## **Supporting Information**

## Bedford and Hartl 10.1073/pnas.0812009106



**Fig. S1.** Comparison of expected and simulated distribution of expression values. The equilibrium distribution of expression values expected from the OU model ( $\sigma = 5.481$  and  $\lambda = 57.038$ ) is shown as a solid line. The equilibrium distribution of expression values obtained from simulation using a strong-selection/weak-mutation model and the fitness landscape from Fig. 3 is shown as a set of discrete points. The simulation used 100,000 steps. In each step, a random mutation (+0.1 expression or -0.1 expression) was drawn and then checked for fixation based upon its selective coefficient. The equilibrium variance predicted by the OU model is 0.263, whereas the variance observed across the 100,000 simulated expression values is 0.265.







**Fig. S3.** As in Fig. S1, the solid line represents the OU expectation of equilibrium variance, given symmetrical mutation rates to higher expression and lower expression. However, in the simulation, the chance of a mutation reducing expression level was twice that of a mutation increasing expression level. Interestingly, asymmetrical mutation appears to shift the mean of the distribution, but not affect its variance. The equilibrium variance predicted by the OU model is 0.263, whereas the variance observed across the 100,000 simulated-expression values is 0.261.



Fig. S4. Normalization reduces gene-expression divergence. The dashed line represents gene-expression variance expected under an OU process, with  $\sigma = 10$  and  $\lambda = 140$ . Each point represents 10,000 realizations of this OU process for a specific time *t*. In each realization, two independent evolutions (arriving at expression values *xA* and *xB*) are taken from a common ancestor [ $x0 \approx N(0, 1)$ ]. Then, the set of *xA* values and the set of *xB* values are independently normalized to have mean 0 and variance 1. Expression variance is measured for each *xA*, *xB* pair, and the mean taken across all pairs. In this scenario, nonlinear regression estimates  $\sigma = 5.506$  and  $\lambda = 57.489$ .



Fig. S5. Species tree for 7 Drosophila species. Branch lengths are proportional to amino acid substitutions per site determined by maximum-likelihood averaged across 5,380 genes. The tree shown here is: (((((dmel: 0.008306, dsim: 0.008237): 0.011678, dyak: 0.015866): 0.046511, dana: 0.063118): 0.034376, dpse: 0.071528): 0.093932, dmoj: 0.060454, dvir: 0.044506).

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Fig. S6. Distribution of expression level across 6,085 *D. melanogaster* genes. Gene expression was determined by log<sub>2</sub> probe intensity, and was normalized to have mean 0 and variance 1. Gene expression distributions for other *Drosophila* species are similar.

## **Other Supporting Information Files**

Dataset S1 (PDF) SI Appendix

DNAS Nd