

## Extensive Sex-Specific Nonadditivity of Gene Expression in *Drosophila melanogaster*

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Manuscript received January 19, 2004

Accepted for publication May 7, 2004

### ABSTRACT

Assessment of the degree to which gene expression is additive and heritable has important implications for understanding the maintenance of variation, adaptation, phenotypic divergence, and the mapping of genotype onto phenotype. We used whole-genome transcript profiling using Agilent long-oligonucleotide microarrays representing 12,017 genes to demonstrate that gene transcription is pervasively nonadditive in *Drosophila melanogaster*. Comparison of adults of two isogenic lines and their reciprocal F<sub>1</sub> hybrids revealed 5820 genes as significantly different between at least two of the four genotypes in either males or females or across both sexes. Strikingly, while 25% of all genes differ between the two parents, 33% differ between both F<sub>1</sub>'s and the parents, averaged across sexes. However, only 5% of genes show overdominance, suggesting that heterosis for expression is rare.

THE null hypothesis in quantitative genetics is the infinitesimal model, that there are an infinite number of genes, that the individual effects of genes are vanishingly small, and that gene effects are predominantly additive (FISHER 1918). While recent work has challenged the assumption of large numbers of genes per trait and the accompanying assumption that allelic effects are small (MACKAY 2001), surprisingly little evidence has been gathered to either support or contradict the assumption of additivity, particularly at the molecular level. The assumption of additivity follows as a parsimonious inference from the observation that phenotypic traits tend to blend in the offspring of divergent parents and is also built into standard mathematical models of complex traits. Yet no genome-wide assessment of the degree of additivity of gene effects has been reported. Gene expression profiling provides one mechanism for performing such a study, recognizing that the path from genotype to phenotype passes through transcript abundance. To address this issue in *Drosophila*, we combined moderate repetition with an experimental platform that provides high measurement repeatability, so as to quantify subtle differences in gene expression.

Both theoretical and empirical studies in a variety of species suggest that with a half dozen replicates of several genotypes (or other treatments), differences in transcript

abundance as small as 1.2-fold can be detected reliably using analysis of variance or Bayesian statistical methods (KERR *et al.* 2000; WOLFINGER *et al.* 2001; EFRON and TIBSHIRANI 2002). Three studies comparing isogenic strains of *Drosophila melanogaster* have demonstrated that of the order of 10–20% of all transcripts significantly differ in abundance between adults or developmental stages (JIN *et al.* 2001; MEIKLEJOHN *et al.* 2003; RIFKIN *et al.* 2003), and a comparable fraction of the transcriptome varies between specific tissue samples of individual fish, mice, and humans (PAVLIDIS and NOBLE 2001; OLEKSIK *et al.* 2002; CHEUNG *et al.* 2003; HSIEH *et al.* 2003). What has not been assessed directly is the additivity or lack thereof of these differences, namely the degree and direction of dominance and epigenetic effects on gene expression.

We approached this question by comparing gene expression profiles of 1-week-old reproductively mature male and female flies of two strains, *OregonR* and *2b*, as well as their reciprocal F<sub>1</sub> offspring. These two well-studied strains (NUZHDIN *et al.* 1993) differ in reproductive and other life history traits and have been used to map QTL for a wide range of traits from bristle number and wing shape to metabolism and fitness in a set of 98 derivative recombinant inbred lines (GURGANUS *et al.* 1998; ZIMMERMAN *et al.* 2000; WAYNE *et al.* 2001; MONTTOOTH *et al.* 2003). The strains show considerable sexual dimorphism for a range of traits, some of which may show more extreme divergence than that observed between two randomly chosen inbred lines of *D. melanogaster*. Also, gene expression profiling of midpupae of the two

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parent strains has been used to narrow down a QTL interval affecting ovariole number to a set of candidate genes (WAYNE and MCINTYRE 2002).

The expression platform that we present is a custom-designed long-oligonucleotide microarray printed *in situ* by Agilent Technologies (HUGHES *et al.* 2001). The array provides nearly comprehensive coverage of the version 3.0 release of the Berkeley Drosophila Genome Project annotation of the Celera genome sequence of *D. melanogaster* (CELNIKER *et al.* 2002). A complete loop design (CHURCHILL 2002; SIMON *et al.* 2002) involving 28 microarrays with seven replicates of each of eight genotype and sex samples was analyzed with gene-specific mixed models using the SAS Microarray Solution. Surprisingly, twice as many genes were observed to differ in gene expression between F<sub>1</sub> flies and their parents as between the two parent strains. We describe this observation in the context of modes of dominance and relative to the contribution of each chromosome and class of gene ontology, as well as the differences between the sexes.

#### MATERIALS AND METHODS

**Agilent microarrays:** Each microarray was synthesized by Agilent Technologies using phosphoramidite chemistry to synthesize 60 mers *in situ* on glass slides (HUGHES *et al.* 2001). Probes were designed using release 3.0 of the BDGP annotation of the Celera *D. melanogaster* genome sequence, and sequences will be available to the public through Agilent as part of the annotation of the commercial release. The 21,929 Drosophila probes represent 12,559 genes with 13,631 unique probes; 3112 of the probes are represented by between two and four replicates that are randomly distributed across the layout, and 3189 of the genes are represented by duplicate probes, 1072 of which are alternate probes. Most probes were designed against a common exon, while the alternate probes typically correspond to an alternate splice variant. Average expression levels derived from seven measurements of each sex and genotype were extremely highly correlated between duplicated probes ( $R^2 > 0.98$ ; data not shown) whereas alternate probes gave uncorrelated measurements, as expected. Oligonucleotide sequences and gene identities are supplied as supplementary information, as is an SAS file containing all fluorescence intensity measurements, at <http://statgen.ncsu.edu/ggibson/SupplInfo/SupplInfo3.htm>.

**Experimental design:** The eight genotype and sex combinations (*OregonR*, *2b* parents and their reciprocal F<sub>1</sub>'s, O2b and 2bO; 7- to 10-day-old mated males and females) were each represented by seven hybridizations on 28 microarrays, according to a loop design (CHURCHILL 2002; Figure 1). Each sample was generated by extracting total RNA from several hundred flies combined from multiple (5–10) rearing vials; three independent amplified RNA-labeling reactions were performed for each fluorescent dye (Cy3 or Cy5, using Agilent kit G2554A according to manufacturer's instructions). To minimize the source of technical error due to labeling, these were pooled and then split into three or four separate hybridization mixes for each of the 16 sex, genotype, and dye samples. The loop design ensured that each sex and genotype was contrasted directly against each other sex and genotype and that the dyes were balanced for sex, genotype, and sex-by-genotype contrasts. Parental and F<sub>1</sub> flies were both reared at a density of ~100–150 larvae per bottle on standard cornmeal medium

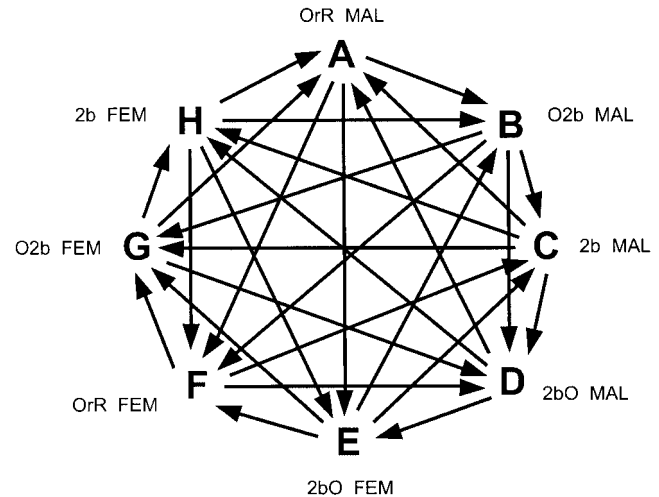


FIGURE 1.—Experimental design. Each of the eight sex and genotype combinations was hybridized to each of the other seven combinations in both directions in a complete loop design that ensured that dyes were balanced within sexes and genotypes. Arrowheads point to sample labeled with Cy5; arrowtails point to sample labeled with Cy3.

(1 kg cornmeal, 200 g yeast, 100 g agar, 700 ml molasses, and 14 liters water, plus tegosept and propionic acid) supplemented with live yeast paste. Adults were maintained in vials on a 12-hr light-dark cycle, separated by sex between days 4 and 7 after eclosion, and collected over a 3-hr midafternoon window to minimize circadian influences, by snap freezing in 70% ethanol at day 7–10. Total RNA was extracted using Trizol reagent. All hybridizations were performed at the Agilent facility in Palo Alto, California, according to standard protocols.

**Statistical analysis:** Slides were scanned using an Agilent scanner (G2565BA), and all analyses were performed on background-subtracted, loess-transformed log base 2 fluorescence intensity measurements. Two-step mixed model analysis of variance (WOLFINGER *et al.* 2001) was performed with SAS Microarray Solution software. Each of the 56 hybridization samples was subjected to linear normalization in PROC MIXED with the model

$$Y_{ij} = \mu + \text{Array}_i + \text{Dye}_j + \text{Array} \times \text{Dye}_{ij} + \text{Residual}_{ij}$$

where  $i$  ( $= 1-28$ ) specifies the microarray number,  $j$  specifies the dye(channel), and  $Y_{ij}$  are the log<sub>2</sub>-transformed expression intensities. The residuals from this model were then analyzed in 21,929 separate mixed models by probe using a model of the form

$$\text{Residual}_{ijkl} = \mu + \text{Array}_i + \text{Dye}_j + \text{Genotype}_k + \text{Sex}_l \\ + \text{Genotype} \times \text{Sex}_{kl} + \text{Error}_{ijkl}$$

where  $k$  specifies the genotype (ORE, O2b, O2b, or 2bO),  $l$  is the sex (male or female), and Array is the only random effect. The significance of each of the 12 specific contrasts of the four genotypes within each sex was then determined using the ESTIMATE option in PROC MIXED (SAS code is available on request). Standardized least-squares means for each sex and genotype were subjected to two-way hierarchical clustering using Ward's method in JMP to generate Figure 2. A total of 15,165 probes showed a difference between the sexes less than the Bonferroni significance cutoff of  $2.3 \times 10^{-6}$  (0.05/21,929 probes) and these correspond to 8775 genes after accounting for duplicate probes and alternate transcripts.

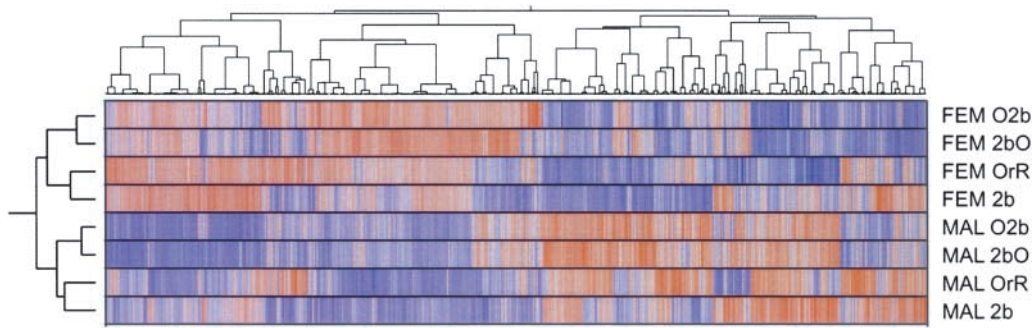


FIGURE 2.—Hierarchical clustering of gene expression by sex and genotype. The least-squares mean measure of relative transcript abundance is represented on a scale from high (red) to low (blue) with genes in columns and genotypes by sex in rows. The first level of clustering separates the two sexes and the next separates each  $F_1$  cluster distinctly from the two parents. The cluster of genes above shows genes with similar inheritance patterns that correspond approximately to the classes shown in Figure 3.

Probes were excluded from further analysis if none of the 12 pairwise genotype-within-sex contrasts showed a significant difference that was  $<1.9 \times 10^{-7}$  [ $0.05/(21,929 \text{ probes} \times 2 \text{ sexes} \times 6 \text{ pairwise comparisons})$ ]. This gave a list of 9557 potentially significant probes, corresponding to 5820 genes. The probes were sorted by gene, and the specific probe for each gene that gave the highest mean significance across comparisons was chosen for subsequent analysis of inheritance patterns. The justification for this is that repeated measures were extremely consistent. This analysis excludes comparison of alternate splice variants. For describing patterns of inheritance, we used mean least squares of the transformed data to estimate transcript abundance. As demonstrated in RANZ *et al.* (2004), slightly different formulation of the mixed models and choice of significance threshold or fitting of a Bayesian approach will alter the categorization of a small percentage of genes. Our analysis thus focuses on the pattern of variation across the genome, rather than on results for specific genes, and on fold changes in transcript abundance, rather than on  $P$ -values, given that all of the genes in the analysis show a highly significant genotype effect across the four parental and  $F_1$  genotypes.

Genes were classified into the inheritance patterns in Figure 4 and Table 1 by computing the difference between least-squares mean expression level estimates for each genotype within sex. Differences  $>1.25$ -fold (a difference in  $\log_2$  intensity of 1.32 or more) typically corresponded to a significance of  $10^{-5}$  and were used to assign genes to categories according to the following intuitive rules using an Excel macro: additive genes are those where both  $F_1$  genotypes are 1.25-fold greater than one parent and less than the other; overdominant (underdominant) genes are 1.25-fold greater (less) than both parents; dominant high (or low) genes are not significantly different from one parent but greater (less) than the other; genes expressed in the  $F_1$  more like the same sex in the parental line that was female (or male) are not significantly different from the female (male) parent of the same sex, but greater than both the reciprocal  $F_1$  and the other parent of the same sex; and genes that are high (low) in one  $F_1$  are greater (less) than each of the other three genotypes (and in some cases, one of the other genotypes might also have been significantly different from the others). Some of these categorizations are somewhat arbitrary, but our analysis tends toward conservative estimation of the number of genes showing nonadditive inheritance patterns. Approximately half of the significant genes did not fit one of the 16 patterns above. These generally showed

differences only between the two most extreme genotypes: many of these may also be additive or show partial dominance that does not satisfy the conservative definitions above. The remaining genes (7519 in females, 8461 in males) did not show significant genotype differences in either sex. Functional categorization of all genes by manually curated FlyBase Gene Ontology (GO) category is indicated in the supplementary table online at <http://www.genetics.org/supplemental/>.

## RESULTS

**Sex-specific generational differences in the transcriptome:** To estimate how many genes show sex and genotype differences, we assessed for each gene separately the significance of the difference between genotype means, relative to the variance in measurement within each genotype. Technical error was minimized by pooling multiple labeling reactions in each hybridization mix. Of the 12,017 genes on the array, 8775 are sexually dimorphic in adults after correction for multiple comparisons, and 5820 showed a significant difference between at least one of the four genotypes in one of the sexes. Two-way hierarchical clustering by gene and by genotype/sex combination reveals which genes share similar patterns of inheritance and provides an overall view of the extent of departure from additivity (Figure 2). The eight rows corresponding to the genes that show significant differences between at least one genotype in one sex cluster at two levels. The deepest branch separates males and females, consistent with previous observations that at least half of the whole adult transcriptome in flies is differentially expressed in the reproductive tissue (JIN *et al.* 2001; ARBEITMAN *et al.* 2002; PARISI *et al.* 2003). In both sexes, a second branch separates the reciprocal  $F_1$ 's from the two parental strains. This is a broad indication that transcription is more divergent between the  $F_1$  and parents than between the parents and, hence, that departures from additivity are apparent for a substantial fraction of the transcriptome.



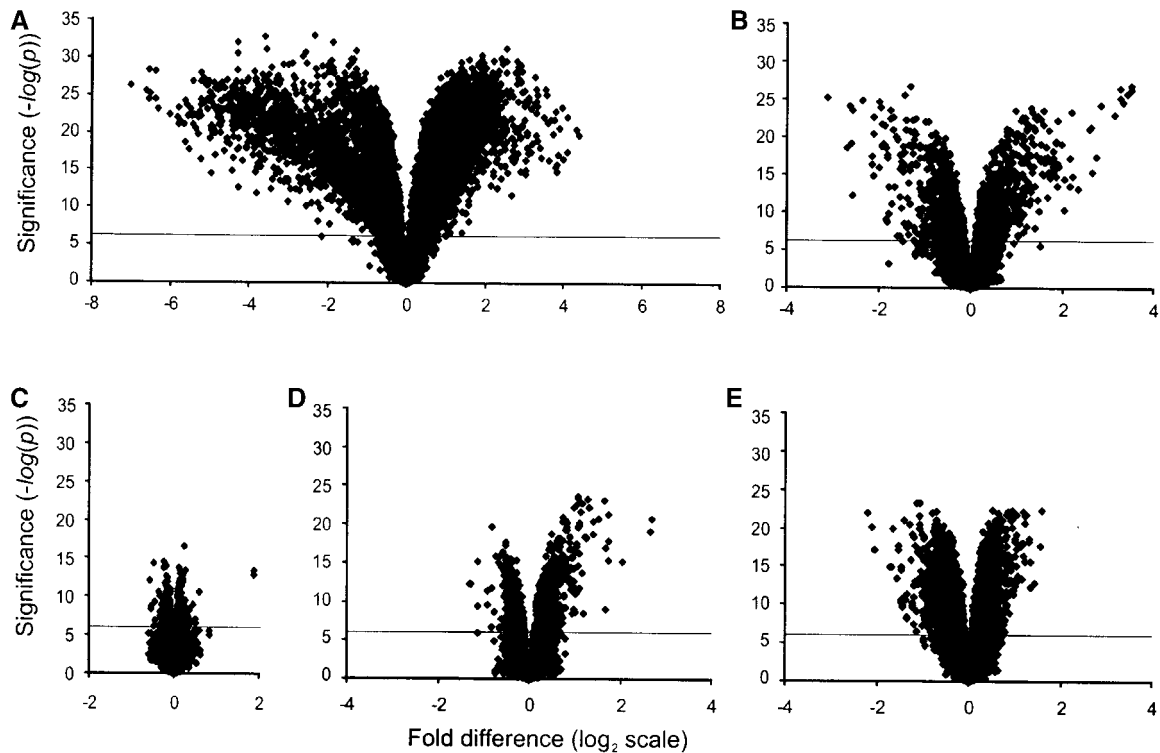


FIGURE 3.—Volcano plots of significance against expression difference. Each plot shows the fold difference in transcript abundance on the abscissa for the indicated contrasts on a  $\log_2$  scale (+/- represents twofold increase or decrease) and significance as the negative logarithm of the  $P$ -value on the ordinate, calculated using the ESTIMATE option in PROC MIXED with SAS. Each of the 21,929 oligonucleotide probes is plotted. Highly significant genes are toward the top, with approximate Bonferroni threshold indicated by the horizontal hairline. (A) Male *vs.* female, showing that more genes are relatively upregulated in females, but fold induction is over a greater range in males. (B) Parental contrast, showing significant differences between *Oregon* and *2b* for  $\sim 20\%$  of the genes averaged across both sexes. (C) Dye effect (*Cy3 vs. Cy5*), which is clearly small relative to the biological differences. (D) Contrast of the reciprocal  $F_1$ 's of both sexes, showing asymmetry favoring elevated expression in the *Oregon* female by *2b* male cross. (E) Contrast of parental average against  $F_1$  average for both sexes, showing that more genes are significantly different from those in the parent contrast although the range is not as great.

Genotype differences are visualized as volcano plots in Figure 3 that contrast significance against magnitude of difference in transcript abundance. Points above the dashed horizontal line in each plot exceed the conservative Bonferroni significance cutoff of  $1.9 \times 10^{-7}$  (see MATERIALS AND METHODS for details). Approximately 25% of the probes differ between the two parents in either sex, 20% between the reciprocal  $F_1$  females and 9% between the reciprocal  $F_1$  males, but an average of 37% between females of either  $F_1$  and either parent or 28% between  $F_1$  and parental males. For comparison, two-thirds of the probes differ between sexes. The percentage of genes differing between inbred lines and sexes is larger than that reported in the above-mentioned studies, but is consistent with their findings given our higher level of replication and the reduced technical error of the Agilent arrays: classification of differences in significance is very much a function of experimental design. Dye effects were small relative to biological effects, with only one gene showing a more than twofold sensitivity to whether *Cy3* or *Cy5* was used in the labeling reaction, although 7% of the probes were significant for the dye effect after Bonferroni correction.

Clusters of genes with similar expression profiles tend to identify patterns of dominance. Each of 16 common modes of inheritance is plotted on a standardized scale of fold change in relative transcript abundance in Figure 4, showing the mean value of each genotype as a boldface line and the range of values as fine lines. Genes showing additive gene expression have transcript abundance estimates intermediate between those of the two parents, whereas dominance is indicated where transcript abundance of the two  $F_1$ 's is similar to one or the other parent. Overdominance is the situation in which transcript abundance is at a higher level in both reciprocal  $F_1$ 's than in both parents, whether or not the parents differ in expression, while underdominance is the reverse situation, with transcript abundance lower in both reciprocal  $F_1$ 's than in the two parents. Female parent-like effects are defined as the case in which the  $F_1$  abundance resembles that of the same sex in the line that donated the female parent, and male parent-like effects are defined as the case in which abundance resembles that of the same sex in the line that donated the male parent. (Note that these are not quite the same as maternal or paternal effects.) Numerous examples where just one

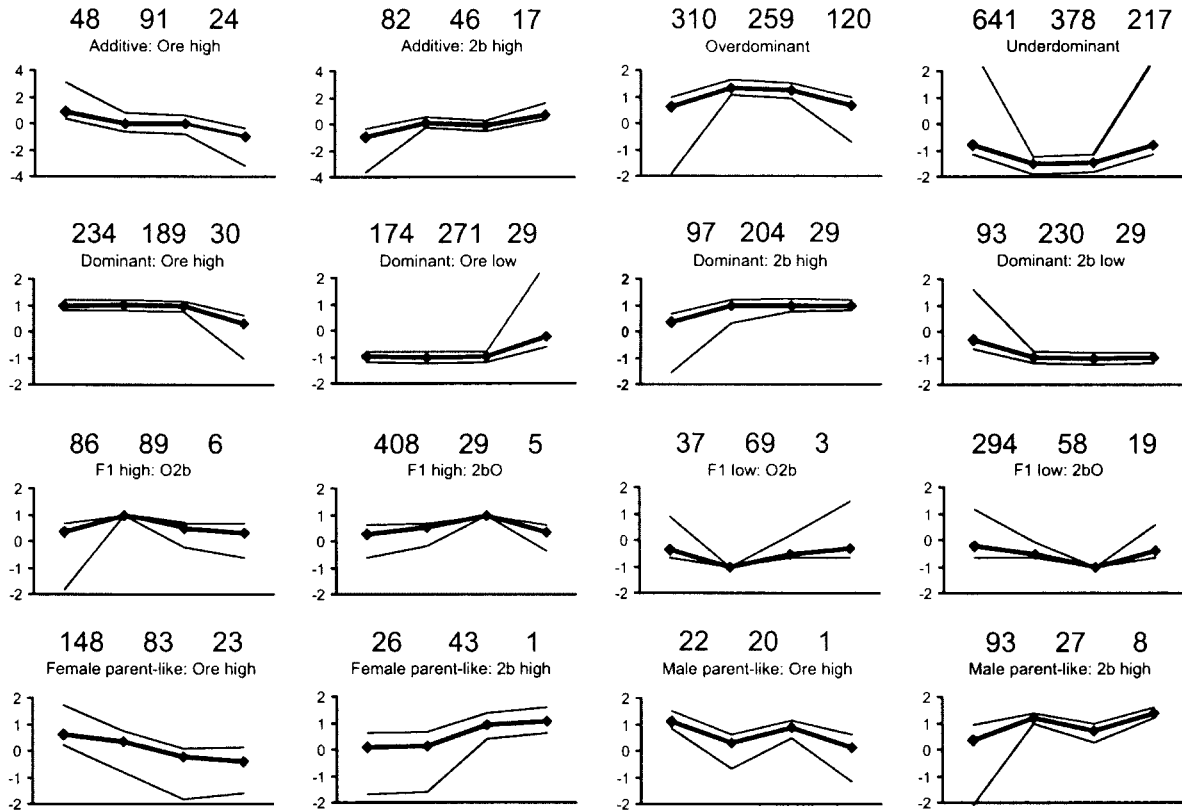


FIGURE 4.—Parallel plots of fold difference by genotype. Each plot illustrates 1 of 16 common patterns of inheritance by showing the average standardized least-squares mean for all of the genes with the indicated pattern in males (boldface lines) as well as the range of least-squares mean values (thin lines). Fold difference is on the ordinate in  $\log_2$  units. Genotypes are plotted in order from left to right: *Oregon* parent, *O2b* F<sub>1</sub> (*Oregon* mother by *2b* male), *2bO* F<sub>1</sub>, and *2b* parent. Numbers above each plot show the number of genes with the particular pattern in females, males, and both sexes, from left to right. The range and mean values in females are very similar. Of the 5820 genes in the analysis, 5040 show genotype differences in females and 4098 show genotype differences in males. In females, 2247 genes and, in males, 2012 genes showed inheritance patterns other than those plotted. As expected if much of the regulation is *cis*-acting, in males only 2 of 47 genes showing “male parent-like” inheritance are X linked while 50 of the 126 “female parent-like” patterns are from the X. (“Male parent-like” means that F<sub>1</sub> expression is more like that in males of the line from which the male parent was derived, so that line’s X chromosome would not be transmitted. The bias was particularly strong for female parental transmission of expression from the *2b* X chromosome: 26 of 43 genes.)

of the F<sub>1</sub> crosses had an extreme high or low expression level were also observed. The three numbers above each plot indicate the number of genes showing the inheritance pattern in females, in males, and in both sexes.

Several features of the analysis of genotype differences stand out. First, 25% more genes are differentially expressed in females than in males, and the total fraction of significant *Drosophila* genes is around one-third of the genome. Second, <2% of the differentially expressed genes are additive in the sense that both F<sub>1</sub>'s are at least 1.25-fold different from both parents. Many of these actually show partial dominance, as indicated in histograms of the ratio of dominance to additivity coefficients for each sex in Figure 5. Third, there is asymmetry in the direction of full dominance. For example, the *2b* parent has higher transcript abundance in more than twice as many genes in males as in females (see numbers above each panel in Figure 4). Fourth, 5% of the genome shows over- or underdominance. Almost half of these effects are observed in both sexes, implying that they may be acting in the soma rather than in germline. Fifth, a re-

markably large number of genes are significantly higher in F<sub>1</sub> females derived from a female *2b* parent than in the other genotypes. Sixth, only half of the differentially expressed genes show these standard patterns of expression: another 2247 genes in females and 2012 in males have less obvious patterns of differential expression between just two of the genotypes. The implications of these findings are discussed below.

**Sex biases in nonadditivity of transcription:** Next we asked whether sex chromosomes and autosomes contribute equally to the patterns of inheritance. Disregarding the genetically depauperate Y chromosome, *D. melanogaster* has six chromosome arms, five of which (X, 2L, 2R, 3L, and 3R) each carry between 15 and 25% of the 13,500 or so genes, while the short chromosome 4 has only ~100 genes. Table 1 suggests that there is a weak bias against genes on the X chromosome showing genotype-specific differences: only 85–90% of the expected number of X chromosome genes are significantly different for any of the common patterns of inheritance given the number of X-linked genes included on the array,

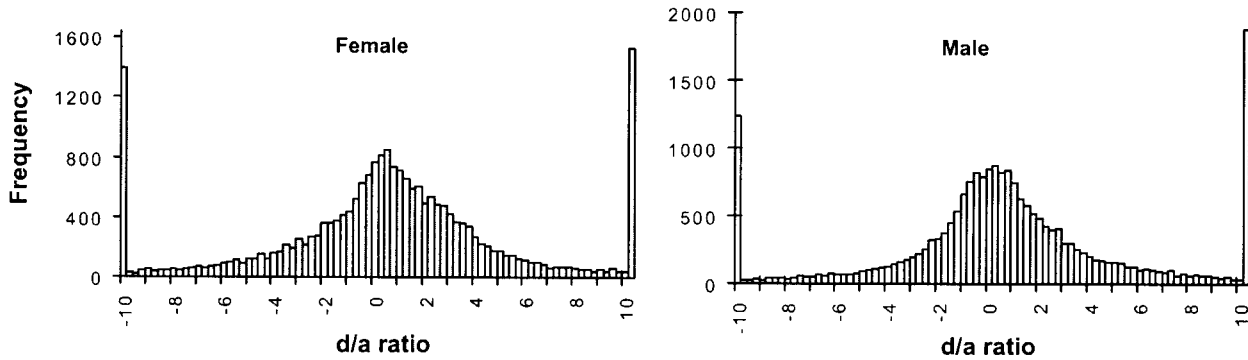


FIGURE 5.—Dominance to additivity ratio by sex. Additivity is the absolute value of half the difference between the parental least-squares mean relative transcript abundance after fitting the array and dye normalization model. Dominance is the difference between the observed  $F_1$  transcript abundance (in this case averaged over the two reciprocal  $F_1$  genotypes) and the midpoint of the two parents. A ratio of zero indicates additivity,  $\pm 1$  indicates full dominance, and values  $>1$  or  $<1$  imply over- or underdominance. This analysis does not take into account measurement variance, so ratios  $>10$  are generally due to extremely similar measurements of the parents that produce estimates of additivity that are smaller than the variance.

whereas each mode of variation for loci on the autosome arms is represented approximately in proportion to its gene density on the microarray. This pattern can be

TABLE 1

Percentage of genes, by chromosome and annotation

Chromosome arm <sup>a</sup>	Males	Females	Array
X	13.9*	14.1*	15.9
2L	18.7	18.3	18.4
2R	21.2	21.3	19.9
3L	19.4	19.9	19.1
3R	25.6	25.3	25.2
4	0.7	0.7	0.6
Unknown	0.5	0.4	0.8
No.	4098	5040	12559
Annotation category <sup>b</sup>	Males	Females	
Behavior	2.1	2.0	
Cell cycle	4.9	4.5	
Defense	2.9	2.6	
Detoxification	4.5	3.9	
Other enzymes	21.7	20.3	
Mitochondrion	4.6	4.5	
Neurotransmission	5.0	5.1	
Proteolysis	8.8	8.5	
Signaling	8.5	9.1	
Structural	5.8	5.3	
Transcription	6.1	7.2	
Translation	6.3	8.5*	
Transport	7.2	6.5	
Other	11.5	12.0	
No. of genes	1119	1521	

\* $P < 0.05$ .

<sup>a</sup> Percentages are based on the total number of genes showing any significant genotype differences in males and/or females, as well as the percentages from each chromosome present on the array and the total number of genes represented on the Agilent microarray (12559).

<sup>b</sup> Percentages are based on the number of genes showing one of the inheritance patterns in Figure 4.

explained by noting that the X chromosome is hemizygous every other generation. Selection is thus more efficient at removing deleterious and fixing beneficial recessive alleles, which should reduce the amount of standing genetic variation due to nonadditive alleles. However, the paucity of variation for expression on the X appears to stem from underrepresentation of additive, not dominant, variation: very few of the “additive” genes are Xlinked (7 of 130 in females, 0 of 137 in males).

The tendency toward over- or underdominance appears to be strongly sex biased. Table 2 shows that among genes that are at least 2-fold different between the sexes, and 1.25-fold different between parents and  $F_1$ , female-biased genes (expression higher in females) are more likely to be expressed at a higher level in the parents than in the  $F_1$ , while the reverse is true for male-biased (expression higher in males) genes. This result holds in both sexes, but is much stronger in males: 80% of all female-biased genes are downregulated in  $F_1$  males, and 63% of male-biased genes are upregulated. The corresponding percentages in  $F_1$  females are 57 and 56%. A similar result is observed in the interspecific hybrid females derived from a cross of *D. melanogaster* and *D. simulans* (RANZ *et al.* 2004) where it has been attributed to a combination of underdevelopment of the ovary and loss of control of male-biased expression. It would seem, though, that there are intrinsic tendencies toward misexpression even in intraspecific hybrids. An alternative explanation might be that expression is at the appropriate level in heterozygous flies, but deviates in isogenic stocks due to deleterious recessive alleles. This in turn implies that misregulation of male-biased genes more frequently causes downregulation, while misregulation of female-biased genes causes upregulation.

#### Functional clustering of differentially expressed genes:

Careful examination of the list of genes within each pattern of inheritance highlights numerous instances of possible coregulation of functionally related genes. Some

**TABLE 2**  
**Sex bias in parental vs. F<sub>1</sub> contrasts**

	Female expression		Male expression	
	F <sub>1</sub> biased	Parent biased	F <sub>1</sub> biased	Parent biased
	All significant probes <sup>a</sup>			
Male biased	<i>1903</i>	1662	<i>2000</i>	1565
Female biased	2995	2996	2687	<i>3304</i>
	Probes with >2-fold sex difference and >1.2-fold parent vs. F <sub>1</sub> difference <sup>b</sup>			
Male biased	<i>230</i>	180	<i>259</i>	152
Female biased	448	<i>608</i>	168	<i>710</i>

See <http://statgen.ncsu.edu/ggibson/SupplInfo/SupplInfo3.htm> for supplementary information on significance and fold change by sex and genotype.

<sup>a</sup>All probes that showed a significant ( $P < 0.05$ ) sex or genotype term in the gene-specific ANOVAs. Here the “biased” category is the one in which expression is higher.

<sup>b</sup>A more restricted subset of highly significant genes that are at least 2-fold different between males and females and 1.2-fold different between the average of the two parental lines and the average of the reciprocal F<sub>1</sub>'s in the indicated sex. Italic numbers highlight overrepresented categories.

of these are located close together on a chromosome as observed by SPELLMAN and RUBIN (2002): for example, a cluster of five odorant-binding proteins in cytological intervals 55 and 56 that show paternal inheritance of expression level from *2b*. In other cases, similar patterns of regulation are observed in genomically distant but functionally related genes, *e.g.*, five unlinked ubiquitin conjugating enzymes on different chromosome arms show underdominant expression. A similar phenomenon may be observed for genes involved in a common biochemical or cellular process; *e.g.*, of 19 differentially expressed genes involved in EGFR signaling, 11 show underdominance and 6 overdominance. It will be interesting to determine whether such relationships hold over a series of crosses involving diverse lines.

We also asked whether any categories of functional annotation had obvious trends with respect to sex dimorphism or particular patterns of inheritance. Table 1 lists the percentage of genes in each of 14 categories based on the GO annotation of the fly genome. Most categories are equally represented in the two sexes, but one deviation stands out. Transcripts encoding proteins involved in translation are overrepresented in the genes that are differentially expressed across genotypes in females, as expected since the nurse cells must prepare the oocyte for rapid protein synthesis upon fertilization. Many of these genes are also strongly female biased. A particularly interesting class of these genes is a collection of 13 aminoacyl-tRNA ligase mRNAs that show significantly elevated levels specifically in F<sub>1</sub> females derived from the cross of *2b* females by *OregonR* males.

## DISCUSSION

Classical quantitative genetic theory is formulated without regard to the molecular events that relate geno-

type to phenotype. Although it has been known for 50 years that this relationship is mediated through transcription, translation, and protein interactions, only recently have the tools been developed that allow the additivity and dominance of gene activity to be addressed in a systematic manner at the transcriptional or proteomic level. Fundamentally, the assumption and very general observation that gene effects are additive intuitively implies that transcript abundance itself is also additive. Dominance at the transcript level but not the trait level would imply modification of the transcriptional effect by other genes, which while plausible is not consistent with basic quantitative genetic models. Similarly, for dominant traits, differences between reciprocal F<sub>1</sub>'s or the two sexes at the transcript level are not generally expected. While we have not attempted in this study to relate transcriptional to phenotypic variation, our results are nevertheless challenging because they indicate extensive nonadditivity at the transcription level, implying that the assumption of a straightforward mapping of genotype onto phenotype is not justified.

Another way of considering this relationship is to consider that genetic polymorphism induces molecular polymorphism, some summation of which results in phenotypic polymorphism at the trait level. The simplest conception of additive effects is that some polymorphisms affect transcript abundance, some affect protein abundance, some affect catalytic rates, and so forth, so that in moving from genotype to trait we would expect a gradual increase in the level of variance at each successive biochemical level. The finding that there is a higher coefficient of variation associated with gene effects at the transcriptional or protein level than at the phenotypic level would not be consistent with this simple conception. It would likely imply that additivity arises as a result of the averaging of a complex web of nonaddi-



tive biochemical effects. We suggest that concerted attention to variability at all levels of biological organization is now warranted, as it has applied and theoretical implications.

As one example, consider that when two inbred strains of any species are crossed together, it is not uncommon for several traits to show improved performance or heterosis. The two most often cited explanations for this phenomenon are unmasking of the effects of deleterious recessive alleles and summation of the dominant effects of multiple loci brought together in the progeny. Neither of these explanations requires over- or underdominance of transcript abundance; nor do they predict it. On the other hand, heterosis and its negative counterpart, outbreeding depression, are so important in plant and animal breeding and so central to evolutionary theory that it is worthwhile to characterize the effect of outcrossing on the expression of genes. We find very little evidence of heterosis for gene expression (only 5% of the transcriptome), although there are multiple other departures from additivity and many instances of altered gene expression in one of the reciprocal  $F_1$ 's or in which the two  $F_1$ 's resemble alternate parents. The percentages of genes in each class may be surprisingly low to some readers and high to others, highlighting the fact that we have little empirical or theoretical evidence on which to base expectations. Comparison of heterosis for expression of specific genes with heterosis for traits regulated by the genes is an obvious research priority.

Whether a transcript shows some degree of dominance for abundance is likely to be influenced by the relative contributions of *cis*- and *trans*-acting factors to expression of the gene. If transcription is controlled predominantly by *cis*-regulatory regions of a particular gene, then transcript abundance might often be expected to be additive in the absence of transvection. By contrast, transcription factors that vary in activity level between the parents are more likely to interact to produce a range of degrees of dominance. Dominance for low-level transcription might be caused by increased repressor activity or, alternatively, by haploinsufficiency for a transcriptional activator. Extreme expression levels in either  $F_1$  or similarity to expression in the same sex of either parental line is more difficult to explain, although *X* linkage and genomic imprinting may contribute. However, genomic imprinting has recently been documented in *Drosophila* (MAGGERT and GOLIC 2002), but only for the *Y* chromosome. Maternal genetic effects are more commonly considered in relation to embryogenesis, but differences in gene expression of adults might also arise as a result of initial discontinuities in the embryo persisting and even amplifying throughout development.

Little theoretical work is available to guide interpretation of departures from additivity of expression. Arguments from biophysical principles relating to the cooperativity of transcription factors (GIBSON 1996) as well

as the structure of regulatory networks (OMHOLT *et al.* 2000) imply that epistasis (*sensu* molecular) and dominance are likely to be natural properties of transcriptional regulation. Recent modeling of development with systems of linear equations (VON DASSOW *et al.* 2000; MEIR *et al.* 2002) demonstrates that regulatory networks can be robust to considerable variation in parameters representing transcript and protein abundance, but has not addressed the causes and consequences of dominance. Empirically, the combination of QTL mapping with expression profiling in recombinant inbred lines of mice (SCHADT *et al.* 2003) has demonstrated that *cis*-acting variation contributes 25% or more of the variation for expression of 15% of genes in the liver. This study also found a number of hotspots for the location of eQTL (QTL affecting gene expression), and these are likely to correspond to the location of *trans*-acting regulatory genes. A similar result was obtained in crosses between two yeast strains (YVERT *et al.* 2003). Our results imply that inclusion of  $F_1$  individuals in studies mapping gene expression differences could greatly assist in the interpretation of sources of variation affecting divergence among lines.

From an evolutionary genetic perspective, documentation of extensive nonadditivity of gene expression has at least two important implications. First, by challenging the assumption that genotype maps directly onto phenotype through transcript abundance, it calls for more attention to the prevalence of epistasis as a pervasive aspect of genetic architecture. The possibility that genotypes produce additive variation for visible traits irrespective of nonadditive variation for transcription and, conversely, that cryptic variation can be hidden at the transcriptional level should be considered explicitly by theoreticians and experimental biologists working with different organisms. Second, the widespread differences between  $F_1$  and parents suggest caution in relation to the use of highly inbred lines to quantify levels of intraspecific variation, at least for gene expression. The degree of differentiation among inbred lines reported here is similar to that documented by others (JIN *et al.* 2001; RIFKIN *et al.* 2003), but more extensive sampling in the form of a diallel cross will be required to assess the generality and covariance of nonadditive gene expression in *Drosophila*.

We thank Russ Wolfinger for providing a beta version of the SAS Microarray Solution software. This work was funded by National Institutes of Health award R24-GM65513.

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Communicating editor: D. WEIGEL

