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. S2. Distributions of gene-specific OU parameter values. On the left, each point represents the maximum-likelihood estimate for a particular gene. The dashed line represents the neutral expectation of equilibrium variance = 1.0. The right shows the distribution of gene-specific estimates of equilibrium variance.
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.
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 $x_A$ and $x_B$) are taken from a common ancestor ($x_0 \sim N(0, 1)$). Then, the set of $x_A$ values and the set of $x_B$ values are independently normalized to have mean 0 and variance 1. Expression variance is measured for each $x_A, x_B$ pair, and the mean taken across all pairs. In this scenario, nonlinear regression estimates $\sigma = 5.506$ and $\lambda = 57.489$. 

Fig. S4.
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).
**Other Supporting Information Files**

Dataset S1 (PDF)
SI Appendix

*Fig. S6.* Distribution of expression level across 6,085 *D. melanogaster* genes. Gene expression was determined by log₂ probe intensity, and was normalized to have mean 0 and variance 1. Gene expression distributions for other *Drosophila* species are similar.
Maximum-likelihood estimation of OU parameters

Here we use the Ornstein-Uhlenbeck process to model gene expression divergence. This document gives a brief tutorial in gene-specific parameter estimation and hypothesis testing.

First, refer to Maximum-likelihood estimation of OU parameters section of Methods.

R code is provided, but the methods will work with any statistical / programming software package. The package mvtnorm (http://cran.r-project.org/web/packages/mvtnorm/index.html) is used to compute the PDF of multivariate normal distributions. Once installed this package is loaded with the command:

```r
library(mvtnorm);
```

### Expression values

Using as an example gene FBgn0011768 with expression vector

```r
eexpr <- c(1.288733, 0.7893953, 0.814975, 0.6608836, 0.3954609, 0.7329379, 0.836787)
```

in *dmel, dsim, dyak, dana, dpse, dmoj*, and *dvir*.

### Distance (t) matrices

The first step is to estimate the evolutionary time points at which gene expression values are sampled. Here, we assume this corresponds to the *Drosophila* phylogeny, with branch-lengths proportional to amino acid distance.
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).

We take the starting point for evolution along this tree as \textit{D. melanogaster}. The distance from \textit{D. melanogaster} to each pair of the other six species is:

tmatrix <- matrix(c(0.016543, 0.044087, 0.137850, 0.180636, 0.263494, 0.247546, 0.044087, 0.035850, 0.145479, 0.188265, 0.271123, 0.255175, 0.137850, 0.145479, 0.129613, 0.235517, 0.318375, 0.302427, 0.180636, 0.188265, 0.235517, 0.172399, 0.255257, 0.299763, 0.247546, 0.255175, 0.302427, 0.310837, 0.299763, 0.239309), nrow=6, byrow=TRUE);

So, the total distance from \textit{dmel} to \textit{dsim} is 0.017, while the total distance in the \textit{dmel}, \textit{dsim}, \textit{dyak} tree is 0.044.

The diagonal of the \textit{tdmatrix} gives $t_i$.

meanslist <- diag(tmatrix);

> meanslist
[1] 0.016543 0.035850 0.129613 0.172399 0.255257 0.239309

The shared distance from \textit{dmel} to each pair of species is:

sdmatrix <- matrix(c(0.016543, 0.008306, 0.008306, 0.008306, 0.008306, 0.008306, 0.008306, 0.008306, 0.035850, 0.019984, 0.019984, 0.019984, 0.019984, 0.019984, 0.066495, 0.066495, 0.066495, 0.066495, 0.066495, 0.172399, 0.100871, 0.100871, 0.100871, 0.100871, 0.194803, 0.008306, 0.019984, 0.066495, 0.100871, 0.194803, 0.239309), nrow=6, byrow=TRUE);

> sdmatrix
[1,] 0.016543 0.008306 0.008306 0.008306 0.008306 0.008306
[2,] 0.008306 0.035850 0.019984 0.019984 0.019984 0.019984
[3,] 0.008306 0.019984 0.129613 0.066495 0.066495 0.066495
[4,] 0.008306 0.019984 0.129613 0.172399 0.100871 0.100871
[5,] 0.008306 0.019984 0.066495 0.066495 0.100871 0.255257
[6,] 0.008306 0.019984 0.066495 0.100871 0.194803 0.239309
**Dsim** and **dyak** share evolution along the **dmel** branch of the phylogeny giving their shared distance as 0.008.

Tracing the total evolutionary distance going from **dsim** through **dmel** to **dyak** requires counting the **dsim** and **dyak** specific branches once and the **dmel** specific branch twice. This can be accomplished by:

```r
> cmatrix <- sdmatrix+tdmatrix;
```

The **cmatrix** gives $t_i + t_j$, while the **sdmatrix** gives $s_{ij}$.

### Multivariate normal PDF

The mean value after time $t$ depends upon the optimum $\mu$ and the selection parameter $\lambda$.

```r
means <- function(x,opt,sel) opt*(1-exp(-meanslist*sel)) + x*exp(-meanslist*sel);
```

The covariance matrix depends upon $t, s, \lambda$ and drift parameter $\sigma$.

```r
covm <- function(sel, drift) (drift^2/(2*sel)) * exp(-sel*cmatrix) * (exp(2*sel*sdmatrix) - 1);
```

For a vector expression values corresponding the seven *Drosophila* species data, the density function of the OU model is given as the log likelihood of drawing **data[1]** from the equilibrium distribution, plus the log likelihood of drawing **data[2:7]** given **data[1]**. Here, the log likelihood is multiplied by $-1$ to aid in later numerical optimization. Additionally, the output is set to 10000 when either $\text{sel}$ or $\text{drift}$ goes below 0. This is to ensure the numerical optimization only returns positive values for $\text{sel}$ and $\text{drift}$.

```r
pdf <- function(param, data) {
  opt <- param[1]; sel <- param[2]; drift <- param[3];
  if ( sel>0 & drift>0 ) {
    -sum(
      dnorm(data[1],mean=opt,sd=drift/sqrt(2*sel),log=TRUE),
      dmvmnorm(data[2:7],opt*(1-exp(-meanslist*sel)) + data[1]*exp(-meanslist*sel),(drift^2/(2*sel)) * exp(-sel*cmatrix) *
      (exp(2*sel*sdmatrix) - 1),log=TRUE)
    )
  } else { 10000 }
}
```
As an example, if \( \text{opt} = 0, \text{sel} = 100 \) and \( \text{drift} = 10 \), then the \(-\log\) likelihood of observing the data is:

\[
\text{pdf(c(0,100,10),expr)}
\]

\[
[1] 8.37288
\]

**Estimation by maximum-likelihood**

Numerical optimization is used get the maximum likelihood estimate of parameter values.

\[
\text{pdfn <- function(param, data) }
\]

\[
\text{opt <- param[1]; sel <- param[2]; sel <- (drift^2)/2 }
\]

\[
\text{if ( sel>0 & drift>0 ) }
\]

\[
\text{-sum( dnorm(data[1], mean=opt, sd=drift/sqrt(2*sel), log=TRUE), dmvmnornorm(data[2:7], opt*(1-exp(-meanslist*sel)) + data[1]*exp(-meanslist*sel),(drift^2/(2*sel)) * exp(-sel*cmatrix) * (exp(2*sel*sdmatrix) - 1), log=TRUE) })
\]

\[
\text{else }
\]

\[
\text{10000 }
\]

>

\[
\text{optim(c(0,1), pdfn, gr=NULL, expr)}
\]

\[
\text{par}
\]

\[
[1] 0.7313387 1.9256308
\]

\[
\text{value}
\]

\[
[1] 4.589143
\]

So, for FBgn0039527, ML estimates \( \mu \) at 0.79, \( \lambda \) at 346.9 and \( \sigma \) at 6.51. This gives as equilibrium variance of:

\[
\text{6.51^2/(2*346.92)}
\]

\[
[1] 0.06108051
\]

**Testing significance of selection vs. neutrality**

Want to compare the log likelihood of a model where \( \mu, \lambda \) and \( \sigma \) are free variables (selection) to a model where the equilibrium variance is constrained to be 1 (neutrality).

The PDF for the neutral model as follows:

\[
pdfn <- function(param, data) { 
\text{opt <- param[1]; sel <- param[2]; sel <- (drift^2)/2 }
\text{if ( sel>0 & drift>0 ) }
\text{-sum( dnorm(data[1], mean=opt, sd=drift/sqrt(2*sel), log=TRUE), dmvmnornorm(data[2:7], opt*(1-exp(-meanslist*sel)) + data[1]*exp(-meanslist*sel),(drift^2/(2*sel)) * exp(-sel*cmatrix) * (exp(2*sel*sdmatrix) - 1), log=TRUE) })
\text{else }
\text{10000 }
}
\]

This gives an ML estimate of:

\[
\text{optim(c(0,1), pdfn, gr=NULL, expr)}
\]

\[
\text{par}
\]

\[
[1] 0.7313387 1.9256308
\]

\[
\text{value}
\]

\[
[1] 4.589143
\]

Because one free parameter differentiates these models, two times the difference between their log likelihoods is expected to be \( \chi^2 \) distributed with one degree of freedom.
Thus, the p-value for the significance of selection on FBgn0011768 is 0.0029.