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Socially-Responsive Gene Expression in Male *Drosophila melanogaster* Is Influenced by the Sex of the Interacting Partner

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ABSTRACT

Behavior is influenced by an organism's genes and environment, including its interactions with same or opposite sex individuals. *Drosophila melanogaster* perform innate, yet socially modifiable, courtship behaviors that are sex specific and require rapid integration and response to multiple sensory cues. Furthermore, males must recognize and distinguish other males from female courtship objects. It is likely that perception, integration, and response to sex-specific cues is partially mediated by changes in gene expression. Reasoning that social interactions with members of either sex would impact gene expression, we compared expression profiles in heads of males that courted females, males that interacted with other males, or males that did not interact with another fly. Expression of 281 loci changes when males interact with females, whereas 505 changes occur in response to male–male interactions. Of these genes, 265 are responsive to encounters with either sex and 240 respond specifically to male–male interactions. Interestingly, 16 genes change expression only when a male courts a female, suggesting that these changes are a specific response to male–female courtship interactions. We supported our hypothesis that socially-responsive genes can function in behavior by showing that *egghead* (*egh*) expression, which increases during social interactions, is required for robust male-to-female courtship. We predict that analyzing additional socially-responsive genes will give us insight into genes and neural signaling pathways that influence reproductive and other behavioral interactions.

BEHAVIORS are complex processes resulting from an organism's ability to integrate sensory cues into physiological and motor outputs. Adding to the complexity of this process are the effects from the organism's genetics and environment, including social interactions, on behavior, brain morphology, and gene expression (Siegel and Hall 1979; Levine *et al.* 2002; Shen *et al.* 2004; Stewart and McLean 2004; Burmeister *et al.* 2005; Kozorovitskiy *et al.* 2006; Yurkovic *et al.* 2006; Carney 2007; Technau 2007; Ellis and Carney 2009).

It is possible to use microarray technology to assess changes in mRNA expression occurring during or in response to behavioral interactions to gain insight into corresponding physiological changes. Several studies, particularly in songbirds, bees, and fruit flies, have examined transcript-level changes in freely behaving animals. In songbirds, 33 genes are regulated by singing behavior, including loci involved in signal transduction and synaptic signaling (Wada *et al.* 2006), and a variety of social environments and stimuli impact honeybee brain gene expression (Grozinger *et al.* 2003; Whitfield *et al.* 2003, 2006; Sen Sarma *et al.* 2009). Similarly, male *Drosophila melanogaster* show rapid changes in transcript levels due to social interactions with females (Carney 2007; Ellis and Carney 2009). However, we do not know if these are specific responses to females or more general responses to interacting with a second individual. Although the signaling cascades mediating changes in mRNA levels due to behavior and social interactions are unclear, by studying these changes we can clarify the intracellular processes affecting nervous system function, physiology, and behavior. An advantage of such studies in Drosophila is that mutant strategies can be employed to characterize behavioral requirements for responsive loci.

The courtship behaviors of male Drosophila are influenced by genetics (reviewed in Billeter *et al.* 2002) and social interactions (Ewing 1983; reviewed in Greenspan and Ferveur 2000; Mehren *et al.* 2004). The somatic sex-determination pathway regulates these behaviors (reviewed in Cline 2005; Shirangi and McKeown 2007) and sexually dimorphic development, including that of the nervous system (Finley *et al.* 1997; Kimura *et al.* 2005; Manoli *et al.* 2005; Stockinger *et al.* 2005; Rideout *et al.* 2007; Sanders and Arbeitman 2008; Mellert *et al.* 2010; Rideout *et al.* 2010; reviewed in Billeter *et al.* 2006). Although target loci of the transcriptional regulatory members of this pathway are known (Burtis *et al.* 1991; Cann *et al.* 2000; Kopp *et al.* 2000; Dauwalder

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The microarray data from this study are available through the Gene Expression Omnibus database via accession no. GSE24167.

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et al. 2002; Fujii and Amrein 2002; Drapeau *et al.* 2003; Arbeitman *et al.* 2004; Goldman and Arbeitman 2007; Lazareva *et al.* 2007; Fujii *et al.* 2008; Dalton *et al.* 2009), few have clearly defined functions in behavior and neural development. Several Drosophila microarray studies were key to identifying most of these downstream targets (Arbeitman *et al.* 2004; Goldman and Arbeitman 2007; Dalton *et al.* 2009), but the strategies used do not allow us to distinguish target genes that affect development of the nervous system from those that impact physiology and behavior post development.

During courtship or other social interactions, males are exposed to sensory information that must be rapidly interpreted to create the appropriate behavioral response (*e.g.*, to continue courtship directed toward that fly or to seek a new mate). In males, interacting with a second individual causes rapid expression-level changes detectable in whole animals (Carney 2007; Ellis and Carney 2009). These rapid responses are likely mediated by signaling in the nervous system, sensory organs, and other tissues that affect neural physiology. Our expression analysis approach has the advantage of using wild-type animals performing behaviors to identify adult-expressed gene products that are impacted by behavior, including target genes of the somatic sex-determination hierarchy.

Since our earlier studies did not address the possibility that some of the loci that respond to male–female interactions might be generally "socially-responsive" genes rather than specifically "courtship-responsive" genes, we examine this possibility in our study by examining gene expression changes occurring in the male head (rather than in the whole body) during interactions with either females or males. We also expanded on our earlier studies by showing that socially-responsive loci can function in behavior. Our data indicate that social interactions cause expression changes in loci expressed in neuronal tissue as well as in non-neuronal adipose tissue that may modulate neural signaling and behavior.

MATERIALS AND METHODS

Microarray analysis: We used an isogenized wild-type *Canton-S* (*CS*) strain and handled flies similarly to Carney (2007) except that the females' genitals were electrically cauterized to prevent mating (non-mateable females). Twenty or fewer virgin isogenic *CS* males were aged collectively for 3 days, and ≤20 virgin isogenic *CS* females were aged collectively for 3 days. On day 4, males were aspirated into individual vials, and females had their genitals cauterized by passing a 4-mA current over two fine tungsten wires on the external genitalia of the female to prevent mating. Females recovered for 1 day in a new vial. All flies were kept on a 12-hr light/dark cycle at 25°, and we performed all procedures within 2 hr of lights on to control for circadian effects on gene expression and behavior.

Analysis of courtship behavior on day 5 included equally dividing males into three groups: (1) courting male, (2) male–male, and (3) control. For the courting male treatment, one cauterized female was aspirated into a male's vial. For the male–male group, a second male was aspirated into the vial of a single male. Control males were treated in the same way except that a second fly was not transferred during the aspiration process. Courtship or male–male exposure lasted for 20 min. In courting-male treatments, the presence of courtship was assessed at 1-min intervals. Only males that courted a female for at least 70% of the observation time were collected for analysis. During this time, brief male–male interactions (lasting only a few seconds) were observed. We did not detect locomotor differences among males in the three treatment groups (two-tailed *t*-test, $P > 0.05$). After 20 min, males were removed from the vials, quick-frozen in liquid nitrogen, and stored at −80° for future RNA extraction.

We separated heads from the rest of the bodies by vortexing quick-frozen flies. For each treatment, 20 male heads were randomly assigned to 1 of 10 groups, giving us 10 RNA preparations for each of the courting-male, male–male, or control treatments. Total head RNA was extracted using Trizol (Invitrogen, Carlsbad, CA) following standard protocols. The University of Kentucky MicroArray Core Facility labeled and hybridized 5 RNA preparations each from courting-male, male–male, and control heads (15 individual samples) to Affymetrix Drosophila 2.0 Genome Arrays following standard Affymetrix (Santa Clara, CA) protocols. Therefore, animals for all three treatment groups were collected and analyzed at the same time, and all 15 microarray hybridizations were carried out concurrently.

We extracted expression values from the microarrays using five algorithms: GeneChip Operating Software (MAS 5.0, Affymetrix), Gene Spring (Agilent, Santa Clara, CA), PM, and PM-MM (dChip; Li and Wong 2001), and GCRMA (R Development Core Team). For paired-data analysis comparing courting male and control treatments, we conducted a Bayesian *t*-test (CyberT; Baldi and Long 2001) and false-discovery rate analyses ($q < 0.05$, Storey and Tibshirani 2003), requiring that $P < 0.001$ in at least three of five algorithms. For a combined analysis of the three data sets, we used the SAS Mixed procedure (SAS Institute, Cary, NC) and identified significantly up- and downregulated socially-responsive genes ($P < 0.05$ in at least three of five algorithms).

Courtship-responsive genes are those for which expression in courting-male heads differs from that in control and male–male heads (control = male–male expression). Male–male-responsive genes are those that differ only in male–male interactions (control = courting male expression). Other genes that respond to interactions with both sexes were placed in the general category of socially-responsive genes.

Quantitative PCR: We validated the microarray results by quantitative PCR (qPCR) analysis on the 5 control and 5 courting male RNA preparations not used for microarray hybridization. cDNA was synthesized from poly$^+$A purified (Oligotex mRNA mini kit, Qiagen) RNA using the SuperScript First-Strand Synthesis System (Invitrogen).

Since few of the socially-responsive loci identified from the paired analysis (courting male compared to control) had known or predicted functions in behavior, primers were designed for a randomly chosen set of six upregulated (*CG9377*, *CG10621*, *egh*, *HLHmβ*, *Lsp2*, *sug*) and three downregulated (*CG31181*, *Rim*, *Sh*) candidate genes. We chose a range of genes with adult expression predicted to be enriched in the brain (*CG9377*, *Rim, Sh*), fat body (*Lsp2*, *sug*), or both tissues [*CG10621*, *CG31181*, *HLHmβ*, *egh* (*egh* expression is very low in fat)] (Chintapalli *et al.* 2007). Genes with low predicted transcript levels in the head were not tested (Chintapalli *et al.* 2007). Of these selected genes, only *egh* and *Sh* had previously described reproductive behavioral roles in females and males, respectively. To control for amplification specificity, primer pairs were designed across introns when possible.

No template controls as well as controls with template but without reverse transcriptase were included.

Using the ABI7500 and its default parameters (Applied Biosystems), each template was run in triplicate, using 2 μl of a 1:4 cDNA dilution and the SYBR Green PCR Mastermix (Applied Biosystems). We used dissociation curve analysis to determine primer-specific amplification and the relative standard curve method (Applied Biosystems) to determine transcript levels. Normalization to *rp49* levels generated relative transcript abundance values for control or courting-male samples. The relative fold change for each gene was measured as the ratio of courting-male relative abundance to control-male relative abundance, and significance was determined by a two-tailed *t*-test. Upregulation of *egh* and *HLHmβ* and downregulation of *CG31181* were confirmed by secondary qPCR analysis.

A regression analysis of microarray mean expression fold changes compared to independent qPCR fold changes indicated a significant positive correlation between results obtained by both methods ($r = 0.68$, $N = 9$, $P = 0.006$).

***In situ* hybridization:** We performed *in situ* hybridization for a subset of socially-responsive genes using cDNA clones for *CG9377* (GH08193), *CG10621* (RE64786), *cwo* (LD15411), *egh* (GH01085), and *sug* (LD36528). Antisense and sense probes were made from the above clones using the digoxigenin (DIG)-labeling kit's standard protocol (Roche, Nutley, NJ). Probes were hydrolyzed into 200-bp fragments and hybridized to dissected male tissues (brains, heads, or abdominal carcasses) as previously described (Arbeitman *et al.* 2004).

To confirm that *fit* expression increased in courting males compared to control males, we generated antisense and sense probes directed against *fit* using the RH40291 clone. Control and courting-male heads were cryosectioned and incubated with *fit* probes as described above. We only detected signal using antisense probes.

Courtship behavior analysis: Flies were maintained on a 12-hr light/dark cycle at 25°, except when noted otherwise. The Bloomington Stock Center supplied *P*-element insertion mutants (egh^{EP804}, $egh^{EY03917}$). Both insertions are located within the first *egh* exon and reduce *egh* expression to barely detectable levels (supporting information, File S1 and Figure S1). For both X-linked *P*-element insertions, we crossed *P*-element females to isogenic *CS* males, and we crossed *P*-element males to isogenic *CS* females, generating experimental and control males, respectively, in genetically similar backgrounds. For behavioral analysis, *P*-element and control males were aged at 25° in individual vials for 4–5 days and *CS* virgin females (≤20) were aged collectively for 3–5 days. All courtship tests with *egh* mutants were performed in dim red-light conditions because mutations in *egh* affect photoreceptor pathfinding (Fan *et al.* 2005) and therefore likely impact eye function. In red-light conditions, fly courtship relies more heavily upon sensory systems other than the eye.

We analyzed courtship behavior under red light at 22°. A male was aspirated into a mating chamber (diameter = 1 cm) and a virgin *CS* female was introduced 2 min later. The pair was video recorded for 10 min. The courtship index (CI)—i.e., the time performing courtship divided by the total observation time—was calculated. CI values were arcsine-transformed, and two-tailed *t*-test comparisons between mutants and controls were calculated to determine significance ($P < 0.05$).

To reduce *egh* specifically in the adult nervous system, we utilized two *egh* RNA interference (RNAi) alleles, egh^{v45160} and egh^{v45161}, from the Vienna Drosophila RNAi Center (VDRC) (Dietzl *et al.* 2007). We used *in situ* hybridization to verify reduced *egh* expression upon activation of each RNAi allele (Figure S1).

We targeted *egh* reduction pan-neuronally with $elav^{c155}$*-Gal4* (Lin and Goodman 1994) and more specifically with ap^{md544}*-Gal4* (Calleja *et al.* 1996), which is expressed in *ap*-expressing neurons in larval and adult nervous systems (Figure S2). We increased the efficiency of the RNAi process by adding one copy of *UAS-Dicer-2* (VDRC). To reduce *egh* specifically in adults, the RNAi alleles were under the control of $UAS\text{-}tubulin\text{-}Gal80^{ts}$ (reviewed in McGuire *et al.* 2004). Crosses were maintained at the permissive temperature. Control males had *UAS-Dicer-2* and $UAS\text{-}tubulin\text{-}Gal80^{ts}$ and either the RNAi allele or the Gal4 driver. We collected virgin males and stored them in individual vials at either 20° or 29°. The courtship objects, *CS* virgin females, were collected and stored collectively at 25°. Behavioral analysis was conducted under red light at the aforementioned temperatures. We used ANOVA and Tukey's post-hoc analysis to determine significant changes in CI due to temperature and genotype.

To restore *egh* expression, we crossed a genomic rescue construct (*eghP2*) to $egh^{EY03917}$ and compared CIs of $egh^{EY03917}$*; eghP2* males to $egh^{EY03917}$ males. To narrow down which cells require *egh* expression for proper courtship behavior, we utilized the rescue construct, *UAS-eghHA* (Soller *et al.* 2006). We crossed *UAS-eghHA* to the ap^{md544}*-Gal4* driver in the $egh^{EY03917}$ background. $egh^{EY03917}$ males with either component of the Gal4/UAS system served as controls. Both rescue experiments were carried out at 22° under red light.

RESULTS

Changes in male gene expression during social interactions with females or males: Within 5 min of male-to-female social interactions, whole-animal transcript profiles are altered in courting males, and there is a differential response to conspecific compared to heterospecific females (Carney 2007; Ellis and Carney 2009). Next, we focused solely on male-head gene expression in response to courtship since the head contains the brain as well as other tissues and sensory organs that impact behavioral and physiological responses to sensory inputs. We extended the courtship interaction period to 20 min to ensure a robust response and used Affymetrix Drosophila 2.0 Genome Arrays to examine ~18,500 transcripts for expression-level changes in males performing courtship toward non-mateable females (referred to as "courting males") compared to males that were not given a female courtship object ("control males") (see materials and methods).

Bayesian CyberT analysis comparing expression values from heads of courting males to those from controls identified 35 loci with altered expression due to male–female interactions (see materials and methods). Sixteen transcripts were upregulated (Table 1) and 19 were downregulated (Table 2) after 20 min of courtship. These changes are not likely due to locomotor differences since courting and control males have similar activity levels during the assay period (two-tailed *t*-test, $P > 0.05$). The small number of loci with altered expression is consistent with results from other behavioral studies (*e.g.*, Lawniczak and Begun 2004; Mack *et al.* 2006; Wada *et al.* 2006) and is partially a consequence of our extremely conservative criteria for identifying responsive genes (see materials and methods).

TABLE 1

Candidate genes upregulated after 20 min of courtship

Gene identifier	Gene name	Average fold change	GO molecular function	GO biological process
CG1897	*Drop* (*Dr*)	1.46	Sequence-specific DNA binding	Nervous system development
CG3850	*sugarbabe* (*sug*)	1.6	Transcription activator activity	Regulation of transcription
CG6494	*hairy* (*h*)	1.52	Transcription repressor activity	Nervous system development
CG6806	*Larval serum protein 2* (*Lsp2*)	1.34	Nutrient reservoir activity	Transport
CG9377		1.7	Serine-type endopeptidase activity	Proteolysis
CG9659	*egghead* (*egh*)	1.36	β-1,4-mannosyltransferase activity	Axon guidance and oogenesis
CG10142	*Ance-5*	1.34	Metallopeptidase activity	Proteolysis
CG10621		1.44	Homocysteine *S*-methyltransferase activity	Unknown
CG10812	*drosomycin-5* (*dro5*)	1.34	Unknown	Defense response to fungus
CG14489	*olf186-M*	1.26	Unknown	Unknown
CG14548	*E(spl) region transcript mβ HLHmβ*	1.58	Transcription repressor activity	Nervous system development
CG14688		1.28	Unknown	Unknown
CG17100	*clockwork orange* (*cwo*)	1.45	Transcription repressor activity	Regulation of circadian rhythm
CG17820	*female-specific independent of transformer* (*fit*)	1.38	Unknown	Unknown
CG18477		1.52	Serine-type endopeptidase activity	Proteolysis
CG42370		1.30	Metalloendo-peptidase activity	Proteolysis

Comparing control male heads to courting male heads revealed that 16 genes are significantly ($P < 0.001$) upregulated in male heads after 20 min of courtship.

We performed a second analysis of our data that included a third comparison to gene expression changes occurring as a consequence of male–male interactions. This strategy allowed us to distinguish loci whose expression changes due to interactions specifically with females from loci that change due to interaction with another individual of either sex (see MATERIALS AND METHODS). A total of 505 genes responded to male–male interactions, while 281 genes responded to male–female interactions. Most expression changes that occur due to male–female interactions also occur as a consequence of male–male interactions (Table S1). The list of 265 genes in Table S1 includes 24 genes present in the original comparison between courting-male and control heads. We also identified 240 genes whose expression changes specifically in response to paired male interactions (Table S2).

Sixteen genes were responsive to male–female interactions but not to male–male interactions and therefore appear to be true courtship-responsive loci (Table 3). Five of these 16 loci [*Drop*, *sugarbabe* (*sug*), *hairy*, *olf186-M*, *HLHmβ*] also were present on the list (Table 1) from the paired comparison of courting males to control males. Six genes with strong statistical support in the initial comparison of courting males and control males [*egh*, *Lsp2*, *clockwork orange* (*cwo*), *cacophony* (*cac*), *CG2217*, *CG4629*] were not present in the new list of genes that responded to encounters with both sexes. However, *cwo* is on the list specific to male–male interactions (Table S2), suggesting that it may be socially-responsive. It is possible that expression changes in the remaining five genes from the paired comparison are also a specific response to courtship rather than a general response to social interactions. In support of this argument, *cac* functions in production of male courtship song. We refer to the broad group of genes identified here as "socially-responsive genes" and refer to specific subcategories (*e.g.*, "courtship-responsive," "male–male-responsive") as appropriate.

qPCR validation of microarray results: To verify our microarray results, we used qPCR to analyze transcript levels from a subset of socially-responsive genes from Tables 1 and 2. We compared expression in control and courting-male head RNA preparations not used for microarray hybridization. The six upregulated and three downregulated socially-responsive genes tested showed the expected trends in expression (Table 4; see also MATERIALS AND METHODS) with fold changes comparable to those from the microarrays. Four of the genes, including the courtship-responsive loci *sug* and *HLHmβ*, showed statistically significant changes in courting males compared to controls. Since all nine genes showed the expected trend by qPCR, this is strong support for the validity of the microarray data. Increasing the sample sizes would likely increase the statistical support.

Socially-responsive genes are expressed in the brain and other head tissues: Because we assayed head tissue, identified loci may be expressed in the brain, sensory structures, the fat body, or a combination of these tissues. Expression of many socially-responsive genes is enriched in the head relative to the brain, indicating higher expression in tissues outside of the brain (CHINTAPALLI *et al.* 2007). Although some socially-responsive genes

TABLE 2

Candidate genes downregulated after 20 min of courtship

Gene identifier	Gene name	Average fold change	GO molecular function	GO biological process
CG1522	*cacophony* (*cac*)	−3.08	Voltage-gated calcium channel activity	Courtship behavior
CG2217		−1.36	Unknown	Unknown
CG3738	*Cyclin-dependent kinase subunit 30A* (*Cks30A*)	−1.36	Cyclin-dependent protein kinase regulator activity	Cyclin catabolic process
CG4269		−3.92	Unknown	Unknown
CG9266		−1.28	Unknown	Unknown
CG9983	*hnRNA-binding protein 1* (*Hrb98DE*)	−1.24	Nucleic acid binding	Unknown
CG10077		−1.3	ATP-dependent helicase activity	Unknown
CG10851	*B52*	−1.3	Nucleic acid binding	Regulation of nuclear mRNA splicing via spliceosome
CG12295	*straightjacket* (*stj*)	−1.3	Voltage-gated calcium channel activity	Synaptic vesicle fusion to presynaptic membrane
CG12348	*Shaker* (*Sh*)	−1.32	Voltage-gated cation channel activity	Regulation of synaptic activity and courtship behavior
CG12449	*Glutamine: fructose-6-phosphate aminotrans-ferase 1* (*Gfat*)	−1.38	Glutamine-fructose-6-phosphate transaminase activity	Carbohydrate biosynthetic process
CG12478	*bruno-3* (*bru-3*)	−1.26	RNA binding	Negative regulation of translation
CG31181		−1.36	Unknown	Unknown
CG31182		−1.44	Unknown	Unknown
CG33197	*muscleblind* (*mbl*)	−1.44	Zinc ion binding	Muscle development
CG33547	*Rim*	−1.44	Small GTPase regulator activity	Regulation of exocytosis
CG42492		−1.34	Unknown	Unknown
CG42670	*pasilla* (*ps*)	−1.34	Unknown	Unknown
CG42698	*pou domain motif 3* (*pdm3*)	−1.38	Unknown	Unknown

Average fold changes, molecular functions, and biological processes are shown for 19 genes that are significantly ($P < 0.001$) downregulated in male heads after 20 min of courtship.

are enriched in the eye, others are enriched in head tissues other than the brain or eye, including the adipose tissue lining the brain (Table S3).

Two socially-responsive genes, *Lsp2* and *female-specific independent of transformer* (*fit*), are expressed in fat surrounding the brain in both sexes (Benes *et al.* 1990; Fujii and Amrein 2002). *fit* was named for its high level of expression in females compared to males and because its expression is regulated by the somatic sex-determination hierarchy gene *Sex-lethal* (Fujii and Amrein 2002).

In an earlier whole-male microarray analysis, we detected a statistically significant increase in *fit* transcripts in courting males; this increase was validated by qPCR (Carney 2007). *fit* also increases in 20-min courting-male heads (Table 1) and is responsive to same-sex interactions as well (Table S1). Since we had not examined the specific tissue in which this increased expression occurs, we examined *fit*'s response to 20-min courtship in sectioned male heads. Although expression is low in virgin males, *fit* levels increased in response to male–female interactions (Figure 1). This increase was detected in the fat body, an adipose tissue previously implicated in modulation of courtship behavior (reviewed in Dauwalder 2008). *In situ* hybridization confirmed that other socially-responsive genes are expressed in the male fat body (*CG10621, sug*), the male brain (*CG9377, egh*), or both tissues (*cwo*) (Figures 2 and 3).

***egghead* is required in the adult male brain for robust courtship:** We hypothesized that genes with altered expression patterns due to male–female interactions likely modulate courtship behavior either by regulating the performance of courtship steps or by making the male a more efficient courter by increasing the efficiency of stimulus processing. This increased efficiency could affect the current courtship interaction or, more likely, subsequent courtship encounters. We predicted that we could identify behavioral functions for these loci by testing mutations in the genes for effects on male courtship behavior.

Therefore, we tested *P*-element insertions or VDRC strains targeting some of the male–female-responsive

TABLE 3

Courtship-responsive genes

Gene identifier	Gene name	Average fold change in courting male heads compared to: Male–male heads	Control male heads	GO molecular function	GO biological process
CG1416		1.12	1.12	ATPase activator activity	Response to stress
CG1897	*Drop* (*Dr*)	1.35	1.44	Sequence-specific DNA binding	Nervous system development
CG3850	*sugarbabe* (*sug*)	1.3	1.61	Transcription activator activity	Regulation of transcription
CG6461		1.1	1.1	Transferase activity	Unknown
CG6494	*hairy* (*h*)	1.63	1.52	Transcription repressor activity	Nervous system development
CG11877		1.11	1.12	Unknown	Unknown
CG13116		1.21	1.25	Unknown	Unknown
CG14489	*olf186-M*	1.18	1.27	Unknown	Unknown
CG14548	*E(spl) region transcript mβ* (*HLHmβ*)	1.39	1.58	Transcription repressor activity	Nervous system development
CG30445	*Tyrosine decarboxyl-ase 1*	1.22	1.24	Tyrosine decarboxylase activity	Unknown
CG31137	*twin*	1.28	1	Transcription regulator activity	Nuclear-transcribed mRNA poly(A) tail shortening
CG4962	*CG4962*	−1.28	−1.20	Unknown	Unknown
CG14210	*CG14210*	−1.23	−1.02	Protein binding	Unknown
CG6081	*Cyp28d2*	−1.11	−1.18	Oxidoreductase activity	Oxidation reduction
CG32491	*modifier of mdg4* [*mod(mdg4)*]	−1.09	−1.16	Transcription factor activity	Induction of apoptosis
CG18525	*Serine protease inhibitor 5* (*Spn5*)	−1.14	−1.11	Serine-type endopeptidase inhibitor activity	Unknown

Eleven upregulated and 5 downregulated genes are courtship-responsive when comparing all three treatment groups (control, courting, and male-exposed) by mixed ANOVA and Tukey's post-hoc analyses ($P < 0.05$).

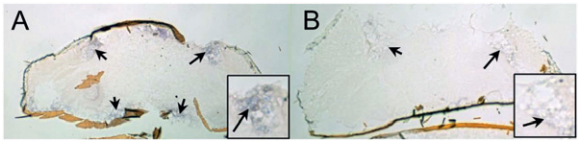
genes from Tables 1 and 2 for effects on male courtship activity (measured as the CI). For several of the alleles, we observed weak phenotypic effects on behavior. However, mutations in *egh* had strong effects on male–female courtship, so we focused our current downstream analysis on this locus. Males with either of two independent insertions in *egh* (egh^{EP804} and $egh^{EY03917}$) performed all standard courtship behaviors but had significantly reduced CI values compared to genetically similar controls (Figure 4, two-tailed *t*-test, $P < 0.001$). We did not observe male–male courtship or aggressive interactions in groups of aged mutant males that were placed

TABLE 4

qPCR confirmation of the microarray results

Gene identifier	Gene symbol	Average relative expression level in control male heads ±SEM	Average relative expression level in courting male heads ±SEM	Relative qPCR fold change ±SEM	Relative array fold change ±SEM
CG9377		0.07 ± 0.01	0.09 ± 0.02	1.25 ± 0.25	1.7 ± 0.07
CG10621		1.95 ± 0.46	2.42 ± 0.63	1.31 ± 0.28	1.44 ± 0.03
CG9659	*egh*	3.2 ± 1.07	6.62 ± 1.72	2.04 ± 0.42	1.36 ± 0.02
CG14548	*HLHmβ*	0.65 ± 0.16	1.92 ± 0.62	3.13 ± 0.82*	1.58 ± 0.05
CG6806	*Lsp2*	0.57 ± 0.17	1.5 ± 0.72	2.79 ± 1.09	1.34 ± 0.03
CG3850	*sug*	3.95 ± 1.2	9.31 ± 2.58	2.36 ± 0.58*	1.6 ± 0.05
CG31181		0.6 ± 0.2	0.15 ± 0.11	−2.7 ± 0.09*	−1.36 ± 0.03
CG33547	*Rim*	0.4 ± 0.09	0.03 ± 0.004	−12.5 ± 0.006	−1.44 ± 0.06
CG12348	*Sh*	0.39 ± 0.09	0.12 ± 0.04	−3.13 ± 0.09*	−1.32 ± 0.04

Average relative expression of nine genes was assessed by qPCR. An asterisk indicates a significant ($P < 0.05$) difference in the average relative expression level in control male heads compared to courting-male heads as determined by qPCR. SEM, standard error of the mean.



C

	- +	++	+++
(A) Courting males	1	10	3
(B) Control males	12	1	0

FIGURE 1.—Courting males show increased *fit* expression in the fat body. A DIG-labeled *fit* RNA antisense probe was made from the RH40291 cDNA clone. *In situ* hybridization was performed on cryosectioned male heads and confirmed that *fit* transcript levels are upregulated in the adipose tissue (arrows) of courting males (A) compared to control males (B). A qualitative assessment of signal intensity in both treatment groups is presented in C.

together in vials and observed over a 2-week period. Therefore, reduced *egh* expression led to an overall reduction in time spent courting a female but did not appear to affect male–male interactions. Reintroduction of a genomic copy of *egh* in the $egh^{EY03917}$ background restored male-to-female courtship activity to wild-type levels (Figure 5, $P < 0.001$), verifying that the courtship phenotype is due to disruption of the *egh* locus.

We selectively reduced *egh* in the adult nervous system with *UAS-egh*-RNAi under the control of *UAS-tubulin-Gal80^{ts}* and neural-expressed *elav^{c155}-Gal4*. This adult-specific decrease in *egh* resulted in significantly reduced CI values for experimental males at the restrictive temperature (29°) compared to all controls (Figure 6).

Larval *egh* expression is required in *ap*-expressing ventral nerve cord neurons for the female Sex-peptide response during adulthood (SOLLER *et al.* 2006). We asked whether this same circuit functioned in male reproductive behavior. Expressing *egh* (via *UAS-eghHA*) under control of *ap^{md544}-Gal4* in $egh^{EY03917}$ mutant males was sufficient to restore male courtship behavior (Figure 7, $P < 0.001$), indicating that Ap neurons modulate reproductive behaviors in both sexes. Although SOLLER *et al.* (2006) attributed modulation of the Sex-peptide response to developmental expression, *ap^{md544}-Gal4* expresses in the male and female adult nervous system (Figure S2). Therefore, we expressed *egh*-RNAi via *ap^{md544}-Gal4* to specifically reduce *egh* expression in adult males (Figure 8). Targeted *egh* reduction significantly decreased courtship activity ($P < 0.001$), confirming that *egh* is needed in Ap neurons during adulthood for proper courtship behavior.

DISCUSSION

Social interactions alter male gene expression: Drosophila perform stereotypical sex-specific courtship behaviors that are influenced by genetics, including the somatic sex-determination pathway, and by environmental cues, including social interactions. Previous studies have shown that male–female social interactions cause rapid (within 5 min) changes in whole-male transcript abundance (CARNEY 2007). In this study we focused on male-head tissue and found that 521 genes are socially-responsive in a 20-min interaction period. Expression of 281 genes changes during male–female interactions, while 505 genes are affected by male–male interactions. At least 16 of these loci are specifically courtship responsive (Table 3). Similarly to genes identified in array studies on songbirds and honeybees responding to behavioral cues (GROZINGER *et al.* 2003; WHITFIELD *et al.* 2003, 2006; WADA *et al.* 2006; SEN SARMA *et al.* 2009), the 16 Drosophila courtship-responsive genes include several loci that regulate gene expression and neural development and signaling, but their specific relationship to behavior is not clear. These loci may control gene cascades important for subsequent courtships, such as those that fine-tune neural connections due to courtship and mating experience. An additional set of five genes identified only in a paired comparison of courting and control males (*Lsp2*, *egh*, *cac*, *CG2217*, and *CG4269*) may also be courtship-responsive since their transcript levels were not affected by male–male interactions. If not specifically courtship responsive, they are likely to be generally socially-responsive or to have behavioral functions. In support of this hypothesis, we showed that *egh* expression is important for robust male–female courtship.

Interestingly, a much larger group of genes is responsive to interactions with both sexes (265 genes, Table S1) or is male–male responsive (240 genes, Table S2). Therefore, social interactions have an impact on gene expression patterns that depends on the sex of the interacting individuals. This result is not surprising since social experience affects a variety of behaviors and morphological phenotypes in flies and other animals

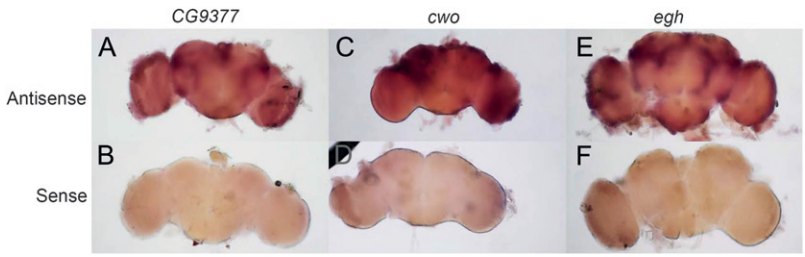


FIGURE 2.—Socially-responsive genes *CG9377*, *cwo*, and *egh* are expressed in the male brain. Antisense (A, C, and E) or sense (B, D, and F) RNA probes were designed for cDNA clones for *CG9377* (A and B), *cwo* (C and D), and *egh* (E and F). *In situ* hybridization to whole-mounted male *CS* tissue reveals that courtship-responsive genes are expressed in male brains. Dark pink staining in A, C, and E indicates expression due to hybridization of antisense probes.

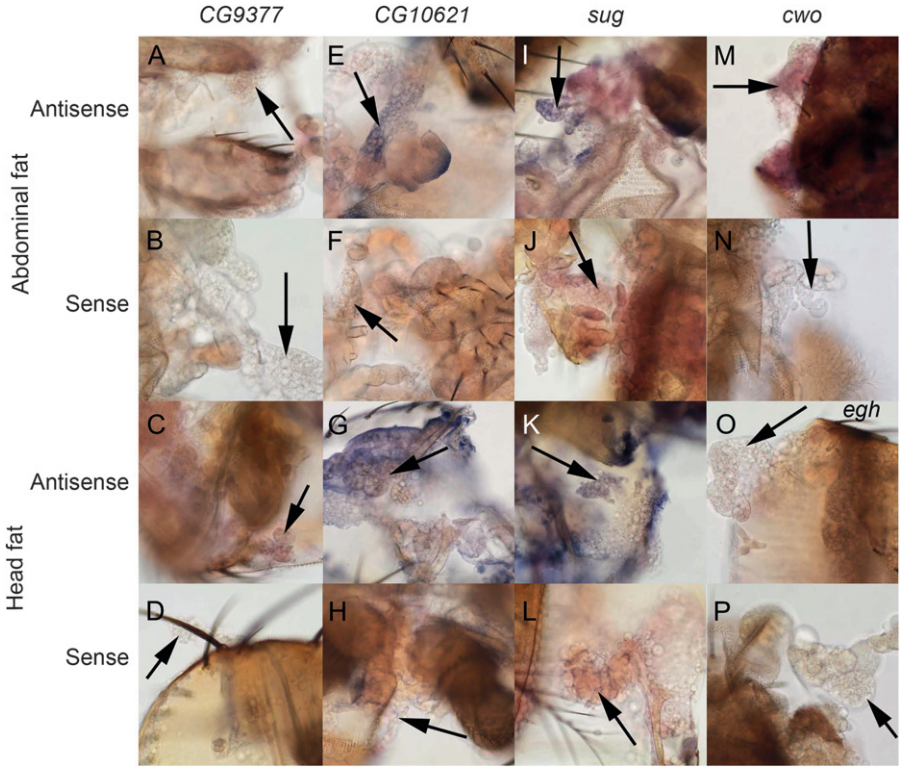


FIGURE 3.—Socially-responsive genes *CG10621*, *sug*, and *cwo* are expressed in male adipose tissue. Antisense (A, C, E, G, I, K, M, and O) or sense (B, D, F, H, J, L, N, and P) RNA probes were designed to cDNA clones for *CG9377* (A–D), *CG10621* (E–H), *sug* (I–L), *cwo* (M and N), and *egh* (O and P). *In situ* hybridization to whole-mounted male *CS* tissue shows candidate gene expression in the fat body tissue (arrows) on abdominal (A, B, E, F, I, J, M–P) or head (C, D, G, H, K, and L) cuticle. Expression is indicated by light (*cwo*) or dark purple (*CG10621*, *sug*).

(SIEGEL and HALL 1979; LEVINE *et al.* 2002; SHEN *et al.* 2004; STEWART and MCLEAN 2004; BURMEISTER *et al.* 2005; KOZOROVITSKIY *et al.* 2006; YURKOVIC *et al.* 2006; TECHNAU 2007). Some of the expression changes identified in our study may underlie observed effects of social interactions on circadian behavior and pheromone profiles (LEVINE *et al.* 2002; KENT *et al.* 2008; KRUPP *et al.* 2008).

The large number of male–male responsive genes was surprising. However, male–male interactions such as those involved in aggressive encounters may have greater effects on gene expression than male–female interactions. Males of many Drosophila species, including *D. melanogaster*, compete for mates and territories, and aggressive behavior is correlated with mating success (DOW and VON SCHILCHER 1975); both factors correlated with genotype (CABRAL *et al.* 2008). Social experience with other males reduces aggressive behavior during competition for territories, and experienced males are more likely to regain territories (HOFFMANN 1990). Although our male–male assays were performed under conditions under which there is predicted to be little male–male competition (*e.g.*, no food source or female), it is likely that sensory processing and gene expression was affected by the brief encounters between individuals. Observed changes in gene expression due to male–male interactions may contribute to the phenotypic plasticity in behaviors important for obtaining territories, food sources, and mates. Further investigation is required to understand fully the importance of the large number of changes that occur due to general social interactions or specifically in response to male–male interactions.

Socially-responsive genes and the sex-determination hierarchy: We predicted that some socially-responsive loci would function as downstream targets of the somatic sex-determination pathway that regulates male courtship behavior. Three transcription factors—Fruitless (Fru), Doublesex (Dsx), and Dissatisfaction (Dsf)—are

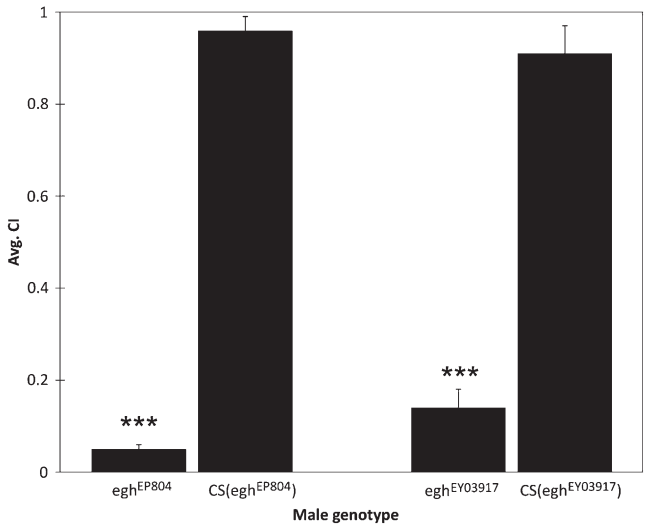


FIGURE 4.—*egh* is required for robust male courtship behavior. Under red light, males with either X-linked *egh* insertion (egh^{EP804} or $egh^{EY03917}$) showed significant (***$P < 0.001$) decreases in CI values compared to control males in a similar genetic background [CS(egh^{EP804}) or CS($egh^{EY03917}$)] under similar conditions. Error bars reflect the SEM. $N = 10$ males for each genotype.

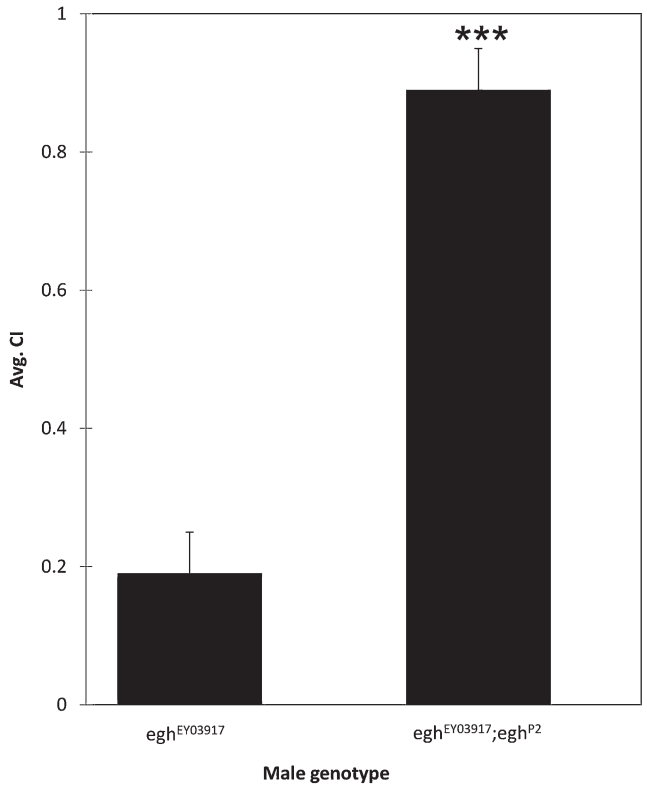


FIGURE 5.—*egh* expression rescues male courtship behavior. Restoring *egh* expression in *egh*-expressing cells (*eghP2*) in the $egh^{EY03917}$ mutant background significantly (***$P < 0.001$) rescued the courtship defect in $egh^{EY03917}$ mutant males. $N = 10$ males for both genotypes.

important regulatory components of this pathway. One courtship-responsive gene, *CG13116*, is negatively regulated by the female-specific Doublesex (Dsx) protein, and one upregulated male–male-responsive gene, *CG16713*, is downstream of *transformer* (*tra*) (GOLDMAN and ARBEITMAN 2007). Four upregulated socially-responsive genes are regulated by the sex-determination pathway. *fit* is regulated by *tra*; *CG9377* is downstream of *fru*; and *CG9837* and *CG8539* are regulated by *dsx* (GOLDMAN and ARBEITMAN 2007). The surprisingly small number of socially-responsive genes that are known sex-determination hierarchy targets may indicate that our lists include many target genes that could not be detected by the strategies used previously to identify output genes of the hierarchy. For example, genes from our study may function downstream of *dsf*, which is expressed in both males and females; transcriptional targets of *dsf* are not known. Another possibility is that the hierarchy does not regulate expression of many socially-responsive genes, indicating an alternative regulation.

Gene expression in the male brain: Since brain gene expression has a clear function in behavior, we expected some socially-responsive genes to be expressed in the brain. *In situ* hybridization showed that *CG9377* and *egh* were expressed in the male brain but were not detected in adipose tissue (Figures 2 and 3). Two downregulated, socially-responsive genes function in courtship behavior and are expressed in the brain. *cac* encodes a calcium voltage-gated channel needed for courtship song production (reviewed in GREENSPAN and FERVEUR 2000; BILLETER *et al.* 2002); *Shaker* (*Sh*) encodes a potassium channel that functions in olfactory memory and learning (reviewed in GREENSPAN and FERVEUR 2000). Other socially-responsive genes (*e.g.*, *Drop*, *egh*, *hairy*, and *Sh*) regulate nervous system development and function (GINIGER *et al.* 1994; HENG and TAN 2003; ZHONG and WU 2004; FAN *et al.* 2005; UEDA and WU 2006; URBACH *et al.* 2006) and may modulate adult neural signaling and behavior.

Changes in brain gene-expression patterns due to social interactions are likely a result of signaling pathways, including G-protein-coupled receptor signaling, functioning within the brain to mediate the perception and integration of sensory cues. Such signaling pathways may coordinate motor output pathways necessary for courtship and relay information to the brain to establish a male brain that is more readily perceptive to courtship cues than a naive male brain.

Gene expression in male adipose tissue: Signals mediating social cues are not likely restricted to the brain, however. Adipose tissue, or the fat body, surrounding the brain and in the thoracic and abdominal cavities is a secretory tissue (reviewed in SCHLEGEL and STAINIER 2007) that could influence neuronal signaling or transmit signals to other reproductively important tissues. Indeed, there is growing evidence that fat body-expressed genes modulate reproductive behaviors (reviewed in DAUWALDER 2008).

fit and *Lsp2* are expressed in the female and male fat body (Figure 1) (BENES *et al.* 1990; FUJII and AMREIN 2002), and *in situ* hybridization confirmed fat body expression of three additional socially-responsive genes (*CG10621*, *cwo*, and *sug*) (Figure 3). *cwo* is also expressed in the male brain, but we did not detect *CG10621* or *sug* transcripts in the male brain. Many socially-responsive genes are enriched in head tissue, including fat body but not including the brain (Figures 2 and 3; Table S3). This suggests that the circuitry responding to and governing social interactions such as courtship likely is modulated by both neuronal and non-neuronal signals. The response to social interactions involves complex and specific changes that may mediate various downstream effects, including neural plasticity.

***egghead* and courtship behavior:** To determine if mutations in candidate genes affected courtship behavior, we measured CI values in various mutants. Our analysis showed that a specific locus, *egh*, is needed for robust male courtship behavior (Figure 4). *egh* encodes a 1,4-mannosyltransferase that regulates glycosphingolipid biosynthesis (WANDALL *et al.* 2003), affects Drosophila neural development and behavior, and is required in Ap neurons for female Sex-peptide response post mating (SOLLER *et al.* 2006).

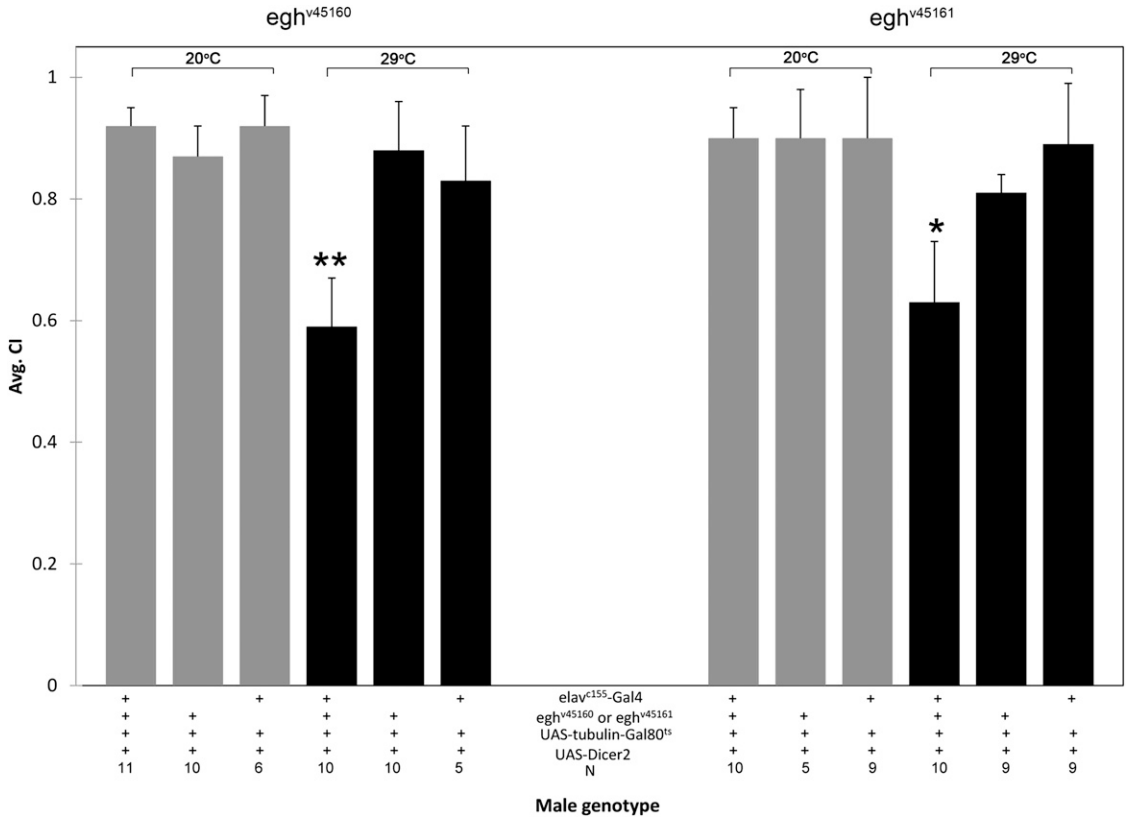


Figure 6.—Male courtship requires *egh* expression in the adult nervous system. Expressing *UAS-egh*-RNAi alleles, egh^{v45160} or egh^{v45161}, in the adult nervous system using $elav^{c155}$*-Gal4*, *UAS-Dicer-2*, and $UAS\text{-}tubulin\text{-}Gal80^{ts}$ at the restrictive temperature (29°, black bars) significantly (**$P < 0.01$; *$P < 0.05$) reduced male courtship activity compared to 29° controls lacking $elav^{c155}$-*Gal4* or *UAS-egh*-RNAi or compared to males at the permissive temperature (20°, gray bars).

Since ap^{md544}*-Gal4* (a *Gal4* insertion in *ap*) is expressed in the adult nervous system of both sexes (Figure S2), we examined whether this neural circuit also functioned in males to regulate courtship behavior. In $egh^{EY03917}$ mutant males, *egh* expression in Ap neurons rescued the courtship defect (Figure 7). Decreased adult *egh* expression (via RNAi) in *ap*-expressing neurons also resulted in decreased courtship (Figure 8). Although Fru neuron expression of the EcR transcription factor is important for courtship behavior (Dalton *et al.* 2009), decreasing EcR in adult Ap neurons did not affect courtship (Figure S3 and Figure S4). Therefore, *egh* appears to have a specific behavioral function in Ap-expressing neurons. Ap is a transcription factor that regulates developmental as well as post-developmental neural gene expression (Benveniste *et al.* 1998). *ap* mutant males also have decreased levels of male-to-female courtship (Ringo *et al.* 1992). Given the similarity between the *ap* and *egh* mutant phenotypes and the requirement for *egh* expression in *ap* neurons for male courtship, the hypothesis that *ap* regulates *egh* expression should be tested in future experiments.

Differences in sex-specific behaviors may be due to dimorphisms in neural architecture, including the number or morphology of neurons, such as those present in the *fruP1* circuit that modulates male courtship behavior (Kimura *et al.* 2005; Stockinger *et al.* 2005; Rideout *et al.* 2007; Clyne and Miesenböck 2008; Datta *et al.* 2008). The same circuit could be coopted by each sex for different behaviors. We hypoth-

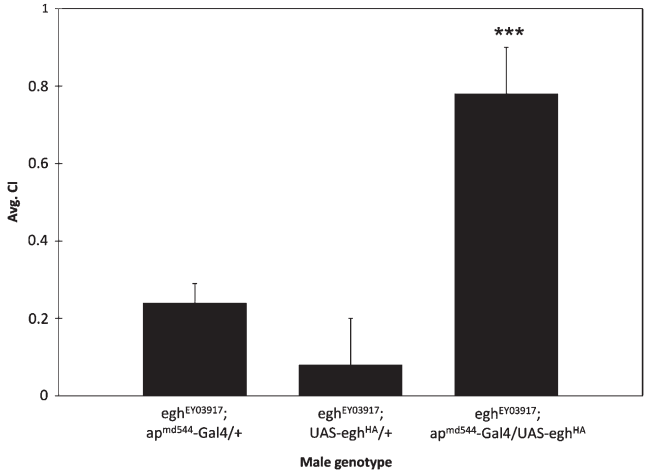


Figure 7.—*egh* expression in *ap*-expressing neurons restores male courtship behavior. Narrowing *egh* expression to *ap* neurons by expressing UAS-*eghHA* under the control of ap^{md544}*-Gal4* in the $egh^{EY03917}$ background significantly (***$P < 0.001$) restored male courtship activity compared to control $egh^{EY03917}$ males lacking either component of the Gal4/UAS system. Ten males of each genotype were tested.

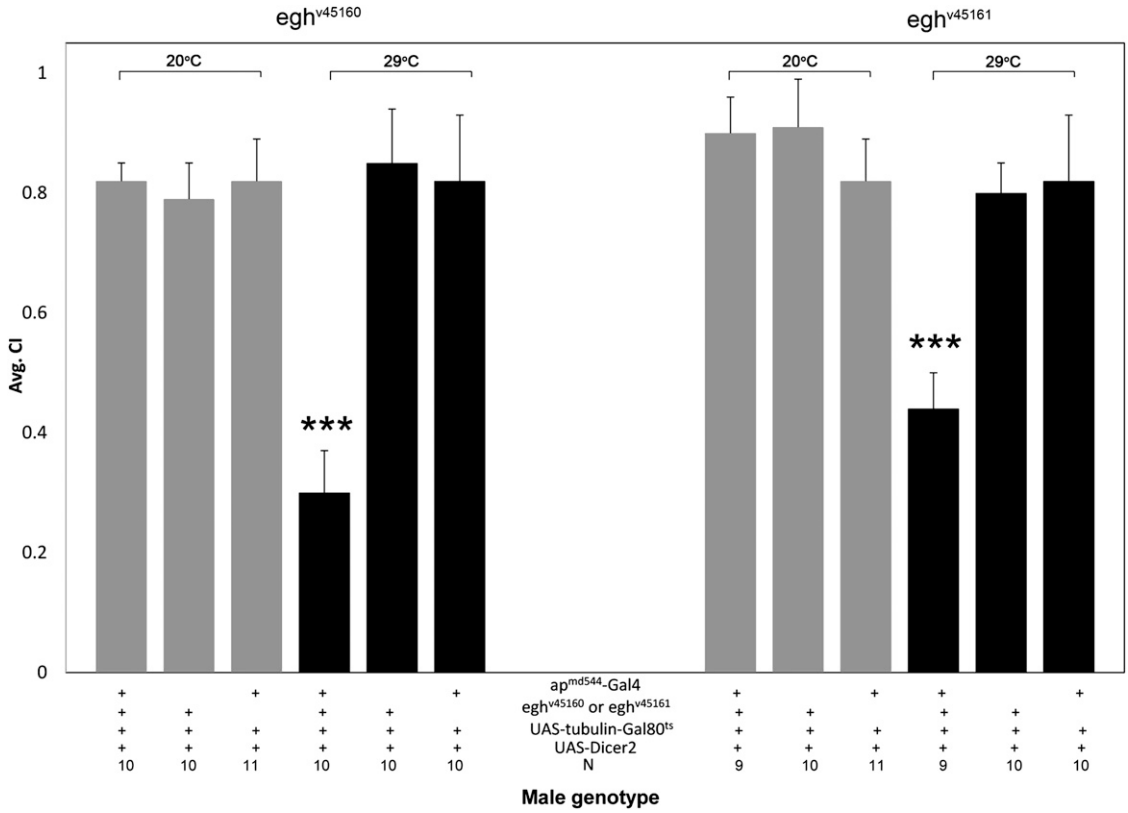


FIGURE 8.—Adult expression of *egh* in *ap*-expressing neurons is necessary for robust courtship behavior. Expressing *UAS-egh*-RNAi alleles, egh^{v45160} or egh^{v45161}, in Ap neurons during adulthood using ap^{md544}*-Gal4*, *UAS-Dicer-2*, and *UAS-tubulin-Gal80*^{ts} at the restrictive (29°, black bars) temperature significantly (***$P < 0.001$) reduced male courtship activity compared to controls lacking the Gal4 or *UAS-egh*-RNAi component or compared to males at the permissive temperature (20°, gray bars).

esize that this is the case for the *egh* circuit. *egh* is required in both male and female Ap neurons but modulates sex-specific reproductive behaviors. This may occur because of changes in neural physiology resulting from the perception of sex-specific cues that trigger different signaling cascades between the sexes. However, it is possible that different subsets of Ap neurons regulate sex-specific behavior. The *egh* circuit important for male behavior does not appear to rely directly upon *fru* neurons since expressing *eghRNAi* in *fru* neurons did not cause the behavioral defects observed in *egh* mutant or ap^{md544}*-Gal4/eghRNAi* males (data not shown). Therefore, *egh* neurons may interact indirectly with *fru* neurons to modulate reproductive behaviors.

Our study strengthens the growing body of work demonstrating that animals respond to social interactions by altering transcript abundance. By investigating the function of these socially-responsive loci, we can clarify the relationship between genetics and the intracellular processes governing behavior and physiology. Additional studies are needed to understand the relationship of courtship-responsive and other socially-responsive loci to the somatic sex-determination hierarchy or other pathways that regulate Drosophila reproductive behaviors.

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GENETICS

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Socially-Responsive Gene Expression in Male *Drosophila melanogaster* Is Influenced by the Sex of the Interacting Partner

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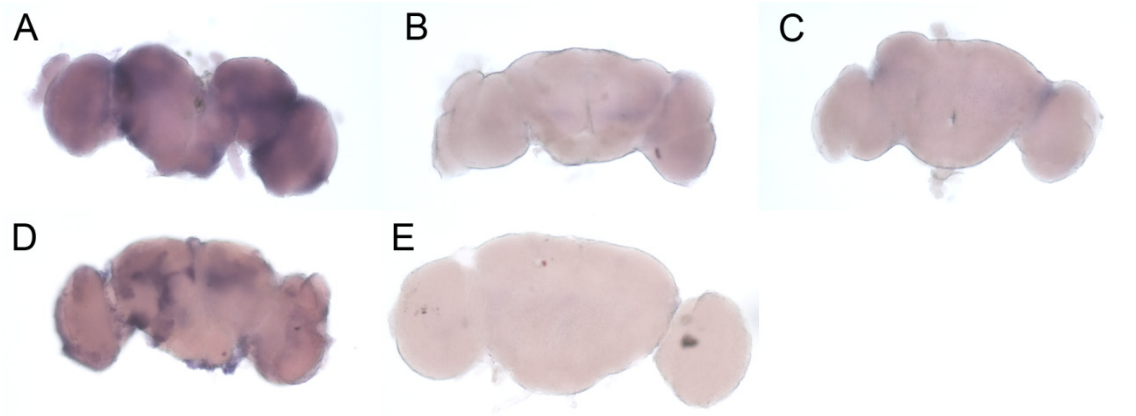

FIGURE S1.—*egh* expression is reduced in P-element and RNAi mutant males. An *egh* antisense probe was designed from the GH01085 cDNA clone and *in situ* hybridization was performed on male brains from *CS* (A), egh^{EP804} (B), $egh^{EY03917}$ (C), egh^{v45160} (D), or ap^{md544}-*Gal4*/egh^{v45160} (E). *CS* controls (A) show increased *egh* expression compared to *egh* P-element insertion mutants (B,C). Decreased *egh* expression is also seen when egh^{v45160} is activated in *ap* neurons (E) compared to control males (D).

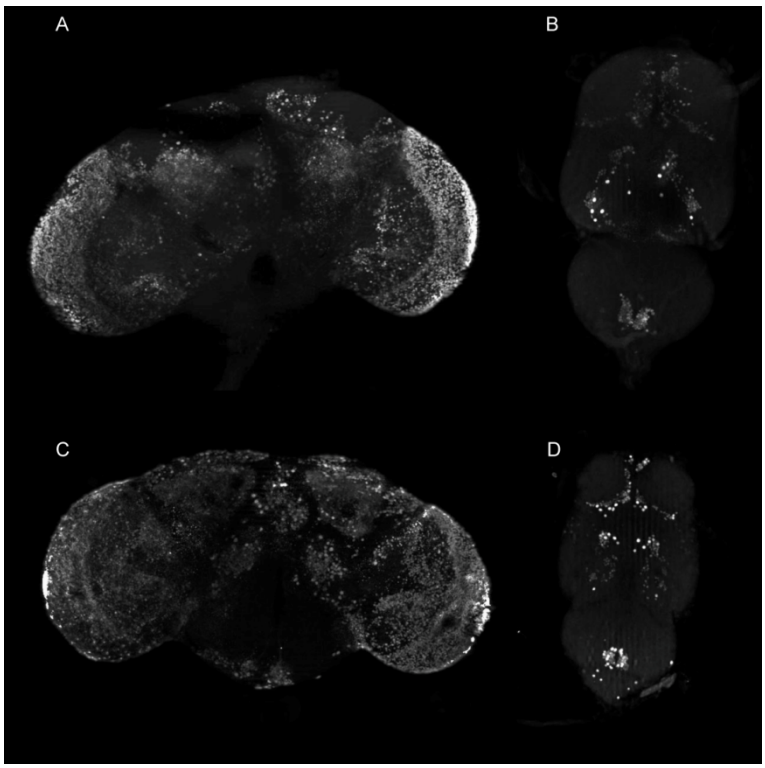

FIGURE S2.—*ap^{md544}-Gal4* drives expression of GFP in the adult nervous system. Using *ap^{md544}-Gal4* to drive expression of GFP reveals *ap^{md544}-Gal4* activity in the adult brain (A, C) and VNC (B, D) of males (A, B) and females (C, D).

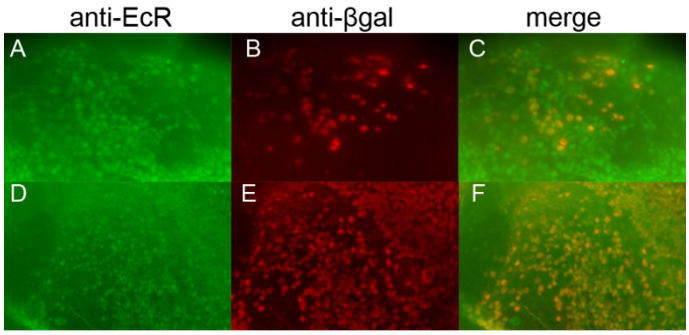

FIGURE S3.—*ap*-expressing neurons also express EcR. *ap^{md544}-Gal4* driven expression of LacZ followed by immunostaining with anti-EcR (A, D) and anti-bgal (B, E) revealed that Ap-expressing neurons in the dorsal brain (A-C) and optic lobe (D-F) also express EcR (C, F). Images are at 40X magnification. Though not shown, co-expression was also detected in male ventral nerve cords.

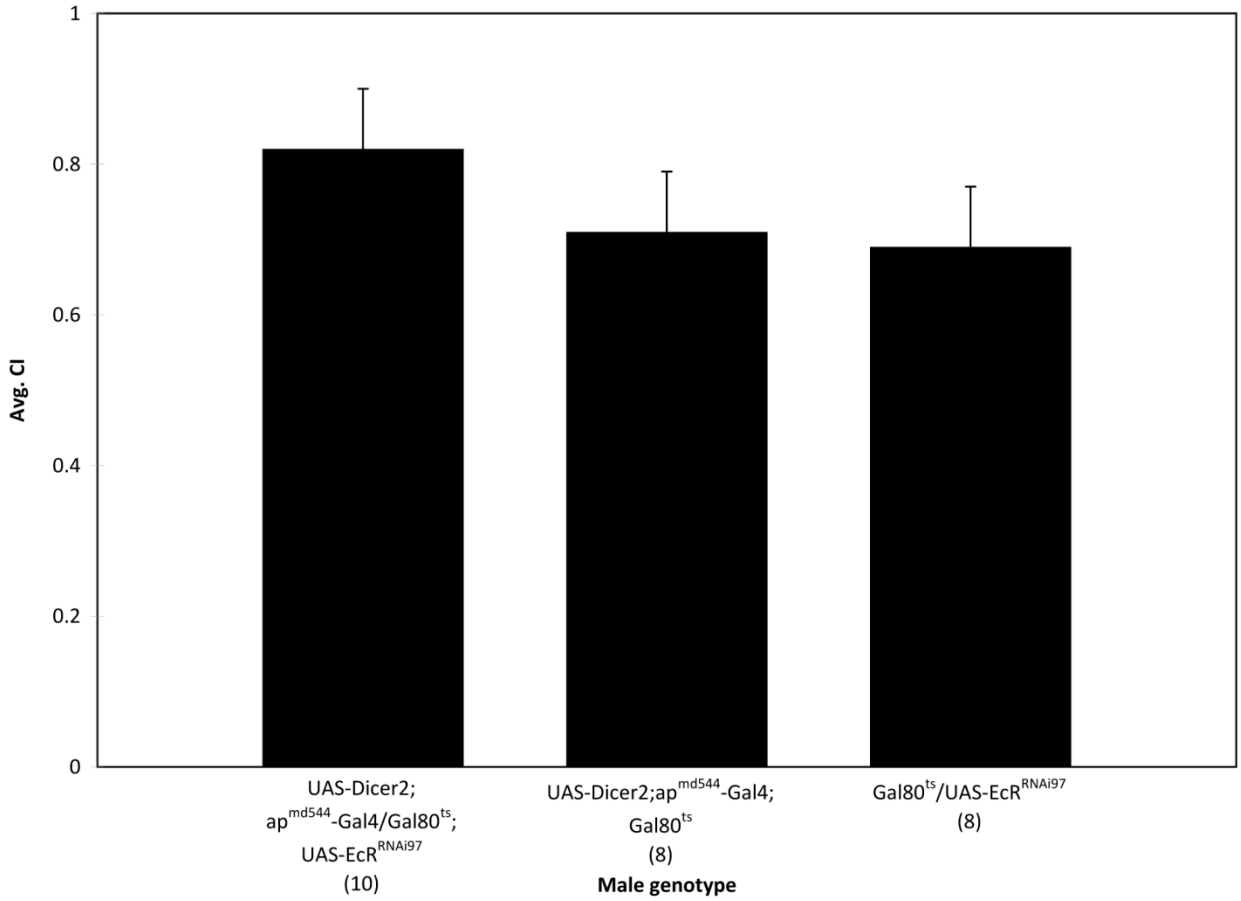

FIGURE S4.—Reduced EcR expression in *ap*-expressing neurons does not affect male courtship behavior. Expressing UAS-EcR$^{RNAi\text{-}97}$ in *ap*-expressing neurons does not reduce courtship activity in experimental males compared to either control ($p>0.05$) at the restrictive temperature of 29°C. (N) reflects the sample size for each group.

FILE S1

Supporting Materials and Methods

Antibody staining

ap^{md544}-Gal4 flies were crossed to flies containing a *UAS-GFPnls* allele. Adult males and females carrying both the Gal4 and UAS alleles were collected. Brains and VNCs were dissected in PBS, fixed in 4% paraformaldehyde and washed in PBS and PBST. We used a 1:50 concentration of anti-GFP in an overnight incubation. After more PBST washes, a 1:1200 concentration of secondary antibody was used.

EcR antibody staining

ap^{md544}-Gal4 flies were crossed to flies containing a *UAS-lacZ.nls* allele. Brains and VNCs were dissected out of adult males carrying both the Gal4 and UAS alleles. The tissues were fixed in 4% paraformaldehyde and washed in PBS and PBST. We used a 1:10,000 concentration of rabbit anti-beta-galactosidase and a 1:5 concentration of anti-EcR AG10.2 (Talbot et al. 1993) in an overnight incubation. The tissues were washed with PBST and a 1:1500 concentration of each secondary antibody was used for fluorescent detection.

EcR behavioral assay

To address whether or not the courtship defect seen in *ap^{md544}-Gal4 eghRNAi* mutants was due to decreased *egh* expression or disruption in *ap*-expressing neural signaling, we reduced expression of *EcR* in Ap neurons. Crosses between *UAS-Dicer2;ap^{md544}-Gal4* to *tubulin-Gal80^{ts};UAS-EcR$^{RNAi-97}$* (Colombani et al. 2005) were maintained at 20°C. Virgin males were collected and housed at 29°C for 5 days. Female courtship objects were collected as virgins and aged for 4 to 5 days at 25°C. Behavioral assays were performed under red light at 29°C as previously described and CI values were analyzed by ANOVA and Tukey's post-hoc analysis.

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TABLE S1

Socially-responsive genes

Gene identifier	Gene name	Avg. fold change of Courting compared to Control	Avg. fold change of Male-male compared to Control	GO Molecular function	GO Biological process
CG1092		1.21	1.43	Protein binding	Unknown
CG1468		1.20	1.31	Unknown	Unknown
CG1662		1.17	1.20	Unknown	Unknown
CG1751	*Spase 25-subunit* (*Spase 25*)	1.11	1.22	Peptidase activity	Signal peptide processing
CG1803	*Regucalcin*	1.25	1.36	Protein binding	Unknown
CG2227	*GIP-like* (*GIP*)	1.18	1.43	Protein binding	Unknown
CG2444		1.40	1.66	Unknown	Unknown
CG2846	*transcript C*	1.10	1.15	Nucleotide binding	Riboflavin biosynthetic process
CG2931		1.09	1.10	Nucleotide binding	Unknown
CG3246		1.21	1.42	Unknown	Unknown
CG3422	*Proteasome 28kD subunit 1* (*Pros8.1*)	1.14	1.20	Hydrolase activity	Ubiquitin-dependent protein catabolic process
CG3431	*Ubiquitin C-terminal hydrolase* (*Uch-L3*)	1.12	1.2	Endopeptidase activity	Protein deubiquitination
CG3455	*Rpt4*	1.1	1.19	Endopeptidase activity	Proteolysis
CG3616	*Cytochrome P450-9c1* (*Cyp9c1*)	1.22	1.35	Oxidoreductase activity	Oxidation reduction
CG3773		1.20	1.22	Unknown	Unknown
CG3835		1.19	1.29	Catalytic activity	Unknown
CG4019		1.13	1.29	Water channel activity	Transport
CG4408		1.26	1.42	Hydrolase activity	Proteolysis
CG4561	*Tyrosyl-tRNA synthetase* (*Aats-tyr*)	1.07	1.07	Aminoacyl-tRNA ligase activity	Translation
CG4716		1.12	1.13	Oxidoreductase activity	Oxidation reduction
CG4721		1.17	1.38	Metalloendopep-tidase activity	Proteolysis
CG5335		1.12	1.31	Oxygen transporter activity	Storage protein import into fat body
CG5378	*Rpn7*	1.11	1.15	Protein binding	Proteolysis
CG6028		1.12	1.27	Catalytic activity	Metabolic process
CG6186	*Transferrin 1* (*Tsf1*)	1.11	1.13	Iron ion transmembrane transporter activity	Defense response

CG6195		1.10	1.15	Hydrolase activity	Unknown
CG7106	*lectin-28C*	1.32	1.39	G-protein coupled receptor activity	Signal transduction
CG7227		1.27	1.62	Scavenger receptor activity	Defense response
CG7275		1.13	1.28	Unknown	Phagocytosis, engulfment
CG7322		1.23	1.47	Oxidoreductase activity	Oxidation reduction
CG7560		1.2	1.55	Methylenetetra-hydrofolate reductase (NADPH) activity	Methionine metabolic process
CG7758	*pumpless* (*ppl*)	1.22	1.32	Lipoic acid binding	Glycine catabolic process
CG7780	*DNaseII*	1.29	1.52	Deoxyribonuclease II activity	DNA metabolic process
CG7993		1.15	1.16	Unknown	Unknown
CG8094	*Hexokinase C* (*Hex-C*)	1.25	1.51	Transferase activity	Glycolysis
CG8151	*TE1 binding factor (Tfb1)*	1.19	1.33	Protein binding	Transcription initiation from RNA polymerase II promoter
CG8525		1.15	1.35	Deoxyribose-phosphate aldolase activity	Metabolic process
CG8539		1.26	1.41	Carboxypeptidase activity	Proteolysis
CG8778		1.14	1.22	Enoyl-CoA hydratase activity	Metabolic process
CG9232		1.13	1.29	Nucleotidyltrans-ferase activity	Carbohydrate metabolic process
CG9377		1.63	1.83	Serine-type endopeptidase activity	Proteolysis
CG9436		1.19	1.27	Aldehyde reductase activity	Oxidation reduction
CG9556	*alien*	1.06	1.08	Protein binding	Negative regulation of transcription, DNA-dependent
CG9837	*CG9837*	1.33	1.43	Protein binding	Unknown
CG10142	*Ance-5*	1.34	1.47	Metallopeptidase activity	Proteolysis
CG10184		1.27	1.55	Catalytic activity	Cellular amino acid metabolic process
CG10621		1.41	1.40	Homocysteine S-methyltransferase activity	Unknown
CG10799		1.20	1.35	Unknown	Unknown
CG10812	*drosomycin-5* (*dro5*)	1.35	1.47	Unknown	Defense response to fungus

CG11315	*Niemann-Pick type C-2h (Npc2h)*	1.17	1.28	Unknown	Unknown
CG11901	*Ef1g*	1.08	1.08	Protein binding	Translation
CG11909	*target of brain insulin (tobi)*	1.37	1.65	Hydrolase activity	Carbohydrate metabolic process
CG12338		1.2	1.51	Oxidoreductase activity	Oxidation reduction
CG12765		1.15	1.20	Unknown	Unknown
CG12811		1.22	1.46	Protein binding	Unknown
CG13086		1.37	1.65	Binding	Unknown
CG13101		1.19	1.17	Unknown	Unknown
CG13176	*washout (wash)*	1.12	1.14	Unknown	Development
CG13189		1.12	1.20	Metal ion transmembrane transporter activity	Metal ion transport
CG13360		1.26	1.62	Unknown	Unknown
CG13922	*Mitochondrial ribosomal protein L46 (mRpL46)*	1.09	1.18	Hydrolase activity	Unknown
CG13912		1.28	1.41	Unknown	Unknown
CG14528		1.35	1.34	Hydrolase activity	Proteolysis
CG14688		1.29	1.29	Protein binding	Unknown
CG15095	*lethal(2)08717*	1.15	1.21	High affinity inorganic phosphate:sodium symporter activity	Transmembrane transport
CG15825	*fondue (fon)*	1.14	1.20	Unknown	Hemolymph coagulation
CG16704		1.21	1.44	Serine-type endopeptidase inhibitor activity	Transport
CG16756		1.2	1.48	Lysozyme activity	Antimicrobial humoral response
CG17224		1.14	1.26	Transferase activity	Nucleoside metabolic process
CG17327		1.08	1.15	Aminoacyl-tRNA hydrolase activity	Translation
CG17820	*female-specific independent of transformer (fit)*	1.37	1.32	Unknown	Unknown
CG17932	*Ugt36Bc*	1.14	1.13	Glucuronosyltrans-ferase activity	Metabolic process
CG18131		1.06	1.09	Protein binding	Unknown
CG18477		1.52	1.53	Serine-type endopeptidase activity	Proteolysis

CG31664		1.34	1.79	Structural constituent of ribosome	Translation
CG33493		1.2	1.4	Unknown	Unknown
CG33503	*Cyp12d1-d*	1.28	1.50	Oxidoreductase activity	Oxidation reduction
CG42235		1.11	1.22	Sodium-dependent multivitamin transmembrane transporter activity	Transmembrane transport
*CG42370**		1.27	1.34	Unknown	Unknown
CG42257		1.19	-1.28	Exonuclease activity	Unknown
CG33250	*AlkB*	-1.1	1.09	Oxidoreductase activity	Oxidation reduction
CG1070	*Alhambra* (*Alh*)	-1.13	-1.29	DNA binding	Regulation of transcription, DNA-dependent
CG1429	*Myocyte enhancer factor 2* (*Mfe2*)	-1.23	-1.53	Transcription factor activity	Muscle fiber development
CG1464	*eyeless (ey)*	-1.16	-1.36	Transcription factor activity	Brain development
CG1664	*small bristles* (*sbr*)	-1.16	-1.28	Nucleotide binding	Adult behavior
CG1677	*CG1677*	-1.21	-1.32	Nucleic acid binding	Unknown
CG1708	*costa* (*cos*)	-1.1	-1.23	Nucleotide binding	Microtubule-based movement
CG1725	*discs large 1* (*dlg1*)	-1.31	-1.11	Guanylate kinase activity	Synaptic transmission
CG1864	*Hormone receptor-like in 38* (*Hr38*)	-1.24	-1.21	Steroid hormone receptor activity	Regulation of transcription
CG1965		-1.12	-1.27	Transcription factor activity	Regulation of transcription
CG2052		-1.15	-1.14	Nucleic acid binding	Unknown
CG2179	*Xe7*	-1.14	-1.30	Protein binding	Unknown
CG2212	*swiss cheese* (*sws*)	-1.13	-1.21	Hydrolase activity	Lipid metabolic process
CG2247		-1.17	-1.10	Protein binding	Unknown
CG2520	*like-AP180* (*lap*)	-1.09	-1.25	Protein binding	Neurotransmitter secretion
CG2621	*shaggy* (*sgg*)	-1.25	-1.81	Protein serine/threonine kinase activity	Olfactory learning
CG2865		-1.16	-1.20	Protein binding	Unknown
CG2993		-1.43	-2.18	Unknown	Phagocytosis, engulfment
CG2999	*unc-13*	-1.11	-1.38	Zinc ion binding	Synaptic transmission
CG3268	*putative homeodomain transcription factor* (*phtf*)	-1.14	-1.43	Transcription regulator activity	Regulation of transcription
CG3361	*martik* (*mrt*)	-1.10	-1.20	Unknown	Learning or memory
CG3443	*pecanex* (*pcx*)	-1.18	-1.41	Unknown	Nervous system development

CG3613	*quaking related 58E-1 (qkr58E-1)*	-1.08	-1.24	mRNA binding	Unknown
CG3654	*Jarid2*	-1.12	-1.24	DNA binding	Unknown
CG3738	*Cyclin-dependent kinase subunit 30A (Cks30A)*	-1.27	-1.12	Cyclin-dependent protein kinase regulator activity	Cyclin catabolic process
CG3848	*trithorax-related (trr)*	-1.21	-1.28	Methyltransferase activity	smoothened signaling pathway
CG3856	*octopamine receptor in mushroom bodies (Oamb)*	-1.15	-1.32	G-protein coupled receptor activity	Octopamine/tyramine signaling pathway
CG3897	*bloated tubules (blot)*	-1.33	-1.16	Neurotransmitter transporter activity	tRNA aminoacylation for protein translation
CG4268	*Pitslre*	-1.13	-1.21	Protein serine/threonine kinase activity	Protein amino acid phosphorylation
CG4294		-1.23	-1.58	Hydrolase activity	Phosphate metabolic process
CG4532	*pod1*	-1.13	-1.33	Actin binding	Unknown
CG4641		-1.18	-1.27	Protein binding	Unknown
CG4672	*TMS1*	-1.08	-1.14	Electron carrier activity	Unknown
CG4795	*Calphotin (Cpn)*	-1.1	-1.27	Calcium ion binding	Visual perception
CG4887		-1.38	-1.30	Nucleotide binding	Unknown
CG4894	*Ca^{+2} channel protein a-1 subunit D (Caa1D)*	-1.15	-1.4	Voltage-gated ion channel activity	Ion transport
CG4952	*dachshund (dac)*	-1.11	-1.21	RNA polymerase II transcription factor activity	Neuron differentiation
CG5237	*unc-79*	-1.16	-1.10	Protein binding	Unknown
CG5403	*retained (retn)*	-1.08	-1.20	Transcription repressor activity	Axon guidance
CG5627	*rab3-GEF*	-1.16	-1.38	Guanyl-nucleotide exchange factor activity	Neurotransmitter secretion
CG5685	*Na/Ca-exchange protein (Calx)*	-1.16	-1.45	Calcium:sodium antiporter activity	Phototransduction
CG5695	*jaguar (jag)*	-1.29	-1.21	Motor activity	Spermatid development
CG5726		-1.44	-1.12	Unknown	Unknown
CG5953		-1.19	-1.22	Protein binding	Unknown
CG6027	*center divider (cdi)*	-1.12	-1.41	Protein serine/threonine kinase activity	Protein amino acid phosphorylation
CG6057	*SMC1*	-1.05	-1.14	Protein binding	Sister chromatid cohesion
CG6181	*Ge-1*	-1.14	-1.24	Protein binding	mRNA catabolic process

CG6222	*suppressor of sable (su(s))*	-1.14	-1.24	Transcription repressor activity	Unknown
CG6282		-1.1	-1.19	Oxidoreductase activity	Lipid metabolic process
CG6383	*crumbs (crb)*	-1.18	-1.12	Calcium ion binding	Nervous system development
CG6700		-1.39	-1.28	Oxidoreductase activity	Metabolic process
CG6844	*nicotinic Acetylcholine Receptor a 96Ab (nAcRa96Ab)*	-1.17	-1.32	Nicotinic acetylcholine-activated cation-selective channel activity	Ion transport
CG6867		-1.05	-1.16	Unknown	Unknown
CG6946	*glorund (glo)*	-1.18	-1.11	Nucleotide binding	Regulation of translation
CG7020	*DISCO Interacting Protein 2 (DIP2)*	-1.13	-1.28	Transcription factor binding	Metabolic process
CG7206		-1.10	-1.37	Protein binding	Unknown
CG7507	*dynein heavy chain 64C (Dhc64C)*	-1.20	-1.22	Microtubule motor activity	Intracellular protein transport
CG7535	*GluClalpha*	-1.19	-1.23	Neurotransmitter receptor activity	Ion transport
CG7736	*Syntaxin 6 (Syx6)*	-1.11	-1.43	SNAP receptor activity	Neurotransmitter secretion
CG7807	*AP-2*	-1.17	-1.14	Transcription factor activity	Regulation of transcription, DNA-dependent
CG8183	*Kinesin-73 (Khc-73)*	-1.14	-1.38	ATP binding	Microtubule-based movement
CG8271	*Silnoon (Sln)*	-1.29	-1.21	Monocarboxylic acid transmembrane transporter activity	Transmembrane transport
CG8301		-1.14	-1.10	Nucleic acid binding	Unknown
CG8318	*Neurofibro-min 1 (Nf1)*	-1.2	-1.7	GTPase activator activity	Unknown
CG8348	*Diuretic hormone (Dh)*	-1.2	-1.46	Hormone activity	Neuropeptide signaling pathway
CG8422	*Diuretic hormone 44 receptor 1 (Dh44-R1)*	-1.18	-1.27	Neuropeptide hormone activity	G-protein coupled receptor protein signaling pathway
CG8585	*Ih channel (Ih)*	-1.07	-1.15	Voltage-gated ion channel activity	Ion transport
CG8639	*Cirl*	-1.16	-1.14	G-protein coupled receptor activity	Neurotransmitter secretion
CG8651	*trithorax (trx)*	-1.15	-1.30	Histone methyltransferase activity (H3-K4 specific)	Regulation of transcription, DNA-dependent
CG8683		-1.21	-1.21	Protein binding	Transport

CG8676	*Hormone receptor-like in 39* (*Hr39*)	-1.12	-1.08	Steroid hormone receptor activity	Regulation of transcription, DNA-dependent
CG8929		-1.11	-1.10	Protein binding	Unknown
CG9056	*tay bridge* (*tay*)	-1.12	-1.36	Unknown	Adult walking behavior
CG9121		-1.10	-1.20	Protein binding	Cytoskeletal anchoring at plasma membrane
CG9266		-1.22	-1.28	Unknown	Unknown
CG9252	*deadlock* (*del*)	-1.16	-1.38	Protein binding	Cell differentiation
CG9351	*falafel* (*flfl*)	-1.19	-1.21	Unknown	DNA repair
CG9811	*Rgk1*	-1.12	-1.12	GTP binding	Signal transduction
CG9983	*Heterogenous nuclear ribonucleo-protein at 98DE* (*Hrb98DE*)	-1.27	-1.12	mRNA binding	Unknown
CG10077		-1.44	-1.41	ATP-dependent helicase activity	Unknown
CG10186		-1.21	-1.24	Peptidase inhibitor activity	Transport
CG10192	*eukaryotic translation factor 4G2* (*eIF4G2*)	-1.12	-1.32	RNA7-methylguanosine cap binding	Spermatogenesis
CG10260	*Pi4KIIa*	-1.38	-1.12	Transferase activity	Phosphoinositide phosphorylation
CG10440		-1.22	-1.28	Voltage-gated potassium channel activity	Potassium ion transport
CG10443	*Leukocyte-antigen-related-like* (*Lar*)	-1.3	-1.92	Protein tyrosine phosphatase activity	Nervous system development
CG10473	*hook-like* (*hkl*)	-1.12	-1.32	Nucleotide binding	Apoptosis
CG10697	*Dopa decarboxylase* (*Ddc*)	-1.15	-1.11	Aromatic-L-amino-acid decarboxylase activity	Courtship behavior
CG10851	*Serine/arginine rich protein 55* (*B52*)	-1.16	-1.28	Nucleic acid binding	Regulation of nuclear mRNA splicing, via spliceosome
CG10986	*garnet* (*g*)	-1.24	-1.37	Protein binding	Vesicle-mediated transport
CG11144	*metabotropic glutamate receptor* (*mGluRA*)	-1.42	-1.22	G-protein coupled receptor activity	Regulation of synaptic transmission, glutamatergic
CG11155		-1.1	-1.18	Extracellular-glutamate-gated ion channel activity	Ion transport
CG11303	*Transmembrane 4 superfamily* (*TM4SF*)	-1.06	-1.12	Unknown	Unknown
CG11325	*Gonadotropin-releasing hormone receptor* (*GRHR*)	-1.14	-1.23	G-protein coupled receptor activity receptor activity	Triglyceride homeostasis

CG11328	*Na+/H+ hydrogen exchanger 3* (*Nhe3*)	-1.29	-1.43	Sodium:hydrogen antiporter activity	Ion transport
CG11376		-1.20	-1.14	GTPase binding	Unknown
CG11638		-1.14	-1.21	Calcium ion binding	Unknown
CG11895	*starry night* (*stan*)	-1.16	-1.10	G-protein coupled receptor activity	Axon guidance
CG12071		-1.15	-1.43	Nucleic acid binding	Phagocytosis, engulfment
CG12076	*YT521-B*	-1.26	-1.27	Unknown	Unknown
CG12191	*dpr20*	-1.11	-1.14	Unknown	Unknown
CG12239		-1.08	-1.2	Unknown	Unknown
CG12295	*straightjacket* (*stj*)	-1.10	-1.30	Voltage-gated calcium channel activity	Synaptic vesicle fusion to presynaptic membrane
CG12348	*Shaker* (*Sh*)	-1.26	-1.10	Voltage-gated cation channel activity	Regulation of synaptic activity and courtship behavior
CG12449	*Glutamine: fructose-6-phosphate aminotransferase 1* (*Gfat1*)	-1.35	-1.69	Glutamine-fructose-6-phosphate transaminase (isomerizing) activity	Carbohydrate metabolic process
CG12478	*bruno-3* (*bru-3*)	-1.19	-1.20	RNA binding	Negative regulation of translation
CG12500	*stoned A* (*stnA*)	-1.1	-1.2	Protein binding	Neurotransmitter secretion
CG13521	*roundabout* (*robo*)	-1.09	-1.18	Receptor activity	Axon guidance
CG13594		-1.27	-1.38	Unknown	Unknown
CG13900		-1.17	-1.21	Nucleic acid binding	Nuclear mRNA splicing, via spliceosome
CG13928		-1.17	-1.12	Protein binding	Unknown
CG14234		-1.11	-1.22	Unknown	Unknown
CG14408		-1.38	-1.22	SH3 domain binding	Unknown
CG14521		-1.34	-1.41	Unknown	Unknown
CG14616	*lethal (1)G0196*	-1.21	-1.48	Acid phosphatase activity	Inositol metabolic process
CG14723	*Histamine-gated chloride channel subunit 1* (*HisCl1*)	-1.09	-1.24	Neurotransmitter receptor activity	Ion transport
CG14755		-1.39	-1.75	Unknown	Unknown
CG14889		-1.15	-1.28	Unknown	Unknown
CG15104	*Topoisomerase I-interacting protein* (*Topors*)	-1.13	-1.24	DNA binding	Regulation of transcription
CG15270	*Axs-like*	-1.10	-1.12	Unknown	Unknown
CG15465		-1.31	-2.32	Unknown	Unknown
CG16777		-1.31	-1.89	Unknown	Unknown

CG16778	*BTB-protein-III*	-1.13	-1.16	DNA binding	Gravitaxis
CG16899	*forkhead domain 85E (fd85E)*	-1.32	-1.76	Transcription factor activity	Regulation of transcription, DNA-dependent
CG17136	*RNA-binding protein 1 (Rbp1)*	-1.29	-1.35	Nucleic acid binding	mRNA splice site selection
CG17360		-1.09	-1.17	Protein binding	Unknown
CG17684		-1.37	-1.28	Serine-type peptidase activity	Proteolysis
CG17686	*DISCO interacting protein 1 (DIP1)*	-1.18	-1.34	Double-stranded RNA binding	Unknown
CG17724		-1.25	-1.37	Zinc ion binding	Axonogenesis
CG17760		-1.27	-1.22	GTPase activity	G-protein coupled receptor protein signaling pathway
CG17762	*tomosyn*	-1.08	-1.23	Syntaxin-1 binding	Neurotransmitter secretion
CG17786		-1.19	-1.66	Unknown	Unknown
CG17977		-1.11	-1.43	Unknown	Unknown
CG18437		-1.14	-1.14	Cation channel activity	Cation homeostasis
CG18769		-1.18	-1.10	Unknown	Unknown
CG30492		-1.17	-1.21	Zinc ion binding	Unknown
CG31064		-1.11	-1.23	Zinc ion binding	Phagocytosis, engulfment
CG31116		-1.15	-1.12	Voltage-gated ion channel activity	Ion transport
CG31132	*BRWD3*	-1.16	-1.22	Unknown	Phagocytosis, engulfment
CG31176		-1.13	-1.41	Protein binding	Unknown
CG31181		-1.13	-1.24	Unknown	Unknown
CG31182		-1.22	-1.24	Unknown	Unknown
CG31349	*polychaetoid (pyd)*	-1.21	-1.54	Oxidoreductase activity	Cell-cell adhesion
CG31361	*dpr17*	-1.17	-1.12	Protein binding	Unknown
CG31534	*CG31534*	-1.12	-1.28	Protein binding	Unknown
CG32149	*RhoGAP71E*	-1.20	-1.32	Hydrolase activity	Phosphate metabolic process
CG32156	*Myosin binding subunit (Mbs)*	-1.16	-1.38	Myosin phosphatase activity	Regulation of compound eye photoreceptor development
CG32174		-1.15	-1.16	Pyrimidine nucleotide sugar transmembrane transporter activity	Carbohydrate transport
CG32217	*Su(Tpl)*	-1.17	-1.11	Transcription elongation regulator activity	Regulation of transcription in response to stress
CG32425		-1.07	-1.15	Protein binding	Unknown
CG32434	*schizo (siz)*	-1.09	-1.28	Guanyl-nucleotide exchange factor activity	Central nervous system development

CG32491	*Modifier of mdg4 (mod(mdg4))*	-1.14	-1.22	Transcription factor activity	Induction of apoptosis
CG32555	*Rp190-RhoGAP*	-1.17	-1.23	GTPase activator activity	Regulation of axonogenesis
CG32813		-1.10	-1.14	Unknown	Unknown
CG32937		-1.13	-1.21	Unknown	Unknown
CG32944		-1.17	-1.36	Nucleotide binding	Protein amino acid phosphorylation
CG33135	*KCNQ potassium channel (KCNQ)*	-1.13	-1.10	Voltage-gated potassium channel activity	Regulation of heart rate
CG33141	*sticks and stones (sns)*	-1.18	-1.42	Unknown	Cell adhesion
CG33143		-1.14	-1.28	Unknown	Unknown
CG33144		-1.16	-1.43	Protein binding	Unknown
CG33174	*inactivation no afterpotential E (inaE)*	-1.36	-1.27	Triacylglycerol lipase activity	Lipid metabolic process
CG33197	*muscleblind (mbl)*	-1.44	-1.14	Zinc ion binding	Muscle development
CG33472	*quiver (qvr)*	-1.14	-1.24	Unknown	Unknown
CG33522	*scaf6*	-1.16	-1.30	Nucleic acid binding	nuclear mRNA splicing, via spliceosome
CG33526	*PNUTS*	-1.12	-1.21	Nucleic acid binding	Regulation of protein amino acid dephosphorylation
CG33547	*Rim*	-1.45	-1.89	Rab GTPase binding	Neurotransmitter secretion
CG33554	*Nipped-A*	-1.22	-1.10	Histone acetyltransferase activity	Regulation of transcription
CG34123		-1.26	-1.6	Ion channel activity	Thermotaxis
CG34319		-1.10	-1.38	Unknown	Unknown
CG34362		-1.09	-1.21	Nucleotide binding	Regulation of alternative nuclear mRNA splicing, via spliceosome
CG34404		-1.10	-1.12	Protein binding	Unknown
CG34413	*Na,K-ATPase Interacting (NKAIN)*	-1.16	-1.22	Protein binding	Regulation of sodium ion transport
CG40498		-1.14	-1.11	Unknown	Unknown
CG42252	*mind-meld (mmd)*	-1.07	-1.37	Metalloendopep-tidase activity	Proteolysis
CG42253	*Na+-driven anion exchanger 1 (Ndae1)*	-1.11	-1.28	Anion transmembrane transporter activity	Proton transport
CG42286		-1.21	-1.77	Protein binding	Unknown
CG42320	*Darkener of apricot (Doa)*	-1.09	-1.22	Protein serine/threonine kinase activity	Nervous system development
CG42492		-1.32	-1.64	Unknown	Unknown

CG42543	*multiplexin (mp)*	-1.16	-1.33	Structural molecule activity	Cell adhesion
CG42555	*tweek*	-1.17	-1.23	Protein binding	Unknown
CG42614	*scribbled* (*scrib*)	-1.21	-1.43	Unknown	Unknown
CG42668		-1.09	-1.14	Oxysterol binding	Lipid transport
CG42670	*pasilla* (*ps*)	-1.84	-2.13	mRNA binding	Nuclear mRNA splicing, via spliceosome
CG42679	*Limpet (Lmpt)*	-1.14	-1.32	Transcription factor activity	Unknown
CG42698	*pou domain motif 3* (*pdm3*)	-1.39	-1.75	Transcription factor activity	Regulation of transcription, DNA-dependent
CG42795		-1.24	-1.10	Rab GTPase activator activity	Regulation of Rab GTPase activity

When comparing all three treatment groups (control, courting and male-exposed) by mixed ANOVA and Tukey's post-hoc analyses ($p<0.05$) 265 genes were found to be socially-responsive. **CG42370* was only significant in 2 of the 5 algorithms.

TABLE S2

Male-male-responsive genes

Gene identifier	Gene name	Avg. fold change of Male-male heads compared to:		GO Molecular function	GO Biological process
		Courting male heads	Control male heads		
CG1041		1.13	1.27	Carnitine O-acetyltransferase activity	Unknown
CG1381	*Ribosomal protein LP0-like* (*RpLP0-like*)	1.17	1.19	Unknown	Ribosome biogenesis
CG1532		1.15	1.23	Unknown	Unknown
CG1665		1.18	1.37	Pyridoxal phosphate binding	Unknown
CG2034		1.11	1.15	Protein binding	Unknown
CG2155	*vermilion* (*v*)	1.23	1.33	Tryptophan 2,3-dioxygenase activity	Oxidation reduction
CG2200		1.19	1.27	Serine-type peptidase activity	Proteolysis
CG2263		1.13	1.12	Aminoacyl-tRNA ligase activity	tRNA aminoacylation for protein translation
CG3097		1.21	1.21	Metallocarboxy-peptidase activity	Proteolysis
CG3322	*Laminin B2* (*LanB2*)	1.16	1.20	Sugar:hydrogen symporter activity	Cell adhesion
CG3353		1.28	1.40	Protein binding	Unknown
CG3460	*Nonsense-mediated mRNA 3* (*Nmd3*)	1.16	1.15	mRNA binding	Ribosomal large subunit export from nucleus
CG3564	*CHOp24*	1.16	1.17	Protein binding	Transport
CG3756		1.12	1.13	Protein dimerization activity	Transcription from RNA polymerase III promoter
CG3790		1.43	1.99	Serine-type endopeptidase inhibitor activity	Transmembrane transport
CG3887		1.14	1.15	Selenium binding	Cell redox homeostasis
CG3999		1.31	1.53	Glycine dehydrogenase (decarboxylating) activity	Oxidation reduction
CG4311	*HMG Coenzyme A synthase* (*Hmgs*)	1.14	1.18	Hydroxymethyl-glutaryl-CoA synthase activity	Metabolic process
CG4389		1.14	1.20	3-hydroxyacyl-CoA dehydrogenase activity	Fatty acid metabolic process
CG4598		1.17	1.40	Dodecenoyl-CoA D-isomerase activity	Metabolic process
CG4645		1.17	1.15	Unknown	Unknown

CG4752		1.26	1.45	5-oxoprolinase (ATP-hydrolyzing) activity	Unknown
CG4821	*Tequila*	1.17	1.36	Serine-type endopeptidase activity	Long-term memory
CG5224		1.23	1.41	Glutathione transferase activity	Unknown
CG5231	*Lipoic acid synthase* (*Las*)	1.11	1.11	Sulfurtransferase activity	Metabolic process
CG5254		1.14	1.26	Transmembrane transporter activity	Autophagic cell death
CG5268	*black pearl* (*blp*)	1.08	1.12	Unknown	Protein transport
CG5431		1.15	1.34	Sulfotransferase activity	Unknown
CG5479	*Mitochondrial ribosomal protein L43* (*mRpL43*)	1.18	1.27	Structural constituent of ribosome	Translation
CG5783		1.20	1.25	N-acetyltransferase activity	Metabolic process
CG6008	*NP15.6*	1.09	1.12	Unknown	Unknown
CG6011	*Prp18*	1.14	1.25	RNA splicing factor activity, transesterification mechanism	Nuclear mRNA splicing, via spliceosome
CG6067		1.14	1.21	Serine-type endopeptidase activity	Proteolysis
CG6895	*Gram-negative bacteria binding protein 1* (*GNBP1*)	1.15	1.23	Lipopoly-saccharide binding	Immune response
CG7265		1.15	1.21	Protein binding	Peptidyl-diphthamide biosynthetic process from peptidyl-histidine
CG7529	*Esterase Q* (*Est-Q*)	1.27	1.45	Carboxylesterase activity	Unknown
CG7554	*comm2*	1.37	1.25	Unknown	Unknown
CG7637		1.24	1.27	Protein binding	Ribosome biogenesis
CG7671		1.20	1.29	Protein binding	Unknown
CG7845		1.11	1.13	Unknown	Unknown
CG8067		1.16	1.11	Methyltransferase activity	Metabolic process
CG8340	*upstream of RpIII128* (*128up*)	1.16	1.21	GTP binding	Unknown
CG8586		1.20	1.41	Serine-type endopeptidase activity	Proteolysis
CG8674	*lethal* (*2*) *k14505*	1.13	1.14	Protein binding	Proton-transporting ATP synthase complex assembly
CG8781	*tsunagi* (*tsu*)	1.11	1.18	Nucleotide binding	mRNA export from nucleus
CG8891		1.20	1.26	Hydrolase activity	Unknown
CG8946	*Sphingosine 1 phosphate lyase* (*Sply*)	1.10	1.16	Catalytic activity	Sphingolipid catabolic process
CG9000	*prenyl protease type I* (*ste24a*)	1.14	1.20	Metalloendopep-tidase activity	Proteolysis

CG9022	*Oligosaccharyltransferase 48kD subunit* (*Ost48*)	1.12	1.24	Dolichyl-diphosphooligo-saccharide-protein glycotransferase activity	Protein amino acid N-linked glycosylation
CG9067		1.19	1.28	Unknown	ER to Golgi vesicle-mediated transport
CG9342	*Microsomal triacylglycerol transfer protein* (*Mtp*)	1.14	1.23	Lipid transporter activity	Triglyceride metabolic process
CG9358	*Pherokine 3* (*Phk-3*)	1.25	1.26	Protein serine/threonine kinase activity	Spermatogenesis
CG9394		1.32	1.56	Glycerophosphodiester phosphodiesterase activity	Carbohydrate metabolic process
CG9629		1.20	1.28	Aldehyde dehydrogenase (NAD) activity	Oxidation reduction
CG9669		1.23	1.23	Protein binding	Unknown
CG9911		1.13	1.12	Protein disulfide isomerase activity	Cell redox homeostasis
CG10038		1.16	1.20	Protein serine/threonine kinase activity	Protein amino acid phosphorylation
CG10226		1.12	1.17	ATPase activity	Transmembrane transport
CG10247	*Cyp6a21*	1.16	1.30	Metal ion binding	Oxidation reduction
CG10587		1.13	1.14	GTPase activity	Proteolysis
CG10685	*lethal(2)37Cg*	1.18	1.18	DNA-directed RNA polymerase activity	Transcription from RNA polymerase I promoter
CG10908	*Derlin-1* (*Der-1*)	1.10	1.18	Peptidase activity	ER-associated protein catabolic process
CG11027	*ADP ribosylation factor 102F* (*Arf102F*)	1.09	1.10	GTPase activity	Neurotransmitter secretion
CG11488	*Mitochondrial ribosomal protein L10* (*mRpL10*)	1.11	1.16	Structural constituent of ribosome	Translation
CG11761	*translin* (*trsn*)	1.14	1.19	Sequence-specific DNA binding	Unknown
CG11780	*b-4-galactosyltran-sferase 7* (*b 4GalT7*)	1.11	1.21	Xylosylprotein 4-b-galactosyltrans-ferase activity	Carbohydrate metabolic process
CG11979	*Rpb5*	1.12	1.21	DNA-directed RNA polymerase activity	Transcription from RNA polymerase II promoter
CG11981	*Proteasome b3 subunit* (*Prosb3*)	1.11	1.19	Threonine-type endopeptidase activity	Cellular response to DNA damage stimulus
CG12582		1.24	1.26	b-mannosidase activity	Carbohydrate metabolic process
CG13090		1.25	1.28	Mo-molybdopterin cofactor sulfurase activity	tRNA processing
CG13369		1.17	1.26	Ribokinase activity	D-ribose metabolic process

CG13698		1.22	1.24	Unknown	Unknown
CG13795		1.23	1.35	Neurotransmitter: sodium symporter activity	Neurotransmitter transport
CG13822		1.20	1.26	Protein binding	Unknown
CG14527		1.12	1.19	Metalloendopeptidase activity	Proteolysis
CG14680	*Cyp12e1*	1.24	1.30	Metal ion binding	Oxidation reduction
CG14823		1.24	1.32	Lysozyme activity	Unknown
CG15199		1.29	1.52	Protein binding	Unknown
CG15201		1.17	1.24	Unknown	Unknown
CG15456		1.27	1.30	Selenium binding	Cell redox homeostasis
CG16711		1.39	1.49	Protein binding	Unknown
CG16713		1.22	1.28	Serine-type endopeptidase inhibitor activity	Unknown
CG17527	*Glutathione S transferase E5* (*GstE5*)	1.24	1.39	Glutathione transferase activity	Unknown
CG17996		1.23	1.23	tRNA-intron endonuclease activity	tRNA splicing, via endonucleolytic cleavage and ligation
CG18591	*Small ribonucleo-protein particle protein SmE* (*SmE*)	1.15	1.21	RNA splicing factor activity, transesterification mechanism	Spliceosome assembly
CG30287		1.14	1.24	Serine-type endopeptidase activity	Proteolysis
CG30349		1.18	1.17	Unknown	Unknown
CG30382		1.15	1.26	Endopeptidase activity	Ubiquitin-dependent protein catabolic process
CG30498	*boca*	1.14	1.23	Unknown	ER to Golgi vesicle-mediated transport
CG31102		1.23	1.28	Protein binding	Unknown
CG31300		1.17	1.21	Protein binding	Unknown
CG32069		1.24	1.31	Unknown	Unknown
CG32115		1.24	1.23	Unknown	Unknown
CG32441		1.09	1.13	Unknown	Unknown
CG34200		1.14	1.16	Unknown	Unknown
CG1316		-1.37	-1.51	Nucleotide binding	Unknown
CG1559	*Upf1*	-1.27	-1.32	Helicase activity	RNA interference
CG1609	*Gcn2*	-1.14	-1.12	Elongation factor-2 kinase activity	Regulation of translation
CG1636		-1.45	-1.49	Protein binding	Unknown
CG1697	*rhomboid-4* (*rho-4*)	-1.23	-1.30	Serine-type endopeptidase activity	DNA repair

CG1725	*discslarge* (*dlg1*)	-1.41	-1.75	Epidermal growth factor receptor binding	Nervous system development
CG1794	*Matrix metalloprotein-ase 2* (*Mmp2*)	-1.36	-1.54	Metalloendopeptidase activity	Proteolysis
CG1836	*Rad23*	-1.10	-1.11	Damaged DNA binding	Nucleotide-excision repair
CG1862	*Ephrin*	-1.52	-1.70	Ephrin receptor binding	Axon guidance
CG1873	*elongation factor 1-a 100E* (*Ef1a100E*)	-1.13	-1.14	GTPase activity	Translation
CG1915	*sallimus* (*sls*)	-1.26	-1.37	Actin binding	Mitosis
CG1945	*fat facets* (*faf*)	-1.54	-1.67	Ubiquitin thiolesterase activity	Visual perception
CG2048	*discs overgrown* (*dco*)	-1.24	-1.32	Protein serine/threonine kinase activity	Regulation of ecdysteroid secretion
CG2096	*flapwing* (*flw*)	-1.11	-1.19	Protein serine/threonine phosphatase activity	Cell adhesion
CG2218		-1.15	-1.12	Ubiquitin-protein ligase activity	Protein ubiquitination
CG2239	*jdp*	-1.60	-1.72	Unfolded protein binding	Protein folding
CG2822	*Shaker cognate w* (*Shaw*)	-1.75	-2.00	Voltage-gated ion channel activity	Ion transport
CG3548		-1.10	-1.14	Protein binding	Unknown
CG3585	*Rabconnectin-3A* (*Rbcn-3A*)	-1.36	-1.41	Acyltransferase activity	Microtubule-based process
CG3665	*Fasciclin II* (*Fas2*)	-1.15	-1.20	Protein binding	Learning or memory
CG3682	*PIP5K59B*	-1.71	-1.89	Phosphatidylinositol phosphate kinase activity	Phosphatidylinositol metabolic process
CG3861	*knockdown* (*kdn*)	-1.13	-1.20	Citrate (Si)-synthase activity	Behavior
CG4049		-1.40	-1.43	Helicase activity	DNA repair
CG4070	*Tis11 homolog* (*Tis11*)	-1.15	-1.15	Nucleic acid binding	RNA interference
CG4128	*nicotinic Acetylcholine Receptor a30D* (*nAcRa30D*)	-1.30	-1.43	Neurotransmitter receptor activity	Ion transport
CG4353	*hemipterous* (*hep*)	-1.34	-1.41	Protein serine/threonine kinase activity	Axon extension
CG4527	*Sterile20-like kinase* (*Slik*)	-1.11	-1.22	Protein serine/threonine kinase activity	Cell proliferation
CG4629		-1.14	-1.22	Protein serine/threonine kinase activity	Protein amino acid phosphorylation
CG4911		-1.52	-1.67	Protein binding	Unknown
CG5067	*capicua* (*cic*)	-1.59	-1.70	Transcription factor activity	Regulation of transcription, DNA-dependent
CG5125	*neither inactivation nor afterpotential C* (*ninaC*)	-1.17	-1.23	Protein serine/threonine kinase activity	Visual perception
CG5559	*Synaptotagmin a* (*Syta*)	-1.47	-1.56	Unknown	Unknown
CG5594	*kazachoc* (*kcc*)	-1.14	-1.25	Amino acid transmembrane transporter activity	Sodium ion transport

CG5629	*Phosphopanto-thenoylcysteine synthetase* (*Ppcs*)	-1.51	-1.61	Unknown	Unknown
CG5683	*Adult enhancer factor 1* (*Aef1*)	-1.26	-1.33	Transcription factor activity	Regulation of transcription
CG5821	*quaking related 58E-2* (*qkr58E-2*)	-1.19	-1.25	mRNA binding	Unknown
CG5915	*Rab-protein 7* (*Rab7*)	-1.12	-1.12	GTPase activity	Endosome to lysosome transport
CG6303	*Bruce*	-1.23	-1.30	Ubiquitin-protein ligase activity	Spermatid development
CG6364		-1.47	-1.47	Transferase activity	Metabolic process
CG6588	*Fasciclin I* (*Fas1*)	-1.76	-2.17	Cell adhesion molecule binding	Axon guidance
CG6619		-1.35	-1.37	Protein binding	Unknown
CG6622	*Protein C kinase 53E* (*Pkc53E*)	-1.34	-1.45	Protein serine/threonine kinase activity	Protein amino acid phosphorylation
CG6772	*Slowpoke binding protein* (*Slob*)	-1.11	-1.15	Protein kinase activity	Regulation of synaptic transmission
CG6998	*cut up* (*ctp*)	-1.17	-1.07	Microtubule motor activity	Spermatogenesis
CG7085	*lethal(2)s5379*	-1.18	-1.22	Protein binding	Unknown
CG7100	*Cadherin-N* (*CadN*)	-1.50	-1.79	b-catenin binding	Axon guidance
CG7134	*cdc14*	-1.46	-1.49	Protein tyrosine/serine/ threonine phosphatase activity	Protein amino acid dephosphorylation
CG7149		-1.08	-1.10	Diacylglycerol cholinephosphotrans-ferase activity	Phagocytosis, engulfment
CG7177	*Wnk*	-1.16	-1.27	Protein serine/threonine kinase activity	Protein amino acid phosphorylation
CG7558	*Actin-related protein 66B* (*Arp66B*)	-1.11	-1.12	Nucleotide binding	Axonal fasciculation
CG7761	*parcas* (*pcs*)	-1.28	-1.30	Protein tyrosine kinase inhibitor activity	Programmed cell death
CG7875	*transient receptor potential* (*trp*)	-1.17	-1.22	Light-activated voltage-gated Calcium channel activity	Olfactory learning
CG7892	*nemo* (*nmo*)	-1.21	-1.32	Protein serine/threonine kinase activity	Gravitaxis
CG7966		-1.20	-1.36	Selenium binding	Unknown
CG8068	*Su(var)2-10*	-1.37	-1.39	Nucleic acid binding	Regulation of protein catabolic process
CG8085	*tre oncogene-related protein* (*RN-tre*)	-1.30	-1.39	Rab GTPase activator activity	Regulation of Rab GTPase activity
CG8224	*baboon* (*babo*)	-1.43	-1.75	G-protein coupled receptor kinase activity	Axon guidance
CG8245		-1.07	-1.12	Unknown	Unknown
CG8250	*Alk*	-1.13	-1.27	Protein tyrosine kinase activity	Axon guidance

CG8386		-1.18	-1.27	Unknown	Unknown
CG8398		-1.23	-1.22	Unknown	Unknown
CG8878		-1.10	-1.20	Protein serine/threonine kinase activity	Protein amino acid phosphorylation
CG9028	*short spindle 2* (*ssp2*)	-1.44	-1.43	Unknown	Mitotic spindle elongation
CG9108	*Regulator of G-protein signalling 7* (*FSG7*)	-1.74	-1.96	Signal transducer activity	G-protein coupled receptor protein signaling pathway
CG9153		-1.16	-1.27	Ubiquitin-protein ligase activity	Protein modification process
CG9279		-1.11	-1.11	Unknown	Microtubule-based movement
CG9375	*Ras oncogene at 85D* (*Ras85D*)	-1.14	-1.16	GTPase activity	Peripheral nervous system development
CG9474	*Synapse protein 24* (*Snap24*)	-1.32	-1.41	Soluble NSF attachment protein activity	Neurotransmitter secretion
CG9739	*frizzled2* (*fz2*)	-1.21	-1.27	G-protein coupled receptor activity	Axon extension
CG9765	*transforming acidic coiled-coil protein* (*tacc*)	-1.32	-1.43	Microtubule binding	Negative regulation of microtubule depolymerization
CG9819	*Calcineurin A at 14F* (*CanA-14F*)	-1.93	-2.44	Protein serine/threonine phosphatase activity	Protein amino acid dephosphorylation
CG31045	*Myosin heavy chain-like* (*Mhcl*)	-1.21	-1.33	ATP binding	Unknown
CG10108	*phyllopod* (*phyl*)	-1.12	-1.14	Protein binding	Peripheral nervous system development
CG10249		-1.08	-1.12	Unknown	Unknown
CG10272	*grappa* (*gpp*)	-1.32	-1.49	Histone-lysine N-methyltransferase activity	Chromatin silencing
CG10542	*Bre1*	-1.16	-1.27	Protein binding	Peripheral nervous system development
CG10624	*sinuous* (*sinu*)	-1.08	-1.14	Unknown	Establishment of endothelial blood-brain barrier
CG10631		-1.13	-1.18	Nucleic acid binding	Unknown
CG10686	*trailer hitch* (*tral*)	-1.41	-1.56	Unknown	ER to Golgi vesicle-mediated transport
CG10701	*Moesin* (*Moe*)	-1.13	-1.16	Actin binding	Olfactory behavior
CG10847	*encore* (*enc*)	-1.19	-1.30	Nucleic acid binding	Mitosis
CG10915		-1.30	-1.37	Unknown	Unknown
CG10946	*dpr14*	-1.29	-1.32	Unknown	Unknown
CG11186	*twin of eyeless* (*toy*)	-1.52	-1.56	Specific RNA polymerase II transcription factor activity	Regulation of transcription, DNA-dependent

CG11526		-1.09	-1.11	Protein binding	Unknown
CG11596		-1.12	-1.01	Unknown	Unknown
CG11760		-1.13	-1.18	Unknown	Unknown
CG11814		-1.23	-1.28	Protein binding	Lysosomal transport
CG12051	*Actin 42A* (*Act42A*)	-1.10	-1.11	Nucleotide binding	Phagocytosis, engulfment
CG12052	*longitudinals lacking* (*lola*)	-1.61	-1.79	RNA polymerase II transcription factor activity binding	Nervous system development
CG12121		-1.12	-1.14	Unknown	Unknown
CG12348	*Shaker* (*Sh*)	-1.50	-1.85	Voltage-gated ion channel activity	Courtship behavior
CG12455		-1.60	-1.61	Voltage-gated calcium channel activity	Unknown
CG12605		-1.32	-1.54	Nucleic acid binding	Unknown
CG12858		-1.60	-1.85	Unknown	Transmembrane transport
CG13253		-1.25	-1.27	Insulin-like growth factor binding	Regulation of cell growth
CG13778	*Menin 1* (*Mnn1*)	-1.58	-1.61	Unknown	Response to stress
CG14180		-1.60	-1.92	Unknown	Unknown
CG14411		-1.19	-1.19	Phosphatase activity	Dephosphorylation
CG14446		-2.15	-2.50	Protein binding	Unknown
CG14562		-1.31	-1.41	Unknown	Unknown
CG14616	*lethal(1)G0196*	-1.24	-1.33	Diphosphoinositol-pentakisphosphate kinase activity	Inositol metabolic process
CG14685	*Chromosome associated protein H2* (*Cap-H2*)	-1.23	-1.32	Protein binding	Chromosome organization
CG14982		-1.37	-1.49	Protein binding	Unknown
CG15630		-1.65	-1.67	Unknown	Unknown
CG16717		-1.26	-1.20	Hydrolase activity	Unknown
CG17100	*clockwork orange* (*cwo*)	-1.33	-1.32	Transcription regulator activity	Peripheral nervous system development
CG17245	*plexin-B* (*plexB*)	-1.28	-1.37	Semaphorin receptor activity	Nervous system development
CG17369	*Vacuolar H+-ATPase 55kD B subunit* (*Vha55*)	-1.10	-1.14	Hydrogen-exporting ATPase activity, phosphorylative mechanism	Ion transport
CG17883		-1.11	-1.16	Rab GTPase activator activity	Regulation of Rab GTPase activity
CG18676	*tipE homolog 3* (*Teh3*)	-1.62	-1.70	Structural constituent of ribosome	Translation
CG18812		-1.21	-1.27	Unknown	Unknown
CG30023	*sprite* (*sprt*)	-1.14	-1.19	Protein binding	Larval heart development

CG30428		-1.40	-1.47	Protein binding	Unknown
CG30483	*Prosap*	-1.34	-1.45	Protein binding	Unknown
CG31151	*winged eye* (*wge*)	-1.19	-1.23	DNA binding	Cell differentiation
CG31243	*couch potato* (*cpo*)	-1.31	-1.47	Nucleotide binding	Olfactory behavior
CG31298	*beat-Vb*	-1.12	-1.15	Unknown	Unknown
CG31760		-1.37	-1.56	G-protein coupled receptor activity	Gamma-aminobutyric acid signaling pathway
CG32082		-1.63	-1.64	signal transducer activity	Filopodium assembly
CG32164		-1.26	-1.25	Protein transporter activity	Intracellular protein transport
CG32464	*lethal(3)82Fd*	-1.36	-1.43	Protein binding	Cell wall macromolecule catabolic process
CG32592	*highwire* (*hiw*)	-1.42	-1.59	Ubiquitin-protein ligase activity	Regulation of synaptic growth at neuromuscular junction
CG32699		-1.44	-1.54	Acyltransferase activity	Phospholipid biosynthetic process
CG33087	*LDL receptor protein 1* (*LRP1*)	-1.28	-1.45	Low-density lipoprotein receptor activity	Unknown
CG33208	*Molecule interacting with CasL* (*Mical*)	-1.47	-1.72	Oxidoreductase activity	Axon guidance
CG33330		-1.45	-1.59	Unknown	Unknown
CG33512	*dpr4*	-1.31	-1.37	Unknown	Unknown
CG33519	*Unc-89*	-1.16	-1.15	Protein serine/threonine kinase activity	Regulation of Rho protein signal transduction
CG33555	*bitesize* (*btsz*)	-1.27	-1.32	Transporter activity	Synaptic vesicle transport
CG33556	*formin 3* (*form3*)	-1.20	-1.30	Actin binding	Actin cytoskeleton organization
CG33967		-1.49	-1.70	Protein binding	Unknown
CG34127		-1.52	-1.79	Carboxylesterase activity	Phagocytosis, engulfment
CG34405	*Na channel protein 60E* (*NaCP60E*)	-1.76	-2.33	Voltage-gated ion channel activity	Olfactory behavior
CG34410	*Rab26*	-1.37	-1.47	GTPase activity	Regulation of exocytosis
CG42236	*Ran-binding protein M* (*RanBPM*)	-1.12	-1.22	Ran GTPase binding	JAK-STAT cascade
CG42278	*cornetto* (*corn*)	-1.29	-1.43	Protein binding	Unknown
CG42281	*bunched* (*bun*)	-1.32	-1.37	Protein homodimerization activity	Peripheral nervous system development
CG42314	*Plasma membrane calcium ATPase* (*PMCA*)	-1.15	-1.30	Calcium-transporting ATPase activity	ATP biosynthetic process
CG42316	*RhoGAP102A*	-1.87	-2.13	Rho GTPase activator activity	signal transduction
CG42328	*C3G*	-1.19	-1.37	Ras guanyl-nucleotide exchange factor activity	small GTPase mediated signal transduction

CG42368		-1.34	-1.35	Testosterone 17-b-dehydrogenase activity	Fatty acid metabolic process
CG42616	*Cullin-3* (*Cul-3*)	-1.39	-1.51	Ubiquitin-protein ligase activity	Ubiquitin-dependent protein catabolic process
CG42768	*Muscle-specific protein 300* (*Msp-300*)	-1.66	-1.75	Rab GTPase activator activity	Regulation of Rab GTPase activity
CG67671	*Argonaute-1* (*AGO1*)	-1.67	-2.00	Nucleic acid binding	RNA interference

Two-hundred forty genes show significant changes (95 up regulated and 145 down regulated) in expression when a male is exposed to a male. Control, courting and male-exposed male heads were compared by mixed ANOVA and Tukey's post-hoc analyses ($p<0.05$).

TABLE S3

Courtship-responsive genes from Tables 1 and 2 are enriched in head tissues including the brain and the fat body

	Total no. of genes	Head	Brain	Eye	Fat body
Up regulated	16	14	6	11	12
Down regulated	19	17	17	16	15

Data was compiled from FlyAtlas (Chintapalli et al., 2007).