common other than that they are all expressed in reproductive tissues. *CG10754* and *Rgl* are potentially involved in neurotransmission through their roles in zinc binding and as a G-protein coupled receptor, respectively.

**Complex genetic architecture of aggressive behavior:** We have identified four novel candidate genes affecting natural variation in aggressive behavior in a small genomic region, in two laboratory strains not selected for aggressive behavior. The QTL regions interact epistatically: it remains to be seen how the candidate genes interact, and what the gene(s) are in the *2b* 97D;100F QTL region that suppress the effect of the *2b* 65D;69D QTL on aggressive behavior. Extensive epistasis poses a serious challenge for understanding the genetic basis of complex traits using linear models of genotype–phenotype associations. The novel candidate genes were not implicated in previous studies that identified large numbers of potential candidate genes affecting aggressive behavior from whole genome expression profiling of genetically divergent strains recently derived from nature (EDWARDS *et al.* 2006, 2008). This is not surprising, since different loci can affect variation in complex traits in different strains. Further, the four loci described in this report may not affect aggression through differences in gene expression; they may differ in expression but these differences were too small to detect in the previous studies; or differences in gene expression at a different developmental stage than young adults (when the transcriptional profiles were obtained) are important. Nevertheless, these results highlight the utility of unbiased genome scans for naturally segregating QTL for identifying novel genes and genetic networks affecting complex behaviors.

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## Quantitative Trait Loci for Aggressive Behavior in *Drosophila melanogaster*

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**TABLE S1**  
**Cytogenetic breakpoints of introgression lines**

Chromosome	Genotype	Line	MAS (SE)
<i>X</i>	1A-3E (B); 4F-20F (O)	<i>20</i>	24.15 (1.69)
	1B (O); 3E-9A (B); 10D-20F (O)	<i>27</i>	17.12 (1.64)
	1B-7E (O); 9A-11D (B), 12E-20F (O)	<i>21</i>	21.75 (1.45)
	1B-9A (O); 10D-16D (B); 17C-20F (O)	<i>43Я</i>	21.85 (1.47)
	1B-11D (O); 12E-20F (B)	<i>76Я</i>	23.7 (1.68)
2	21A-30D (B); 33E-60F (O)	<i>78Я</i>	19.0 (2.34)
	21A-21E (O); 22F-33E (B); 34EF-60F (O)	<i>18</i>	26.3 (1.67)
	21A-27B (O); 29F-38E (B); 39A-60F (O)	<i>16</i>	9.08 (1.82)
	21A-30D (O); 33E-50F (B); 57C-60F (O)	<i>81Я</i>	23.4 (2.22)
	21E (O); 22F (B); 27B-50B (O); 50D-60F (B)	<i>6Я</i>	17.3 (2.04)
	21A-50F (O); 57C-60F (B)	<i>22</i>	22.6 (1.57)
3	61A-63A (O); 65A-100F (B)	<i>34Я</i>	11.55 (1.43)
	61A-67D (O); 68B-100F (B)	<i>31Я</i>	13.65 (1.72)
	61A-68C (B); 69D-91D (O); 92A-96A (B); 96F-100A (O)	<i>15</i>	18.6 (1.50)
	61A-65A (O); 65D-69D (B); 70C-100A (O)	<i>88Я</i>	34.05 (1.89)
	61A-67D (O); 68B-96F (B); 97D-100A (O)	<i>5</i>	18.4 (1.65)
	61A-68B (O); 68C-94D (B); 96A-100F (O)	<i>22</i>	19.2 (1.69)
	61A-69D (O); 70C-91D (B); 92A-100F (O)	<i>16</i>	18.95 (1.24)
	61A-89B (O); 91A-100F (B)	<i>17</i>	13.67 (1.71)
61A-93B (O); 94D-100A (B)	<i>27</i>	14.95 (1.95)	

The portion of the genome deriving from each of the parental lines, *2b* (B) and *Oregon-R* (O), is described for each of the 20 introgression lines, along with the Mean Aggression Score (MAS) of each line.

**TABLE S2**

**Mutant Complementation Tests**

Table S2 is available as an Excel file at <http://www.genetics.org/cgi/content/full/genetics.109.101691/DC1>.