ABSTRACT

SAMBANDAN, DEEPA. The Genetic Architecture of Odor-Guided Behavior in *Drosophila melanogaster*. (Under the direction of Dr. Robert R. H. Anholt.)

Understanding the genetic architecture of complex traits requires identification of the underlying genes and characterization of gene-by-gene and genotype by environment interactions (GEI). Behaviors that mediate interactions between organisms and their environment are complex traits that are especially sensitive to environmental conditions. *Drosophila melanogaster* presents an opportunity to systematically dissect epistasis and GEI, since large numbers of genetically identical individuals can be reared under defined environmental conditions. The olfactory system of Drosophila and its behavioral response to odorants have been well characterized. Previous studies on olfactory behavior have shown that the genetic architecture of this model behavior depends on epistatic networks of pleiotropic genes. I have used *P*-element mutagenesis in a co-isogenic background to identify genes that contribute to olfactory behavior. I have demonstrated that the effects of the transposon insertions are often dependent on developmental stage and that hypomorphic mutations in developmental genes can elicit profound adult behavioral deficits. I also assessed epistasis among these genes by constructing all possible double heterozygotes and measuring avoidance responses at two odorant concentrations. I observed enhancer and suppressor effects among subsets of these genes, and surprisingly, these epistatic interactions shifted with changes in the concentration of the olfactory stimulus. I then assessed variation in olfactory behavior in a population of 41 wild-derived inbred lines and asked to what extent different larval rearing environments would influence adult olfactory behavior and whether GEI is a minor or major contributing source of phenotypic variation. My results show that

about 50% of phenotypic variation in adult olfactory behavior is attributable to GEI. In contrast, transcriptional analysis revealed that only 20 genes show GEI at the level of gene expression (FDR<0.05), some of which are associated with physiological responses to environmental chemicals. Quantitative complementation tests with *piggyBac*-tagged mutants for two of these genes (*CG9664* and *Transferrin 1*) demonstrate that genes that show transcriptional GEI are candidate genes for olfactory behavior, and that GEI at the level of gene expression is correlated with GEI at the level of phenotype.

The Genetic Architecture of Odor-Guided Behavior in *Drosophila melanogaster*

by
Deepa Sambandan

A dissertation submitted to the Graduate Faculty of
North Carolina State University
in partial fulfillment of the
requirements for the Degree of
Doctor of Philosophy

Genetics

Raleigh, North Carolina

2008

APPROVED BY:

Patricia A. Estes	Christina M. Grozinger
Trudy F.C. Mackay Co-Chair of Advisory Committee	Robert R. H. Anholt Chair of Advisory Committee

DEDICATION

My love and soul mate Bharani Ramaswamy whose selfless sacrifices, words of encouragement and firm support made this possible.

BIOGRAPHY

Born in Chennai, India, a bustling metropolis, I was fortunate to have the privilege of being raised in a city with private school education and lots of exposure to the outside world. Growing up I watched and learned from my parents, the most passionate and career driven couple I have ever seen till today, to love my career. My interest in biology was a natural one. As a child I remember studying biology more than any other subject. My very first college level course in genetics was the trigger that set me forward until my Ph.D. I had always known that I wanted to do Drosophila Genetics. I feel extremely fortunate to have achieved what I desired.

ACKNOWLEDGEMENTS

I would like to first acknowledge my advisers Dr. Robert. R. H. Anholt and Dr. Trudy F.C. Mackay for their extraordinary guidance and direction during my entire stay at North Carolina State University. Robert is one of a kind adviser who takes extra effort in making sure all of lab members past and present are progressing towards success in their scientific career. He has not only taught me how to do science but also how to write and talk science. It is truly an honor to work with Trudy. It is remarkable how Trudy can break the most complex concepts into simple bits that anyone can follow. I consider myself extremely privileged to be a part of their laboratories. Undoubtedly, without their advice and support, I would not have had success with my Ph.D.

I would like to thank the Department of Genetics, North Carolina State University for providing me with this golden opportunity. Especially, I would like to thank Dr. Stephanie E. Curtis for taking time to make sure that every student feels secure and is doing well in the department.

I would like to thank my committee members Dr. Pat Estes and Dr. Christina Grozinger for their valuable suggestions.

I would like to thank Dr. Richard Lyman for patiently teaching me to program in SAS and readily offering all the help I needed with my statistical analyses. I feel extremely fortunate to have had his help and suggestions throughout.

Dr. Aki Yamamoto for all the cheerful support and advice he has offered me with flies, starting from basics like collecting virgins to more complicated crosses. Dr. Juan Jose Fanara for performing the *P*-element screen for olfactory behavior, from which I selected fly lines for my project.

Dr. Mary Anna Carbone for help and advice with molecular techniques. Dr. Tanya Morozova for everything from giving me rides to offering to take care of my flies. Julien Ayroles for his help in statistical analyses. Gunjan Arya, Ping Wang, Steven West, and Shanshan Zhou for being great sports and making my lab life great. I would like to thank Faye Lawrence for her tireless enthusiasm in preparing fly food. Laura, Charlene, Mike, Katie, Susan, Harland, Elizabeth, Beth, Alexis and Kultaran for making my days in the department a joyful one.

I would like to specifically acknowledge my husband Bharani Ramaswamy for his tremendous patience and the incredible confidence he has in me. He has never failed to lift my spirits, boost my confidence and provide me the warmth, care and affection that I quite often need especially during my low times. It is not an exaggeration to say that without his firm support, the sacrifices he has made throughout, this career goal of mine would not have had the slightest possibility. Mere words cannot describe my gratefulness to him. I hope I make him proud. I would like to acknowledge our cute little baby girl, Iniya, for all the joy and the wonderful feelings that she unknowingly is offering our family. Last but most certainly not the least my parents who have always provided me with the best in life and have always wished nothing but the best for me.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER ONE

INTRODUCTION

Chemical ecology

Chemical ecology is a rapidly growing field that studies behavioral responses of animals to chemical cues in their environment. The fast growing field of genomics, availability of sequenced genomes, and application of quantitative behavioral genetics has further enhanced the growth of chemical ecology.

Most behaviors are mediated via successful chemical communication. Such behavioral responses may be innate, learned or phenotypically plastic depending on the environment. Chemical communication is prevalent in the plant kingdom too. Plants produce defensive semiochemicals to defend themselves (BIRKETT, 2000; AGRAWAL AND KARBAN, 1999). From insects to humans, olfaction is one of the core modalities that ensure the survival and procreation of species by responding appropriately to food, foe and mates, by avoiding toxins, and by recognizing kin and members of the species. Chemoreception is dependent on several factors such as environment (air, humidity, and distance), physiology (levels of hormones) and sex.

Insects especially rely on their highly sensitive olfactory systems to perceive and respond to biologically relevant odor cues in their environments. The female mosquito *Anopheles gambiae* relies chiefly on odor cues to localize its human host (TAKKEN, 1996) thus spreading malaria. Male moths *Manduca sexta* use their olfactory system to recognize pheromones and navigate towards females (HILDEBRAND, *et al.* 1997) and in honey bees, *Apis mellifera*, olfactory learning contributes to foraging behavior (GALIZIA AND MENZEL,

2000). Thus, chemical communication is a complex phenomenon which involves several processes such as the production of pheromones, recognition of odorants by odorant receptors, signal transduction, transfer of the signal to the central nervous system, and signal integration all which ultimately lead to the elicitation of olfactory behavior (VOSSHALL AND STOCKER, 2007). The olfactory system has to perform accurately by not only detecting and discriminating odor cues appropriately but also by integrating relevant information into the nervous system, thus ensuring the elicitation of the best suitable behavioral response. My research has focused on the genetic underpinnings that enable these processes to take place.

Drosophila as a model organism to study olfactory behavior

Drosophila melanogaster presents an attractive model system for elucidating the genetic architecture of olfactory behavior. Its genome is fully sequenced, a large genomic data base is available (http://flybase.bio.indiana.edu/) and behavioral traits can be quantified by designing sensitive and reproducible behavioral assays. It also has a short generation time, there is no recombination in males, and genetically identical individuals can be easily generated and reared under controlled environmental conditions. This facilitates the analysis of complex traits. Furthermore, mutations can be stably propagated and chromosomal substitutions and manipulations can be done due to the availability of balancer chromosomes. There are extensive resources available for genomic studies on Drosophila, and national stock centers make resources such as deficiency lines and transposon insertion lines available to the scientific community. Finally, the Drosophila olfactory system is very well characterized at the anatomical, molecular and cellular levels.

Anatomy and function of the *Drosophila* olfactory system

The functional organization of the olfactory system in Drosophila parallels that of mammals. However, when compared with mammals the olfactory system in flies is less complex with 10,000-100,000 times fewer neurons and 30 times fewer glomeruli. Glomeruli are spherical ensembles of neuropil where the first synapses between the axons of the ORNs and output neurons are made (LAISSUE, *et al.* 1999). Flies have two main olfactory organs, the antennae and maxillary palps. There are ~ 1,200 olfactory receptor neurons (ORNs) distributed in the basiconic, coeloconic and trichoid sensilla of the third antennal segment and ~ 120 ORNs in the basiconic sensilla of the maxillary palp (SHANDBHAG, *et al.* 1999). The three types of sensilla differ in size and morphology. There is sexual dimorphism in the antennal sensillum number, with males having 20% lesser basiconic and 30% higher trichoid sensilla (STOCKER, 2001). Each sensillum has one to four neurons surrounded by perilymph. The axons of ORNs project into glomeruli within the antennal lobe of the brain (STOCKER, 1994; LAISSUE, *et al.*1999). Axons of ORNs in Diptera bifurcate and project bilaterally to corresponding glomeruli in the antennal lobes. Neurons that express the same receptor send their axons to the same glomeruli (STOCKER, 1994). Axons of ORNs expressing the same OR converge on one or sometimes two glomeruli (VOSSHALL, *et al.* 2000; GAO, *et al.* 2000). Glomeruli are innervated by the dendrites of second order projection neurons (PNs). ORNs synapse with these dendrites. Individual PNs innervate only selected glomeruli (JEFFERIS, *et al.* 2001; WONG, *et al.* 2002; MARIN, *et al.* 2002). Glomeruli contain synaptic contacts with local interneurons which are intrinsic to the antennal lobe and are primarily inhibitory. Interneurons branch in multiple glomeruli to process information between glomeruli (STOCKER, 1990; STOCKER, *et al.* 1994). Output neurons from the antennal lobe in turn

project to higher brain centers such as the mushroom bodies and the lateral horn of the protocerebrum in the central brain (ITO, *et al.* 1998).

Each ORN expresses a single or rarely a few odorant receptor (OR) genes (CLYNE, *et al.* 1999; VOSSHALL, L.B. 1999; VOSSHALL, *et al.* 2000). In insects, along with the expression of one of 60 OR genes that bind odorants, the *Or83b* receptor, is also expressed. *Or83b* is a conserved member of the insect OR gene family (LARRSON, *et al.* 2004; KRIEGER, *et al.* 2003; JONES, *et al.* 2005; PITTS, 2004) and is required for the stabilization of ORs (NAKAGAWA, *et al.* 2005). Or83b forms a heterodimer with the neuron specific OR and this dimerization is essential for the transport and insertion of ORs in the chemosensory dendritic membranes (BENTON, *et al.* 2006). Also, it has been proposed to open a cation channel upon activation by odorants (SATO, et al. 2008). ORs are located on the plasma membrane of the ORN dendrite (ELMORE AND SMITH, 2001; DOBRITSA, *et al.* 2003). *Or* genes in flies like in mammals (BUCK AND AXEL, 1991) are G-protein coupled receptors, a superfamily of proteins with diverse sequences but with a common structure composed of seven transmembrane domains (CLYNE, *et al.* 1999; KIM, *et al.* 2000). The 60 OR genes are located in small clusters throughout the genome (CLYNE, *et al.*1999; VOSSHALL, *et al.* 1999; ROBERTSON, *et al.* 2003). Two of these genes alternatively splice thus producing a total of 62 odorant receptor proteins (ROBERTSON, *et al.* 2003). OR proteins are highly diverse with less than 20% sequence identity with each other and no similarity to mammalian odorant receptor proteins. In comparison, the mouse genome contains a multigene family of ~ 1,000 OR genes (BUCK and AXEL 1991) that has undergone a rapid evolution through gene duplication and diversification (HUGHES AND HUGHES 1993; BEN-ARIE, *et al.* 1993; ISSEL-TARVER AND RINE, 1997; ROUQUIER, *et al.* 1998). The identification of a three amino acid difference between

two tightly linked genes *Or19a* and *Or19b* suggests that *Or* gene clusters might have arisen through recent genome duplication in Drosophila (ROBERTSON, *et al.* 2003).

The molecular response profile of several ORs expressed in the maxillary palps (DE BRUYNE, *et al.*1999) and in the antennae (DE BRUYNE, *et al.*2001, DOBRITSA, *et al.*2003) have been assessed. In an attempt to functionally assess the molecular response profile of the antennal repertoire of *Or* genes, the mutant ab3A neuron that lacks the *Or22a/b* gene was used to characterize the response of *Or* genes to a panel of >100 odorants by electrophysiology (HALLEM and CARLSON, 2004). In addition to determining a receptor to neuron olfactory map, this research also confirmed that the receptors dictate the properties of ORN. Individual ORs differ in their tuning breadth to different odorants. The expression of the OR gene determines the characteristics of the ORN such as response dynamics, firing rate, and odor response spectrum (HALLEM, *et al.* 2004). In Drosophila many odor stimuli elicit responses from multiple receptors (HALLEM AND CARLSON, 2004). More receptors are activated at higher odorant concentrations and different odorants activate different sets of ORs. For example *Or7a* in the maxillary palp responded strongly to E2-hexenal whereas *Or47b* responded weakly to most odorants except to 1- hexanol and 1-octen 3-ol (HALLEM AND CARLSON, 2004). Surprisingly, the responses of the trichoid ORs are strongly inhibited by food odors and other odorants suggesting that the ORs that are expressed in the trichoid ORNs may be involved in pheromone recognition (HALLEM AND CARLSON, 2006).

In addition to ORs, odorant binding proteins (OBPs) play a role in olfaction. These are carrier proteins for odorants that float in the perilymph (PIKIELNY, *et al.* 1994; MCKENNA, *et al.* 1994). There are 51 OBPs encoded in the Drosophila genome. Single nucleotide polymorphisms (SNPs) in some of these OBPs are associated with naturally

occurring variation in olfactory responses to the odorant benzaldehyde (WANG *et al.* 2007).

Larval olfactory system

Larval olfactory behavior is different from that of the adult as larvae experience a different environment. The larval olfactory system is much simpler and consists of 21 ORNs as opposed to 1,300 ORNs in adults. Twenty five ORs are expressed in larvae of which 14 are larva specific and 11 are common to both adults and larvae (FISHILEVICH, *et al.* 2005). Most of these ORs bind either aromatic or aliphatic compounds found in their food (KREHER *et al.* 2005). Though different in peripheral organization, the olfactory system in larvae is similar to that of the adults in its central projection pathways. Larvae in which a single pair of functional ORNs has been ablated show disrupted chemotaxis behavior to few odorants. More surprisingly, larvae with only a single pair of functional ORNs still show robust chemotaxis behavior. Thus ORNs functions in a combinatorial mode, but in larvae are also capable of functioning independently to initiate chemotaxis behavior (FISHILEVICH, *et al.* 2005).

Benzaldehyde and its ecological significance

Benzaldehyde is a well characterized odorant for olfactory behavior at electrophysiological, neuroanatomical and behavioral levels. It is a naturally found compound in several fruits such as strawberries, peaches, and raspberries. In its natural form benzaldehyde is an effective antifungal agent for plants (VAUGHN, *et al.* 1993). Flies survive on decaying fruit. Benzaldehyde is released in undamaged and intact fruits in high quantities.

The amount of benzaldehyde is reduced in decaying fruits thus making the fruit a more suitable survival medium for flies. In Drosophila electroantennograms have shown that Or7a, Or85f, Or10a (HALLEM, *et al.* 2004) and Or43a (STÖRTKUHL, *et al.* 2005) responsd to benzaldehyde.ORNs that express Or43a project into the DA4 glomerulus in the fly antennal lobe (STÖRTKUHL, *et al.* 2005).

Genetic Architecture of olfactory behavior

Understanding the genetic architecture of a complex trait requires first and foremost identifying the genes that contribute to manifestation of the trait. One approach for achieving this is by performing mutagenesis screens. A second layer of analysis is determining how genes implicated in the manifestation of the trait form functional ensembles, either through additive or non-linear interactions (ANHOLT AND MACKAY 2004; ANHOLT 2004). Assessing the temporal and spatial dynamics of such interactions as a function of genotype by environment interactions remains a challenging problem.

Behaviors are genetically complex and are controlled by epistatic networks of pleiotropic genes with allelic effects that are highly sensitive to the environment (ANHOLT AND MACKAY, 2004). Several discoveries indicate the involvement of pleiotropic genes in regulating behaviors. For example, the gene *dunce* is implicated in learning and memory (DUDAI *et al.*1976) circadian behavior (LEVINE, *et al.* 1994) and mating behavior (BELLEN AND KIGER, 1987). Another example is the gene *Catsup* which affects lifespan (MACKAY, *et al*. 2005), starvation resistance (HARBISON, *et al*. 2005) and sensory bristle number (STATHAKIS, *et al*. 1999). An example of a sex-specific behavior comes from the *fruitless* gene, which is implicated in mating behavior (RYNER, *et al.* 1996; GAILEY AND HALL 1989;

Ito, *et al.* 1996), and in aggression (LEE AND HALL 2000). Three male specific Fru^M transcripts work together in an "additive" manner to elicit courtship behavior (BILLETER, *et al.* 2006). The gene *fruitless* is expressed in ~100-150 ORNs in the trichoid sensilla of antennae and in four ORNs in each maxillary palp. Silencing ORNs that express *fruitless* significantly reduced courtship behavior (STOCKINGER, 2005).

Olfactory behavior is a complex trait that shows genetic variation in natural population (MACKAY, *et al.* 1996; WANG, *et al.* 2007). Apart from odorant receptor genes and odorant binding proteins such as *Lush* (KIM, *et al.* 1998) and *pinocchio* $^{(smi21F)}$, a putative odorant binding protein (ROLLMANN, *et al.* 2005), several other genes have been implicated in olfactory behavior, such as 14 *P*[*lArb*] tagged *smell-impaired* (*smi*) loci (ANHOLT, *et al.* 1996) that include the DSCI sodium channel (Kulkarni, *et al.* 2002) and *scribble* (GANGULY, *et al.* 2003). Other genes implicated in olfactory behavior include *Vanaso,* an allele of *discs lost* (FANARA, *et al.* 2002) and *Calreticulin* (STOLTZFUS, *et al.* 2003) to name a few.

Electrophysiological studies have clarified the functions of several other genes in various aspects of olfactory processing, including *retinal degeneration B*, *norpA* (WOODARD, *et al.* 1992; RIESGO-ESCOVAR, *et al.* 1994, 1995), an inositol 1,4,5-trisphosphate receptor (DESHPANDE, *et al.* 2000), an integrin (AYYUB, *et al.* 2000), the Na^{2+} channel *paralytic* (LILLY, *et al.* 1994), and a Ca^{2+} channel (STORTKUHL, *et al.* 1999).

Epistasis

The formation of extensive interacting ensembles of genes has been accounted by the observation of genome-wide modulation of transcript abundance of co-regulated genes as a

consequence of the introduction of a single *P*-element in a co-isogenic background (ANHOLT, *et al.* 2003). The importance of epistasis and genotype by environment interactions in the genetic architecture for olfactory behavior is discussed in great detail in the following sections.

Genes that contribute to the variation in trait are likely to functionally interact. Epistasis is defined as the deviation of phenotypic values from additivity between segregating alleles at two or more loci (FALCONER AND MACKAY, 1996). Analysis of epistatic interactions between candidate genes will allow the identification of gene pathways that regulate behaviors. Epistasis is evident when the effect of a variation at one locus is either suppressed or enhanced by the genotype at another locus.

One method of identifying epistatic interactions is to screen for mutations in unlinked loci that enhance or suppress mutant effects of a known member in the pathway. The important requirement here is that the background genotype needs to be strictly controlled to enable the identification of small phenotypic effects. Thus a controlled genetic background confirms that the interactions between the mutations are only due to epistasis and are not non-additive interactions among alleles segregating between the background genotypes in which the mutations were induced.

Pervasive epistasis appears to be a characteristic feature of the genetic architecture of complex traits in Drosophila (MACKAY 2004). It has been postulated previously that epistatic networks for behavioral traits are modulated by sex, the physical and social environment, and developmental history (ANHOLT 2004). A method to identify interacting genes was applied successfully to *P*-element insertional mutations in a common co-isogenic background by generating all possible double heterozygote genotypes in a half-diallel design, where each

mutant line is crossed with every other mutant line (ANHOLT AND MACKAY, 2004; ANHOLT, 2004). Of 12 *P*-element insertional mutations affecting olfactory behavior, eight formed an interacting network, in addition to two independently interacting mutations (FEDOROWICZ, *et al.* 1998). *Alcohol dehydrogenase* (*Adh*) expression and protein level are traits that show natural variation in populations of *Drosophila melanogaster*. Three polymorphic sites that affect natural variation of the Adh protein level were identified in the intronic 2.3 kb region of *Adh*. Functional testing of the effects of each polymorphic trait associated with the trait individually and in combination revealed significant epistasis between these sites (STAM AND LAURIE, 1996). By constructing simple crosses of all possible double heterozygotes for eight pairs of *P*-element insertions, epistatic effects with the same magnitude as that of main effects were identified also for metabolic traits (CLARK AND WANG 1997). Examination of a simple loss of co-ordination behavior demonstrated the plasticity of epistatic interactions between 16 mutations in different genetic contexts based on their interactions at two genetic context, the presence or absence of a mutation in *Syntaxin 1A* (VAN SWINDEREN AND GREENSPAN, 2005). In the presence of the *Syntaxin 1A* mutation 44 significant interactions were found out of 120 possible interactions. In the absence of the *Syntaxin 1A* mutation 43 interactions were significant. Even though the total number of interactions between the two genetic backgrounds did not differ much, the nature of the interactions that emerged was different (VAN SWINDEREN AND GREENSPAN, 2005). Epistatic interactions were also identified between a *P*-element mutant at the *Tre1-Gr5A* locus and insulin growth factors *foxo* and *trbl* for starvation resistance and life span (ROLLMANN, *et al.* 2006). Genome-wide pair wise interaction analyses have demonstrated the presence of epistatic interactions that contribute to circadian behavior in mammals (SHIMOMURA, *et al.* 2001). Fourteen pairs of

loci in particular allelic combinations had greater effect on circadian behavior than individual loci (SHIMOMURA *et al.* 2001).

Epistatic interactions between quantitative trait loci (QTLs)

Finding interactions among QTLs is one other way of identifying the presence of epistasis. The effect of epistasis is an important component of trait variation. High and low lines derived by artificial selection for sternopleural bristle number in Drosophila accounted for 40% significant interactions between pairs of QTLs. Some of these showed negative epistasis, indicating that the trait is under stabilizing selection (GURGANUS *et al.*1999). Detection of epistasis between pairs of QTLs by ANOVA indicated that 11% affected abdominal bristle numbers, most of which due to synergistic epistasis, and 9% of interactions were significant for sternopleural bristle numbers, most of which were diminishing epistasis as expected for traits under stabilizing selection (DILDA AND MACKAY, 2002). Sex-specific epistatic interactions of the same magnitude as the main effect were identified between intervals affecting bristle number trait with significant epistasis between third chromosome intervals for abdominal bristle numbers (LONG, 1995). The genetic basis of pigmentation between two sister species, *Drosophila yakuba* and *Drosophila santomea* identified epistatic interactions that were sex-specific (CARBONE, *et al.* 2005). Significant epistatic interactions were identified between all four QTLs in males and only one pair of QTL interacted in females (CARBONE, *et al.* 2005). Epistasis and genotype by environment interactions also contribute to the genetic architecture of mating behavior of the house fly (MEFFERT AND HAGENBUCH, 2005).

Genotype by environmental interactions (GEI)

Phenotypic plasticity is a hallmark of many quantitative traits, as the ability to adapt to changes in the environment is essential for survival. Phenotypic plasticity itself, however, can vary depending on genotype. Genotype by environment interaction (GEI) occurs when there is variation among genotypes in the rank order or relative magnitude of effects in different environments (FALCONER and MACKAY, 1996).

Behaviors are complex traits that mediate interactions between an organism and its environment. Behavioral phenotypes are thus expected to be especially susceptible to genotype by environment interactions.

Genetic variation observed in a natural population is the product of segregating genes that produce the trait and is conditional on the environment (FALCONER AND MACKAY, 1996). Given that olfactory behavior is controlled by a vast number of genes with a gamut of functions, allelic variations and differences in expression levels of any of these genes would contribute to the genetic variation in natural populations (ANHOLT, AND MACKAY, 2001). In fact, olfactory behavior shows considerable variation within a population (MACKAY, *et al.* 1996; WANG, *et al.* 2007).

GEI studies for other traits

Drosophila studies performed to evaluate the extent to which QTLs exhibit GEI (MACKAY AND LYMAN, 1998; DILDA AND MACKAY, 2000; VIEIRA, *et al.* 2000; LEPIS AND MACKAY 2000; GURGANUS, *et al.* 1998; GILLESPIE AND TUELLI, 1989) indicate that GEI is pervasive (ANHOLT AND MACKAY, 2006). In Drosophila, virtually all traits show GEI. Several studies have demonstrated GEI for fitness (FRY, *et al.* 1996; KONDRASHOV AND

HOULE, 1994; FERNANDEZ AND LOPEZ-FANJUL, 1996), GEI for bristle numbers (CALIGARI AND MATTER, 1975; GURGANUS, *et al.* 1998; MACKAY, *et al.* 1998; GEIGER-THORNSBERRY, *et al.* 2002; MORETEAU, *et al.* 2003; MORETEAU AND DAVID, 2005). GEI studies for other traits include pigmentation (GIBERT, *et al.* 2004), morphometric traits such as body size and shape, wing size and shape (GIBERT, *et al.* 2004; WAYNE, *et al.* 2005; DAVID, *et al.* 2006; CARREIRA, *et al.* 2006), viability growth rate and developmental time (FANARA, *et al.* 2006). Studies on the magnitude of GEI between organic and conventional farming for traits such as milk production in cows (NAUTA, *et al.* 2006) found moderate GEI for yield traits. Such studies on GEI are of economic importance. Generally, studies on GEI have been mostly descriptive. The question whether underlying rules that govern GEI exist requires a systematic analysis.

Relevance of epistasis and GEI to human diseases

One of the major challenges in studying the molecular basis of human diseases is to understand and analyze the genetic and environmental influence of disease causing alleles. Human diseases are often caused by several interacting genes with subtle effects whose expression are highly influenced by the environment. Pharmacogenomics is a fast growing field that attempts to develope personalized drug treatments to account for variation in patients' responses to drugs. The major goal of research in this area is to determine genetic variation in drug responses by identifying candidate genes, interactions between genes, and the effects of environmental factors in gene expressions. At this point, accounting for all if these factors in human studies remains challenging.

As my work on olfactory behavior as a model trait in *Drosophila melanogaster*, a

genetic model organism, may reveal generally applicable principles that may impact future studies by human geneticists, I will briefly highlight the importance of epistasis and GEI for mapping disease susceptibility loci.

Epistasis and diseases

Often human disease susceptibility is a result of allelic combinations at multiple loci. Many disease susceptibility alleles are ineffectual when separately isolated, however they produce pronounced effects when they interact with other alleles (RISCH, 2000). Epistasis contributes to the variability of complex phenotypes. A better understanding of epistatic interactions will be essential to fully understand the complex nature of diseases. Six QTLs for genetically determined susceptibility to UV induced immunosuppression, which is a risk factor for skin cancer, were identified in mouse. Two QTLs on chromosomes 14 and 19 were identified as interacting with one of the QTLs on chromosome 1 (CLEMENS, *et al.* 2000). Among four new loci (*Sluc* genes) that affect susceptibility of lung cancer in mice models of human cancer, two significant epistatic interactions between *Sluc1* (on chromosome 19) that interacts with *Sluc2* (chromosome 2), and *Sluc3* (chromosome 6) that interacts with *Sluc4* (chromosome 11) were uncovered (FIJNEMAN *et al.* 1996). Epistatic interactions between type 2 diabetes susceptibility loci on chromosomes 1q21-25 and 10q23-26 have also been identified (WILTSHIRE, *et al*. 2006). Further proof that interacting networks of multiple genes may contribute to diseases was shown by genome-wide detection of epistasis for disease like diabetes (STYLIANOU, *et al*. 2006). This study led to the identification of a single QTL on chromosome 19 as the center for a network of 8 interacting QTLs. A QTL on chromosome 4 is the hub for six interacting genes and a QTL on chromosome 17 is the center of four

interacting genes. In addition, four pairs of interactions were detected outside this network (STYLIANOU, *et al*. 2006). Thus, epistatic interactions between disease susceptibility alleles seem to be a common phenomenon that further complicates mapping human disease causing alleles.

GEI and diseases

The importance of GEI has been documented in several studies on human disease susceptibility. GEI in which early experience influences adult disease susceptibility has been documented for asthma. This study showed that cytokine response profiles are influenced by early exposure to immune challenges (HOFFJAN *et al.* 2005). Furthermore, a variable number tandem repeat in the promoter of the monoamine oxidase A gene is associated with violent behavior, but only in individuals who were abused as children (CASPI *et al*. 2002). Another study showed that the short allele of the serotonin transporter predisposes to depression, but that this effect is more pronounced in individuals who have experienced stressful events (CASPI *et al.* 2003). A more recent study on the effect of environment such as lifestyle and geographical location on gene expression in three Moroccan populations found that specific factors of the immune system and respiratory genes were differentially expressed among urban and desert dwelling individuals (IDAGHDOUR, *et al.* 2008). This shows that it is important to account for the effects of environment on disease susceptibility studies. Thus, GEI is pervasive and remains a confounding factor in the analysis of the genetics of complex traits, including the identification of risk alleles for human diseases.

Project overview and aims

Understanding the genetic architecture of any complex trait requires addressing the following questions: (1) What are the genes that mediate in the manifestation of the trait and what are the subset of these genes that contribute to the natural phenotypic variation; (2) To what extent do these genes interact as functional ensembles and are such networks stable or dynamic; and (3) What is the nature of the interplay between the genome and the environment in shaping expression of the trait.

Here, for my doctoral research I report the identification of ten *P*-element insertion lines in *Drosophila melanogaster* with aberrant olfactory avoidance behavior to the odorant benzaldehyde from a screen of 1,339 co-isogenic lines that contain a single marked *p[GT1]* gene-trap transposon (LUKACSOVICH *et al*. 2001; BELLEN *et al*. 2004). The *p[GT1]* gene trap element is a versatile transposable element designed to insert in or near target genes and contains an enhancer trap *GAL4* cassette that can drive the expression of *GAL4* under an endogenous promoter thereby enabling potential transgene expression under *UAS* promoters for genetic manipulations of cells in which the target gene is expressed (LUKACSOVICH *et al*. 2001). Most of the *p[GT1]*-tagged candidate genes included in my study have been implicated in early development of the nervous system. Null mutants in these genes often result in developmental defects that will not allow the maturation of a healthy adult animal, thereby precluding assessment of the effects of such genes on adult behavior. The hypomorphic *P*-element induced mutations that I used for this study, however, allow ostensibly normal development, but have profound effects on adult olfactory avoidance behavior.

Since all *p[GT1]*-elements are in a co-isogenic background it is possible to assess

epistatic interactions among them and ask whether epistatic networks are invariant or dynamic under different environmental conditions. I have performed this analysis with ten *p[GT1]*-element insertion lines and have shown that the manifestation of epistatic interactions between candidate genes depends on the odorant concentration that elicits the avoidance response.

My results show that the composition of epistatic networks is more dynamic than generally appreciated. These studies led to proposal of a model in which the genetic architecture of olfactory behavior depends on genetic networks that comprise dynamic epistatic interactions with few stable hubs and with varying enhancer/suppressor effects that are manifested under different environmental conditions.

I extended these studies by investigating variation in adult olfactory responsiveness as a result of prior environmental exposure during larval development for 41 wild derived inbred genotypes and I constructed reaction norms to estimate phenotypic plasticity and GEI. I performed whole-genome transcript analysis on a subset of eight lines that differed in patterns of phenotypic plasticity and GEI to identify genes that showed corresponding variations in transcript abundance. This revealed a surprisingly small group of 20 genes associated with the manifestation of GEI for adult olfactory behavior. Finally, I performed quantitative complementation tests to demonstrate that genes that show transcriptional GEI are indeed candidate genes for olfactory behavior, and that GEI at the level of gene expression is correlated with GEI at the level of phenotype.

CHAPTER 2

MATERIALS AND METHODS

Drosophila stocks

All flies were reared on an agar yeast-molasses medium in vials maintained at 25°C and under a 12 h light/dark cycle.

P-element tagged lines

p[GT1] insertion lines (LUKACSOVICH *et al*. 2001), constructed in co-isogenic *Canton-S* background – B as a resource for the Berkeley Drosophila Genome Project, were obtained from Dr. Hugo Bellen (Baylor College of Medicine, Houston, TX; BELLEN *et al*. 2004). These lines are homozygous viable *p[GT1]* insertion lines.

Wild-derived inbred lines

Drosophila melanogaster lines were derived from 41 isofemale lines collected from a Raleigh natural population by 20 generations of full-sib mating. The lines are viable and fertile, and do not contain detectable residual heterozygosity as determined by whole genome marker analysis. I reared larvae on three different media, based on a previous study in which the effect of genotype by environment interactions on fitness was documented (FRY *et al.* 1996), standard cornmeal-molasses medium**,** standard medium supplemented with 9% ethanol (FRY *et al.* 1996), and standard medium enriched with tomato paste (340g of tomato paste/l of standard medium; FRY *et al.* 1996). After eclosion, I transferred all flies to standard medium at 25°C under a 12:12 hour light/dark cycle prior to characterization of behavioral responses to benzaldehyde and transcription profiling.

Behavioral assay

Flies are taken off their food source for a period of 45 min before testing. Five adult flies (3-7 day old) of a single sex are introduced into a plastic vial divided into two marked compartments (Figure 2.1). The odorant benzaldehyde is introduced via a cotton swab into the plastic vial. After a period of 15 s, the number of flies in the compartment farthest away from the source of the odorant is scored every 5 s for a period of 60 s and the average of the ten scores is calculated. This corresponds to a single replicate. Ten such replicates per sex per line are performed. The mean olfactory response score of the line is calculated as the mean of the 10 replicates and ranges between 0 (complete attraction) to 5 (complete avoidance). Behavioral assays were conducted in an environmental chamber (25°C, 70% humidity) between 9 a.m. and 12 a.m. in a randomized design, in which measurements on individual lines were collected over multiple days to average environmental variation. In the case of *P*-element insertion lines avoidance scores of appropriate control lines were also determined on each day.

Quantitative RT-PCR for *P*-element tagged lines

I characterized ten *P*-element tagged mutant lines, all derived in the *Canton* S-B isogenic background. I quantified mRNA levels in these lines by quantitative RT- PCR, using an ABI-7900 sequence detector with a SYBR green detection method, according to the protocol from Applied Biosystems, Inc. (Foster City, CA) with glyceraldehyde-3-phosphate dehydrogenase as internal standard. Independent triplicates of total RNA were isolated from female Canton S-B control and mutant flies using the Trizol reagent (Gibco BRL, Gaithersburg, MD) and cDNA was generated from 150-200ng of total RNA by reverse

transcription. Transcript-specific primers were designed to amplify approximately 60bp-100bp regions of all 11 genes using the primer express program from Applied Biosystems, Inc. Primers were designed to encompass common regions of alternative transcripts. Negative controls without reverse transcriptase were used for all genes to exclude potential genomic DNA contamination. Statistical significance for differences in gene expression levels between *P*-element insertion lines and the control line was determined by two-tailed Student's *t*-tests.

To examine developmental stage specific deficiencies in gene expression levels in mutant lines, relative levels of expression were analyzed in the same way after extraction of triplicate RNA samples from embryos between 13 h and 16 h after oviposition, third instar larvae, pupae, and adult female heads.

Diallel crosses and statistical analyses

I analyzed epistasis among the ten co-isogenic *p[GT1]* insertion lines with impaired olfactory behavior exactly as described previously (FEDOROWICZ *et al.* 1998) by crossing homozygous mutant parental strains to construct all 45 possible double heterozygous F1 genotypes with two *P*-elements at different loci (excluding reciprocal crosses) in a half diallel cross design according to Method 4, Model 1 of GRIFFING (1956). Since four of the mutant lines had *p[GT1]* insertions on the *X* chromosome, I restricted my analysis of epistasis to females. Avoidance responses of transheterozygotes to 0.1% (v/v) and 0.3% (v/v) benzaldehyde were quantified with 20 replicate assays (100 flies per cross) between 8:00 and 11:00 am contemporaneously with the *Canton S*-B control. Avoidance scores of the transheterozygote genotypes were analyzed by a two-way fixed effects ANOVA according to

the model $Y = \mu + G + E + G \times E + \varepsilon$, where G denotes transheterozygote genotype, E is the benzaldehyde concentration (environment), and ε is the variance between individuals within each genotype and benzaldehyde concentration. I also ran reduced analyses separately for each concentration of benzaldehyde.

To analyze epistatic effects between the ten loci, I first estimated the average heterozygous effect of each mutation in combination with all other mutations as the general combining ability (*GCA*), which reflects its average avoidance score as a transheterozygote when combined with all other mutations, expressed as the deviation from the overall mean (SPRAGUE and TATUM 1942). Since the lines are co-isogenic, it is then possible to estimate the expected phenotypic value of each transheterozygote based on the *GCA* values of both parents under the null hypothesis that there is no epistasis among the two loci. Epistasis is inferred if the observed phenotypic value deviates significantly from the predicted value. Thus, the specific combining ability (*SCA*) of a transheterozygous genotype is defined as the difference between the observed avoidance score of the genotype, $X_{i,j}$ (where i and j denote two different mutations), and the score expected from the sum of the corresponding *GCA*s of mutants i and j. The *GCA* for each mutant was estimated as

$$GCA_i = T_i/(n-2) - \sum T/n(n-2),$$

where T_i is the sum of mean avoidance score values (averaged over all replicates) of heterozygotes with the ith mutation, $\sum T$ is twice the sum of mean avoidance score values of all heterozygotes, and n is the number of mutant lines (see also FALCONER and MACKAY 1996). The *SCA* effects were computed using the method of GRIFFING (1956) for each heterozygous genotype as

$$SCA_{ij} = X_{ij} - (T_i + T_j)/(n-2) + \sum T/(n-1)(n-2).$$

Standard errors of individual *GCA* and *SCA* effects were computed according to the formulae given by Griffing (1956) as

$$\sqrt{\{(T_c/df/r) \times (n-3)/(n-1)\}},$$

where T_c is the corrected total, *df* the degrees of freedom, and *r* the number of replicates. I used the Diallel-SAS05 program (ZANG *et al.* 2005) to partition variance among transheterozygous genotypes into variance attributable to *GCA* and *SCA*; to partition the $G \times E$ interaction variance into variance attributable to $GCA \times E$ and $SCA \times E$; and to estimate individual *GCA* and *SCA* effects and their standard errors.

Estimation of Genotype by environment interactions

I used factorial analysis of variance to partition variance among the genotypes for olfactory behavior into sources attributable to line (*L*; random effect), sex (*S*; fixed effect), concentration of benzaldehyde (*C*; fixed effect) and food (*F*; fixed effect). Variance components were calculated using SAS GLM and VARCOMP programs (SAS Institute, Cary, NC), according to the model $Y = \mu + L + S + F + C + L \times S + L \times F + L \times C + S \times F + S \times C + F \times C + L \times S \times F + L \times S \times C + S \times F \times C + L \times C \times F + L \times S \times C \times F + \varepsilon$, where μ indicates the overall mean and ε the environmental variance between replicates. The term $L \times F$ indicates GEI. $L \times C$ indicates differences in the dose-response relationship to benzaldehyde among the lines. $S \times F$ and $S \times C$ indicate differences in olfactory behavior between the sexes, which depend on the larval rearing environment and the concentration of benzaldehyde, respectively. $F \times C$ reflects variation in the dose-response to benzaldehyde as a result of the larval rearing environment. $L \times S \times F$ and $L \times S \times C$ indicate differences in the dependence of

sexual dimorphism in the behavioral response as a function of the larval food medium and the odorant concentration, respectively. $S \times F \times C$ assesses the dependence of sexual dimorphism in the behavioral response on larval rearing environment and benzaldehyde concentration. $L \times F \times C$ indicates the dependence of the effect of line on benzaldehyde concentration and growth medium. $L \times S \times C \times F$ indicates the dependence of the effect of line on sex, benzaldehyde concentration and growth medium.

I performed reduced ANOVAs for the two stimulus concentrations of benzaldehyde separately pooled across sexes and larval food source based on the model $Y = \mu + L + S + F + L \times S + L \times F + S \times F + L \times S \times F + \varepsilon$; for three larval food sources pooled across sexes and concentration of benzaldehyde based on the model $Y = \mu + L + S + C + L \times S + L \times C + S \times C + L \times S \times C + \varepsilon$; for two concentrations of benzaldehyde and three larval food sources pooled across sexes based on the model $Y = \mu + L + S + L \times S + \varepsilon$; and, for two concentrations of benzaldehyde and three larval food sources by sex based on the model $Y = \mu + L + \varepsilon$.

I estimated cross environmental genetic correlations (r_{GE}) between adult olfactory responses and larval food media from variance components as $r_{GE} = \sigma^2_{\mathrm{L}} / \sigma^2_{\mathrm{L}} + \sigma^2_{\mathrm{X}}$, where σ^2_{L} is the variance among the lines and σ^2_{X} denotes the total variance of all significant line interaction terms.

Line selection and expression analysis

I used hierarchical clustering analysis by using the TREE procedure for Centroid Hierarchical cluster analysis (SAS PROC VARCLUS program) to group the 41 genotypes based on their similarity in olfactory behavior and defined eight clusters. I selected one

genotype from each cluster for whole genome expression analysis. I collected two replicates of 3-5 day old flies from each genotype for each of the three rearing conditions with an equal number of males and females for each sample. I extracted total RNA from the 48 samples (eight lines × two replicates × three rearing conditions) using the Trizol reagent (GIBCO BRL). Biotinylated cRNA probes were hybridized to high density oligonucleotide microarrays (Affymetrix, Inc. Drosophila GeneChip 2.0) and visualized with a streptavidin-phycoerythrin conjugate, as described in the Affymetrix GeneChip Expression Analysis Technical Manual (2000), using internal references for quantification.

Microarray data analysis

The 18,800 probe sets on the Affymetrix Drosophila GeneChip 2.0 are represented by 14 perfect-match (PM) and 14 mismatch (MM) pairs. The quantitative estimate of expression of each probe set is the Signal (Sig) metric, as described in the Affymetrix Microarray Suite, Version 5.0. The Sig metric is computed using the weighted log (PM-MM) intensity for each probe set, and was scaled to a median intensity of 500. A detection call of Present, Absent, or Marginal is also reported for each probe set. I assigned a score of "1" to Present and Marginal calls and assigned "0" to Absent calls. As I had 48 arrays in total, I then excluded from the analysis probe sets with detection scores less than 5. This filter eliminated probe sets with very low and/or insignificant expression levels. I analyzed the remaining probe sets with two-way fixed effect ANOVA of the Signal metric, based on the model $Y = \mu + L + F + L \times F + \varepsilon$, where μ indicates the overall mean, L denotes lines, F denotes larval food environment, $L \times F$ denotes GEI and ε is the variance between replicate arrays. I corrected the p-values computed in these ANOVAs for multiple tests using a false discovery rate of FDR <

0.05 (BENJAMINI AND HOCHBERG, 1995). All statistical analyses were performed using SAS procedures. Gene ontology categories were annotated with the functional annotation tool, DAVID 2.0 Bioinformatics resources 2007, NIAID/NIH (http://david.abcc.ncifcrf.gov/home.jsp).

Quantitative RT-PCR for genes significant for GEI from microarray analyses

For the 48 RNA samples used for microarray analysis, cDNA was generated from 200ng of total RNA by reverse transcription. I quantified mRNA levels of six genes, *CG9664*, *Transferrin 1*, *Dopamine transporter*, *geko*, *Turandot A* and *Pherokine-1*, using an ABI-7900 sequence detector with a SYBR green detection method (Applied Biosystems, Inc. Foster City, CA). Transcript-specific primers were designed to amplify 50-60 bp regions of all six genes using the primer express program from Applied Biosystems. Primers were designed to encompass common regions of alternative transcripts (Table 2.1). I used negative controls without reverse transcriptase for all genes to exclude potential genomic DNA contamination.

Quantitative complementation tests

I obtained *P*-element stocks for *CG9664* and *Transferrin 1* from the Bloomington Drosophila stock center. *CG9664* is on the second chromosome (*w[1118]; PBac{w[+mC]=PB}CG9664[c00321]*) and *Transferrin 1* is on the *X* chromosome (*w[1118] PBac{w[+mC]=WH}Tsf1[f05108]*). I backcrossed these stocks to the isogenic *Canton-S* B genotype for three generations and made homozygous *P*-element and control stocks for each of the two genes. Virgin females from each of the two mutant and two control stocks were

then mated with males of each of the eight lines used for expression analysis and larvae were reared on standard, tomato, or alcohol-supplemented media. The F1 (3-7 days old) was measured for adult olfactory behavior to 0.3% (v/v) benzaldehyde.

I used ANOVA to analyze complementation with mutants of *CG9664* based on the model $Y = \mu + L + F + S + G + L \times S + L \times F + L \times G + S \times F + S \times G + F \times G + L \times S \times F + L \times S \times G + S \times F \times G + L \times G \times F + L \times S \times G \times F + \varepsilon$. Since *Transferrin 1* is on the *X* chromosome, only female olfactory responses were analyzed according to the ANOVA model $Y = \mu + L + F + G + L \times F + L \times G + F \times G + L \times G \times F + \varepsilon$. Here L denotes the wild-derived inbred line, G is the genotype (mutant or control), S is sex, F denotes larval rearing medium and ε is the error variance. Significant $L \times G$, $L \times G \times S$, or $L \times S \times F \times G$ interaction terms indicate quantitative failure to complement for GEI. Least square means were calculated for the $L \times G$ term for the two genes for controls and mutants using the LSMEANS program of SAS.

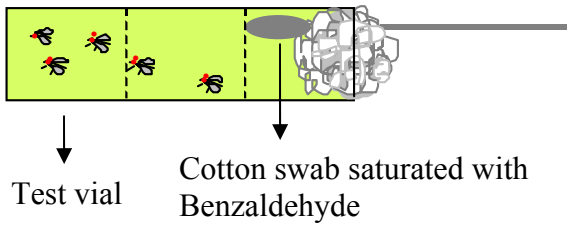
TABLE 2.1

Transcript specific qRT-PCR primers for six genes.

Gene	Primers	
	Forward	Reverse
CG9664	5’ GTAGCTGCCCACACAATCCA	5’ CCGCCCATTATGTTCTTCGA
geko	5’ GGCGTTGTCGCCTCATG	5’ AAAAAGATCGCCCACAAGGA
Turandot A	5’ CGATTCAGGGAGGTCGAAAG	5’ GCCCTTCACACCTGGAGATACA
Transferrin 1	5’ CTGCTCATCCAGCCGAATG	5’ TGGGATCGATGGCAGCAT
Pherokine1	5’ GGGCAGGATCTCCTTCAACA	5’ CTGGAGGGCACAGGTCCTT
Dopamine Transporter	5’CGATCCAGCAGATGGAAGAAA	5’ TGGTCGGTTTGGCCAGTT

FIGURE 2.1

Diagrammatic representation of the olfactory avoidance behavior assay



CHAPTER 3

RESULTS

Effects of *p[GT1]* insertions on expression of candidate genes implicated in odor-guided behavior

I selected ten *p[GT1]* insertion lines with large impairments in olfactory behavior for my analysis. In six of these lines the transposon is located either within the immediate vicinity of the transcription initiation site or within an intron of the candidate gene (*innexin2*; *CG32556*, a predicted gene of unknown function; the transcriptional regulators *pipsqueak* and *escargot*; *CG16708*, which encodes a D-erythrosphingosine kinase; and, *Semaphorin-5C*). In three lines the *p[GT1]* element has inserted in an exon (*Merlin*, *Calreticulin*, and *neuralized*). In one case the transposon has inserted in the vicinity of two predicted genes of unknown function, in the exon of *CG14782,* located immediately upstream of the 3' end of the neighboring gene, *CG14781*. (Figure 3.1)

Among these candidate genes, *pipsqueak* (*psq*) is a transcriptional regulator associated with early development (WEBER et al. 1995:LEHMANN *et al.* 1998; SIEGMUND and LEHMANN 2002), and *escargot* (*esg*) is a transcriptional regulator, associated with development of the nervous system, including sensory bristles (WHITELEY *et al.* 1992; HAYASHI *et al.* 1993; ADELILAH-SEYFRIED *et al*. 2000; NORGA *et al.* 2003). *Semaphorin 5C* (*Sema-5C*) has been implicated in early embryogenesis (KHARE *et al.* 2000) and *neuralized* (*neur*) encodes a ubiquitin ligase (LAI *et al*. 2001; YEH *et al.* 2001), which regulates neurogenesis mediated by the Notch-Delta signaling pathway (BOULIANNE *et al*. 1991; YEH *et al*. 2000) and has been implicated in the development of olfactory sensilla (JHAVERI *et al.*

2000). *Merlin* (*Mer*) is a homologue of the neurofibromatosis 2 tumor suppressor factor, implicated in axis specification during early embryonic development (MACDOUGALL *et al.* 2001), and *innexin2 (inx2*) encodes a gap junction protein essential for epithelial morphogenesis (BAUER *et al*. 2002 and 2004). The D-erythrosphingosine kinase, encoded by *CG16708,* has been implicated in autophagic cell death (GORSKI *et al*. 2003). *Calreticulin* (*Crc*) encodes a calcium binding protein involved in intracellular protein transport and exocytosis, as well as development of the nervous system (PROKOPENKO *et al.* 2000), and *Crc* mutants are defective in olfactory avoidance behavior to 4-methylcyclohexanol, 3-octanol, and benzaldehyde (STOLTZFUS *et al.* 2003). *CG14872* has been implicated as a putative guanyl nucleotide exchange factor with a developmental function, whereas the functions of the gene products encoded by *CG14781* and *CG32556* remain unknown.

I performed quantitative RT-PCR to assess to what extent insertions of the *p[GT1]*-elements disrupt expression of these candidate genes. First, I performed assays of control and mutant lines side-by-side on independent triplicate RNA samples extracted from whole flies (Figure 3.2A). Statistically significant effects on gene expression ranged from reductions in the case of *Mer, inx2, CG14781 CG14782* and *CG32556,* to significant increases in gene expression as in the case of *esg*. However, gene expression levels were not significantly different in the case of the remaining transposon-tagged genes such as *Sema-5c*, *psq*, *neur*, *Crc* and *CG16708* in whole bodies of adult female flies.

I assessed whether differences in expression levels that occur during earlier developmental stages could account for the aberrant behavioral phenotypes observed in adults. Surprisingly, *p[GT1]*-induced disruptions of gene expression showed a marked dependence on developmental stage (Figure 3.2B). Only *inx2* and *CG14782* showed reduced

expression from embryonic stages throughout development. Expression of *Mer* is reduced in all developmental stages, except pupae. *CG16708* shows a profound reduction in expression only in the larval stage, while *CG32556* is reduced in both embryos and larvae. After puparium formation *CG16708* and *CG32556* expression levels are indistinguishable from control levels. Effects of the *p[GT1]* insertion in *esg* are especially intriguing, showing reduction in embryos and larvae, but a significant increase in transcription in adult heads, consistent with the expression profile observed in adult whole bodies (Figure 3.2A). The expression profile of *esg* is mimicked by *psq*, albeit less extreme. Disruption of *neur* expression is especially pronounced in embryos and larvae. In the case of *Sema-5C,* expression is decreased in embryos, larvae and especially during puparium formation, but is restored to near control levels in adult flies (Figures 3.2A and B). Both *CG14781* and its neighboring gene *CG14782* are affected by the insertion of the *p[GT1]* element. However, whereas both gene products are overexpressed in embryos, and show reduced expression in larvae and adults, expression of *CG14781* is upregulated in pupae, whereas that of *CG14782* is downregulated during puparium formation. The reduction in expression in whole bodies for these genes is much larger than that observed in heads only (compare Figures 3.2A and B), suggesting that *CG14781* and *CG14782* are also expressed abundantly elsewhere in the body. Thus, the transcriptional effects exerted by the transposons are surprisingly diverse and heterogeneous during development, possibly reflecting effects on different promoter/enhancer elements that are recruited for regulation of gene expression at different developmental stages. My results indicate that, at least in some cases, hypomorphic disruptions that occur in early development may account for the observed aberrant adult behavioral phenotype.

Dose-response relationships of olfactory avoidance behavior in *p[GT1]* insertion lines

To further characterize the phenotypic effects of *P*-element insertions on the olfactory avoidance response to benzaldehyde and to establish a discriminating concentration range for detection of enhancer or suppressor effects in the analysis of epistasis, I measured dose-response relationships over a wide range of benzaldehyde concentrations from 0.01 to 3.0 % (v/v) (Figure 3.3). There was no linear relationship between the magnitude of reduction of transcript abundance in adult flies (Figure 3.3) and reduction in the avoidance response to benzaldehyde. For example, the *p[GT1]* insertion near *Sema-5C* has no detectable effect on transcript abundance in the adult, but profoundly affects olfactory behavior. Whereas odor-guided behavior is restored to control levels at saturating concentrations of benzaldehyde in *CG32556*, *psq*, *esg*, and *neur,* in all other cases avoidance responses are statistically significantly reduced over the entire range of concentrations in mutants compared to the *Canton-S* control (Figure 3.3). Based on the dose-response relationships, I selected 0.1% and 0.3% (v/v) benzaldehyde as stimulus concentrations for our subsequent analysis of epistatic interactions, as this concentration range elicited a significant behavioral response above background, yet was below saturation for the *Canton S*-B control and discriminated responses of all mutant lines from the control.

Epistasis among ten co-isogenic *p[GT1]*-insertion lines with transposon insertions at candidate genes implicated in odor-guided behavior

Since the transposons in the ten *p[GT1]* insertion lines were introduced in the same genetic background, it is possible to examine epistasis among them by separating

heterozygous effects from epistasis in double heterozygotes using a half-diallel crossing design (Table 3.1; FEDOROWICZ *et al.* 1998). The average dominance effect for each transposon-tagged gene in combination with all other *p[GT1]* insertion lines can be estimated as its general combining ability (*GCA*). Knowledge of the *GCA* values of both parents can predict the avoidance score expected for the transheterozygous offspring. Any statistically significant deviation of the observed value from the predicted value (*SCA*) is in this scenario attributable to epistasis. Enhancer effects will result in the transheterozygote showing an avoidance score that is more biased toward the mutant phenotype than predicted, whereas suppressor effects will reflect an avoidance score that is more wild-type than predicted from the *GCA*s of the parents.

Analysis of variance showed significant variation in avoidance scores between the two stimulus environments and among double heterozygous genotypes as well as a significant genotype by environment interaction term (Table 3.2). The effect of genotype was also highly significant ($P < 0.0001$) when analyses were performed separately for each environment (Table 3.2). Next, I asked whether I could observe environment-dependent variation in *GCA* and *SCA* values. Analysis of variance revealed significant variation in *GCA* and *SCA* values when pooled over both concentrations of benzaldehyde (Table 3.3). In addition to variation due to the environment *per se*, both the *GCA* by environment and *SCA* by environment interaction terms were statistically significant (Table 3.3) and I also observed statistically significant variation in both *GCA* and *SCA*, when analyses were performed separately for each concentration of benzaldehyde (Table 3.3). As expected from the steeper rise in the dose response curve at 0.1% (v/v) benzaldehyde than at 0.3% (v/v) benzaldehyde, which is near the inflection point that approaches the response maximum, variation in mean

avoidance scores among the double heterozygotes was greater at the lower stimulus concentration (Table 3.1, Figure 3.4). The genotype by environment interaction is evident both from the analysis of variance (Table 3.2) and from the crossing over of reaction norms (Figure 3.4).

Based on previous observations (FEDOROWICZ *et al*. 1998), it was likely that epistatic interactions for at least some of the transheterozygotes among the ten *P*-element insertion lines could be assessed. To assess to what extent the manifestation of such epistatic networks could be influenced by subtle changes in the chemosensory environment I measured avoidance responses for transheterozygotes and established *GCA* values at the two stimulus concentrations of 0.1% (v/v) and 0.3% (v/v) benzaldehyde (Tables 3.1 and 5; Table 3.4). I used the *GCA* values to estimate *SCA* values that showed statistically significant deviations from expected values based on the *GCA* estimates of both parents (Table 3.3 and Table 3.5).

When the analysis was performed with the two stimulus concentrations pooled, I observed three epistatic interactions averaged over both stimulus concentrations: an enhancer effect between *Mer* and *neur*, and suppressor effects between *Mer* and *inx 2*, and *Crc* and *neur* (Figure 3.5A). However, the pattern of epistatic interactions was greatly enriched in the two individual chemosensory environments. I identified epistatic interactions in six transheterozygous genotypes when avoidance responses were quantified at 0.1% (v/v) benzaldehyde (Figure 3.5B). Enhancer effects were observed between *Mer* and *Crc*, and between *Mer* and *neur*, whereas suppressor effects were evident between *Crc* and *neur*, *Mer* and *inx2, Mer* and *CG14781/CG14782*, and *Crc* and *esg* (Figure 3.5B). Surprisingly, the epistatic network shifted when avoidance responses were measured at a 3-fold higher benzaldehyde concentration (0.3% v/v). Under this condition epistatic interactions comprised

seven transheterozygous genotypes and included two enhancer effects, *Mer - CG32556* and *esg – inx2*, and five suppressor effects, *Sema-5C – CG32556*, *inx2 – CG32556*, *psq – CG14781/CG14782, Mer – inx2,* and *Mer – esg* (Figure 3.5C). Thus, a dynamic epistatic network is evident in which a small number of genes, such as *Mer* and *inx2*, emerge as nodes around which enhancer/suppressor effects are shaped depending on the chemosensory environment.

Variation in olfactory behavior in the wild derived inbred lines

I assessed the magnitude of variation in olfactory behavior in a population of wild-derived inbred lines and asked to what extent larval exposure to different nutritional rearing environments would influence adult olfactory behavior, and whether GEI is a minor or major contributing source of phenotypic variation. I reared flies on different food sources during larval development and evaluated the effects of larval exposure to standard, tomato or alcohol-supplemented medium on adult olfactory behavior and gene expression (Figure 3.6).

I measured olfactory responses to a standard odorant, benzaldehyde, at two submaximal concentrations (0.1% v/v and 0.3% v/v) for optimal resolution of variation in the behavioral response. Both the full model and reduced models ANOVA showed significant variation among the lines (Table 3.6-3.10), in agreement with a previous study on the response to benzaldehyde in the same population (WANG *et al.* 2007). Sexual dimorphism in the response between males and females was small and did not contribute significantly to the total variance. Sex effects were mostly evident as a significant $L \times S$ interaction at lower concentrations of benzaldehyde or for flies reared as larvae under adverse nutritional conditions (alcohol-supplemented medium). To estimate the contribution of GEI to the total

variance, I estimated the cross environmental genetic correlation. For the full model ANOVA r_{GE} =0.47, which indicates that GEI contributes about 50% to the observed variation in adult olfactory behavior when flies are reared on different food sources as larvae. The extent of GEI is unexpectedly high, considering that it arises entirely from differences in larval, but not adult rearing environments. GEI among the lines is readily visualized as extensive crossing-over of reaction norms (Figure 3.7).

I calculated the broad sense heritability to be H^2 = 0.37 according to the full model ANOVA with estimates ranging from 0.20 to 0.57 for reduced model analyses (Table 3.10). This value indicates both substantial genetic and environmental contributions to phenotypic variation in olfactory behavior, consolidating our notion that olfactory behavior in Drosophila is an appropriate model for studies on GEI.

Variation in transcriptional response

Since GEI contributes about half of the observed genetic variation in adult olfactory behavior (Table 3.6; Figure 3.7), I asked whether this large GEI effect at the level of phenotype would be mirrored by a large GEI effect at the level of transcription. To answer this question I compared whole genome transcript abundance profiles of adult flies from lines reared on different larval food sources. To reduce the scope of the experiment to within manageable limits, I selected a representative sample of lines from among the 41 wild-derived inbred strains. First, I performed a hierarchical clustering analysis for olfactory behavior measured at 0.3% (v/v) benzaldehyde (Table 3.11) and grouped the lines according to similarity in olfactory behavior under the different larval rearing conditions (Figure 3.8). I identified eight distinct clusters. Lines within each cluster were more similar in their olfactory response to

0.3% (v/v) benzaldehyde to each other than to lines in other clusters. To capture the extent of variation in GEI among the lines, it was therefore sufficient to analyze the transcriptional profiles of eight lines, one randomly chosen from each cluster. Genetic correlations for olfactory behavior among these eight lines across sexes and odorant concentrations were r_{GE} = 0.24 for standard medium, r_{GE} = 0.37 for tomato medium, and r_{GE} = 0.36 for ethanol-supplemented medium.

I used ANOVA to quantify statistically significant differences in transcript levels for the probe sets on the array and calculated a false discovery rate to correct for multiple testing (BENJAMINI and HOCHBERG 1995). At FDR < 0.05, we found 6,940 probe sets which varied in expression among the lines (approximately half of the genome), 329 probe sets with variation in expression attributable to the medium on which the larvae were reared (phenotypic plasticity), and 22 probe sets, representing 20 genes, in which variation in expression due to the larval growth medium was dependent on genotype. This surprisingly restricted number of genes is associated with the manifestation of GEI in adult flies (Table 3.12). No additional probe sets associated with GEI were evident when the FDR criterion was relaxed to 0.2.

Transcripts which varied in abundance among the lines included transcripts for two odorant receptors, *Or42a* and the ubiquitously expressed odorant receptor *Or83b*, and 27 odorant binding proteins. The latter account for about half of the family of odorant binding proteins, but, since about half of the genome shows transcriptional variation among the lines, this multigene family is not significantly over-represented. The paucity of odorant receptor transcripts that show transcriptional variation is due to the low levels of transcription of these genes below or near the detection limit of our expression microarrays. In addition to odorant

binding proteins and odorant receptor transcripts, other genes previously associated with olfactory behavior in *Drosophila melanogaster* also showed significant variation in expression among the lines, including *scribble* (FEDOROWIC *et al.* 1998), and *Semaphorin 5C* (ROLLMANN *et al.* 2007). Interestingly, this also includes the genes that I identified and characterized for olfactory behavior namely, *escargot*, *innexin 2*, *Merlin*, *CG32556*, *CG16708* and *pipsqueak.* Altered transcript abundance was also observed for several genes involved in olfactory learning and memory, and genes implicated both in olfactory behavior and in response to ethanol, including the odorant binding protein *lush* (KIM *et al.* 1998) and *protein kinase A* (MOORE *et al.* 1998; Table 3.11).

Genes that showed phenotypic plasticity in the behavioral response to benzaldehyde, as reflected by a significant main effect of the Food term in the ANOVA, included *Obp49a*, *Pbprp1*(*Obp69a*), and *Calreticulin* (STOLTZFUS *et al.* 2003), previously identified as a candidate gene for olfactory behavior, and genes implicated in olfactory learning and memory, phototaxis and male courtship behavior (*no-on-transient A,* CAMPESAN 2001; GREENSPAN and FERVEUR 2000), and circadian rhythm (*pigment dispersing factor*, WILLIAMS 2001; MERTENS *et al.* 2005; Table 3.13).

Transcripts for which the Line×Food interaction term in the ANOVA was significant demonstrate GEI. This group contained only 22 probe sets representing 20 genes (Table 3.12). Genes that show GEI for transcript abundance in adult flies following larval growth on different food sources include *CG9664,* associated with lipid metabolism; *Dopamine transporter*, implicated in regulation of sleep and arousal in insects (KUME *et al.* 2005); *geko*, implicated in olfactory behavior and response to ethanol (SHIRAIWA *et al.* 2000); *Transferrin 1* (NICHOL *et al.* 2002), involved in iron transport and homeostasis; *ryan express*, which

plays a role in male meiosis and spermatogenesis (MUKAI *et al.* 2006); *Jonah 66C*, involved in intracellular signaling and proteolysis (ROSS *et al.* 2003); *Ugt36Bb*, involved in defense response and polysaccharide metabolism (THEOPOLD *et al.* 1999); and several other genes with predicted transcripts of unknown function. Some of these gene products appear to have in common a role in responses to and metabolism of hydrophobic xenobiotics. Interestingly, no genes encoding odorant receptors or odorant binding proteins showed GEI at the level of transcription.

Confirmation of variation in transcript abundance by quantitative RT-PCR

In order to confirm the reliability of variation in transcript abundance measured on the microarrays, I performed quantitative RT-PCR analysis for a sample of six genes that includes *CG9664*, *Transferrin 1*, *Dopamine transporter, geko* and *Pherokine 1.* All of these genes showed significant variation among lines. Five of them also showed GEI, *i.e.* they were significant for the Line×Food term in the ANOVA, *CG9664*, *Transferrin 1*, *Dopamine transporter* and *geko* at FDR< 0.05, and *Pherokine1* at FDR<0.1.

Quantitative RT-PCR measurements were highly correlated with expression microarray signal amplitudes for *CG9664 (P*<0.0001*)*, *Transferrin 1 (P*=0.0003*)*, *Turandot A (P*<0.0001*)*, *Pherokine 1 (P*=0.0094*)* and *geko (P*<0.0001*)* (Figure 4). The correlation between the microarray signal and quantitative RT-PCR signal was marginal for *Dopamine transporter (P*=0.0614*)*, most likely due to low expression levels (Figure 3.9).

Quantitative tests for complementation of GEI

I hypothesized that genes that show GEI at the level of transcription would also be implicated in GEI at the level of olfactory behavior. To determine to what extent GEI at the level of gene expression correlates with GEI at the level of phenotype, I performed quantitative complementation tests for GEI, using available mutant stocks from the Bloomington Drosophila stock center. Based on the availability of suitable stocks, I selected two genes, *CG9664* and *Transferrin 1,* tagged by a *piggyBac* transposon. For each of these genes, I generated control and mutant stocks and crossed them to each of the eight wild-derived inbred lines on which expression microarrays had been performed and reared the larvae on the three different food media. I then measured olfactory behavior at 0.3% (v/v) benzaldehyde of adults that were maintained on standard medium (Tables 3.14 and 3.15). Since *Transferrin 1* is located on the *X* chromosome, quantitative complementation tests for this gene were limited to females.

GEI can be inferred when the $F \times G$ interaction term in the ANOVA is significant, as this indicates that the effect of the rearing environment on the behavioral response to benzaldehyde is significantly different when the wild-derived inbred line is crossed to the mutant compared to its control, *i.e.* failure to complement. ANOVA of the behavioral responses showed a significant $F \times G$ interaction term for all but two (774 and 786) of the eight lines crossed to the *CG9664* mutant, and for all but two (786 and 859) of the eight lines crossed to the *Transferrin 1* mutant (Tables 3.16 and 3.17).

A comparison of least square means for the $L \times G$ terms among the wild-derived lines in combination with *CG9664* or *Transferrin 1* shows substantial diversity in the nature of the GEI (Figure 3.10). First, the magnitude and direction of line means differ for both mutants

crossed to the inbred lines. Second, the inbred lines differ widely in the manifestation of GEI with, in the case of *CG9664*, in some instances antagonistic effects between the sexes (Figure 3.10A). My results demonstrate that genes that show transcriptional GEI for the trait can be implicated as candidate genes that contribute to its manifestation, and that GEI at the level of gene expression can be correlated with GEI at the level of the trait phenotype.

TABLE 3.1

Diallel cross between ten *p[GT1]* insertion lines with aberrant olfactory avoidance behavior.*

(A)	*CG 32556*	*CG 16708*	*CG14781 /CG14782*	*Crc*	*inx2*	*Mer*	*psq*	*neur*	*esg*	*Sem a-5c*	T	*GCA*
CG32556		4.17	4.26	4.40	4.29	3.68	4.51	4.38	4.22	4.31	38.21	0.049
CG16708			4.33	4.20	4.22	3.62	4.17	4.24	4.27	4.06	37.26	-0.070
CG14781/ CG14782				4.30	4.37	3.55	4.39	4.34	4.54	3.98	38.04	0.028
Crc					4.44	4.06	4.53	4.15	4.21	4.15	38.42	0.075
inx2						3.47	4.30	4.23	4.29	4.20	37.78	-0.005
Mer							3.84	4.26	4.03	3.59	34.09	-0.466
psq								4.53	4.41	4.42	39.08	0.157
neur									4.60	4.20	38.90	0.135
esg										4.49	39.04	0.153
Sema-5c											37.375	-0.055

(B)	*CG 32556*	*CG 16708*	*CG14781/ CG14782*	*Crc*	*inx2*	*Mer*	*Psq*	*neur*	*esg*	*Sem a-5c*	T	*GCA*
CG32556		4.62	4.75	4.64	4.29	4.57	4.90	4.71	4.57	4.22	41.26	-0.001
CG16708			4.45	4.66	4.65	4.53	4.76	4.78	4.60	4.59	41.62	0.043
CG14781/ CG14782				4.67	4.53	4.50	4.53	4.83	4.55	4.41	41.22	-0.006
Crc					4.64	4.48	4.90	4.71	4.81	4.58	42.08	0.100
inx2						4.10	4.79	4.49	4.77	4.58	40.81	-0.057
Mer							4.53	4.41	4.18	4.08	39.35	-0.239
psq								4.76	4.83	4.66	42.64	0.171
neur									4.75	4.64	42.06	0.099
esg										4.43	41.48	0.026
Sema-5c											40.18	-0.136

* The table lists avoidance scores for transheterozygous females and calculated *GCA* values at 0.1% v/v (panel A) and 0.3% v/v (panel B) benzaldehyde. Parental homozygous *p[GT1]*

TABLE 3.1 (Continued).

insertion lines are indicated on the top row and first column of each panel. The means of avoidance scores are derived from 20 replicate measurements for each hybrid cross. T is the sum of avoidance scores used to compute the *GCA* values.

TABLE 3.2

Analyses of variance of avoidance response of double heterozygote genotypes to two concentrations of benzaldehyde

Analysis	Source	d.f.	SS	*F*	*P*
0.1% (v/v) and 0.3% (v/v) Pooled	Environment	1	66.083	255.46	<0.0001
	Genotype	44	74.369	6.53	<0.0001
	Genotype × Environment	44	23.961	2.11	<0.0001
	Error	1710	442.352		
0.1% (v/v)	Genotype	44	64.597	5.24	<0.0001
	Error	855	239.499		
0.3% (v/v)	Genotype	44	33.732	3.23	<0.0001
	Error	855	202.853		

TABLE 3.3

Analyses of variance of general and specific combining abilities of double heterozygote genotypes at two concentrations of benzaldehyde

Analysis	Source	d.f.	SS	*F*	*P*
0.1% (v/v) and 0.3% (v/v) Pooled	Environment (*E*)	1	66.083	255.46	<0.0001
	GCA	9	61.424	26.38	<0.0001
	SCA	35	12.944	1.43	0.0501
	GCA × *E*	9	7.599	3.26	0.0006
	SCA × *E*	35	16.362	1.81	0.0027
	Error	1710	442.352		
0.1% (v/v)	*GCA*	9	48.027	19.05	<0.0001
	SCA	35	16.570	1.69	0.0080
	Error	855	239.499		
0.3% (v/v)	*GCA*	9	20.996	9.83	<0.0001
	SCA	35	12.737	1.53	0.0258
	Error	855	202.853		

TABLE 3.4

Estimates of General Combining Abilities*

	0.1% and 0.3% (v/v) benzaldehyde		0.1% (v/v) benzaldehyde		0.3% (v/v) benzaldehyde	
Genotype	*GCA*	*P*	*GCA*	*P*	*GCA*	*P*
CG32556	0.0238	0.4162	0.0490	0.2174	–0.0015	0.9675
CG16708	–0.0134	0.6457	–0.0698	0.0792	0.0428	0.2407
CG14781/CG14782	0.0103	0.7239	0.0278	0.4847	–0.0071	0.8457
Crc	**0.0878**	**0.0027**	0.0746	0.0604	**0.1010**	**0.0058**
inx2	–0.0312	0.2849	–0.0054	0.8923	–0.0571	0.1183
Mer	**–0.3528**	**<0.0001**	**–0.4660**	**<0.0001**	**–0.2396**	**<0.0001**
psq	**0.1644**	**<0.0001**	**0.1571**	**<0.0001**	**0.1716**	**<0.0001**
neur	**0.1172**	**<0.0001**	**0.1353**	**0.0007**	**0.0991**	**0.0068**
esg	**0.0897**	**0.0022**	**0.1528**	**0.0001**	0.0266	0.4661
Sema-5c	**–0.0957**	**0.0011**	–0.0554	0.1634	**–0.1359**	**0.0002**

* significant *GCA* values are indicated in bold font.

TABLE 3.5

Estimates of Specific Combining Abilities *

		0.1% and 0.3% benzaldehyde		0.1% (v/v) benzaldehyde		0.3% (v/v) benzaldehyde	
Parent 1	Parent 2	*SCA*	*P*	*SCA*	*P*	*SCA*	*P*
CG32556	*CG16708*	–0.0114	0.8817	–0.0113	0.9142	–0.0116	0.9038
CG32556	*CG14781/82*	0.0773	0.3142	–0.0188	0.8575	0.1734	0.0714
CG32556	*Crc*	0.0148	0.8470	0.0744	0.4763	–0.0447	0.6415
CG32556	*inx2*	–0.0986	0.1993	0.0444	0.6708	**–0.2416**	**0.0121**
CG32556	*Mer*	0.0579	0.4507	–0.1050	0.3147	**0.2209**	**0.0217**
CG32556	*psq*	0.1233	0.1086	0.1019	0.3293	0.1447	0.1323
CG32556	*neur*	0.0079	0.9176	–0.0113	0.9142	0.0271	0.7776
CG32556	*esg*	–0.1121	0.147	–0.1838	0.0787	–0.0404	0.6745
CG32556	*Sema-5c*	–0.0592	0.4408	0.1094	0.2950	**–0.2278**	**0.0179**
CG16708	*CG14781/82*	–0.0055	0.9430	0.1650	0.1143	–0.1760	0.0673
CG16708	*Crc*	–0.0405	0.5982	–0.0119	0.9094	–0.0691	0.4720
CG16708	*inx2*	0.0811	0.2913	0.0881	0.3987	0.0740	0.4412
CG16708	*Mer*	0.0451	0.5569	–0.0463	0.6578	0.1365	0.1556
CG16708	*psq*	–0.0845	0.2712	–0.1244	0.2337	–0.0447	0.6420
CG16708	*neur*	0.0101	0.8951	–0.0275	0.7922	0.0478	0.6191
CG16708	*esg*	–0.0349	0.6499	–0.0150	0.8858	–0.0547	0.5689
CG16708	*Sema-5c*	0.0405	0.5983	–0.0169	0.8716	0.0978	0.3088
CG14781/82	*Crc*	–0.0067	0.9300	–0.0044	0.9666	–0.0091	0.9245

TABLE 3.5 (Continued).

		0.1% and 0.3% benzaldehyde		0.1% (v/v) benzaldehyde		0.3% (v/v) benzaldehyde	
Parent 1	Parent 2	*SCA*	*P*	*SCA*	*P*	*SCA*	*P*
CG14781/82	*inx2*	0.0748	0.3301	0.1406	0.1782	0.0090	0.9253
CG14781/82	*Mer*	–0.0261	0.7338	**–0.2138**	**0.0409**	0.1615	0.0930
CG14781/82	*psq*	–0.1108	0.1494	–0.0019	0.9857	**–0.2197**	**0.0224**
CG14781/82	*neur*	0.0589	0.4434	–0.0300	0.7738	0.1478	0.1243
CG14781/82	*esg*	0.0514	0.5036	0.1575	0.1317	–0.0547	0.5689
CG14781/82	*Sema-5c*	–0.1133	0.1404	–0.1944	0.0629	–0.0322	0.7377
Crc	*inx2*	0.0873	0.2557	0.1638	0.1170	0.0109	0.9098
Crc	*Mer*	0.1364	0.0759	**0.2444**	**0.0194**	0.0284	0.7676
Crc	*psq*	0.0642	0.4031	0.0913	0.3822	0.0372	0.6986
Crc	*neur*	**–0.1686**	**0.0283**	**–0.2619**	**0.0123**	–0.0754	0.4329
Crc	*esg*	–0.0636	0.4076	**–0.2194**	**0.0359**	0.0921	0.3377
Crc	*Sema-5c*	–0.0233	0.7619	–0.0763	0.4652	0.0297	0.7572
inx2	*Mer*	**–0.2296**	**0.0028**	**–0.2656**	**0.0111**	**–0.1935**	**0.0443**
inx2	*psq*	0.0158	0.8372	–0.0538	0.6067	0.0853	0.3746
inx2	*neur*	–0.1246	0.1050	–0.1069	0.3061	–0.1422	0.1390
inx2	*esg*	0.0754	0.3261	–0.0644	0.5375	**0.2153**	**0.0253**
inx2	*Sema-5c*	0.1183	0.1237	0.0538	0.6067	0.1828	0.0573
Mer	*psq*	–0.0226	0.7681	–0.0531	0.6109	0.0078	0.9351
Mer	*neur*	**0.1770**	**0.0213**	**0.3888**	**0.0002**	–0.0347	0.7177

TABLE 3.5 (Continued).

		0.1% and 0.3% benzaldehyde		0.1% (v/v) benzaldehyde		0.3% (v/v) benzaldehyde	
Parent 1	Parent 2	*SCA*	*P*	*SCA*	*P*	*SCA*	*P*
Mer	*esg*	–0.0255	0.7400	0.1413	0.1763	**–0.1922**	**0.0457**
Mer	*Sema-5c*	–0.1126	0.1426	–0.0906	0.3855	–0.1347	0.1613
psq	*neur*	–0.0351	0.6473	–0.1009	0.2937	–0.1009	0.2937
psq	*esg*	–0.0301	0.6947	0.0466	0.6279	0.0466	0.6279
psq	*Sema-5c*	0.0799	0.2981	0.0436	0.6497	0.0436	0.6497
neur	*esg*	0.0720	0.3486	0.0390	0.6847	0.0390	0.6847
neur	*Sema-5c*	0.0024	0.9756	0.0916	0.3407	0.0916	0.3407
esg	*Sema-5c*	0.0674	0.3806	–0.0509	0.5961	–0.0509	0.5961

* significant *SCA* values are indicated in bold font.

TABLE 3.6

ANOVA of olfactory behavior of a population of 41 wild-derived inbred lines.*

Source	df	MS	F	*P*	σ^2
Line (*L*)	40	14.539	6.55	**0.0001**	**0.0937**
Sex (*S*)	1	2.674	4.11	**0.0493**	----
Food (*F*)	2	4.034	1.28	0.2841	----
Concentration (*C*)	1	251.194	139.11	< **0.0001**	----
L×*S*	40	0.650	0.85	0.6882	0.0000
L×*F*	80	3.155	1.22	0.2003	0.0207
L×*C*	40	1.806	0.63	0.9437	0.0000
S×*F*	2	1.784	3.35	**0.0399**	----
S×*C*	1	0.225	0.28	0.5974	----
F×*C*	2	13.609	5.19	**0.0076**	----
L×*S*×*F*	80	0.532	0.95	0.5866	0.0000
L×*S*×*C*	40	0.795	1.42	0.0909	0.0048
S×*F*×*C*	2	0.894	1.60	0.2082	----
L×*F*×*C*	80	2.623	4.69	< **0.0001**	**0.0887**
L×*S*×*F*×*C*	80	0.559	1.44	**0.0066**	**0.0166**
Error	4428	0.388			0.3879

TABLE 3.6 (Continued).

* df designates degrees of freedom; MS designates mean squares; significant *P* and σ^2 values are in bold font. Correlation of line means across sexes, concentrations and food sources is $r_{GE} = 0.4708$

TABLE 3.7

Analyses of variance of olfactory behavior of 41 wild-derived inbred lines for two concentrations of benzaldehyde. *

Concentration (v/v)	Source	df	MS	F	*P*	σ^2
0.1%	Line (*L*)	40	8.298	2.35	**0.0007**	**0.0795**
	Sex (*S*)	1	2.226	3.65	0.0634	----
	Food (*F*)	2	1.419	0.40	0.6694	----
	L×*S*	40	0.610	1.02	0.4636	0.0003
	L×*F*	80	3.517	5.86	**<0.0001**	**0.1458**
	S×*F*	2	0.093	0.16	0.8566	----
	L×*S*×*F*	80	0.600	1.73	**<0.0001**	**0.0253**
	Error	2214	0.347			0.3475
0.3%	Line (*L*)	40	8.047	3.09	**<0.0001**	**0.0907**
	Sex (*S*)	1	0.673	0.81	0.3745	----
	Food (*F*)	2	16.224	7.18	0.0014	----
	L×*S*	40	0.835	1.70	**0.0221**	**0.0115**
	L×*F*	80	2.261	4.61	**<0.0001**	**0.0885**
	S×*F*	2	2.585	5.27	**0.0071**	----
	L×*S*×*F*	80	0.491	1.15	0.1816	0.0062
	Error	2214	0.428			0.4284

TABLE 3.7 (Continued).

* df designates degrees of freedom; MS designates mean squares; significant *P* and σ^2 values are in bold font. Correlation of line means across sexes and food sources: 0.1% (v/v) benzaldehyde, $r_{GE} = 0.32$; 0.3% (v/v) benzaldehyde, $r_{GE} = 0$.

TABLE 3.8

Analyses of variance of olfactory behavior of 41 wild-derived inbred lines for three larval food sources.*

Food	Source	df	MS	F	*P*	σ^2
Standard	Line (*L*)	40	5.794	2.85	**0.0008**	**0.0940**
	Sex (*S*)	1	0.354	0.51	0.4778	----
	Concentration (*C*)	1	89.491	47.31	**<0.0001**	----
	L×*S*	40	0.690	1.26	0.2356	0.0071
	L×*C*	40	1.892	3.45	**<0.0001**	**0.0672**
	S×*C*	1	1.851	3.38	0.0736	----
	L×*S*×*C*	40	0.548	1.30	0.0995	0.0127
	Error	1476	0.421			0.4212
Tomato	Line (*L*)	40	6.770	4.35	**0.0001**	**0.1254**
	Sex (*S*)	1	2.648	5.21	**0.0278**	----
	Concentration (*C*)	1	28.265	16.13	**0.0003**	----
	L×*S*	40	0.508	0.72	0.8477	0.0000
	L×*C*	40	1.752	2.49	**0.0024**	**0.0573**
	S×*C*	1	0.132	0.19	0.6678	----
	L×*S*×*C*	40	0.705	1.90	**0.0007**	**0.0236**
	Error	1476	0.371			0.3710

TABLE 3.8 (Continued).

Alcohol	Line (*L*)	40	8.285	2.54	**0.0031**	**0.1219**
	Sex (*S*)	1	3.240	6.28	0.0164	----
	Concentration (*C*)	1	160.657	47.15	**<0.0001**	----
	L×*S*	40	0.516	0.78	0.7786	0.0000
	L×*C*	40	3.407	5.17	**<0.0001**	**0.1410**
	S×*C*	1	0.031	0.05	0.8301	----
	L×*S*×*C*	40	0.659	1.77	**0.0022**	**0.0216**
	Error	1476	0.372			0.3716

* df designates degrees of freedom; MS designates mean squares; significant *P* and σ^2 values are in bold font. Correlation of line means across sexes and concentrations: Standard medium, $r_{GE} = 0.58$; Tomato medium, $r_{GE} = 0.61$; Alcohol supplemented medium, $r_{GE} = 0.43$.

TABLE 3.9

Analyses of variance of olfactory behavior of 41 wild-derived inbred lines for two concentrations of benzaldehyde and three larval food sources. *

Concentration	Food	Source	df	MS	F	P	σ^2
0.1%	Standard	Line (L)	40	4.139	10.11	**<0.0001**	**0.1727**
		Sex (S)	1	0.293	0.72	0.3979	----
		L×S	40	0.686	1.67	**0.0064**	**0.0276**
		Error	738	0.410			0.4096
	Tomato	Line (L)	40	3.090	9.85	**<0.0001**	**0.1195**
		Sex (S)	1	0.799	2.55	0.1109	----
		L×S	40	0.700	2.23	**<0.0001**	**0.0386**
		Error	738	0.314			0.3138
	Alcohol	Line (L)	40	8.103	25.39	**<0.0001**	**0.3839**
		Sex (S)	1	1.320	4.14	0.0423	----
		L×S	40	0.425	1.33	0.0849	0.0106
		Error	738	0.319			0.3191
0.3%	Standard	Line (L)	40	3.547	8.20	**<0.0001**	**0.1497**
		Sex (S)	1	1.912	4.42	0.0359	----
		L×S	40	0.553	1.28	0.1207	0.0120
		Error	738	0.433			0.4328
	Tomato	Line (L)	40	5.431	12.68	**<0.0001**	**0.2459**
		Sex (S)	1	1.981	4.63	0.0318	----
		L×S	40	0.513	1.20	0.1898	0.0085
		Error	738	0.428			0.4282
	Alcohol	Line (L)	40	3.590	8.46	**<0.0001**	**0.1420**
		Sex (S)	1	1.951	4.60	0.0323	----
		L×S	40	0.750	1.77	**0.0028**	**0.0326**
		Error	738	0.424			0.4241

* df designates degrees of freedom; MS designates mean squares; significant *P* and σ^2 values are in bold font. Correlation of line means across sexes: 0.1% (v/v) benzaldehyde:

TABLE 3.9 (Continued).

Standard medium, $r_G = 0.86$; Tomato medium, $r_G = 0.76$; Alcohol supplemented medium, $r_G = 1$; 0.3% (v/v) benzaldehyde: Standard medium, $r_G = 1$; Tomato medium, $r_G = 1$; Alcohol supplemented medium, $r_G = 0.81$.

TABLE 3.10

Analyses of variance of olfactory behavior of 41 wild-derived inbred lines by sex, for two concentrations of benzaldehyde and three larval food sources.*

Concentration	Food	Sex	Source	df	MS	F	P	σ^2	H^2
0.1%	Standard	F	Line	40	2.552	6.17	**<0.0001**	0.2138	0.3406
			Error	369	0.414			0.4139	
		M	Line	40	2.273	5.61	**<0.0001**	0.1868	0.3155
			Error	369	0.405			0.4053	
	Tomato	F	Line	40	1.514	4.69	**<0.0001**	0.1191	0.2693
			Error	369	0.323			0.3232	
		M	Line	40	2.276	7.48	**<0.0001**	0.1972	0.3931
			Error	369	0.304			0.3044	
	Alcohol	F	Line	40	4.591	14.25	**<0.0001**	0.4269	0.5699
			Error	369	0.322			0.3222	
		M	Line	40	3.937	12.45	**<0.0001**	0.3621	0.5339
			Error	369	0.316			0.3161	
0.3%	Standard	F	Line	40	1.714	3.53	**<0.0001**	0.1229	0.2021
			Error	369	0.485			0.4851	
		M	Line	40	2.386	6.27	**<0.0001**	0.2005	0.3452
			Error	369	0.380			0.3804	
	Tomato	F	Line	40	2.719	6.37	**<0.0001**	0.2293	0.3496
			Error	369	0.427			0.4266	
		M	Line	40	3.225	7.50	**<0.0001**	0.2796	0.3941
			Error	369	0.430			0.4298	
	Alcohol	F	Line	40	1.981	5.05	**<0.0001**	0.1589	0.2884
			Error	369	0.392			0.3921	
		M	Line	40	2.359	5.17	**<0.0001**	0.1903	0.2944
			Error	369	0.456			0.4561	

* df designates degrees of freedom; MS designates mean squares.

TABLE 3.11

Olfactory response scores of the eight lines selected for transcriptional profiling at 0.3% (v/v) benzaldehyde averaged over ten replicates.*

Line		Standard		Tomato		Alcohol	
		Male	Female	Male	Female	Male	Female
158	Mean	3.46	3.44	2.50	2.74	3.59	3.48
	SEM	0.08	0.14	0.24	0.22	0.28	0.24
304	Mean	3.42	3.56	3.50	3.75	3.45	2.93
	SEM	0.15	0.31	0.17	0.14	0.24	0.20
362	Mean	3.37	3.53	1.87	2.44	2.90	3.63
	SEM	0.09	0.27	0.23	0.16	0.24	0.16
379	Mean	4.16	3.57	3.75	3.66	3.59	3.71
	SEM	0.13	0.22	0.19	0.19	0.23	0.22
380	Mean	3.74	2.86	3.74	3.26	3.23	3.55
	SEM	0.07	0.26	0.34	0.19	0.15	0.29
774	Mean	2.09	2.36	2.23	2.67	2.52	3.02
	SEM	0.20	0.19	0.17	0.18	0.09	0.08
786	Mean	3.65	2.61	4.13	3.92	4.18	4.08
	SEM	0.17	0.25	0.11	0.16	0.20	0.15
859	Mean	3.69	3.71	3.50	3.32	4.13	4.03
	SEM	0.15	0.19	0.09	0.26	0.09	0.22

* SEM, standard error of the mean

TABLE 3.12

Genes with significant GEI effects

Gene	Biological Function Gene Ontology
CG1486	Carboxylic acid metabolic process
CG15434	Mitochondrial electron transport, NADH to ubiquinone
CG17177	Cell communication and signal transduction
CG17821	Lipid metabolic process and fatty acid metabolic process
CG31091	Lipid metabolic process
CG32238	Protein modification process, C-terminal protein-tyrosinylation and protein metabolic process
CG32496	
/CG6788	Defense response to bacterium, cell adhesion, signal transduction
CG4835	Chitin metabolic process
CG9664	Lipid metabolic process and lipid transport
Dopamine	
transporter	Neurotransmitter transport, regulates sleep and arousal in insects, target for cocaine addiction
geko	Implicated in olfactory behavior and response to ethanol
Jonah 66C	Proteolysis, intracellular signaling cascade, cyclic nucleotide biosynthetic process
ryan express	Transcription initiation from RNA polymerase II promoter, male meiosis, spermatogenesis
Transferrin1	Iron ion transport and homeostasis, defense response
Ugt36Bb	
	Polysaccharide metabolic process, defense response, steroid metabolic process, response to toxin
CG13532	Unknown function
CG14872	Unknown function
CG15649	Unknown function
CG30447	Unknown function
E protein	Unknown function

TABLE 3.13

Genes with significant effects of the Line and Food terms in the ANOVA of transcript abundance of the expression microarrays.

Term	Category	Genes
Line	Odorant receptors	*Or42a* and *Or83b*
	Odorant binding proteins	*Obp18a, 19a, 22a, 44a, 49a, 50c, 50d, 51a, 56a, 56d, 56e, 56f, 56g, 56h, 56i, 57a, 57b, 57c, 8a, 99a, 99b, 99c, 99d, PBprp1(Obp69a), PBprp3(Obp83a), PBprp4(Obp84a), PBprp5(Obp28a)*
	Olfactory learning and memory	*cAMP-dependent protein kinase R* (MEHREN *et al.* 2004*), transient receptor potential* (AL-ANZI *et al*. 2006)*, dunce* and *rutabaga* (MARTIN *et al.* 2001)*, Enhancer of sevenless-2* (PHILIP *et al.* 2001)*, Fasciclin II* (HAMLIN *et al.* 2004, CHENG 2001*), Leonardo* (GASQUE *et al.* 2006)*, Minibrain* (SCHOLZ *et al.* 2000)*, neurofibromatosis type1* (ZARS 2000)*, Protein phosphatase 1* (ASZTALOS *et al.* 1993), *volado* (CONNOLLA and TULLY 1998), *dare* (FREEMAN *et al.* 1999) *and gp210 , murashka , nord , martik, quo vadis, rogdi, veinlet* and *visgun* (DUBNAU *et al.* 2003).
	Olfactory behavior and response to ethanol	*lush* (KIM *et al.* 1998) and *protein kinase A* (MOORE *et al.* 1998).
	Olfactory behavior	*Semaphorin 5C* (SAMBANDAN *et al*. 2006; ROLLMANN *et al.* 2007)*, pipsqueak, escargot, innexin 2, Merlin, CG32556, CG16708* (SAMBANDAN *et al*. 2006)*, scribble* (FEDOROWICH *et al.* 1998), *dare* (FREEMAN *et al.* 1999)*, martik* (DUBNAU *et al.* 2003)*, CG30033*, *Pbprp3, OS-9*
Food	Odorant binding proteins	*Obp49a*, *Pbprp1(Obp69a)*
	Olfactory behavior	*Calreticulin* (STOLTZFUS *et al.* 2003)
	Learning and memory	*neurofibromatosis type1*(GUO 2000; ZARS 2000), *quo vadis* (DUBNAU 2003)
	Olfactory learning	*Enhancer of sevenless-2B* (PHILIP *et al.* 2001)
	Response to stimulus	*no-on-transient A* (CAMPESAN 2001)
	Circadian rhythm	*pigment dispersing factor* (WILLIAMS 2001; MERTENS *et al.* 2005)

TABLE 3.14

Olfactory response scores for F1 from crosses between *CG9664* stocks (control and mutant) to the eight selected lines used for transcriptional profiling reared on standard, tomato-enriched or alcohol-supplemented media at 0.3% (v/v) benzaldehyde averaged over ten replicates.*

CG9664 - Control												
	Standard				Tomato				Alcohol			
	Male		Female		Male		Female		Male		Female	
Line	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
158	3.63	0.24	3.05	0.12	3.25	0.229	2.78	0.1	3.07	0.16	3.07	0.237
304	3.75	0.24	3.34	0.15	3.25	0.129	3.39	0.17	3.34	0.13	3.58	0.123
362	3.59	0.17	3.2	0.22	3.32	0.225	3.13	0.1	2.6	0.19	3.21	0.177
379	3.5	0.16	3.65	0.17	3.275	0.132	2.99	0.1	2.65	0.21	3.45	0.104
380	3.9	0.13	3.36	0.14	3.23	0.119	3.08	0.13	3.4	0.09	3.39	0.131
774	3.36	0.33	3.04	0.12	3.19	0.189	2.58	0.09	3.4	0.2	3.43	0.132
786	3.39	0.11	2.81	0.14	3.24	0.161	2.92	0.13	3.21	0.21	3.34	0.156
859	2.73	0.28	3.11	0.13	2.56	0.164	2.85	0.08	2.8	0.18	3.55	0.13

TABLE 3.14 (Continued).

CG9664 - Mutants												
	Standard				Tomato				Alcohol			
	Male		Female		Male		Female		Male		Female	
Line	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
158	3.58	0.14	3.15	0.14	3.95	0.13	3.43	0.11	3.87	0.14	3.11	0.08
304	3.77	0.11	3.91	0.20	3.77	0.08	3.76	0.15	3.67	0.11	3.01	0.08
362	3.98	0.23	3.26	0.12	4.31	0.09	3.61	0.09	2.64	0.08	3.53	0.12
379	3.83	0.12	3.15	0.20	3.79	0.06	3.31	0.09	3.33	0.09	2.66	0.21
380	4.03	0.19	3.85	0.11	4.05	0.12	3.36	0.08	3.82	0.09	3.13	0.09
774	3.54	0.17	3.00	0.16	3.21	0.08	3.08	0.15	3.49	0.11	3.26	0.12
786	3.53	0.11	3.17	0.10	3.17	0.12	3.50	0.12	3.71	0.11	3.20	0.14
859	2.47	0.30	2.95	0.12	3.62	0.12	3.59	0.10	2.52	0.09	3.55	0.23

* SEM, standard error of the mean

TABLE 3.15

Olfactory response scores for F1 females from crosses between *Transferrin 1* stocks (control and mutant) to the eight selected lines used for transcriptional profiling reared on standard, tomato-enriched or alcohol-supplemented media at 0.3% (v/v) benzaldehyde averaged over ten replicates.*

	Transferrin 1- Control					
	Standard		Tomato		Alcohol	
Line	Mean	SEM	Mean	SEM	Mean	SEM
158	2.49	0.27	2.05	0.23	3.18	0.25
304	2.74	0.22	2.44	0.31	3.50	0.17
362	2.86	0.15	2.88	0.09	3.00	0.19
379	2.85	0.14	3.19	0.21	3.27	0.19
380	2.45	0.10	3.40	0.16	3.43	0.16
774	1.56	0.13	2.82	0.12	2.78	0.22
786	1.54	0.12	2.89	0.17	2.77	0.12
859	2.58	0.24	3.33	0.24	2.95	0.11

TABLE 3.15 (Continued).

Transferrin 1- Mutants						
	Standard		Tomato		Alcohol	
Line	Mean	SEM	Mean	SEM	Mean	SEM
158	2.95	0.23	3.27	0.12	3.02	0.21
304	3.32	0.12	3.21	0.17	3.29	0.17
362	3.37	0.23	3.28	0.15	2.39	0.18
379	3.73	0.23	3.75	0.14	2.90	0.12
380	3.86	0.18	3.64	0.27	2.79	0.14
774	3.14	0.21	3.51	0.22	2.32	0.06
786	2.62	0.21	3.72	0.26	3.27	0.15
859	3.69	0.17	4.25	0.11	3.82	0.11

* SEM, standard error of the mean

TABLE 3.16

ANOVA for olfactory behavior to test for failure to complement pooled across three larval food sources for *CG9664* (sexes pooled; A) and *Transferrin 1* (females only; B).*

A. *CG9664*

Source	df	MS	F	*P*
Line (*L*)	7	3.531	1.15	0.422
Sex (*S*)	1	5.177	2.08	0.1921
Food (*F*)	2	1.8	1.18	0.3349
Genotype (*G*)	1	13.419	39.69	**0.0004**
L×*S*	7	2.484	2.9	0.127
L×*F*	14	1.52	2.71	0.183
L×*G*	7	0.338	0.61	0.7086
S×*F*	2	2.562	3.32	0.0659
S×*G*	1	2.981	4.17	0.0805
F×*G*	2	5.394	12.83	**0.0007**
L×*S*×*F*	14	0.77	1.22	0.3558
L×*S*×*G*	7	0.714	1.13	0.3962
S×*F*×*G*	2	1.436	2.28	0.1390
L×*F*×*G*	14	0.419	0.67	0.7715
L×*S*×*F*×*G*	14	0.63	2.39	**0.0029**
Error	864	0.264		

TABLE 3.16 (Continued).

B. ***Transferrin 1***

Source	Df	MS	F	P
Line (*L*)	7	4.273	1.81	0.1878
Food (*F*)	2	5.402	2.65	0.1055
Genotype (*G*)	1	30.805	26.53	**0.0013**
L×*F*	14	2.036	2.45	0.0523
L×*G*	7	1.16	1.4	0.2803
F×*G*	2	12.964	15.62	**0.0003**
L×*F*×*G*	14	0.829	2.42	**0.0029**
Error	432	0.343		

* df designates degrees of freedom; MS designates mean squares; significant *P* values are in bold font.

TABLE 3.17

ANOVA of olfactory behavior by *line* across all three larval food media for *CG9664* (pooled both sexes) and *Transferrin1* (females only).*

Line	Gene	Sex (S)	Food (F)	(G)	SxF	SxG	FxG	SxFxG
158	*CG9664*	**<0.0001**	0.7912	**0.0003**	0.851	0.2726	**0.0309**	0.1523
	Tsf1	----	0.1107	**0.0074**	----	----	**0.0119**	----
304	*CG9664*	0.333	**0.0492**	**0.0335**	0.4837	0.3872	**0.0491**	**0.0105**
	Tsf1	----	**0.0227**	**0.0254**	----	----	**0.0447**	----
362	*CG9664*	0.3712	**<0.0001**	**<0.0001**	**<0.0001**	0.3167	**0.0285**	0.1951
	Ttsf1	----	**0.0301**	0.4755	----	----	**0.0028**	----
379	*CG9664*	**0.0474**	**<0.0001**	0.0938	0.1767	**<0.0001**	**0.0305**	**0.0105**
	Tsf1	----	0.1017	**0.016**	----	----	**0.0024**	----
380	*CG9664*	**<0.0001**	**0.0001**	**<0.0001**	0.9179	0.0575	**0.0405**	**0.0104**
	Tsf1	----	**0.0488**	**0.0243**	----	----	**<0.0001**	----
774	*CG9664*	**0.0044**	**0.0125**	0.3505	0.3827	1	0.4879	0.2615
	Tsf1	----	**<0.0001**	**<0.0001**	----	----	**<0.0001**	----
786	*CG9664*	0.0764	0.4217	**0.0487**	0.356	0.9593	0.9032	**0.0298**
	Tsf1	----	**<0.0001**	**<0.0001**	----	----	0.2739	----
859	*CG9664*	**<0.0001**	**0.0118**	0.0654	**0.0081**	0.9193	**<0.0001**	0.4457
	Tsf1	----	**0.0018**	**<0.0001**	----	----	0.7706	----

* Values in the Table represent *P* values for each of the ANOVA terms. Significant *P* values are in bold font.

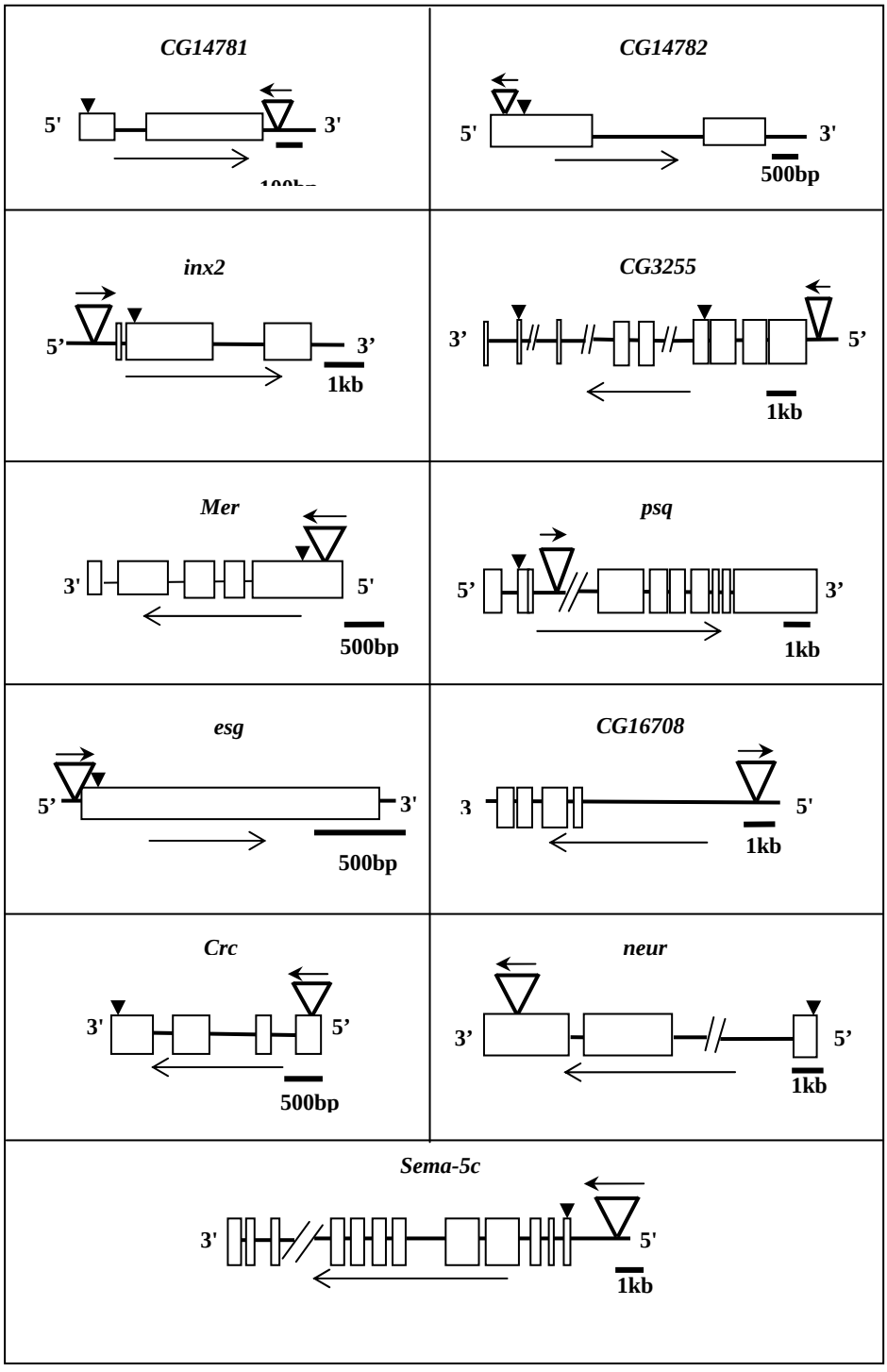

CG14781
5'
3'
CG14782
5'
3'
500bp
inx2
5'
3'
1kb
CG3255
3'
5'
1kb
Mer
3'
5'
500bp
psq
5'
3'
1kb
esg
5'
3'
500bp
CG16708
3
5'
1kb
Crc
3'
5'
500bp
neur
3'
5'
1kb
Sema-5c
3'
5'
1kb

FIGURE 3.1

Diagrammatic representation of *p[GT1]* insertion sites in candidate genes. Inverted triangles indicate the *P*-element insertion sites. Boxes indicate exons. Orientation of the candidate gene and the *P*-element are indicated by the long arrows below each diagram and the small arrows above the inverted triangles, respectively. Arrowheads indicate the position of the translation initiation ATG site of the coding sequence.

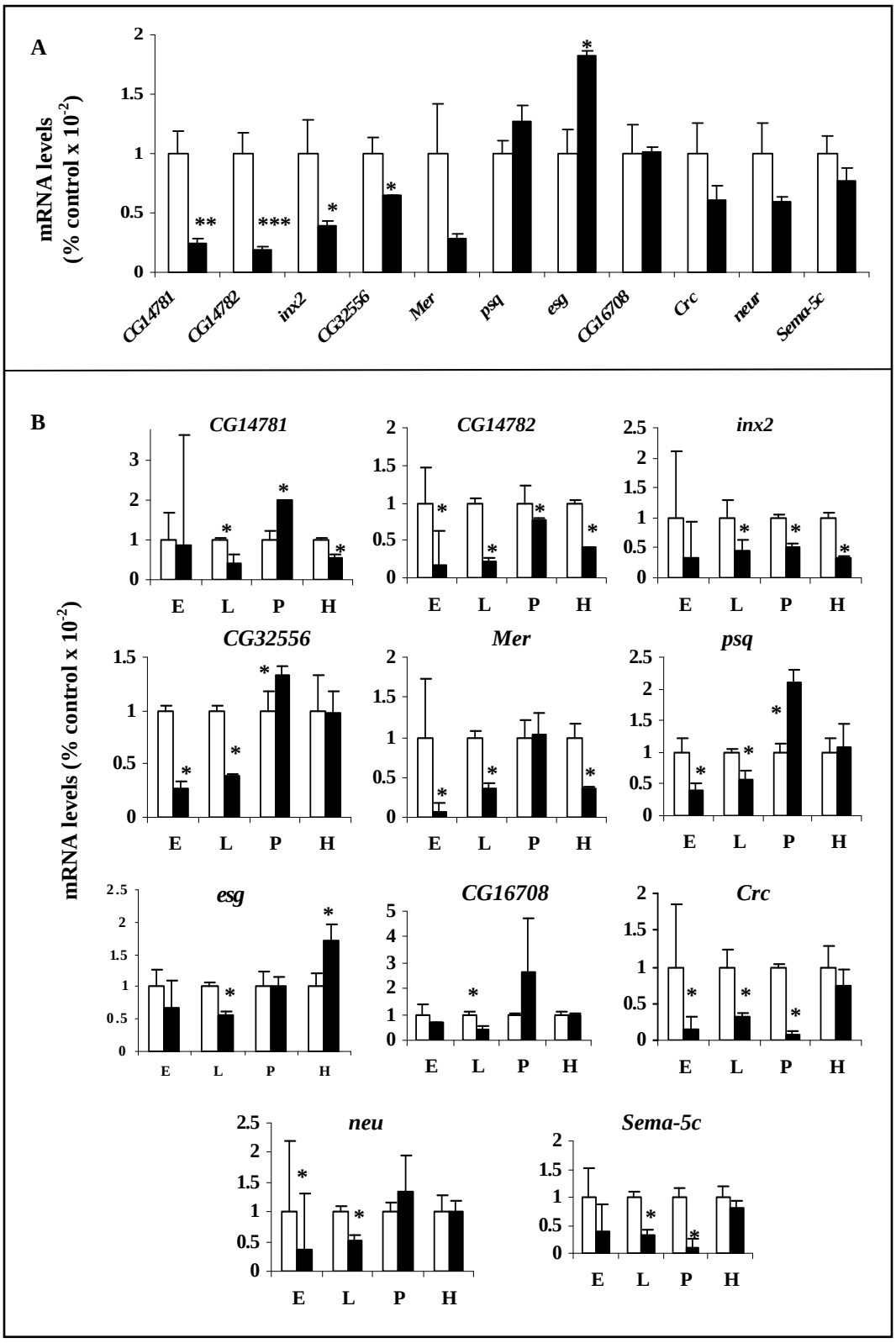

A
mRNA levels (% control x 10-2)
CG14781
CG14782
inx2
CG32556
Mer
psq
esg
CG16708
Crc
neur
Sema-5c
B
mRNA levels (% control x 10-2)
CG14781
CG14782
inx2
CG32556
Mer
psq
esg
CG16708
Crc
neu
Sema-5c
E
L
P
H

FIGURE 3.2

Quantitative RT-PCR analyses of candidate gene expression levels in whole female flies (A) and at four different developmental stages (B). mRNA expression levels are standardized against the *Canton S* B control (open bars). Solid bars show the relative expression levels in the *p[GT1]* insertion lines. E designates embryos; L, larvae; P, pupae; H, adult female heads. In panel A, significance levels for differences between mutants and the control were estimated with two-tailed Student's *t* test. *, $P<0.05$; **, $P<0.01$; ***, $P<0.001$. In panel B, differences in gene expression levels were considered significant, indicated by the asterisks, when 95% confidence intervals around the mean value of the mutant and that of the control were non-overlapping.

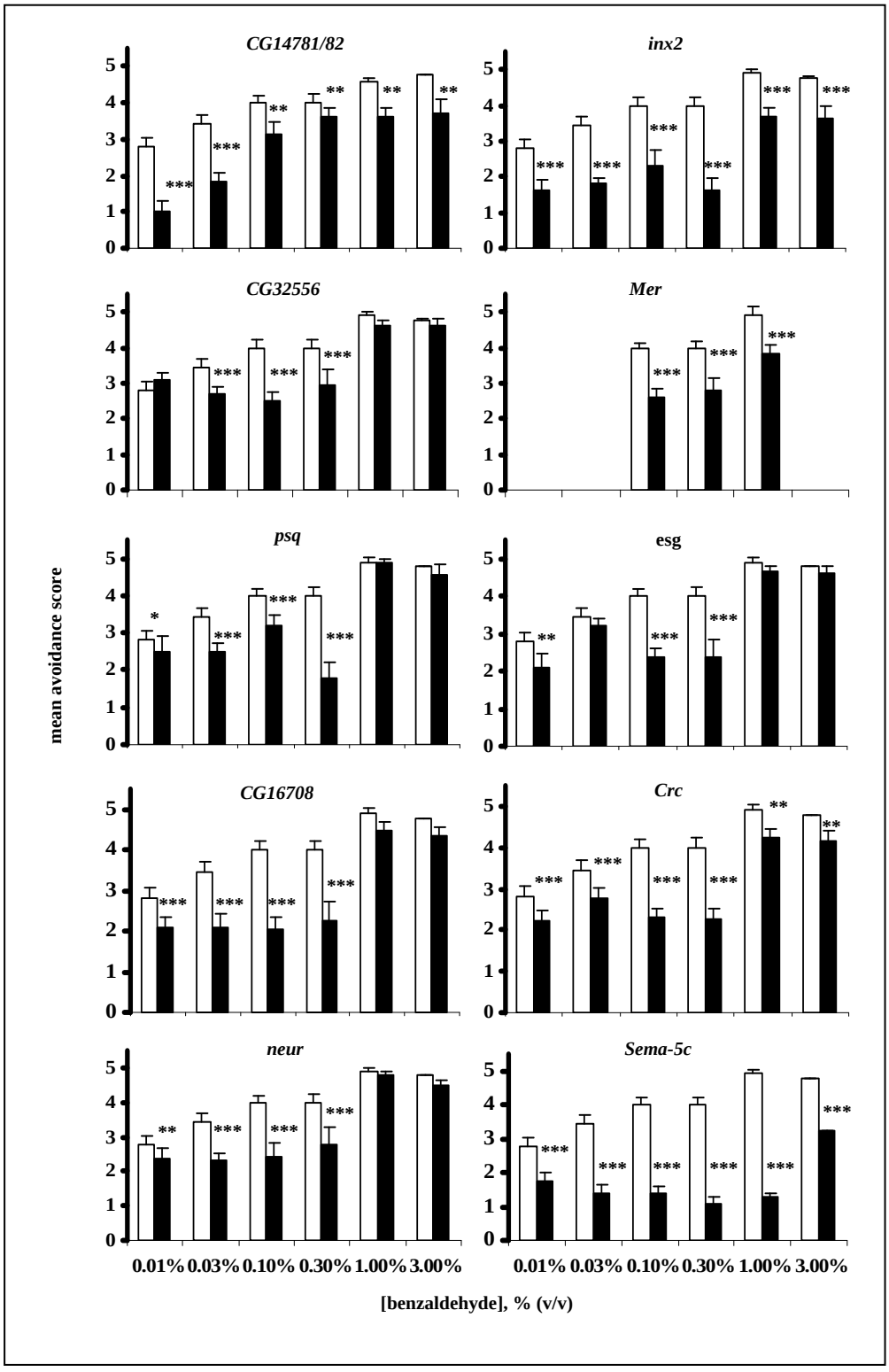

CG14781/82
inx2
CG32556
Mer
psq
esg
CG16708
Crc
neur
Sema-5c
mean avoidance score
0.01% 0.03% 0.10% 0.30% 1.00% 3.00%
[benzaldehyde], % (v/v)

FIGURE 3.3

Dose responses for olfactory avoidance behavior to benzaldehyde of female flies. Solid bars correspond to *p[GT1]*-element insertion lines and open bars to the *Canton S*-B control. Significance levels for differences between mutants and the control were estimated with Student's *t* test. *, $P<0.05$; **, $P<0.01$; ***, $P<0.001$

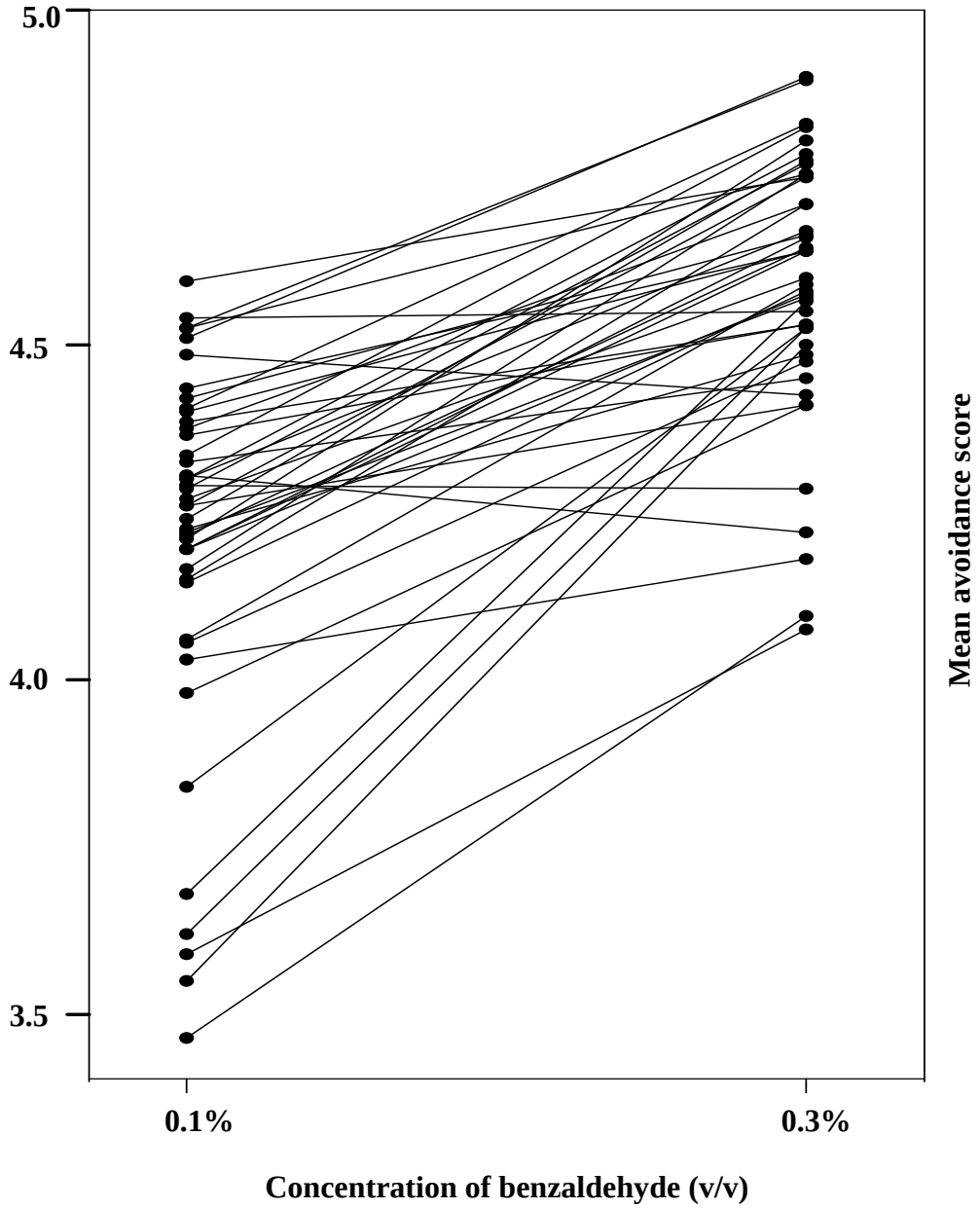

5.0
4.5
4.0
3.5
Mean avoidance score
0.1%
0.3%
Concentration of benzaldehyde (v/v)

FIGURE 3.4

Variation in mean avoidance scores of transheterozygotes at two concentrations of benzaldehyde. Notice the greater variation in scores at the lower stimulus concentration and the crossing over of reaction norms, which reflects genotype by environment interactions.

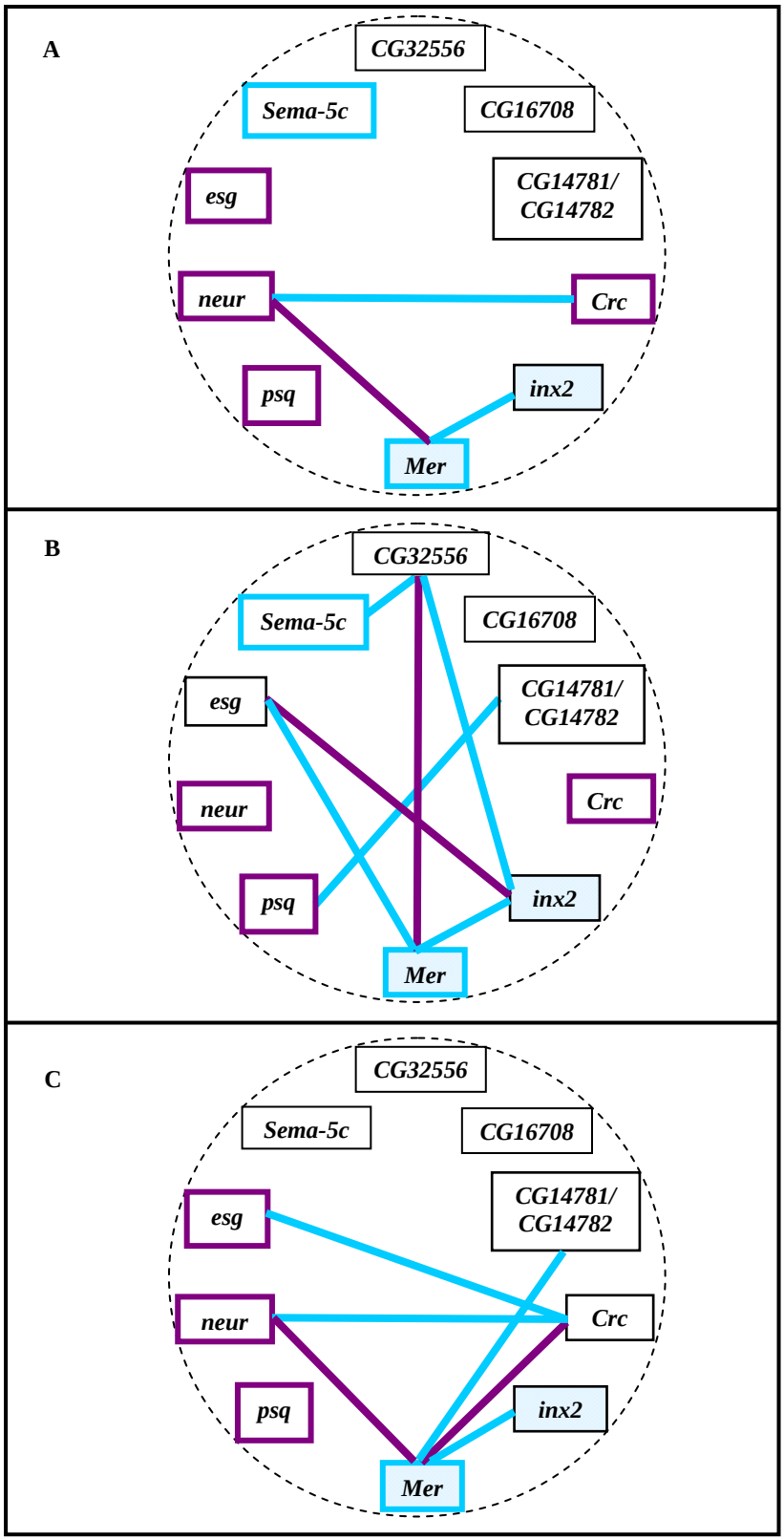

A
CG32556
Sema-5c
CG16708
esg
CG14781/
CG14782
neur
Crc
psq
inx2
Mer
B
CG32556
Sema-5c
CG16708
esg
CG14781/
CG14782
neur
Crc
psq
inx2
Mer
C
CG32556
Sema-5c
CG16708
esg
CG14781/
CG14782
neur
Crc
psq
inx2
Mer

FIGURE 3.5

Epistatic interactions between ten pGT1 insertion lines. Panel A shows a diagrammatic representation of enhancer (violet lines) and suppressor (blue lines) effects among transposon tagged candidate genes, when the analysis is performed with data pooled from both stimulus concentrations. Calculated *SCA* values for significant epistatic interactions are 0.1770 (*Mer-neur*), -0.2296 (*Mer-inx2*) and -0.1686 (*Crc-neur*). Panel B shows a diagrammatic representation of enhancer (violet lines) and suppressor (blue lines) effects among transposon tagged candidate genes observed at 0.1% (v/v) benzaldehyde. Calculated *SCA* values for significant epistatic interactions are -0.2138 (*Mer-CG14781/CG14782*), 0.2444 (*Mer-Crc*), -0.2619 (*Crc-neur*), -0.2194 (*Crc-esg*), -0.2656 (*Mer-inx2*), and 0.3888 (*Mer-neur*). Panel C shows a diagrammatic representation of enhancer (violet lines) and suppressor (blue lines) effects among transposon tagged candidate genes observed at 0.3% (v/v) benzaldehyde. Calculated *SCA* values for significant epistatic interactions are -0.2416 (*CG32556-inx2*), 0.2209 (*CG32556-Mer*), -0.2278 (*CG32556-Sema-5C*), -0.2197 (*CG14781/CG14782-psq*), -0.1935(*Mer-inx2*), 0.2153 (*inx2-esg*), and -0.1922 (*Mer-esg*). *Mer* and *inx 2* are highlighted against a blue background to emphasize their central positions and the invariant interaction between them in both environments. Violet and blue bordered boxes indicate significant positive and negative *GCA* values of the indicated candidate gene, respectively.

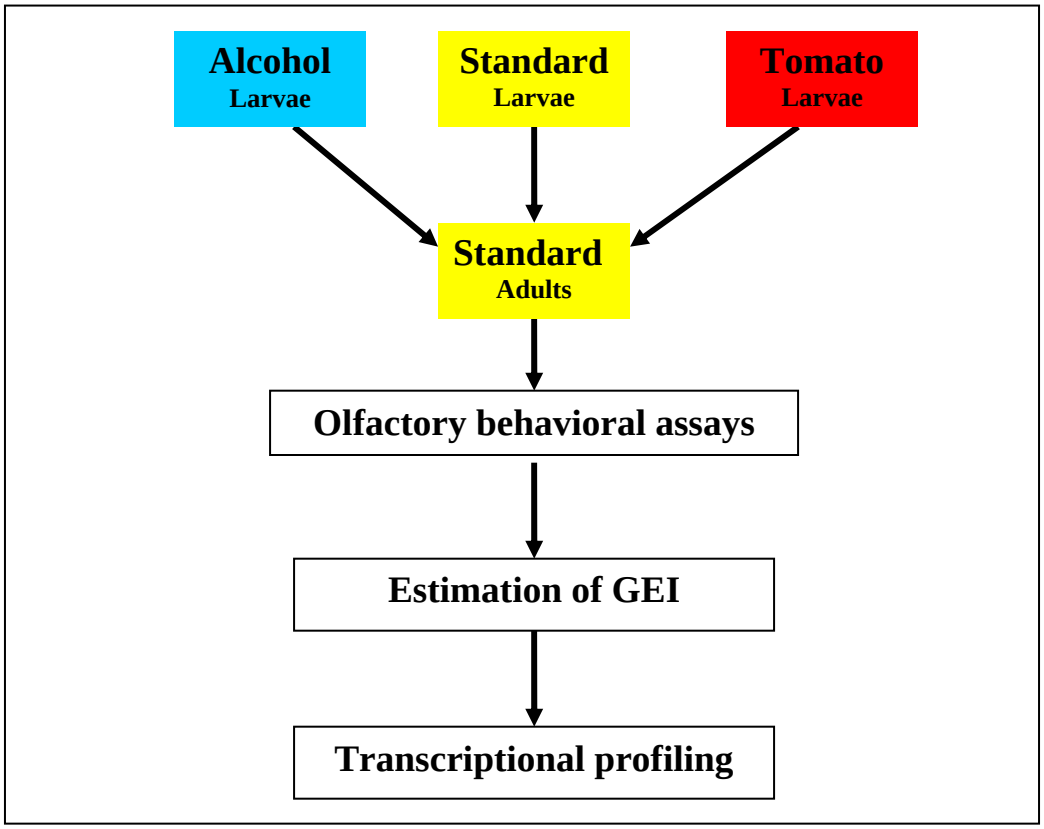
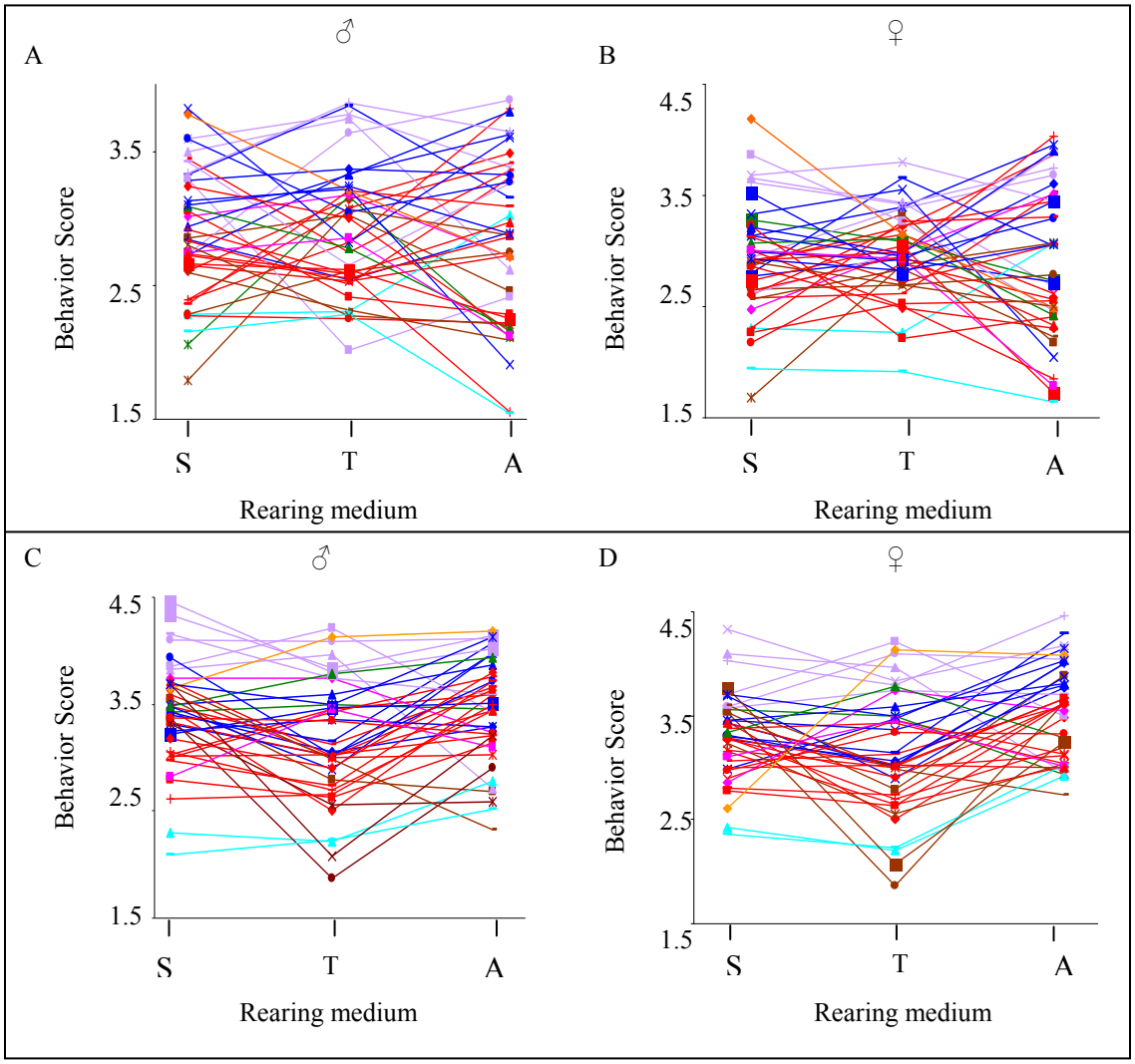


FIGURE 3.6

Diagrammatic representation of the experimental design.

FIGURE 3.7

Reaction norms for olfactory behavior of adult flies reared in different larval environments among wild-derived inbred lines. Crossing over of reaction norms indicates GEI. The olfactory behavior scores are plotted on the Y axis. The X-axis designates the rearing environments. S, T and A denote standard, tomato and alcohol larval food sources, respectively. Panels A and B show behavior scores obtained at 0.1% (v/v) benzaldehyde, and panels C and D show behavior scores obtained at 0.3 % (v/v) benzaldehyde. Panels A and C show reaction norms for males. Panels B and D show reaction norms for females. Lines are color coded according to their hierarchical clustering profile shown in Figure 3.8.


A
♂
Behavior Score
4.5
3.5
2.5
1.5
S
T
A
Rearing medium
B
♀
Behavior Score
4.5
3.5
2.5
1.5
S
T
A
Rearing medium
C
♂
Behavior Score
4.5
3.5
2.5
1.5
S
T
A
Rearing medium
D
♀
Behavior Score
4.5
3.5
2.5
1.5
S
T
A
Rearing medium

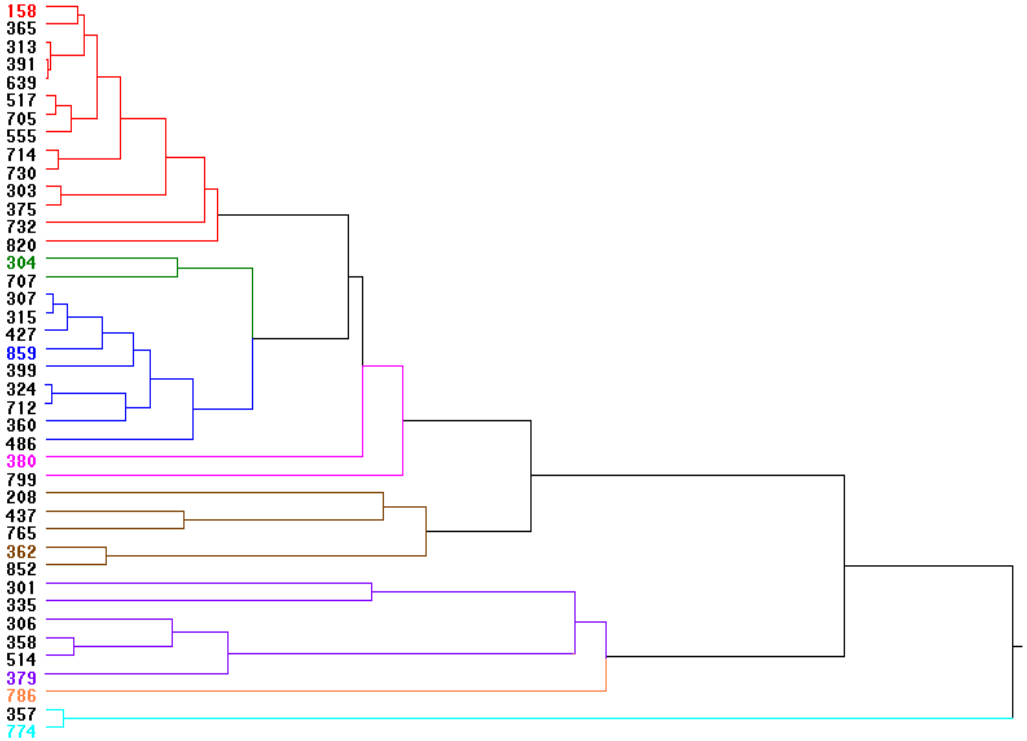


FIGURE 3.8

Hierarchical clustering for olfactory behavioral measurements at 0.3% (v/v) benzaldehyde pooled for sexes. The eight major clusters are color coded to the reaction norms in figure 3.7. The numbers of the eight lines chosen from each cluster for the microarray analysis are highlighted in color.

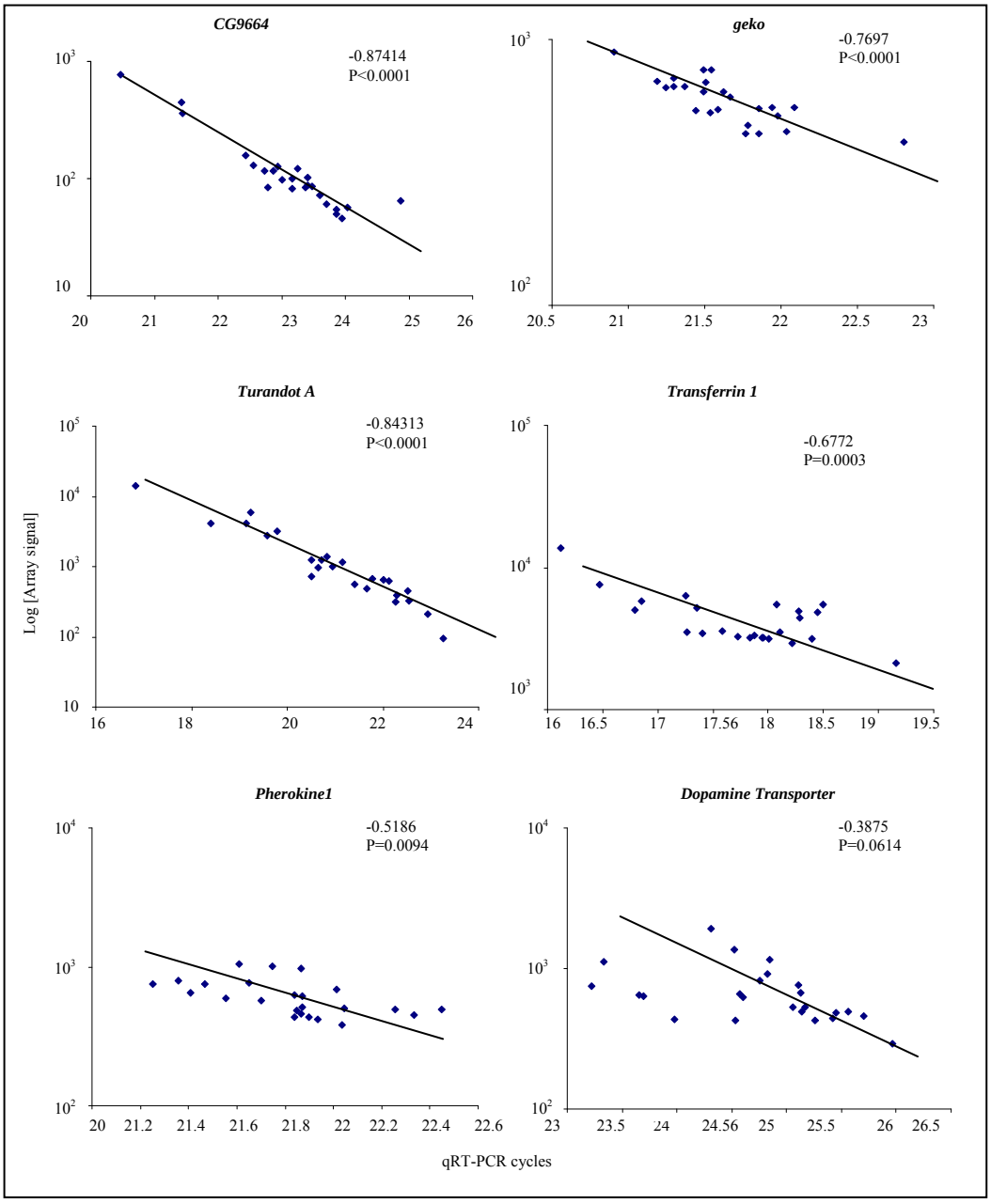


FIGURE 3.9

Correlations between qRT-PCR and expression microarray signals. The Y axis shows the log transformed raw microarray signal values for the six genes. The X-axis shows the number of PCR cycles needed to reach the SYBR green detection threshold.

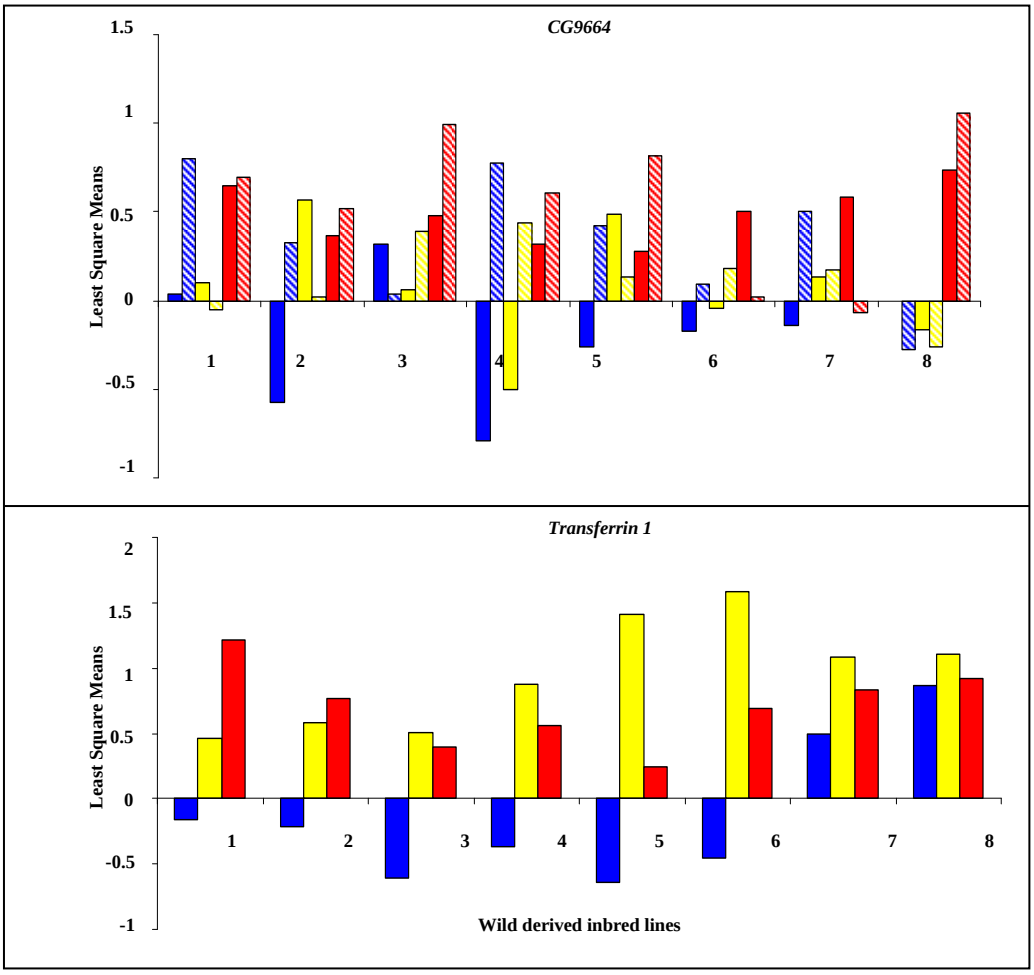


FIGURE 3.10

Quantitative complementation tests for GEI for *Transferrin 1* and *CG9664* mutants. Least mean squares (mutant – control) calculated for the Line×Genotype interaction term is plotted along the Y axis. The numbers designate the eight lines used for expression microarray analysis (see also Figure 9). Blue, yellow and red bars indicate behavior of flies reared on alcohol, standard and tomato food sources. Solid bars indicate female olfactory behavior and thatched bars indicate male olfactory behavior.

CHAPTER 4

DISCUSSION

Identification and expression of novel candidate genes for olfactory behavior

The *p[GT1]* dual gene-trap element was designed to insert in target genes (LUKACSOVICH *et al*. 2001). Indeed, in most instances this transposon has inserted either in exons, introns or in close proximity to promoter/enhancer regions of the candidate genes (Figure 3.1). Rigorous evidence that the tagged gene is indeed responsible for the mutant phenotype is generally provided by demonstrating phenotypic rescue by *P*-element excision or introduction of a wild-type transgene in the mutant background. Transgenic rescue is technically challenging in the case of hypomorphic mutants where - as in this case - either under- or overexpression can result in aberrant behavioral phenotypes. The advantage of the *p[GT1]* transposon is that the intragenic location of the insertion itself and its corresponding effects on gene expression strongly implicate the candidate gene as causal to the phenotype, although additional effects on neighboring genes cannot be excluded. For example, one *P*-element insertion affects expression of both *CG14781* and *CG14782*. Both genes encode transcripts of unknown function and it remains to be determined whether disruption of either one or both of these genes contributes to the observed aberrant olfactory avoidance responses.

One striking observation is how much the effects of single *P*-element insertions on gene expression depend on developmental stage. This phenomenon most likely reflects differential disruption by the transposon of distinct promoter elements that are active at different developmental stages. It should also be noted that several genes previously

implicated only in early development, such as *Sema-5C* (KHARE *et al*. 2000) and *esg* (WHITELEY *et al.* 1992; HAYASHI *et al.* 1993; ADELILAH-SEYFRIED *et al*. 2000), are expressed also in adults and likely perform as yet unknown functions that may either recapitulate or be distinct from their functions in embryonic or larval stages. In cases in which gene expression in adult heads is similar in mutants and the wild-type control, adverse effects on odor-guided behavior are likely the consequence of early disruptions in the animal's developmental blueprint. Such disruptions are predicted to be subtle, as healthy adults emerge without apparent morphological defects. In cases in which gene expression is altered in adults as well as in prior developmental stages, it is not discernable whether aberrant olfactory behavior is a remnant of early developmental defects or directly related to functional disruption of the gene product in the adult brain. Future detailed neuroanatomical studies will be needed to determine whether alterations in neuronal connectivity or structure can be resolved or whether the precise spatial distribution of gene expression has changed in the mutant versus its control at different developmental stages.

Dynamic environment dependant epistatic interactions among candidate genes

Availability of a co-isogenic collection of *P*-element insertion lines enables identification of epistatic effects by generating double heterozygotes according to the half-diallel crossing scheme of GRIFFING (1956) and statistically separating heterozygous effects from epistasis. A previous screen of 379 co-isogenic lines with autosomal *p[lArB]*-element insertions identified 14 *smell-impaired* (*smi*) loci (ANHOLT *et al*. 1996). Since all these lines were in the same genetic background, except for the differential insertion site of a single *P*-element, it was possible to assess the extent of epistasis among them. This was accomplished

by generating all possible double heterozygotes of 12 *smi* lines in a half-diallel design and separating average heterozygous dominance effects from epistatic enhancer and suppressor effects (FEDOROWICZ *et al*. 1998). The surprising result of that study revealed an extensive network of epistatic interactions among 8 of the 12 genes, which was not a predicted result especially when it comes from a limited number of independently isolated mutants. Transcriptional profiling of 5 of these *smi* loci showed that disruption by a *P*-element of its target gene results in changes in gene expression of on average about 125 other genes. Quantitative complementation tests between mutants of genes with altered transcriptional regulation and the original *smi* mutants showed that transcriptional epistasis is significantly correlated with epistasis at the level of phenotype (ANHOLT *et al*. 2003).

Here, I analyzed epistatic interactions among ten *p[GT1]*-insertion lines and asked whether epistatic networks between these loci are invariant or dynamic. It should be noted, however, that the half diallel crossing design is limited in practice, as the number of measurements to be performed increases exponentially with each additional mutant (ANHOLT and MACKAY 2004). Furthermore, the epistatic interactions that I observe are likely sensitive to the sex environment. However, here, the analysis was limited to females only, as four of the transposon tagged candidate genes were located on the *X*-chromosome. Despite the restricted scope of this experimental design, epistatic interactions between the candidate genes could be resolved. Surprisingly, the nature of enhancer and suppressor effects was highly dynamic and depended strongly on the concentration of the stimulus used to elicit the olfactory avoidance response (Figure 3.5). Two candidate genes, *Mer* and *inx2*, engage in epistatic interactions at both concentrations of benzaldehyde (Figure 3.5). It is thus tempting to hypothesize that these genes may represent focal points around which a dynamic epistatic

network revolves. It is thus also possible for *Mer* which is implicated in axis formation and *inx2* a gap junction protein to interact to form neuronal networks during development that subserve epistatic interactions that regulate olfactory behavior. However, as the size of the networks considered here and the number of genes involved is small, this assessment is at present speculative. Nevertheless, the plasticity of genetic networks that mediate complex traits, illustrated here, suggests a dynamic continuum of epistatic partnerships which comprise the full spectrum from highly stable hubs to fragile, fleeting interactions. Such dynamic genetic architectures are expected to be capable of exquisitely adapting transcriptional regulation to continually changing environmental conditions.

Significance of Genotype by Environment Interactions studies

GEI presents a confounding factor in human association studies. It is difficult to quantify the extent of GEI in human populations due to genetic background differences and uncontrolled environmental conditions. Consequently, only a handful of studies have demonstrated GEI in human genetics studies and these analyses were limited to allelic effects of single genes (CASPI *et al*. 2002 and 2003; HOFFJAN *et al.* 2005). Model organisms, in which genetic backgrounds and environmental conditions can be controlled, present a more favorable scenario for the analysis of GEI. QTL analyses have demonstrated GEI for life history traits, including age at maturity, fertility, egg size and growth rate, for *Caenorhabditis elegans* reared at different temperatures (GUTTELING *et al.* 2007); inflorescence development in *Arabidopsis thaliana* in different photoperiod environments (UNGERER *et al.* 2003); GEI for fitness in *D. melanogaster* (FRY *et al.* 1996; KONDRASHOV and HOULE 1994; FERNANDEZ and LOPEZ-FANJUL 1996), sensory bristle number in *D. melanogaster* in different

temperature environments (GURGANUS *et al*. 1998); longevity in *D. melanogaster* under conditions of different temperatures (VIEIRA *et al.* 2000) and larval densities (LEIPS *et al*. 2000); and, ovariole number in *D. melanogaster* (WAYNE and MACKAY 1998). Furthermore, a single nucleotide polymorphism at the *Delta* locus is associated with GEI in sensory bristle number in *Drosophila* (GEIGER-THORNSBERRY and MACKAY, 2002).

GEI regulates olfactory behavior

GEI for olfactory behavior, an essential survival trait, has not been previously analyzed. Here, I have not only detected the presence of GEI, but also quantified the extent of GEI both in terms of the fraction of total phenotypic variance contributed by GEI and the number of genes implicated in its manifestation. My data suggests that GEI accounts for a substantial amount of the total phenotypic variation, as much as 50%, in adult olfactory behavior, but that this variation is accompanied by transcriptional GEI of only a small number of genes. It should be noted, however, that genes that were not detected in this study because of low transcript levels might also contribute to GEI. Similarly, environment-dependent posttranslational modifications could also affect to GEI. The results presented here, however, are in concordance with a previous study on yeast, where six strains of *Saccharomyces cereviciae* were grown in four different nutritional environments; transcriptional profiling showed that only 5% of the genes in the genome contributed to the observed GEI (LANDRY *et al.* 2006). Thus, a general underlying rule for the genetic architecture of complex traits may be that relatively few genes may give rise to extensive GEI.

The GEI effects on adult olfactory behavior detected in this study may depend not only on those genes with genotype dependent transcript plasticity which were detected using adult samples for expression microarrays, but may also arise from genetic effects during the larval stage on adult development (or possibly during the pharate stage post-eclosion before adult flies were collected). Thus, the subset of genes that give rise to GEI may be an underestimate of the total fraction of the genome. Nonetheless, compared to the vast number of transcripts that vary in expression among the lines and the 329 transcripts that show environmental plasticity, my assessment that only a limited number of genes give rise to GEI is likely to remain valid.

The genes that give rise to GEI in olfactory behavior are not *a priori* associated with olfaction, but fulfill diverse functions, many of which appear to be related to defense responses aimed at metabolizing lipids, which would include most odorants. I have validated the accuracy of detection of the microarrays by quantitative RT-PCR studies on a sample of these genes. Furthermore, quantitative complementation tests show that at least two of these genes, *CG9664* and *Transferrin 1*, influence the behavioral phenotype directly.

Major challenges in the field of behavioral genetics

Previous studies have indicated that olfactory behavior is mediated by epistatic networks of pleiotropic genes (FEDOROWICZ *et al.* 1998; ANHOLT, 2004). Critical challenges lie in elucidating if epistatic interactions between genes that mediate olfactory behavior change for different odorants and between avoidance and attraction? Do these networks differ between sexes and between larvae and adults? Are changes in these genetic networks accompanied by alterations in the expression levels and patterns of ORs and OBPs? Will the

occurrence of SNPs in these genes alter network patterns? What are the correlations between genetic networks and neuronal networks?

Individual SNPs in the *Catsup* which is associated with bristle number (STATHAKIS, *et al*. 1999), starvation resistance (HARBISON, *et al*. 2005), and lifespan (MACKAY, *et al*. 2005) were associated with individual quantitative traits (CARBONE, *et al.* 2006). Most of the ten genes that I have characterized and assessed epistatic interactions for are pleiotropic with known functions in development. Two genes, namely *Merlin* and *innexin2,* act as central hubs around which genetic networks shift with different chemosensory environments. By sequencing these two genes in a wild-derived inbred natural population it can be assessed if any of the naturally occurring variation in these genes is associated with olfactory behavior to benzaldehyde and also estimate if SNPs associated with olfactory behavior are also associated with other quantitative traits, such as locomotion, alcohol sensitivity, aggression and life span.

The *GAL4* cassette in the *P*-element enables the expression of *LacZ* when the mutant lines are crossed with the *UAS-LacZ* lines. This will enable observation of spatial expression of the ten candidate genes that are implicated in olfactory behavior in adults. Previous research has shown that a hypomorphic mutation in the *Semaphorin 5C* gene impacts on brain morphology. Morphometric studies revealed a reduction in the size of the ellipsoid body in the *P*-element tagged *Semaphorin 5C* mutant (ROLLMANN, *et al.* 2007). Examining the neuroanatomy of the ten lines will reveal if the observed olfactory impairment is associated with subtle alterations in neural structures.

The realization of pervasive pleiotropy raises two central questions for future studies. Do the same genes that contribute GEI for olfactory behavior also give rise to genotype-

dependent phenotypic plasticity in other traits? and, would the same genes account for GEI in different populations? If indeed a core set of relatively few genes accounts for GEI for multiple traits across populations and possibly across species, identification of this subset of genes becomes feasible and may provide insights that will help manage the confounding effects of GEI both in studies on model organisms and in human populations.

LITERATURE CITED

ABDELILAH-SEYFRIED, S., CHAN, Y. M., ZENG, C., JUSTICE, N. J., YOUNGER-SHEPHERD, S. *et al.,* 2000 A gain-of-function screen for genes that affect the development of the Drosophila adult external sensory organ. Genetics 155: 733-752.

AGRAWAL, A A., AND KARBAN, R. 1999 Induced plant responses and information content about risk of herbivory. Trends Ecol Evol. 14(11):443-447.

ANHOLT, R. R. H. 2004 Genetic modules and networks for behavior: lessons from Drosophila. Bioessays 26:1299-1306.

ANHOLT, R. R. H ., DILDA, C.L., CHANG, S., FANARA, J.J., KULKARNI, N.H. *et al*., 2003 The genetic architecture of odor-guided behavior in Drosophila: epistasis and the transcriptome. Nat. Genet. 35:180-184.

ANHOLT, R. R. H., LYMAN, R. F., AND MACKAY, T.F.C. 1996 Effects of single *P*-element insertions on olfactory behavior in *Drosophila melanogaster*. Genetics 143: 293-301.

ANHOLT, R. R. H., AND MACKAY, T.F.C. 2004 Quantitative genetic analyses of complex behaviours in Drosophila. Nat. Rev. Genet. 5: 838-849.

ASZTALOS, Z., VON WEGERER, J., WUSTMANN, G., DOMBRADI, V., GAUSZ, J. *et al.,* 1993 Protein phosphatase 1-deficient mutant Drosophila is affected in habituation and associative learning. J. Neurosci. 13: 924-930.

AYYUB, C., RODRIGUES, V, HASAN, G., AND SIDDIQI, O. 2000 Genetic analysis of olfC demonstrates a role for the position-specific integrins in the olfactory system of *Drosophila melanogaster*. Mol Gen Genet. 263(3):498-504.

BAUER, R., LEHMANN, C., FUSS, B., ECKARDT, F., AND HOCH, M. 2002 The Drosophila gap junction channel gene innexin 2 controls foregut development in response to Wingless signaling. J. Cell Sci. 115: 1859-1867.

BAUER, R., LEHMANN, C., MARTINI, J., ECKARDT, F., AND HOCH, M. 2004 Gap junction channel protein innexin2 is essential for epithelial morphogenesis in the Drosophila embryo. Molec. Biol. Cell 15: 2992-3004.

BELLEN, H.J., AND KIGER, J. A. JR. 1987 Sexual hyperactivity and reduced longevity of dunce females of Drosophila melanogaster. 115(1):153-160.

BELLEN, H.J., LEVIS, R.W., LIAO, G., HE, Y., CARLSON, J.W. *et al.* 2004 The BDGP gene disruption project: single transposon insertions associated with 40% of Drosophila genes. Genetics 167: 761-781.

BEN-ARIE, N., KHEN, M., AND LANCET, D. 1993 Glutathione S-transferases in rat olfactory epithelium: purification, molecular properties and odorant biotransformation. Biochem J. 292 :379-384.

BENJAMINI, Y., AND Y. HOCHBERG, 1995 Controlling the false discovery rate: a practical and powerful approach to multiple testing. J. Roy. Statist. Soc. Ser. B 57: 289-300.

BENTON, R., SACHSE, S., MICHNICK, S.W., AND VOSSHALL, L.B. 2006 Atypical membrane topology and heteromeric function of *Drosophila* odorant receptors in vivo. PLoS Biol 4: e20.

BHALERAO, S., SEN, A., STOCKER, R., AND RODRIGUES, V. 2003 Olfactory neurons expressing identified receptor genes project to subsets of glomeruli within the antennal lobe of *Drosophila melanogaster*. J. Neurobiol. 54: 577-592.

BILLETER, J. C., VILLELLA, A., ALLENDORFER, J.B., DORNAN, A.J., RICHARDSON, M., *et al.* 2006 Isoform-Specific Control of Male Neuronal Differentiation and Behavior in Drosophila by the fruitless Gene Current Biology 16, 1063–1076.

BIRKETT, M.A., CAMPBELL, C.A., CHAMBERLAIN, K., GUERRIERI, E., HICK, A.J., *et al.* 2000 New roles for cis-jasmone as an insect semiochemical and in plant defense. Proc Natl Acad Sci U S A. 97:9329-9334.

BOULIANNE, G.L., DE LA CONCHA, A., CAMPOS-ORTEGA, J.A., JAN, L.Y., AND JAN, Y.N. 1991 The Drosophila neurogenic gene *neuralized* encodes a novel protein and is expressed in precursors of larval and adult neurons. EMBO J. 10: 2975-2983.

BUCK, L., AND AXEL, R. 1991 A novel multigene family may encode odorant receptors: a molecular basis for odor recognition. Cell 65(1):175-187.

CALIGARI, P.D., AND MATTER, K. 1975 Genotype--environment interaction. III. Interactions in Drosophila melanogaster. Proc R Soc Lond B Biol Sci. 191(1104):387-411.

CAMPESAN, S., DUBROVA, Y. HALL, J. C., AND KYRIACOU, C. P. 2001 The *nonA* gene in Drosophila conveys species-specific behavioral characteristics. Genetics 158: 1535-1543.

CARBONE, M.A., LLOPART, A., DEANGELIS, M., COYNE, J.A., AND MACKAY, T.F. 2005 Quantitative trait loci affecting the difference in pigmentation between Drosophila yakuba and D. santomea. Genetics 171(1):211-225.

CARREIRA, V. P., SOTO, I.M., HASSON, E., AND FANARA J.J. 2006 Patterns of variation in wing morphology in the cactophilic *Drosophila buzzatii* and its sibling *D. koepferae*. J Evol Biol. 19(4):1275-1282.

CASPI, A., MCCLAY, J., MOFFITT, T.E., MILL, J., MARTIN, J. *et al.* 2002 Role of genotype in the cycle of violence in maltreated children. Science 297: 851-854.

CASPI, A., SUGDEN, K., MOFFITT, T.E., TAYLOR, A., CRAIG, I. W. *et al.* 2003 Influence of life stress on depression: moderation by a polymorphism in the 5-HTT gene. Science 301: 386-389.

CHENG, Y., ENDO, K., WU, K., RODAN, A.R., HEBERLEIN, U. *et al.* 2001 Drosophila *fasciclinII* is required for the formation of odor memories and for normal sensitivity to alcohol. Cell 105: 757-768.

CLARK, A.G., AND WANG, L 1997 Epistasis in measured genotypes: Drosophila P-element insertions. Genetics 147:157-163.

CLEMENS, K. E., CHURCHILL, G., BHATT, N., RICHARDSON, K., AND N OONAN, F.P. 2000 Genetic control of susceptibility to UV-induced immunosuppression by interacting quantitative trait loci Genes Immun. 1:251-259.

CLYNE, P., WARR, C., FREEMAN, M., LESSING, D., KIM, J., *et al.*, 1999 A novel family of divergent seven-transmembrane proteins: candidate odorant receptors in *Drosophila*. Neuron 22: 327-338.

CONNOLLY, J.B. AND TULLY, T. 1998 Integrins: a role for adhesion molecules in olfactory memory. Curr. Biol. 8: 386-389.

DANIELSON-FRANCOIS, A.M., KELLY, J. K., AND GREENFIELD, M.D. 2006 Genotype x environment interaction for male attractiveness in an acoustic moth: evidence for plasticity and canalization. J Evol Biol. 19:532-542.

DAVID, J.R., LEGOUT, H., AND MORETEAU, B. 2006 Phenotypic Plasticity of body size in a temperate population of *Drosophila melanogaster*: when the temperature-size rule does not apply. J Genet. 85:9-23.

DE BRUYNE, M., CLYNE , P., CARLSON, J. 1999 Odor coding in a model olfactory organ: the *Drosophila* maxillary palp. J. Neurosci. 19: 4520-4532.

DE BRUYNE, M., FOSTER, K., AND CARLSON, J. 2001 Odor coding in the Drosophila antenna. Neuron 30: 537-552.

DESHPANDE, M., VENKATESH, K., RODRIGUES, V, AND HASAN, G. 2000 The inositol 1,4,5-trisphosphate receptor is required for maintenance of olfactory adaptation in Drosophila antennae. J Neurobiol. 43:282-288.

DILDA, C.L. AND MACKAY, T.F.C. 2002 The genetic architecture of Drosophila sensory bristle number. Genetics 162: 1655-1674.

DOBRITSA, A. A., VAN DER GOES VAN NATERS, W., WARR, C.G., STEINBRECHT, R.A., CARLSON, J.R. 2003 Integrating the molecular and cellular basis of odor coding in the *Drosophila* antenna. Neuron 37: 827-841.

DUBNAU, J., CHIANG, A.S., GRADY, L., BARDITCH, J., GOSSWEILER, S., *et al.,* 2003 The staufen/pumilio pathway is involved in Drosophila long-term memory. Curr. Biol. 13: 286-296.

DUDAI, Y., JAN, Y. N., BYERS, D., QUINN, W. G., AND BENZER, S. 1976 dunce, a mutant of Drosophila deficient in learning. Proc Natl Acad Sci U S A. 73:1684-1688.

ELMORE, T., AND SMITH D. P. 2001 Putative Drosophila odor receptor OR43b localizes to dendrites of olfactory neurons. Insect Biochem Mol Biol. 31:791-798.

FALCONER, D. S., AND T. F. C. MACKAY, 1996 *Introduction to Quantitative Genetics*, Ed. 4, Addison-Wesley Longman, Harlow, UK.

FANARA, J. J., ROBINSON, K.O., ROLLMANN, S.M., ANHOLT, R.R.H., AND MACKAY, T.F.C. 2002 *Vanaso* is a candidate quantitative trait gene for Drosophila olfactory behavior. Genetics 162: 1321-1328.

FANARA, J.J., FOLGUERA, G., IRIARE, P.E., MENSCH, J., AND HASSON, E. 2006 Genotype by environment interactions in viability and developmental time in populations of cactophilic Drosophila. J Evol Biol. 19(3):900-908.

FEDOROWICZ, G.M., FRY, J.D., ANHOLT, R.R.H., AND MACKAY, T.F.C. 1998 Epistatic interactions between smell-impaired loci in *Drosophila melanogaster*. Genetics 148: 1885-1891.

FERNANDEZ, J., AND LOPEZ-FANJUL, C. 1996 Spontaneous mutational variances and covariances for fitness-related traits in *Drosophila melanogaster*. Genetics 143: 829-837.

FIJNEMAN, R. J., DE VRIES, S.S., JANSEN, R.C., AND DEMANT, P. 1996 Complex interactions of new quantitative trait loci, Sluc1, Sluc2, Sluc3, and Sluc4, that influence the susceptibility to lung cancer in the mouse. Nat Genet. 14:465-467.

FISHILEVICH, E. DOMINGOS, A.I., ASAHINA, K., NAEF, F., VOSSHALL, L.B., AND LOUIS, M. 2005 Chemotaxis behavior mediated by single larval olfactory neurons in Drosophila. Curr Biol. 15:2086-2096.

FRANKEL W. N., AND SCHORK, N.J. 1996 Who's afraid of epistasis? Nat Genet. 1996 14:371-373.

FREEMAN, M. R., DOBRITSA, A., GAINES, P., SEGRAVES, W.A., AND CARLSON, J.R. 1999 The dare gene: steroid hormone production, olfactory behavior, and neural degeneration in Drosophila. Development 126: 4591-4602.

FRY, J. D., HEINSOHN, S. L., AND MACKAY T.F.C. 1996 The contribution of new mutations to genotype-environment interaction for fitness in *Drosophila melanogaster*. Evolution 50: 2316-2327.

GAILEY, D.A., AND HALL, J. C. 1989 Behavior and cytogenetics of fruitless in Drosophila melanogaster: different courtship defects caused by separate, closely linked lesions. Genetics 121:773-85.

GALIZIA C. G., AND MENZEL, R. 2000 Odour perception in honeybees: coding information in glomerular patterns. Curr Opin Neurobiol. 10:504-510.

GANGULY, I., MACKAY, T.F.C., AND ANHOLT, R.R.H. 2003 *scribble* is essential for olfactory behavior in *Drosophila*. Genetics 164: 1447-1457.

GAO, Q., AND CHESS, A. 1999 Identification of candidate *Drosophila* olfactory receptors from genomic DNA sequence. Genomics 60: 31-39.

GAO, Q., YUAN, B., AND CHESS, A. 2000 Convergent projections of *Drosophila* olfactory neurons to specific glomeruli in the antennal lobe. Nat. Neurosci. 3: 780-785.

GASQUE, G., LGABARCA, P., DELGADO, R., AND DARSZON, A. 2006 Bridging behavior and physiology: ion-channel perspective on mushroom body-dependent olfactory learning and memory in Drosophila. J. Cell Physiol. 209: 1046-1053.

GEIGER-THORNSBERRY, G.L., AND MACKAY, T.F.C. 2002 Association of single-nucleotide polymorphisms at the *Delta* locus with genotype by environment interaction for sensory bristle number in *Drosophila melanogaster*. Genet. Res. 79:211-218.

GIBERT, P., CAPY, P., IMASHEVA, A., MORETEAU, B, MORIN, J.P., *et al.* 2004 Comparative analysis of morphological traits among *Drosophila melanogaster* and *D. simulans*: genetic variability, clines and phenotypic plasticity. Genetica 120:165-179.

GILLESPIE, J.H., AND TURELLI, M. 1989 Genotype-Environment Interactions and the Maintenance of Polygenic Variation. Genetics 121:129-138.

GORSKI, S. M., CHITTARANJAN, S., PLEASANCE, E.D., FREEMAN, J.D., ANDERSON, C.L. *et al.* 2003 A SAGE approach to discovery of genes involved in autophagic cell death. Curr. Biol. 13: 358-363.

GREENSPAN, R. J., AND FERVEUR, J.F. 2000 Courtship in Drosophila. Annu. Rev. Genet 34: 205-232.

GURGANUS, M.C., FRY, J.D., NUZHDIN, S.V., PASYUKOVA, E.G., LYMAN, R.F., *et al.* 1998 Genotype-environment interaction at quantitative trait loci affecting sensory bristle number in *Drosophila melanogaster*. Genetics 49:1883-1898.

GURGANUS, M.C., NUZHDIN, S. V., LEIPS, J. W., AND MACKAY, T. F. 1999 High-resolution mapping of quantitative trait loci for sternopleural bristle number in Drosophila melanogaster. Genetics 152:1585-1604.

GUO, H. F., TONG, J., HANNAN, F., LUO, L., AND ZHONG, Y. 2000 A neurofibromatosis-1-regulated pathway is required for learning in Drosophila. Nature 403: 895-898.

GUTTELING, E.W., RIKSEN, J.A., BAKKER, J., AND KAMMENGA, J.E. 2007 Mapping phenotypic plasticity and genotype-environment interactions affecting life-history traits in *Caenorhabditis elegans*. Heredity 98: 28-37.

HALLEM, E., HO, M., AND CARLSON, J. 2004 The molecular basis of odor coding in the *Drosophila* antenna. Cell 117: 965-979.

HALLEM, E. A., AND CARLSON, J.R. 2006 Coding of odors by a receptor repertoire. Cell 125: 143-160.

HAMLIN, J.A., FANG, H., AND SCHWOB, J.E. 2004 Differential expression of the mammalian homologue of fasciclin II during olfactory development *in vivo* and *in vitro*. J. Comp. Neurol. 474:438-452.

HARBISON, S.T., CHANG, S., KAMDAR, K.P., AND MACKAY, T.F.C. (2005). Quantitative genomics of starvation stress resistance in Drosophila. Genome Biol. 6, R36.

HAYASHI, S., HIROSE, S., METCALFE, T., AND SHIRRAS, A.D. 1993 Control of imaginal cell development by the escargot gene of Drosophila. Development 118: 105-115.

HILDEBRAND, J. G., ROSSLER, W., AND TOLBERT, L.P. 1997 Postembryonic development of the olfactory system in the moth Manduca sexta: primary-afferent control of glomerular development. Semin Cell Dev Biol. 8:163-170.

HOFFJAN, S., NICOLAE, D., OSTROVNAYA, I., ROBERG, K., EVANS, M, *et al.* 2005 Gene-environment interaction effects on the development of immune responses in the 1st year of life. Am. J. Hum. Genet. 76: 696-704.

HUGHES A.L., AND HUGHES M. K. 1993 Adaptive evolution in the rat olfactory receptor gene family. J Mol Evol. 36:249-254.

IDAGHDOUR, Y, STOREY J.D., JADALLAH, S.J., AND GIBSON, G. 2008 A genome-wide gene expression signature of environmental geography in leukocytes of Moroccan Amazighs. PLoS Genet. 4:e1000052.

ISSEL-TARVER, L., AND RINE, J. 1997 The evolution of mammalian olfactory receptor genes Genetics 145:185-95.

ITO, H., FUJITANI, K., USUI, K., SHIMIZU-NISHIKAWA, K., TANAKA, S. *et al*. 1996 Sexual orientation in Drosophila is altered by the satori mutation in the sex-determination gene fruitless that encodes a zinc finger protein with a BTB domain. Proc Natl Acad Sci U S A. 93:9687-9692.

ITO, K., SUZUKI,K., ESTES,P., RAMASWAMI, M., AND YAMAMOTO, D. *et al.* 1998 The organization of extrinsic neurons and their implications in the functional roles of the mushroom bodies in *Drosophila melanogaster* Meigen. Learn. Mem. 5: 52-77.

JHAVERI, D., SEN, A., AND RODRIGUES, V. 2000 Mechanisms underlying olfactory neuronal connectivity in Drosophila - The atonal lineage organizes the periphery while sensory neurons and glia pattern the olfactory lobe. Dev. Biol. 226: 73-87.

JEFFERIS, G. S., MARIN, E.C., STOCKER, R.F., LUO, L. *et al.* 2001 Target neuron prespecification in the olfactory map of Drosophila. Nature 414: 204-208.

JONES, W.D., NGUYEN, T.A., KLOSS, B., LEE, K.J., AND VOSSHALL, L.B., 2005 Functional conservation of an insect odorant receptor gene across 250 million years of evolution. Curr Biol. 15(4).

KHARE, N., FASCETTI, N., DAROCHA, S., CHIQUET-EHRISMANN, R., AND BAUMGARTNER, S. 2000 Expression patterns of two new members of the semaphorin family in Drosophila suggest early functions during embryogenesis. Mech. Dev. 91: 393-397.

KIM, M. S., REPP, A., AND SMITH, D.P. 1998 LUSH odorant-binding protein mediates chemosensory responses to alcohols in *Drosophila melanogaster*. Genetics 150: 711-721.

KIM, M. S., AND SMITH, D. P. 2001 The invertebrate odorant-binding protein LUSH is required for normal olfactory behavior in Drosophila. Chem Senses. 26:195-199.

KREHER, S. A, KWON, J. Y., AND CARLSON, J. R. 2005 The molecular basis of odor coding in the Drosophila larva. Neuron. 2005 46:445-456.

KRIEGER, J., KLINK, O., MOHL, C., RAMING, K., AND BREER, H. 2003 A candidate olfactory receptor subtype highly conserved across different insect orders. J Comp Physiol A Neuroethol Sens Neural Behav Physiol. 189:519-526.

KONDRASHOV, A. S., AND HOULE, D. 1994 Genotype-environment interactions and the estimation of the genomic mutation rate in *Drosophila melanogaster*. Proc. Biol. Sci. 258: 221-227.

KONOPKA, R. J., AND BENZER, S. 1971 Clock mutants of Drosophila melanogaster. Proc Natl Acad Sci U S A. 68: 2112-2116.

KULKARNI, N. H., YAMAMOTO, A., ROBINSON, K.O., MACKAY, T.F.C., AND ANHOLT, R.R.H. 2002 The DSC1 channel, encoded by the *smi60E* locus, contributes to odor-guided behavior in *Drosophila melanogaster*. Genetics 161: 1507-1516.

KUME, K., KUME, S., PARK, S.K., HIRSH, J., AND JACKSON, F.R. 2005 Dopamine is a regulator of arousal in the fruit fly. J. Neurosci. 25: 7377-7384.

LAISSUE, P., REITER, C., HIESINGER, P., HALTER, S., FISCHBACH, K. *et al.* 1999 Three-dimensional reconstruction of the antennal lobe in *Drosophila melanogaster.* J. Comp. Neurol. 405: 543-552.

LANDRY, C.R., OH, J., HARTL, D. L., AND CAVALIERI, D. 2006 Genome-wide scan reveals that genetic variation for transcriptional plasticity in yeast is biased towards multi-copy and dispensable genes. Gene 366: 343-351.

LARSSON, M., DOMINGOS, A., JONES, W., CHIAPPE, M., AMREIN, H., *et al.* 2004 *Or83b* encodes a broadly expressed odorant receptor essential for *Drosophila* olfaction. Neuron 43: 703-714.

LEE, G., AND HALL, J.C. 2000 A newly uncovered phenotype associated with the fruitless gene of Drosophila melanogaster: aggression-like head interactions between mutant males. Behav Genet. 30(4):263-75.

LEHMANN, M., SIEGMUND, T., LINTERMANN, K.G., AND KORGE, G. 1998 The pipsqueak protein of *Drosophila melanogaster* binds to GAGA sequences through a novel DNA-binding domain. J. Biol. Chem. 273: 28504-28509.

LEIPS, J., AND MACKAY, T.F.C. 2000 Quantitative trait loci for life span in *Drosophila melanogaster*: interactions with genetic background and larval density. Genetics 155: 1773-1788.

LEVINE, J.D., CASEY, C.I., KALDERON, D.D., AND JACKSON, F.R. 1994 Altered circadian pacemaker functions and cyclic AMP rhythms in the Drosophila learning mutant dunce. Neuron 13:967-74.

LILLY, M., KREBER, R., GANETZKY, B., AND CARLSON, J. R. 1994 Evidence that the Drosophila olfactory mutant smellblind defines a novel class of sodium channel mutation. Genetics. 1994 136:1087-1096.

LONG, A.D., MULLANEY, S.L., REID, L.A., FRY, J.D., AND LANGLEY, C. H. 1995 High resolution mapping of genetic factors affecting abdominal bristle number in Drosophila melanogaster Genetics 139:1273-1291.

LUKACSOVICH T, Z. ASZTALOS , W. AWANO , K. BABA , S. KONDO, *et al.* 2001 Dual-tagging gene trap of novel genes in *Drosophila melanogaster*. Genetics 157: 727-742.

MACDOUGALL, N., LAD, Y., WILKIE, G.S., FRANCIS-LANG, H., SULLIVAN, W., DAVIS, I. 2001 *Merlin*, the Drosophila homologue of neurofibromatosis-2, is specifically required in posterior follicle cells for axis formation in the oocyte. Development 128: 665-673.

MACKAY, T. F. C., 2004 The genetic architecture of quantitative traits: lessons from Drosophila. Curr.Opin.Genet.Dev. 14:253-257.

MACKAY, T. F. C., HACKETT, J. B., LYMAN, R.F., WAYNE, M.L., AND ANHOLT, R.R.H. 1996 Quantitative genetic variation of odor-guided behavior in a natural population of Drosophila melanogaster. Genetics 144:727-735.

MACKAY, T.F.C., AND LYMAN, R.L. 1998 Polygenic mutation in Drosophila melanogaster: genotype x environment interaction for spontaneous mutations affecting bristle number. Genetica 102-103:199-215.

MACKAY, T.F.C., ROSHINA, N.V., LEIPS, J.W., AND PASYUKOVA, E.G. (2005). Complex genetic architecture of Drosophila longevity. In Handbook of the Biology of Aging, Sixth Edition, E.J. Masaro and S.N. Austad, eds. (Burlington: Elsevier Press), pp. 181–216.

MARIN, E. C., JEFFERIS, G.S., KOMIYAMA, T., ZHU, H., AND LUO, L. 2002 Representation of the glomerular olfactory map in the Drosophila brain. Cell 109: 243-255.

MARTIN, F., CHARRO, M.J., AND ALCORTA, E. 2001 Mutations affecting the cAMP transduction pathway modify olfaction in Drosophila. J. Comp. Physiol. 87: 359-370.

MCKENNA, M. P., HEKMAT-SCAFE, D.S., GAINES, P., AND CARLSON, J.R. 1994 Putative Drosophila pheromone-binding proteins expressed in a subregion of the olfactory system J Biol Chem. 269:16340-16347.

MEFFERT L.M., AND HAGENBUCH K.L. 2005 The genetic architecture of house fly mating behavior. Curr. Top Dev Biol. 66:189-213.

MERTENS, I., VANDINGENEN, A., JOHNSON, E.C., SHAFER, O.T., LI. W., *et al.* 2005 PDF receptor signaling in Drosophila contributes to both circadian and geotactic behaviors. Neuron 48: 213-219.

MOORE, M.S., DEZAZZO, J., LUK, A.Y., TULLY, T., SINGH, C.M. *et al.* 1998 Ethanol intoxication in Drosophila: Genetic and pharmacological evidence for regulation by the cAMP signaling pathway. Cell 93: 997-1007.

MORETEAU, B., AND DAVID, J. R. (2005) Phenotypic plasticity and reaction norms of abdominal bristle number in *Drosophila melanogaster*. J Biosci. 30:689-697.

MORETEAU, B., GIBERT, P., DELPUECH, J.M., PETAVY, G., AND DAVID, J.R. 2003 Phenotypic plasticity of sternopleural bristle number in temperate and tropical populations of *Drosophila melanogaster*. Genet Res. 81:25-32.

MUKAI, M., KITADATE, Y., ARITA, K., SHIGENOBU, S., KOBAYASHI, S. 2006 Expression of meiotic genes in the germline progenitors of Drosophila embryos. Gene Expr. Patterns 6:256-266.

NAGASE, K., MAO, J.H., DE KONING, J.P., MINAMI, T., AND BALMAIN, A. 2001 Epistatic interactions between skin tumor modifier loci in interspecific (spretus/musculus) backcross mice. Cancer Res. 61:1305-1308.

NAKAGAWA, T., SAKURAI, T., NISHIOKA, T., TOUHARA, K. 2005 Insect sex-pheromone signals mediated by specific combinations of olfactory receptors. Science 307:1638-42.

NAUTA, W.J., VEERKAMP, R.F., BRASCAMP, E.W., AND BOVENHUIS, H. 2006 Genotype by environment interaction for milk production traits between organic and conventional dairy cattle production in The Netherlands. J Dairy Sci. 89:2729-2737.

NG, M., ROORDA, R., LIMA, S., ZEMELMAN, B., MORCILLO, P. *et al.* 2002 Transmission of olfactory information between three populations of neurons in the antennal lobe of the fly. Neuron 36: 463-474.

NICHOL, H., LAW, J. H., AND WINZERLING, J.J. 2002 Iron metabolism in insects. Annu. Rev. Entomol. 47: 535-559.

NORGA, K. K., GURGANUS, M.C., DILDA, C.L., YAMAMOTO, A., LYMAN, R.F. *et al.*, 2003 Quantitative analysis of bristle number in Drosophila mutants identifies genes involved in neural development. Curr. Biol. 13: 1388-1397.

PHILIP, N., ACEVEDO, S.F., SKOULAKIS, E.M. 2001 Conditional rescue of olfactory learning and memory defects in mutants of the 14-3-3zeta gene *leonardo*. J. Neurosci. 21: 8417-8425.

PIKIELNY, C. W. HASAN, G, ROUYER, F., ROSBASH, M. 1994 Members of a family of Drosophila putative odorant-binding proteins are expressed in different subsets of olfactory hairs. Neuron. 12:35-49.

PITTS, R. J., FOX, A. N., AND ZWIEBEL, L.J. 2004 A highly conserved candidate chemoreceptor expressed in both olfactory and gustatory tissues in the malaria vector Anopheles gambiae. Proc Natl Acad Sci U S A. 101:5058-5063.

PROKOPENKO, S.N., HE, Y., LU, Y., AND BELLEN, H.J. 2000 Mutations affecting the development of the peripheral nervous system in Drosophila: A molecular screen for novel proteins. Genetics 156: 1691-1715.

REIFSNYDER, P.C., CHURCHILL, G., LEITNER, E.H. 2000 Maternal environment and genotype interact to establish diabesity in mice. Genome Res. 10(10):1568-1578.

RENAULT, A.D., STARZ-GAIANO , M., AND LEHMANN, R. 2002 Metabolism of sphingosine 1-phosphate and lysophosphatidic acid: a genome wide analysis of gene expression in Drosophila. Mech. Dev. 119: S293-S301.

RIESGO-ESCOVAR, J., RAHA, D., AND CARLSON, J.R. 1995 Requirement for a phospholipase C in odor response: overlap between olfaction and vision in Drosophila. Proc Natl Acad Sci U S A. 92:2864-2868.

RIESGO-ESCOVAR, J., WOODARD, C., AND CARLSON, J.R. 1994 Olfactory physiology in the Drosophila maxillary palp requires the visual system gene rdgB. J Comp Physiol [A]. 175:687-693.

RISCH, N. 2000 Searching for genes in complex diseases: lessons from systemic lupus erythematosus. J Clin Invest. 105:1503-1506.

ROBERTSON, H. M., WARR, C. G., AND CARLSON, J. R. 2003 Molecular evolution of the insect chemoreceptor gene superfamily in Drosophila melanogaster. Proc Natl Acad Sci U S A. 100 Suppl 2:14537-14542.

ROLLMANN, S. M., MACKAY, T.F.C., AND ANHOLT, R.R.H. 2005 Pinocchio, a novel protein expressed in the antenna, contributes to olfactory behavior in *Drosophila melanogaster*. J. Neurobiol. 63: 146-158.

ROLLMANN S. M., YAMAMOTO, A., GOOSSENS, T., ZWARTS, L., CALLAERTS-VÉGH, Z. *et al.* 2007 The early developmental gene *Semaphorin 5c* contributes to olfactory behavior in adult Drosophila. Genetics 176: 947-956.

ROSS, J., JIANG, H., KANOST, M.R., AND WANG, Y. 2003 Serine proteases and their homologs in the *Drosophila melanogaster* genome: an initial analysis of sequence conservation and phylogenetic relationships. Gene 304: 117-131.

ROUQUIER, S., TAVIAUX, S., TRASK, B.J., BRAND-ARPON, VB., VAN DEN ENGH, G., *et al.* 1998 Distribution of olfactory receptor genes in the human genome. Nat Genet. 18:243-50.

RYNER, L.C., GOODWIN, S. F., CASTRILLON, D. H., ANAND, A., VILLELLA, A., BAKER, B.S., *et al.*1996 Control of male sexual behavior and sexual orientation in Drosophila by the fruitless gene Cell 87:1079-89.

SATO, K., PELLEGRINO, M., NAKAGAWA, T., NAKAGAWA, T., VOSSHALL, L.B., AND TOUHARA, K. 2008 Insect olfactory receptors are heteromeric ligand-gated ion channels. 452:1002-1006.

SCHOLZ, F., RAMOND, J., SINGH, C.M., HEBERLEIN, U. 2000 Functional ethanol tolerance in Drosophila. Neuron 28: 261-271.

SHANDBHAG, S., MULLER, B., AND STEINBRECHT, A. 1999 Atlas of olfactory organs of *Drosophila melanogaster*. 1. Types, external organization, innervation and distribution of olfactory sensilla. Int. J. Insect Morphol. Embryol. 28: 377-397.

SHIMOMURA, K., LOW-ZEDDIES, S. S., KING, D. P. STEEVES, T.D., WHITELE, A. *et al.* 2001 Genome-wide epistatic interaction analysis reveals complex genetic determinants of circadian behavior in mice. Genome Res. 11:959-980.

SHIRAIWA, T., NITASAKA, E., AND YAMAZAKI, T. 2000 *Geko*, a novel gene involved in olfaction in *Drosophila melanogaster*. J. Neurogenet. 14: 145-164.

SIEGMUND, T., AND LEHMANN, M. 2002 The Drosophila Pipsqueak protein defines a new family of helix-turn-helix DNA-binding proteins. Dev. Genes Evol. 212: 152-157.

SPRAGUE, G. F., AND TATUM, L.A. 1942 General *vs.* specific combining ability in single crosses of corn. J. Amer. Soc. Agron. 34: Acad. 923–932.

STAM, L.F., AND LAURIE, C.C .1996 Molecular dissection of a major gene effect on a quantitative trait: the level of alcohol dehydrogenase expression in Drosophila melanogaster. Genetics 144:1559-1564.

STATHAKIS, D.G., BURTON, D.Y., MCIVOR, W.E., KRISHNAKUMAR, S., WRIGHT, T.R., AND O'DONNELL, J.M. (1999). The Catecholamines up (Catsup) protein of Drosophila melanogaster functions as a negative regulator of tyrosine hydroxylase activity. Genetics 153, 361–382.

STOCKER, R. F., 2001 Drosophila as a focus in olfactory research: mapping of olfactory sensilla by fine structure, odor specificity, odorant receptor expression, and central connectivity. Microsc Res Tech. 55:284-296.

STOCKER, R. F., LIENHARD, M. C., BORST , A., AND FISCHBACH, K. F. 1990 Neuronal architecture of the antennal lobe in Drosophila melanogaster Cell Tissue Res. 262:9-34.

STOCKER, R. F. 1994 The organization of the chemosensory system in *Drosophila melanogaster*: Cell Tissue Res. 275: 3-26.

STOCKINGER, P. KVITSIANI, D., ROTKOPF, S., TIRIAN, L., AND DICKSON, B.J. 2005 Neural circuitry that governs Drosophila male courtship behavior. Cell 121: 795-807.

STOLTZFUS, J. R., HORTON, W. J., AND GROTEWIEL, M.S. 2003 Odor-guided behavior in Drosophila requires calreticulin. J. Comp. Physiol. A. 189: 471- 483.

STORTKUHL, K. F., HOVEMANN, B. T., CARLSON, J. R. 1999 Olfactory adaptation depends on the Trp Ca^{2+} channel in Drosophila. J Neurosci. 19:4839-46.

STÖRTKUHL, K.F., KETTLER, R., FISCHER, S. AND HOVEMANN, B.T. 2005 An Increased Receptive Field of Olfactory Receptor Or43a in the Antennal Lobe of *Drosophila* Reduces Benzaldehyde-driven Avoidance Behavior Chemical Senses 30:81-87

STYLIANOU, I.M., KORSTANJE, R., LI, R., SHEEHAN, S., PAIGEN, B., AND CHURCHILL, G.A. 2006 Quantitative trait locus analysis for obesity reveals multiple networks of interacting loci. Mamm Genome. 17:22-36.

TAKKEN W., 1996 Synthesis and future challenges: the response of mosquitoes to host odours Ciba Found Symp. 200:302-312

THEOPOLD, U., RISSLER, M., FABBRI, M., SCHMIDT, O., AND NATORI, S.1999 Insect glycobiology: A lectin multigene family in *Drosophila melanogaster*. Biochem. Biophys. Res. Commun. 261: 923-927.

UNGERER, M.C., HALLDORSDOTTIR, S.S., PURUGGANAN, M.D., AND MACKAY, T.F.C. 2003 Genotype by environment interactions at quantitative trait loci affecting inflorescence development in *Arabidopsis thaliana*. Genetics 165: 353-365.

VAN SWINDEREN, B., AND GREENSPAN, R. J. 2005 Flexibility in a gene network affecting a simple behavior in *Drosophila melanogaster*. Genetics 169: 2151-2163.

VAUGHN, S.F., SPENCER, G.F., AND SHASHA, B.S. 1993 Volatile Compounds from Raspberry and Strawberry Fruit Inhibit Postharvest Decay Fungi. Journal of Food Science 58 793-796.

VIEIRA, C., PASYUKOVA, E.G., ZENG, Z.B., HACKETT, J.B., LYMAN, R.F., *et al.* 2000 Genotype-environment interaction for quantitative trait loci affecting life span in *Drosophila melanogaster*. Genetics 154: 213-327.

VOSSHALL, L., AMREIN, H., MOROZOV, P., RZHETSKY, A., AND AXEL, R. 1999 A spatial map of olfactory receptor expression in the *Drosophila* antenna. Cell 96: 725-736.

VOSSHALL, L.B., AND STOCKER, R.F. 2007 Molecular architecture of smell and taste in Drosophila. Annu Rev Neurosci.30:505-533.

VOSSHALL, L., WONG, A., AND AXEL, R. 2000 An olfactory sensory map in the fly brain. Cell 102: 147-159.

WANG, P., LYMAN, R.F., SHABALINA, S.A., MACKAY, T.F.C., AND ANHOLT, R.R.H., 2007 Association of polymorphisms in odorant-binding protein genes with variation in olfactory response to benzaldehyde in Drosophila. Genetics 177: 1655-1665.

WANG, J., WONG, A., FLORES, J., VOSSHALL, L., AND AXEL, R. 2003 Two-photon calcium imaging reveals an odor-evoked map of activity in the fly brain. Cell 112: 271-282.

WAYNE, M.L., KOROL, A., AND MACKAY, T.F. 2005 Microclinal variation for ovariole number and body size in *Drosophila melanogaster* in 'Evolution Canyon'. Genetica. 123:263-270.

WAYNE, M.L., AND MACKAY, T.F. 1998 Quantitative genetics of ovariole number in *Drosophila melanogaster*. II. Mutational variation and genotype-environment interaction. Genetics 148: 201-210.

WEBER, U., SIEGEL, V., AND MLODZIK, M. 1995 *pipsqueak* encodes a novel nuclear protein required downstream of seven-up for the development of photoreceptor R3 and R4. EMBO J. 14: 6247-6257.

WHITELEY, M., NOGUCHI, P. D., SENSABAUGH, S. M., ODENWALD, W. F., AND KASSIS, J. A. 1992 The Drosophila gene *escargot* encodes a zinc finger motif found in snail-related genes. Mech. Dev. 36: 117-127.

WILLIAMS, J.A., SU, H. S., BERNARDS, A., FIELD, J., AND SEHGAL, A. 2001 A circadian output in Drosophila mediated by *Neurofibromatosis-1* and Ras/MAPK. Science 293: 2251-2256.

WITSHIRE, S., BELL, J.T., GROVES, C.J., DINA, C., HATTERSLEY, A.T. *et al.* 2006 Epistasis between type 2 diabetes susceptibility Loci on chromosomes 1q21-25 and 10q23-26 in northern Europeans. Ann Hum Genet. 70:726-737

WOODARD, C., ALCORTA, E., AND CARLSON, J. 1992 The rdgB gene of Drosophila: a link between vision and olfaction. J Neurogenet. 8:17-31.

WONG, A. M., WANG, J.W., AND AXEL, R. 2002 Spatial representation of the glomerular map in the Drosophila protocerebrum. Cell 109: 229-241.

YEH, E., DERMER, M., COMMISSO, C., ZHOU, L., MCGLADE, C.L., AND BOULIANNE, G.L. 2001 Neuralized functions as an E3 ubiquitin ligase during Drosophila development. Curr. Biol. 11: 1675-1679.

YEH, E., ZHOU, L., RUDZIK, N., AND BOULIANNE, G.L. 2000 *neuralized* functions cell autonomously to regulate Drosophila sense organ development. EMBO J. 19: 4827-4837.

ZARS, T., 2000 Behavioral functions of the insect mushroom bodies. Curr. Opin. Neurobiol. 10: 790-795.

ZHANG, Y., KANG, M.S., AND LAMKEY, K.R. 2005 DIALLEL-SAS05: A Comprehensive Program for Griffing's and Gardner–Eberhart Analyses. Agron. J. 97:1097–1106.