Strain-Dependent Differences in Several Reproductive Traits Are Not Accompanied by Early Postmating Transcriptome Changes in Female Drosophila melanogaster

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Manuscript received December 10, 2008
Accepted for publication February 16, 2009

ABSTRACT

Upon mating, Drosophila melanogaster females undergo numerous alterations in their behavior and reproductive physiology that are accompanied by small-magnitude transcript-level changes in up to 1700 genes. Many of these postmating transcriptome changes are the direct result of the sperm and seminal fluid proteins (Acps) that females receive from their mates. To begin to determine if the genetic background of the female’s mate contributes to the previously described gene expression changes, we assessed whether interactions between the genotypes of two commonly used laboratory strains of D. melanogaster (Canton-S and Oregon R) influence the female’s postmating transcriptome as well as several pre- and postcopulatory phenotypes. We find negligible differences in the female’s transcriptome at 1–3 hr postmating regardless of the strain of the male with whom she mated. However, a male × female genotype interaction significantly influenced mate selection, and, in some cases, fecundity, fertility, and hatchability. Our data support previous work suggesting that many of the early postmating changes observed in D. melanogaster females are not caused by large modifications of transcript levels. Instead, early postmating phenotypes result from preexisting receptors or pathways that are already in place upon sexual maturity.

With few exceptions, reproduction in most sexual animals relies on contributions from both the male and the female. In such animals, both sexes not only contribute gametes, but also participate behaviorally and molecularly in mate choice decisions, courtship, copulation, and postcopulatory processes (Andersson 1994). Historically, most behavioral and molecular studies of mating have attempted to understand reproductive dynamics by focusing measurements on a single sex at a time, perhaps largely for technical reasons. However, theory and data reiterate (Eberhard 1996) that it is the complex interactions between the sexes, and not the marginal variability within either sex, that ultimately predict the outcome of sexual events and the evolution of sexual traits.

Studies of sperm competition and related postcopulatory processes in Drosophila have made invaluable contributions toward our understanding of the extent and nature of male × female interactions. Studies using chromosome extraction lines have shown an effect of male genotype (Clark et al. 1995; Fiumera et al. 2005, 2006, 2007b), female genotype (Clark and Begun 1998), and an interaction between male and female genotypes (Clark et al. 1999) on the outcome of sperm competition. Furthermore, interpopulation crosses have demonstrated that different combinations of male and female genotypes can result in variable, and often theoretically unpredictable, outcomes in postcopulatory traits such as the size of the insemination reaction mass (Knowles and Markow 2001), female fitness, remating rate, and sperm competition (Long et al. 2006). In addition to affecting reproductive success, interactions between the genotypes of the sexes during mating can also have important ecological consequences (Knowles et al. 2004).

Despite the growing body of evidence that interactions between male and female genotypes contribute to the outcome of reproduction, the basis of the genetic variation that leads to these differences is largely unknown. In Drosophila melanogaster males, naturally occurring variation (SNPs or indels) in several genes encoding seminal fluid proteins derived from the male’s accessory glands (Acps) has been associated with variation in some postcopulatory phenotypes (Clark et al. 1995; Fiumera et al. 2005, 2006, 2007a,b). In addition to Acps, a quantitative genetic and microarray study identified at least 27 additional (non-Acp) genes with variable intrapopulation mRNA levels in males that correlate with sperm competition phenotypes (Drnevich et al. 2004). In females, the genetic variation that contributes
to differential mating success is largely unknown and genes that contribute to postmating responses are only beginning to be identified. Several microarray studies (Lawniczak and Beun 2004; McGraw et al. 2004, 2008) have identified >1700 genes whose expression levels in whole bodies of females were altered by courtship or mating. Of the genes whose transcript levels were altered by mating, many were regulated by sperm or Acps that females receive from their mates (McGraw et al. 2004, 2008). An additional microarray study identified 539 transcripts that are differentially expressed in the female reproductive tract at several time points after mating (Mack et al. 2006; Kapelnikov et al. 2008). Although these microarray experiments did not attempt to identify genetic variation in the genes detected, the methodologies of those studies provide an excellent framework for beginning to uncover genetic variation in gene expression profiles that depend on male × female interactions.

The goal of this study was to assess whether the genetic background of two commonly used laboratory strains of D. melanogaster [Canton-S (CS) and Oregon R (OR)] that have been maintained separately, but under identical conditions in the laboratory for >15 years contributes to differences in postmating gene expression profiles in females. In addition, since precopulatory interactions may affect gene expression changes that occur within the mated female and postcopulatory outcomes may depend on these changes, we also assessed how the genetic background of the sexes affected several pre- and postcopulatory traits. Using interstrain crosses, we examined gene expression profiles in whole-body mated females between 1 and 3 hr after the end of copulation. We demonstrate that shortly following mating, the strain of the male to whom the female has mated has negligible impact on the female’s transcriptome. Despite the paucity of strain-dependent transcriptome responses, one precopulatory phenotype and several postcopulatory phenotypes that we measured differed, depending on the strain of the male and female in the mating pair.

METHODS

Drosophila stocks and crosses: Flies used in all crosses originated from inbred, wild-type stocks of D. melanogaster strains, CS and OR. Stocks were reared on standard yeast/dextrose medium at 25°C under a 12:12-hr light:dark cycle. Virgin flies were collected within 4 hr of eclosion and allowed to age for 3–4 days in vials without the addition of live yeast. Except for the few cases noted, within 3 hr of the beginning of their light cycle, single virgin females were paired with single virgin males of the appropriate genotype and allowed to mate. CS females (F) were mated either to CS males (M) (CSF × CSM) or to OR males (CSF × ORM). OR females were mated either to OR males (ORF × ORM) or to CS males (ORF × CSM). All matings were observed and females that copulated for < ~18–20 min were not used for further analysis.

Affymetrix GeneChips hybridization and data analysis: Microarray studies were performed as in McGraw et al. (2004). Briefly, females were mated to males of the appropriate genotype and flash-frozen in liquid nitrogen 1–3 hr after the end of copulation. Total RNA was collected from whole female flies using Trizol (Invitrogen, Carlsbad, CA) and purified with the RNeasy mini kit (Qiagen, Valencia, CA). Labeling and hybridization were performed according to the Affymetrix GeneChip Expression Analysis Technical Manual. Four replicate Drosophila Genome 1.0 arrays were hybridized for each type of male × female cross (CSF × CSM, CSF × ORM, ORF × ORM, or ORF × CSM).

Because of potentially confounding effects of different hybridization affinities of cDNAs from the two strains to the GeneChips, arrays from cDNA of mated CS females were analyzed independently from those from cDNA of mated OR females. Data were analyzed using two separate approaches. First, a mixed-model ANOVA was performed in SAS (Sall et al. 2003; Gott et al. 2004). Individual perfect-match (PM) probe measurements were normalized by subtracting the log2-transformed value for all probes on the array. Normalized probe data were fitted to gene-specific models, where

\[
\log_2(PM_{ijk}) = T_i + P_j + TP_{ij} + A_k + e_{ijk},
\]

and PM_{ijk} is the PM expression measurement of the ith probe (i = 1, 2, ..., 16) for the jth treatment (j = 1, 2) on the kth array (k = 1, 2, ..., 4). T and P represent the fixed effects of treatments and probes and A represents the random effects of individual arrays. Treatment in this case refers to the contrast between the two males (OR vs. CS) mated to the females whose transcripts are being quantified. Random effects were assumed to be normally distributed with a mean of zero and a variance of \(\sigma^2\). The mean and unexplained error are represented by \(\mu\) and \(e\), respectively. Once expression values were generated for each gene over all treatments, the DIFFS procedure in PROC MIXED was used to estimate the magnitude and significance in expression levels between treatments. Statistical significance was assessed at the testwise threshold of \(P \leq 0.05\). We also adjusted for multiple comparisons by calculating the 5% false discovery rate (FDR) and subsequent \(q\)-value (Storey and Tibshirani 2003).

Data were also analyzed using GeneTraffic UNO 3.2 (Iobion Informatics; Stratagene, La Jolla, CA). Probe-level data were normalized using the robust multichip average (RMA) method (Irizarry et al. 2003). The intensities were log2-transformed and statistical significance of differences in gene expression between treatments was calculated using two-sided Student’s \(t\)-tests. Statistical significance was assessed at the testwise threshold of \(P \leq 0.05\) and \(q\)-values (Storey and Tibshirani 2003) were calculated.
performed with CS females by adding N visible under 6× magnification. Preference tests were performed with CS females by adding N females to a mating chamber containing N blue-colored CS males and N red-colored OR males (where N ranged from 20 to 30 flies and flies were maintained at a 1:1:1 ratio). Copulating pairs were aspirated from the mating chamber and male color was verified under a microscope at 6× magnification. Two independent replicates were performed with one color scheme and an additional two independent replicates were performed with dye colors reversed. Analogous experiments were performed with OR females, using the same experimental design. A simple dye-swap experiment showed that food coloring had no effect on mate preference (data not shown) and only in two circumstances was the dye color of the male ambiguous. These males were excluded from the analysis.

**Quantifying latency to copulation and copulation duration:** In observed matings, we recorded the time at which a male was added to a vial with a single female, the time at which the male mounted the female, and the time at which the male dismounted. All time observations are accurate to ~30 sec.

**Quantifying the amount of sperm transferred to the female and stored in her seminal receptacle:** Three- or 4-day-old virgin CS or OR females were mated to 3- or 4-day-old virgin males as described above. To estimate the number of sperm transferred to a female and to quantify the amount of sperm stored in the female’s seminal receptacle, sperm were orcein stained and counted as described in McGraw et al. (2007).

**Quantifying fertility, fecundity, and hatchability:** Upon completion of copulation, males were removed from the vials. For 10 days following mating, females were transferred to fresh vials, without additional dry yeast, at approximately the same time each day. Fecundity was determined by counting the total number of eggs laid per female. Fertility was determined by counting the total number of progeny produced per female. Hatchability is the proportion of eggs that result in progeny. Three replicate experiments were performed for each of the four crosses (CSF × CSM, CSF × ORM, OFR × ORM, and ORF × CSM).

Egg counts and progeny counts for each day differed significantly across replicates, likely due to daily fluctuations in the surface texture of the media (Chiang and Hodson 1950; Rockwell and Grossfield 1978); however, the total number of eggs and total number of progeny over all 10 days did not differ between replicates. Thus, total egg counts and total progeny counts over 10 days from replicates were pooled for analyses.

**Female refractoriness:** Refractoriness is defined as the proportion of females who do not remate with a subsequent male on the day after an initial mating. CS females and OR females were mated to either a CS male or an OR male. On the following day, they were placed in a vial with a new single male and observed for 6 hr to determine if they would remate. These crosses were performed in eight different F × M combinations (CSF × CSM, CSF × ORM, CSF × ORM, CSF × ORM, ORF × ORM, ORF × ORM, ORF × ORM, ORF × CSM, and ORF × ORM; female strain is written first followed by the order of males to whom she was mated).

**Statistical analysis of postmating phenotypes:** All analyses (except for microarray data analyses described below) were analyzed with JMP 5.1 (SAS Institute, Cary, NC). Differences in latency to copulation, copulation duration, fertility, fecundity, and hatchability between treatments were determined using Wilcoxon/Kruskal–Wallis rank sums tests. Chi-square tests were used to determine the probabilities that mating preference deviated from random mating. Student’s t-tests were used to determine differences in the number of sperm transferred to and stored by females across treatments.

### RESULTS

**Male strain had negligible effect on the female’s postmating transcriptome:** We assessed differential gene expression using two different microarray analysis methods and three significance cutoffs (summarized in Table 1). Using a nominal, uncorrected P-value cutoff of 5%, we identified 361 probes in CS females and 196 probes in OR females.
probes in OR females common to both methods of data analysis whose transcript levels differed depending on the strain of the female’s mate; however, a larger set of probes was detected only in a single analysis method (Figure 1). Within the sets of genes at the uncorrected 5% significance cutoff, the magnitude of expression differences was quite small with only 13 probes exhibiting twofold or greater differences in mRNA abundance (Figure 1). However, after controlling for a 5% FDR, only 12 probes in total stand up to a highly conservative statistic. Results of each analysis method can be viewed in online supplementary materials. Thus, at the time point we examined (1–3 hr postmating), it appears that the genetic background of the male does not make a profound contribution to the previously reported postmating changes in the female’s transcriptome at 1–3 hr postmating (Lawiczak and Begun 2004; Mack et al. 2006; McGraw et al. 2004, 2008).

**Male × female interactions affected the outcome of four behavioral phenotypes:** Of the two precopulatory phenotypes examined, only mate selection, but not latency to copulation, depended on an interaction between the genetic backgrounds of the male and female. Both CS females and OR females were significantly more likely to mate with CS males than with OR males ($\chi^2 = 0.36, P < 0.05$). However, it is not possible to distinguish whether females prefer to mate with CS males or whether CS males are generally more successful in coercing females to mate with them. Despite this apparent bias toward mating with CS males in both strains of females, when males and females were tested in pairs, females did not commence copulation sooner with CS males (CS, $n = 81, P = 0.27$; OR, $n = 93, P = 0.76$) nor did they mate longer with CS males (CS, $n = 81, P = 0.89$; OR, $n = 93, P = 0.85$; Table 2).

All of the postcopulatory traits that we measured could be influenced by the amount of sperm that males transfer to and have stored by females. In *D. melanogaster*, females store sperm for up to 2 weeks within specialized sperm storage organs and the numbers of sperm within these storage sites can directly affect the female’s fertility and fecundity as well as egg hatchability. Furthermore, the mere presence of sperm within the storage organs contributes to the “sperm effect” (Manning 1962; Gromko et al. 1984). As long as sperm are present within the storage organs, the female’s propensity to remate is drastically reduced. Thus, to take into account effects of sperm numbers on postcopulatory traits, we measured whether a male × female strain interaction affected the number of sperm males transferred to females or a male’s ability to have his sperm stored within the female’s seminal receptacle. However, neither the number of sperm transferred (CS, $t_{17} = -0.16, P = 0.87$; OR, $t_{17} = -0.84, P = 0.41$) nor the number of sperm stored within the female’s seminal receptacle (CS, $t_{26} = 2.00, P = 0.06$; OR, $t_{28} = 0.285, P = 0.78$) differed, depending on the strain of the male with whom she was mated (see Table 2). Consistent with the observation that sperm transfer and sperm storage did not have a genotypic component, female remating refractoriness, a trait that is in part influenced by sperm storage (Manning 1962; Gromko et al. 1984), was also unaffected by the male’s genotype. Regardless of the order of the males that females mated with, neither CS nor OR females differed in their propensity to remate [CS, $\chi^2(9, 4) = 12.00, P > 0.05$; OR, $\chi^2(9, 4) = 12.00, P > 0.05$].

Although we detected no effect of male × female genotype interactions on sperm transfer, sperm storage,
or remating refractoriness, we did find that in some cases, fecundity, fertility, and hatchability depended on the genotype of the male to whom the female mated. CS females laid significantly fewer eggs after mating to CS males (272.86 ± 7.70 SE) vs. OR males (304.09 ± 8.46 SE; n = 156, P = 0.0033; Figure 2A, supplemental Figure 1). CS females also produced fewer progeny after mating to CS males (257.59 ± 8.22 SE) vs. OR males (292.47 ± 9.67 SE; n = 156, P = 0.0014; Figure 2C, supplemental Figure 1). Despite these differences, hatchability was unaffected by male strain (n = 156, P = 0.72; Figure 2E). Like CS females, OR females also laid significantly more eggs (n = 172, P = 0.049; Figure 2B, supplemental Figure 2) after mating to OR males (244.63 ± 10.45 SE) than after mating to CS males (209.33 ± 12.46 SE). However, the number of progeny produced by OR females did not differ (n = 172, P = 0.61; Figure 2D, supplemental Figure 2) when the females were mated either to OR (207.57 ± SE) or to CS males (191.86 ± 12.58 SE). In fact, eggs of OR females had higher hatchability after females mated to CS males (n = 172, P = 0.025; Figure 2F).

### DISCUSSION

We assessed whether the genetic background of two commonly used laboratory strains of *D. melanogaster* (CS and OR) affects the female’s postmating transcriptome and a suite of pre- and postcopulatory reproductive phenotypes. Using the Affymetrix microarray platform and two separate methods of data analysis, we find negligible detectable differences in the female’s transcriptome at 1–3 hr postmating despite our findings that many of the reproductive phenotypes that we measured are dependent upon interactions between the male and female genotypes.

In this context, our findings suggest that although a large number of small-magnitude transcript-level changes are known to occur in the female upon mating (Lawniczak and Begun 2004; McGraw et al. 2004, 2008; Mack et al. 2006), the genetic background of the female’s mate does not profoundly influence these changes. However, while we were unable to detect transcriptome differences across treatments, these findings do not necessarily preclude a role for a male × female genotype interaction in influencing transcript abundance in the mated female. In previous studies that detected postmating transcriptome changes in whole-body female flies, while a large number of genes had altered transcript levels, the magnitude of these changes was quite small (Lawniczak and Begun 2004; McGraw et al. 2004, 2008). Allelic differences between the two strains examined in the present study may not be expected to generate large transcript-level differences across treatments. However, even subtle variation in genetic backgrounds between strains may be enough to generate slight alterations in the mated females’ transcriptome that may be undetectable using the experimental design and microarray platform in this study. For example, using whole-body females to measure genomewide transcript abundance may have diluted our ability to detect small, but biologically relevant tissue-specific alterations in transcript abundance.

Consistent with this idea, several recent studies have demonstrated that the expression of complex behavioral phenotypes is rarely derived via a single gene, but instead emerges via complicated networks of interacting, pleiotropic genes (Anholt et al. 2003; Anholt 2004; Van Swinderen and Greenspan 2005; Rollmann et al. 2008). In other words, slight allelic variants have the potential to have subtle impacts on the transcriptome and the expression of complex behaviors by affecting transcript levels of numerous genes within an epistatic network. A hypothetical scenario by which male × female genotypes may interact to generate the phenotypic differences that we have detected in this study would be if strain-specific alleles in genes specifying male-derived molecules such as cuticular hydrocarbons or seminal fluid proteins introduced structural variants in these molecules that affected their ability to bind to

### TABLE 2

<table>
<thead>
<tr>
<th></th>
<th>CSF × CSM</th>
<th>CSF × ORM</th>
<th>ORF × ORM</th>
<th>ORF × CSM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Latency to copulation</td>
<td>12.37 ± 2.21</td>
<td>17.49 ± 3.26</td>
<td>24.32 ± 4.28</td>
<td>18.20 ± 2.50</td>
</tr>
<tr>
<td>P</td>
<td>0.27</td>
<td>0.76</td>
<td>0.89</td>
<td>0.85</td>
</tr>
<tr>
<td>Copulation duration</td>
<td>20.18 ± 0.53</td>
<td>21.12 ± 0.39</td>
<td>20.42 ± 0.32</td>
<td>20.38 ± 0.34</td>
</tr>
<tr>
<td>P</td>
<td>0.87</td>
<td>0.85</td>
<td>0.87</td>
<td>0.85</td>
</tr>
<tr>
<td>No. sperm transferred</td>
<td>808.89 ± 41.32</td>
<td>818.30 ± 40.12</td>
<td>823.10 ± 46.36</td>
<td>770.00 ± 43.00</td>
</tr>
<tr>
<td>P</td>
<td>0.87</td>
<td>0.85</td>
<td>0.87</td>
<td>0.85</td>
</tr>
<tr>
<td>No. sperm stored</td>
<td>322.40 ± 13.07</td>
<td>279.60 ± 16.92</td>
<td>269.93 ± 19.33</td>
<td>277.67 ± 19.02</td>
</tr>
<tr>
<td>P</td>
<td>0.06</td>
<td>0.85</td>
<td>0.85</td>
<td>0.85</td>
</tr>
</tbody>
</table>

Latency to copulation and copulation duration are given in minutes. Sperm transferred and stored represent numbers of sperm.
receptors in conspecific females. Differences in receptor binding may then alter expression of pathways downstream of the receptor, resulting in the emergence of strain-dependent phenotypic differences. Future studies employing more sensitive transcriptomics technologies such as tissue-specific quantification of mRNA levels using next-generation sequencing technologies will aid in a more thorough understanding of whether minute shifts in the transcriptome network are responsible for the strain-dependent interactions between sexual partners that generate the phenotypic differences that we observe in this study.

Our findings that large transcript-level changes do not coincide with the phenotypic differences we observed across treatments lend further support to the idea that prior to mating, sexually mature females are molecularly "poised" to immediately initiate postmating events such as sperm storage and ovulation and to commence behavioral changes such as refractoriness to remating (Heifetz and Wolfner 2004; McGraw et al. 2004, 2008; Mack et al. 2006; Kapelnikov et al. 2008). Instead of relying on large-scale alterations of the transcriptome, the act of courtship, copulation, and the introduction of male-derived cells and molecules into the female’s reproductive tract likely activate preexisting receptors, triggering pathways that lead to the rapid onset of postmating alterations in the female’s behavior and physiology. The small magnitude of transcript-level changes that is observed shortly after copulation likely contributes to maintaining the postmating reproductive state (Heifetz and Wolfner 2004; McGraw et al. 2004, 2008; Mack et al. 2006; Kapelnikov et al. 2008). In this study, the strain-dependent behavioral and physiological phenotypes that we observe also appear to be uncoupled.
from any detectable transcript-level changes and also likely arise via pathways that are already in place but are sensitive to allelic differences between the two strains.

In conclusion, although only mated females were considered in this study, previous experiments examining whole-body female flies have demonstrated that up to 1700 transcript-level changes occur in females by 1–3 hr postmating relative to virgin females, although most detected changes were small in magnitude (Lawrence and Begun 2004; McGraw et al. 2004, 2008; Mack et al. 2006). In this study, we compared the postmating transcriptome and several pre- and postcopulatory traits in females from two laboratory strains of *D. melanogaster*. Females within each of the two strains differed only by the strain of the male to whom they were mated. We found that in both CS and OR females, the male’s strain had no effect on the female’s transcriptional profile measured 1–3 hr postmating. However, we also demonstrated that some, but not all, of the pre- and postcopulatory traits that we measured [in addition to sperm competition that was previously demonstrated (Clark et al. 1995, 1999; Clark and Begun 1998)] were influenced by the strain of the male to whom the female was mated.

Like other behaviors (Anholt et al. 2003; Anholt 2004; van Swinderen and Greenspan 2005; Rollmann et al. 2008), the female’s response to mating is a complex, quantitative trait involving the interactions of many genes and their products. This study suggests that like other behavioral traits, extensive amounts of pleiotropy and epistasis underlie the networks of genes that govern a female’s postmating behavior. For example, we previously found mating-induced modulation of many female genes that were found to function only during development (McGraw et al. 2004). Unlike other behaviors, however, the female’s mating response not only depends on interrelationships among her own network of genes, but also is dependent upon the behaviors, genes, and gene products of her mates, as well as the subset of tissues in which the changes occur.

We thank the University of Kentucky Microarray Core Facility for processing the GeneChips. This work was supported by National Institutes of Health (NIH) grant HD38921 to M.F.W., National Science Foundation grant DEB-0108965 to A.G.C., and a traineeship to L.A.M. from NIH training grant T32GM07117.

LITERATURE CITED


Communicating editor: J. A. Birchler