ABSTRACT

ZHOU, SHANSHAN. Genetic Basis of Phenotypic Plasticity in *Drosophila melanogaster*. (Under the direction of Robert R. H. Anholt and Trudy F.C. Mackay).

The ability to produce distinct phenotypes under different environmental conditions is referred to as phenotypic plasticity. Phenotypic plasticity is advantageous in that it allows organisms to adapt rapidly to environmental changes. However, excessive plasticity caused by dramatic environmental perturbations in otherwise robust traits can lead to undesirable changes in development and behaviors, and increase susceptibility to diseases, which leads to reduction in population fitness. Understanding the genetic basis of phenotypic plasticity will greatly advance our understanding of adaptive evolution as well as the nature of constraints on adaptation.

In this dissertation I focus on environmental effects on regulation of transcript abundance in *Drosophila melanogaster*, as the transcriptome directly reflects genome regulation and is organized as dynamic regulatory networks that are influenced by and interact with the environment. I also associate environmental variation at the transcript level with environmental variation of different fitness traits, in order to understand the environmental impact of genetic regulation on organismal fitness.

Chemosensation is an essential modality for evaluating the environment, is implicated in guiding social interactions, and also affects longevity. The chemosensory system of *Drosophila melanogaster* is one of the best studied

sensory systems. Chemosensation is mediated by large families of chemoreceptor genes. I constructed customized cDNA microarrays representing all classical *Or*-type odorant receptors, gustatory receptors and odorant binding protein genes of Drosophila. I investigated their expression patterns under different developmental stages, physiological status and social conditions, using a laboratory inbred line. I found that chemoreceptor genes that are clustered cytologically in the genome undergo independent transcriptional regulation at different developmental stages and between sexes. Expression of distinct subgroups of chemoreceptor genes is sensitive to reproductive state and social interactions. Furthermore, exposure of flies only to odor of the opposite sex results in altered transcript abundance of chemoreceptor genes. These transcripts are distinct from those that show transcriptional plasticity when flies are allowed physical contact with same or opposite sex members. Finally, clustering analysis revealed small covariant modules of chemoreceptor transcripts suggesting fine tuning of the expression of the chemoreceptor repertoire in response to environmental and physiological conditions.

To expand the scope of my study on a genome-wide scale I constructed a synthetic outbred population using 40 inbred lines derived from a natural population with sequenced genomes and published transcriptome data and exposed this population to 20 different rearing conditions. I found that over 15% of the transcriptome is highly responsive to environmental changes while the majority of transcripts show robust expression that are consistent across conditions. Among the environmentally sensitive transcripts, 1249 are highly significant in the ANOVA for treatment. These transcripts are associated with response to heat shock, xenobiotic and energy metabolism, immune defense, proteolysis, transcription, and also include non-coding RNAs and computationally predicted transcripts of unknown function. They responded differentially to different environments and could be grouped in 103 highly correlated covariant modules. Environmentally sensitive transcripts associated with different organismal traits have distinct regulatory patterns, and are different from those associated with the same traits across the 40 genotypes under a standard rearing environment. I identified an additional 970 environmentally sensitive transcripts that were not detected by ANOVA due to high within-treatment variation. These transcripts have little genetic variation, show extensive sexual dimorphism, and gene ontology analysis shows that they are enriched for reproduction and post-mating behaviors. Comparisons of sequence conservation across 12 Drosophila species shows that environmentally sensitive genes are more rapidly evolving than those with robust expression under different environments.

Genetic Basis of Phenotypic Plasticity in *Drosophila melanogaster*

by
Shanshan Zhou

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

Zoology

Raleigh, North Carolina

2011

APPROVED BY:

Robert R. H. Anholt	Trudy F. C. Mackay
Chair of Advisory Committee	Co-Chair of Advisory Committee
John R. Godwin	Kevin Gross

DEDICATION

献给我的爸爸，妈妈。

To my parents, who give me confidence and show me the beauty of life.

BIOGRAPHY

I was born March 8th, 1983 in Beijing, China. I feel very lucky to have a home town with such rich culture and history, and am also glad to experience the dramatic changes of China's reformation and opening up to the world since the late 70s. I went to Beijing No.4 High School (Beijing Sizhong) from 1998 to 2001, which is one of the oldest public schools in Beijing founded in the Qing Dynasty. There, I accumulated not only knowledge, but also learned how to conduct myself to be an upright person. Unlike most other Chinese schools, at Sizhong I had a lot of free time to expand my interests in sports and music. After the college entrance exams, which most Chinese students would recall as the toughest time of their school years - and it was a real challenge for knowledge, mind and body - I was lucky to be accepted to my dream college, Tsinghua University. There I studied Biology, the field I was always interested in ever since I was a curious kid. Tsinghua is an amazing place that opened my eyes and introduced me to many new concepts, and in my undergraduate studies I developed strong interests in genetics and ecology. Interestingly, Tsinghua University was first founded in the early 19th century as a preparatory school for students who were later sent to study in the United States; and here I am, after I got my Bachelor's degree, I decided to continue my study in the USA. I began my doctoral study in 2005 under the guidance of Dr. Robert Anholt and Dr. Trudy Mackay at North Carolina State University, and studied phenotypic plasticity using fruit flies. Here I learned how to be a scientist. Conducting research reflects my life philosophy; it needs diligence, rigor, honesty and creativity.

ACKNOWLEDGMENTS

I would like to first and foremost thank my advisors, Drs. Robert Anholt and Trudy Mackay, for their extraordinary guidance and support. I cannot thank you enough for how much you taught me. You have always been patient and supportive, and encouraged me to follow my ideas. You introduced me to the world of behavioral genetics, and fascinated me with its beauty. Robert is like my dad, he takes care of me not just in science but also in everyday life, and it is not hard to tell that we share common genes in food. It is always fun to listen to Robert and Trudy telling stories about their horses. I will miss you so much after I leave the lab. You are truly great mentors that will continue to influence me throughout my life with your passion, your wisdom, your kindness, and your humor.

I want to thank Mike Magwire for his love, support and patience, especially during those stressful writing days. You and Dixie always make me laugh, make me happy and make wherever you are feel like home.

I also like to thank my committee members, Drs. John Godwin and Kevin Gross for their precious feedback on my research. Dr. Eric Stone, it is a pleasure to work with you, thanks for your advice and your work is a great example that shows connections between science and art.

Dr. Harold Heatwole (Hal) and Audry Heatwole, thank you for the opportunity that made my graduate study possible and your understanding of my decision. Thank you for taking care of me in those hard days when I first got to the US. Thank you for

bringing me to Antarctica, one of the most beautiful places in the world that I will remember forever. The world lost a great artist, a kind mom, an inspiring wife, when Audry left us.

I want to thank all members of the W. M. Keck Center for Behavioral Biology, who work so hard to make it lively. The Keck Center provides all kinds of great opportunities for developing our careers from social evening discussions to inviting prestigious speakers from all over the world. I am grateful to be involved in all those events.

I want to thank both the Biology and Genetics department staff, especially Susan Marschalk, Meredith Henry and Suzanne Quick. They have helped me tremendously with all kinds of paper work and procedures that are complicated because of my international status.

Last, but definitely not the least; I really want to thank everyone in the Mackay-Anholt lab. You have been part of my life. I appreciate all your help, and all the laughter and tears we shared.

TABLE OF CONTENTS

LIST OF TABLES

CHAPTER TWO

CHAPTER THREE

LIST OF FIGURES

CHAPTER ONE

CHAPTER TWO

CHAPTER THREE

CHAPTER FOUR

CHAPTER ONE

INTRODUCTION

Early in the history of genetics, it was realized that most traits do not conform to the Mendelian model; instead, they seemed to be controlled by many genes with small effects (Falconer and Mackay 1996). The realization of the existence of these “quantitative” traits opened up the field of quantitative genetics, the focus of which has been to understand the genetic underpinnings of complex traits. This endeavor is often complicated due to pleiotropy and epistasis that make it difficult to connect a phenotype with causal genes. In the case of pleiotropy, one gene or locus can affect multiple traits. Epistasis is due to non-additive interactions between genes or loci affecting a trait, and can be visualized as non-parallel reaction norms of different haplotypes (Falconer and Mackay 1996).

In addition to the complexity that arises from pleiotropy and non-additive interactions, environmental influences are another major confounding factor. Environmental effects, however, are commonly ignored and treated as the source of noise to be standardized and minimized by experimental designs that use laboratory organisms reared under tightly controlled conditions, or/and by statistical approaches that partition genetic effects and relegate environmental effects to the error term. The environmental factor, however, as well as the interaction between genotype and environment remains a confounding source limiting the detection power of genetic studies on complex traits. Especially in human association studies, inability to control for the developmental background of individuals and population structures add additional difficulty in teasing apart genetic and environmental influences in order to find the causal alleles.

The ability of individual genotypes to produce different phenotypes when exposed to different environmental conditions is called phenotypic plasticity (Bradshaw 1965). Phenotypic plasticity plays an important role in adaptive evolution, as it allows organisms to respond to changing environments by changing their phenotypes in order to increase survival under different conditions. These changes are generally fast and efficient without time lag and the cost tag associated with natural selection.

In this dissertation I address the genetic basis of phenotypic plasticity. I will describe two comprehensive interrelated studies, which together will give new insights in genome-wide transcriptional responses to environmental changes. In the first study, described in Chapter 2, I investigate phenotypic plasticity of the chemoreceptor repertoire of *Drosophila melanogaster*, since Drosophila, like many animals, relies foremost on chemosensory input to gain information about its external milieu. The second study, described in Chapter 3, expands the scope of this analysis to the entire genome and presents a comprehensive analysis of phenotypic plasticity of the Drosophila transcriptome under 20 different environmental treatments using a synthetic outbred population generated by a round robin cross design of 40 inbred wild-derived lines with fully sequenced genomes and documented transcriptional profiles, obtained under a standard growth condition. The final Chapter of this dissertation will discuss the implications and limitations of my studies and outline avenues for future investigation.

In this introductory chapter I will first provide a detailed overview of the chemosensory system of *Drosophila melanogaster*, including a detailed description of odorant receptors, gustatory receptors and odorant binding proteins, which will provide context for the data presented in Chapter 2. I will then provide a detailed discussion of phenotypic plasticity to provide the conceptual framework for the studies presented in Chapter 3.

THE CHEMOSENSORY SYSTEM IN DROSOPHILA

Olfactory and gustatory systems are essential for the survival of most animals. Chemosensation directs animals to locate food sources, mates, oviposition sites, and to avoid aversive environments, thereby insuring survival and reproductive success. The fruit fly, *Drosophila melanogaster*, is a valuable model organism for studying the genetic and neural basis of chemosensation. *Drosophila melanogaster* is amenable to genetic manipulation, and accessible to molecular, neuroanatomical and electrophysiological techniques. The availability of complete genome sequence (Adams et al. 2000) facilitates comprehensive bioinformatics analyses (Clyne et al. 1999; Clyne et al. 2000; Hekmat-Scafe et al. 2002), and comparative studies with other Drosophila species provide insights in the role chemoreception plays in genetic isolation and evolutionary divergence (Dekker et al. 2006; Matsuo 2008).

Drosophila melanogaster exhibits robust responses to odors, and chemosensory behaviors can be studied with simple, quantitative assays under controlled laboratory conditions. Furthermore, the short generation time enables the production of large numbers of genetically identical individuals that can be reared under controlled environmental conditions. Finally, Drosophila shares a similar functional organization of chemosensory systems with mammals (Smith 2007), but the numerical simplicity of fewer cells facilitates studies on olfactory and gustatory information processing.

OLFACTORY RECOGNITION IN ADULT FLIES

Functional Characterization of Olfactory Organs

Drosophila has two symmetrical pairs of functional 'noses', the maxillary palps and the third segments of the antennae. In Drosophila, each antenna has ~1200 olfactory receptor neurons (ORNs), and each maxillary palp has ~120 ORNs. Unlike mammalian ORNs located throughout the olfactory epithelium, small numbers of fly ORNs are compartmentalized in hair-like structures, called sensilla. There are three morphologically distinct types of sensilla, basiconic (large and small), trichoid and coeloconic sensilla (Fig.1.1) (Stocker 1994). All three types of sensilla are found on the antenna, but the maxillary palps contain only basiconic sensilla. The sensilla are hair-like, hollow, fluid-filled structures; each of them contains one to four ORNs (Hallem et al. 2006), whose dendrites are bathed in sensillar lymph secreted by support cells that surround the ORNs. Odorants enter the sensilla through pores in the cuticular wall, dissolve in the sensillar lymph, and activate ORNs. The axons of ORNs project to the brain, where they form connections with projection neurons (PN) in the antennal lobes (AL), the functional homologues of the mammalian olfactory bulbs. Each AL contains about 43 identifiable spherical structures of neuropil called “glomeruli” (Laissue et al. 1999). Individual ORNs project bilaterally, sending axons to only one or few glomeruli in each AL (Stocker 1994), and each PN typically innervates only a single glomerulus (Marin et al. 2002). Unlike antennal ORNs which project their axons directly to the AL, the axons of maxillary palp ORNs travel through the subesophageal ganglion (SOG) to reach the AL and terminate close to gustatory neurons in the labial nerve (Vosshall and Stocker 2007).

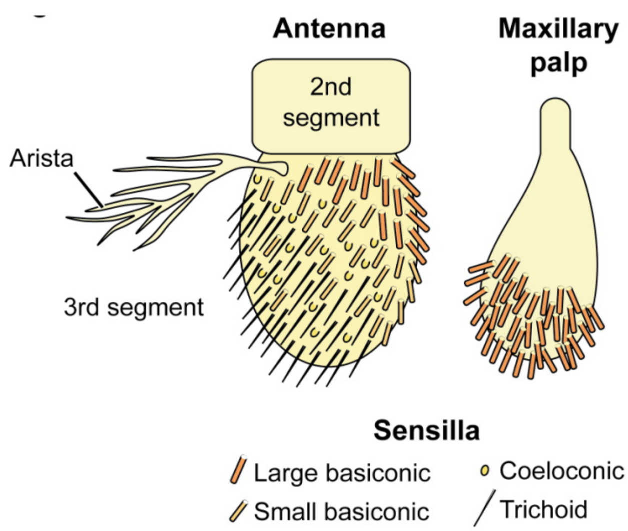

Figure 1.1 Distributions of different types of sensilla on the 3rd segment of the antenna and the maxillary palp. Figure adapted from Benton et al. (2006).

Single-unit electrophysiological recording has been widely used to study responses of insect ORNs to odorants, for example in moths, honeybees, mosquitoes, and flies (Clyne et al. 1997; Meijerink and van Loon 1999; Shields and Hildebrand 2000; Meijerink et al. 2001; Laurent et al. 2002). Large-scale single-unit electrophysiological recordings allowed classification of odor response spectra of an entire set of olfactory sensilla and olfactory neurons (de Bruyne et al. 1999; de Bruyne et al. 2001). Different functional classes of sensilla and ORNs were identified according to the different electrophysiological response patterns of each sensillum. These studies revealed six different functional classes of ORNs housed in three types of basiconic sensilla in the maxillary palp. The sensilla are designated pb1 through pb3, and the ORNs within them have been designated pb1A, pb1B, pb2A, pb2B, pb3A, and pb3B. Similarly, 18 classes of ORNs housed in three types of large basiconic sensilla and five types of small basiconic sensilla are found in the antenna. Antennal sensilla have been designated ab1 through ab8. The ab1 sensillum contains four ORNs, and the other sensilla each contain two ORNs (de Bruyne et al. 1999; de Bruyne et al. 2001; Elmore et al. 2003).

Identification of Olfactory Receptors

Olfactory receptors (*Or*s) were first discovered in the rat (Buck and Axel 1991) based on the assumption that olfactory receptors belong to a G-protein coupled receptor superfamily, with high diversity within the family, and would be expressed exclusively in olfactory epithelium (Buck and Axel 1991). A few years later large families of olfactory receptors were found in *C. elegans* (Troemel et al. 1995). Drosophila *Ors* were identified in 1999 through bioinformatic (Clyne et al. 1999; Gao and Chess 1999; Vosshall et al. 1999) and molecular (Vosshall, 99) approaches, by searching for a family of seven transmembrane domain proteins selectively expressed in ORNs. A bioinformatics search of the *Drosophila melanogaster* genome identified 60 olfactory receptor genes that encode 62 OR proteins through alternative splicing (Robertson et al. 2003).

The *Or* gene family is distributed across all three major chromosomes in Drosophila, and many are found in small clusters of two or three genes. Whereas some show evidence of recent duplication events, most are only distantly related (Robertson et al. 2003). *Or* genes show an overall amino acid homology of about 20% across the family, suggesting that they share an ancient origin which gave rise to clusters of *Or* genes following duplication events (Robertson et al. 2003). Unlike vertebrate and *C. elegans Or* genes, Drosophila *Or* genes show no homology to other members of the G-protein coupled receptor superfamily (Vosshall et al. 1999). *Or* genes between different insect species, including *Drosophila melanogaster*, the mosquito *Anopheles gambiae*, and the moth *Heliothis virescens* (Hill et al. 2002; Krieger et al. 2002) show low sequence homology, indicating that *Or* genes experienced rapid divergence and that different gene families evolved to accommodate unique chemosensory requirements in different species (Vosshall et al. 1999). The only exception is the *Or83b* receptor which is conserved across insect species, with 65% amino acid identity between Drosophila, mosquito, and moth, indicating an essential universal role for this receptor.

There is an emerging view that insect olfactory receptors function as ion channels, in contrast to mammalian odorant receptors. Indeed, insect *Ors* are distinct from mammalian *Ors* in many aspects. Drosophila *Or* genes show no homology with known GPCR families (Vosshall et al. 1999). Each ORN expresses one or a small number of *Ors* together with the universal *Or83b* (Larsson et al. 2004). Fly OR proteins have a reverse configuration across the membrane compared to classical GPCRs, with an intracellular N-terminus and an extracellular C-terminus. ORs interact physically with OR83b through intracellular loops forming OR/OR83b dimers (Benton et al. 2006). Thus, insect olfactory receptors activate signaling pathways through a different mechanism than mammalian olfactory receptors. Patch clamp recording studies on heterologous cells expressing OR/OR83b complexes reveal that they show similar properties with other known voltage-independent, non-selective cation channels (Sato et al. 2008; Wicher et al. 2008). However, it is still being argued whether G-proteins and cAMP are involved in the downstream events of OR activation to reinforce the activation (Sato et al. 2008; Wicher et al. 2008). Discrepancies could be due to the differences in time scale on which measurements were taken (Chesler and Firestein 2008).

In addition to the classical Ors that are seven transmembrane G-protein coupled receptors, a new family of chemosensory receptors in Drosophila has been identified that are *ionotropic receptors* (*IR*s) with a similar modular organization to canonical families of iGluRs (AMPA, kainite, NMDA, or delta), although they are not closely related to any of the ionotropic glutamate receptors in sequence (Benton et al. 2009). *IR*s have a highly divergent extracellular ligand-binding domain, and unlike iGluRs, they lack one or more residues that directly contact the glutamate ligand (Benton et al. 2009). The *IR* gene family contains 61 predicted members and two pseudogenes, distributed throughout the genome, and they are expressed in coeloconic sensilla. *IR* genes are also highly divergent with an overall amino acid sequence identity of 10%-70%. Large comparative genomic analysis of *IR* repertoires indicates that at least 14 *IR*s from the antennal subfamily are conserved across insects, while 45 *IR*s from a divergent subfamily are largely specific to Drosophila, and *IR25a* has the most similar primary sequence to iGluRs (Croset et al. 2010).

Expression of Olfactory Receptors

In situ hybridization, immunohistochemistry, and reporter gene analysis have revealed expression patterns of Drosophila *Or* genes. In contrast to *C. elegans* where individual ORNs express a large number of odorant receptors (Troemel 1999), in Drosophila, like in mammals, individual ORNs only express one or a small number of *Or* genes together with the universal receptor, *Or83b* (Larsson et al. 2004; Fishilevich and Vosshall 2005; Vosshall and Stocker 2007). Each *Or* is expressed in a subpopulation of ORNs and this expression pattern is conserved between individual flies (Vosshall et al. 1999), There are 12 larva-specific *Ors*, identified from in situ hybridization studies (Fishilevich and Vosshall 2005; Vosshall and Stocker 2007); however, some of these *Or* genes are also expressed in adults, an observation based on hybridizing adult and larval RNA samples to customized chemosensory gene cDNA microarrays (Zhou et al. 2009).

Among the *Or* genes expressed in adults, seven *Or* genes are expressed in maxillary palps, 31 *Or* genes are expressed in basiconic and trichoid sensilla in the antenna and only one *Or* gene (*Or35a*) is expressed in coeloconic sensilla in the antenna (Elmore and Smith 2001; Dobritsa et al. 2003). The common *Or83b* receptor gene is co-expressed with other *Or* genes and is required for their trafficking and function in ciliated dendrites (Fig.1.2) (Larsson et al. 2004).

Using the UAS-Gal4 binary expression system, ORN projections can be studied by driving GFP expression under the control of specific *Or* promoters. These studies revealed that ORNs expressing the same olfactory receptor converge onto a single or small number of glomeruli in the antenna lobes, and the projection patterns are conserved between individual flies (Vosshall et al. 2000). This basic single neuron--single receptor--common glomerulus, organization is conserved from insects to mammals. Most mammalian ORNs also express only one or few *Ors* (Malnic et al. 1999), and ORNs expressing the same *Or* gene converge onto the same glomeruli in the olfactory bulb (Mombaerts et al. 1996).

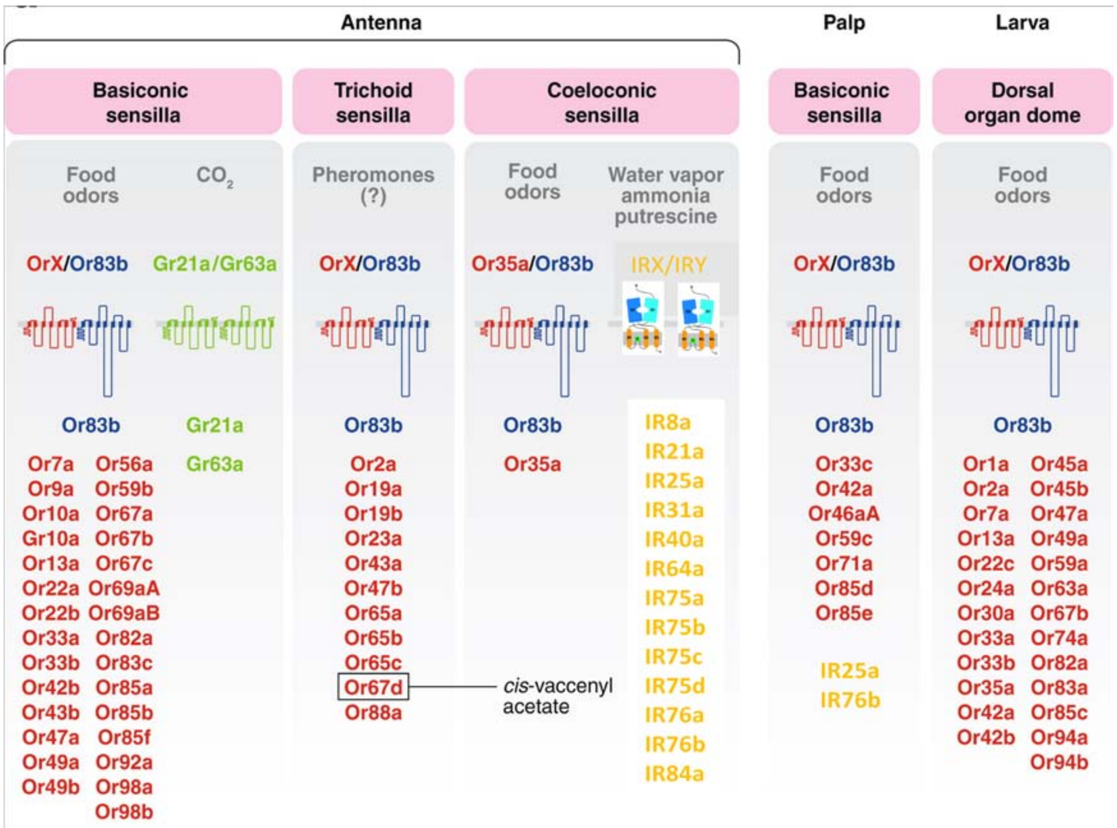

Figure 1.2 Distribution and tuning of olfactory receptors: A comprehensive list of *Or*s, *Gr*s, and *IR*s expressed in each olfactory organ subdivided by functional class and sensillum. Note that expression of only 15 members of the newly discovered *IR* genes has been confirmed in coeloconic sensilla and proboscis with two or three *IR*s being coexpressed in individual neurons. *Or83b* is an obligatory co-receptor for all the *Or*s in olfactory neurons, but not for *Gr*s and *Ir*s. Figure adapted from Vosshall et al. (2007) and Benton et al. (2008) and modified.

Among 61 predicted *IR* genes, expression of 15 has been shown in the antenna exclusively (Fig. 1.2). They are not expressed in basiconic and trichoid sensilla, and are also not coexpressed with *Or83b*, with one exception: both *IR76b* and

Or35a/Or83b are coexpressed in a subpopulation of coeloconic ORNs (Couto et al. 2005; Benton et al. 2009). *IR* genes are expressed in different subpopulations of coeloconic sensilla, as well as in a feather-like projection called the arista, and in neurons within a three-chamber pocket called the sacculus, while *IR*25a and *IR76a* are also expressed in the proboscis (Benton et al. 2009). Double and triple RNA *in situ* hybridization revealed that individual neurons express between one and three different *IR* genes and are organized into four distinct clusters of two or three neurons in the antenna. These four clusters of *IR* expressing neurons are consistent with the four types of coeloconic sensilla (ac1-ac4) identified by electrophysiological recording (Yao et al. 2005). *IR* genes are expressed in both the cell body and in the ciliated dendrites, suggesting a role for *IRs* in sensory detection, and the existence of a transport mechanism independent of *Or83b* to concentrate IR protein at the cilia (Benton et al. 2009).

Two closely related *IR*s, *IR25a* and *IR8a*, have broad expression in antennal *IR*-expressing OSNs (Benton et al. 2009; Croset et al. 2010). Double immunofluorescence revealed heterogeneous expression in distinct but partially overlapping neuron populations; *IR25a*-expressing neurons innervate the first and second chambers of the sacculus, while *IR8a* is strongly expressed in neurons innervating the third chamber (Abuin, 2011). In addition, they are expressed throughout the main body of the antenna (Benton, 2009).

Receptor Mapping

The receptor-to-neuron maps of the antenna and maxillary palp revealed that *Or* genes expressed in the same sensillum are not more closely related to each other than to the rest of the *Or* genes in sequence and genomic location, and that olfactory receptors show high redundancy in their odorant responses. Misexpression of individual receptors in the empty neuron, a mutant ORN lacking its own receptors, also revealed that it is the olfactory receptor that determines the properties of an olfactory receptor neuron, including the firing rate, signaling mode (excitatory or inhibitory), and dynamics (i.e. prolonged or quickly terminating response) (Hallem et al. 2004). Little is known about how ORNs select which receptors to express from a repertoire of 60 *Or* genes. It has been shown that relatively short DNA sequences are often sufficient to recapitulate endogenous receptor gene expression and comparisons of upstream regions of *D. melanogaster* maxillary palp *Or* genes and orthologs in *D. pseudoobscura* which diverged tens of millions of years ago indicated conserved elements (*cis*-regulatory elements, CREs) (Ray et al. 2008). Subsequent analysis of these conserved elements across another 10 Drosophila species identified the most conserved element for each of five *Or* genes. Each gene also contains at least one unique element that is not shared with other *Ors*. Functional studies of these elements revealed that maxillary palp neurons use a combinatorial code of unique and shared *cis*-acting elements such that 'positive' elements regulate the expression of *Or*s in a subpopulation of ORNs while 'negative' elements restrict the expression of *Or*s to a single class of ORNs (Fig. 1.3) (Ray et al. 2008).

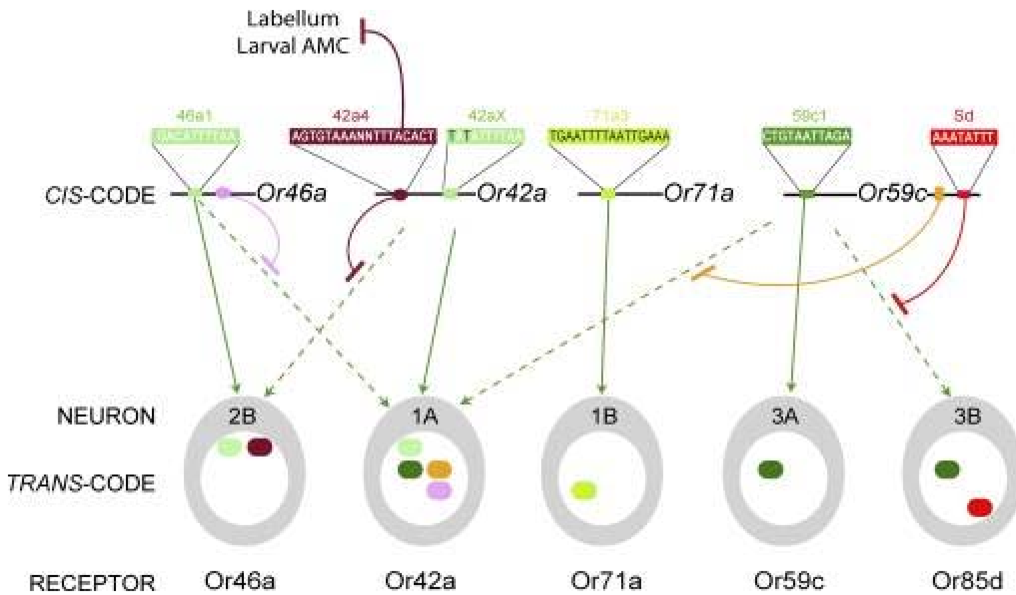

Figure 1.3 Model for combinatorial coding of odorant receptor gene choice in the maxillary palp. Conserved gene specific regulatory elements are color coded. Green elements are positive regulatory elements, red, pink and orange are negative elements. Gray balls represent different maxillary palp ORNs. Expression of a single receptor in individual neurons is determined by *cis* and *trans* regulation through combination of different factors acting upon different regulatory elements. Figure adapted from Ray et al. (2009).

The POU-domain transcription factor abnormal chemosensory jump 6 (*acj6*) has been implicated in receptor choice in ORNs, as *acj6* mutants lack expression of a subset of *Or* genes (Clyne et al. 1999). However, several *Or* promoter reporters only incompletely recapitulate endogenous *Or* expression, indicating additional necessary regulatory elements (Stocker 2001). vidence has been found in several peripheral sensory tissues (not including olfactory tissue) that loss of the microRNA *miR-9a* results in the formation of additional sensory organ precursors through reduced suppression of transcriptional regulation of sensory organ precursor specification (Li et al. 2006).

Similar to receptor choice, little is known in Drosophila about the molecular cues that guide ORN axons to their target glomeruli. Unlike in mammals, where the odorant receptor itself has been implicated in this process (Wang et al. 1998), Drosophila ORNs of which *Or* genes have been deleted or which express ectopic *Or* genes still target axons to their cognate glomeruli (Dobritsa et al. 2003). The adaptor protein Dock and the serine-threonine kinase Pak have been implicated in proper routing of ORN axons in the AL (Ang et al. 2003). Furthermore, two POU-domain transcription factors, *acj6* and *drifter* are necessary for PNs to target their dendrites in the AL, and for axonal arborization in the upper level brain lateral horn (LH) (Komiyama et al. 2003). Finally, the cell adhesion molecule N-cadherin has a role in proper branching of ORN axons and PN dendrites within the AL. Like *acj6, N-cadherin* is required for proper branching of PN axons in the mushroom body (MB) and the LH (Zhu and Luo 2004).

Functional Characterization of Receptors

The first insect *Or* to be functionally characterized was the *Drosophila melanogaster* antennal odorant receptor *Or43a*. Overexpression of *Or43a* in the antenna and heterologous expression of *Or43a* in *Xenopus laevis* oocytes, identified the odorants cyclohexanone, cyclohexanol, benzaldehyde, and benzyl alcohol as ligands for

Or43a (Stortkuhl and Kettler 2001; Wetzel et al. 2001). Later, *Or22a/b* and *Or43b* were characterized by electrophysiological recording of corresponding deletion mutants (Dobritsa et al. 2003; Elmore et al. 2003). *Or22a* neurons are sensitive to volatile odors of *Morinda citrifolia* (Noni fruit), which is a preferred feeding host for *D. sechellia*, but is toxic for most other Drosophila species. It was found that in *D. sechellia* the proportion of ab3 sensilla which contain *Or22a*-expressing neurons, is increased at the expense of ab1 and ab2 sensilla compared to *D. melanogaster,* suggesting that this specialization for odorant detection has occurred as an adaptation to feeding on Noni fruit (Dekker et al. 2006). In addition, the *Or22a/b* mutant also provided a useful tool for subsequent large-scale analyses of all Drosophila odorant receptors. *Or22a* and *Or22b* are closely related and are coexpressed in the ab3A antennal neuron (Dobritsa et al. 2003). A deletion mutant that lacks *Or22a* and *Or22b* provided an *in vivo* expression system for other olfactory receptors, as the absence of these genes results in a mutant ab3A neuron – the "empty neuron" – that is unresponsive to odors (Fig. 1.4). Individual odorant receptors can be expressed in this empty neuron under an *Or22a* promoter, and odor response spectra can be identified by electrophysiological recording and compared with well characterized response spectra of different types of sensilla (Hallem et al. 2004).

Or83b is a ubiquitous receptor that is coexpressed with conventional receptor genes in all 120 maxillary palp neurons and ~70-80% of antennal ORNs. *Or83b* mutant antennae show no odorant evoked action potentials. In *Or83b* mutants, Or22a/b and Or43b receptor proteins cannot be detected in dendrites. Thus, in addition to the structural function of forming ion channel with conventional *Ors*, these observations also suggest that *Or83b* is required for localizing conventional *Or* expression to the site of odor interaction in the dendrites (Larsson et al. 2004).

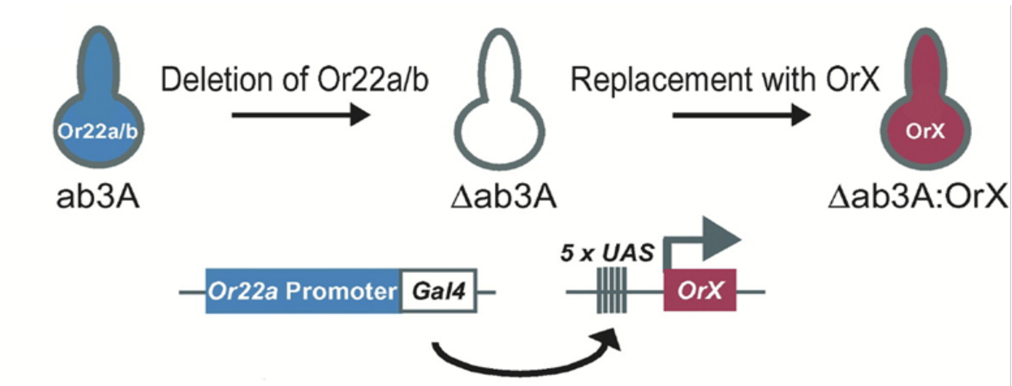

Figure 1.4 In vivo expression system using empty neuron. *Or*s can be specifically introduced into the ab3A neuron lacking *Or22a* and *Or22b* (empty neuron), using the Gal4-UAS system. Figure adapted from Hallem et al. (2004).

Genetic tracing studies revealed that 23 glomeruli are innervated by antennal basiconic ORNs, eight are innervated by antennal trichoid ORNs, six are innervated by maxillary palp basiconic ORNs, and eight glomeruli are innervated by antennal coeloconic ORNs (based on coeloconic ORN-specific genetic markers) leaving four glomeruli unassigned (Couto et al. 2005; Vosshall and Stensmyr 2005). Projections from different sensilla types tend to cluster in the AL, with antennal basiconic neurons at the medial edge, antennal trichoid neurons at the lateral edge, maxillary palp basiconic neurons at the anterior middle portion, and coeloconic neurons at the ventral middle region (Couto et al. 2005). Two large glomeruli innervated by antennal trichoid sensilla are *fruitless*-positive (Manoli et al. 2005) and sexually dimorphic (Kondoh et al. 2003). One of these expresses *Or67d* which responds specifically to a male-specific pheromone, 11-cis-vaccenyl acetate (cVA) (Ha and Smith 2006), suggesting that this projection processes pheromone cues rather than general food odors (Vosshall and Stocker 2007). In contrast to such narrowly tuned chemical stimuli, many individual odorants or behaviorally important odor blends are detected by combinations of receptors, activating different combinations of glomeruli in the AL (Hallem and Carlson 2006).

Whether there is a chemotopic arrangement of glomeruli so that glomeruli that are stimulated by similar odorants are close to each other, is still controversial (Couto et al. 2005; Vosshall and Stensmyr 2005; Hallem and Carlson 2006). However, based on a study of responses of 24 odorant receptors expressed in the empty neuron to 110 odorants, including complex fruit odors, at different concentrations, a 24 dimensional space was constructed in which each axis represents the response of one of the 24 receptors in spikes/s (Hallem and Carlson 2006). Each odorant could be mapped to a particular position in this space, and there is a trend that odorants of the same chemical class or odorants that are structurally similar often cluster together, suggesting that odorants that map far apart are easier for flies to discriminate. The position of an odorant in the odor space also depends on its molecular complexity and concentration (Hallem and Carlson 2006).

D. melanogaster is attracted by low concentrations of apple cider vinegar which is a complex volatile blend. Stimulation by apple cider vinegar induces activation of six glomeruli in the AL, while only two glomeruli, DM1 and VA2, which are innervated by *Or42a* and *Or92a* expressing ORNs, affect the attractant behavior. Blocking DM1 results in a complete loss of attraction and blocking VA2 results in a 30% reduction in attraction (Semmelhack and Wang 2009). Interestingly, recruitment of a single glomerulus innervated by *Or85a* expressing ORNs switches attractant behavior at a low concentration of vinegar to avoidance behavior at a high concentration (Semmelhack and Wang 2009).

The functional characterization of the novel family of ionotropic receptors contributes an additional dimension to the *Drosophila* olfactory system. The broad expression and localization to dendritic cilia of *IR8a* and *IR25a* indicate widespread roles for these two receptors in odor detection. Indeed, testing seven odorants on individual *IR8a* and *IR25a* mutants showed loss of multiple distinct ligand-evoked responses suggesting that these two receptors function as co-receptors that act in combinations with different subsets of odorant-specific *IR*s (Abuin et al. 2011). *IR84a* which is co-expressed with *IR8a* in ac4 sensilla, is no longer localized to the distal dendritic segment of the cilium in the *IR8a* mutant, but instead its localization is restricted to the inner dendritic segment very close to the cell body. Reciprocally, *IR8a* cilium co-localization is abolished in the *IR84a* mutant. Similar observations have been found between *IR8a* and *IR64a*, which suggests co-dependency of the common receptor *IR8a* and neuronal specific *IR*s in promoting proper cilia targeting and odor-evoked responses (Abuin et al. 2011). Evidence from optical imaging of fluorescent protein-tagged receptors revealed a similar structure for many types of iGluR in which each *IR* complex contains up to two subunits, each with two co-receptors (Abuin et al. 2011). In addition, one unique case showed a requirement for co-expression of three receptors of *IR25a*, *IR76a* and *IR76b* (Abuin et al. 2011).

Gr21a/Gr63a neurons in the antennae mediate avoidance behavior of *Drosophila* to CO_2 (Suh et al. 2004; Jones et al. 2007). However, when *Gr21a/Gr63a* neurons are inactivated, flies still show avoidance behavior towards CO_2 at concentrations higher than 5%, and recent research showed that the DC4 glomerulus is responsive only to high concentrations of CO_2 (Ai et al. 2010). DC4 is innervated by *IR64a* expressing neurons which also innervate another glomerulus, DP1m. DC4 responds specifically to protons which are produced when high concentrations of CO_2 are dissolved in the sensillar lymph fluid. In addition, *IR64a* mutant flies show significant decreases in avoidance to acid. Interestingly, apple cider vinegar which contains 5% acetic acid induces attractant behavior in contrast to avoidance behavior induced by pure 5% acetic acid, and in both cases the DC4 glomerulus is activated. These findings suggest that apple cider vinegar contains at least one attractive odorant that is capable of overcoming DC4 mediated avoidance by activating additional olfactory receptors, possibly *Or42a* and *Or92a* (Semmelhack and Wang 2009; Ai et al. 2010).

Olfactory Pathways in Higher Brain Levels

Single odorants can activate multiple ORNs while single ORNs can respond to multiple odorants; one odorant can be excitatory for some ORNs, but inhibitory for others while single ORNs can be excited by some odorants, but inhibited by others; one odorant may elicit a quickly terminating response in some ORNs, but a prolonged response in others; an individual neuron may show an abruptly terminating response to some odorants, but sustained response to others. This complex combinatorial interaction between odorants and ORNs expands the coding capacity of the Drosophila olfactory system that relies on only ~60 olfactory receptors (de Bruyne et al. 2001). The information perceived by ORNs is transferred to the AL in a one-to-one fashion so that a single class of ORNs expressing the same *Ors*, converges their axons to single or in few cases small numbers of glomeruli. The nature and concentration of the odorant is represented in spatial and temporal patterns of activated glomeruli (Fig. 1.5) (Vosshall et al. 1999). In the antennal lobe, local interneurons (LN) interact with ORNs providing “horizontal” connections among glomeruli, and cholinergic projection neurons interact with ORNs forming glomeruli and providing “vertically” projecting connections to the mushroom body and the lateral horn (LH) of the protocerebrum (Stocker 1994). This provides the possibility of modification of olfactory information in the AL before passing information to the MB and LH. Most of the LNs are GABAergic (Wilson and Laurent 2005) and receive excitatory input from ORNs and PNs. They establish inhibitory synapses with PNs. Recently, cholinergic LNs have also been found in the AL (Shang et al. 2007), which may function in interglomerular excitation of PNs. The possible functions of this neural circuitry could be to synchronize PN activity either within a given glomerulus or between PNs that innervate different glomeruli, thereby widening the range of odorant sensitivities in PNs (Krieger et al. 2002) and generating a modified odor image reflected both by temporal and spatial PN activity. PNs project their axons to both the calyx in the MB and the LH. PNs that innervate the same glomerulus reveal strikingly similar axonal topography, whereas PNs from different glomeruli show very different projection patterns to both the MB and LH (Wong et al. 2002). A topographic map of olfactory information is retained in the two higher olfactory centers, but the characteristics of the maps differ from the one in the AL, with diffuse and extensive projections (Wong et al. 2002).

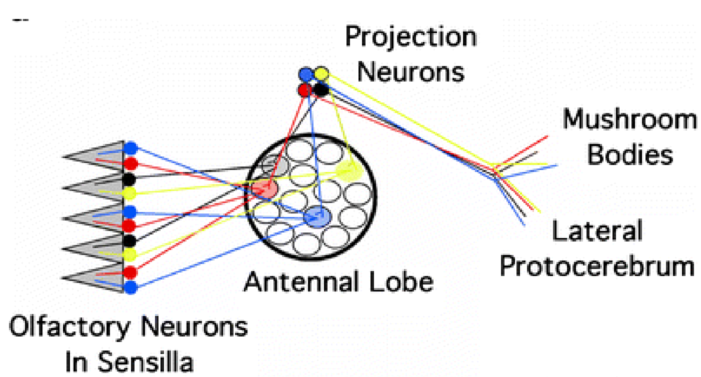

Figure 1.5 Functional organization of the Drosophila olfactory system. Figure adapted from Smith (2007).

GUSTATORY RECEPTION IN ADULT FLIES

Gustatory Organs

Unlike vertebrate taste, which is restricted to a single gustatory region in the head, insects have taste organs distributed over their entire body surface (Fig. 7). In Drosophila, the primary taste organ for evaluating food quality before and after ingestion is the proboscis, the homologue of the mammalian tongue. There are also taste sensilla distributed on the wing margins, the tarsi, and the vaginal plate at the tip of the female abdomen (Stocker 1994). The functional unit of the taste organs is the taste sensillum, which has a single pore at the tip. There are two morphological types of taste sensilla, the taste hairs or bristles located on the surface of the fly body, and the taste pegs located on the inner labial palps. There are 31 taste bristles, each housing between two and four gustatory receptor neurons (GRNs) as well as a single mechanosensory neuron, distributed on each labellum (labial palp) of the proboscis. In addition, each labellum also has ~30 taste pegs housing one GRN each. The pharynx of the inner proboscis contains three bilaterally symmetric aligned internal taste organs, the labral sense organ (LSO), the ventral cibarial sense organ (VCSO), and the dorsal cibarial sense organ (DCSO) (Gendre et al. 2004). The LSO has nine sensilla, three of which are taste sensilla with a total of ten GRNs, the VCSO has three sensilla with eight GRNs, and the DCSO has two sensilla with three GRNs each (Gendre et al. 2004). The labella and pharynx allow flies to evaluate food quality both before and after it is ingested, but before digestion.

There is sexual dimorphism in the number of taste bristles on the first tarsus, with males having more bristles than females. This is due to the presence of male-specific sensilla that detect female pheromones and are important for sexual behavior (Bray and Amrein 2003). There are 30 taste bristles and 32 taste bristles on each second and third tarsus in both males and females. Each of these sensilla houses two to four GRNs. There are 40 taste bristles on the wing margin, where each bristle houses four GRNs. The female vaginal plates carries ~10 sensilla, which are still poorly characterized.

Taste bristles have been analyzed by the tip-recording technique, in which electrophysiological recording of taste neurons are recorded after applying a drop of taste solution containing electrolyte to the pore at the tip of the sensillum. This resulted in the characterization of four types of GRNs housed in s-type (small), l-type (long) and i-type (intermediate) taste sensilla, which are categorized by size, distribution and number of GRNs (Amrein and Thorne 2005). S-type and l-type sensilla on the labellum each house four neurons tuned to different tastants; the S cell is tuned to sugar, the W cell is activated by water, the L1 cell is tuned to low salt, and the L2 cell is tuned to high salt (Amrein and Thorne 2005). The i-type sensillum has two GRNs; one neuron combines both L1 and S cell activity, while the other neuron has L2 cell properties (Hiroi et al. 2004).

Identification of Gustatory Receptors

After the identification of olfactory receptors in Drosophila, gustatory receptors (*Grs*) were identified through a similar bioinformatics approach, aimed at finding putative G-protein coupled taste receptors (Clyne et al. 2000). A thorough search through the complete *Drosophila melanogaster* genome yielded a gene family of 68 receptors encoded by 60 *Gr* genes (Robertson et al. 2003). GR proteins are even more divergent in sequence than ORs; they share as little as 8% amino acid identity (Robertson et al. 2003). Like *Ors*, *Gr* genes are dispersed throughout the genome, and about two-thirds are in clusters of two to six genes (Robertson et al. 2003).

Expression of Gustatory Receptors

The expression of many *Gr* genes has been confirmed by RT-PCR (Clyne et al. 2000). However, unlike *Or* genes, *Gr* genes express a low level of mRNA in small groups of taste neurons; moreover, many appendages expressing *Gr* genes, like wings and legs, are not readily amenable to *in situ* hybridization, which makes it difficult to capture the complete expression pattern of all putative *Gr* genes (Dunipace et al. 2001). Driving reporter genes under putative *Gr* promoters provides an alternative way to analyze *Gr* gene expression (Thorne et al. 2004; Wang et al. 2004; Weiss et al. 2011). This approach together with RNA *in situ* hybridization reveals a complex expression profile of the *Gr* gene family (Dunipace et al. 2001; Scott et al. 2001; Thorne et al. 2004; Wang et al. 2004; Weiss et al. 2011). Individual *Gr* genes are expressed in subsets of chemosensory neurons in one or more of the gustatory organs. Some *Grs* are widely expressed in multiple tissues, whereas expression of most *Gr* genes is restricted to a few neurons in one or two taste organs. While most of the detected *Gr* genes are expressed in gustatory organs, there are three *Gr* genes – *Gr10a*, *Gr21a*, and *Gr63a* – that are expressed in olfactory neurons in the antenna, suggesting that these *Gr* genes might serve olfactory functions (Scott et al. 2001). Unlike olfactory neurons, many taste neurons express multiple gustatory receptors. Two *Gr* genes were identified that are expressed in non-overlapping subpopulations of GRNs. The *Gr5a* gene is expressed in taste neurons in the labellum and tarsi; *Gr66a* is expressed in one-fourth of the neurons in the proboscis, that do not express the Gr5a receptor (Chyb et al. 2003; Thorne et al. 2004; Wang et al. 2004). Thus, fly gustatory neurons can be characterized according to the gustatory receptors they express. Neurons that express the *Gr5a* receptor are sugar neurons, which detect trehalose, sucrose, glucose and other sugars (Wang et al. 2004) and neurons that express the *Gr66a* receptor are neurons that recognize compounds identified by humans as bitter (Thorne et al. 2004; Wang et al. 2004).

A recent study from Carlson's group systematically analyzed the expression patterns of all 68 *Gr* family members using *Gr-GAL4* lines (Weiss et al. 2011). They further confirmed the non-overlapping pattern between sugar receptors (*Gr5a, Gr61a, Gr64e and Gr64f*) and *Gr66a,* and found that, in contrast to universal expression of sugar neurons in all labellar sensilla, Gr66a neurons or bitter compound responsive neurons are restricted only to S (except S_4 and S_8) and I sensilla. Five *Grs, Gr32a, Gr33a, Gr39a.a, Gr66a* and *Gr89a* are expressed in all bitter neurons (Fig. 1.6) (Weiss et al. 2011).

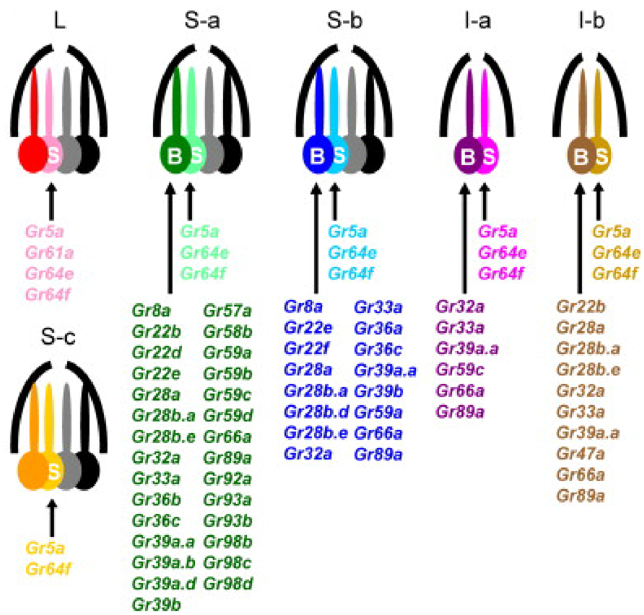

Figure 1.6 Expression of Drosophila gustatory receptors. Figure adapted from Weiss, et al (2011).

Functional Characterization of Gustatory Receptors

Ablation of *Gr5a* neurons results in behavioral taste defects to different sugars (Wang et al. 2004). Artificial activation of *Gr5a* neurons by capsaicin via expression of a mammalian capsaicin receptor under control of the *Gr5a* promoter induces acceptance behavior. Capsaicin is a neutral taste compound for wild type Drosophila. These observations show that *Gr5a* neurons are hardwired for acceptance behavior (Marella et al. 2006). The fact that the *Gr5a* receptor is not required for the response to many sugars other than trehalose, m-α-glucoside, glucose, and melezitose (Dahanukar et al. 2001; Chyb et al. 2003; Dahanukar et al. 2007) suggests that *Gr5a* is not the only receptor expressed in sugar neurons. The search of additional sugar receptors started by examining *Gr* genes that are most close related to *Gr5a*, namely *Gr61a* and the *Gr64* gene cluster (Dahanukar et al. 2007). *Gr64a* mutants show defects in responses to many sugars including fructose, stachyose, maltotriose, maltose and sucrose. These sugars do not overlap with those detected by *Gr5a* (Dahanukar et al. 2007). In addition, *Gr64f* is coexpressed with *Gr5a* in sugar neurons (Dahanukar et al. 2007), and *Gr64f* mutants have impaired responses to both *Gr5a* and *Gr64a* ligands including sucrose, maltose and glucose (Jiao et al. 2008). Introduction of *Gr5a* or *Gr64a* in *Gr5a* or *Gr64a* mutant neurons is not sufficient to restore the response to sugar, whereas coexpression of *Gr5a* with *Gr64f* or *Gr64a* with *Gr64f* restores the response to the sugars normally detected by *Gr5a* or *Gr64a* (Jiao et al. 2008). However, ectopic coexpression of these pairs of genes is not sufficient to elicit sugar responses in bitter neurons, indicating that there are additional components uniquely expressed in sugar neurons that are necessary for sugar detection (Jiao et al. 2008). Thus, *Gr5a* and *Gr64a* are the primary sugar receptors in the labellum, and each of them is capable of mediating responses to a subset of sugars independent of the other. These two sugar receptors are able to evaluate caloric value in food sources, with the assistance of *Gr64f* and some unknown components specific to sugar neurons.

Similar to sugar neurons, bitter taste neurons marked by *Gr66a* are hardwired for avoidance behavior towards bitter compounds (Marella et al. 2006). One gene that is coexpressed with *Gr66a* is *Gr93a*. Both *Gr66a* mutants and *Gr93a* mutants are defective exclusively in detecting caffeine, but have normal responses to many other bitter compounds and normal preference for sugars. Like *Gr64a* and *Gr5a*, coexpressing *Gr93a* and *Gr66a* in sugar neurons does not elicit behavior or electrophysiological responses to caffeine (Lee et al. 2009), indicating that additional factors are needed to support avoidance behavior together with *Gr93a* and *Gr66a* in bitter taste neurons. Another receptor that is coexpressed with *Gr66a* is *Gr32a*. All labellar neurons that express *Gr32a* also express *Gr66a*, but in the tarsi *Gr32a* and *Gr66a* are expressed in distinct, non-overlapping sets of GRNs (Miyamoto and Amrein 2008). These *Gr32a* expressing GRNs are associated with courtship behavior. In contrast to wild type males, mutant males when given a choice between males and mated or virgin females frequently courted male flies and mated females in addition to virgin females. However, when placed together with a virgin female only, *Gr32a* mutants show normal courtship behavior. This suggests that Gr32a is important for suppressing courtship towards males and mated females. Thus, Gr32a is required for discriminating between the two sexes and the female mating status, through sensing inhibitory male pheromones that are released from male flies and are received by females after copulation (Miyamoto and Amrein 2008). In addition to *Gr32a*, another putative pheromone receptor, *Gr68a*, is exclusively expressed in neurons of about ten male-specific taste bristles in the tarsi. Reduction in expression of *Gr68a* protein levels results in inefficient courtship behavior and reduced mating success, indicating a role for this receptor in detecting attractant pheromones transmitted from the female flies (Bray and Amrein 2003).

A systematic functional analysis of all bitter receptors in Drosophila revealed a receptor-to-neuron-to-response map towards a panel of different bitter compounds (Weiss et al. 2011). A two-choice feeding assay between sucrose and sucrose mixed with a bitter compound was used to test 14 selected bitter compounds at different concentrations. Different compounds elicited different degrees of aversive behavior, for example, denatonium benzoate elicited the strongest avoidance, two orders of magnitude greater than the weakest avoidance, elicited by escin (Weiss et al. 2011). One of the underlying mechanisms may rely on the differential distributions of responsive bitter neurons across different labellar sensilla. L sensilla and S_4 and S_8 sensilla do not respond to any of the bitter compounds. I_0 to I_6 sensilla exclusively respond to 5 compounds (including denatonium benzoate), I_7 to I_{10} sensilla only respond to another 3 compounds, while all S sensilla (except S_4 and S_8) respond broadly to most of the tested compounds (Weiss et al. 2011). Based on the responsiveness towards different compounds, labellar sensilla are divided into five functional classes: broadly tuned sensilla (S-a, S-b), narrowly tuned sensilla (I-a and I-b) and a class that does not respond to any tested bitter compounds (L, S-c). In addition to the neuronal distribution, the electrophysiological properties are different for different compounds as well. Different compounds elicit responses with delays of different lengths of the interval between the time at which tastants are introduced and the onset of spike discharge. The same compound can also elicit responses with different latencies across different sensilla (Weiss et al. 2011).

By combining functional expression analyses, it is possible to predict the functions of certain receptors. For example, only one receptor, *Gr59c,* is expressed in I-a but not I-b sensilla. I-a shows strong responses to berberine chloride, denatonium benzoate and lobeline hydrochloride, but I-b sensilla do not respond to any of these compounds. This suggests that *Gr59c* plays a role in responding to these compounds; this has been confirmed by misexpression of *Gr59c* in different sensilla (Weiss et al. 2011).

In addition to the sugar neurons and bitter neurons, marked by *Gr5a* and *Gr66a* respectively, an additional group of taste peg neurons in the labellum, was identified through a Gal4 enhancer trap screen (Fischler et al. 2007). These neurons, designated as E409, are specialized for detecting low concentrations of carbon dioxide. E409 neurons like sugar neurons are hardwired for acceptance behavior towards carbon dioxide, but this attractant behavior is not as pronounced as that mediated by sugar neurons toward sugars, suggesting that carbon dioxide might improve taste preference in conjunction with other food components without providing nutrition on its own (Fischler et al. 2007). Interestingly, two gustatory receptors, *Gr21a* and *Gr63a*, mediate avoidance behavior towards carbon dioxide vapors and are expressed exclusively in abC1 neurons in the antenna that project to the V glomeruli in the AL, in the absence of coexpression with *Or83b* (Jones et al. 2007). The *Gr63a* and *Gr21a* homologues *GPRGR24* and *GPRGR22* in the mosquito *Anopheles gambiae* are coexpressed in neurons in the maxillary palp where they also serve as a CO_2 sensor; however, for mosquitoes carbon dioxide is an attractant that aids host-seeking behavior (Jones et al. 2007). Together, E409 and *Gr21a*/*Gr63a* neurons sense different concentrations of carbon dioxide over short and long ranges, and mediate opposite behaviors through distinct pathways.

Gustatory Projections

Gr5a positive sugar neurons, *Gr66a* positive bitter neurons and E409 neurons define three classes of GRNs that mediate the detection of different taste qualities and direct different behaviors. *Gr5a*, *Gr66a* and E409 neurons send axons to distinct areas of the subesophageal ganglion (SOG), *Gr5a* neurons project ipsilaterally to the lateral region of the SOG, *Gr66a* neurons project to a ring-like web in the medial SOG that is slightly anterior to targets of *Gr5a* (Thorne et al. 2004; Wang et al. 2004), and E409 neurons terminate in snake-like projections in the anterior SOG that do not cross the midline and are spatially segregated from *Gr5a* and *Gr66a* projections (Fischler et al. 2007). Recent studies on sensory motor control of taste behavior identified a group of neurons, designated E49 neurons, that, when silenced, generate a specific motor defect in proboscis extension in response to tastant (Gordon and Scott 2009). E49 silent flies fail to execute the full motor program in proboscis extension. They do not lift the rostrum of the head while other movements remain intact. Expressing exogenous channels in E49 neurons has shown that activation of E49 motor neurons is sufficient to generate this rostrum lifting subprogram (Gordon and Scott 2009). In addition there is no simple monosynaptic connection between sensory neurons and E49 motor neurons, indicating that there are unidentified higher order neurons in this taste circuit (Gordon and Scott 2009).

OLFACTORY AND GUSTATORY RECEPTION IN LARVAE

Larvae have a similar but simplified chemosensory system compared to the adult system. There are three major sense organs in the head, the dorsal organ, terminal organ and ventral organ, as well as three pharyngeal organs (Gendre et al. 2004). These sense organs consist of multiple sensilla, each housing one to several sensory neurons. The dorsal organ is innervated by 21 ORNs as well as GRNs while the terminal and ventral organs are mainly innervated by GRNs (Vosshall and Stocker 2007). Compared to adult flies, which have ~1300 ORNs and ~300 GRNs, larva only have 21 ORNs and ~80 GRNs with a dramatically reduced number of chemosensory neurons. Interestingly, GRNs outnumber ORNs in the larva, consistent with an expected predominant short range chemical perception due to a lifestyle embedded in food (Vosshall and Stocker 2007). Though the olfactory and gustatory systems share peripheral organs in the larva, they are separated into distinct pathways in the brain. The larval olfactory system resembles the organization of the adult system, with single input from individual ORNs to corresponding glomeruli in the AL. Axons of ORNs project to 21 glomeruli and interacts with LNs and PNs, PNs then project to the MB calyx. With a reduced number of ORNs and glomeruli, larvae show lower dimensions and less redundancy in olfactory sensing than adults (Vosshall and Stocker 2007). The larval gustatory system is less well studied; nevertheless, it is known that there are four major target subregions in the larval SOG and they receive projections according to the location of the projecting GRNs in the peripheral organs (Colomb et al. 2007). Unlike adult *Gr66a* neurons, which project bilaterally to the medial SOG, larval *Gr66a* neurons project ipsilaterally, suggesting that these afferents mediate attractant responses (Heimbeck et al. 1999).

ODORANT-BINDING PROTEINS

Odorant binding proteins (OBPs) contain six cysteines with conserved spacing between cysteines 2 and 3 (three residues) and cysteines 5 and 6 (eight residues) which is the defining feature of the family (Vogt et al. 1991). Both vertebrates and invertebrates have OBPs in their olfactory system, but they are not related according to the X-ray crystal structure data (Bianchet et al. 1996). There are 51 genes in the Drosophila *Obp* gene family. These genes are often found in large clusters in the genome; however, despite the proximity of genes within a cluster, they have strikingly different expression patterns (Galindo and Smith 2001). OBPs have been proposed to solubilize odors in the sensillar lymph, to transport odorants through the lymph to the odorant receptors, to remove deleterious compounds from the lymph, and to contribute to deactivation of odorants following receptor activation (Hallem et al. 2006). In addition to their expression in the sensillar lymph, *Obp* mRNA can be detected also in many other non-chemosensory tissues (Chintapalli et al. 2007), for example the accessory gland, indicating additional roles for OBP proteins.

Lush is one of the best characterized *Obp*s. *Lush* null mutants display abnormal olfactory behavioral responses to a subset of odorants including ethanol, propanol, butanol, and benzaldehyde (Kim et al. 1998). More interestingly, in *lush* mutants, activation of *Or67d* neurons, located in T1 trichoid sensilla, by a specific pheromone cVA is abolished. cVA is a male-specific pheromone, present on the cuticle and is secreted by the ejaculatory duct and transferred to females upon mating (Smith 2007). cVA elicits sexually dimorphic behaviors in males and females. cVA can induce male aggregation behavior (Wang and Anderson 2010) and suppress male courtship towards females as well as males (Kurtovic et al. 2007). In females, cVA activates the same sensory neurons to promote receptivity to males (Kurtovic et al. 2007). Infusion of bacterially expressed, recombinant LUSH protein directly into the sensillar lymph of *lush* mutants can restore the response to cVA (Xu et al. 2005). In addition to the cVA response, the spontaneous activity of *Or67d* neurons in *lush* mutants is also reduced, suggesting that the LUSH protein itself is directly responsible for spontaneous activity (Fig. 1.7) (Xu et al. 2005). However, *lush* and

Or67d are not the only factors required to mediate detection of cVA; Another protein specific to trichoid sensilla, sensory neuron membrane protein (SNMP), a homologue of silk moth SNMP (Rogers et al. 1997), which is expressed in dendrites of pheromone sensitive olfactory neurons (Rogers et al. 1997), appears crucial for the cVA response of *Or67d* neurons (Jin et al. 2008).

In addition to the increased proportion of ab3 sensilla, as mentioned previously, two OBPs have been identified as critical mediators of host plant preference on *Morinda citrifolia*. *Obp57d* and *Obp57e* are responsible for behavioral differences between species in their responses to hexanoic acid and octanoic acid (Matsuo et al. 2007). *Drosophila sechellia* shows a preference for and resistance to the ripe fruit of *M*. *citrifolia*, whereas its closely related species, *Drosophila melanogaster*, is a generalist who dies upon contact with *M*. *citrifolia*, and, therefore, avoids the fruit. A 4bp insertion in the upstream region of *Obp57e* in *Drosophila sechellia* accounts for the differences in expression of *Obp57e* in the tarsi between *D. sechellia* and *D. melanogaster*. Knock-outs of *Obp57d* and/or *Obp57e* in *Drosophila melanogaster*, show altered preference to toxic fruit. However, knock-out flies retained their behavioral response to hexanoic acid and octanoic acid, suggesting that Obp57d/e do not function as adaptors for hexanoic acid and octanoic acid (like Lush). Rather, they seem to modulate dose-dependent responses to hexanoic acid and octanoic acid (Matsuo et al. 2007).

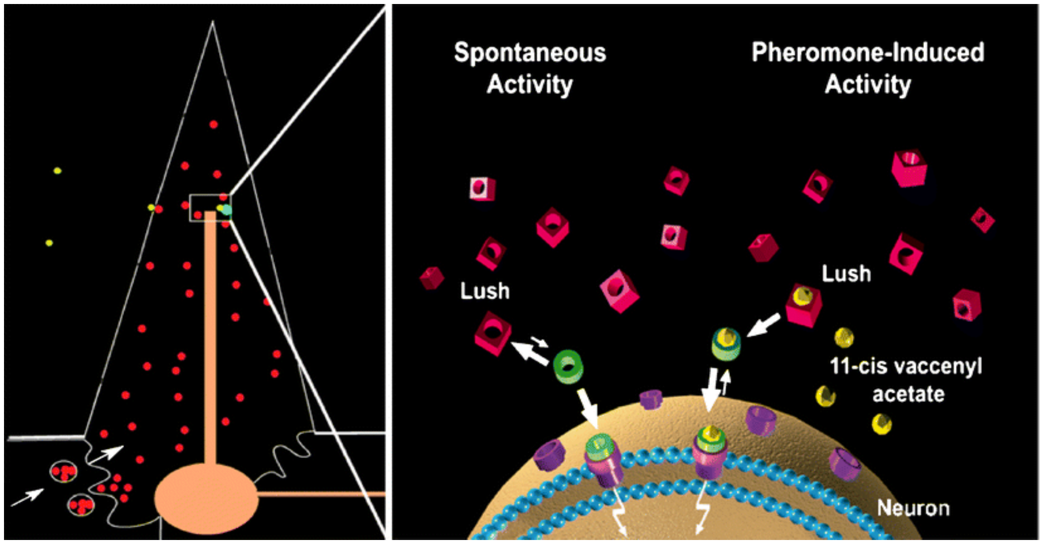

Figure 1.7 Functional model of LUSH in T1 sensilla. LUSH protein (red) changes its conformation (green) when it interacts with the pheromone cVA (yellow), in order to activate the *Or67d* receptor (purple). However, there are spontaneous conformational changes from the inactive form to the active form of the LUSH protein, producing a signature of spontaneous activity in wild type *Or67d* neurons when LUSH protein is present. Note, SNMP is not shown in this figure for simplicity. Figure adapted from Smith (2007).

In addition to olfactory functions, many *Obp* genes are expressed in non olfactory tissues. For example, *Obp56e*, *Obp56f*, *Obp56g* and *Obp56i* are enriched in the accessory gland (www.flyaltas.org), and expression of *Obp56g* is genetically correlated with expression of three *accessory gland protein* (*Acp*) genes, which are involved in male-induced postmating behavior, in a transcriptional network affecting fitness (Ayroles et al. 2009). In addition, genes of the *Obp56* cluster show extensive plasticity under different physiological conditions and social context, with sex and developmental stage specificity (Zhou, 2009).

CHEMOSENSORY BEHAVIORS

Behaviors are complex traits that mediate interactions with the environment. There are several chemosensory behavior assays that are simple, robust and quantitative. They can be grouped into two classes: choice assays that involve a choice made by the animal and require orientation behavior, and assays that only include responses to a single odor or taste stimulus (Devaud 2003).

Behavior Assays

Feeding preference tests and T-maze or Y-maze tests are the most widely used choice assays. Feeding preference tests consist of offering two food sources with different color dyes simultaneously, with one colored food source containing the chemical to be tested and the other solvent or agar as control (Fig. 1.8a). The choice made by the flies can be easily determined through visual inspection of the food color in their abdomens. This assay has been used to demonstrate that *Gr5a* mutants show a selective trehalose taste defect (Dahanukar et al. 2001). T-maze and Y-maze tests are designed to measure odor preference. Flies are introduced into the choice point and make a choice between two compartments which contain either the test odor or a control or two different odors (Fig. 1.8b, c). The preference for the odor can be evaluated by calculating the proportion of flies that make the choice to walk into the odor compartment. T-maze assays have recently been adapted to study behavioral avoidance of Drosophila stress odor (dSO), of which CO_2 is one component (Suh et al. 2004).

Another group of assays is more straightforward and involves a simple response to only one stimulation source. The proboscis extension reflex (Vaysse and Medioni 1973) is a widely used taste assay together with the feeding preference test (Fig. 1.8d). It is easily performed by touching the tarsus or labellum with a sugar solution; in addition, bitter compounds can be added to the sugar solution to test their ability to suppress the response to sugar. This assay is commonly used for separating responses from sugar and bitter taste neurons, and identifying taste specificities for individual gustatory receptors (Marella et al. 2006; Dahanukar et al. 2007; Fischler et al. 2007).

Different assays have also been designed for measuring olfactory behavior. A 'dipstick' avoidance assay can measure the avoidance of flies to a certain odor (Fig. 1.8f). The response is quantified by counting the number of flies that migrate to a part of the vial remote from the odor source at successive intervals (Anholt et al. 1996). This assay has been used to identify a large number of *smell impaired* (*smi*) mutants in a forward genetic screen (Anholt et al. 1996). In the fly trap assay (Woodard et al. 1989) groups of flies are introduced in a petri dish containing the trap, and the proportion of flies attracted into the trap is counted after a sufficient time (Fig. 1.8g). The chemosensory jump assay exploits the startle response when flies encounter an odor (Fig. 1.8e) (McKenna et al. 1989). This assay led to the identification of the *acj6* mutant in which expression of a subset of the *Or* genes is disrupted (Helfand and Carlson 1989; Clyne et al. 1999). Chemotaxis assays have been used in functional studies of larval ORNs (Fishilevich and Vosshall 2005; Louis et al. 2008).

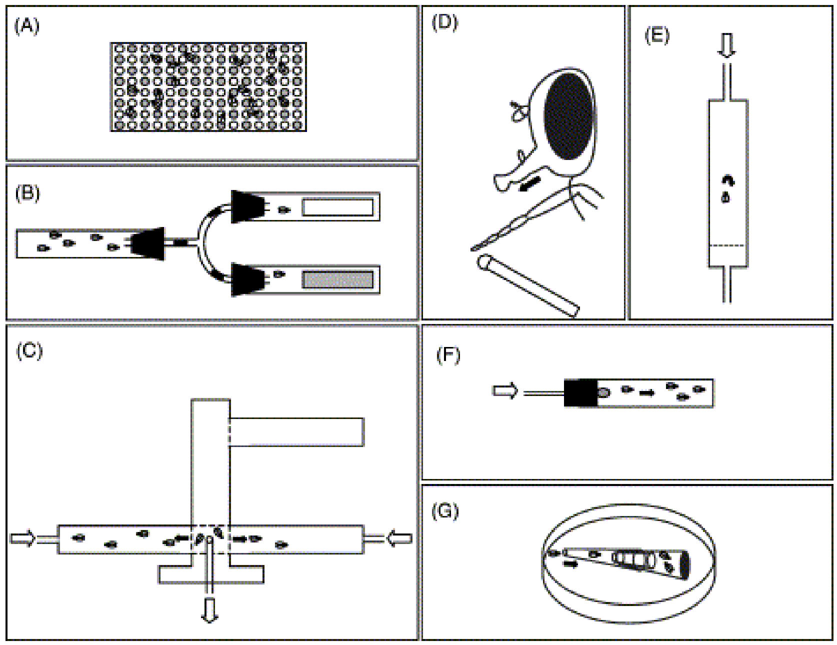

Figure 1.8 Olfactory behavior paradigms. (a) Feeding preference test, flies are allowed to choose feeding on two different food sources with different color dye. (b) Y maze, flies are introduced into a start tube which is connected to two separate tubes with filter paper soaked with either solvent or odorant. (c) T maze, flies are introduced from the upright start tube to the central chamber in the vertical elevator. The flies can choose between the compartments with different odorants. (d) Proboscis extension reflex assay, stimulation of fly tarsi or the labellum with a drop of sugar solution will provoke proboscis extension. (e) Jump assay, a single fly is introduced in a vertical tube; it jumps when a pulse of odorant is drawn through the tube. (f) Avoidance assay, 5 single sex flies are introduced into a horizontal tube; repellent odorant is introduced at one end using a cotton swab and the avoidance response is recorded by calculating the numbers of flies that migrate to the opposite end of the tube. (g) Trap assay, flies are introduced into a petri dish or a bottle containing a trap with

an attractant odor source; attraction is assessed from the proportion of trapped flies. Figure adapted from the review by Devaud (2003).

For proper interpretation of the results, behavior assays should be under careful control. Chemosensory behaviors are complex traits that are determined by networks of genes that are pleiotropic and epistatic. The effect of a gene on behavior can change when the genetic background changes. Hence, a proper control of the same genetic background for mutants and control is essential to exclude any confounding background effects on behavior. Sexual dimorphism is prevalent in behavioral traits (Anholt and Mackay 2004) including chemosensory behavior. Thus, assays should be done with females and males separately. This also prevents interactions between the sexes during the assay (Devaud 2003). Electrophysiological data show that antennal responses to odors decrease with age (Devaud 2003), indicating that olfactory performance is age-dependent. Therefore, controlling the ages of tested flies is necessary when comparing results between different experiments. Furthermore, one caveat of many behavior assays is that the phenotypic defect may be due to a defect in locomotor activity. Hence, multiple assays should be used to evaluate different aspects of the behavior. In addition, locomotor activity changes throughout the day (Ashmore and Sehgal 2003), and the sensitivity of the antennae also displays circadian variations (Krishnan et al. 1999). Thus, behavioral assays should be done at the same time of the day, when measurements are collected over several days. Finally, appropriate sample size depends on the amount of phenotypic variation, including genetic and environmental variation. Because of the environmental effect, measurements of the same behavior for individuals that have the same genotype will not be identical. Flies reared in the same common environment (reared in the same vial, measured on the same day and by the same handler) show a more similar phenotype than flies reared in different environments. Many individuals need to be measured to get a statistically accurate estimate of the genotypic effect, and the larger the phenotypic variation, the more individuals need to be measured. Environmental variation can be accounted for by strict randomization. Alternatively, common environmental variation can be evaluated by examining the phenotypic variation of flies with the same genotype (Anholt and Mackay 2004). Finally, olfactory and gustatory behaviors are highly dependent on the concentration of the stimulus. Chemosensory behavior can change dramatically at different stimulus concentrations. Flies can shift from attraction to avoidance, and reciprocally from avoidance to attraction, when facing different doses of chemicals (Devaud 2003). Thus, for proper interpretation of the results from chemosensory assays, a dose-response relationship should be documented.

Genetic Approaches in the Study of Chemosensory Behavior

Single gene mutants have provided valuable information in chemosensory behavior studies. Several null mutants with large genetic effects have been generated through chemically induced mutations. The *acj6* mutant is one of the best studied olfactory mutants that showed a defect in its response to benzaldehyde (Helfand and Carlson

1989) and encodes a transcription factor that plays a role in olfactory receptor choice of ORNs and in ORN axon guidance (Clyne et al. 1999). However, mutations of large effects often cause homozygous lethality or sterility, *P*-element insertion has become the preferred strategy for the induction of mutations with small effects. Highly efficient systematic screens can be performed with co-isogenic *P*-element insertion lines. Screening of single *P*-element insertion lines led to the identification of 14 olfactory genes (*smell impaired, smi*, which showed significantly reduced avoidance response to benzaldehyde and did not include *Or*, *Gr* and *Obp* genes (Anholt et al. 1996). Among these olfactory mutants, *smi21F, also known as Pinocchio (Pino*), was identified as a ligand odorant binding protein for the clearance of xenobiotics (including odrants) from the perilymph (Rollmann et al. 2005)*; smi60E* encodes a sodium channel gene *dsc1* (Kulkarni et al. 2002), and *smi97B* is an allele of a pleiotropic gene, *scribble* (*scrib*), which is a component of postsynaptic densities (Ganguly et al. 2003). Significant epistatic interactions were identified among these independently isolated *smi* genes. Epistasis is evident when the double heterozygote of recessive mutations has the same loss-of-function phenotype as the single homozygous mutations as a result of combined haplo-insufficiency of function. Among 14 co-isogenic *P*-element insertion lines, 12 were crossed in a half-dialle design to obtain all possible double heterozygous genotypes. Eight *smi* genes including *smi27F* and *smi97B* form an interaction network with large epistatic effects, suggesting coordinated modulation of olfactory behavior by multiple gene loci (Fedorowicz et al. 1998). Similarly, a larger scale screen of 1339 co-isogenic *P*-

element insertion lines identified 55 new candidate genes that affect olfactory avoidance behavior towards benzaldehyde. Ten *P*-element insertion lines with large effects on olfactory behavior were selected to evaluate epistatic interactions under two different odorant concentrations. This study revealed highly dynamic epistatic interactions under different stimulus environments (Sambandan et al. 2006). Since interactions between the organism and its chemical environment change rapidly, chemosensory behavior is expected to show high plasticity with genotype by environment interactions (GEI). Sambandan et al. used 41 wild derived inbred lines to quantify the extent of GEI and found that GEI accounts for as much as 50% of the observed variation in adult olfactory avoidance behavior toward two different odorant concentrations, when flies are reared on different food sources. However, whole genome transcriptional profiling of 8 inbred lines reared on the same food sources, showed that only 20 genes contributed to the observed transcriptional GEI, suggesting that relatively few genes may give rise to extensive GEI in olfactory behavior (Sambandan et al. 2008).

As discussed above, *Drosophila melanogaster* has a well characterized olfactory system which shares a similar functional organization with mammals, but is much simpler in terms of the number of ORNs. The molecular and neuronal basis of chemosensory systems has been well studied in Drosophila; however, little is known about how regulation of gene expression modulates and adapts the chemosensory system during development and under different physiological and social conditions.

In my studies described in Chapter 2, I assess environmental effects on the transcriptional adjustments in expression levels of chemoreceptors in response to changing environmental conditions. I compared transcriptional profiles of chemosensory genes (*Or*s, *Gr*s, *Obp*s) at different developmental stages, under different physiological conditions and in different social contexts. I found high plasticity of chemoreceptor genes in expression across different conditions and dramatic sex dimorphism within each condition using a single isogenic Drosophila line.

These studies provide strong evidence of expression plasticity in chemosensory system, which mediating the external environmental input to the subsequent phenotypes. In the second part of the Introduction, I will review on phenotypic plasticity and focus on its genetic basis which is the drive of my work described in Chapter 3.

GENETIC BASIS OF PHENOTYPIC PLASTICITY

Genetic differences and environmental influences together determine the uniqueness of each individual and each species, and the biodiversity we see today is the fruit of billions of years of evolution, shaped by natural selection, and increasingly influenced by human activity. The macro- and micro- environment of an organism are changing constantly, and this raises questions of how organisms adapt to changing environments. To answer these questions, it is important to understand the relationship between genes and environment.

PHENOTYPIC PLASTICITY

Phenotypic plasticity is beneficial in predictably changing environments, for example mammals change their fur type, density and length to adapt to seasonal temperature changes and birds drop their body temperature at night to save energy expenditure. Phenotypic plasticity also allows a population to shift from one environment to the other while providing opportunities for long term natural selection through changes in allele frequency, which will lead to an increase in population fitness in the new environment. This process has been described by Waddington as genetic assimilation (Waddington 1959). Phenotypic plasticity is a common property of the reaction norm of a genotype responding across environments (Fig. 1.9) and non-parallel reaction norms of different genotypes indicate the presence of genotype by environment interaction (Fig. 1.9e) (Bradshaw 1965).

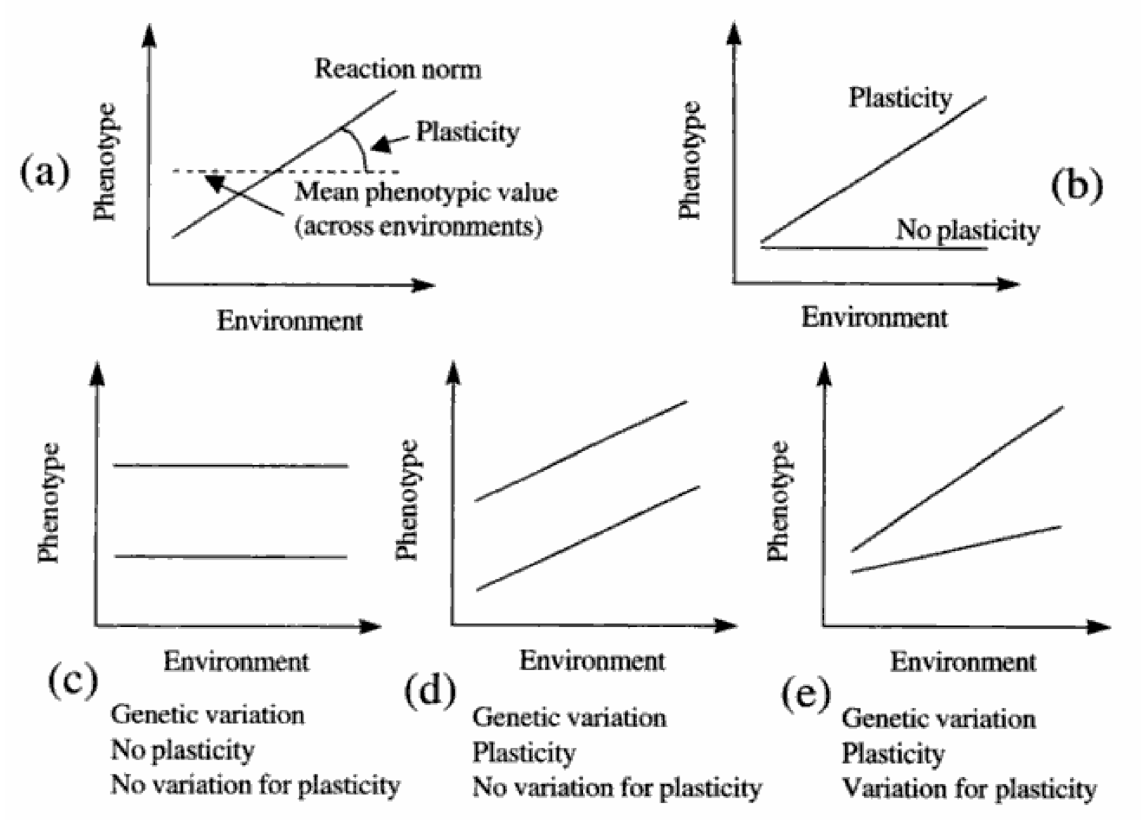

Figure 1.9 Reaction norms under different scenarios. Figure adapted from Pigliucci, (2001).

Phenotypic plasticity occurs in many traits ranging from morphology through developmental biology to physiology and behavior, and occurs in all classes of organism (Pigliucci 2001). The first documented phenotypic plasticity study traces back to 1909 when Woltereck described an odd phenomenon in *Daphnia* even though Woltereck at that time did not really understand the environmental role causing the phenomenon. Woltereck found that *Daphnia* develop a “helmet” or crest in the presence of a predator, and its formation could be induced simply by the release of specific chemicals in the water without the actual physical presence of the predator. Furthermore, the helmet reduces the predation rate; however, it comes at the cost of reducing either metabolic or swimming efficiency, which presumably explains why *Daphnia* that are not exposed to the predator cue do not develop the helmet (Dodson 1989).

Many phenotypic plasticity studies have been focused on plants, since phenotypic plasticity plays an important role in plant survival, as plants cannot voluntarily choose their location to avoid aversive environmental conditions or move towards favorable conditions. Plants exhibit a remarkable capacity to adjust their morphology, physiology, and reproduction to particular environmental conditions by means of phenotypic plasticity (Matesanz et al. 2010). *Arabidopsis thaliana* exposed to mechanical stimulation grows shorter compared to the same genotype grown without mechanical stimulation. This plasticity to mechanical stimulation can be an adaptive response to wind, rain precipitation and/or attacks by insects (Braam and Davis 1990). Insects have been a good model for studies of behavioral plasticity. In the crickets, *Gryllus pennsylvanicus* and *Allonemobius fasciatus,* higher temperatures increase the chirp rate of courtship song, while decreasing inter-chirp interval and chirp duration. These characters are also affected by circadian rhythm as well as male spatial density, indicating multiple environmental effects affecting this single trait in an integrative manner (Ciceran et al. 1994; Olvido and Mousseau 1995). Plasticity of song production is not confined to invertebrates; Marler and Nelson distinguished between memory-based and action-based song learning in three species of sparrow (*Melospiza georiana*, *Spizella pusilla*, and *Zonotrichia leucophrys*). Memory-based song learning includes a sensory phase in which the bird learns a song by hearing it and memorizing it, and a motor phase in which the bird matches its own song to the tutor model via auditory feedback (Marler and Nelson 1992; Nelson and Marler 1994). However, in the action-based mode, the young male bird can already produce a variety of songs, and it will select one to be crystallized as a mature song and discard all other songs. This selection process is highly influenced by its social interactions and this plasticity of song production is restricted to a certain age window (Marler and Nelson 1992; Nelson and Marler 1994).

Not all plastic responses to given environments are necessarily adaptive. However, if the organism achieves greater fitness in the new environment or maintains fitness in a stressful environment, as a consequence of plasticity, then plasticity is adaptive (Bradshaw 1965; Matesanz et al. 2010). Human-induced environmental change is expanding and leads to loss of habitat, reductions in habitat quality, pollutions, climate changes, and invasion of alien species. Plastic responses may adapt phenotypes to these changes without genetic changes, buffering the strength of selection and preventing loss of genetic variation in the population. Even though an optimum phenotype may not be produced in a novel environment, plasticity may allow organisms to survive and have a chance to adapt under the new conditions during successive generations (Price et al. 2003; Strauss et al. 2006; Hendry et al. 2008).

In the early 1960's, Bradshaw pointed out that phenotypic plasticity, like other traits, is under genetic control and therefore is subject to evolutionary mechanisms such as natural selection or drift (Bradshaw 1965). The butterfly *Bicyclus anynana* is characterized by two seasonal forms, a wet-seasonal form with eye spots on its wings, and a dry-seasonal form which is spotless. They were tested for susceptibility to bird predation under different background colors, and the results support the idea that there is selection against the eye spots in the dry season and that eye spots are favored in the wet season. The antagonistic selection forces may maintain this adaptive phenotypic plasticity (Lyytinen et al. 2004). Research in recent decades has focused on finding the genetic basis of phenotypic plasticity, and trying to prove empirically that phenotypic plasticity is a heritable trait, like other developmental, physiological and behavioral traits.

GENOTYPE BY ENVIRONMENT INTERACTION

Shown in Fig. 1.9e are two genotypes with reaction norms that differ not only in their height, but also in their degree of plasticity. This illustrates genetic variation for plasticity which is the genetic basis of evolution of phenotypic plasticity. Different genotypes that vary in their environmental sensitivity are the hallmark of genotype by environment interaction (GxE) (Falconer and Mackay 1996). There is increasing experimental evidence that GxE is prevalent. A classic demonstration comes from an experiment, in which eight different inbred strains of mice were tested in six behavior assays at three different locations. While test protocols and many environmental variables were rigorously controlled, strains differed markedly in all behaviors and showed significant genotype by environment interaction (Crabbe et al. 1999). Naturally occurring alleles of the *foraging* (*for*) gene of *Drosophila melanogaster*, give rise to the rover and sitter behavioral morphs. As larvae, rovers move more and feed less in the presence of food than sitters, while they show no difference in the absence of food (Osborne et al. 1997). However, studies on adult rovers and sitters exposed to well-fed and food deprived conditions revealed a significantly greater response of rovers to food changes than sitters for a majority of behavioral, metabolic, and gene expression traits (Kent et al. 2009). There is also evidence that GxE plays an important role in human psychiatric disorders. For example, a functional polymorphism in the gene encoding the neurotransmitter metabolizing enzyme *monoamine oxidase A* (*MAOA*) can modulate the impact of traumatic early life events on the propensity for engaging in violence as an adult. Maltreated children with low *MAOA* expression were more likely to develop antisocial problems as adults (Caspi et al. 2002). Later studies also showed that low expression of *MAOA*, together with traumatic early life events, confer a high risk for predisposition to physical aggression for both psychiatric patients and healthy adults (Caspi and Moffitt 2006; Kim-Cohen et al. 2006). These examples indicate that the environment is a factor that cannot be ignored, even for traits that used to be considered to be completely genetically determined.

Genetic heritability is measured as the ratio between genetic variation and phenotypic variance, and is related to the speed with which a trait will respond to selection (Falconer and Mackay 1996). Fig. 1.9e illustrates that the heritability of a trait depends on, among other things, the environment in which it is measured, as given genotypes may show different phenotypic variation under different environmental conditions. As an extreme example, two genotypes have the same phenotype in environment 1; therefore, genetic variation is zero resulting in a heritability of zero. Under conditions in which the two genotypes have different degrees of plasticity, the phenotypic difference will increase under environment 2, resulting in higher genetic variation and heritability larger than zero (Fig. 1.9e). An important consequence of this environment-dependent genetic heritability is that selection in one environment could be ineffective, but could be effective in a different environment. This was demonstrated empirically in *Drosophila melanogaster*. Variation in embryonic development rate is lower at the natural temperature range (17^o-27^o) compared to that at more extreme temperatures (27^o-32^o). Selection for faster development is usually ineffective in *D. melanogaster*. Successful selection for faster development at extreme temperatures proved the above principle (Neyfakh and Hartl 1993).

Since plasticity can be measured reliably and is variable within populations, it can also have heritability (Pigliucci 2001). One simple way of estimating the heritability of phenotypic plasticity is to use parent-offspring regression. Scheiner and Lyman proposed a definition of the heritability of plasticity based on analysis of variance (Scheiner and Lyman 1989). They proposed an equation that is analogous to the general equation for heritability $h_{pl}^2 = \sigma_{GxE}^2/\sigma_P^2$, where σ_{GxE}^2 is the variance of the genotype-environment interaction in an analysis of variance, and σ_P^2 is the total phenotypic variance (Scheiner and Lyman 1989). In addition, they calculated the heritability of phenotypic plasticity of thorax size in response to temperature in *D. melanogaster* and found significant heritability of plasticity (Scheiner and Lyman 1989).

To analyze GxE, a typical ANOVA model can be written as $\sigma_P^2 = \sigma_G^2 + \sigma_E^2 + \sigma_{GxE}^2 + \sigma_{err}^2$; $\sigma_G^2$is the portion of variance that is attributable to differences among genotypes, irrespective of the environment, as shown in the reaction norms, representing the average spread of the height of reaction norms across environments. Similarly, σ_E^2 is the portion of variance that is attributable to differences across environments, irrespective of the genotypes, as shown in the reaction norms, representing the average phenotypic value across genotypes under each environment. σ_{GxE}^2 is the interaction variance, associated with variation due to the fact that some genotypes might respond to the environment in a different way than others, as shown in the reaction norms, giving rise to different slopes of reaction norms. It is worth to note that under certain situations, for example perfect cross-over reaction norms for different genotypes, σ_G^2 and σ_E^2 could yield no variation because of the symmetry of the spread across environments. However, dramatic differences of slopes of reaction norms provide evidence for significant genotype by environment interaction (Pigliucci 2001).

In the early 1990s, Scheiner summarized the three main models that could explain the genetic basis of phenotypic plasticity: overdominance, pleiotropy and epistasis. In contrast to heterozygous effects that reduce and buffer the environmental influence, overdominance may enhance it. Conditional pleiotropy for some genes in certain environments can result in plasticity of affected traits across different environments. Epistasis, which is due to gene-by-gene interactions that can change under different conditions, can also contribute to phenotypic plasticity (Scheiner 1993). In reality, a combination of all three mechanisms is likely to underlie phenotypic plasticity of complex traits.

THE BALANCE BETWEEN PLASTICITY AND ROBUSTNESS

On the one hand, plasticity enables organisms to respond in an adaptive manner to different conditions, on the other hand, variation in developmental processes and excessive phenotypic plasticity come with costs. Inconsistency in development will cause a reduction in fitness and high variation within a population around its most fit phenotypes will reduce population fitness. For practical applications reducing phenotypic plasticity is important for the breeding industry. Inconsistent yields under different weather conditions and growing locations can cause economic damage. It is Waddington who in 1942 developed the concept of canalization, which he described as a lack of plasticity in developmental processes under minor environmental variations, which is a general phenomenon in most organisms (Waddington 1942). However, canalization can be expanded to a variety of traits, including developmental, physiological and behavioral traits, and in addition to environmental perturbations, it can be applied also to genetic perturbations (Gibson and Wagner 2000). In other words, canalization is a process that results in phenotypic robustness, and is opposite to phenotypic variation which comprises genetic variation and environmental plasticity. Organisms would optimally adapt when they reach a balance between plasticity and robustness in their most frequent environmental conditions, and this balancing point would be different under different conditions. It is further noted that decanalization in human populations may be a possible cause of highly increased risk to common diseases. This hypothesis was postulated by Greg Gibson and states that rapid evolution of the human genome combined with dramatic environmental and cultural perturbations in the past two generations might cause decanalization which contributes to the epidemic nature of common genetic diseases. Environmental and cultural changes, including dietary shifts, pollution, psychological stresses, and altered pathogen exposures, shifted the human population away from its optimum, thereby releasing hidden genetic variation. This can result from an increase in epistatic interactions, which will augment phenotypic variation and reduce population fitness (Gibson 2009). The genome contains millions of SNPs; however, only a small fraction is associated with different phenotypic traits. It is possible that most of the genetic variation is suppressed by canalization and will not contribute to variation in phenotype. Fig. 1.10, panel b illustrates how environmental or/and genetic disruptions can change genetic architecture by releasing genetic variation.

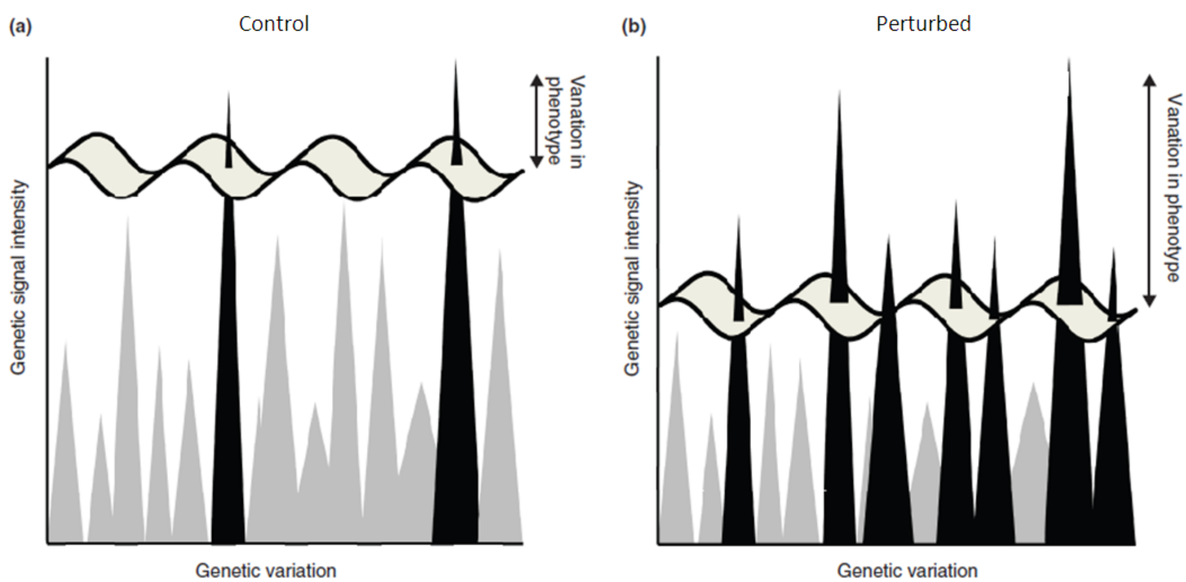

Figure 1.10 Decanalization of genetic variation under genetic or environmental perturbations. a) Genetic variation and phenotypic variation of a population under the control condition; few genes are expressed and phenotypic variation is low. b) Perturbation caused decanalization; more genetic variation contributes to phenotypic variation in the population. Note the means of the phenotypes of the same population under different conditions are very different. Figure adapted from Sato and Siomi, (2010).

Finding the genes that control the balance between canalization and plasticity is crucial in confirming the existence of canalization and understanding the connection between canalization and plasticity. One of the best studied genes associated with canalization is *Hsp83/Hsp90* which encodes the Hsp90 protein. Hsp90 is a chaperone and heat shock protein. It occupies a highly connected node in many genetic circuits and is highly environmentally sensitive. Impaired Hsp90 protein results in a dramatic increase in morphological variation in Drosophila and Arabidopsis under different environmental conditions (Rutherford and Lindquist 1998; Sangster et al. 2008). In Arabidopsis, this increase in phenotypic variation by Hsp90 dysfunction is genotype dependent (different genetic backgrounds have different effects) (Queitsch et al. 2002; Sangster et al. 2008). It is still not completely clear what the mechanisms are that underlie the canalization function of Hsp90. One possible mechanism relates to its chaperone function, in which lack of Hsp90 will reduce stability of other proteins (Salathia and Queitsch 2007). Hsp90 is also involved in the biogenesis of Piwi-interacting RNA (piRNA), which is a class of germ line specific RNA. Hsp90 mutations can generate new phenotypes by affecting piRNA silencing of transposons that could induce morphological mutants (Salathia and Queitsch 2007; Specchia et al. 2010). It is interesting that temperature alone could produce the Hsp90-dependent phenotypes in a strain-specific manner, suggesting a possible genetic basis for environmental perturbations. However, Hsp90 is not the only capacitor maintaining phenotypic robustness, as only certain traits are affected and decanalized by dysfunction of Hsp90, while other traits remain stable. A study of microRNAs suggests that networks of different miRNAs also function as canalization capacitors by preventing expression of leaky transcripts and buffering genetic noise (Hornstein and Shomron 2006).

APPROACHES IN STUDYING PHENOTYPIC PLASTICITY

Studies on phenotypic plasticity involve environmental manipulations that expose organisms to two or more levels of a particular environmental variable, frequently are temperature, food availability, water, light condition and nutrients. In addition, biotic factors, especially social context, like density and interspecific competition are also used though not as common as biotic factors (Pigliucci 2001). Studies of phenotypic plasticity require large numbers of experimental subjects compared to common garden (single environment) studies to the extent that a block design may be necessary to account for inevitable variation due to micro-environmental effects. An approach commonly used in plants to study phenotypic plasticity is transplantation. This approach assumes that a genetically specialized population has evolved that is adapted to a specific local environment, and that transplanting the same genotype to a different location will result in plasticity of different traits as well as a change in fitness (Pigliucci 2001). In contrast to the environmental manipulations, phenotypic manipulation is designed to manipulate genetic component, or physiological conditions to induce phenotypic response which is not appropriate to its rearing condition but mimicking the response towards other environmental conditions. For example, Daphnia can produce a prominent helmet as a defense against predators. It is, however, also possible to induce the same morphological changes without predator by presenting Daphnia with a chemical compound released by the predator. This exposure will trigger regulation of the same developmental pathways (Dodson 1989). Another example of phenotypic manipulation involves two experiments that are performed 50 years apart. Waddington in the mid 50's, showed that decanalization of *Ultrabithorax* (*Ubx*) by selection under ether treatment will increase haltere-to-wing transformation (Waddington 1956). Fifty years later, studies showed that misexpression of a Hox cluster miRNA, *miR-iab-405p* in the haltere disc phenocopies the decanalized Ubx phenotype (Ronshaugen et al. 2005).

If phenotypic plasticity has a genetic basis, like other complex traits, it should respond to selection. Response to artificial selection in turn will validate the genetic component of plasticity. Selection for phenotypic plasticity has been studied in Arabidopsis. *Arabidopsis thaliana* showed a significant response in variation of leaf number at bolting towards different light quality and photoperiod conditions. It has also been suggested that leaf number at bolting, and the light quality and photoperiod-induced plasticity of leaf number are either genetically independent or have a complicated relationship (Callahan and Pigliucci 2005).

One critical question is: What is the molecular mechanism that drives phenotypic plasticity and what are the genes that regulate environmental plasticity of different traits? Quantitative trait loci (QTL) mapping has been used to study the genetic basis of phenotypic plasticity in natural or laboratory populations, with especially important applications for animal and plant breeding. QTL mapping of 13 traits related to inflorescence development patterns in *Arabidopsis thaliana* reared under different photoperiod environments using two recombinant inbred mapping populations revealed that 12 out of 44 and 32 out of 62 QTLs, respectively, exhibited a significant QTL by environment interaction (Ungerer et al. 2003). Similarly, genome-wide mapping of four fitness related traits in a recombinant panel of *Caenorhabditis elegans* reared at different temperatures revealed that 5 QTLs associated with age at maturity, fertility and growth rate showed significant QTL by environment interaction. These QTLs, which harbor genes that contribute to variation of a trait under different environmental conditions are also co-localized with QTLs for trait variation under a single condition. This observation supports the allelic sensitivity model which suggests genes associated with genetic variation and the phenotypic plasticity of a trait are the same (Via et al. 1995; Gutteling et al. 2007). However, more detailed analyses of individual QTL are needed to determine whether the genes regulating trait plasticity and variation in the trait itself are the same. Alternatively, it is possible that specific genes expressed only in certain environments determine the environmentally sensitivity of a trait. These two hypotheses are, however, not mutually exclusive (Via et al. 1995; Mackay and Anholt 2007).

INTEGRATIVE BIOLOGY OF PHENOTYPIC PLASTICITY

Until recently, there was a gap between between studies of phenotypic plasticity from ecological and evolutionary perspectives and studies from a molecular and genetic angle. This gap can be bridged by an integrating different disciplines, so that molecular techniques can further our understanding of gene functions and molecular mechanisms underlying ecologically important traits, and, conversely, ecological studies can inform functional genomics (Aubin-North, 2009). Development of different expression microarrays and next generation sequencing technologies provide the ability to examine the expression of large numbers of genes simultaneously under different environmental conditions and to define “genomic reaction norms”. For example, different populations of *Drosophila mojavensis* larvae grow on different species of cactus. Raising populations on two different cactus hosts showed that 7% of the genome was differentially expressed (Matzkin, 2006). In addition to environmental changes, changes during aging induce different gene expression and behavior. The honeybee (*Apis mellifera*) faces distinct environments and novel tasks at each of its life stages. Studies on variation in gene expression in the brain of the nurse bee and the forager bee showed that ~40% of the genome was differentially expressed suggesting that large changes in regulation of gene expression are required to support the dramatic changes in the bee’s physiology and behavior when they face different environmental challenges (Grozinger et al. 2003; Whitfield et al. 2006). Simple and comprehensive studies on yeast have examined the expression profile changes at different time points along the yeast life cycle, and across many stress conditions and novel environments (Gasch et al. 2000).

Large scale functional genomics studies produce large amounts of data. Data reduction and information integration is crucial to give insights in expression patterns that may underlie molecular interactions that could lead to phenotypic plasticity. We can gain insights in the function of a gene by analyzing other genes that are co-expressed and coregulated. A new clustering method, modulated modularity clustering, provides an unbiased method to analyze cross-genotype or cross-environment expression correlations between transcripts to construct modules that group correlated transcripts and order them based on the level of correlation (Stone and Ayroles 2009). This clustering tool is especially useful for GxE studies, as it allows visualization of co-regulated changes in genetic networks under different environmental conditions. Gene ontology analysis of clustered genes provides further insights in the molecular functions of environmentally plastic genes (Yon Rhee et al. 2008).

Most studies on the molecular basis of plastic traits focus on transcript levels, as it is the easiest and most direct measurement for assessing variation in gene expression. However, transcript abundance is only a proxy for the quantification of protein abundance, as posttranscriptional and posttranslational modulations are prevalent. Whereas direct determination of variation in protein abundance under environmental challenges is desirable, proteomic studies for assessing plasticity are not common due to the high sensitivity to sample quality, the high variation of quantification between samples and the high cost of the methodology. Still several studies have provided insights in phenotypic plasticity at the protein level. During parasite host co-evolution parasites evolve to optimize their transmission while hosts evolve to minimize parasite-induced fitness loss. The malaria parasite *Plasmodium falciparum* manipulates the behavior of its mosquito vector *Anopheles gambiae* by increasing the biting rate on vertebrate hosts when they are ready to be transmitted, and decreasing contact between vector and vertebrate host when they are at the oocyst stage and not transmissible to reduce the risk of death of the vector (Rossignol et al. 1984; Anderson and Roitberg 1999). Studies on the head proteomes of malarial parasite infected and control mosquitoes revealed altered levels of 12 proteins belonging to the energy metabolism category as well as a heat shock protein, suggesting that the parasites alter vector behavior through altering their energy regulation (Lefevre et al. 2007).

Studies on both long and short ncRNAs (snoRNA, miRNA, siRNA, piRNA), revealed their regulatory functions (Lu et al. 2008; De Lucia and Dean 2010). Such regulatory ncRNAs open up a new dimension in the study of phenotypic plasticity, made possible through advanced technologies. One of the mechanisms through which these regulatory ncRNAs may act may involve regulation of epigenetic components, for example, regulating DNA methylation (De Lucia and Dean 2010; Meaney and Ferguson-Smith 2010). DNA methylation of upstream elements of a coding sequence can reduce gene expression by physically interrupting the binding of transcription factors or recruiting histone modifiers to change histone conformation

(Meaney and Ferguson-Smith 2010). Methylation is responsive to environmental variations and could have sustained effects on gene expression (Pomraning et al. 2009). High and low maternal care off rat pups results in different responses to the stress as adults. Methylation patterns are unmodified in rats from low-care mothers and these results in a long-term lowering of expression of the glucocorticoid receptor in the hippocampus, which in turn results in a highly reactive stress response (Weaver et al. 2004; Meaney and Szyf 2005). Another example of the involvement of epigenetic modification in phenotypic plasticity is the effect of juvenile hormone on the development of a honeybee larva into a queen (Hartfelder and Engels 1998). When methylation of specific genes was induced honeybees developed into workers, but when methylation was reduced through RNAi, a majority of larvae became queen-like (Kucharski et al. 2008).

Thus, different approaches and integration of different concepts will be necessary to shed light on the molecular mechanisms underlying ecologically important adaptive plasticity, and to describe the complex relationships that connect environmental cues, genes, the brain and plastic behavior in physiological, developmental and evolutionary contexts.

CONTRIBUTIONS OF THIS DISSERTATION

Although phenotypic plasticity has been extensively documented, its detailed genetic underpinnings is still poorly understood. In Chapter 2 of this dissertation I document phenotypic plasticity of the chemosensory receptor repertoire in a standard laboratory strain. This study was motivated by the notion that chemoreception is for many organisms the principal sensory modality that conveys information about the environment. In the study described in Chapter 3, I present the most comprehensive genome-wide study performed to date on phenotypic plasticity of the Drosophila transcriptome. I performed a systematic search for environmentally sensitive transcripts across 20 environments and treatments. Those 20 rearing conditions included temperature, diet, chemical exposures, stress conditions, as well as normal physiological changes, like aging and mating status. Instead of using the laboratory Canton-S(B) strain used in Chapter 2, I chose to use an outbred population re-constructed from 40 inbred wild derived lines that are fully sequenced and have records of whole-genome transcriptome profiles under a standard growth condition. This enabled me to compare genetic variation across these lines as well as environmental variation across conditions for the same transcripts to provide insights in the nature of environmentally sensitive transcripts. In this study, I addressed several fundamental questions that we are only able to answer with the specific experimental design described in this dissertation. I asked what the proportion of the genome comprises environmentally sensitive transcripts; is most of the genome robust under environmental perturbations or are many or all genes environmentally sensitive? Do the same transcripts vary under all different conditions, or are they sensitive to specific treatments? What are the environmentaly sensitive transcripts? What are their biological functions? Are transcripts associated with plasticity of different traits the same as those associated with these traits under standard growth conditions? And, importantly, what are the genetic properties of these transcripts; are they different from canalized transcripts? This dissertation presents - if not complete - at least partial answers to these critical questions.

REFERENCES

Abuin, L., B. Bargeton, et al. (2011). "Functional architecture of olfactory ionotropic glutamate receptors." *Neuron* **69**(1): 44-60.

Adams, M. D., S. E. Celniker, et al. (2000). "The genome sequence of Drosophila melanogaster." *Science* **287**(5461): 2185-2195.

Ai, M., S. Min, et al. (2010). "Acid sensing by the Drosophila olfactory system." *Nature* **468**(7324): 691-695.

Amrein, H. and N. Thorne (2005). "Gustatory perception and behavior in Drosophila melanogaster." *Curr Biol* **15**(17): R673-684.

Anderson, R. A. and B. D. Roitberg (1999). "Modelling trade-offs between mortality and fitness associated with persistent blood feeding by mosquitoes." *Ecology Letters* **2**(2): 98-105.

Ang, L. H., J. Kim, et al. (2003). "Dock and Pak regulate olfactory axon pathfinding in Drosophila." *Development* **130**(7): 1307-1316.

Anholt, R. R. H., R. F. Lyman, et al. (1996). "Effects of single P-element insertions on olfactory behavior in Drosophila melanogaster." *Genetics* **143**(1): 293-301.

Anholt, R. R. H. and T. F. C. Mackay (2004). "Quantitative genetic analyses of complex behaviours in Drosophila." *Nat Rev Genet* **5**(11): 838-849.

Ashmore, L. J. and A. Sehgal (2003). "A fly's eye view of circadian entrainment." *J Biol Rhythms* **18**(3): 206-216.

Ayroles, J. F., M. A. Carbone, et al. (2009). "Systems genetics of complex traits in Drosophila melanogaster." *Nat Genet* **41**(3): 299-307.

Benton, R., S. Sachse, et al. (2006). "Atypical membrane topology and heteromeric function of Drosophila odorant receptors in vivo." *PLoS Biol* **4**(2): e20.

Benton, R., K. S. Vannice, et al. (2009). "Variant ionotropic glutamate receptors as chemosensory receptors in Drosophila." *Cell* **136**(1): 149-162.

Bianchet, M. A., G. Bains, et al. (1996). "The three-dimensional structure of bovine odorant binding protein and its mechanism of odor recognition." *Nat Struct Biol* **3**(11): 934-939.

Braam, J. and R. W. Davis (1990). "Rain-, wind-, and touch-induced expression of calmodulin and calmodulin-related genes in Arabidopsis." *Cell* **60**(3): 357-364.

Bradshaw, A. D. (1965). "Evolutionary Significance of Phenotypic Plasticity in Plants." *Advances in Genetics* **13**: 115-155.

Bray, S. and H. Amrein (2003). "A putative Drosophila pheromone receptor expressed in male-specific taste neurons is required for efficient courtship." *Neuron* **39**(6): 1019-1029.

Buck, L. and R. Axel (1991). "A novel multigene family may encode odorant receptors: a molecular basis for odor recognition." *Cell* **65**(1): 175-187.

Callahan, H. S. and M. Pigliucci (2005). "Indirect consequences of artificial selection on plasticity to light quality in Arabidopsis thaliana." *Journal of Evolutionary Biology* **18**(6): 1403-1415.

Caspi, A., J. McClay, et al. (2002). "Role of genotype in the cycle of violence in maltreated children." *Science* **297**(5582): 851-854.

Caspi, A. and T. E. Moffitt (2006). "Gene-environment interactions in psychiatry: joining forces with neuroscience." *Nat Rev Neurosci* **7**(7): 583-590.

Chesler, A. and S. Firestein (2008). "Neuroscience: current views on odour receptors." *Nature* **452**(7190): 944.

Chintapalli, V. R., J. Wang, et al. (2007). "Using FlyAtlas to identify better Drosophila melanogaster models of human disease." *Nat Genet* **39**(6): 715-720.

Chyb, S., A. Dahanukar, et al. (2003). "Drosophila Gr5a encodes a taste receptor tuned to trehalose." *Proc Natl Acad Sci U S A* **100 Suppl 2**: 14526-14530.

Ciceran, M., A.-M. Murray, et al. (1994). "Natural variation in the temporal patterning of calling song structure in the field cricket Gryllus pennsylvanicus: effects of temperature, age, mass, atime of day, and nearest neighbour." *Canadian Journal of Zoology* **72**: 38-42.

Clyne, P., A. Grant, et al. (1997). "Odorant response of individual sensilla on the Drosophila antenna." *Invert Neurosci* **3**(2-3): 127-135.

Clyne, P. J., S. J. Certel, et al. (1999). "The odor specificities of a subset of olfactory receptor neurons are governed by Acj6, a POU-domain transcription factor." *Neuron* **22**(2): 339-347.

Clyne, P. J., C. G. Warr, et al. (2000). "Candidate taste receptors in Drosophila." *Science* **287**(5459): 1830-1834.

Clyne, P. J., C. G. Warr, et al. (1999). "A novel family of divergent seven-transmembrane proteins: candidate odorant receptors in Drosophila." *Neuron* **22**(2): 327-338.

Colomb, J., N. Grillenzoni, et al. (2007). "Architecture of the primary taste center of Drosophila melanogaster larvae." *J Comp Neurol* **502**(5): 834-847.

Couto, A., M. Alenius, et al. (2005). "Molecular, anatomical, and functional organization of the Drosophila olfactory system." *Curr Biol* **15**(17): 1535-1547.

Crabbe, J. C., D. Wahlsten, et al. (1999). "Genetics of mouse behavior: interactions with laboratory environment." *Science* **284**(5420): 1670-1672.

Croset, V., R. Rytz, et al. (2010). "Ancient protostome origin of chemosensory ionotropic glutamate receptors and the evolution of insect taste and olfaction." *PLoS Genet* **6**(8): e1001064.

Dahanukar, A., K. Foster, et al. (2001). "A Gr receptor is required for response to the sugar trehalose in taste neurons of Drosophila." *Nat Neurosci* **4**(12): 1182-1186.

Dahanukar, A., Y. T. Lei, et al. (2007). "Two Gr genes underlie sugar reception in Drosophila." *Neuron* **56**(3): 503-516.

de Bruyne, M., P. J. Clyne, et al. (1999). "Odor coding in a model olfactory organ: the Drosophila maxillary palp." *J Neurosci* **19**(11): 4520-4532.

de Bruyne, M., K. Foster, et al. (2001). "Odor coding in the Drosophila antenna." *Neuron* **30**(2): 537-552.

De Lucia, F. and C. Dean (2010). "Long non-coding RNAs and chromatin regulation." *Curr Opin Plant Biol*.

Dekker, T., I. Ibba, et al. (2006). "Olfactory shifts parallel superspecialism for toxic fruit in Drosophila melanogaster sibling, D. sechellia." *Curr Biol* **16**(1): 101-109.

Devaud, J. M. (2003). "Experimental studies of adult Drosophila chemosensory behaviour." *Behav Processes* **64**(2): 177-196.

Dobritsa, A. A., W. van der Goes van Naters, et al. (2003). "Integrating the molecular and cellular basis of odor coding in the Drosophila antenna." *Neuron* **37**(5): 827-841.

Dodson, S. (1989). "Predator-induced Reaction Norm." *BioScience* **39**(7): 6.

Dunipace, L., S. Meister, et al. (2001). "Spatially restricted expression of candidate taste receptors in the Drosophila gustatory system." *Curr Biol* **11**(11): 822-835.

Elmore, T., R. Ignell, et al. (2003). "Targeted mutation of a Drosophila odor receptor defines receptor requirement in a novel class of sensillum." *J Neurosci* **23**(30): 9906-9912.

Elmore, T. and D. P. Smith (2001). "Putative Drosophila odor receptor OR43b localizes to dendrites of olfactory neurons." *Insect Biochem Mol Biol* **31**(8): 791-798.

Falconer, D. S. and T. F. C. Mackay (1996). *Introduction to quantitative genetics*. Harlow, Addison Wesley Longman, Harlow, Essex, UK.

Fedorowicz, G. M., J. D. Fry, et al. (1998). "Epistatic interactions between smell-impaired loci in Drosophila melanogaster." *Genetics* **148**(4): 1885-1891.

Fischler, W., P. Kong, et al. (2007). "The detection of carbonation by the Drosophila gustatory system." *Nature* **448**(7157): 1054-1057.

Fishilevich, E. and L. B. Vosshall (2005). "Genetic and functional subdivision of the Drosophila antennal lobe." *Curr Biol* **15**(17): 1548-1553.

Galindo, K. and D. P. Smith (2001). "A large family of divergent Drosophila odorant-binding proteins expressed in gustatory and olfactory sensilla." *Genetics* **159**(3): 1059-1072.

Ganguly, I., T. F. Mackay, et al. (2003). "Scribble is essential for olfactory behavior in Drosophila melanogaster." *Genetics* **164**(4): 1447-1457.

Gao, Q. and A. Chess (1999). "Identification of candidate Drosophila olfactory receptors from genomic DNA sequence." *Genomics* **60**(1): 31-39.

Gasch, A. P., P. T. Spellman, et al. (2000). "Genomic expression programs in the response of yeast cells to environmental changes." *Mol Biol Cell* **11**(12): 4241-4257.

Gendre, N., K. Luer, et al. (2004). "Integration of complex larval chemosensory organs into the adult nervous system of Drosophila." *Development* **131**(1): 83-92.

Gibson, G. (2009). "Decanalization and the origin of complex disease." *Nat Rev Genet* **10**(2): 134-140.

Gibson, G. and G. Wagner (2000). "Canalization in evolutionary genetics: a stabilizing theory?" *Bioessays* **22**(4): 372-380.

Gordon, M. D. and K. Scott (2009). "Motor control in a Drosophila taste circuit." *Neuron* **61**(3): 373-384.

Grozinger, C. M., N. M. Sharabash, et al. (2003). "Pheromone-mediated gene expression in the honey bee brain." *Proc Natl Acad Sci U S A* **100 Suppl 2**: 14519-14525.

Gutteling, E. W., J. A. Riksen, et al. (2007). "Mapping phenotypic plasticity and genotype-environment interactions affecting life-history traits in Caenorhabditis elegans." *Heredity* **98**(1): 28-37.

Ha, T. S. and D. P. Smith (2006). "A pheromone receptor mediates 11-cis-vaccenyl acetate-induced responses in Drosophila." *J Neurosci* **26**(34): 8727-8733.

Hallem, E. A. and J. R. Carlson (2006). "Coding of odors by a receptor repertoire." *Cell* **125**(1): 143-160.

Hallem, E. A., A. Dahanukar, et al. (2006). "Insect odor and taste receptors." *Annu Rev Entomol* **51**: 113-135.

Hallem, E. A., M. G. Ho, et al. (2004). "The molecular basis of odor coding in the Drosophila antenna." *Cell* **117**(7): 965-979.

Hartfelder, K. and W. Engels (1998). "Social insect polymorphism: hormonal regulation of plasticity in development and reproduction in the honeybee." *Curr Top Dev Biol* **40**: 45-77.

Heimbeck, G., V. Bugnon, et al. (1999). "Smell and taste perception in Drosophila melanogaster larva: toxin expression studies in chemosensory neurons." *J Neurosci* **19**(15): 6599-6609.

Hekmat-Scafe, D. S., C. R. Scafe, et al. (2002). "Genome-wide analysis of the odorant-binding protein gene family in Drosophila melanogaster." *Genome Res* **12**(9): 1357-1369.

Helfand, S. L. and J. R. Carlson (1989). "Isolation and characterization of an olfactory mutant in Drosophila with a chemically specific defect." *Proc Natl Acad Sci U S A* **86**(8): 2908-2912.

Hendry, A. P., T. J. Farrugia, et al. (2008). "Human influences on rates of phenotypic change in wild animal populations." *Mol Ecol* **17**(1): 20-29.

Hill, C. A., A. N. Fox, et al. (2002). "G protein-coupled receptors in Anopheles gambiae." *Science* **298**(5591): 176-178.

Hiroi, M., N. Meunier, et al. (2004). "Two antagonistic gustatory receptor neurons responding to sweet-salty and bitter taste in Drosophila." *J Neurobiol* **61**(3): 333-342.

Hornstein, E. and N. Shomron (2006). "Canalization of development by microRNAs." *Nat Genet* **38 Suppl**: S20-24.

Jiao, Y., S. J. Moon, et al. (2008). "Gr64f is required in combination with other gustatory receptors for sugar detection in Drosophila." *Curr Biol* **18**(22): 1797-1801.

Jin, X., T. S. Ha, et al. (2008). "SNMP is a signaling component required for pheromone sensitivity in Drosophila." *Proc Natl Acad Sci U S A* **105**(31): 10996-11001.

Jones, W. D., P. Cayirlioglu, et al. (2007). "Two chemosensory receptors together mediate carbon dioxide detection in Drosophila." *Nature* **445**(7123): 86-90.

Kent, C. F., T. Daskalchuk, et al. (2009). "The Drosophila foraging gene mediates adult plasticity and gene-environment interactions in behaviour, metabolites, and gene expression in response to food deprivation." *PLoS Genet* **5**(8): e1000609.

Kim-Cohen, J., A. Caspi, et al. (2006). "MAOA, maltreatment, and gene-environment interaction predicting children's mental health: new evidence and a meta-analysis." *Mol Psychiatry* **11**(10): 903-913.

Kim, M. S., A. Repp, et al. (1998). "LUSH odorant-binding protein mediates chemosensory responses to alcohols in Drosophila melanogaster." *Genetics* **150**(2): 711-721.

Komiyama, T., W. A. Johnson, et al. (2003). "From lineage to wiring specificity. POU domain transcription factors control precise connections of Drosophila olfactory projection neurons." *Cell* **112**(2): 157-167.

Kondoh, Y., K. Y. Kaneshiro, et al. (2003). "Evolution of sexual dimorphism in the olfactory brain of Hawaiian Drosophila." *Proc Biol Sci* **270**(1519): 1005-1013.

Krieger, J., K. Raming, et al. (2002). "A divergent gene family encoding candidate olfactory receptors of the moth Heliothis virescens." *Eur J Neurosci* **16**(4): 619-628.

Krishnan, B., S. E. Dryer, et al. (1999). "Circadian rhythms in olfactory responses of Drosophila melanogaster." *Nature* **400**(6742): 375-378.

Kucharski, R., J. Maleszka, et al. (2008). "Nutritional control of reproductive status in honeybees via DNA methylation." *Science* **319**(5871): 1827-1830.

Kulkarni, N. H., A. H. Yamamoto, et al. (2002). "The DSC1 channel, encoded by the smi60E locus, contributes to odor-guided behavior in Drosophila melanogaster." *Genetics* **161**(4): 1507-1516.

Kurtovic, A., A. Widmer, et al. (2007). "A single class of olfactory neurons mediates behavioural responses to a Drosophila sex pheromone." *Nature* **446**(7135): 542-546.

Laissue, P. P., C. Reiter, et al. (1999). "Three-dimensional reconstruction of the antennal lobe in Drosophila melanogaster." *J Comp Neurol* **405**(4): 543-552.

Larsson, M. C., A. I. Domingos, et al. (2004). "Or83b encodes a broadly expressed odorant receptor essential for Drosophila olfaction." *Neuron* **43**(5): 703-714.

Laurent, S., C. Masson, et al. (2002). "Whole-cell recording from honeybee olfactory receptor neurons: ionic currents, membrane excitability and odourant response in developing workerbee and drone." *Eur J Neurosci* **15**(7): 1139-1152.

Lee, Y., S. J. Moon, et al. (2009). "Multiple gustatory receptors required for the caffeine response in Drosophila." *Proc Natl Acad Sci U S A* **106**(11): 4495-4500.

Lefevre, T., F. Thomas, et al. (2007). "Malaria Plasmodium agent induces alteration in the head proteome of their Anopheles mosquito host." *Proteomics* **7**(11): 1908-1915.

Li, Y., F. Wang, et al. (2006). "MicroRNA-9a ensures the precise specification of sensory organ precursors in Drosophila." *Genes Dev* **20**(20): 2793-2805.

Louis, M., T. Huber, et al. (2008). "Bilateral olfactory sensory input enhances chemotaxis behavior." *Nat Neurosci* **11**(2): 187-199.

Lu, Y. D., Q. H. Gan, et al. (2008). "Roles of microRNA in plant defense and virus offense interaction." *Plant Cell Rep* **27**(10): 1571-1579.

Lyytinen, A., P. M. Brakefield, et al. (2004). "Does predation maintain eyespot plasticity in Bicyclus anynana?" *Proc Biol Sci* **271**(1536): 279-283.

Mackay, T. F. C. and R. R. H. Anholt (2007). "Ain't misbehavin' ? Genotype-environment interactions and the genetics of behavior." *Trends Genet* **23**(7): 311-314.

Malnic, B., J. Hirono, et al. (1999). "Combinatorial receptor codes for odors." *Cell* **96**(5): 713-723.

Manoli, D. S., M. Foss, et al. (2005). "Male-specific fruitless specifies the neural substrates of Drosophila courtship behaviour." *Nature* **436**(7049): 395-400.

Marella, S., W. Fischler, et al. (2006). "Imaging taste responses in the fly brain reveals a functional map of taste category and behavior." *Neuron* **49**(2): 285-295.

Marin, E. C., G. S. Jefferis, et al. (2002). "Representation of the glomerular olfactory map in the Drosophila brain." *Cell* **109**(2): 243-255.

Marler, P. and D. A. Nelson (1992). "Action-Based Learning: a New Form of Developmental Plasticity in Bird Song." *Netherlands Journal of Zoology* **43**: 91-103.

Matesanz, S., E. Gianoli, et al. (2010). "Global change and the evolution of phenotypic plasticity in plants." *Ann N Y Acad Sci* **1206**: 35-55.

Matsuo, T. (2008). "Rapid evolution of two odorant-binding protein genes, Obp57d and Obp57e, in the Drosophila melanogaster species group." *Genetics* **178**(2): 1061-1072.

Matsuo, T., S. Sugaya, et al. (2007). "Odorant-binding proteins OBP57d and OBP57e affect taste perception and host-plant preference in Drosophila sechellia." *PLoS Biol* **5**(5): e118.

McKenna, M., P. Monte, et al. (1989). "A simple chemosensory response in Drosophila and the isolation of acj mutants in which it is affected." *Proc Natl Acad Sci U S A* **86**(20): 8118-8122.

Meaney, M. J. and A. C. Ferguson-Smith (2010). "Epigenetic regulation of the neural transcriptome: the meaning of the marks." *Nat Neurosci* **13**(11): 1313-1318.

Meaney, M. J. and M. Szyf (2005). "Maternal care as a model for experience-dependent chromatin plasticity?" *Trends Neurosci* **28**(9): 456-463.

Meijerink, J., M. A. Braks, et al. (2001). "Olfactory receptors on the antennae of the malaria mosquito Anopheles gambiae are sensitive to ammonia and other sweat-borne components." *J Insect Physiol* **47**(4-5): 455-464.

Meijerink, J. and J. J. van Loon (1999). "Sensitivities of antennal olfactory neurons of the malaria mosquito, Anopheles gambiae, to carboxylic acids." *J Insect Physiol* **45**(4): 365-373.

Miyamoto, T. and H. Amrein (2008). "Suppression of male courtship by a Drosophila pheromone receptor." *Nat Neurosci* **11**(8): 874-876.

Mombaerts, P., F. Wang, et al. (1996). "Visualizing an olfactory sensory map." *Cell* **87**(4): 675-686.

Nelson, D. A. and P. Marler (1994). "Selection-based learning in bird song development." *Proc Natl Acad Sci U S A* **91**(22): 10498-10501.

Neyfakh, A. A. and D. L. Hartl (1993). "Genetic Control of the Rate of Embryonic Development: Selection for Faster Development at Elevated Temperatures." *Evolution* **47**(5): 1625-1631.

Olvido, A. E. and T. A. Mousseau (1995). "Effect of rearing environent on calling-song plasticity in the striped ground cricket. ." *Evolution* **49**: 1271-1277.

Osborne, K. A., A. Robichon, et al. (1997). "Natural behavior polymorphism due to a cGMP-dependent protein kinase of Drosophila." *Science* **277**(5327): 834-836.

Pigliucci, M. (2001). *Phenotypic Plasticity: Beyond Nature and Nurture*. Baltimore, The Johns Hopkins University Press, Baltimore, Maryland, US.

Pomraning, K. R., K. M. Smith, et al. (2009). "Genome-wide high throughput analysis of DNA methylation in eukaryotes." *Methods* **47**(3): 142-150.

Price, T. D., A. Qvarnstrom, et al. (2003). "The role of phenotypic plasticity in driving genetic evolution." *Proc Biol Sci* **270**(1523): 1433-1440.

Queitsch, C., T. A. Sangster, et al. (2002). "Hsp90 as a capacitor of phenotypic variation." *Nature* **417**(6889): 618-624.

Ray, A., W. van der Goes van Naters, et al. (2008). "A regulatory code for neuron-specific odor receptor expression." *PLoS Biol* **6**(5): e125.

Robertson, H. M., C. G. Warr, et al. (2003). "Molecular evolution of the insect chemoreceptor gene superfamily in Drosophila melanogaster." *Proc Natl Acad Sci U S A* **100 Suppl 2**: 14537-14542.

Rogers, M. E., M. Sun, et al. (1997). "Snmp-1, a novel membrane protein of olfactory neurons of the silk moth Antheraea polyphemus with homology to the CD36 family of membrane proteins." *J Biol Chem* **272**(23): 14792-14799.

Rollmann, S. M., T. F. C. Mackay, et al. (2005). "Pinocchio, a novel protein expressed in the antenna, contributes to olfactory behavior in Drosophila melanogaster." *J Neurobiol* **63**(2): 146-158.

Ronshaugen, M., F. Biemar, et al. (2005). "The Drosophila microRNA iab-4 causes a dominant homeotic transformation of halteres to wings." *Genes Dev* **19**(24): 2947-2952.

Rossignol, P. A., J. M. Ribeiro, et al. (1984). "Increased intradermal probing time in sporozoite-infected mosquitoes." *Am J Trop Med Hyg* **33**(1): 17-20.

Rutherford, S. L. and S. Lindquist (1998). "Hsp90 as a capacitor for morphological evolution." *Nature* **396**(6709): 336-342.

Salathia, N. and C. Queitsch (2007). "Molecular mechanisms of canalization: Hsp90 and beyond." *J Biosci* **32**(3): 457-463.

Sambandan, D., M. A. Carbone, et al. (2008). "Phenotypic plasticity and genotype by environment interaction for olfactory behavior in Drosophila melanogaster." *Genetics* **179**(2): 1079-1088.

Sambandan, D., A. Yamamoto, et al. (2006). "Dynamic genetic interactions determine odor-guided behavior in Drosophila melanogaster." *Genetics* **174**(3): 1349-1363.

Sangster, T. A., N. Salathia, et al. (2008). "HSP90-buffered genetic variation is common in *Arabidopsis thaliana*." *Proc Natl Acad Sci U S A* **105**(8): 2969-2974.

Sangster, T. A., N. Salathia, et al. (2008). "HSP90 affects the expression of genetic variation and developmental stability in quantitative traits." *Proc Natl Acad Sci U S A* **105**(8): 2963-2968.

Sato, T., T. Fujikado, et al. (2008). "Direct effect of electrical stimulation on induction of brain-derived neurotrophic factor from cultured retinal Muller cells." *Invest Ophthalmol Vis Sci* **49**(10): 4641-4646.

Scheiner, M. S. and R. F. Lyman (1989). "The genetics of phenotypic plasticity I. Heritability." *J. evol. Biol.* **2**: 95-107.

Scheiner, S. M. (1993). "Genetics and Evolution of Phenotypic Plasticity." *Annual Review of Ecology and Systematics* **24**: 35-68.

Scott, K., R. Brady, Jr., et al. (2001). "A chemosensory gene family encoding candidate gustatory and olfactory receptors in Drosophila." *Cell* **104**(5): 661-673.

Semmelhack, J. L. and J. W. Wang (2009). "Select Drosophila glomeruli mediate innate olfactory attraction and aversion." *Nature* **459**(7244): 218-223.

Shang, Y., A. Claridge-Chang, et al. (2007). "Excitatory local circuits and their implications for olfactory processing in the fly antennal lobe." *Cell* **128**(3): 601-612.

Shields, V. D. and J. G. Hildebrand (2000). "Responses of a population of antennal olfactory receptor cells in the female moth Manduca sexta to plant-associated volatile organic compounds." *J Comp Physiol [A]* **186**(12): 1135-1151.

Smith, D. P. (2007). "Odor and pheromone detection in Drosophila melanogaster." *Pflugers Arch* **454**(5): 749-758.

Specchia, V., L. Piacentini, et al. (2010). "Hsp90 prevents phenotypic variation by suppressing the mutagenic activity of transposons." *Nature* **463**(7281): 662-665.

Stocker, R. F. (1994). "The organization of the chemosensory system in Drosophila melanogaster: a review." *Cell Tissue Res* **275**(1): 3-26.

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**(5): 284-296.

Stone, E. A. and J. F. Ayroles (2009). "Modulated modularity clustering as an exploratory tool for functional genomic inference." *PLoS Genet* **5**(5): e1000479.

Stortkuhl, K. F. and R. Kettler (2001). "Functional analysis of an olfactory receptor in Drosophila melanogaster." *Proc Natl Acad Sci U S A* **98**(16): 9381-9385.

Strauss, S. Y., J. A. Lau, et al. (2006). "Evolutionary responses of natives to introduced species: what do introductions tell us about natural communities?" *Ecol Lett* **9**(3): 357-374.

Suh, G. S., A. M. Wong, et al. (2004). "A single population of olfactory sensory neurons mediates an innate avoidance behaviour in Drosophila." *Nature* **431**(7010): 854-859.

Thorne, N., C. Chromey, et al. (2004). "Taste perception and coding in Drosophila." *Curr Biol* **14**(12): 1065-1079.

Troemel, E. R. (1999). "Chemosensory signaling in C. elegans." *Bioessays* **21**(12): 1011-1020.

Troemel, E. R., J. H. Chou, et al. (1995). "Divergent seven transmembrane receptors are candidate chemosensory receptors in C. elegans." *Cell* **83**(2): 207-218.

Ungerer, M. C., S. S. Halldorsdottir, et al. (2003). "Genotype-environment interactions at quantitative trait loci affecting inflorescence development in Arabidopsis thaliana." *Genetics* **165**(1): 353-365.

Vaysse, G. and J. Medioni (1973). "[First experiments on tarsal gustation in Drosophila melanogaster: stimulation with sucrose]." *C R Seances Soc Biol Fil* **167**(3): 560-564.

Via, S., R. Gomulkiewicz, et al. (1995). "Adaptive phenotypic plasticity: consensus and controversy." *Trends Ecol Evol* **10**(5): 212-217.

Vogt, R. G., G. D. Prestwich, et al. (1991). "Odorant-binding-protein subfamilies associate with distinct classes of olfactory receptor neurons in insects." *J Neurobiol* **22**(1): 74-84.

Vosshall, L. B., H. Amrein, et al. (1999). "A spatial map of olfactory receptor expression in the Drosophila antenna." *Cell* **96**(5): 725-736.

Vosshall, L. B. and M. C. Stensmyr (2005). "Wake up and smell the pheromones." *Neuron* **45**(2): 179-181.

Vosshall, L. B. and R. F. Stocker (2007). "Molecular architecture of smell and taste in Drosophila." *Annu Rev Neurosci* **30**: 505-533.

Vosshall, L. B., A. M. Wong, et al. (2000). "An olfactory sensory map in the fly brain." *Cell* **102**(2): 147-159.

Waddington, C. H. (1942). "Canalization of development and the inheritance of acquired characters." *Nature* **150**(3811): 563-565.

Waddington, C. H. (1956). "Genetic Assimilation of the Bithorax Phenotype." *Evolution* **10**(1): 1-13.

Waddington, C. H. (1959). "Canalization of development and genetic assimilation of acquired characters." *Nature* **183**(4676): 1654-1655.

Wang, F., A. Nemes, et al. (1998). "Odorant receptors govern the formation of a precise topographic map." *Cell* **93**(1): 47-60.

Wang, L. and D. J. Anderson (2010). "Identification of an aggression-promoting pheromone and its receptor neurons in Drosophila." *Nature* **463**(7278): 227-231.

Wang, Z., A. Singhvi, et al. (2004). "Taste representations in the Drosophila brain." *Cell* **117**(7): 981-991.

Weaver, I. C., N. Cervoni, et al. (2004). "Epigenetic programming by maternal behavior." *Nat Neurosci* **7**(8): 847-854.

Weiss, L. A., A. Dahanukar, et al. (2011). "The molecular and cellular basis of bitter taste in Drosophila." *Neuron* **69**(2): 258-272.

Wetzel, C. H., H. J. Behrendt, et al. (2001). "Functional expression and characterization of a Drosophila odorant receptor in a heterologous cell system." *Proc Natl Acad Sci U S A* **98**(16): 9377-9380.

Whitfield, C. W., Y. Ben-Shahar, et al. (2006). "Genomic dissection of behavioral maturation in the honey bee." *Proc Natl Acad Sci U S A* **103**(44): 16068-16075.

Wicher, D., R. Schafer, et al. (2008). "Drosophila odorant receptors are both ligand-gated and cyclic-nucleotide-activated cation channels." *Nature* **452**(7190): 1007-1011.

Wilson, R. I. and G. Laurent (2005). "Role of GABAergic inhibition in shaping odor-evoked spatiotemporal patterns in the Drosophila antennal lobe." *J Neurosci* **25**(40): 9069-9079.

Wong, A. M., J. W. Wang, et al. (2002). "Spatial representation of the glomerular map in the Drosophila protocerebrum." *Cell* **109**(2): 229-241.

Woodard, C., T. Huang, et al. (1989). "Genetic analysis of olfactory behavior in Drosophila: a new screen yields the ota mutants." *Genetics* **123**(2): 315-326.

Xu, P., R. Atkinson, et al. (2005). "Drosophila OBP LUSH is required for activity of pheromone-sensitive neurons." *Neuron* **45**(2): 193-200.

Yao, C. A., R. Ignell, et al. (2005). "Chemosensory coding by neurons in the coeloconic sensilla of the Drosophila antenna." *J Neurosci* **25**(37): 8359-8367.

Yon Rhee, S., V. Wood, et al. (2008). "Use and misuse of the gene ontology annotations." *Nat Rev Genet* **9**(7): 509-515.

Zhou, S., E. A. Stone, et al. (2009). "Plasticity of the chemoreceptor repertoire in Drosophila melanogaster." *PLoS Genet* **5**(10): e1000681.

Zhu, H. and L. Luo (2004). "Diverse functions of N-cadherin in dendritic and axonal terminal arborization of olfactory projection neurons." *Neuron* **42**(1): 63-75.

CHAPTER TWO

PLASTICITY OF THE CHEMORECEPTOR REPERTOIRE IN *DROSOPHILA MELANOGASTER*

Plasticity of the Chemoreceptor Repertoire in *Drosophila melanogaster*

Shanshan Zhou [1,2], Eric A. Stone [2,3,4], Trudy F. C. Mackay [2,4] and Robert R. H. Anholt [1,2,4*]

1 Department of Biology, North Carolina State University, Raleigh, North Carolina, United States of America, **2** W. M. Keck Center for Behavioral Biology, North Carolina State University, Raleigh, North Carolina, United States of America, **3** Department of Statistics, North Carolina State University, Raleigh, North Carolina, United States of America, **4** Department of Genetics, North Carolina State University, Raleigh, North Carolina, United States of America.

Reference:
Zhou S, Stone EA, Mackay TFC, Anholt RRH, 2009 Plasticity of the Chemoreceptor Repertoire in *Drosophila*
melanogaster. PLoSGenet 5(10):e1000681. doi:10.1371/journal.pgen.1000681

INTRODUCTION

Responses to the chemical environment play an important role in animal survival, as chemical cues direct foraging behavior and food selection, predator avoidance, and, in insects, host plant recognition for oviposition and larval feeding. Chemical signals are also essential for the selection of mating partners, maternal behavior, and kin recognition. As a consequence of the profound importance of chemosensation for survival and reproduction, several large families of chemosensory genes have evolved through repeated processes of gene duplication and diversification (Robertson et al. 2003; Aloni et al. 2006; Niimura and Nei 2006; Kambere and Lane 2007), including genes that encode odorant receptors (*Or*s)(Buck and Axel 1991; Clyne et al. 1999; Gao and Chess 1999; Vosshall et al. 1999; Robertson et al. 2003), gustatory receptors (*Gr*s) (Clyne et al. 2000; Robertson et al. 2003), and, in insects, odorant binding proteins (*Obp*s) (Galindo and Smith 2001; Graham and Davies 2002; Hekmat-Scafe et al. 2002). In addition, large multigene families aimed at eliminating toxic chemicals have evolved, most prominently the cytochrome P450 superfamily (Nebert et al. 1991). Detoxification of plant defense chemicals together with development of chemosensors that enable fine tuning to host plants has been instrumental in the establishment of specialized insect-host plant relationships (Berenbaum 2002). For example, the black swallowtail butterfly, *Papilio polyxenes*, has developed cytochrome P450s that can metabolize toxic furanocoumarins, which allows it to feed and oviposit on plants of the Umbelliferae family(Wen et al. 2006). Similarly, *Drosophila sechellia*'s host plant, *Morinda citrifolia*, is toxic to other

Drosophila species. A 4 bp insertion in the upstream regulatory region of the *D. sechellia Obp57e* gene eliminates expression of this odorant binding protein, which elicits avoidance of the Morinda fruit in Drosophila species in which the gene is intact (Matsuo et al. 2007).

The rapid evolution of these large chemoreceptor gene families has generated functional redundancy between receptors and their ligands (Malnic et al. 1999; Hallem and Carlson 2006), which confers sensitivity and robustness to the chemical recognition process. Animals, however, interact differently with their chemosensory environments under different developmental, physiological and social conditions. Therefore, it stands to reason that expression of the chemosensory repertoire would be dynamically regulated. This raises several fundamental questions: (1) Is the expression of chemoreceptor genes that are organized as clusters in the genome independently regulated or do genes within a cluster act as co-regulated functional ensembles? (2) Are all chemoreceptor genes equally sensitive to environmental fluctuations or is a core group of chemoreceptor genes particularly responsive to environmental or physiological changes? (3) Are certain chemoreceptor genes frequently co-regulated when environmental or physiological conditions change? (4) Is the expression of particular chemoreceptor genes upregulated or downregulated as a function of sex (males *versus* females), development (*e.g.* in larval stages, adult stages and during senescence), reproductive state (*e.g.* virgin or mated) or social context (*e.g.* solitary or group reared)?

To answer these questions we focused on the chemoreceptor families of *Drosophila melanogaster*, where both the olfactory and gustatory systems have been well characterized (Clyne et al. 1999; Gao and Chess 1999; Vosshall et al. 1999; Clyne et al. 2000; Galindo and Smith 2001; Graham and Davies 2002; Hekmat-Scafe et al. 2002; Robertson et al. 2003; Vosshall and Stocker 2007). *D. melanogaster* provides an advantageous genetic model as inbred individuals can be readily generated and grown under controlled conditions, enabling control over both the genotype and the environment (Anholt and Mackay 2004). We constructed expression microarrays that enable us to survey simultaneously expression of all *Obp*, *Or* and *Gr* genes. We analyzed chemoreceptor expression as a function of sex, development, reproductive state, and social environment, and obtained a systematic description of the plasticity of the chemosensory window through which the fly experiences its chemical environment. We found that genes in clusters are independently regulated in the two sexes, during different developmental stages, and under different physiological and social conditions. Whereas many chemosensory genes showed plasticity in expression, a smaller number of exceptionally plastic genes was evident. Analysis of covariance of transcript levels across all environmental conditions showed that the chemosensory subgenome is structured as a mosaic of 20 small modules of highly correlated transcripts. This finely pixilated modular organization of the chemosensory transcriptome allows finely tuned phenotypic plasticity of expression of the chemoreceptor repertoire under different environmental conditions.

RESULTS

Construction and Characterization of the cDNA Microarrays

To assess to what extent transcription of chemosensory genes responds to changing conditions, we constructed cDNA expression arrays that represent 50 *Odorant binding protein* (*Obp*), 59 *Odorant receptor* (*Or*), and 59 *Gustatory receptor* (*Gr*) genes, four genes that encode other antenna-specific proteins, and four control genes. To prepare cDNA probes, primer sets were designed to generate unique 400-600bp amplicons. All amplification products were sequenced and the sequences analyzed using the BLAST algorithm to ensure absence of cross-hybridizing sequences. Cross-hybridization is likely to occur in only two cases. Amplicons for *Gr64d* and *Gr64e* do not overlap, but these genes have partially overlapping transcripts and, therefore, could cross-hybridize. In addition, *Or19a* and *Or19d* are located 50kb apart in opposite orientation and share the same sequences, rendering them indistinguishable. The extent of dye effects was assessed by hybridization of a mixture of equal amounts of Cy3 and Cy5 labeled RNA of the same sample. There was generally a close correlation between Cy3 and Cy5 hybridization intensities (Fig. 2.1), indicating overall minor dye effects.

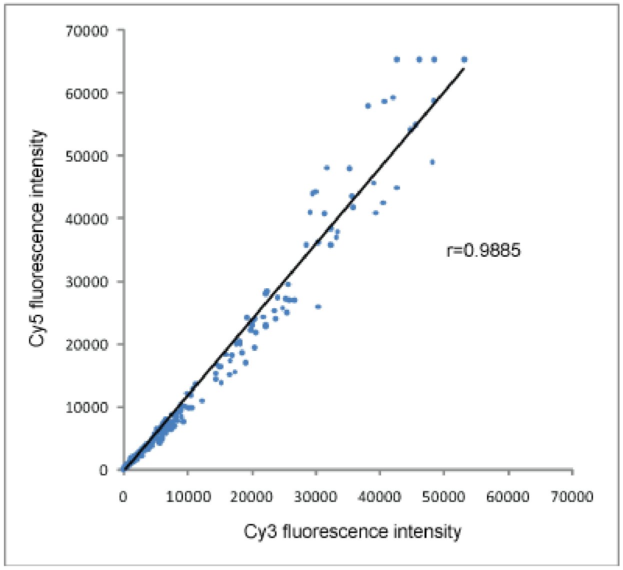

Figure 2.1 Correlation between Cy3 and Cy5 hybridization intensities. To assess dye effects we performed hybridization with a mixture of equal aliquots from the same RNA sample, extracted from an equal number of male and female flies, labeled separately with Cy3 and Cy5. There were four replicates of each cDNA probe on the array. Note the close correlation between Cy3 and Cy5 hybridization intensities with only minor dye effects, skewed towards Cy3 at low signal intensities and towards Cy5 at high fluorescent intensities.

Among the 168 chemosensory genes represented on the microarray, we detected expression of 50 *Obp* genes, 54 *Or* genes, and 52 *Gr* genes, in at least one experimental condition. Expression levels of *Obp* genes were generally at least one order of magnitude higher than those of *Or* and *Gr* genes, which is not surprising, as odorant binding proteins are secreted proteins. Expression of chemoreceptor genes on our customized EST microarrays correlated well with previously obtained transcriptional profiles of chemosensory genes represented on high density oligonucleotide microarrays from Affymetrix, Inc. (Morozova et al. 2006) (Fig. 2.2; r = 0.818, n = 174), but resolution for detection of chemoreceptor gene expression was substantially improved. We were not able to detect expression of *Gr22b, Gr58c, Gr59c, Gr77a, Gr93b, Gr93c, Gr93d, Or10a, Or24a, Or85b*, *Or85c* and *Or85d*, possibly due to highly localized expression of rare transcripts.

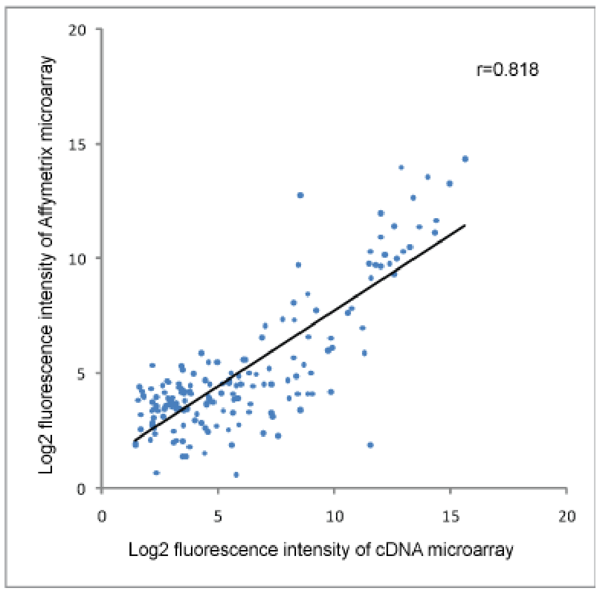

Figure 2.2 Correlation between chemoreceptor gene hybridization signal intensities on Affymetrix and cDNA microarrays. The figure shows the correlation between fluorescence intensities of an Affymetrix microarray and our customized cDNA microarray for independent RNA samples extracted from young mated adult male flies. The Affymetrix microarray data are obtained from Morozova, 2006. The scatter diagram includes 174 comparisons, excluding the *lacZ* and *GAL4* genes, which were included on the cDNA microarrays as background controls.

Modulation of Chemoreceptor Gene Expression during Development

To assess modulation of chemoreceptor gene expression during development we compared expression of *Obp*, *Or* and *Gr* genes in third instar larvae (mixed sexes) and in virgin adult males and females. We also assessed changes in chemoreceptor gene expression during senescence in males and females. Pairwise comparisons between larvae and adults showed that relative expression of 28 chemoreceptor genes was biased in or specific to larvae at a Bonferroni corrected significance threshold of $P < 5.68E\text{-}5$ (corrected for multiple testing at a nominal significance level of $P < 0.01$) with a 2-fold change filter; conversely, 35 chemoreceptor genes showed adult-biased or adult-specific relative expression (Fig. 2.3; Table 2.1). To validate our microarray observations, we amplified transcripts of the *Obp58* and *Obp99* gene clusters in larvae and adults. *Obp99c* was highly expressed in larvae and adults, whereas *Obp99b* showed strong adult-biased expression (Fig. 2.4). Similarly, *Obp58c* and *Obp58d* were virtually undetectable in larvae, but expressed in adults with especially strong adult-specific expression of *Obp58c*. The results of the microarray analysis showed good concordance with results from RT-PCR experiments (Fig. 2.4).

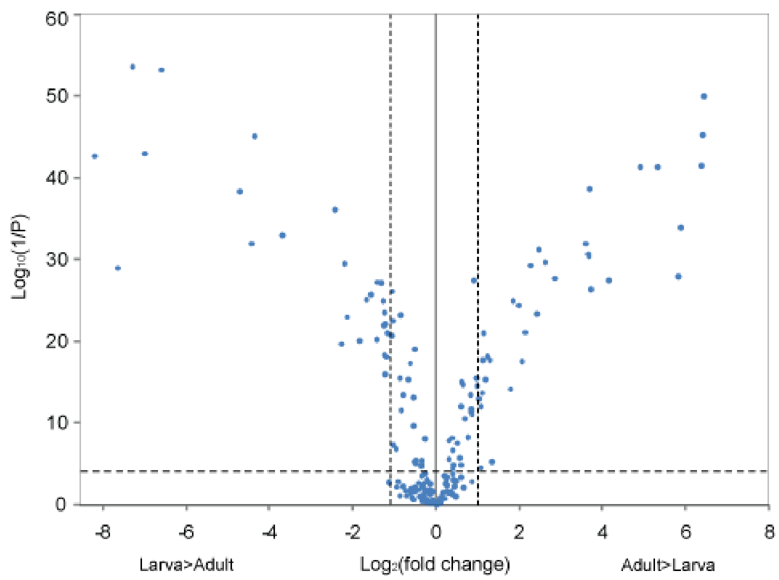

Figure 2.3 Volcano plot of differences in transcript abundance between larvae and adult flies. The figure illustrates differences in transcript expression levels between RNA extracted from third instar larvae and from an equal mixture of virgin adult males and females. Each dot represents a probe on the array. The horizontal dashed line shows the Bonferroni-corrected significance threshold of P = 5.2E-5. The vertical dashed lines show 2-fold enrichment boundaries between the samples.

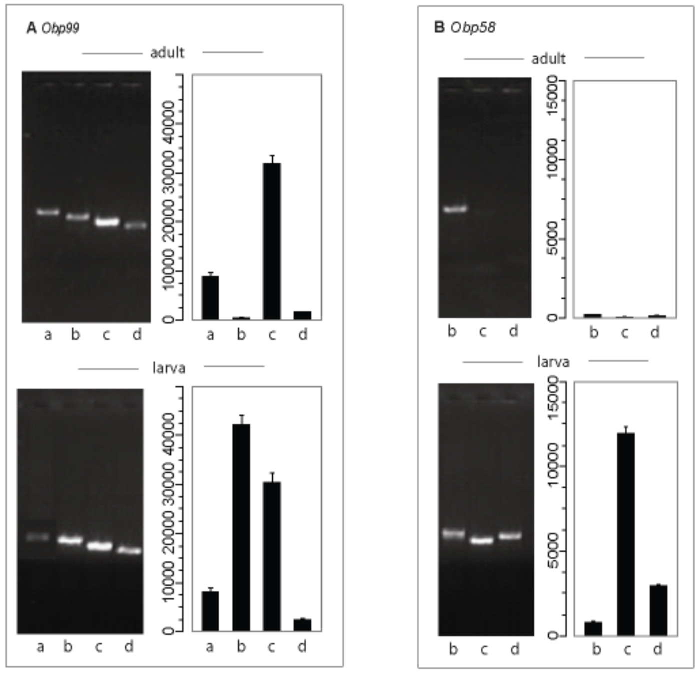

Figure 2.4 Confirmation of microarray expression data by RT-PCR. Fragments of cDNA corresponding to transcripts of *Obp* genes within the *Obp58* (panel A) and *Obp99* (panel B) clusters were amplified from adult (top panels) or larval (bottom panels) RNA samples using the same primers used to construct the corresponding microarray probes. Amplification was done after 2 min denaturation at 94^{o}C by 30s denaturation at 94^{o}C, 30s annealing at 55^{o}C, and 1 min extension at 72^{o}C for 30 cycles followed by 4 min incubation at 72^{o}C. Intensity of ethidium bromide stained bands on agarose gels are compared to fluorescence intensities after hybridization of labeled RNA samples to the microarrays (bar graphs in each panel). Quantitative comparisons are not precise at low levels of expression. Note, however, that absence of *Obp58c* in adults and high intensities of *Obp58c* in larvae on the arrays are matched by the appearance of the corresponding RT-PCR products. Similarly, high intensity levels of *Obp99c* in adults and *Obp99b* and *Obp99c* in larvae correspond between fluorescent intensity levels on the microarrays and staining of the corresponding RT-PCR products.

Since many chemoreceptors occur in clusters in the genome (Robertson et al. 2003), we asked whether individual members of a cluster show coordinated or independent regulation during development. We examined chemoreceptor gene clusters without intervening genes, including the *Gr22a-e* cluster, the *Obp19a-d*, *Obp50a-e*, *Obp56a-f*, and *Obp57a-c* clusters, and the *Or43a-b* cluster (Fig. 2.5). There were extensive differences between larvae and adults in chemoreceptor gene expression. *Gr22d*, *Gr22e*, *Obp50d*, *Obp56a-d*, and *Or43a* showed larva-biased expression. Especially striking was the high larva-specific expression of *Gr22d*, as well as *Gr22e.* In contrast, expression of some chemoreceptor genes was observed only in adults, for example the *Obp19a-d* and *Obp57a-c* gene clusters and *Obp56f*.

When we compared relative expression of the same chemoreceptor genes in males and females, we observed extensive sexual dimorphism in transcript abundance levels. Male-biased expression was evident for *Obp50c*, *Obp56d*, and *Obp56f*, whereas female-biased expression was observed for *Obp19a*, *Obp19c*, *Obp56a*, *Obp56e*, *Obp57a*, and *Or43b* (Fig. 2.5; Table 2.2). These results show that expression of chemoreceptor genes that are located within gene clusters can be regulated independently at different developmental stages and between the sexes.

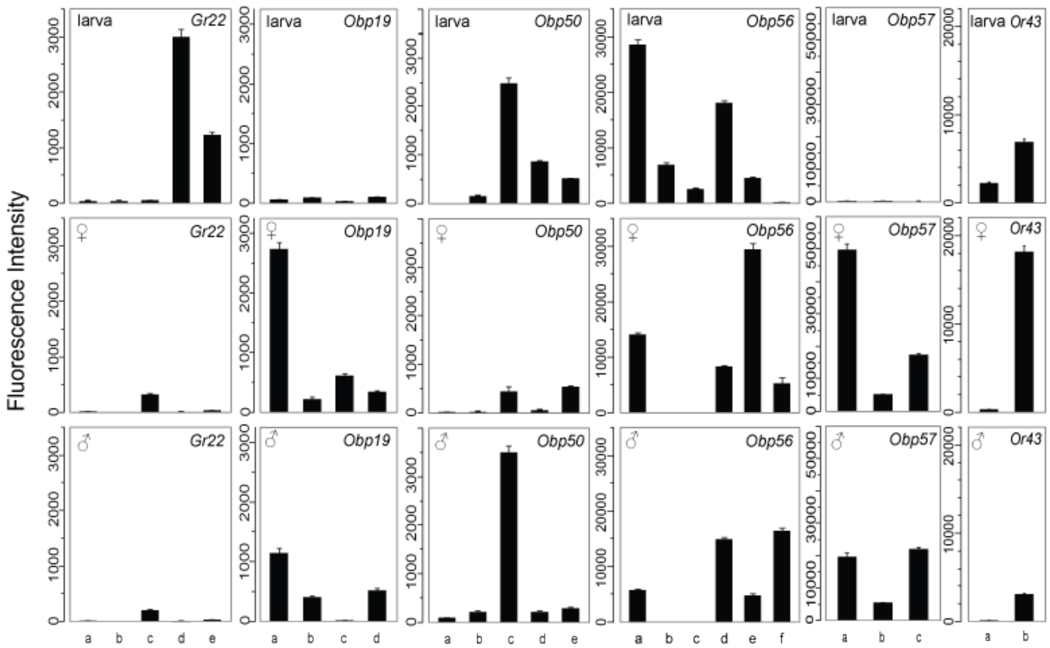

Figure 2.5 Differential and sexually dimorphic expression of chemoreceptor genes between larvae and adults. Average fluorescent intensities are shown corresponding to expression of chemosensory genes within the *Gr22*, *Obp19*, *Obp50*, *Obp56*, *Obp57*, and *Or43* gene clusters in larvae (top panels), adult virgin females (center panel), and adult virgin males (bottom panels). None of the clusters shown contain intervening genes. Note the dramatic differences in expression patterns between larvae and adults with strict larva-specific expression of *Gr22d*, *Obp56b* and *Obp56c*, the extensive sexual dimorphism among adults, and the apparently independent regulation among genes within the same cluster.

Next, we asked whether chemosensory gene expression levels are stable throughout adult life or are subject to age-dependent plasticity. We compared transcript abundance levels in 10-day old and 6-week-old virgin males and females maintained under carefully controlled standard laboratory conditions, and found extensive age-dependent changes in transcriptional regulation in all classes of chemosensory genes (Fig. 2.6). We found 104 chemosensory genes with altered transcriptional regulation in one or both sexes. Many genes with altered expression during senescence were shared between males and females. However, sexual dimorphism in age-dependent chemoreceptor gene regulation was pervasive. Interestingly, in males 15 *Gr* genes and 19 *Or* genes showed alterations in expression levels during aging (Fig. 2.6B), while in females only three *Gr* genes and four *Or* genes changed expression levels during senescence (Fig. 2.6A). The ubiquitous odorant receptor *Or83b* showed decreased expression levels with age in both sexes, whereas expression of *Or1a* and *Gr98a* was upregulated in both sexes during aging. Extensive differences among transcript abundance levels of *Obp* genes in young and old flies were especially prevalent for both sexes. *Obp51a*, *Obp56e*, *Obp56g*, *Obp57a*, *Obp57c*, and *Obp99b* showed altered expression levels during aging in both sexes, but in opposite directions (Fig. 2.6). Again, expression of genes within a cluster appears to be regulated independently from other genes in the same cluster during senescence.

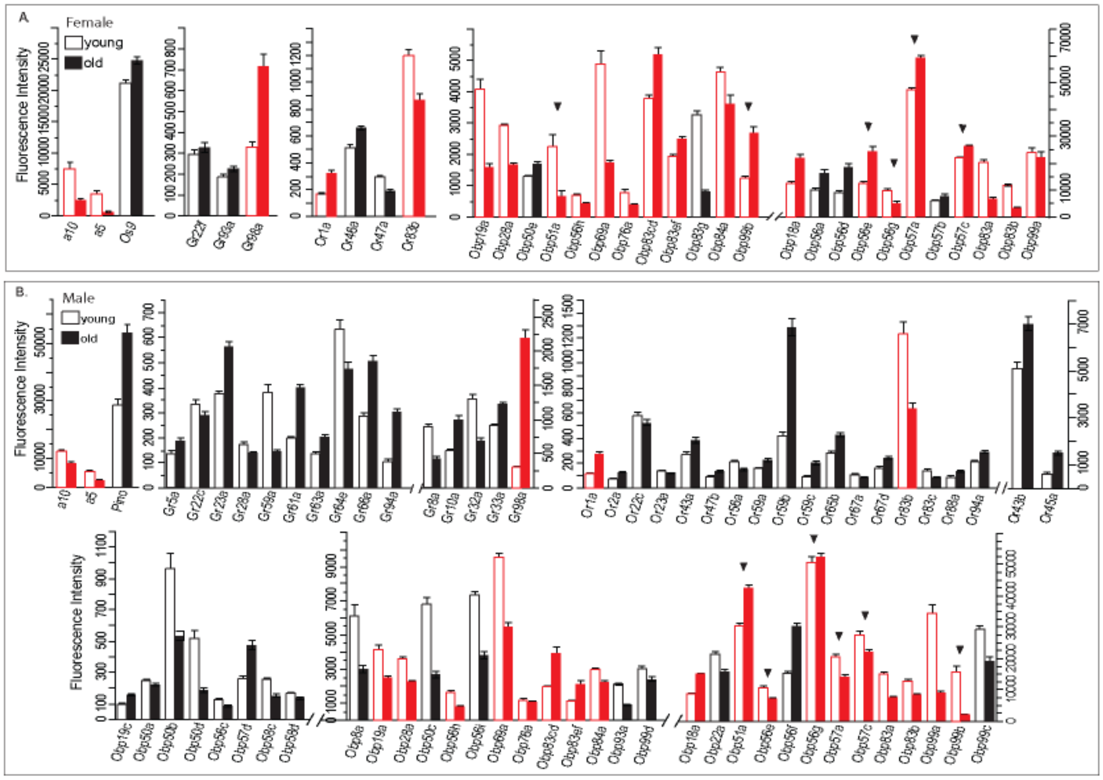

Figure 2.6 Differential expression of chemoreceptor genes during senescence. Only chemoreceptor genes of which expression levels change significantly after Bonferroni correction between 7-10 day-old (open bars) and 6 week-old (solid bars) virgin females (panel A) or males (panel B) are shown. Red bars indicate chemoreceptor genes with altered expression during senescence in both sexes. Note the preponderance of *Obp* genes. Arrowheads indicate chemoreceptor genes of which expression changes in opposite directions between males and females (*Obp51a, Obp99b, Obp56e, Obp57a* and *Obp57c*).

Modulation of Chemoreceptor Gene Expression by Reproductive State

Next, we asked to what extent changes in physiological condition affect expression of the chemoreceptor repertoire. Mating results in profound physiological changes in females (Wolfner 2002), although post-mating physiological and behavioral changes in males have not been documented. We compared transcript abundance levels of chemosensory genes in virgin males and females reared separately to those of individuals that were allowed to mate (Fig. 2.7). Following mating, only females showed a reduction in transcript levels of a suite of four *Gr* and 12 *Or* genes. In contrast, changes in *Obp* transcript abundance were seen in both sexes. Here, 16 out of 23 *Obp* genes with altered transcriptional regulation showed up-regulation in mated females (Fig. 2.7A). Substantial changes in transcript abundance of *Obp* genes and *Pino* (a.k.a. *smi21F*), a putative odorant binding protein (Rollmann et al. 2005), were also evident in mated males (Fig. 2.7B). Twelve *Obp* genes showed altered expression in both sexes, and among these five showed antagonistic changes in expression levels between the sexes (Fig. 2.7). Thirteen out of 19 *Obp* genes with altered transcript abundance in mated males showed a reduction in transcript abundance, in contrast to the predominant up-regulation of *Obp* expression levels seen in mated females. Thus, mating caused profound changes in subsets of chemosensory genes in both sexes. The identities of the chemosensory genes affected or the effect on their transcript levels were distinct between males and females, indicating a profound sexually dimorphic change in the functional composition of the chemoreceptor repertoire after mating.

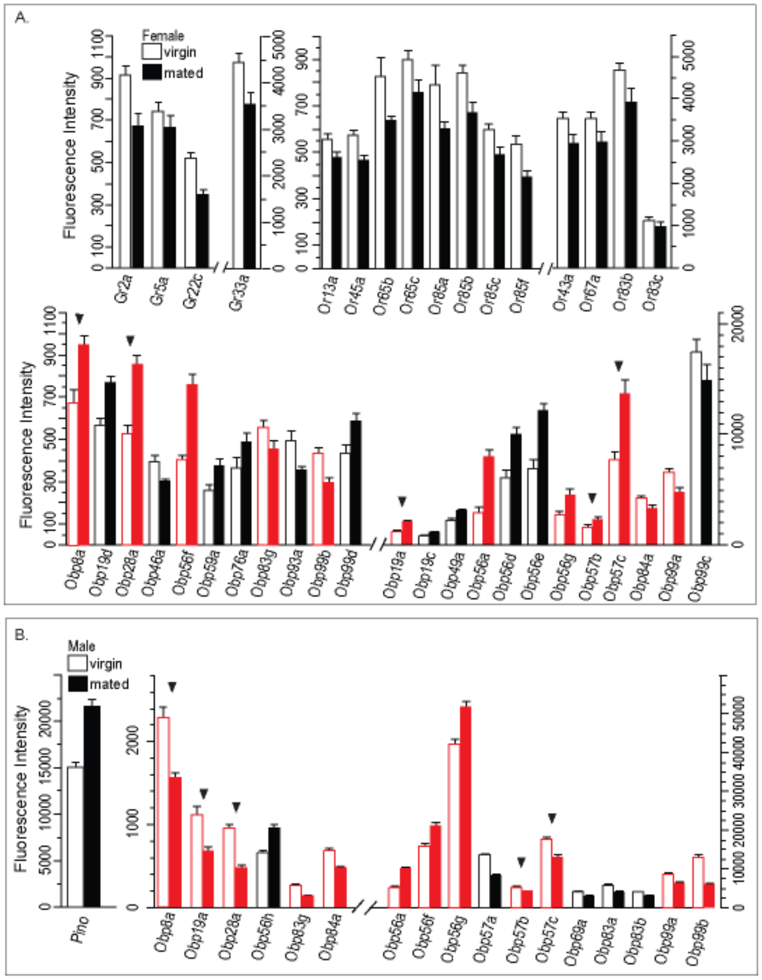

Figure 2.7 Differential expression of chemoreceptor genes after mating. Only chemoreceptor genes of which expression levels change significantly after Bonferroni correction between virgin (open bars) and mated (solid bars) females (panel A) or males (panel B) are shown. Red bars indicate chemoreceptor genes with altered expression after mating in both sexes. Note the preponderance of *Obp* genes. Differential expression of *Gr* and *Or* genes after mating is only observed in females. Arrowheads indicate chemoreceptor genes of which expression changes in opposite directions between males and females (*Obp8a*, *Obp19a*, *Obp57b* and *Obp57c*).

Modulation of Chemoreceptor Gene Expression by Social Context

Our observation that the expression of the chemosensory repertoire is modified dramatically by social contact during reproduction led us to ask whether social context *per se* can elicit altered expression of distinct chemosensory genes. We compared transcript abundance levels in male and female flies that were reared as single isolated individuals to those of virgin flies reared in corresponding single sex groups. We observed changes in expression levels of few *Gr* or *Or* genes under these conditions (Fig. 2.8). However, in females transcript abundance levels of seven *Obp* genes and *Pino* were down-regulated when individuals were reared in isolation, whereas two *Obp* genes were up-regulated (Fig. 2.8A). In males transcription of five *Obp* genes was down-regulated when individuals were reared in isolation, whereas three *Obp* genes were upregulated (Fig. 2.8B). Compared to our other experimental conditions, we found less overlap between genes with altered regulation in males and females. Different members of the *Obp56* gene cluster featured prominently among transcripts with altered regulation in each sex. Only *Obp56e*, however, showed down-regulation in isolated individuals in both sexes and *Obp57b* was down-regulated in females and up-regulated in males when flies were reared in isolation (Fig. 2.8).

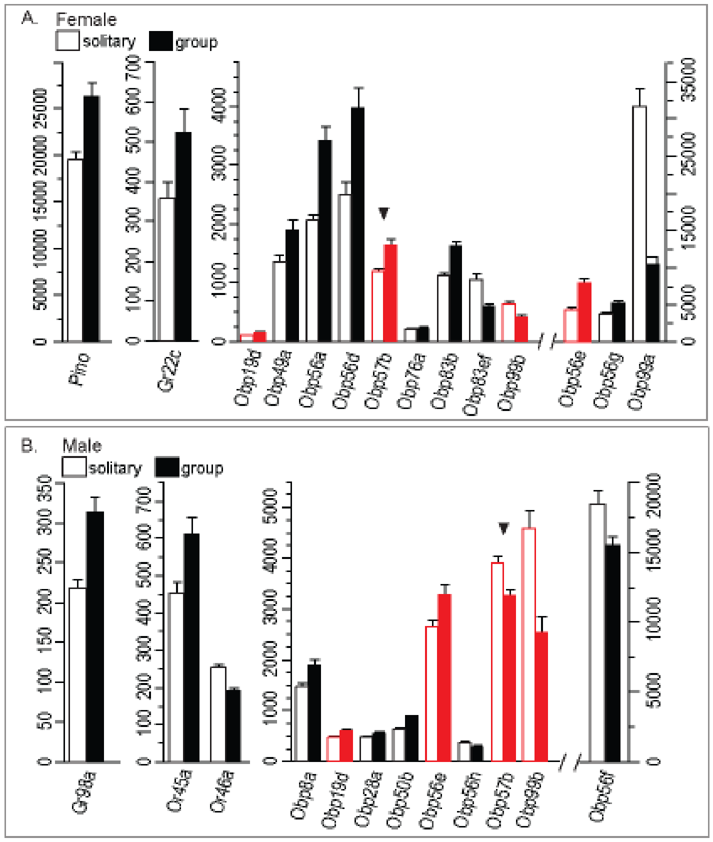

Figure 2.8 Regulation of chemoreceptor gene expression by social context. Only chemoreceptor genes of which expression levels change significantly after Bonferroni correction when flies are reared solitary (open bars) or in same sex groups (solid bars) are shown for females (panel A) and males (panel B). Note the preponderance of *Obp* genes. Red bars indicate Obp56e and *Obp57b* which show altered expression in different social contexts in both sexes. Arrowheads indicate chemoreceptor genes of which expression changes in opposite directions between males and females in opposite directions.

Chemoreceptors have been implicated in the detection of both volatile (van der Goes van Naters and Carlson 2007) and non-volatile (Ferveur 2005) social chemical signals. We wanted to assess whether exposure to social odor cues alone could result in altered transcriptional regulation of chemosensory genes. Therefore, we separated single flies from groups of same-sex or opposite sex flies with a double cheesecloth partition that would allow the transmission of olfactory cues, but would prevent any other social sensory input or interaction (it should be noted that *Canton S* w^{-} flies used in these experiments are visually impaired). When single flies were maintained for five days under conditions in which they were exposed to same-sex group odors, there were virtually no changes in transcript patterns of chemosensory genes. Only expression of *Obp57c* was increased in females exposed to female group odor (Fig. 2.9A), whereas expression of *Obp84a* and *Obp83b* was increased in males exposed to male group odor (Fig. 2.9B). In contrast, we saw more extensive changes in transcript levels when we exposed single flies to opposite sex group odor for the same time period. Here, nine chemosensory genes in females showed altered regulation, including seven *Obp* genes and the antenna-specific *a5* and *a10* genes (Fig. 2.9C). With the exception of *Obp19c*, all of these genes were down-regulated when a single female was exposed to male group odor. In single males exposed to female group odor, six *Obp* genes and a gustatory receptor gene (*Gr2a*) showed altered transcriptional regulation (Fig. 2.9D). Remarkably, there was no overlap between the subsets of chemosensory genes that underwent altered regulation when single males or females were exposed to opposite sex group odor.

Notably, members of the *Obp56* gene cluster (Fig. 2.8) did not show altered expression under these conditions. The lack of concordance between transcriptional regulation of chemosensory genes when isolated individuals were compared to group reared individuals (Fig. 2.8) and when isolated individuals were limited only to same sex group olfactory exposure Fig. 2.9A and B) shows that physical interactions are instrumental in determining expression of the chemoreceptor repertoire within same sex groups.

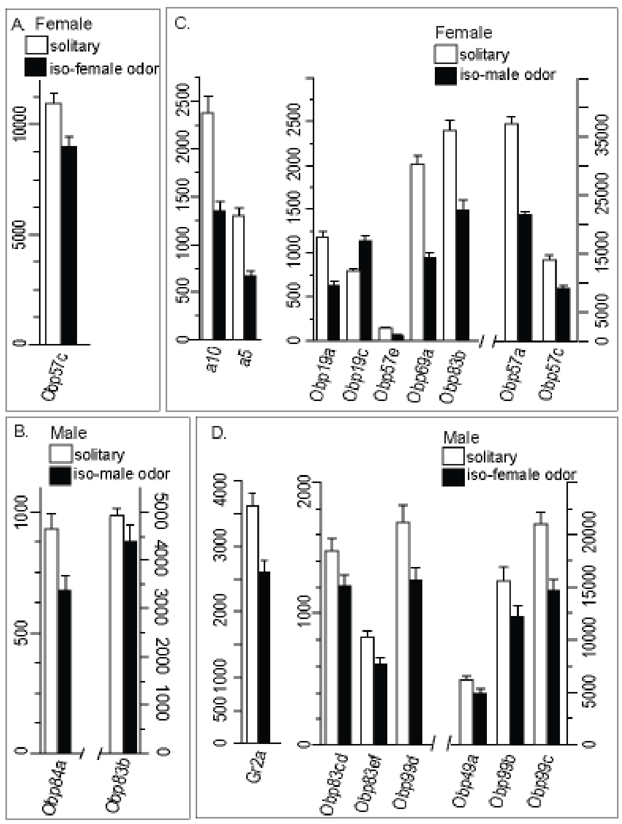

Figure 2.9 Social odor induced differential expression of chemoreceptor genes. Only chemoreceptor genes of which expression levels change significantly after Bonferroni correction between single fly controls (solid bars) and single flies exposed to same sex odor or opposite sex odor (open bars) are

shown for females (panel A, exposed to female group odor; and C, exposed to male group odor) and males (panel B, exposed to male group odor; and D, exposed to female group odor). Red bars indicate *Obp83b* which shows altered expression in both sexes when single flies are exposed to male group odor, but in opposite directions between the sexes (arrowhead). Note that differential chemoreceptor gene expression is more prominent when flies are exposed to opposite sex group odor than to same sex group odor.

Correlated Phenotypic Plasticity of Chemoreceptor Gene Transcripts

We noticed that environmental plasticity of expression was heterogeneous among chemosensory genes, with certain members of the chemoreceptor ensemble responding more frequently to environmental changes than others. Therefore, we decided to investigate whether groups of chemosensory genes showed correlated transcriptional regulation across all experimental conditions. We analyzed transcript levels using the modulated modularity clustering method. This unbiased, self-organizing paradigm is based on correlations of transcript abundance levels between different conditions, and sorts transcripts into modules such that transcript abundance levels among members within each module are more closely correlated than with members outside that module (Ayroles JF 2009; Stone EA 2009). The resulting pairwise correlation matrix can be represented graphically such that modules of correlated transcripts are organized in a matrix, with color-coding indicating the strength of each pairwise correlation (Ayroles JF 2009; Stone EA 2009) (Fig 2.10). This analysis revealed 20 covariant ensembles (Fig 2.10; Table 2.3), indicating that transcriptional regulation of the chemoreceptor repertoire is indeed modular. At the same time, however, the large number of modules and their small sizes reflect the overall heterogeneity in transcriptional regulation of chemosensory genes. Whereas genes that are members of the same cluster were by and large independently regulated (*e.g.* Fig 2.4), in some instances genes in close proximity to each other within a cluster appeared to co-vary in expression levels. This was the case for *Obp58b* and *Obp58c* (located 376 bp apart in different orientations; Module 3), *Obp56b* and *Obp56c* (located 855 bp apart; Module 8), *Obp83cd* and *Obp83ef* (which have a 56bp overlap with different orientations; Module 5), *Obp 99b* and *Obp99d* (located 1298 bp apart in different orientation with one intervening gene, *Dup99B*; Module 15), *Or42a* and *Or42b* (located 4231 bp apart with one intervening gene, *Tsp42A*; Module 19) and *Or33a* and *Or33b* (located 464 bp apart; Module 14)*.* Strong negative correlations that reflect the antagonistic regulation of chemoreceptor gene expression described above were also observed, e.g. in Module 8. *Obp76a* (a.k.a. *Lush*), which binds the courtship pheromone *cis*-vaccenyl acetate (Xu et al. 2005) shows a strong positive correlation with *Or67d*, the transcript that encodes the receptor for *cis*-vaccenyl acetate (Ha and Smith 2006; Kurtovic et al. 2007), and a strong negative correlation with *Gr64a* and *Gr64c* (Module 4). With some exceptions, it appears that by and large *Obp* genes are segregated in modules that are distinct from modules that contain *Or* and *Gr* genes (*e.g.* Modules 7, 8, 15), and *Or* and *Gr* genes are frequently intermixed within covariant ensembles, *e.g.* Modules 14, 19 and 20 (Fig 2.10; Table 2.3). The transcript that encodes the ubiquitous odorant co-receptor *Or83b* (Vosshall et al.

1999) clustered in module 17. The CO_2 co-receptor *Gr63a* (Jones et al. 2007) clusters in Module 16, while its counterpart *Gr21a* (Suh et al. 2004; Jones et al. 2007) forms part of Module 13, indicating that *Gr63a* and *Gr21a* expression is not closely correlated in the range of environmental conditions investigated in this study. Interestingly, *Gr32a* and *Gr68a*, which have both been implicated in pheromone recognition during the Drosophila courtship ritual (Bray and Amrein 2003; Miyamoto and Amrein 2008) cluster together in Module 16 (Fig 2.10; Table 2.3).

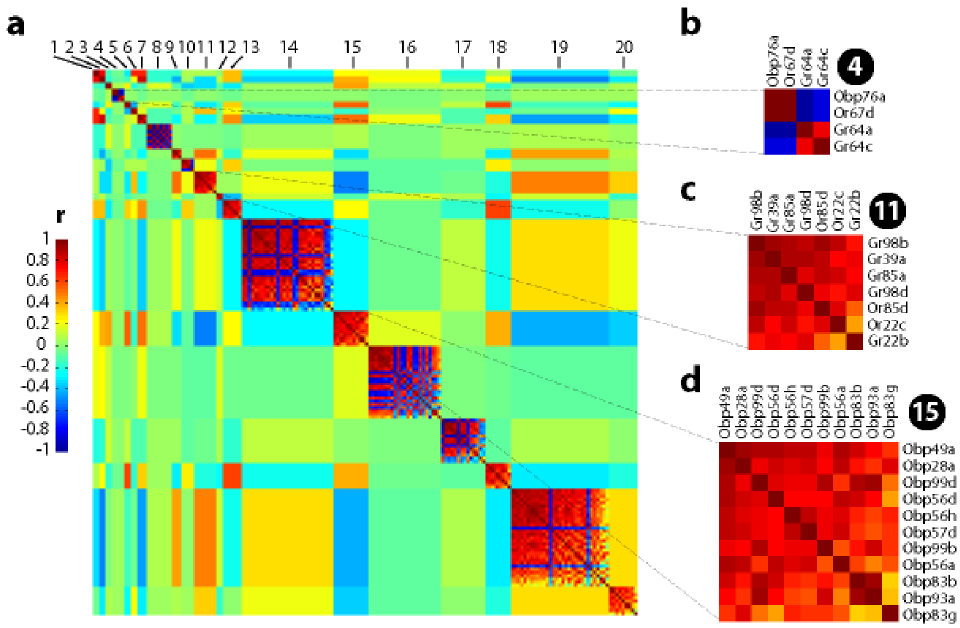

Figure 2.10 Correlated transcriptional response in phenotypic plasticity (a) Clustering of 172 genes into 20 modules that show correlated transcriptional responses across environmental conditions. The modules populate the main diagonal and are ordered by decreasing strength from the upper left; the genes are ordered from left to right and from top to bottom as in Supplementary Table 3. Color has been used to indicate strength of correlation as illustrated in the legend. Within a module, each

colored square reports the correlation in transcriptional response between a pair of genes. Pairs of genes that do not share a module are given the color that corresponds to the average absolute pairwise correlation between genes from those modules. (b-d) Magnification of Module 4 (b), Module 11 (c), and Module 15 (d) with gene labels.

DISCUSSION

The olfactory and gustatory systems in *Drosophila melanogaster* have been well characterized (Clyne et al. 1999; Gao and Chess 1999; Vosshall et al. 1999; Clyne et al. 2000; Galindo and Smith 2001; Graham and Davies 2002; Hekmat-Scafe et al. 2002; Robertson et al. 2003; Vosshall and Stocker 2007), but the central problem of how ecologically relevant environmental conditions affect transcriptional variation in expression of the chemoreceptor repertoire has not been addressed previously in a systematic manner. As chemoreceptors are distributed over the entire body of the fly, including the third antennal segment, maxillary palps, proboscis, cibarial taste organs, tarsi, wing margins and the female abdominal reproductive plate, we chose to use for a comprehensive analysis whole flies rather than heads. Consequently, some differences in expression between the sexes may be due to expression of chemoreceptors in non-chemosensory tissues. It is of interest to note that expression of odorant receptors in non-chemosensory organs has been observed using similar customized cDNA microarrays in both mice (Zhang et al. 2004) and humans (Zhang et al. 2007).

Here, we have shown that transcriptional profiles of chemosensory genes in *D. melanogaster* are highly plastic during early development and senescence, as a result of mating, and in social contexts. Expression of chemoreceptor genes is highly sexually dimorphic and frequently sexually antagonistic, and the extent of transcriptional responses to changing conditions is heterogeneous among the chemoreceptor repertoire. Examination of the FlyAtlas expression data base indicates that *Obp50c*, *Obp56d Obp99a* and *Gr32a* are expressed in testes, *Obp8a*, *Obp22a*, *Obp51a*, *Obp56e*, *Obp56f*, *Obp56g*, *Obp56i* and *Or59b* in the accessory gland, *Obp19c* in the ovaries and *Pino* in both ovaries, testes and accessory glands, which suggests pleiotropic functions of these chemoreceptors and may account in part for the observed sexually dimorphic expression patterns (Chintapalli et al. 2007). In this study we have not included an analysis of expression of the recently discovered family of ionotropic odorant receptor (*IR*) genes, which are expressed in coeloconic sensilla of the antenna and respond, among others, to water and amines (Benton et al. 2009), and which were not represented on our microarrays. It will be of interest to investigate in future studies whether these genes show similar plasticity in expression as observed for the classical chemosensory genes.

A previous study used *in situ* hybridization to detect GFP expression of odorant receptors in larvae under the control of odorant receptor-specific promoters (Fishilevich and Vosshall 2005) through the *GAL4-UAS* binary expression system (Brand and Perrimon 1993). This study showed expression of 25 odorant receptors in the Drosophila larval olfactory system and reported that 14 of these receptors were larval-specific (Fishilevich and Vosshall 2005). Although most of the larval expressed *Or* transcripts reported in this study were also identified on our arrays, the majority of these *Or* transcripts was also detectable in adults. There was some agreement with specificity of odorant receptor expression in larvae and adults (*e.g. Or33a* was found to show larval-biased expression and 10 *Or* genes were found to be expressed in adults as well as larvae both by us and others). However, the concordance between larval specificity detected by *GAL4-UAS* mediated expression of GFP in olfactory tissues and direct measurements of transcript abundance on our arrays from whole flies was generally poor. This can be due to expression of chemoreceptors in adult tissues not examined by previous *in situ* hybridization or reporter gene expression, differences in detection thresholds between the techniques used, differences in the strengths of *GAL4*-linked odorant receptor promoters in larvae and adults, or possibly differences in genetic backgrounds between strains used in the two studies.

A previous study reported sexually dimorphic expression of *Obp99a* and *Obp99b* (Anholt et al. 2003). Here we showed that sexual dimorphism in expression of chemosensory genes is widespread. This is especially evident among *Obp* genes, but the apparent prevalence of sexual dimorphism among these genes may be caused by their higher expression levels compared to those of *Or* and *Gr* genes. These broad sex-dependent differences in levels of expression of chemosensory genes suggest that males and females experience, interact with, and adapt to their chemical environments differently. Furthermore, transcript profiles change drastically after mating, not only in females but also in males. The altered transcript abundance of *Obp19d*, *Obp28a, Obp56a*, *Obp56g*, and *Obp99c* that we observe in mated females (Fig. 2.7) is consistent with a previous study which compared mating-induced changes of whole genome transcript profiles on high density oligonucleotide microarrays (McGraw et al. 2004). It is of interest that some odorant binding proteins, including *Obp56e, Obp56f*, *Obp56g* and *Obp56i* are highly expressed in the male accessory glands (Chintapalli et al. 2007). Thus, in addition to a function in olfaction, these odorant binding proteins may function also (or primarily) as carriers for physiologically active ligands that are transferred from the male into the female during copulation. Whereas chemically-induced physiological and behavioral changes in females upon mating have been well characterized (Chen 1996; Wolfner 1997), biological consequences of mating in mated males have not been documented previously.

Both volatile chemicals and cuticular hydrocarbons signal social information in Drosophila. The gustatory receptor *Gr68a*, which is expressed in chemosensory cells in the male tarsi, has been implicated in tactile chemosensation during courtship (Bray and Amrein 2003), together with *Gr32a* (Miyamoto and Amrein 2008). Recognition of the courtship pheromone, 11-*cis*-vaccenyl acetate, is mediated via the odorant binding protein Lush (*Obp76a*) and the *Or67d* receptor (Xu et al. 2005; Ha and Smith 2006; Kurtovic et al. 2007). The expression of transcripts for *Obp76a* and *Or67d* is highly correlated across the range of environments studied here, as is expression of transcripts for *Gr32a* and *Gr68a.* A large ensemble of chemoreceptor genes, however, is sensitive to the social environment and modulated based on social context and, especially, opposite sex group odor (Fig. 2.9). The identities of the odorants that are instrumental in mediating social interactions are not known, neither are the mechanisms that give rise to alterations in chemosensory gene expression levels.

POU-domain transcription factors, such as *acj-6*, have been implicated in mediating expression of odorant receptors in Drosophila olfactory neurons (Certel et al. 2000; Tichy et al. 2008). A phylogenetic analysis of conserved regulatory elements among sequenced genomes of 12 Drosophila species has identified regulatory elements that act combinatorially to promote or repress the expression of specific odorant receptors in the olfactory sensilla of the maxillary palp (Ray et al. 2008). A similar array of regulatory elements acted on by various transcription factors may also regulate *Or* gene expression in the antenna. Similar elements that regulate expression of *Obp* genes or *Gr* genes have not yet been identified. It is not clear whether transcriptional regulators and their binding sites that fine-tune transcription of *Or* genes in response to environmental changes are the same as those that control *Or* gene expression during development. Our results show that such fine tuning is exquisite in that genes that are located in close proximity within clusters can undergo independent transcriptional regulation (*e.g.* Fig. 2.5).

Elegant electrophysiological studies have provided a detailed characterization of the molecular response profiles of a large number of odorant receptors in *D. melanogaster* (Hallem et al. 2004; Hallem and Carlson 2006). Although these studies have greatly contributed to our understanding of odor coding in the Drosophila olfactory system, the nature of naturally occurring ecologically relevant chemical signals that are discriminated by these receptors and the functional relationships between odorant binding proteins and odorant receptors and/or gustatory receptors remain largely unknown.

Our focused analysis of the chemoreceptor gene families using cDNA microarrays that provide enhanced resolution over previously used Affymetrix GeneChips revealed that the ensemble of chemosensory genes fractionates into 20 relatively small environmentally correlated modules (Fig. 2.10). This observation shows that plastic transcriptional responses of chemoreceptor genes to a range of environments is modular, but at the same time indicates a great capacity of groups of chemosensory genes to alter their expression levels independently under a wide range of external environmental conditions.

METHODS

Drosophila Rearing

Isogenic *Drosophila melanogaster Canton S (B) w⁻* flies were used for all experiments and grown under standard culture conditions (cornmeal-molasses-agar-medium, 25^{o}C, 60-75% relative humidity, 12-hr light-dark cycle) for 4-5 days, unless otherwise specified. Larvae were collected at the 3rd instar stage. Sexes were reared separately after eclosion, except where indicated otherwise.

Modulation of Gene Expression during Development

Chemoreceptor gene expression was compared between larval and adult samples, prepared by pooling an equal number of females and males. In addition, we compared young flies (10-day old) and old flies (6 week-old), transferred to fresh food every two days.

Sexual Dimorphism and Modulation of Gene Expression after Mating

Chemoreceptor gene expression was compared between virgin females and virgin males, between virgin and mated females, and between virgin and mated males. To ensure that males had mated, we placed single males in vials with two females and collected males for microarray analysis when they were 5 days old, if females had oviposited.

Modulation of Gene Expression by Social Context

Chemoreceptor gene expression was compared between flies reared in isolation and reared in a group of 25 same sex flies. To assess to what extent modulation of gene expression was dependent on social odor cues, we exposed single males or females to the odor from groups of flies of the same sex or opposite sex. Single flies were separated from groups of flies behind a screen of two layers of cheese cloth that prevented physical interactions (visual contact does not occur as our *Canton S (B)* strain carries a *white* mutation that renders them blind).

cDNA Microarrays

We amplified 400-600 bp fragments from genomic DNA or cDNA corresponding to exon sequences of 50 *Obp* genes, 59 *Or* genes, 59 *Gr* genes, four genes encoding antennal specific proteins (*a5*, *a10, smi21F, Os9*), plus two housekeeping genes as positive controls (*Gapdh1* and *actin-5C*), and *Gal4* and *LacZ* as negative controls (Table S4). The identities of all amplicons were verified by sequencing and arrays were printed on a Genetix QArray2 microarray printer at the Genomic Sciences Laboratory at North Carolina State University. Experiments comparing gene expression between larvae and adult flies used arrays with four technical replicates per slide; all other experiments used arrays containing eight technical replicates per slide.

For hybridization to the arrays, fly samples were collected and frozen between 1:00 and 3:00 pm. RNA samples were extracted from 25 flies per biological replicate, subjected to one round of amplification using the MessageAmp aRNA kit from Ambion Biosystems, Inc. (Foster City, CA) and 5 μg of each RNA sample was labeled with Cy3 or Cy5 fluorescent dyes (Amersham, Pharmacia, Piscataway, NJ; cat. # PA23001 and 25001). Labeled samples were purified using the QIAquick PCR Purification Kit (Qiagen, Inc., Valencia, CA). Six biological replicates of each sample were used for each experiment and included dye swaps to control for possible dye effects. Hybridization was performed for 60 h in a water bath at 42^{o}C in the dark.

Arrays were scanned in a GenePix 4000B scanner, and raw data gathered by GenePix Pro software.

Microarray Data Analysis

The raw data were subjected to log2 transformation and first normalized using a mixed ANOVA model accounting for dye, array, technical replicates (nested within array), and dye×array effects, where array, rep (array) and dye×array are random effects. Residuals were then extracted from the model and used for further ANOVA analyses to assess significant differences in gene expression among the samples. We used factorial, mixed model ANOVA according to the model: Residual = μ + dye + array + rep (array) + stage/sex/condition + ε, where μ represents the overall mean value and ε the error variance, to further partition variation of transcriptional expression between dye (fixed), array (random), technical replicates nested within array (rep (array) random) and stage (or sex, or treatment) terms by gene for each experiment. We also extracted residuals from raw data across all experiments after mixed model normalization to account for technical variation for cluster analysis. We used Modulated Modularity Clustering (MMC) (Stone EA 2009) to organize the 172 genes into modules of correlated transcripts. MMC returned 20 modules as illustrated in Figure 8. Statistically significant differences were determined following normalization of the data by mixed model ANOVA. Bar graphs in the figures show fluorescent intensities of the raw data standardized for average array intensity and dye effect by adjusting fluorescent intensities based on the overall mean fluorescent intensities across arrays and between dyes. Comparisons of chemoreceptor gene expression between virgin females, mated females, virgin males and mated males employed a loop design. The data normalization procedure and analysis were identical except for an additional *post-hoc* pairwise comparison Student's t-test. A detection threshold was established based on two standard deviations from the mean *lacZ* signal intensity of the negative *lacZ* control. A Bonferroni corrected significance threshold of $P < 5.68E\text{-}05$ was established as a criterion for statistical significance.

Table 2.1 Genes that are differentially expressed in larvae and adult flies

Larva>Adult	*P-Value*	Fold Change	Adult>Larva	*P-Value*	Fold Change
Gr22d	1.0E-43	127.48	*Gr22c*	1.0E-12	2.10
Gr33a	1.0E-25	2.43	*Gr2a*	5.0E-16	2.25
Obp28a	8.0E-21	3.59	*Gr59f*	5.0E-32	5.47
Obp47a	1.0E-29	201.97	*Gr8a*	8.0E-22	4.36
Obp47b	8.0E-19	2.30	*Gr93b*	2.9E-05	2.10
Obp49a	7.0E-27	2.09	*Gr98a*	7.0E-15	3.42
Obp50d	7.0E-37	5.44	*Obp18a*	2.0E-30	6.10
Obp50e	7.0E-26	3.19	*Obp19a*	4.0E-42	39.51
Obp56a	6.0E-28	2.67	*Obp19b*	4.0E-42	29.74
Obp56b	5.0E-54	97.56	*Obp19c*	4.0E-27	13.04
Obp56c	1.0E-32	21.62	*Obp19d*	2.0E-39	12.69
Obp56h	7.0E-46	20.62	*Obp22a*	1.0E-28	55.84
Obp57d	2.0E-26	2.99	*Obp50b*	5.0E-30	4.75
Obp58b	2.0E-20	4.86	*Obp51a*	1.0E-50	85.82
Obp58c	2.0E-54	156.65	*Obp56e*	1.0E-21	2.18
Obp58d	4.0E-39	26.28	*Obp56g*	5.0E-46	83.24
Obp83cd	3.0E-30	4.61	*Obp57a*	1.0E-34	57.88
Obp83ef	1.0E-23	4.42	*Obp57b*	3.0E-28	17.51
Obp83g	1.0E-33	12.96	*Obp57c*	3.0E-42	81.58
Obp85a	5.0E-19	2.37	*Obp59a*	4.0E-31	12.58
Obp99a	6.0E-21	2.69	*Obp69a*	2.0E-31	12.42
Obp99b	2.0E-43	296.24	*Obp83b*	2.0E-18	2.13
Or30a	1.0E-16	2.38	*Obp84a*	3.0E-18	4.14
Or43a	3.0E-24	2.38	*Obp8a*	1.0E-32	12.03
Or67a	8.0E-23	2.36	*Or1a*	4.0E-25	3.95
Or83b	2.0E-21	2.12	*Or22a*	1.0E-13	2.03
Or83c	1.0E-22	2.42	*Or22b*	2.0E-14	2.13
Os9	5.3E-08	2.04	*Or45a*	2.0E-28	7.15
			Or47a	6.2E-06	2.52
			Or59b	4.0E-24	5.28
			Or63a	1.0E-25	3.58
			Or92a	7.0E-19	2.32
			Or9a	2.0E-18	2.43

Table 2.2 Genes that show sexual dimorphic expression

Female>Male	*P-Value*	Fold Change	Male>Female	*P-Value*	Fold Change
Gr2a	1.2E-05	1.93	*Gr22f*	1.0E-11	4.77
Gr5a	1.4E-07	3.40	*Gr59a*	9.0E-42	20.05
Gr10b	2.0E-19	1.67	*Gr59f*	4.0E-23	8.07
Gr21a	1.0E-49	3.88	*Gr64c*	3.0E-06	2.05
Gr22a	2.5E-05	2.03	*Gr64d*	5.0E-15	2.80
Gr22c	2.0E-11	2.33	*Gr64e*	9.4E-08	2.24
Gr22d	1.3E-05	2.48	*Gr65a*	1.0E-11	5.77
Gr22e	2.6E-05	1.28	*Gr98a*	1.9E-05	6.49
Gr23a	6.0E-54	12.85	*Obp8a*	3.0E-17	1.85
Gr33a	2.0E-68	4.14	*Obp19b*	1.8E-07	1.28
Gr36b	3.0E-12	3.25	*Obp19d*	7.3E-08	1.26
Gr36c	8.0E-17	5.39	*Obp22a*	4.0E-46	9.03
Gr43a	3.0E-23	3.45	*Obp50a*	2.0E-14	4.77
Gr58a	3.3E-07	3.36	*Obp50b*	8.0E-50	22.65
Gr59e	6.0E-21	4.09	*Obp50c*	4.0E-55	21.61
Gr61a	6.0E-23	4.14	*Obp50d*	6.0E-26	13.34
Gr68a	3.0E-16	5.96	*Obp51a*	2.0E-25	11.57
Gr77a	2.8E-09	1.36	*Obp56c*	5.1E-05	2.25
Gr85a	1.6E-09	1.59	*Obp56d*	2.0E-29	1.71
Obp18a	5.0E-13	2.32	*Obp56f*	1.0E-48	8.34
Obp19a	4.0E-14	2.48	*Obp56g*	5.0E-51	8.08
Obp19c	2.0E-55	180.71	*Obp56i*	5.0E-42	14.11
Obp28a	3.8E-08	1.69	*Obp57b*	1.0E-16	1.22
Obp46a	4.0E-21	12.53	*Obp57d*	4.0E-06	1.83
Obp47a	7.4E-07	2.31	*Obp58b*	2.0E-27	7.29
Obp47b	1.7E-07	4.10	*Obp58c*	8.0E-27	14.73
Obp50e	5.0E-13	1.63	*Obp59a*	2.0E-17	3.07
Obp56a	8.0E-21	2.42	*Obp76a*	2.0E-30	1.93
Obp56e	2.0E-28	7.21	*Obp83a*	7.2E-09	1.28
Obp57a	2.0E-58	5.34	*Obp83b*	9.0E-28	1.80
Obp57c	6.0E-13	1.47	*Obp93a*	2.0E-17	5.69
Obp83cd	5.0E-59	3.19	*Obp99b*	9.0E-48	9.73
Obp83ef	4.0E-40	2.65	*Obp99d*	2.0E-13	2.26
Obp83g	5.0E-05	1.61	*Or45a*	9.0E-17	4.04
Obp84a	2.0E-69	5.15	*Or45b*	6.0E-16	3.95
Obp99a	1.0E-40	3.01	*Or46a*	6.0E-10	3.64
Or7a	4.7E-07	2.59	*Or47a*	3.3E-05	2.20
Or24a	3.1E-06	2.13	*Or56a*	1.0E-26	5.45
Or43a	3.1E-05	2.27	*Or59b*	2.0E-25	10.07
Or43b	1.0E-55	5.33	*Or63a*	8.0E-19	3.59
Or49a	8.9E-08	1.83	*Or67c*	8.0E-10	4.20
Or59c	2.0E-12	1.72	*Or94a*	7.0E-08	3.36
Or65a	6.3E-08	1.43			
Or83b	5.0E-21	2.21			
Os9	8.0E-18	2.08			
smi21F	1.0E-13	1.98			

Table 2.3 MMC analysis of array data

Gene	Module	Average Degree[1]	Degree[2]
Obp56f	1	0.97628	0.97628
Obp56i	1	0.97628	0.97628
Gr59f	2	0.95915	0.95915
Obp56g	2	0.95915	0.95915
Obp58b	3	0.91491	0.91491
Obp58c	3	0.91491	0.91491
Obp76a	4	0.89548	0.93586
Or67d	4	0.89548	0.93569
Gr64a	4	0.89548	0.89577
Gr64c	4	0.89548	0.8146
Obp83cd	5	0.89485	0.89485
Obp83ef	5	0.89485	0.89485
Gr65a	6	0.87775	0.87775
Obp50a	6	0.87775	0.87775
Obp8a	7	0.86127	0.90711
Obp59a	7	0.86127	0.84196
Obp83a	7	0.86127	0.83473
Gr22d	8	0.84434	0.8756
Obp56b	8	0.84434	0.87505
Obp57c	8	0.84434	0.87286
Obp56c	8	0.84434	0.86766
Obp19a	8	0.84434	0.86038
Obp47a	8	0.84434	0.85761
Obp57a	8	0.84434	0.79842
Obp58d	8	0.84434	0.7471
Or74a	9	0.81246	0.86926
Or82a	9	0.81246	0.81075
Or67b	9	0.81246	0.75735
Gr23a	10	0.80828	0.83235
Gr22c	10	0.80828	0.82706
Obp84a	10	0.80828	0.80657
Or23a	10	0.80828	0.76713
Gr98b	11	0.78903	0.85634
Gr39a	11	0.78903	0.84736
Gr85a	11	0.78903	0.83532
Gr98d	11	0.78903	0.80146
Or85d	11	0.78903	0.79327
Or22c	11	0.78903	0.72362
Gr22b	11	0.78903	0.66587
Or1a	12	0.77267	0.77267
Or22a	12	0.77267	0.77267
Gr10b	13	0.77114	0.82454
Gr21a	13	0.77114	0.79357
Or65b	13	0.77114	0.79189

Table 2.3 Continued

Gr10a	**13**	**0.77114**	**0.78332**
Gr66a	13	0.77114	0.76062
Or67c	13	0.77114	0.67293
Gr36a	14	0.73899	0.84691
Or33b	14	0.73899	0.8377
Gr64e	14	0.73899	0.81836
Gr39b	14	0.73899	0.8135
Or94b	14	0.73899	0.81179
Or30a	14	0.73899	0.80825
Or35a	14	0.73899	0.79944
Gr58a	14	0.73899	0.79711
Or98b	14	0.73899	0.7866
Or85c	14	0.73899	0.78646
Or24a	14	0.73899	0.78493
Obp57b	14	0.73899	0.78248
Or13a	14	0.73899	0.7761
Or65c	14	0.73899	0.77282
Gr22a	14	0.73899	0.77125
Gr47b	14	0.73899	0.76015
Obp19d	14	0.73899	0.75852
Or46a	14	0.73899	0.74523
Gr93b	14	0.73899	0.74277
Or98a	14	0.73899	0.73602
Gr92a	14	0.73899	0.70441
Gr5a_Tre	14	0.73899	0.69869
Or85b	14	0.73899	0.69341
Or33a	14	0.73899	0.68639
Gr57a	14	0.73899	0.68179
Or88a	14	0.73899	0.66493
Gr64f	14	0.73899	0.63018
Obp19b	14	0.73899	0.61431
Or47a	14	0.73899	0.32024
Obp49a	15	0.7337	0.82903
Obp28a	15	0.7337	0.78494
Obp99d	15	0.7337	0.78315
Obp56d	15	0.7337	0.75847
Obp56h	15	0.7337	0.75561
Obp57d	15	0.7337	0.74906
Obp99b	15	0.7337	0.74092
Obp56a	15	0.7337	0.71767
Obp83b	15	0.7337	0.71028
Obp93a	15	0.7337	0.67891
Obp83g	15	0.7337	0.56262
Obp50b	16	0.68812	0.7942
Obp50c	16	0.68812	0.79415
Gr59a	16	0.68812	0.78853
Obp51a	16	0.68812	0.78398
Gr32a	16	0.68812	0.78212

Table 2.3 Continued

Or45a	**16**	**0.68812**	**0.7612**
Obp22a	16	0.68812	0.74552
Obp50d	16	0.68812	0.73714
Obp47b	16	0.68812	0.7126
Or94a	16	0.68812	0.71181
Obp19c	16	0.68812	0.71056
Obp46a	16	0.68812	0.70693
Gr22f	16	0.68812	0.70133
Or59b	16	0.68812	0.69108
Gr68a	16	0.68812	0.68581
Gr43a	16	0.68812	0.6841
Or63a	16	0.68812	0.65644
Gr61a	16	0.68812	0.62775
Gr98a	16	0.68812	0.62122
Or71a	16	0.68812	0.62071
Or56a	16	0.68812	0.60949
Or45b	16	0.68812	0.53513
Obp57e	16	0.68812	0.36495
Obp69a	17	0.68311	0.79334
Obp85a	17	0.68311	0.78867
Gr22e	17	0.68311	0.78686
Or67a	17	0.68311	0.78044
Gr93d	17	0.68311	0.76745
Gr2a	17	0.68311	0.74634
Gr58c	17	0.68311	0.73206
Or65a	17	0.68311	0.71128
Obp18a	17	0.68311	0.69962
Or43a	17	0.68311	0.6489
Or83c	17	0.68311	0.63321
Or83b	17	0.68311	0.53475
Gr8a	17	0.68311	0.4869
Or85f	17	0.68311	0.4537
smi21F/Pino	18	0.67328	0.72713
Obp99a	18	0.67328	0.72134
Os9/Hf	18	0.67328	0.72091
Or43b	18	0.67328	0.71255
Obp56e	18	0.67328	0.70537
Obp99c	18	0.67328	0.69636
Obp50e	18	0.67328	0.61174
Gr33a	18	0.67328	0.49081
Gr59c	19	0.66409	0.7831
Or19b	19	0.66409	0.76974
Or42b	19	0.66409	0.76252
Gr59e	19	0.66409	0.75722
Gr94a	19	0.66409	0.75511
Or42a	19	0.66409	0.75475
Or19a	19	0.66409	0.74705
Or2a	19	0.66409	0.74437

Table 2.3 Continued

Or9a	**19**	**0.66409**	**0.73575**
Gr77a	19	0.66409	0.73205
Gr59b	19	0.66409	0.72961
Or7a	19	0.66409	0.72145
a10	19	0.66409	0.71798
Or92a	19	0.66409	0.71301
Gr98c	19	0.66409	0.70764
Gr59d	19	0.66409	0.6871
Or22b	19	0.66409	0.68527
Gr58b	19	0.66409	0.68412
Or83a	19	0.66409	0.67207
Or59a	19	0.66409	0.6674
Gr28b	19	0.66409	0.66623
Gr97a	19	0.66409	0.65741
Or85a	19	0.66409	0.65662
Gr36b	19	0.66409	0.65297
a5	19	0.66409	0.63082
Or10a	19	0.66409	0.58843
Gr93c	19	0.66409	0.58634
Or49a	19	0.66409	0.51798
Or59c	19	0.66409	0.42858
Gr47a	19	0.66409	0.40584
Gr28a	19	0.66409	0.26819
Or33c	20	0.56638	0.64981
Or69a	20	0.56638	0.64164
Or49b	20	0.56638	0.62421
Gr63a	20	0.56638	0.62267
Gr64b	20	0.56638	0.61222
Gr93a	20	0.56638	0.6032
Gr36c	20	0.56638	0.57009
Or47b	20	0.56638	0.48975
Gr64d	20	0.56638	0.28386

[1]Average absolute correlation between all gene pairs in the module, i.e. $\frac{1}{n(n-1)}\sum_{i\neq j}|r_{ij}|$ for a module of size n

[2]Average absolute correlation between a gene and the rest of its module, i.e. $\frac{1}{n-1}\sum_{i\neq j}|r_{ij}|$ is the degree of gene i

Table 2.4 Primer pairs of array probes

Gene	FlybaseID	5' PRIMER	3' PRIMER
Gr2a	FBgn0027796	GGACACGCTGAGAGCTCTGG	CGCACGAGCTGAGTGGCG
Gr5a/Tre	FBgn0003747	GTGCAATCGTGCTGTTCG	GATGCTCAGCAGGTGTTC
Gr8a	FBgn0030108	CGTGTGGCTGACGTATCT	CGTATCGGCTGATGCAGG
Gr10a	FBgn0045502	GAGCCACCGCCTCCAGCTC	CTTCAAGTTCTGCCTGATC
Gr10b	FBgn0030297	GGTCGCTGTAGGTGTCGTGC	CTACAATCCCACTCGGCGG
Gr21a	FBgn0041250	GGTAGTGCCGATGCTGAA	CAGGAGAAGACGCACAGCG
Gr22a	FBgn0045501	CGGTCAGGATCTACGCTA	GATCCTGGGTCCTAGGAC
Gr22b	FBgn0045500	GGACACCGACATGGTAGTGC	GGATCCTGGCTACTCGGT
Gr22c	FBgn0045499	GTTGCCAGGCAGTCCGTAG	CATCACGTAGCGACTTGCAG
Gr22d	FBgn0045498	GGCAGTCGTAGCAATCGG	CCAGTCTCCTTATTCTGATG
Gr22e	FBgn0045497	GGATGCTATCGACCTGTTC	GTCCATCCGCTCAGACTC
Gr22f	FBgn0041249	CTTGAGGAGCTTAGATGG	GACCCTCTACGCATCCTG
Gr23a	FBgn0041248	GGTGCAGCACGTCATGGA	CGCAGTGAGCAGTCCAAT
Gr28a	FBgn0041247	GAGGTAGCACGTGGCAGC	CGCTACTCACATCGCCTAC
Gr28b	FBgn0045495	CGAATGCTGCTGCAGAGG	GGACGTGCAACTCCAGAC
Gr32a	FBgn0041246	CGTGCAAGGCGAGATCAC	CTCCTTCGACTTGGCATAC
Gr33a	FBgn0032416	CACGTGTCGGTCATCTCGC	GCAGCATAGTCGAAGCAC
Gr36a	FBgn0045487	GCTCAAGACAGAGGTGCG	GCAGCAGATGAACTGCGA
Gr36b	FBgn0045486	GTGGATGCATTGGACCG	CTCCAGTCGAATGTCCAAG
Gr36c	FBgn0045485	GGCTTGCAGTACTGGATG	GGCTTCTTCGAGGCGGAC
Gr39a	FBgn0041244	GCAGATCAGCCGTGTCCTG	CTAAGTAGGCTTGCAAGG
Gr39b	FBgn0041245	CCATCAGATGACACTCTAG	CCTTTCATCCGTACCTC
Gr43a	FBgn0041243	CGGAGAAGTCAGCATCTAC	CAATGCAACCAGTGCCATC
Gr47a	FBgn0041242	CAGCTTGCAGTTCTCAGA	CTGCGATGGTAGCAGTCT
Gr47b	FBgn0041241	GAGTGCCTCAGTTGCTCGGTC	GGCAGATAACGTACACG
Gr57a	FBgn0041240	CGAACTGGATGAGTATGAGC	GCTGCAGCAACTGCTACAGC
Gr58a	FBgn0041239	CTACTGACCGTCCTGCCCT	CTTCTCGGACTAATCGCTG
Gr58b	FBgn0041238	GATGCTGCTATTGCTGTAC	CCTTAGAGCCATCGCTGT
Gr58c	FBgn0041237	CGTGGTGGCTCTGACCAA	CACTGGAAGATGTCAACG
Gr59a	FBgn0045483	GCAGTCGCGGATTACTCG	CTCCAAGTCCATCGATTG
Gr59b	FBgn0045482	CGTGACGCTCATCATGTTGC	GCTGTCTTGGTGAGGCAG
Gr59c	FBgn0041235	GAGTGATCACTGAGCTTAG	GGTGCAGGTGATTGGGCT
Gr59d	FBgn0041236	GATCACGAGCAGGTTATTCC	GGAGCTGGTCAGTCGATG
Gr59e	FBgn0041233	CTGCAGACCTCCAACTCG	GACGTGGTCAACTATGAC
Gr59f	FBgn0041234	GAGTTGGATGATTCTGTGG	GAGCAGTAGGATGGAGTA
Gr61a	FBgn0035167	GGTGGACATTCTGATGCT	GATCAGCGAGTACCAGAAG
Gr63a	FBgn0035468	GGTCAACTTGCTCAGTCG	CACATTGTGCGCTCCCTG
Gr64a	FBgn0045479	CGAATCTGGACGAGAACGG	CTCCAGTAGCTCGCACATC
Gr64b	FBgn0045478	CTGGCATGGAGTGGAGCA	CAGCGAATCCTAAGGAGT
Gr64c	FBgn0045477	GCTCTGATCCTGTTCGTC	CGAATCCTCTGCCAGAAT
Gr64d	FBgn0035486	GCTGAGGAGCCATTTGTC	GGTATACAGCTGAAGGCC
Gr64e	FBgn0045476	CATGATCACCATCGTGGC	GTGGTCGCTTGGACTCATC
Gr64f	FBgn0052255	GCTGGTCTTGATAGTGGC	CTCAGCAACTGGACGCAA
Gr65a	FBgn0041232	GGATCTATTGAGGACACT	GTAGTGCTCATTGCCACG
Gr66a	FBgn0035870	CTCAGGTAGATGAGGTAC	GAGGAACTGGTGGACACG
Gr68a	FBgn0041231	GAGCAACCTCATAGAGGT	GATAGGTAGTAACAGCCG
Gr77a	FBgn0045474	GATCATCCTTGAGTATCTG	CTCAGCTCGGATACATTC
Gr85a	FBgn0045473	CAGCGACAGCCATTCGAT	CCATGAACTACCTGCTGT
Gr92a	FBgn0045471	CGTGTGGCTGACGTATCT	GCCAATCAAGGCAATTAGT
Gr93a	FBgn0041229	GTGGCGCTGCATTGTGGT	CACACTCGTCCAACTCAT
Gr93b	FBgn0045470	GCAGAATGGCGTGGTAGGAA	GCTGTCTGATCTGCAGCG
Gr93c	FBgn0045469	CATTCGTGAACTACTGACC	CTGGTCTACGAACTGTAC
Gr93d	FBgn0045468	GCGTCTGCGATACACGAT	GGAGTTCTCTATGCTGAG
Gr94a	FBgn0041225	CGCCACCACTCTGTACAG	CCACCGTTATTCCGAGCA
Gr97a	FBgn0041224	CCTCGTTGATGTAGTGAT	CAACTATGTGACCCAATG
Gr98a	FBgn0039520	GCACCATCGACCTGAGCA	GTGCTCAACTCTGCTGAG
Gr98b	FBgn0046887	GATGGTACTTGATGACTG	GAGCTTGAAGCATGTCCT
Gr98c	FBgn0046886	CGACTCCGACTGACGCTT	CAGCTGTAGGCAAGCATC
Gr98d	FBgn0046885	GCCTGTGGATAGTGTTGC	GAATGCAGAACTCGCTGT
Obp8a	FBgn0030103	GGAGATCACAGATCGGTT	GCTATGTCACAATAGCTAG
Obp18a	FBgn0030985	CTGTGATGAAGCCCATTG	GCATAGCTGTACTATGGA
Obp19a	FBgn0031109	GGTCTGGAGTCTGGAATC	CACCTGTTCCGAATGCCA
Obp19b	FBgn0031110	GATGCAGTGCAGCCGAATG	GGAGAACATGCAATGGTG
Obp19c	FBgn0031111	GCTGGCGACTGATGCACTGG	GAAGCCATCCACTCCAGT

Table 2.4 Continued

Obp19d/Pbprp2	**FBgn0011280**	**CCAGAGCTGTTGATCGAT**	**CCTGTTGATCTCCTCGTG**
Obp22a	FBgn0043539	GTGGCACATGTCGCGTTG	GCGAGTGTTGCTGGCTTT
Obp28a/Pbprp5	FBgn0011283	CCTCGCAGTGATCATCCG	CACCGACCTAGCATCATG
Obp46a	FBgn0033508	GCGCTTGGTGGGACAGTG	GTGCTCCCAACTGTTCGC
Obp47a	FBgn0033573	CGAGTTCTAGTGCTATTGC	GCCACGAATCGCGTGGCA
Obp47b	FBgn0033614	GCTCAAGATCACGATAACG	GCCAAGCCACGATTGATTG
Obp49a	FBgn0050052	CATGTCAGTGCACTGCTG	CGCAGCTATTACTGCTCG
Obp50a	FBgn0050067	CAAGCGAGCATACGCACA	GGCGTATACTTGTTGCTT
Obp50b	FBgn0050073	GTCTTCGGTGCTGCATCT	GTGCAACTTGACAGCAAT
Obp50c	FBgn0050072	CCTGCTGATCTGCTCATT	GCGATGACTAGAACCACG
Obp50d	FBgn0050074	GCTTCACAAGCTGACTTG	GCGAATGCTGTATTCCCT
Obp50e	FBgn0033931	GCAACGGAACATGAGGCA	GGAATGTTCGTTGGCGTC
Obp51a	FBgn0043530	CACCAAGTAACCGAGCTC	GCTGTCACTACGCTGTCA
Obp56a	FBgn0034468	CTCAACATGAACTCCTAC	CCCGAATCACAATTTGCC
Obp56b	FBgn0046880	GTCACAGGTGGCGGCTGA	CTTATCTACTTGTTGGTTG
Obp56c	FBgn0046879	GACTTAGCGGTGGTCATT	GTTCCAGATCCGCCAAATG
Obp56d	FBgn0034470	CGGAGATTTGGCGCTTAG	GCAGGAGGGAATCACCAA
Obp56e	FBgn0034471	CAGCAGATTACGATCAGC	GCACGCCTATTTGGGAAG
Obp56f	FBgn0043533	TGCTATCTGGCTCCAAGC	GCTTGGTAATGGACTGAT
Obp56g	FBgn0034474	GGCAGTGTCACAGGCATT	GGCTACATTCGCATTGAC
Obp56h	FBgn0034475	CCGGACTTTCGCCAAATA	CTGGAATGGCCTTGTGCT
Obp56i	FBgn0043532	CACCTGCTGTGCATTATTG	CCTCTGTGATTTCGAGTT
Obp57a	FBgn0043535	GGAAAGCCAACCCTTTGA	AAACAGATCCGCCTGCTG
Obp57b	FBgn0043534	GTTCATCTACAGACTTGT	GCACACATCACCAGTCTA
Obp57c	FBgn0034509	GAGGTGCTACTGTGCTAG	GACCTCATCGCTCTGCTC
Obp57d	FBgn0043536	TCTTGCCTTAAACAATTGAA	ATTGCCGATTCTAACGATCC
Obp57e	FBgn0050145	CGCAAGCTTCACATCCTTG	CCAACTTACACTGTGTTTG
Obp58b	FBgn0034768	GGACTCGTGGCTGTCCGA	CAGCACATCCTTGCACTT
Obp58c	FBgn0034769	GGCACTTCTTGCTGAAGG	GATTGCGAGAATACGGAG
Obp58d	FBgn0034770	GGACGTTCCTAATTCTGG	CGCACATATCCTTAGTTCC
Obp59a/Obp58a	FBgn0034766	GCAACTGTATGCATCGCC	GAAGAGCATGTTGCCGTG
Obp69a/Pbprp1	FBgn0011279	GCTGCGAATGCGATGCTT	GCTCTTGGGAATCGTGGA
Obp76a/lush	FBgn0020277	CATCATTTCCGGGGGAAC	GGCGTGCGAGTGTGTTAC
Obp83a/Pbprp3/Os-F	FBgn0011281	CATGCACAACTCGCCTAC	CCTTGTCGCTGCTCAGCG
Obp83b/Os-E	FBgn0010403	GTAGTGGACAGGATCAGC	CAAATACCCACTGATACTAC
Obp83cd	FBgn0046878	CGAACGTCTAGAACGATTC	GCGTAGCAGGTGAACTGT
Obp83ef	FBgn0046876	CTACTGCTCTTCGTTGTCC	CGGTATTCATCTTGGACG
Obp83g	FBgn0046875	CCTGTAAGGCGTACAGTG	CACATTCGCACTTGAGCC
Obp84a/Pbprp4	FBgn0011282	GATAAGTCCGAGATGTGAC	GAGCTTGTGCTGTCATTG
Obp85a	FBgn0037589	GCTCGCATTTCCGATACG	CCCTGGGTCGGTGGTATT
Obp93a	FBgn0038859	CCACATCGCTGTGCAATC	CTGCGATCACGCAAAGCA
Obp99a	FBgn0039678	GTCTTCGCTCGATCGCTG	GAATCGAGAAGGAATGCT
Obp99b	FBgn0039685	GACAAGCGATCAGATGGT	CATACATCAGCATGAAGG
Obp99c	FBgn0039682	CATCATGCACTGGTGTCC	CTCAGTTCAGAACCTCAAC
Obp99d	FBgn0039684	GAATCACTTGAGACTGGAG	GACTCATGAAGTCCTTGAC
Or1a	FBgn0029521	GATGCACGGACTACAGAT	CCACAAATGCGATCTCCA
Or2a	FBgn0023523	CAGTGCTGTGTACTACCAC	CGTGAGCAGGCAGAGAAAC
Or7a	FBgn0030016	CACTGGCACTGGAAGATG	GCTATGCCATCTACTCCT
Or9a	FBgn0030204	CATCATGATGTACGACACG	GCACTCTGCGTGGACTCG
Or10a	FBgn0030298	GCTGAACGAGTCGACGTT	CACCCTTCAATATGACTA
Or13a	FBgn0030715	CGTGGTACGTCATTGCAT	CTGCGATTCGTTGGTGTA
Or19a	FBgn0041626	CAGCAGAGTAAGCATGGT	CACTTGTCCTCAATCTGA
Or19b	FBgn0062565	CCTTCCACATCTGCATCT	CTGGTGGTCGTGGATGTA
Or22a	FBgn0026398	CTCGAGATTGGAGTCAAC	GACCCAGTACAGTACCGA
Or22b	FBgn0026397	GAGACAGGAGGCTAAGATG	CTGCATAGATACGCAGGAC
Or22c	FBgn0026396	CCATCTGCTCTTCGTGTT	CCTGCGAATGTCCTGCTG
Or23a	FBgn0026395	GGTACTGGAGTTGGTCGA	GCAGTTGACCAGATCCTG
Or24a	FBgn0026394	GCACAAGTGTAGACCACA	CACTGGCAACGCAACTAAC
Or30a	FBgn0032096	GGTAGCACTCTGAAGCTA	GCAACACTCGACACATGA
Or33a	FBgn0026392	CAAGTCTTCAGGAGTCTG	GTTGGCTAAGATGTAAAG
Or33b	FBgn0026391	GGATGTCTGCAAGGGTCT	GTGATCCTCGATGCTTTCG
Or33c	FBgn0026390	GGATCTGCATGCGATTGC	GACCACAAATGGACATGTC
Or35a	FBgn0028946	GTCGCTGCATCGAAGTGA	CAAGAGGGACTTGCAGTTG
Or42a	FBgn0033041	GTAGGAAGTACATGAGTG	GCGATCTTCATTGGAAGG
Or42b	FBgn0033043	CTTATGTTGCTGCTCATGG	CTGGCAGCTGTACAATCC
Or43a	FBgn0026389	GAGTTCCTGCCTCGTACC	GTCGGTGCTCTGGTATCG
Or43b	FBgn0026393	CTGCAGGAACTGGATGAC	CTGGACGGACTACTGCAC
Or45a	FBgn0033404	CTCATCCTGTCCTTGATC	CTCCGCCATTCAGATCCT
Or45b	FBgn0033422	CGTGGCTTCACCTCTTGT	GGCGTACACAGTGACCTG
Or46a	FBgn0026388	CTACTGCTATCAGTGTCTG	CAAGGTCGAGTGAAGGCG

Table 2.4 Continued

Or47a	**FBgn0026386**	**CTGCTGTCAGCTTGGATAC**	**CATTGTGATGTAGGACATCG**
Or47b	FBgn0026385	CTGGTGGAAGCGAACCAC	TACATGCATTTGCGTTGC
Or49a	FBgn0033727	GCTATCTGCTTGTGCGAG	GCTTGAGACGCACATCAT
Or49b	FBgn0028963	GAGTACGAGAGTCCGTAC	CCCTCATCATCATGAACA
Or56a	FBgn0034473	CCATCTCCTCCACTCGCT	GCATCGATTTGGCTTAGCTG
Or59a	FBgn0026384	GAAGTACATCCGTGCCAT	GGATGTGCTGGAGATGGA
Or59b	FBgn0034865	CGATCAGCATGTTGCAGG	CGACAGCGATCGCGAGAGG
Or59c	FBgn0034866	CGATGATCCTGAATGCAG	GCTCGGACTGAGCCTCAC
Or63a	FBgn0035382	GCGTGAGTAGCTTGGCAT	GTGACCTCATAAGTCCCA
Or65a	FBgn0041625	GTCAATCTGGGACGAGATC	GGTTGCAGCGATCTGTAAG
Or65b	FBgn0041624	GTGATCAGCGATCTGGAC	GTCCTTCCGAGCCTCCAC
Or65c	FBgn0041623	CCTATCGTTCCTCTTGGC	GCACACAAGCCAAGTCAG
Or67a	FBgn0036009	TGGATAACGTCGCGGAAA	CAACCAGGAGTCGCGAAA
Or67b	FBgn0036019	GTAGAGCAGCATGCAGATC	GGAACTGCTGAGCTCAGT
Or67c	FBgn0036078	GATACGACATGCTGATGAC	CTGGATCATGTACTGGGA
Or67d	FBgn0036080	GAAGCGAATCCGCATTACAT	AGCCGCAAAAGGTGTAGAGA
Or69a	FBgn0041622	CGTAGCACGCATCATCAG	CTCGAACTCACAGCAGTTG
Or71a	FBgn0036474	CTGTTGCTTGAGTCGCTG	CAGCATACCGCCGATGTC
Or74a	FBgn0036709	CTCGAGTTCAGTTGGATG	GGTGCTCAACTTCTGGGT
Or82a	FBgn0041621	CCGATAGTACCGCACTCA	GCAACAACGACGACAGTG
Or83a	FBgn0037322	CACTGCGAACATCCTTCA	GTGGCCTGCATATGGTGC
Or83b	FBgn0037324	CACGATCTGGACGAAGGT	CTCGCTGGACACCTACAG
Or83c	FBgn0037399	CCACTCAACTCATACCAG	CTACGTAGTCACGTACATG
Or85a	FBgn0037576	GCAGCCTTATGCAATCGTG	CGCAATCGCTGCTCAGCT
Or85b	FBgn0037590	CGAAGCAGATGACGAACG	GGAAGCAGTCACTGCGTT
Or85c	FBgn0037591	GACGATAGCGAATGTCAG	GTGCTGCTGTTCTGTCAA
Or85d	FBgn0037594	CCTACGATCACAAGTACAG	CATGTCAGCTGCTAGCTG
Or85f	FBgn0037685	GTGCAGTACAGCTACGAG	GATGCTGATGTAGATCCA
Or88a	FBgn0038203	CAGCATGGTGAACGCATC	GTCCCTGCATCACATTGA
Or92a	FBgn0038798	GCAGTTCGGTCTCACAGT	CACGAGCGATGCGGCAAT
Or94a	FBgn0039033	CTATCGCTTCCTGCTCCA	GAAGATGGCACGCAACTGC
Or94b	FBgn0039034	GTACTGCACCTTCCACTG	GGTCTGGCTTTCTCTTCG
Or98a	FBgn0039551	CGAGACAGCACTTATGCT	GTCAATCTGTCAGCTATG
Or98b	FBgn0039582	GCAGCATCTGCTGCATTC	GGCAATCTGGAATAGGGC
Os9/Hf	FBgn0014000	CATGTTGATCAGACAGTC	GTACTTCGAGGAGCTCGA
smi21F/Pino	FBgn0016926	GAGCACTATGCACATTAG	GTTGTGCAGCGTTGAGTC
a5	FBgn0011294	GGATAACGACGAGAATGTC	CACTACTCACTGACACCA
a10	FBgn0011293	GAAGCTTCTCTGCAGTTT	GCATACGATCGATTCGAA
Act5C	FBgn0000042	GTTGCTGCTCTGGTTGTC	GGTCAGGATCTTCATCAG
Gapdh1	FBgn0001091	CTCCTCGACCTTAGCCTT	CAGAAGATCACCGTGTTC
Gal4	/	TGACGAGCAAGGTCTTCG	GCCAAGGGTGTTCCTTCA
LacZ	/	CGATCGCGTCACACTACG	CCGTGGCCTGATTCATTC

REFERENCES

Aloni, R., T. Olender, et al. (2006). "Ancient genomic architecture for mammalian olfactory receptor clusters." *Genome Biol* **7**(10): R88.

Anholt, R. R. H., C. L. Dilda, et al. (2003). "The genetic architecture of odor-guided behavior in Drosophila: epistasis and the transcriptome." *Nat Genet* **35**(2): 180-184.

Anholt, R. R. H. and T. F. C. Mackay (2004). "Quantitative genetic analyses of complex behaviours in Drosophila." *Nat Rev Genet* **5**(11): 838-849.

Ayroles JF, C. M., Stone EA, Jordan KW, Lyman RF et al. (2009). "Systems genetics of complex traits in Drosophila melanogaster." *Nat Genet* **In press**.

Benton, R., K. S. Vannice, et al. (2009). "Variant ionotropic glutamate receptors as chemosensory receptors in Drosophila." *Cell* **136**(1): 149-162.

Berenbaum, M. R. (2002). "Postgenomic chemical ecology: from genetic code to ecological interactions." *J Chem Ecol* **28**(5): 873-896.

Brand, A. H. and N. Perrimon (1993). "Targeted gene expression as a means of altering cell fates and generating dominant phenotypes." *Development* **118**(2): 401-415.

Bray, S. and H. Amrein (2003). "A putative Drosophila pheromone receptor expressed in male-specific taste neurons is required for efficient courtship." *Neuron* **39**(6): 1019-1029.

Buck, L. and R. Axel (1991). "A novel multigene family may encode odorant receptors: a molecular basis for odor recognition." *Cell* **65**(1): 175-187.

Certel, S. J., P. J. Clyne, et al. (2000). "Regulation of central neuron synaptic targeting by the Drosophila POU protein, Acj6." *Development* **127**(11): 2395-2405.

Chen, P. S. (1996). "The accessory gland proteins in male Drosophila: structural, reproductive, and evolutionary aspects." *Experientia* **52**(6): 503-510.

Chintapalli, V. R., J. Wang, et al. (2007). "Using FlyAtlas to identify better Drosophila melanogaster models of human disease." *Nat Genet* **39**(6): 715-720.

Clyne, P. J., C. G. Warr, et al. (2000). "Candidate taste receptors in Drosophila." *Science* **287**(5459): 1830-1834.

Clyne, P. J., C. G. Warr, et al. (1999). "A novel family of divergent seven-transmembrane proteins: candidate odorant receptors in Drosophila." *Neuron* **22**(2): 327-338.

Ferveur, J. F. (2005). "Cuticular hydrocarbons: their evolution and roles in Drosophila pheromonal communication." *Behav Genet* **35**(3): 279-295.

Fishilevich, E. and L. B. Vosshall (2005). "Genetic and functional subdivision of the Drosophila antennal lobe." *Curr Biol* **15**(17): 1548-1553.

Galindo, K. and D. P. Smith (2001). "A large family of divergent Drosophila odorant-binding proteins expressed in gustatory and olfactory sensilla." *Genetics* **159**(3): 1059-1072.

Gao, Q. and A. Chess (1999). "Identification of candidate Drosophila olfactory receptors from genomic DNA sequence." *Genomics* **60**(1): 31-39.

Graham, L. A. and P. L. Davies (2002). "The odorant-binding proteins of Drosophila melanogaster: annotation and characterization of a divergent gene family." *Gene* **292**(1-2): 43-55.

Ha, T. S. and D. P. Smith (2006). "A pheromone receptor mediates 11-cis-vaccenyl acetate-induced responses in Drosophila." *J Neurosci* **26**(34): 8727-8733.

Hallem, E. A. and J. R. Carlson (2006). "Coding of odors by a receptor repertoire." *Cell* **125**(1): 143-160.

Hallem, E. A., M. G. Ho, et al. (2004). "The molecular basis of odor coding in the Drosophila antenna." *Cell* **117**(7): 965-979.

Hekmat-Scafe, D. S., C. R. Scafe, et al. (2002). "Genome-wide analysis of the odorant-binding protein gene family in Drosophila melanogaster." *Genome Res* **12**(9): 1357-1369.

Jones, W. D., P. Cayirlioglu, et al. (2007). "Two chemosensory receptors together mediate carbon dioxide detection in Drosophila." *Nature* **445**(7123): 86-90.

Kambere, M. B. and R. P. Lane (2007). "Co-regulation of a large and rapidly evolving repertoire of odorant receptor genes." *BMC Neurosci* **8 Suppl 3**: S2.

Kurtovic, A., A. Widmer, et al. (2007). "A single class of olfactory neurons mediates behavioural responses to a Drosophila sex pheromone." *Nature* **446**(7135): 542-546.

Malnic, B., J. Hirono, et al. (1999). "Combinatorial receptor codes for odors." *Cell* **96**(5): 713-723.

Matsuo, T., S. Sugaya, et al. (2007). "Odorant-binding proteins OBP57d and OBP57e affect taste perception and host-plant preference in Drosophila sechellia." *PLoS Biol* **5**(5): e118.

McGraw, L. A., G. Gibson, et al. (2004). "Genes regulated by mating, sperm, or seminal proteins in mated female Drosophila melanogaster." *Curr Biol* **14**(16): 1509-1514.

Miyamoto, T. and H. Amrein (2008). "Suppression of male courtship by a Drosophila pheromone receptor." *Nat Neurosci* **11**(8): 874-876.

Morozova, T. V., R. R. H. Anholt, et al. (2006). "Transcriptional response to alcohol exposure in Drosophila melanogaster." *Genome Biol* **7**(10): R95.

Nebert, D. W., D. R. Nelson, et al. (1991). "The P450 superfamily: update on new sequences, gene mapping, and recommended nomenclature." *DNA Cell Biol* **10**(1): 1-14.

Niimura, Y. and M. Nei (2006). "Evolutionary dynamics of olfactory and other chemosensory receptor genes in vertebrates." *J Hum Genet* **51**(6): 505-517.

Ray, A., W. van der Goes van Naters, et al. (2008). "A regulatory code for neuron-specific odor receptor expression." *PLoS Biol* **6**(5): e125.

Robertson, H. M., C. G. Warr, et al. (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., T. F. Mackay, et al. (2005). "Pinocchio, a novel protein expressed in the antenna, contributes to olfactory behavior in Drosophila melanogaster." *J Neurobiol* **63**(2): 146-158.

Stone EA, A. J. (2009). "Modulated modularity clustering as an exploratory tool for functional genomic inference " *PLoS Genetics* **In press**.

Suh, G. S., A. M. Wong, et al. (2004). "A single population of olfactory sensory neurons mediates an innate avoidance behaviour in Drosophila." *Nature* **431**(7010): 854-859.

Tichy, A. L., A. Ray, et al. (2008). "A new Drosophila POU gene, pdm3, acts in odor receptor expression and axon targeting of olfactory neurons." *J Neurosci* **28**(28): 7121-7129.

van der Goes van Naters, W. and J. R. Carlson (2007). "Receptors and neurons for fly odors in Drosophila." *Curr Biol* **17**(7): 606-612.

Vosshall, L. B., H. Amrein, et al. (1999). "A spatial map of olfactory receptor expression in the Drosophila antenna." *Cell* **96**(5): 725-736.

Vosshall, L. B. and R. F. Stocker (2007). "Molecular architecture of smell and taste in Drosophila." *Annu Rev Neurosci* **30**: 505-533.

Wen, Z., S. Rupasinghe, et al. (2006). "CYP6B1 and CYP6B3 of the black swallowtail (Papilio polyxenes): adaptive evolution through subfunctionalization." *Mol Biol Evol* **23**(12): 2434-2443.

Wolfner, M. F. (1997). "Tokens of love: functions and regulation of Drosophila male accessory gland products." *Insect Biochem Mol Biol* **27**(3): 179-192.

Wolfner, M. F. (2002). "The gifts that keep on giving: physiological functions and evolutionary dynamics of male seminal proteins in Drosophila." *Heredity* **88**(2): 85-93.

Xu, P., R. Atkinson, et al. (2005). "Drosophila OBP LUSH is required for activity of pheromone-sensitive neurons." *Neuron* **45**(2): 193-200.

Zhang, X., O. De la Cruz, et al. (2007). "Characterizing the expression of the human olfactory receptor gene family using a novel DNA microarray." *Genome Biol* **8**(5): R86.

Zhang, X., M. Rogers, et al. (2004). "High-throughput microarray detection of olfactory receptor gene expression in the mouse." *Proc Natl Acad Sci U S A* **101**(39): 14168-14173.

CHAPTER THREE

PHENOTYPIC PLASTICITY OF THE DROSOPHILA TRANSCRIPTOME

Phenotypic Plasticity of the Drosophila Transcriptome

Shanshan Zhou[1,3], Terry G Campbell[2,3], Eric A Stone[2,3], Trudy F C Mackay[2,3] & Robert R H Anholt[1,2,3]

[1] Department of Biology, North Carolina State University, Raleigh, NC 27695, USA.

[2] Department of Genetics, North Carolina State University, Raleigh, NC 27695, USA.

[3] W. M. Keck Center for Behavioral Biology, North Carolina State University, Raleigh, NC 27695, USA.

INTRODUCTION

Phenotypic plasticity is the ability of a genotype to give rise to different phenotypes (Bradshaw 1965). Phenotypic plasticity represents the counterpoint of Waddington's concept of environmental canalization (Waddington 1942; Waddington 1959). Whereas phenotypic plasticity allows organisms to adapt rapidly to changing environmental conditions without time lag from natural selection on allelic variants and without cost of selection, environmental canalization buffers environmental perturbations and produces robust phenotypes by reducing phenotypic plasticity and phenotypic variation across different genotypes. The balance between plasticity and robustness is crucial for optimal fitness (Waddington 1959; Gibson 2009). The genetic principles that underlie phenotypic plasticity remain poorly understood. Elucidating the genetic underpinnings of phenotypic plasticity requires that we determine what fraction of the transcriptome is environmentally sensitive, which genes respond to the same or different environmental perturbations, how expression of environmentally sensitive genes is correlated with plasticity of organismal phenotypes, what the relationship is between genetic variance and phenotypic plasticity, whether the same genes affecting phenotypic plasticity for a trait also affect genetic variation for that trait, and whether environmentally plastic and environmentally robust genes evolve at different rates.

Here, we examine environmental plasticity of the Drosophila transcriptome in a synthetic outbred population generated by a round-robin crossing design of 40 inbred wild-derived *D. melanogaster* lines with sequenced genomes and documented whole-genome transcriptional profiles (Ayroles et al. 2009). The majority of the genome shows robust expression across a range of environmental challenges, including different nutritional rearing conditions, physical stress conditions, chemical exposures, social crowding during larval or adult stages, and aging. About 15% of the transcriptome is variable across a wide range of rearing conditions, and this portion of the transcriptome consists of two classes of transcripts. One is genetically highly variable across the 40 original genotypes under standard growth conditions and comprises gene families dedicated to abiotic response mechanisms, as well as a cadre of transcriptional regulators. We describe associations of these transcripts with four fitness-related phenotypes, including development time, life span, starvation stress resistance, and chill coma recovery. The second class shows robust expression across different genotypes. These transcripts are characterized by greater variation both within and across treatments. They are enriched in reproductive functions, show extensive sexual dimorphism, and are rapidly evolving. These transcripts may undergo decanalization as a result of outbreeding and environmental perturbations to increase offspring survival.

RESULTS

Phenotypic Plasticity of the Transcriptome

We collected transcriptional profiles of flies exposed to 20 treatments, including different nutrient or drug supplements, exposure to different physical and social environments, and maintenance at different reproductive states to identify environmentally sensitive transcripts. Of the 18,800 transcripts represented on the microarray, 14,400 (76.6%) generated signal intensities above background under at least one treatment, similar to the proportion of the transcriptome detected in a previous study, in which transcript profiles were obtained separately for the 40 individual genotypes that gave rise to our synthetic outbred population (Ayroles et al. 2009). Analysis of variance of microarray intensity signals at a false discovery rate of 0.05 revealed 1,249 transcripts that showed a significant treatment effect (8.7%), 6,745 transcripts that showed a sex effect (46.8%), and 200 transcripts with a significant treatment by sex interaction term (1.4%). These results indicate that the bulk of the transcriptome is remarkably robust in the face of diverse environmental changes.

We grouped 1,133 transcripts that showed a significant treatment effect, but no treatment by sex interaction, according to their relative expression levels across the 20 conditions (Fig. 3.1). Highest relative transcript abundance levels were observed in flies exposed to low temperature, dopamine, nicotine or high sugar, whereas the lowest relative levels were seen in flies exposed to heat shock or grown on high yeast medium. Surprisingly, overall relative transcript abundance levels are either uniformly higher or lower (more than 70% show higher abundance levels than the median) across the 20 conditions, while starvation stress resistance, aging, larval crowding and exposure to high temperature, result in substantial variation among relative expression levels (Fig. 3.1).

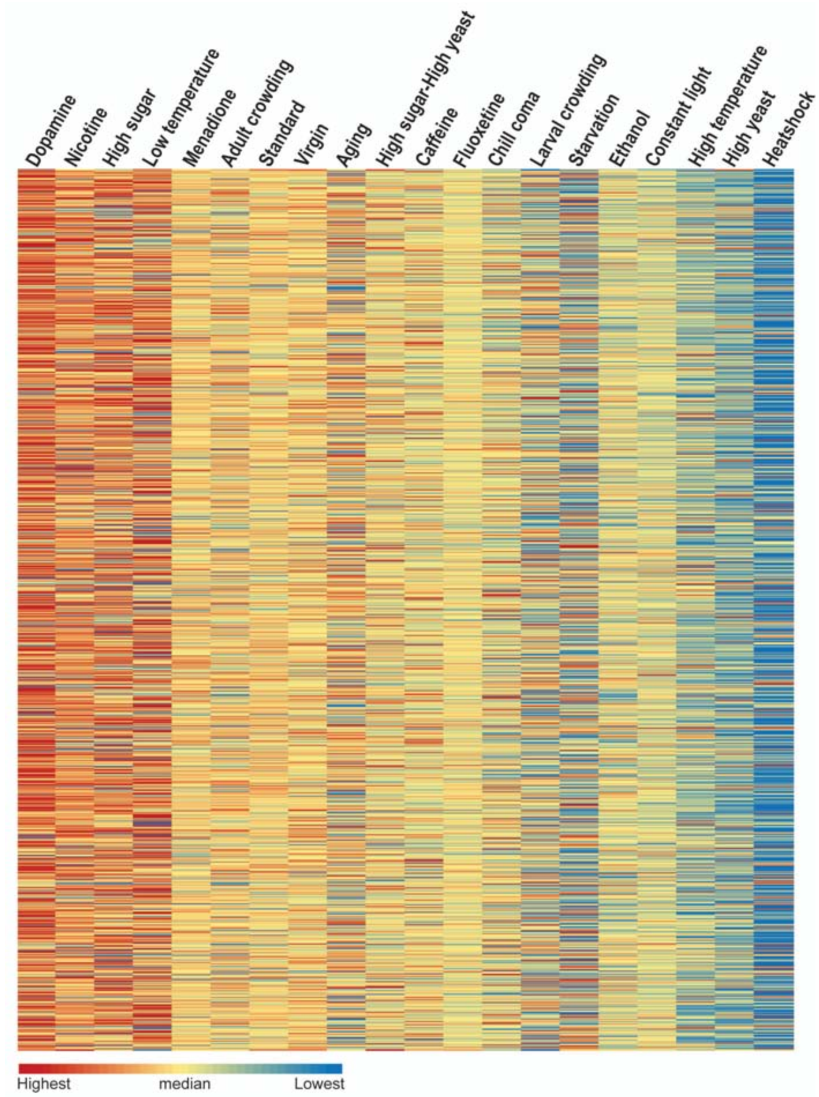

Figure 3.1 Variation of transcript abundance across 20 rearing conditions. The diagram represents 1133 transcripts that show significant differences in expression levels across conditions. An additional 116 transcripts with a significant sex-by-environment interaction have been excluded from the diagram. Transcripts represented in the figure are sex centered. The color scale reflects the rank

order of expression for individual genes across the 20 conditions with red and blue intensities indicating higher and lower expression levels, respectively. The 20 conditions from left to right, are sorted based on the number of genes with transcript levels higher than their median across conditions.

To further examine the relationship between transcript abundance and environmental exposure, we arbitrarily compared transcript abundance levels under the different treatments to the standard rearing condition with post hoc Least Significant Difference (LSD) tests ($p<0.05$). This analysis revealed that heat shock has the greatest impact on transcript levels (589 transcripts), whereas treatment with fluoxetine resulted in changes in expression of only four transcripts (Fig. 3.2a). Many transcripts show altered expression under multiple treatments. For example, 167 transcripts show altered expression both after heat shock and exposure to starvation (Fig. 3.2a). In contrast to these environmentally sensitive transcripts, the majority of transcripts do not undergo significant changes compared to the standard rearing condition (Fig. 3.2b).

Among the 1,133 environmentally sensitive transcripts, 691 encode computationally predicted transcripts of unknown function, 14 probe sets correspond to intergenic regions, non-coding RNAs and transposons, and 428 are annotated transcripts.

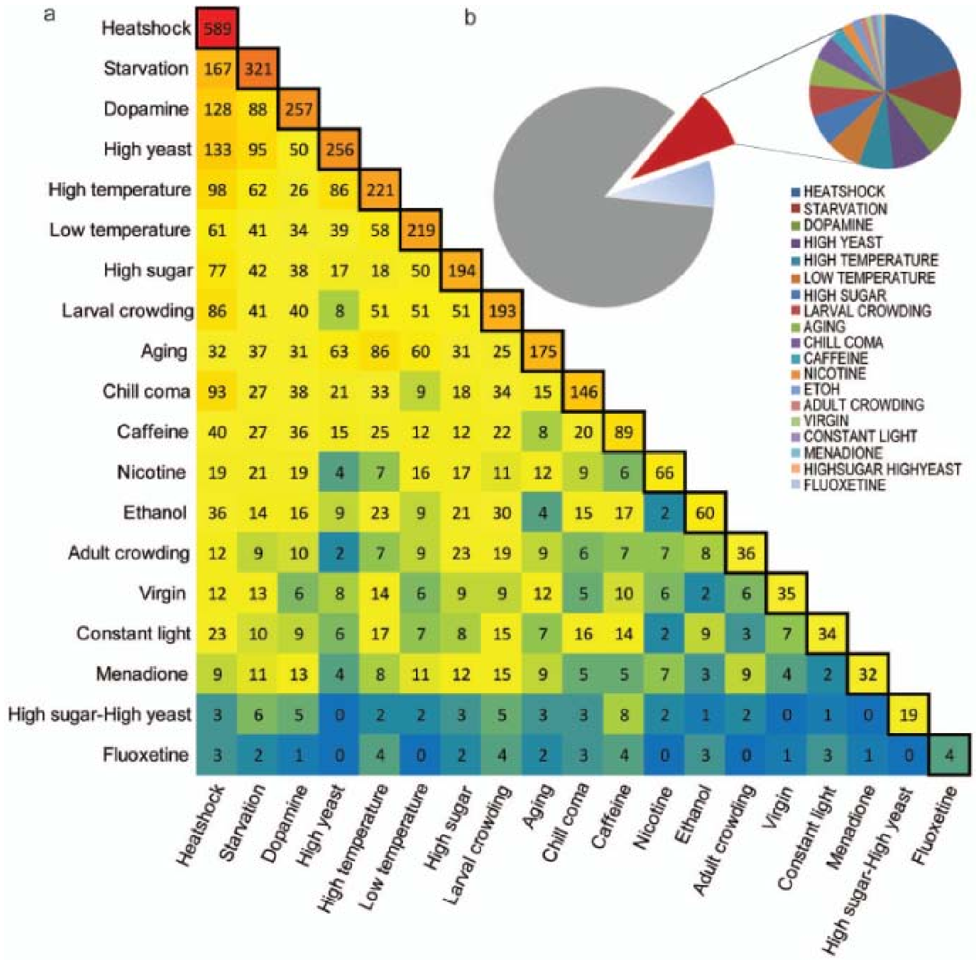

Figure 3.2 Environmentally responsive transcripts across 19 treatments compared to the standard rearing condition. (a) Transcripts with differential expression levels under different experimental treatments compared to their expression under the standard condition. The blue-red color scale accentuates increasing numbers of transcripts. Pair-wise comparisons indicate the number of overlapping transcripts with differential expression under two conditions. (b) Proportion of environmentally responsive transcripts. The gray area of the pie chart indicates the proportion of the transcriptome that does not undergo altered expression under 20 different environmental conditions. The red slice indicates the proportion of Class I transcripts that show differential expression

compared to their expression under the standard rearing condition, and the pie-chart insert shows the proportion of those genes that are affected by each of the 19 treatments. The blue slice indicates the Class II environmentally sensitive transcripts (see also Fig. 3.7)

We assessed changes in transcript abundance levels for each condition relative to the standard growth condition. About half of the environmentally sensitive transcripts show altered expression after heat shock, including 13 *Hsp* transcripts (Fig. 3.3), 60 proteases, 17 members of the cytochrome P450 family (Fig. 3.4), along with two glutathione-S-transferases (Fig. 3.4) and six UDP-glucose glycoprotein glycosyltransferases, (Fig. 3.4) and several immune defense annotated transcripts, including six immune-induced molecules (*IM*) (Fig. 3.3). The 13 *Hsp* transcripts, including *Hsp83,* which complexes with other *Hsp* gene products, e.g. *Hsp70,* are upregulated after heat shock and the majority of these transcripts are also upregulated after induction of chill coma (Fig. 3.3).

Proteases feature prominently among environmentally responsive transcripts. For example, 11 Jonah proteases are downregulated during starvation stress and several are also downregulated following heat shock, treatment with dopamine, and growth on high yeast, whereas nicotine treatment results in upregulation of three members of this protease family (Fig. 3.3).

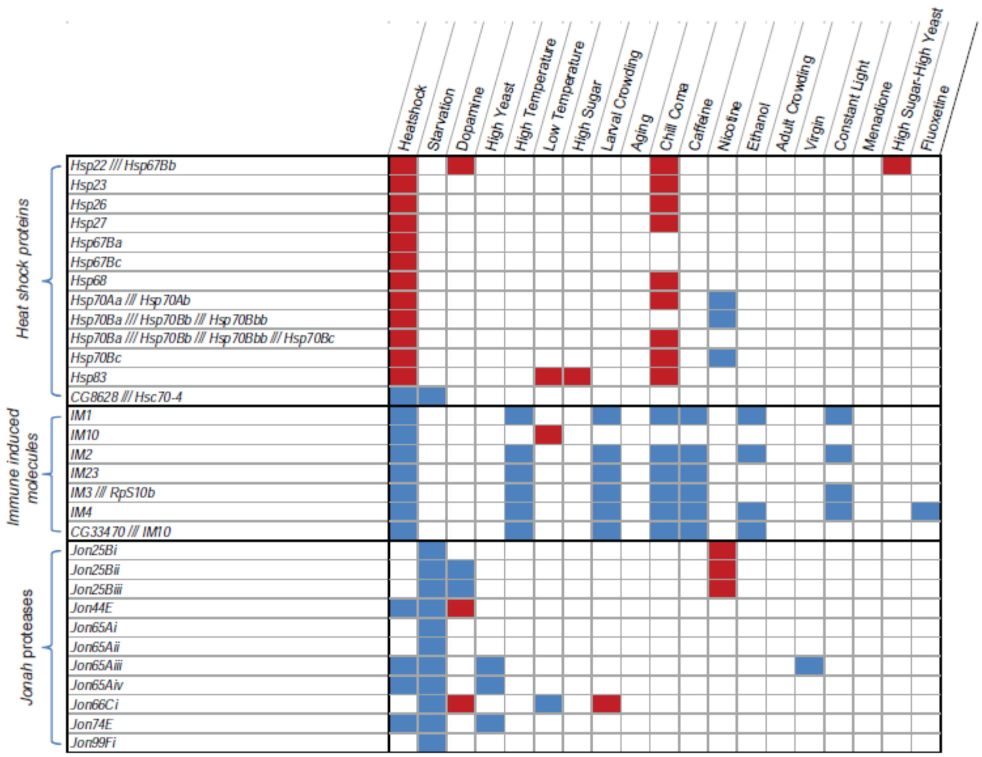

Figure 3.3 Examples of environmentally sensitive gene families. The diagram illustrates up-regulation or down-regulation, indicated by red and blue boxes, respectively, of members of the *Hsp*, *IM*, and *Jon* families under different treatments compared to the standard growth condition.

Members of the family of “immune induced molecules (*IM*)” are down-regulated in response to heat shock, growth at high temperature, larval crowding, chill coma, treatment with caffeine or ethanol, and growth under constant light (Fig. 3.3).

Prominent transcripts that show plasticity in response to different growth conditions are associated with xenobiotic metabolism, including 24 members of the Cytochrome P450 family, 11 Glutathione-S-transferases, and six UDP-glucose-glycoprotein glucosyltransferases (Fig. 3.4). Members of these gene families show environmental plasticity under virtually all conditions, except maintenance of flies as virgins, growth under constant light, and treatment with fluoxetine. Under environmental stress xenobiotic transcripts are primarily downregulated. This is especially notable for heat shock, but also apparent for starvation, growth at high or low temperature, conditions of larval crowding, and chill coma exposure (Fig. 3.4). Whereas members of the *Cyp*, *Gst* and *Ugt* families are upregulated upon chemical exposure, including dopamine, caffeine and nicotine, different transcripts are upregulated under different chemical exposures (Fig. 3.4). *Cyp309a1*, *GstD5* and *Ugt37b1* show altered regulation when flies are grown on ethanol. *Cyp309a1* is also implicated in the response to heat shock. It is of interest that heat shock exposure can induce tolerance to subsequent exposure to ethanol (Scholz et al. 2005). *Cyp4ad1* and *Cyp4d1* show enhanced expression during aging (Fig. 3.4). Seven additional members of the *Cyp* family (*Cyp4ac3*, *Cyp4p2*, *Cyp6g1*, *Cyp9b1*, *Cyp9b2*, *Cyp12a5*, and *Cyp12c1*) show sex-dependent modulation of expression across environments.

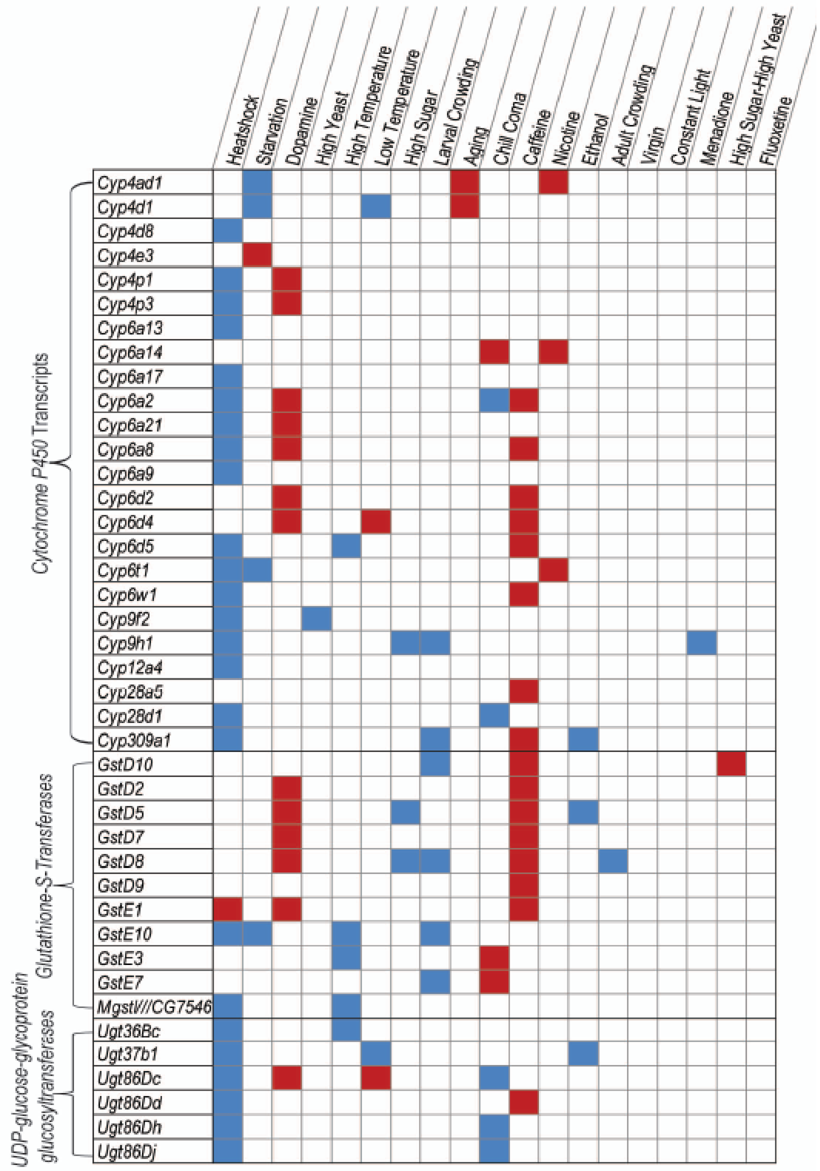

Figure 3.4 Environmentally sensitive transcripts associated with xenobiotic metabolism. Up-regulation or down-regulation of members of the Cytochrome P450, Glutathione-S-transferase and UDP-glucose-glycoprotein glucosyltransferase families under different treatments compared to the standard growth condition is indicated by red and blue boxes, respectively.

A variety of additional transcripts in diverse gene ontology (GO) categories also respond to environmental challenges (Table 3.1). When flies are grown on high yeast environmentally responsive transcripts appear in GO categories related to cellular respiration and energy metabolism, including acetyl-CoA metabolism, the tricarboxylic acid cycle, and electron transport. Growth at 28^{o}C does not induce *Hsp*s, but results in altered abundance of transcripts associated with immune response and energy metabolism. Flies grown at 18^{o}C develop slower and grow larger. Here, transcripts associated with polysaccharide, aminoglycan and chitin metabolism are enriched among environmentally responsive genes. Interestingly, both *period* and *timeless* are modulated under this condition. Transcripts involved in cell morphogenesis and neuron development feature as prominent GO categories when flies are grown on high sugar-supplemented medium. Similar to growth at low temperature, larval crowding also results in altered transcript regulation, related to polysaccharide and aminoglycan metabolism along with immune response related transcripts. Aging modulates expression of genes associated with energy metabolism.

Most transcripts that show altered regulation during adult crowding or when flies are maintained as virgins, under constant light, or after exposure to menadione-induced oxidative stress are unannotated. It is of interest that *per* and *tim* transcripts do not change under constant light compared to the standard 12h light/dark cycle. This reflects most likely the fact that all flies were collected during the light phase. The high yeast-high sugar condition and exposure to fluoxetine elicit small transcriptional responses, not readily amenable to GO analyses.

Modules of Correlated Environmentally Sensitive Transcripts

A previous study on the 40 DGRP lines from which our outbred population is derived demonstrated that the transcriptome (10,096 transcripts) is highly genetically intercorrelated and can be subdivided into 241 co-regulated modules identified by modulated modularity clustering (MMC) (Ayroles et al. 2009; Stone and Ayroles 2009). Here, we used MMC to identify transcripts that co-vary across different treatments (Fig. 3.5a). This analysis partitioned the 1,133 environmentally sensitive genes into 103 relatively small, but highly correlated transcriptional modules (average $|r| > 0.56$). Extensive cross-module correlations are also evident. Negative correlations are rare, in agreement with the overall uniform up- or down-regulation of transcripts (Fig 3.1). All seven *IM* transcripts group together in module 71 (see also Fig 3.3). A putative *IM*, *CG15065*, which is genetically correlated with *IM1* and *IM3*, is also contained (Ayroles et al. 2009) in this module. The *Cyp* transcripts are distributed across multiple modules, which may reflect their specialized functions. However, *Cyp6a21*, *Cyp6a2*, and *Cyp6d5,* along with *Ugt86Dd*, group in module 18; *Cyp6w1* and *Cyp6a8*, co-vary in module 49; *Cyp6a17*, *Cyp12a4*, and *Cyp9f2* cluster in module 68; and, *Cyp4d1* and *Cyp4ad1*, both associated with aging, co-vary in module 101. *GstD2* and *GstD9* cluster with *Cyp6d2* in module 45.

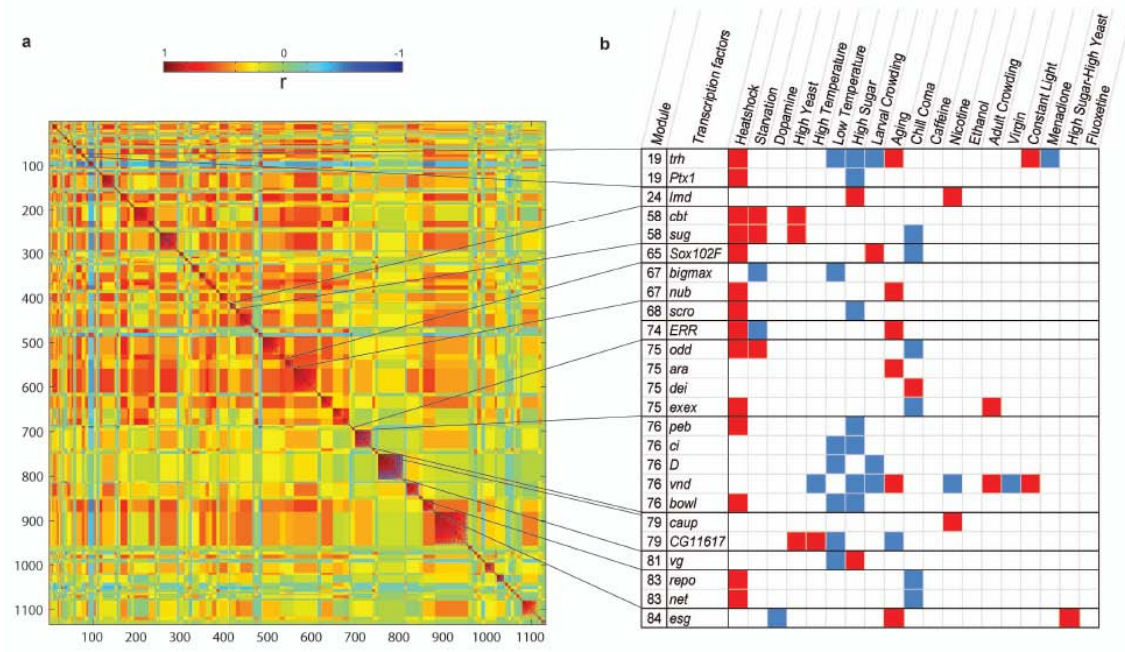

Figure 3.5 Modular organization of the environmentally sensitive transcriptome. (a) Partitioning of the 1,133 environmentally variable genes, shown in Fig. 3.1, into 103 covariant modules by MMC (Stone and Ayroles 2009). The modules populate the diagonal and are ordered by decreasing strength from the upper left to the lower right. Note the pervasiveness of cross-module correlations. (b) Environmentally sensitive transcription factors. The diagram lists 25 environmentally sensitive transcripts that encode transcriptional regulators and the black lines connect these transcription factors to the modules that contain them in panel a (only modules that contain multiple transcription factors are indicated). Red boxes and blue boxes designate up-regulation and down-regulation, respectively, for each treatment compared to the standard growth condition.

Environmentally Sensitive Transcription Factors

What are the cellular mechanisms that regulate transcriptional responses to environmental changes? Among the environmentally responsive transcripts, we identified 26 transcripts that encode transcriptional regulators and 25 of them were differentially expressed compared to the standard growth condition (Fig. 3.5b, Fig. 3.6). Among these transcription factors, five pairs occur together in covariant transcriptional modules, along with two covariant groups of four and five transcription factors (Fig. 3.5b). Fourteen transcription factors are upregulated following heat shock. Six of these (*sug*, *Sox102F*, *odd*, *exex*, *repo* and *net*) are also implicated in the transcriptional response to chill coma, but in this case, are downregulated. Three transcription factors implicated in the heat shock response (*trh*, *nub* and *ERR*) also show increased expression in aged flies. The covariant transcripts for *cbt* and *sug* are upregulated following heat shock, exposure to starvation stress and by growth on high yeast medium (Fig. 3.5b, Fig. 3.6a).

Each transcription factor can exert wide-ranging effects on networks of interacting genes that include other regulatory genes, non-regulatory genes and miRNAs (Bushati and Cohen 2007; Roy et al. 2010) (Fig. 3.6b). Extensive interactions of transcription factors with miRNAs suggest that the latter may also contribute to regulation of environmental plasticity of the transcriptome (Bushati and Cohen 2007; Roy et al. 2010). In addition, seven long non-coding RNAs and intergenic regions show environmental sensitivity and genes that encode several environmentally sensitive transcripts contain overlapping or flanking sequences for short non-coding RNAs. Finally, we note that predicted transcripts of unknown function may also play a role as well as transcriptional regulators that themselves show no change in transcript abundance level, but may be regulated by environment-sensitive posttranslational modifications.

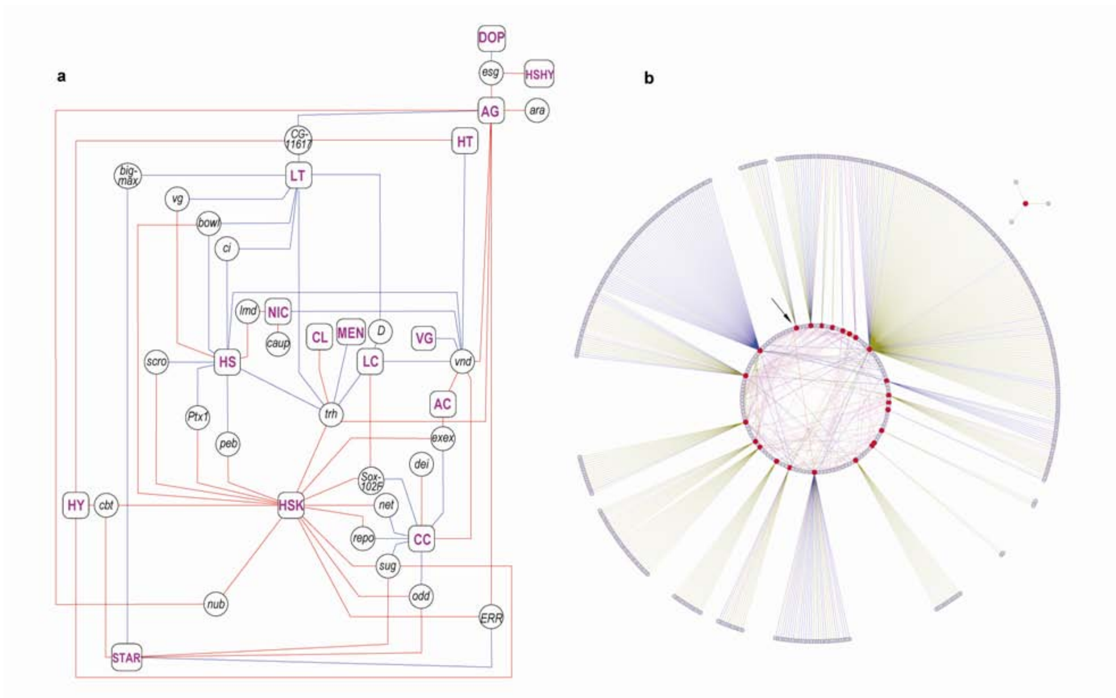

Figure 3.6 Environmentally sensitive transcription factors. (a) Diagram of the relationship between transcription factor regulation and rearing conditions. The 25 environmentally sensitive transcription factor transcripts are shown in circles and 16 treatment conditions are shown in cyan font in rectangular boxes. Red and blue lines designate up- and down- regulation of the transcription factor, respectively, under different treatments. Designations are: HSK, heatshock; STARV, starvation; DOP, dopamine (DOP); HY, high yeast; HT, high temperature; LT, low temperature; HS, high sugar; LC, larval crowding; AG, aging; CC, chill coma; NIC, nicotine: AC, adult crowding; VG, virgin;

CL, constant light; MEN, menadione; HSHY, high sugar-high yeast. (b) Interaction networks of environmentally sensitive transcription factors. Interaction networks of the 25 transcription factors (red nodes) were analyzed through the Drosophila Interaction Database (DroID) (Yu et al. 2008; Murali et al. 2011). The diagram shows protein-protein interactions (green edges), genetic interactions (blue edges), protein-DNA interactions (red edges), interactions that involve both protein-protein, genetic and/or protein-DNA interactions (cyan edges), and interactions with miRNAs (pink edges). Interactions with single transcription factors are shown around the periphery, whereas multiple interactions between transcription factors and interactions between genes (or micro RNAs) and multiple transcription factors are shown inside the circle. Transcription factors are clockwise starting from the arrow: *bowl, ara, caup, cbt, exex, CG1617, trh, ci, net, ERR, lmd, repo, ptx1, scro, Sox102F, sug, vg, D, vnd, dei, odd, nub, esg*, and *peb*. Only three interactions are documented for *bigmax*, shown as a separate diagram.

A second Class of Environmentally Sensitive Transcripts

We compared the previously reported genetic variance and micro-environmental variation (within-line variation) across the original 40 inbred lines (Ayroles et al. 2009) reared under the standard growth condition with the cross-treatment and within-treatment variation of the same transcripts in the reconstructed outbred population. This analysis revealed an additional group of transcripts - not identified by our initial ANOVA due to high within-treatment variation - with high cross-treatment variation, but low genetic variance (Fig. 3.7a, b). We refer to this class of environmentally-sensitive transcripts as Class II transcripts and to the transcripts described above with high genetic variance, high cross-treatment variation, but low within-treatment variation, as Class I transcripts. Class II transcripts show sexual dimorphism, with females (Fig. 3.7b, purple dots) showing higher genetic variance than males (Fig. 3.7a, green dots). We resolved 970 Class II transcripts after filtering; 683 transcripts survived the filtering criteria in males, and 424 in females (Fig. 3.7a, b).

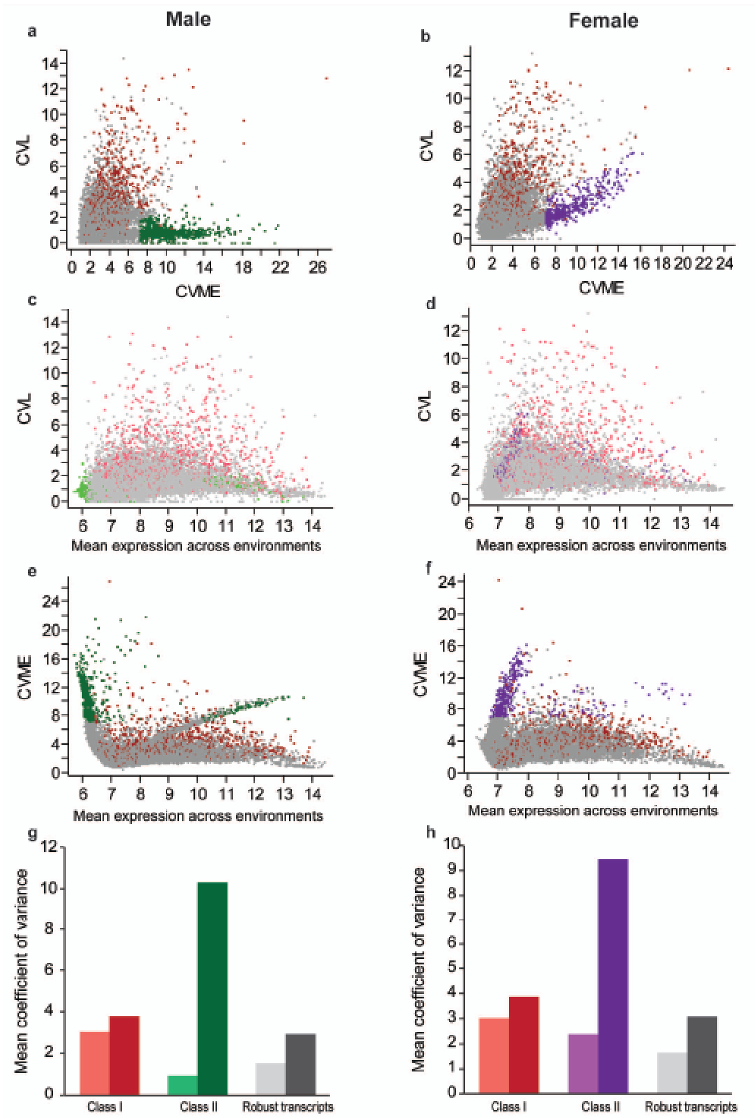

Figure 3.7 Variance analysis and classification of environmentally sensitive transcripts. (a,b) Relationships between coefficients of genetic variance across lines (CVL) and coefficients of

environmental variance across treatments (CVME) in males (a) and females (b). (c,d) Random distributions of coefficients of genetic variance across mean transcript expression levels in males (c) and females (d). (e,f) Correlation structures between coefficients of environmental variance and mean transcript expression levels in males (e) and females (f). Class II transcripts explain the majority of the correlation structure. Red dots indicate Class I transcripts, green and purple dots indicate Class II transcripts in males (a,c,e) and females (b,d,f), and grey dots indicate robust transcripts. (g,h) Average coefficients of genetic variance (light shades) and environmental variance (dark shades) of each transcript class in males (g) and females (h).

Class I transcripts have greater genetic variation for both sexes than environmentally robust transcripts, which are stably expressed both across genotypes and treatments (Fig. 3.7g, h and Fig. 3.8). In males the average genetic variance of Class II transcripts is lower than both Class I and robust transcripts (Fig. 3.7g, Fig. 3.8a), while in females the average genetic variance of Class II transcripts is lower than the Class I but higher than the robust transcripts (Fig. 3.7h, Fig. 3.8b). In contrast to the genetic variance, the average variation across treatments of Class II transcripts is about two times higher than that of Class I and robust transcripts for both sexes, and Class I transcripts have a greater average across-treatment variation than robust transcripts (Fig. 3.7g, h, Fig. 3.8d, e).

Whereas the genetic variation of environmentally robust transcripts is randomly distributed across expression levels, the cross-treatment variance and other environmental variables of Class II transcripts are correlated with their expression levels (Fig. 3.7,c-f, Fig. 3.9a-g). To exclude that this observation is an artifact due to array quality, we examined the correlation between the previously published mean expression levels of transcripts across the 40 inbred lines (Ayroles et al. 2009) and the mean expression level in the reconstituted outbred population across conditions. Transcript means were highly correlated between the two experiments (r =0.960 and r =0.963, for females and males, respectively; Fig. 3.9g, h).

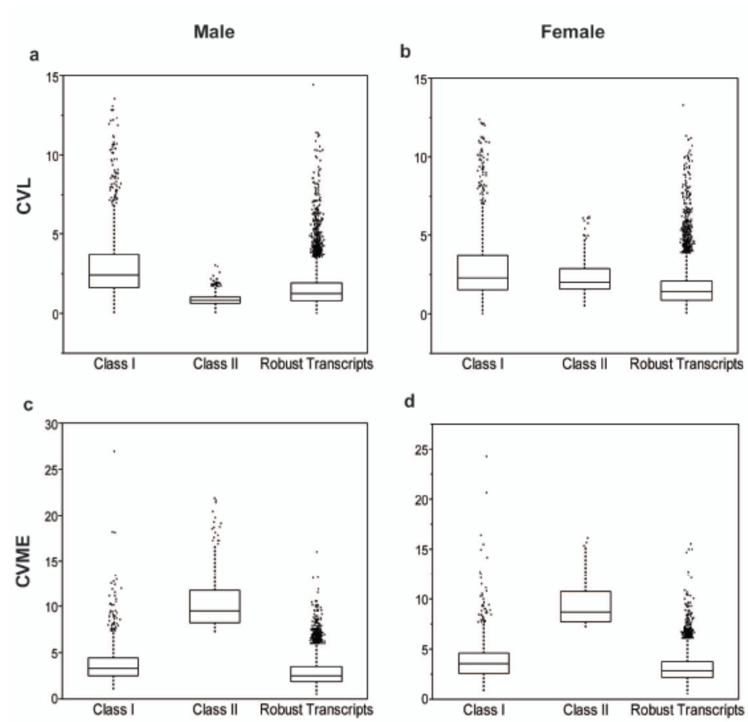

Figure 3.8 Distribution of genetic and environmental variation between transcript classes. (a,b) Box plots of coefficients of genetic variation (CVL) across inbred lines of Class I, Class II and robust transcripts in males (a) and females (b). (c,d) Box plots of coefficients of cross treatment variation (CVME) of the three transcript classes in males (c) and females (d).

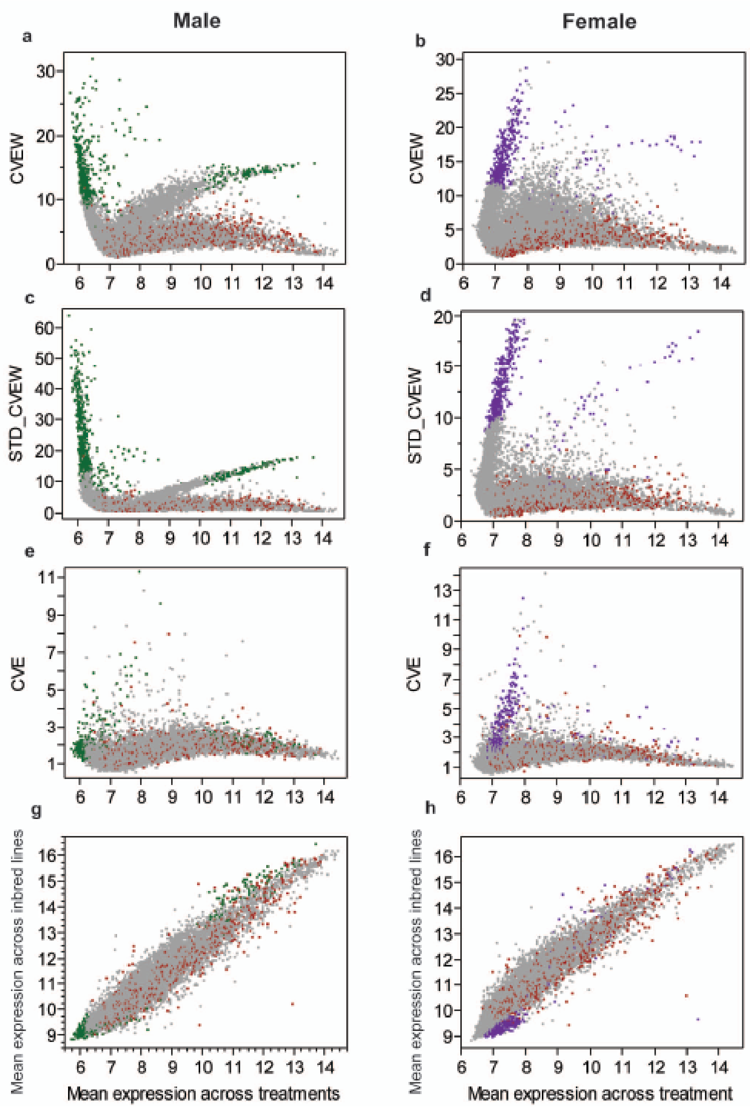

Figure 3.9 Variance analysis and classification of environmentally sensitive transcripts. (a,b) Correlation structures between cross-treatment means of the coefficient of within-treatment variance (CVEW) and mean transcript expression levels in males (a) and females (b). (c,d) Correlation structures between standard deviations of the coefficient of within-treatment variance (STD_CVEW) and mean transcript expression levels in males (c) and females (d). Class I transcripts have significant lower within-treatment variation than Class II transcripts. Transcripts associated with correlation structures that are not explained by Class II transcripts are also distinct from Class I transcripts with

higher within-treatment variation (CVEW) and variance of the within-treatment variations (STD_CVEW). (e,f) Relationships between coefficients of within-line variation (CVE) and mean transcript expression levels in males (e) and females (f). (g,h) Correlations between mean transcript expression across the original 40 inbred lines and the mean transcript expression across the 20 treatments of the reconstituted outbred population in males (g) and females (h). Red dots indicate Class I transcripts, green and purple dots indicate Class II transcripts in males (a,c,e,g) and females (b,d,f,h), respectively, and grey dots indicate robust transcripts.

Class II transcripts can be further classified as high expressed and low expressed transcripts. High expressed transcripts in females overlap only with low expressed transcripts in males, and GO analysis shows that these 32 transcripts encode yolk proteins and chorion proteins and are enriched for oogenesis and sexual reproduction (Table 3.2). Similarly, low expressed transcripts in females overlap only with high expressed transcripts in males, and GO analysis shows that these 100 transcripts encode male-specific proteins, accessory gland proteins and hormones which affect mating and post-mating behaviors (Table 3.3). Further GO analyses indicate that female-specific class II transcripts are enriched in mitochondria- and muscle-related functions, whereas male-specific transcripts are enriched in functions of cuticular structure and DNA replication in meiosis (Table 3.4, 3.5).

Environmental Plasticity of Organismal Phenotypes

We next asked to what extent genetically variable and environmentally sensitive transcripts (Class I transcripts) would be associated with environmental plasticity of organismal phenotypes. We first determined environmental plasticity of four organismal phenotypes that serve as life history and fitness indicators: development time, lifespan, starvation stress resistance and chill coma recovery time. Development is exquisitely sensitive to environmental conditions (Sambucetti et al. 2006; Mensch et al. 2008) (Fig. 3.10a) and is accelerated when flies are grown on medium supplemented with high yeast, and delayed when the medium is supplemented with high sugar. When grown on both high sugar and high yeast medium, development time is identical to that under the standard growth condition. Growth at 28^{o}C also accelerates development, but reduces survival, whereas 18^{o}C delays development about 2-fold. Larval crowding and exposure to the chemical oxidative stress agent menadione bisulfate results in delayed development along with reduced survival. A smaller effect on development time and survival is observed when flies are reared on medium supplemented with 10% ethanol. All other treatments lead to slower development compared to the standard growth condition. We did not observe significant sex differences in environmental plasticity of development time (Fig. 3.10a).

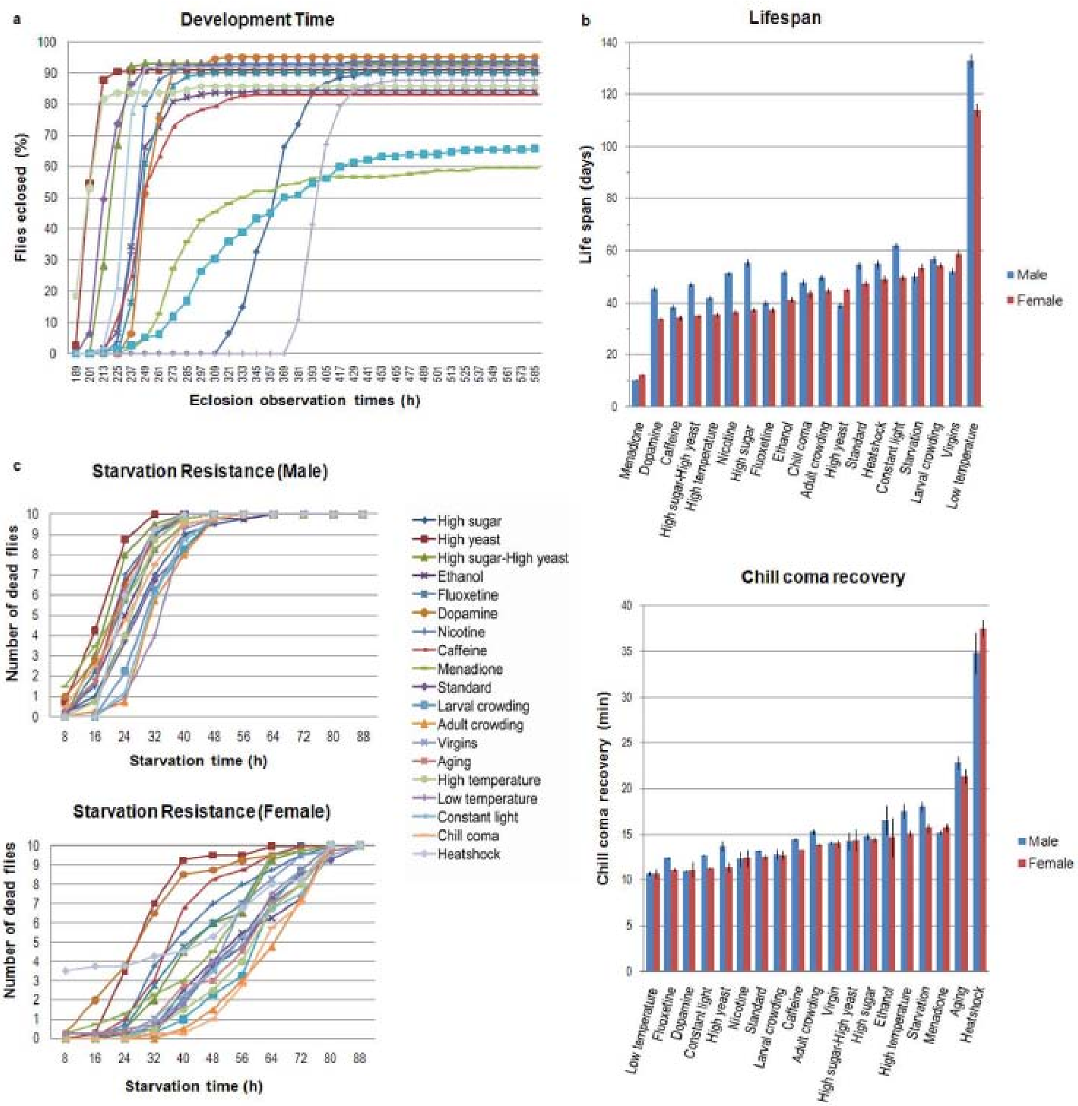

Figure 3.10 Variation for organismal phenotypes under various treatment conditions. (a) Development latency. Development time was assessed under 14 conditions (adult stage treatments were excluded). The X-axis indicates eclosion times after egg collection for sexes pooled. (b) Lifespan. Average survival times were measured for flies reared under 19 different experimental treatments. (c) Starvation stress resistance. The number of dead flies was counted at different times following food deprivation under 19 different treatment conditions. (d) Chill coma recovery time. Average recovery times from chill coma were measured for flies reared under 19 different

experimental treatments. Blue and red bars indicate males and females, respectively. Error bars, s.e.m.

In addition to prolonging development, growth at $18^{o}C$ results in a two-fold increase in lifespan (Fig. 3.10b). Furthermore, virgin females live longer than mated females, as expected (Chapman et al. 1995). Growth at $28^{o}C$, on caffeine-supplemented medium, or on menadione bisulfate reduces life span in both sexes. Female lifespan is shortened when flies are reared on dopamine- or nicotine-supplemented medium, or medium supplemented either with high sugar or with high sugar and high yeast, whereas the lifespan of males is shortened on medium supplemented with high yeast or fluoxetine.

Resistance to starvation stress shows extensive sexual dimorphism, as reported previously (Harbison et al. 2005). When reared under standard growth conditions, subsequent food deprivation allows females to survive twice as long as males (Fig. 3.10c). Starvation stress resistance is reduced in both sexes for flies reared on medium supplemented with dopamine, nicotine, caffeine, or fluoxetine, or on high yeast medium. Previous exposure to heat shock reduces starvation stress resistance in females, whereas exposure to menadione, aging, or previous growth on high yeast/high sugar containing medium reduces survival of males during food deprivation. Survival curves indicate a trend towards longer survival times for both sexes when flies have been maintained at high density, either as larvae or adults, or at 18^{o}C. Exposure to chill coma tends to increase survival time during subsequent food deprivation in females (Fig. 3.10c).

In contrast to starvation stress resistance, ANOVA does not show a significant sex by environment effect for chill coma recovery time. Rearing at 18^{o}C or rearing on dopamine supplemented medium leads to faster recovery after chill-induced coma (Fig. 3.10d). Previous exposure to heat shock extends the chill coma recovery time. Recovery is also delayed as a result of aging, growth at 28^{o}C, previous exposure to starvation stress, growth on ethanol-, menadione-, or high sugar-supplemented medium, and maintenance at high density as adults (Fig. 3.10d).

We found little overlap (~3%) between genetically variable transcripts associated with lifespan, starvation resistance, and chill coma recovery under the standard growth condition (Ayroles et al. 2009) and transcripts associated with environmental plasticity of these traits.

Finally, we observed correlations between the four fitness traits. A longer development time correlates with reduced lifespan and reduced resistance to starvation stress, except when flies are grown at 18^{o}C, 28^{o}C, or on high yeast medium, which prolongs or reduces, respectively, both development and lifespan (Fig. 3.11).

	Development time		Life span		Starvation resistance		Chill coma recovery	
	F	M	F	M	F	M	F	M
High Sugar	⇑	⇑	⇓				⇑	⇑
High Yeast	⇓	⇓		⇓	⇓	⇓		
High Sugar-High Yeast			⇓			⇓		
Ethanol	⇑	⇑					⇑	⇑
Fluoxetine	⇑	⇑		⇓	⇓	⇓		
Dopamine	⇑	⇑	⇓		⇓	⇓	⇓	⇓
Nicotine	⇑	⇑	⇓		⇓	⇓		
Caffeine	⇑	⇑	⇓	⇓	⇓	⇓		
Menadione	⇑	⇑	⇓	⇓		⇓	⇑	⇑
Standard								
Larval Crowding	⇑	⇑			⇑			
Adult Crowding	NA	NA			⇑	⇑	⇑	⇑
Virgins	NA	NA	⇑					
Aging	NA	NA	NA	NA		⇓	⇑	⇑
High Temperature	⇓	⇓	⇓	⇓			⇑	⇑
Low Temperature	⇑	⇑	⇑	⇑		⇑	⇓	⇓
Constant Light	⇑	⇑				⇑		
Chill Coma	NA	NA			⇑		NA	NA
Heat Shock	NA	NA			⇓		⇑	⇑
Starvation	NA	NA			NA	NA	⇑	⇑

Figure 3.11 Comparisons between responses of different organismal traits to different environments. Changes in phenotypic values of development time, life span, chill coma recovery and starvation resistance under different treatment conditions compared to the standard growth condition are indicated by arrows. Values for females (F) and males (M) are shown separately by red and blue arrows, respectively. Grey boxes indicate no significant differences from the standard growth condition. Arrows within grey boxes indicate suggestive trends that did not reach statistical significance.

Transcriptional Modules Associated with Phenotypic Plasticity

We regressed transcript abundance levels of Class I environmentally responsive genes on values of organismal phenotypes and used MMC to construct covariant modules(Stone and Ayroles 2009) (Fig. 3.12). In contrast to the large modules observed for transcriptional variation across genotypes within a single environment(Ayroles et al. 2009), the environmentally responsive transcriptome is highly fragmented in modules that contain on average about 7 covariant transcripts, and the modular organization associated with each trait is distinct. Variation in development time is associated with 426 transcripts, of which 411 (average $|r| > 0.5$) are co-regulated into 36 modules (Fig. 3.12a). Among 186 genes associated with variation in lifespan, 103 (average $|r| > 0.5$) group into 12 modules (Fig. 3.12b). Variation in starvation stress resistance is associated with 320 genes and 309 are highly co-regulated (average $|r| > 0.5$) into 30 modules (Fig. 3.12c). Similarly, transcripts associated with variation in chill coma recovery show a high degree of covariance. Clustering of 440 genes reveals 23 modules (Fig. 3.12d). Modules associated with each trait show high degrees of inter-correlation, and there is evidence for cross-module clustering, indicating hierarchical co-regulation of the environment-sensitive genes (Fig. 3.12).

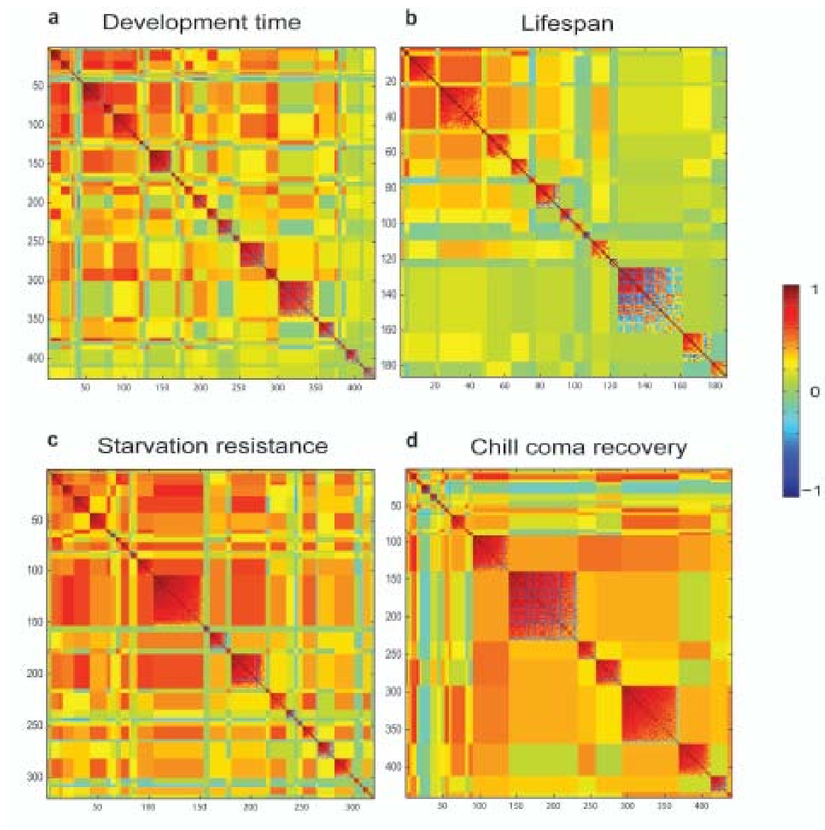

Figure 3.12 Partitioning of correlated environmentally responsive transcripts associated with organismal phenotypes across different rearing conditions by MMC (Stone and Ayroles 2009). (a) Clustering of 426 genes significantly associated with variation in developmental latency into 116 modules. (b) Clustering of 186 genes significantly associated with variation in lifespan into 16 modules. (c) Clustering of 320 genes significantly associated with variation in starvation resistance into 32 modules. (d) Clustering of 440 genes significantly associated with variation in chill coma recovery into 23 modules. The modules populate the diagonal and are ordered by decreasing strength from the upper left to the lower right.

Comparisons of the modular compositions of phenotype-associated environmentally sensitive transcriptional networks show that some modules associated with different organismal phenotypes share covariant transcripts, indicating pleiotropy (Fig. 3.13). For example, the composition of module 17 of the transcriptional signature associated with variation in chill coma recovery shows overlap with multiple modules associated with variation in developmental latency and variation in starvation stress resistance. Whereas pleiotropy at the level of covariant transcriptional modules is prominent between chill coma recovery, starvation stress resistance and development time, it is sparser between lifespan and the other traits (Fig. 3.13).

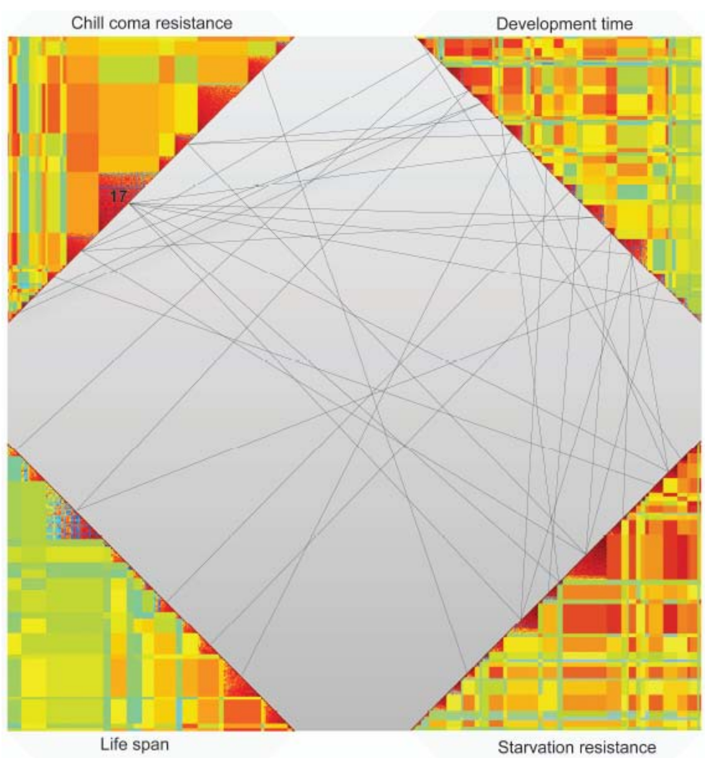

Figure 3.13 Pleiotropy between covariant transcriptional modules associated with four organismal phenotypes. Strength of connectivity within modules along the diagonals increases in a clockwise

direction. Black lines connect modules with similar composition of covariant environmentally responsive transcripts, associated with variation in development latency, lifespan, starvation stress resistance and chill coma recovery time, shown in Fig. 3.10. The significance of modular overlap was determined by a hypergeometric probability test with Bonferroni correction for multiple testing. Modules with fewer than three pleiotropic transcripts were not considered. Module 17 in the chill coma resistance diagram is highlighted as an example of a module that contains a large number of pleiotropic transcripts.

We surveyed the FlyAtlas database (Chintapalli et al. 2007) to assess to what extent expression of covariant Class I transcripts would be tissue-specific (Fig. 3.14). For all organismal phenotypes, covariant transcripts are generally expressed in multiple, but not all tissues. Expression in testes or ovaries is observed only infrequently, whereas expression in the digestive tract (*e.g.* both the larval and adult midgut) is prominent. Expression of some modules is observed in spermatheca. Similarly, the fat body features as a prominent organ for expression of Class I covariant transcripts. Some modules show enriched expression in brain, heart and salivary gland. Thus, the signature of the environmentally responsive transcriptome is not confined to specific tissues, but generally widely distributed (Fig. 3.14).

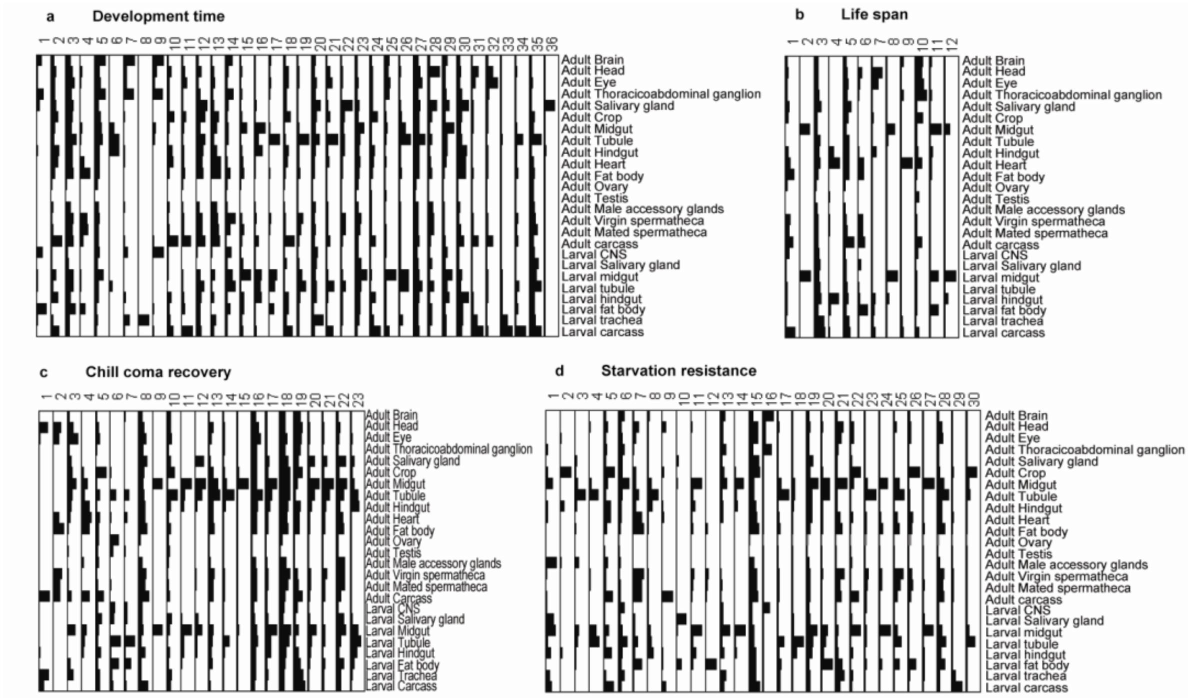

Figure 3.14 Distribution of expression patterns of modules associated with development time, life span and starvation resistance. Tissue specific expression patterns of modules associated with (a) development time, (b) life span, (c) chill coma recovery and (d) starvation resistance were analyzed using the FlyAtlas database.

Conservation of Environmentally Sensitive Genes

To assess to what extent environment sensitive genes are evolutionary conserved compared to the rest of the genome, we surveyed 12 Drosophila species (Clark et al. 2007; Larracuente et al. 2008) (Fig. 3.15). Whereas Class I genes are less conserved across the Drosophila clade than the environmentally robust genome, Class II genes show a dramatically faster rate of evolution as evident both from the percentage of homologues across Drosophila species and the frequency distribution of ω (Fig. 3.15). Thus, environmentally plastic genes appear especially responsive to natural selection.

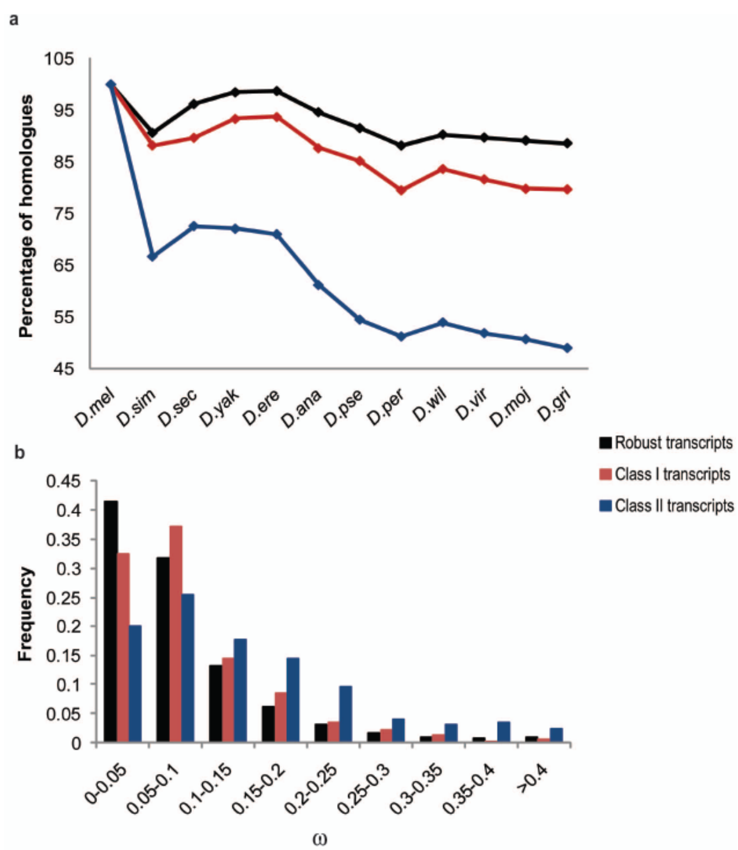

Figure 3.15 Cross species analyses of transcript classes. (a) Percentage of homologues across 12 Drosophila species. (b) Frequency distribution of ω (d_N/d_S). Both Class I (G=34.33, P<0.0001) and Class II (G=334.48, P<0.00001) have significant different distributions from that of the robust transcripts.

DISCUSSION

Genome-wide transcriptional analysis of flies reared under 20 conditions shows that the environmentally sensitive transcriptome comprises about 15% of expressed transcripts. The remainder is environmentally robust. We found two classes of environmentally responsive transcripts. Class I transcripts are genetically and environmentally variable and enriched in functions of detoxification, metabolism, proteolysis and heat shock proteins. Class I transcripts also encode gene products of unknown function, including non-coding RNAs, which may contribute to modulation of chromatin structure and transcriptional regulation.

Class II transcripts have low genetic variance, but greater environmental variation. The high environmental plasticity despite low genetic variation of these transcripts may be due to overdominance and/or epistasis. The reconstituted outbred population has undergone random mating for over 40 generations, resulting in extensive heterozygosity. In contrast to buffering environmental influences, overdominance will increase phenotypic plasticity both within treatment and across treatments. It is possible that the generation of heterozygosity as a result of outbreeding releases cryptic genetic variation due to decanalization (Gibson and Dworkin 2004), thereby increasing epistatic interactions that can lead to increased phenotypic plasticity. Such effects could not be resolved when one examines inbred genotypes in a single environment. High within-treatment variation compared to within-line variation across the genome can be explained by general heterogeneity of the outbred population.

Analysis of covariant regulation among Class I environmentally sensitive transcripts revealed a fragmented modular organization. We assessed the relationship between these transcripts and environmental sensitivity of four fitness related organismal phenotypes. We found distinct transcriptional signatures for development time, lifespan, starvation stress resistance, and chill coma recovery.

Bioinformatics analysis of tissue expression of Class I transcripts indicates broad expression. Modules that comprise transcripts associated with development are frequently expressed in brain, whereas enrichment in brain is less evident for the other traits (Fig. 3.14). Module 8, associated with development time, is enriched exclusively in larval trachea under the standard growth condition, but shows differential expression under various environmental challenges in adults (Fig. 3.14c). These genes could be potential targets for cryptic genetic variation, which only contribute to a phenotype that arises as a result of environmental changes or the introduction of novel alleles (Gibson and Dworkin 2004).

Enrichment of Class II transcripts for reproductive functions suggests that the high environmental responsiveness of these transcripts might affect survival of the next generation.

In a previous study we documented plasticity of the Drosophila chemoreceptor repertoire (Zhou et al. 2009). This study employed customized cDNA arrays, since odorant and gustatory receptor genes were poorly represented on the Affymetrix exon arrays. Along with chemoreceptor genes a vast number of environmentally responsive transcripts belong to rapidly evolving multigene families (Fig. 3.15). Such rapid evolution may involve gene duplication and subfunctionalization, as is evident within the *Cyp* gene family (Ranson et al. 2002; Claudianos et al. 2006; Feyereisen 2006; Richards et al. 2008; Strode et al. 2008). For example, we demonstrated selective induction of *Cyp6a2* and *Cyp6a8* in response to caffeine. The same *Cyp* transcripts have been implicated previously in response to caffeine exposure (Bhaskara et al. 2006). Exposure to DDT results in induction of *Cyp6g1,* and - in the absence of *Cyp6g1* - also *Cyp6a8* (French-Constant et al. 2004).

The similarity in the transcriptional response of *Hsp*s to heat shock and chill coma may reflect a similar need for protection of protein folding under either temperature extreme. Whereas caloric restriction extends lifespan, a single 24h food deprivation period does not affect lifespan. The increase in starvation stress resistance following chill coma recovery may be due to slowing of intermediary metabolism during chill coma and, consequently, preservation of metabolic energy.

Previous studies have characterized genome-wide circadian fluctuations in transcription (Claridge-Chang et al. 2001; McDonald and Rosbash 2001; Storch et al. 2002; Ueda et al. 2002). Since we collected samples for analysis at a single contemporaneous time point, it is possible that additional environmentally responsive transcripts could be identified at different times during the light/dark cycle and that the transcriptional signatures associated with the four organismal phenotypes might reveal dynamic circadian changes. Studies on transcriptional responses to environmental changes in yeast sampled at multiple circadian time points showed responses in expression of about 14.5% of the transcriptome (Gasch et al. 2000; Causton et al. 2001).

Our analysis has not extended to the proteome, nor can we make inferences about the impact of posttranslational protein modifications on the organismal response to environmental challenges. However, the abundance of heat shock proteins and proteases encoded by environmentally sensitive genes may reflect substantial environmental effects on the composition of the proteome.

Our observations indicate that transcriptional responses to environmental changes comprise a broad environmentally responsive group of transcripts together with transcripts tuned to specific treatments, whereas most of the transcriptome shows stable expression under different environments. It has been proposed that Hsp90/Hsp83 may buffer environmental variation and confer phenotypic robustness. Compromised Hsp90 function unleashes cryptic genetic variation resulting in increased phenotypic variation (Rutherford and Lindquist 1998; Ruden et al. 2005; Sangster et al. 2008; Jarosz and Lindquist 2010; Sgro et al. 2010). Our observations vastly extend these notions and suggest that an environmentally responsive segment of the transcriptome may serve as a homeostatic mechanism to confer robustness to the bulk of the transcriptome.

Finally, although we assessed the transcriptional response to a vast variety of conditions and treatments, additional treatments, *e.g.* different chemical exposures or sleep deprivation, might reveal additional features of the environmentally sensitive transcriptome. Nonetheless, our comprehensive analysis has revealed general principles of the response of the Drosophila transcriptome to environmental variation.

METHODS

Fly Rearing

We generated a synthetic outbred population by round-robin crossing of 40 wild derived inbred lines of the Drosophila Genetic Reference Panel (Ayroles et al. 2009), followed by random mating for over 47 generations. For age-synchronization, we randomly collected 1000 females and 1000 males and allowed oviposition for 12h on grape agar plates supplemented with yeast paste. Unless indicated otherwise, 55 eggs were collected and subjected to different treatments throughout development. The standard rearing condition (cornmeal (65g/L) -molasses (45ml/L) -yeast (13g/L)-agar medium at 25^{o}C, 60-75% relative humidity and a 12h light-dark cycle) resulted in hatching of ~50 larvae. Adults were collected immediately after eclosion, and placed at a density of 25 females and 25 males under the desired condition. Flies were transferred onto fresh medium every two days.

Experimental Treatments

For nutritional and pharmacological treatments, flies were reared on standard medium supplemented with 225ml/L molasses (‘high sugar’), 65g/L yeast (‘high yeast’), 225ml/L molasses and 65g/L yeast (‘high sugar-high yeast’), 10% (v/v) ethanol, 200µM fluoxetine hydrochloride, 47mM dopamine, 1mM nicotine, 2mM caffeine or 4mM menadione sodium bisulfite. Different physical environments included constant light, 28^{o}C (‘high temperature’), 18^{o}C (‘low temperature’), and exposure to different stresses, including heat shock (37 ^{o}C for 1h; 1h recovery prior to RNA extraction), chill coma (3h on ice; 1h recovery prior to RNA extraction), and 24h starvation. Different social environments included larval crowding (300 eggs/vial) and adult crowding (80 females and 80 males were pooled in each vial immediately after eclosion). To compare mated with non-mated flies, 50 single sex virgins were reared separately. Aged flies were 30 days old.

Whole Genome Transcript Analysis

We used Affymetrix Drosophila 2.0 arrays to assess whole genome transcriptional profiles. Males and females (3-5 days old) were collected between 1:00 - 3:00 pm by aspiration and immediately frozen on dry ice. RNA was extracted from three independent samples (30 flies/sex/condition), and 10 µg of biotinylated, fragmented cRNA was hybridized to each microarray. RNA extraction, labeling and hybridization across samples was randomized. Raw data were $\log_2$ transformed and normalized across sexes and conditions using a median standardization. For each probe set, we used the median $\log_2$ signal intensity as the measurement of expression. We used negative control probe sets to estimate background intensity. Probe sets with hybridization intensities below background under all different treatment conditions were removed from the analysis.

We analyzed array data using a Generalized Linear Model (GLM) in SAS to partition phenotypic variation between sexes (S, fixed), environments/treatments (E, fixed), the S x E interaction (fixed) and the error variance (ε). To identify environmentally responsive Class I transcripts we used an FDR < 0.05 to correct for multiple tests. Post-hoc LSD tests were used to identify transcripts with a significant environment term. Signal intensities for those transcripts were sex-centered by subtracting the female or male mean across all conditions for each gene. Transcripts with a significant interaction term were excluded. To resolve Class II transcripts, we applied two arbitrary filters. First, we selected transcripts with cross-treatment variance >95th percentile of the cross-treatment variance distribution of the Class I transcripts (coefficients of cross-treatment variation >7.06 and >7.12 for females and males, respectively). We filtered these transcripts further by applying a ratio of coefficient of cross-treatment variance and coefficient of genetic variance >23rd percentile of the ratio of distributions of transcripts that survived the first filtering procedure (ratio>2.47 and >4 for females and males, respectively). Finally, we removed overlapping transcripts between Class I and Class II.

The Modulated Modularity Clustering (MMC) algorithm (Stone and Ayroles 2009) was used to group transcripts into covariant modules. Gene ontology analysis was done using the DAVID bioinformatics database, using the Benjamini correction of $P < 0.05$ as criterion for enrichment (Huang da et al. 2009; Huang da et al. 2009).

Organismal Phenotypes

To measure development time, we allowed flies to lay eggs for 3 hours (10:00am-1:00pm), after which 55 eggs were collected and placed under 14 different growth conditions (300 eggs were collected to assess development time under the larval crowding condition). We counted flies, sexes separately, that eclosed every 12 hours (N=4 vials/condition). Life span was measured by collecting three females and three males immediately after eclosion, transferring them to fresh vials every two days, and recording survival daily (N=26 replicates/condition). To measure starvation stress resistance, we placed ten 3-5 days old flies in vials containing 1.5% agar, and scored survival every 8 hours (N = 4x10/sex/condition). To measure chill coma recovery, we placed 3-7 day-old flies in empty vials on ice for 3 hours, and determined their subsequent recovery time at room temperature by their ability to recover upright posture (N = 2x50 flies/sex/condition).

Transcript-Phenotype Associations

We used regression to identify transcripts with variation in expression levels that associated with organismal phenotypic variation ($P<0.05$). For traits with a significant sex by environment interaction, regression was applied to sexes separately ($Y=\mu + Exp + \varepsilon$, where Exp denotes the covariate median $\log_2$ expression level). For traits without a significant sex by environment interaction, we used sex-centered measures of deviations from female or male means for both expression and organismal phenotypes. We used the residuals from the regressions ($Y= \mu + T + \varepsilon$, where T denotes the trait covariate) to compute environmental correlations between transcripts significantly associated with each organismal phenotype for construction of covariant modules using MMC (Stone and Ayroles 2009).

Table 3.1 Gene ontology analysis of Class I environmental sensitive genes using DAVID

Category	Term	Count	%	PValue	Benjamini
GOTERM_BP_FAT	proteolysis	118	10.8	3.52E-15	5.65E-12
GOTERM_BP_FAT	polysaccharide metabolic process	37	3.4	3.78E-10	3.01E-07
GOTERM_BP_FAT	aminoglycan metabolic process	33	3.0	8.16E-09	4.33E-06
GOTERM_BP_FAT	chitin metabolic process	24	2.2	4.20E-06	1.67E-03
GOTERM_BP_FAT	oxidation reduction	83	7.6	1.07E-05	2.83E-03
GOTERM_BP_FAT	polysaccharide catabolic process	13	1.2	1.06E-05	3.36E-03
GOTERM_BP_FAT	peptidoglycan metabolic process	8	0.7	4.89E-05	9.68E-03
GOTERM_BP_FAT	peptidoglycan catabolic process	8	0.7	4.89E-05	9.68E-03
GOTERM_BP_FAT	aminoglycan catabolic process	12	1.1	4.53E-05	1.02E-02
GOTERM_BP_FAT	defense response	30	2.7	6.12E-05	1.08E-02
GOTERM_BP_FAT	glycosaminoglycan catabolic process	8	0.7	9.03E-05	1.43E-02
GOTERM_BP_FAT	response to temperature stimulus	17	1.6	1.22E-04	1.75E-02
GOTERM_BP_FAT	carbohydrate catabolic process	19	1.7	2.01E-04	2.63E-02
GOTERM_BP_FAT	response to heat	16	1.5	2.93E-04	3.52E-02
GOTERM_CC_FAT	extracellular region	84	7.7	3.40E-13	9.15E-11
GOTERM_CC_FAT	vesicular fraction	26	2.4	1.91E-08	1.71E-06
GOTERM_CC_FAT	microsome	26	2.4	1.91E-08	1.71E-06
GOTERM_CC_FAT	membrane fraction	29	2.7	1.42E-08	1.91E-06
GOTERM_CC_FAT	insoluble fraction	29	2.7	3.57E-08	2.40E-06
GOTERM_CC_FAT	cell fraction	29	2.7	6.88E-08	3.70E-06
GOTERM_CC_FAT	extracellular region part	31	2.8	3.67E-07	1.64E-05
GOTERM_CC_FAT	extracellular space	20	1.8	1.14E-06	4.37E-05
GOTERM_CC_FAT	extrinsic to membrane	27	2.5	2.36E-04	7.04E-03
GOTERM_CC_FAT	basement membrane	7	0.6	2.33E-04	7.82E-03
GOTERM_CC_FAT	extracellular matrix part	7	0.6	4.02E-04	1.08E-02
GOTERM_CC_FAT	contractile fiber part	8	0.7	4.62E-04	1.12E-02
GOTERM_CC_FAT	contractile fiber	8	0.7	9.67E-04	2.14E-02
GOTERM_MF_FAT	serine-type endopeptidase activity	68	6.2	1.75E-18	3.73E-16
GOTERM_MF_FAT	serine hydrolase activity	73	6.7	1.30E-18	4.16E-16
GOTERM_MF_FAT	serine-type peptidase activity	73	6.7	7.86E-19	5.04E-16
GOTERM_MF_FAT	peptidase activity	118	10.8	1.32E-17	2.11E-15
GOTERM_MF_FAT	peptidase activity, acting on L-amino acid peptides	112	10.2	4.33E-17	5.55E-15
GOTERM_MF_FAT	endopeptidase activity	87	8.0	9.12E-15	9.73E-13
GOTERM_MF_FAT	polysaccharide binding	30	2.7	5.47E-08	5.01E-06
GOTERM_MF_FAT	pattern binding	30	2.7	5.47E-08	5.01E-06
GOTERM_MF_FAT	carbohydrate binding	39	3.6	2.27E-07	1.82E-05
GOTERM_MF_FAT	electron carrier activity	41	3.7	1.40E-06	8.16E-05
GOTERM_MF_FAT	solute:sodium symporter activity	20	1.8	1.64E-06	8.77E-05
GOTERM_MF_FAT	glutathione transferase activity	15	1.4	1.38E-06	8.87E-05
GOTERM_MF_FAT	heme binding	32	2.9	1.25E-06	8.90E-05
GOTERM_MF_FAT	tetrapyrrole binding	32	2.9	1.25E-06	8.90E-05
GOTERM_MF_FAT	glucosidase activity	10	0.9	9.15E-06	4.51E-04
GOTERM_MF_FAT	chitin binding	22	2.0	1.15E-05	5.28E-04
GOTERM_MF_FAT	iron ion binding	45	4.1	1.64E-05	6.99E-04
GOTERM_MF_FAT	alkaline phosphatase activity	8	0.7	8.39E-05	3.36E-03
GOTERM_MF_FAT	alpha-glucosidase activity	8	0.7	8.39E-05	3.36E-03
GOTERM_MF_FAT	peptidoglycan binding	8	0.7	8.39E-05	3.36E-03
GOTERM_MF_FAT	glucuronosyltransferase activity	12	1.1	9.76E-05	3.67E-03
GOTERM_MF_FAT	solute:cation symporter activity	20	1.8	1.10E-04	3.90E-03
GOTERM_MF_FAT	anion:cation symporter activity	13	1.2	1.28E-04	4.32E-03
GOTERM_MF_FAT	metallopeptidase activity	30	2.7	1.46E-04	4.67E-03
GOTERM_MF_FAT	sodium:iodide symporter activity	6	0.5	1.56E-04	4.76E-03
GOTERM_MF_FAT	monovalent anion:sodium symporter activity	6	0.5	1.56E-04	4.76E-03
GOTERM_MF_FAT	symporter activity	20	1.8	1.89E-04	5.50E-03
GOTERM_MF_FAT	anion transmembrane transporter activity	21	1.9	2.45E-04	6.80E-03
GOTERM_MF_FAT	transferase activity, transferring alkyl or aryl (other than methyl) groups	16	1.5	2.69E-04	7.16E-03
GOTERM_MF_FAT	N-acetylmuramoyl-L-alanine amidase activity	7	0.6	4.71E-04	1.20E-02
GOTERM_MF_FAT	hydrolase activity, acting on carbon-nitrogen (but not peptide) bonds, in linear amides	15	1.4	9.09E-04	2.22E-02
GOTERM_MF_FAT	exopeptidase activity	20	1.8	1.39E-03	3.26E-02
GOTERM_MF_FAT	glycosaminoglycan binding	8	0.7	1.46E-03	3.28E-02
KEGG_PATHWAY	Metabolism of xenobiotics by cytochrome P450	24	2.2	1.94E-08	1.69E-06
KEGG_PATHWAY	Drug metabolism	24	2.2	4.10E-08	1.78E-06
KEGG_PATHWAY	Starch and sucrose metabolism	21	1.9	6.69E-07	1.94E-05
KEGG_PATHWAY	Folate biosynthesis	11	1.0	5.22E-05	1.14E-03
KEGG_PATHWAY	Retinol metabolism	12	1.1	4.42E-04	6.40E-03
KEGG_PATHWAY	Limonene and pinene degradation	21	1.9	4.07E-04	7.06E-03
KEGG_PATHWAY	Ascorbate and aldarate metabolism	11	1.0	7.88E-04	9.76E-03
KEGG_PATHWAY	Glutathione metabolism	16	1.5	2.73E-03	2.35E-02
KEGG_PATHWAY	Galactose metabolism	10	0.9	2.54E-03	2.43E-02
KEGG_PATHWAY	Glycine, serine and threonine metabolism	9	0.8	2.52E-03	2.71E-02
KEGG_PATHWAY	Pentose and glucuronate interconversions	11	1.0	6.06E-03	4.69E-02

Table 3.2 Gene ontology analysis of overlapping Class II transcripts between high expressed transcripts in females and low expressed transcripts in males using DAVID

Category	Term	Count	%	PValue	Benjamini
GOTERM_BP_FAT	GO:0048610~reproductive cellular process	19	55.88	2.97E-17	7.36E-15
GOTERM_BP_FAT	GO:0019953~sexual reproduction	19	55.88	7.91E-15	9.77E-13
GOTERM_BP_FAT	GO:0007292~female gamete generation	16	47.06	1.54E-12	1.27E-10
GOTERM_BP_FAT	GO:0030703~eggshell formation	10	29.41	3.67E-12	2.28E-10
GOTERM_BP_FAT	GO:0007304~chorion-containing eggshell formation	10	29.41	3.67E-12	2.28E-10
GOTERM_BP_FAT	GO:0003006~reproductive developmental process	15	44.12	2.52E-11	1.25E-09
GOTERM_BP_FAT	GO:0007276~gamete generation	16	47.06	5.90E-11	2.44E-09
GOTERM_BP_FAT	GO:0048609~reproductive process in a multicellular organism	16	47.06	1.63E-10	5.77E-09
GOTERM_BP_FAT	GO:0032504~multicellular organism reproduction	16	47.06	1.63E-10	5.77E-09
GOTERM_BP_FAT	GO:0048477~oogenesis	14	41.18	4.61E-10	1.43E-08
GOTERM_BP_FAT	GO:0030707~ovarian follicle cell development	10	29.41	6.71E-09	1.85E-07
GOTERM_BP_FAT	GO:0007306~eggshell chorion assembly	7	20.59	6.55E-08	1.62E-06
GOTERM_BP_FAT	GO:0010927~cellular component assembly involved in morphogenesis	7	20.59	3.56E-07	8.03E-06
GOTERM_BP_FAT	GO:0007028~cytoplasm organization	6	17.65	7.79E-07	1.61E-05
GOTERM_BP_FAT	GO:0007296~vitellogenesis	4	11.76	2.03E-06	3.87E-05
GOTERM_BP_FAT	GO:0030704~vitelline membrane formation	4	11.76	3.24E-06	5.74E-05
GOTERM_BP_FAT	GO:0007305~vitelline membrane formation in chorion-containing eggshell	4	11.76	3.24E-06	5.74E-05
GOTERM_BP_FAT	GO:0030198~extracellular matrix organization	4	11.76	1.63E-05	2.70E-04
GOTERM_BP_FAT	GO:0007338~single fertilization	4	11.76	5.44E-05	8.43E-04
GOTERM_BP_FAT	GO:0009566~fertilization	4	11.76	6.38E-05	9.31E-04
GOTERM_BP_FAT	GO:0007343~egg activation	3	8.82	4.34E-04	5.96E-03
GOTERM_BP_FAT	GO:0001775~cell activation	3	8.82	5.57E-04	7.24E-03
GOTERM_BP_FAT	GO:0043062~extracellular structure organization	4	11.76	1.98E-03	2.43E-02
GOTERM_CC_FAT	GO:0042600~chorion	7	20.59	1.03E-10	3.72E-09
GOTERM_CC_FAT	GO:0030312~external encapsulating structure	7	20.59	1.55E-10	2.78E-09
GOTERM_CC_FAT	GO:0005576~extracellular region	12	35.29	2.70E-06	3.24E-05
GOTERM_MF_FAT	GO:0005213~structural constituent of chorion	6	17.65	3.24E-11	1.78E-09
GOTERM_MF_FAT	GO:0005198~structural molecule activity	13	38.24	8.82E-10	2.43E-08
GOTERM_MF_FAT	GO:0008316~structural constituent of vitelline membrane	3	8.82	6.36E-05	1.17E-03
INTERPRO	IPR005649:Chorion 2	3	8.82	2.46E-05	1.25E-03
INTERPRO	IPR013818:Lipase, N-terminal	4	11.76	5.52E-05	1.41E-03
INTERPRO	IPR000734:Lipase	4	11.76	5.52E-05	1.41E-03
INTERPRO	IPR013135:Vitelline membrane cysteine-rich domain	3	8.82	1.22E-04	2.08E-03
SP_PIR_KEYWORDS	Secreted	12	35.29	1.93E-12	8.69E-11
SP_PIR_KEYWORDS	signal	13	38.24	1.21E-10	2.73E-09
SP_PIR_KEYWORDS	sulfation	3	8.82	1.12E-04	1.68E-03
UP_SEQ_FEATURE	signal peptide	13	38.24	5.72E-07	4.92E-05
UP_SEQ_FEATURE	domain:VM	3	8.82	1.40E-04	6.01E-03

Table 3.3 Gene ontology analysis of overlapping Class II transcripts between low expressed transcripts in females and high expressed transcripts in males using DAVID

Category	Term	Count	%	PValue	Benjamini
GOTERM_BP_FAT	GO:0045297~post-mating behavior	8	8.25	4.77E-12	9.53E-10
GOTERM_BP_FAT	GO:0019098~reproductive behavior	8	8.25	2.75E-08	2.75E-06
GOTERM_BP_FAT	GO:0007320~insemination	5	5.15	1.77E-07	1.18E-05
GOTERM_BP_FAT	GO:0007618~mating	7	7.22	5.02E-07	2.51E-05
GOTERM_BP_FAT	GO:0048609~reproductive process in a multicellular organism	15	15.46	5.45E-07	2.18E-05
GOTERM_BP_FAT	GO:0032504~multicellular organism reproduction	15	15.46	5.45E-07	2.18E-05
GOTERM_BP_FAT	GO:0007620~copulation	5	5.15	8.31E-07	2.77E-05
GOTERM_BP_FAT	GO:0007610~behavior	11	11.34	3.47E-06	9.90E-05
GOTERM_BP_FAT	GO:0046692~sperm competition	4	4.12	9.16E-06	2.29E-04
GOTERM_BP_FAT	GO:0033057~reproductive behavior in a multicellular organism	5	5.15	9.12E-05	2.02E-03
GOTERM_BP_FAT	GO:0007321~sperm displacement	3	3.09	9.33E-05	1.86E-03
GOTERM_BP_FAT	GO:0007283~spermatogenesis	6	6.19	4.09E-04	7.42E-03
GOTERM_BP_FAT	GO:0048232~male gamete generation	6	6.19	4.09E-04	7.42E-03
GOTERM_BP_FAT	GO:0045434~negative regulation of female receptivity, post-mating	3	3.09	8.55E-04	1.42E-02
GOTERM_BP_FAT	GO:0007621~negative regulation of female receptivity	3	3.09	8.55E-04	1.42E-02
GOTERM_BP_FAT	GO:0046008~regulation of female receptivity, post-mating	3	3.09	1.36E-03	2.08E-02
GOTERM_BP_FAT	GO:0060180~female mating behavior	3	3.09	1.99E-03	2.80E-02
GOTERM_BP_FAT	GO:0018991~oviposition	3	3.09	1.99E-03	2.80E-02
GOTERM_BP_FAT	GO:0045924~regulation of female receptivity	3	3.09	1.99E-03	2.80E-02
GOTERM_CC_FAT	GO:0005576~extracellular region	14	14.43	3.58E-07	1.61E-05
GOTERM_MF_FAT	GO:0008235~metalloexopeptidase activity	7	7.22	3.77E-08	2.64E-06
GOTERM_MF_FAT	GO:0004177~aminopeptidase activity	7	7.22	3.77E-08	2.64E-06
GOTERM_MF_FAT	GO:0030145~manganese ion binding	7	7.22	2.82E-07	9.89E-06
GOTERM_MF_FAT	GO:0005179~hormone activity	6	6.19	5.75E-06	1.34E-04
GOTERM_MF_FAT	GO:0008238~exopeptidase activity	7	7.22	7.20E-06	1.26E-04
GOTERM_MF_FAT	GO:0008237~metallopeptidase activity	7	7.22	9.39E-05	1.31E-03
INTERPRO	IPR008283:Peptidase M17, leucyl aminopeptidase, N-terminal	7	7.22	3.44E-13	1.76E-11
INTERPRO	IPR011356:Peptidase M17, leucyl aminopeptidase	7	7.22	3.44E-13	1.76E-11
INTERPRO	IPR000819:Peptidase M17, leucyl aminopeptidase, C-terminal	7	7.22	2.56E-12	6.53E-11
INTERPRO	IPR006611:Protein of unknown function DUF1431, cysteine-rich, Drosophila	6	6.19	6.74E-10	1.15E-08
INTERPRO	IPR009392:Drosophila ACP53EA	3	3.09	7.56E-05	9.64E-04
INTERPRO	IPR007702:Janus/Ocnus	3	3.09	1.51E-04	1.54E-03
KEGG_PATHWAY	dme00480:Glutathione metabolism	7	7.22	2.11E-08	8.43E-08
PIR_SUPERFAMILY	PIRSF002545:fruit fly testis-specific protein	5	5.15	2.47E-08	1.98E-07
PIR_SUPERFAMILY	PIRSF001116:cytosol aminopeptidase	4	4.12	3.09E-06	1.24E-05
SMART	SM00689:DM6	6	6.19	2.47E-11	1.73E-10
SP_PIR_KEYWORDS	behavior	11	11.34	3.20E-14	1.22E-12
SP_PIR_KEYWORDS	Aminopeptidase	7	7.22	4.10E-07	7.80E-06
SP_PIR_KEYWORDS	Secreted	11	11.34	3.46E-06	4.39E-05
SP_PIR_KEYWORDS	polymorphism	8	8.25	2.34E-05	2.22E-04
SP_PIR_KEYWORDS	signal	11	11.34	8.19E-04	6.21E-03
UP_SEQ_FEATURE	sequence variant	11	11.34	4.20E-07	2.01E-05
UP_SEQ_FEATURE	signal peptide	11	11.34	8.57E-05	2.06E-03

Table 3. 4 Gene ontology analysis of female specific Class II transcripts using DAVID

Category	Term	Count	%	PValue	Benjamini
GOTERM_CC_FAT	GO:0044455~mitochondrial membrane part	8	2.90	1.89E-03	4.50E-02
GOTERM_CC_FAT	GO:0005740~mitochondrial envelope	12	4.35	8.02E-04	4.77E-02
GOTERM_CC_FAT	GO:0031966~mitochondrial membrane	12	4.35	4.23E-04	5.03E-02
GOTERM_CC_FAT	GO:0044429~mitochondrial part	15	5.43	1.76E-03	5.24E-02
GOTERM_CC_FAT	GO:0019866~organelle inner membrane	10	3.62	2.77E-03	5.48E-02
GOTERM_CC_FAT	GO:0005743~mitochondrial inner membrane	10	3.62	1.53E-03	6.04E-02
GOTERM_MF_FAT	GO:0004867~serine-type endopeptidase inhibitor activity	8	2.90	4.46E-05	9.98E-03
INTERPRO	IPR018248:EF hand	8	2.90	1.06E-04	8.33E-03
INTERPRO	IPR011992:EF-Hand type	9	3.26	9.97E-05	1.17E-02
INTERPRO	IPR002048:Calcium-binding EF-hand	8	2.90	7.99E-05	1.88E-02
INTERPRO	IPR018249:EF-HAND 2	8	2.90	3.85E-04	2.26E-02
INTERPRO	IPR014044:SCP-like extracellular	5	1.81	6.63E-04	3.09E-02
INTERPRO	IPR001283:Allergen V5/Tpx-1 related	5	1.81	6.63E-04	3.09E-02
INTERPRO	IPR001715:Calponin-like actin-binding	5	1.81	1.48E-03	5.69E-02
INTERPRO	IPR000215:Protease inhibitor I4, serpin	5	1.81	1.87E-03	6.13E-02
SMART	SM00054:EFh	8	2.90	6.22E-05	3.17E-03
SMART	SM00198:SCP	5	1.81	5.93E-04	1.50E-02
SMART	SM00093:SERPIN	5	1.81	1.68E-03	2.12E-02
SMART	SM00033:CH	5	1.81	1.33E-03	2.24E-02

Table 3.5 Gene ontology analysis of male specific Class II transcripts using DAVID

Category	Term	Count	%	PValue	Benjamini
GOTERM_CC_FAT	GO:0030312~external encapsulating structure	8	2.24	9.06E-08	7.16E-06
GOTERM_CC_FAT	GO:0042600~chorion	8	2.24	5.64E-08	8.92E-06
GOTERM_CC_FAT	GO:0005576~extracellular region	23	6.44	4.96E-04	2.58E-02
GOTERM_CC_FAT	GO:0032993~protein-DNA complex	6	1.68	9.71E-04	3.77E-02
GOTERM_MF_FAT	GO:0005214~structural constituent of chitin-based cuticle	11	3.08	3.28E-05	9.47E-03
GOTERM_MF_FAT	GO:0042302~structural constituent of cuticle	11	3.08	6.65E-05	9.60E-03
GOTERM_MF_FAT	GO:0015267~channel activity	12	3.36	1.02E-03	4.81E-02
GOTERM_MF_FAT	GO:0022803~passive transmembrane transporter activity	12	3.36	1.02E-03	4.81E-02
INTERPRO	IPR000618:Insect cuticle protein	11	3.08	2.18E-05	7.95E-03
KEGG_PATHWAY	dme03030:DNA replication	6	1.68	1.95E-04	7.01E-03

REFERENCES

Ayroles, J. F., M. A. Carbone, et al. (2009). "Systems genetics of complex traits in Drosophila melanogaster." *Nat Genet* **41**(3): 299-307.

Bhaskara, S., E. D. Dean, et al. (2006). "Induction of two cytochrome P450 genes, Cyp6a2 and Cyp6a8, of *Drosophila melanogaster* by caffeine in adult flies and in cell culture." *Gene* **377**: 56-64.

Bradshaw, A. D. (1965). "Evolutionary Significance of Phenotypic Plasticity in Plants." *Advances in Genetics* **13**: 115-155.

Bushati, N. and S. M. Cohen (2007). "microRNA functions." *Annu Rev Cell Dev Biol* **23**: 175-205.

Causton, H. C., B. Ren, et al. (2001). "Remodeling of yeast genome expression in response to environmental changes." *Mol Biol Cell* **12**(2): 323-337.

Chapman, T., L. F. Liddle, et al. (1995). "Cost of mating in Drosophila melanogaster females is mediated by male accessory gland products." *Nature* **373**(6511): 241-244.

Chintapalli, V. R., J. Wang, et al. (2007). "Using FlyAtlas to identify better *Drosophila melanogaster* models of human disease." *Nat Genet* **39**(6): 715-720.

Claridge-Chang, A., H. Wijnen, et al. (2001). "Circadian regulation of gene expression systems in the Drosophila head." *Neuron* **32**(4): 657-671.

Clark, A. G., M. B. Eisen, et al. (2007). "Evolution of genes and genomes on the Drosophila phylogeny." *Nature* **450**(7167): 203-218.

Claudianos, C., H. Ranson, et al. (2006). "A deficit of detoxification enzymes: pesticide sensitivity and environmental response in the honeybee." *Insect Mol Biol* **15**(5): 615-636.

Feyereisen, R. (2006). "Evolution of insect P450." *Biochem Soc Trans* **34**(Pt 6): 1252-1255.

Ffrench-Constant, R. H., P. J. Daborn, et al. (2004). "The genetics and genomics of insecticide resistance." *Trends Genet* **20**(3): 163-170.

Gasch, A. P., P. T. Spellman, et al. (2000). "Genomic expression programs in the response of yeast cells to environmental changes." *Mol Biol Cell* **11**(12): 4241-4257.

Gibson, G. (2009). "Decanalization and the origin of complex disease." *Nat Rev Genet* **10**(2): 134-140.

Gibson, G. and I. Dworkin (2004). "Uncovering cryptic genetic variation." *Nat Rev Genet* **5**(9): 681-690.

Harbison, S. T., S. Chang, et al. (2005). "Quantitative genomics of starvation stress resistance in Drosophila." *Genome Biol* **6**(4): R36.

Huang da, W., B. T. Sherman, et al. (2009). "Bioinformatics enrichment tools: paths toward the comprehensive functional analysis of large gene lists." *Nucleic Acids Res* **37**(1): 1-13.

Huang da, W., B. T. Sherman, et al. (2009). "Systematic and integrative analysis of large gene lists using DAVID bioinformatics resources." *Nat Protoc* **4**(1): 44-57.

Jarosz, D. F. and S. Lindquist (2010). "Hsp90 and environmental stress transform the adaptive value of natural genetic variation." *Science* **330**(6012): 1820-1824.

Larracuente, A. M., T. B. Sackton, et al. (2008). "Evolution of protein-coding genes in Drosophila." *Trends Genet* **24**(3): 114-123.

McDonald, M. J. and M. Rosbash (2001). "Microarray analysis and organization of circadian gene expression in Drosophila." *Cell* **107**(5): 567-578.

Mensch, J., N. Lavagnino, et al. (2008). "Identifying candidate genes affecting developmental time in *Drosophila melanogaster*: pervasive pleiotropy and gene-by-environment interaction." *BMC Dev Biol* **8**: 78.

Murali, T., S. Pacifico, et al. (2011). "DroID 2011: a comprehensive, integrated resource for protein, transcription factor, RNA and gene interactions for Drosophila." *Nucleic Acids Res* **39**(Database issue): D736-743.

Ranson, H., C. Claudianos, et al. (2002). "Evolution of supergene families associated with insecticide resistance." *Science* **298**(5591): 179-181.

Richards, S., R. A. Gibbs, et al. (2008). "The genome of the model beetle and pest *Tribolium castaneum*." *Nature* **452**(7190): 949-955.

Roy, S., J. Ernst, et al. (2010). "Identification of functional elements and regulatory circuits by Drosophila modENCODE." *Science* **330**(6012): 1787-1797.

Ruden, D. M., L. Xiao, et al. (2005). "Hsp90 and environmental impacts on epigenetic states: a model for the trans-generational effects of diethylstibesterol on uterine development and cancer." *Hum Mol Genet* **14 Spec No 1**: R149-155.

Rutherford, S. L. and S. Lindquist (1998). "Hsp90 as a capacitor for morphological evolution." *Nature* **396**(6709): 336-342.

Sambucetti, P., V. Loeschcke, et al. (2006). "Developmental time and size-related traits in *Drosophila buzzatii* along an altitudinal gradient from Argentina." *Hereditas* **143**(2006): 77-83.

Sangster, T. A., N. Salathia, et al. (2008). "HSP90-buffered genetic variation is common in *Arabidopsis thaliana*." *Proc Natl Acad Sci U S A* **105**(8): 2969-2974.

Scholz, H., M. Franz, et al. (2005). "The hangover gene defines a stress pathway required for ethanol tolerance development." *Nature* **436**(7052): 845-847.

Sgro, C. M., B. Wegener, et al. (2010). "A naturally occurring variant of Hsp90 that is associated with decanalization." *Proc Biol Sci* **277**(1690): 2049-2057.

Stone, E. A. and J. F. Ayroles (2009). "Modulated modularity clustering as an exploratory tool for functional genomic inference." *PLoS Genet* **5**(5): e1000479.

Storch, K. F., O. Lipan, et al. (2002). "Extensive and divergent circadian gene expression in liver and heart." *Nature* **417**(6884): 78-83.

Strode, C., C. S. Wondji, et al. (2008). "Genomic analysis of detoxification genes in the mosquito *Aedes aegypti*." *Insect Biochem Mol Biol* **38**(1): 113-123.

Ueda, H. R., A. Matsumoto, et al. (2002). "Genome-wide transcriptional orchestration of circadian rhythms in Drosophila." *J Biol Chem* **277**(16): 14048-14052.

Waddington, C. H. (1942). "Canalization of development and the inheritance of acquired characters." *Nature* **150**(3811): 563-565.

Waddington, C. H. (1959). "Canalization of development and genetic assimilation of acquired characters." *Nature* **183**(4676): 1654-1655.

Yu, J., S. Pacifico, et al. (2008). "DroID: the Drosophila Interactions Database, a comprehensive resource for annotated gene and protein interactions." *BMC Genomics* **9**: 461.

Zhou, S., E. A. Stone, et al. (2009). "Plasticity of the chemoreceptor repertoire in *Drosophila melanogaster*." *PLoS Genet* **5**(10): e1000681.

CHAPTER FOUR

CONCLUSIONS AND FUTURE PERSPECTIVES

TUNING THE CHEMOSENSORY WINDOW

Accurate perception of chemical signals from the environment is critical for the fitness of most animals. *Drosophila melanogaster* experiences its chemical environment through families of chemoreceptors that include olfactory receptors, gustatory receptors and odorant binding proteins. Its chemical environment, however, changes during its life cycle and the interpretation of chemical signals is dependent on dynamic social and physical surroundings. Phenotypic plasticity of gene expression of the chemoreceptor repertoire allows flies to adjust the chemosensory window through which they “view” their world and to modify the ensemble of expressed chemoreceptor proteins in line with their developmental and physiological state and according to their needs to locate food and oviposition sites under different social and physical environmental conditions. Furthermore, males and females differ in their expression profiles of chemoreceptor genes. Thus, each sex experiences its chemical environment via combinatorial activation of distinct chemoreceptor ensembles. The remarkable phenotypic plasticity of the chemoreceptor repertoire raises several fundamental questions. What are the mechanisms that translate environmental cues into regulation of chemoreceptor gene expression? How are gustatory and olfactory cues perceptually integrated? What is the relationship between ensembles of odorant binding proteins and odorant receptors? And, what is the significance of co-regulated chemoreceptor transcriptional networks?

PLASTICITY OF CHEMORECEPTOR EXPRESSION

Throughout the life cycle of a fly, chemical signals serve critical roles. When larvae hatch, they depend on chemosensory information for feeding behavior and growth. Following pupation and eclosion, different chemical signals guide the behaviors of each sex. To identify mating partners, males depend on chemical cues to distinguish males from females, and to distinguish conspecific receptive females from non-receptive females (Amrein 2004). During mating, transfer of proteins from the male's accessory gland with his sperm into the female profoundly changes her physiology and behavior. A change in receptiveness towards other males and stimulation of egg production and oviposition gives rise to dependence on different chemosensory cues (Fig. 4.1) (Wolfner 1997; McGraw et al. 2004). The dynamic interplay between changes in the chemical environment and changes in the perceived importance of chemical signals under different developmental, social and physiological conditions requires adaptation of chemosensory recognition and behavior to maximize the chance for survival and reproduction, i.e. individual fitness (Jones 2005). Whereas experience-dependent changes in olfactory behavior have been well documented in laboratory studies and are mediated by central pathways that include the mushroom bodies (Tully 1996), genome-wide transcriptional regulation of chemoreceptors under different developmental, social and physiological conditions has been studied only recently (Morozova et al. 2006; Zhou et al. 2009).

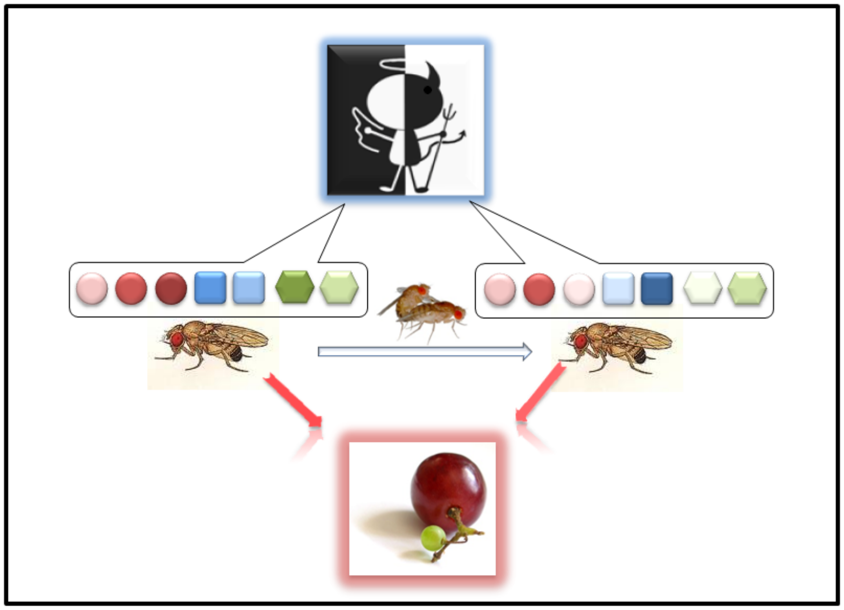

Figure 4.1 Phenotypic plasticity of chemoreceptor gene expression. Female flies change their chemosensory perception after mating, being less attracted to males and focusing on oviposition site selection and egg laying. These behavioral changes are accompanied by modulation of the expression of different combinations of chemoreceptor genes, Obps, Ors and Grs, represented by circles, squares and hexagons, respectively, with color intensities indicating relative expression levels. Following mating, females show a reduction in transcript levels of four Gr and 12 Or genes, as well as changes in transcript levels of 23 Obp genes (Zhou et al. 2009).

Whereas the evolutionary history of the chemoreceptor gene repertoire provides the organism with a diverse “chemosensory toolkit”, changing environments during the animal’s lifetime and changes in its physiological state require modulation of the expression of chemoreceptor genes (Zhou et al. 2009). By measuring expression levels of all chemosensory genes simultaneously, I identified transcripts with altered expression at different developmental stages, during aging, in males and females, following mating, and under different social conditions. These experiments showed that chemosensory genes that are located in close proximity to one another on the chromosome are often regulated independently at different developmental stages and between the sexes. Of special interest are the *Gr22d* and *Gr22e* genes, which are highly expressed in larvae, but undetectable in adults, and likely serve a larval specific feeding function. Dramatic changes in expression levels of *Or*, *Gr* and *Obp* genes occur during aging and are more extensive in males than in females. After mating, four *Gr* genes and 12 *Or* genes show altered expression, only in females, whereas members of the *Obp* gene family undergo altered regulation in both sexes.

Different complements of *Obp* transcripts respond most prominently to changes in social environment, i.e. flies reared in isolation versus flies reared in single sex groups. When single flies are separated from same or opposite sex groups by a cheesecloth barrier, which allows olfactory signals to be exchanged but prevents tactile stimulation, changes in chemoreceptor gene expression are few when isolated flies are paired with same sex groups, but more extensive when they are exposed to odor of the opposite sex. Again, modulation of *Obp* transcripts stands out, and, in this case, there is no overlap between *Obp* transcripts that show altered regulation when single males are exposed to female group odor and when single females are exposed to male group odor. The extensive sexual dimorphism and often antagonistic regulation, in which certain transcripts are upregulated in males and downregulated in females, or *vice versa*, under different conditions, suggests that a large segment of the chemoreceptor gene repertoire is utilized differently in males and females to extract information from the chemical environment. Moreover, much of the adjustment of the chemosensory window appears to be due to altered regulation of expression of *Obp* genes.

Environmental plasticity of chemoreceptor expression is heterogeneous, with certain members of the chemoreceptor ensemble responding more frequently to environmental changes than others. This raised the question of whether some chemosensory genes might show correlated transcriptional regulation across all experimental conditions. This question was answered with a statistical analysis using the modulated modularity clustering method, an unbiased, self-organizing paradigm based on correlations of transcript abundance levels between different conditions, which sorts transcripts into modules such that transcript abundance levels among members within each module are more closely correlated than with members outside that module (Stone and Ayroles 2009). This analysis organized the chemoreceptor repertoire in 20 covariant ensembles, revealing an underlying modular organization of the phenotypic plasticity of the chemosensory receptor repertoire. There are indications that this modular organization reflects biological significance. For example, four odorant receptors that all respond electrophysiologically to alcohols and aliphatic esters are contained within the same module, as are *Obp76a* [a.k.a. *Lush*]) and *Or67d*, which encode an odorant binding protein and an odorant receptor, respectively, that respond to the pheromone 11-*cis*-vaccenyl acetate (Ha and Smith 2006). The finely pixilated modular organization of the chemosensory subgenome, which consists of a large number of relatively small, albeit highly correlated modules, enables fine tuning of the expression of the chemoreceptor repertoire in response to ecologically relevant environmental and physiological conditions.

MECHANISM OF PLASTICITY OF CHEMORECEPTOR EXPRESSION

The mechanism by which external cues modulate sensory systems is a question of fundamental biological importance. In a broad sense, phenotypic plasticity of the chemoreceptor repertoire in *Drosophila* is reminiscent of modulation of brain circuits during the acquisition of birdsong. Here, expression of the immediate early gene transcription factor zenk is induced by auditory experience of a tutor song (Mello et al. 2004). Similarly, the mechanisms that translate environmental cues into regulation of chemoreceptor gene expression must involve environment-dependent regulation of transcription factors. The identity of these transcription factors and their mode of regulation, remain, however, unknown. Elegant studies by Carlson and colleagues have demonstrated that the POU domain transcription factors acj6 and pdm3 control expression of odorant receptors in subsets of olfactory sensory neurons (Tichy et al. 2008). The acj6 transcription factor is essential for expression of *Or* genes in the pb1A neurons in the maxillary palps, which respond to the odorant E2-hexenal, and interacts genetically with pdm3 (Tichy et al. 2008). Flies that carry a *pdm3* mutation do not express the Or42a receptor in antennal ab1A sensory neurons, whereas three other neurons contained within the same sensilla appear to be unaffected (Tichy et al. 2008). Combinations of transcription factors acting on *cis*-regulatory elements either promote or inhibit the expression of specific *Or* genes (Ray et al. 2008). Such transcription factors include lozenge and Scalloped, respectively, but it is reasonable to predict that a wide range of additional as yet unannotated transcription factors may contribute to controlling the expression of chemoreceptor genes. An examination of enrichment of known transcription factor binding motifs among transcriptionally coregulated chemoreceptor genes did not yield readily interpretable results, attesting to the complexity of transcriptional regulation of chemoreceptor genes during the animal's lifetime not only within but also among classes of chemoreceptor genes.

It is possible that differences in chemoreceptor gene expression between the larval and adult stages are largely controlled by hard-wired genetic programs, but that variation in chemoreceptor gene expression as a function of environmental experience is due to upregulation of chemoreceptor genes in neural circuits that are more frequently activated, while chemoreceptor genes that are expressed in neurons that undergo less frequent stimulation are downregulated. This mechanism of phenotypic plasticity would empower neurons that are most important for sensing relevant chemical information at a particular place and time. If such an adaptive mechanism indeed occurs, it begs the question as to whether peripheral signal transduction at the level of the olfactory sensory neuron is sufficient to modulate transcriptional regulation of chemoreceptor gene expression, or whether central feedback plays a role, which would suggest that perception of the chemical milieu at the level of the mushroom bodies or lateral protocerebrum might regulate the sensitivity of peripheral chemosensory organs.

A second fundamental biological question is how gustatory and olfactory cues are integrated perceptually? The perception of carbon dioxide via receptors expressed in the antenna or receptors expressed in the proboscis results in different behavioral responses. Antennal perception of carbon dioxide is interpreted as an alarm signal and generates an avoidance response (Jones et al. 2007). Whereas the perception of carbon dioxide via taste receptors on the proboscis is interpreted as the presence of a fermenting food source and elicits an attractant response (Fischler et al. 2007). These observations and behavioral responses to pheromones would suggest that inputs from specialized chemosensory systems are processed via independent labeled lines. Nevertheless, one cannot exclude that input from olfactory receptors and gustatory receptors might often be integrated, a notion that is supported by covariant transcriptional modules that contain both *Gr* and *Or* transcripts (Zhou et al. 2009).

A similar issue concerns the relationship between Obps and Ors. On the one hand distinct odorant response profiles can be documented electrophysiologically for a wide range of *Or* genes expressed in the same “empty” neuron, suggesting that the role of Obps is of limited importance (Hallem et al. 2004). On the other hand, behavioral studies have shown that Obps are critical for activation of the *Or67d* receptor which is stimulated by the Lush odorant binding protein after it binds the courtship pheromone 11-*cis*-vaccenyl acetate (Ha and Smith 2006). Furthermore, a 4 bp insertion/deletion in *Obp57e* determines differences in host plant specificity between *D. sechellia* and *D. melanogaster*. Inactivation of the *Obp57e* and *Obp57d* genes abolishes repulsion by hexanoic and octanoic acid, toxins produced by *Morinda citrifolia*, the host plant for *D. sechellia* (Matsuo et al. 2007). In addition, SNPs in the *Obp99a-d* cluster contribute to phenotypic variation in olfactory behavior in response to benzaldehyde and acetophenone in a population of wild-derived *D. melanogaster* lines (Wang et al. 2007).

Whereas the connection between *Lush* and *Or67d* might suggest a simple relationship between distinct Obps and Ors, there appear to be no similarities in expression patterns between *Obp* and *Or* genes in chemosensory tissues (Chintapalli et al. 2007). One can speculate that odor recognition in insects is a two step process, in which combinatorial recognition proceeds sequentially through two moderate-size chemoreceptor gene families, the *Obp* and *Or* families; such a system might achieve similar discrimination power as the much larger vertebrate odorant receptor gene families, which consist of hundreds of genes. One might predict that multiple Obps in the sensillar perilymph would provide distinct, yet broad odorant binding specificities that serve as a filter to modulate interactions with Ors that have more circumscribed (albeit still broad) ligand binding characteristics. How covariant regulation is coordinated between *Obp*, *Or* and *Gr* transcripts in response to environmental or physiological changes is a question of profound importance that cannot be answered at present. Thus, the remarkable phenotypic plasticity where males and females adjust their chemosensory windows by modulating expression of different subsets of chemoreceptor genes illustrates the complexities of the genes-brain-behavior paradigm and the many unanswered questions of the mechanisms by which environmental effects modulate gene expression even in as "simple" an organism as a fly.

TOWARDS A COMPREHENSIVE UNDERSTANDING OF PHENOTYPIC PLASTICITY

Phenotypic plasticity as a common phenomenon has been observed since the beginning of civilization. People kept track of the seasonal and regional changes of their crops and livestock to improve farming, and observed changes in animal behaviors to predict natural disasters. Adaptive changes of organisms to their surroundings have been recorded extensively. Only in the last several decades, however, has phenotypic plasticity been brought to the center of research, and is no longer considered a nuisance that reduces the reproducibility of genetic studies, but as an important heritable trait in its own right that promotes adaptive evolution under environmental changes.

Studies on the genetic basis of phenotypic plasticity have generally followed the experimental design used to study other complex traits. However, introducing phenotypic variation as a function of environmental perturbations, adds an additional layer of complexity. Large scale studies that include many genotypes and many environments are extremely hard to perform in higher order organisms, both logistically and in terms of statistical analysis. In my study described in Chapter 3, I tried to solve this problem by adopting a model that studies genetic and environmental components in large scale settings separately. This allows us to collect comprehensive information on both genetic variation and environmental variation under similar controlled genetic backgrounds. First I was able to tease apart environment specific effects on the transcriptome. I could then use this as a filter for a second stage of analysis to examine gene by environment interactions at a reduced scale with multiple genotypes and multiple environments.

Many fundamental questions about the genetic basis of phenotypic plasticity have not been answered, such as whether genes controlling a trait are the same as those controlling plasticity of that trait? Will regulation of expression of these genes change under different environmental conditions? Or will the organization of genetic networks change under different conditions? Note that we are considering two levels of plasticity, one is at the level of expressed phenotypes, and the other is at the level of the genetic components that control the traits or plasticity of the traits, which can result in plasticity of the expressed phenotype.

Further, one main reason we want to understand the genetic basis of phenotypic plasticity is to understand the underlying mechanisms of adaptive evolution. In this context, studies of phenotypic plasticity are of critical importance. Rather than relying on purely theoretical studies, empirical studies of closely related species under reciprocally adapted ecological conditions are desirable, as they may give us direct insights in the causal genetic parameters that underlie the differentiation of species under different selection pressures.

As I discussed in the Introduction, phenotypic plasticity and robustness are two sides of the coin. One cannot simply isolate one from the other as they are interconnected. However, most studies have considered them individually, which provides only a partial picture. Canalized genes are canalized with help from plastic genes (for example Hsp90), and decanalization causes a higher level of plasticity. Hence, we must keep the connection between plasticity and robustness in mind in future studies. It is also worth to note that not all the sequence variations that exist between different genotypes contribute to phenotypic variation, due to genetic buffering by canalization. However, environmental or genetic perturbations could decanalize the robust genome and reveal cryptic genetic variation, which means that genetic interactions could be changed. This is important, since when we refer to genetic networks, interactions, or pathways, we generally consider static models, while in reality these interactions are dynamic. Future approaches in systems biology along with the rapid development of advanced technologies as well as extensive collaboration across disciplines, will provide us with the ability to integrate different endophenotypes with genetic variation for an integrated view of the causality of a particular organismal trait. Similarly, applying systems biology approaches to different environmental conditions will allow us to investigate the dynamics of genetic interactions.

In addition to genome wide approaches, focusing on the plasticity of a subset of genes can be highly informative. I have illustrated this in Chapter 2 by focusing on the phenotypic plasticity of the olfactory system. Studying plasticity of sensory systems will provide us with a wealth of information about how organisms interact with the environment and how peripheral sensory input directs changes in gene expression, protein synthesis and eventually long term changes in physiology and behaviors.

Finally, in reality environment factors do not act on organisms in isolation, but different environmental conditions will exert their effects in combination. Just like one environmental change can switch a genetically controlled phenotype, adding in another environmental component could enhance, weaken, or even reverse the effects of the first environmental factor. For example, I have shown in my study that high yeast-supplemented and high sugar supplemented media affect Drosophila development in opposite ways, but effects on development time are diminished when flies are reared on a high yeast-high sugar diet. Thus, a future direction in phenotypic studies should focus on environment by environment effects which will provide us with insights in how complex environmental information is integrated to modulate the organization of genetic networks that underlie phenotypic plasticity.

REFERENCES

Amrein, H. (2004). "Pheromone perception and behavior in Drosophila." *Curr Opin Neurobiol* **14**(4): 435-442.

Chintapalli, V. R., J. Wang, et al. (2007). "Using FlyAtlas to identify better Drosophila melanogaster models of human disease." *Nat Genet* **39**(6): 715-720.

Fischler, W., P. Kong, et al. (2007). "The detection of carbonation by the Drosophila gustatory system." *Nature* **448**(7157): 1054-1057.

Ha, T. S. and D. P. Smith (2006). "A pheromone receptor mediates 11-cis-vaccenyl acetate-induced responses in Drosophila." *J Neurosci* **26**(34): 8727-8733.

Hallem, E. A., M. G. Ho, et al. (2004). "The molecular basis of odor coding in the Drosophila antenna." *Cell* **117**(7): 965-979.

Jones, C. D. (2005). "The genetics of adaptation in Drosophila sechellia." *Genetica* **123**(1-2): 137-145.

Jones, W. D., P. Cayirlioglu, et al. (2007). "Two chemosensory receptors together mediate carbon dioxide detection in Drosophila." *Nature* **445**(7123): 86-90.

Matsuo, T., S. Sugaya, et al. (2007). "Odorant-binding proteins OBP57d and OBP57e affect taste perception and host-plant preference in Drosophila sechellia." *PLoS Biol* **5**(5): e118.

McGraw, L. A., G. Gibson, et al. (2004). "Genes regulated by mating, sperm, or seminal proteins in mated female Drosophila melanogaster." *Curr Biol* **14**(16): 1509-1514.

Mello, C. V., T. A. Velho, et al. (2004). "Song-induced gene expression: a window on song auditory processing and perception." *Ann N Y Acad Sci* **1016**: 263-281.

Morozova, T. V., R. R. H. Anholt, et al. (2006). "Transcriptional response to alcohol exposure in Drosophila melanogaster." *Genome Biol* **7**(10): R95.

Ray, A., W. van der Goes van Naters, et al. (2008). "A regulatory code for neuron-specific odor receptor expression." *PLoS Biol* **6**(5): e125.

Stone, E. A. and J. F. Ayroles (2009). "Modulated modularity clustering as an exploratory tool for functional genomic inference." *PLoS Genet* **5**(5): e1000479.

Tichy, A. L., A. Ray, et al. (2008). "A new Drosophila POU gene, pdm3, acts in odor receptor expression and axon targeting of olfactory neurons." *J Neurosci* **28**(28): 7121-7129.

Tully, T. (1996). "Discovery of genes involved with learning and memory: an experimental synthesis of Hirschian and Benzerian perspectives." *Proc Natl Acad Sci U S A* **93**(24): 13460-13467.

Wang, P., R. F. Lyman, et al. (2007). "Association of polymorphisms in odorant-binding protein genes with variation in olfactory response to benzaldehyde in Drosophila." *Genetics* **177**(3): 1655-1665.

Wolfner, M. F. (1997). "Tokens of love: functions and regulation of Drosophila male accessory gland products." *Insect Biochem Mol Biol* **27**(3): 179-192.

Zhou, S., E. A. Stone, et al. (2009). "Plasticity of the chemoreceptor repertoire in Drosophila melanogaster." *PLoS Genet* **5**(10): e1000681.