Supporting Information

Wang *et al.* 10.1073/pnas.0801327105

SI Text

Cumulative Probability Distribution (CDP) Analysis for Fig. S2. The most sensitive criterion for fighting is that a given pair of flies exhibits at least one lunge during the 20-min observation period. However, arbitrarily less sensitive (more stringent) criteria can also be used. We wished to examine systematically how criteria of different stringencies affected the comparison between housing conditions and genotypes. To do this, we first measured the total number of lunges exhibited by each pair of flies, during the 20-min observation period. We then plotted the data as a cumulative probability distribution curve, for each genotype and housing condition (heterozygous/single-housed; homozygous/single-housed; heterozygous/group-housed; homozygous/group-housed). Due to different genetic backgrounds, separate comparisons were made for P-element heterozygotes vs. homozygotes ($Cyp6a20^{+/-}$ vs. $Cyp6a20^{-/-}$, **A**), and Df heterozygotes vs. Df/P-element homozygotes (*Df(2R)BSC11*/+ vs. *Cyp6a20*/*Df*, **B**).

In each case, group-housed heterozygous controls (**A**, $Cyp6a20^{+/-}$ and **B**, *Df(2R)BSC11*/+) were chosen as the "reference" datasets for comparison to other conditions/genotypes. For each stringency criterion (lunge number), a Kruskal–Wallis ANOVA (significance level = 0.05) was performed on each dataset from the four curves. If there was significant difference between the four datasets, then a Mann–Whitney *U* test (significance level = 0.05) was performed between the "reference" dataset and the three other "test" datasets. This analysis permitted a comparison of the aggressiveness of different genotypes under different housing conditions, at progressively higher stringencies.

At the origin of each curve, when $X = 0$, the group-housed heterozygous controls (**A**, $Cyp6a20^{+/-}$ Group housing, and **B**, *Df(2R)BSC11*/+ Group housing) are significantly different from all other groups. As the lunge threshold criterion (X) increases, the difference between the four datasets becomes gradually indistinguishable. This is to be expected, because if the lunge threshold were set arbitrarily high (e.g., at least 1,000 lunges in 20 min), then none of the pairs in any condition or genotype would be scored as having a fight (fighting frequency = 0), and therefore all of the curves would be statistically indistinguishable. The question is, how do the different curves behave as they approach this point of statistical equivalence? The asterisks above the reference curve represent points that are statistically different from "test" datasets in some or all of the other three curves. "N.S." above the remaining curves represents the point after which these curves become indistinguishable from the reference curve.

Four major conclusions can be drawn from this analysis:

1. The behavior of the three "test" curves with respect to each other is clearly different in **A** and **B**. In general, flies containing the *Df(2R)BSC11* deficiency chromosome (**B**) are more aggressive than those containing the P-element insertion chromosome (**A**). This difference could reflect a difference in the genetic backgrounds of CS and *Df(2R)BSC11*/*Cyo* flies, or haplo-insufficiency of genes other than *Cyp6a20* that are encompassed by the deficiency. Therefore, the most conservative conclusions are those that can be drawn from the analysis of the P-element insertion data (**A**).
2. The three "test" datasets in **A** and **B** are indistinguishable from each other by Kruskal-Wallis ANOVA, in the range $X = 0$–19. In addition, all "test" datasets are significantly different from the "reference" (heterozygous/group-housed) dataset for threshold criteria $X = 0$ (**A**) or $X = 0$–4 (**B**), at each of the individual lunge threshold values within these intervals. This indicates that the *Cyp6a20* homozygous mutation renders flies equally aggressive under group-housed and single-housed conditions, and that under both housing conditions, these mutant flies are more aggressive than group-housed heterozygous controls. This supports the conclusion that the suppressive effect of enriched social experience on aggressiveness is mediated, at least in part, by *Cyp6a20*.
3. The curves for homozygous P-element mutant flies, in either the single- or group-housing conditions, become statistically indistinguishable from the reference curve at the same lunge criterion ($X = 1$, $Cyp6a20^{-/-}$ group housing, and $Cyp6a20^{-/-}$ single housing, **A**). This indicates that single-housed homozygous flies are no more aggressive than group-housed homozygous flies, compared with group-housed heterozygous flies. This would suggest that most or all of the difference in aggressiveness between single and group-housed heterozygous flies is due to *Cyp6a20,* and therefore that this gene is the primary, if not the exclusive, mediator of the effect of social experience on aggressiveness. However, in experiments using the deficiency-containing chromosome (**B**), the two homozygous curves reach statistical equivalence with the "reference" curve at different points ($X = 5$ for *Cyp6a20*/*Df*, group housing, $X = 11$ for *Cyp6a20*/*Df*, single housing, **B**). This indicates that under single housing conditions, the homozygous mutants are still somewhat more aggressive than they are under group housing conditions; subject to the caveats in 1), this would imply that additional genes besides *Cyp6a20* mediate the effect of social experience on aggressiveness.
4. The curves for single-housed flies (homozygous or heterozygous) become indistinguishable from the reference curve at the same point in **A** ($X = 1$, $Cyp6a20^{-/-}$, single housing and $Cyp6a20^{+/-}$, single housing). This implies that single-housed flies are no more aggressive in the presence or absence of *Cyp6a20*, compared with group-housed heterozygous flies. This would suggest that *Cyp6a20* exclusively functions in mediating the effect of group housing to suppress aggressiveness. However, in **B**, the two single-housing curves reach statistical equivalence with the reference curve at different points ($X = 8$ for *Df(2R)BSC11*/+, single housing, $X = 11$ for *Cyp6a20*/*Df,* single housing). This suggests a difference of aggressiveness between single-housed heterozygous (*Df*/+) and homozygous (*Df*/*P-element*) *Cyp6a20* mutants. If so, then subject to the caveats in 1), *Cyp6a20* may function not only in mediating the effect of group housing on aggressiveness, but also in controlling the level of aggressiveness under single housing conditions.

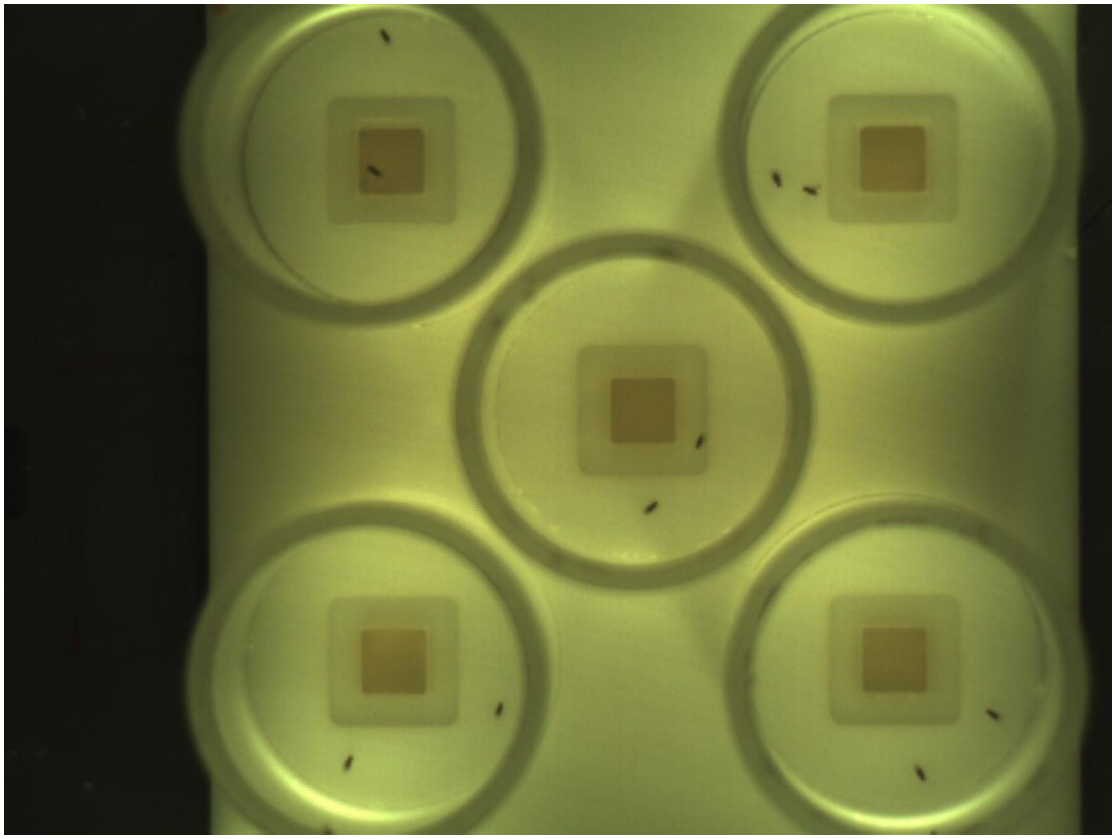

Fig. S1. Aggression arena. The apparatus was modified from an original design by Hoyer SC *et al.* [Hoyer SC, *et al.* (2008) Octopamine in male aggression of *Drosophila Curr Biol* 18:156–167]. The dimensions of each arena were 5.0 cm (*D*) × 11.4 cm (*H*). The center of the arena contains an inner square well (1.2 cm × 1.2 cm) filled with sucrose-apple juice-agar medium, and an outer square well (2.2 cm × 2.2 cm) filled with 1% agar medium. Fluon was painted on the inner surface of the wall of each arena, to prevent flies from climbing out. Arenas were covered by transparent plastic lids to prevent flies from escaping and to facilitate video capture.

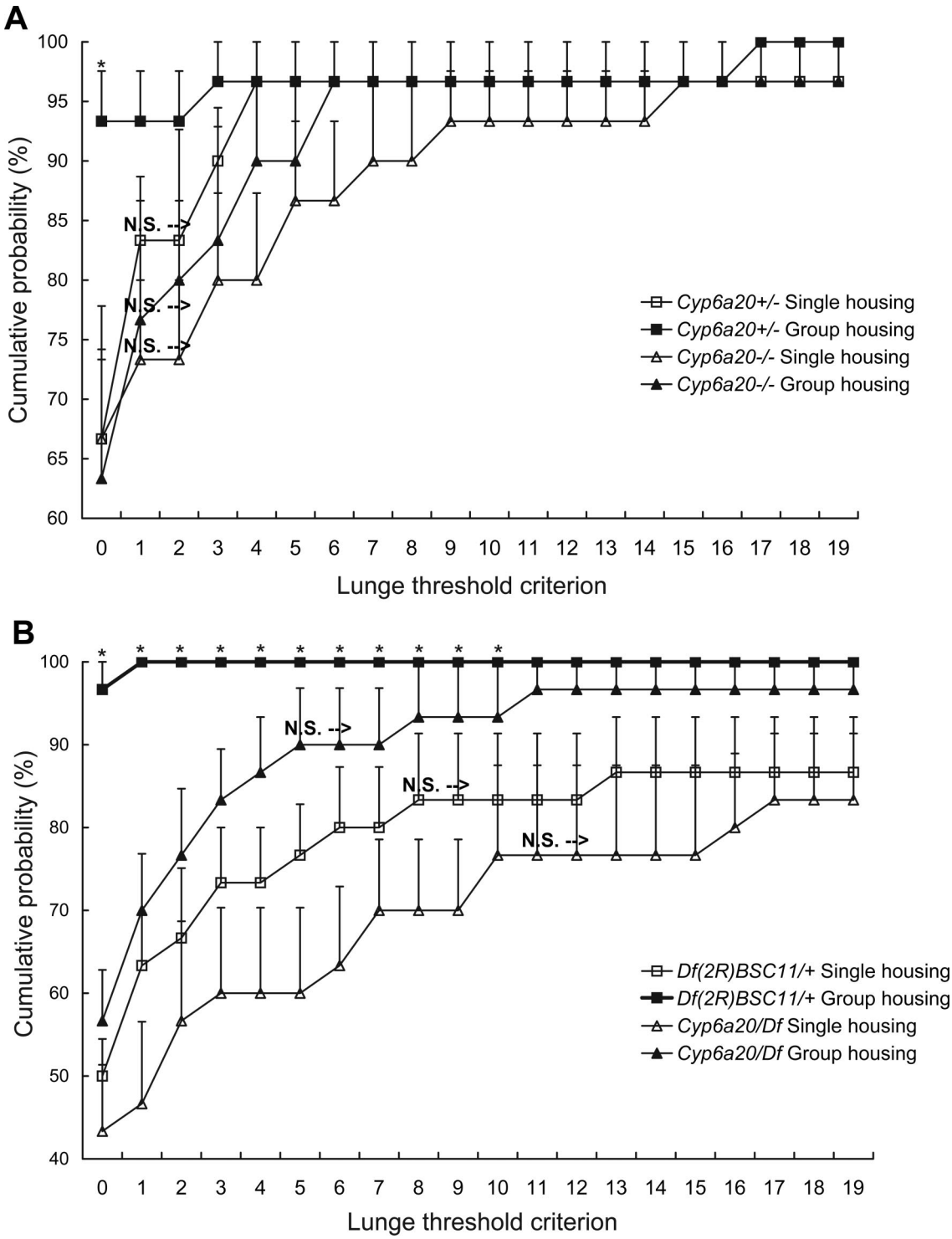

Fig. S2. Cumulative probability distributions of lunging thresholds for *Cyp6a20* mutant analysis. For each lunge threshold criterion (*x* axis), the cumulative probability (*y* axis) represents the cumulative percentage of pairs that can be accounted for by *X* lunges or less (in 20 min). (*A*) Cumulative probability distributions for $Cyp6a20^{+/-}$ and $Cyp6a20^{-/-}$ ($n = 6$, same datasets used in Fig. 3, error bars represent SEM). (*B*) Cumulative probability distributions for $Cyp6a20^{Df/+}$ and $Cyp6a20^{Df/-}$ ($n = 6$, same data used in Fig. 3, error bars represent SEM). Curves that approach 100% more rapidly indicate a lower level of aggressiveness. Squares represent homozygous mutants ($Cyp6a20^{-/-}$ in *A* and *Cyp6a20/Df* in *B*), triangles represent heterozygous controls ($Cyp6a20^{+/-}$ in *A* and *Df(2R)BSC11/+* in *B*). Open and solid symbols indicate single- and group-housing conditions, respectively (refer to *SI Text*).

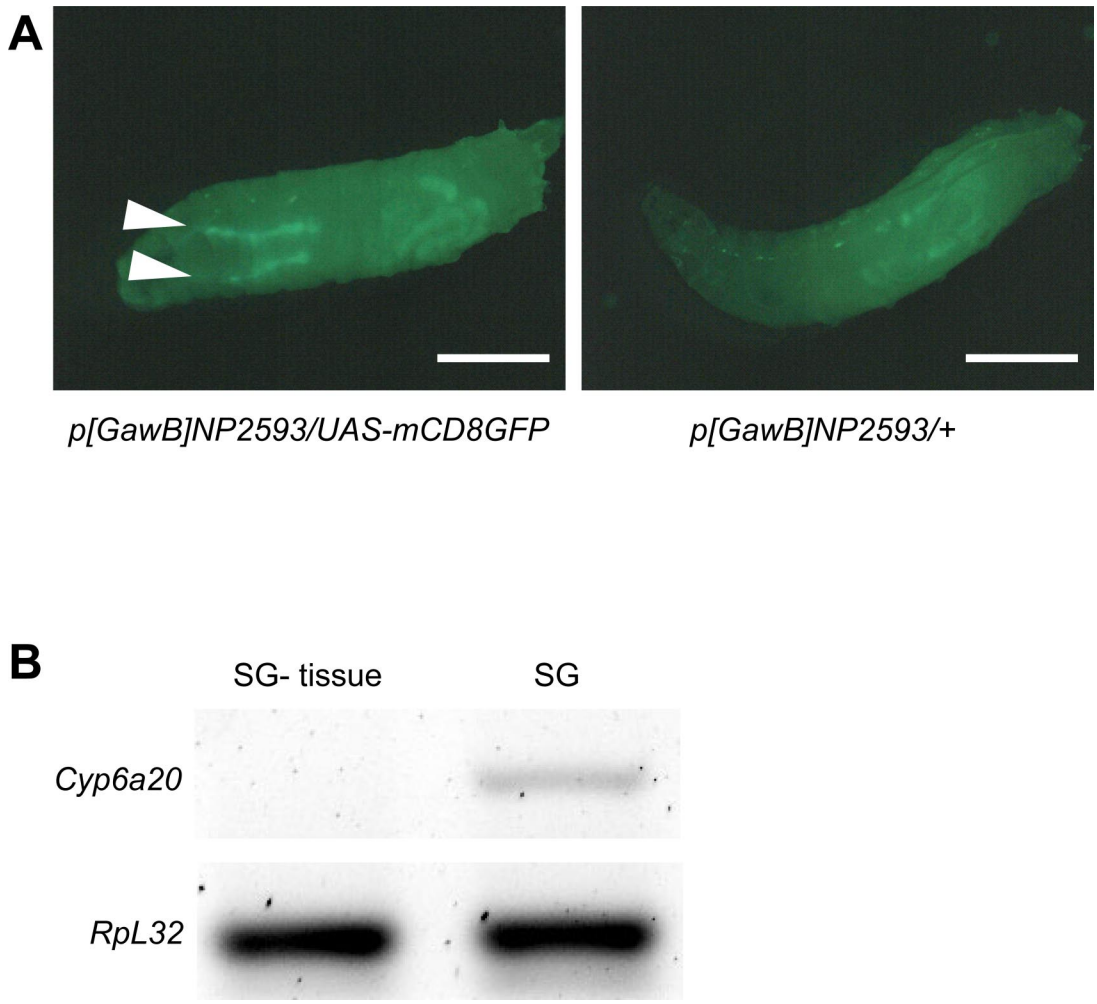

Fig. S3. Larval *Cyp6a20* expression is enriched in salivary gland (SG). For B>, C-S third instar larvae were dissected in PBS and both SG and SG-removed larval tissue were collected. Equal amount of RNA extracted from both tissues were reverse-transcribed and simultaneously amplified using *RpL32* and *Cyp6a20* primers, producing 423 bp and 962 bp, respectively. *RpL32* (CG7939) was used as an internal control of gene expression. PCR products were loaded on 1.2% agarose gel and visualized by ethidium bromide. (*A*) GFP expression was seen in SG of *P[GawB]NP2593/UAS-mCD8GFP* (*Left*, arrow head), but not in SG of *P[GawB]NP2593/+* (*Right*) larva. (Scale bar: 1 mm.) (*B*) RT-PCR confirmed *Cyp6a20* transcripts were enriched in SG.

Other Supporting Information Files

Dataset S1