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Functional Genomics of Odor-guided Behavior in *Drosophila melanogaster*

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Abstract

The avoidance response to repellent odorants in *Drosophila melanogaster*, a response essential for survival, provides an advantageous model for studies on the genetic architecture of olfactory behavior. Transposon tagging in a highly inbred strain of flies in combination with a rapid and simple statistical behavioral assay enables the identification of not only large phenotypic effects, but also small aberrations from wild-type avoidance behavior. The recent completion of the sequence of the *Drosophila* genome facilitates the molecular characterization of transposon-tagged genes and correlation between gene expression and behavior in *smell-impaired* (*smi*) mutant lines. Quantitative genetic analyses of a collection of *smi* lines in a co-isogenic background revealed an extensive network of epistatic interactions among genes that shape the olfactory avoidance response. Candidate genes for several of these transposon-tagged *smi* loci implicate genes that mediate odorant recognition, including a novel odorant binding protein; signal propagation, including a voltage-gated sodium channel; and a protein containing multiple leucine rich repeats and PDZ domains likely to be involved in postsynaptic organization in the olfactory pathway. Several novel genes of unknown function have also been implicated, including a novel tyrosine-regulated protein kinase. The discovery and characterization of novel gene products that have major, hitherto unappreciated effects on olfactory behavior will provide new insights in the generation and regulation of odor-guided behavior. The identification and functional characterization of proteins encoded by *smi* genes that form part of the olfactory subgenome and correlation of polymorphisms in these genes with variation in odor-guided behavior in natural populations will advance our understanding of the genetic architecture of chemosensory behavior.

Introduction

Odor-guided behavior is essential for the survival and reproduction of most animals. In recent years considerable progress has been made in elucidating the molecular mechanisms that underlie odor recognition and odor-guided behavior. In vertebrates, nematodes and insects large gene families encoding G protein-coupled odorant receptors have been identified (Buck and Axel, 1991; Ngai *et al.*, 1993; Troemel *et al.*, 1995; Sengupta *et al.*, 1996; Clyne *et al.*, 1999; Gao and Chess, 1999; Vosshall *et al.*, 1999). A surprisingly large percentage of the genome comprises genes that mediate olfaction. In vertebrates, odorant receptor genes alone have been estimated to comprise up to 1% of the genome (Buck and Axel, 1991). In *Drosophila melanogaster* it has been estimated that ~4% of the genome contributes directly to the avoidance response of the repellent odorant, benzaldehyde (Anholt *et al.*, 1996). Our studies are aimed at understanding the genetic architecture of odor-guided behavior using *Drosophila* as a model system.

Drosophila melanogaster as a model system

Odor-guided behavior is a complex trait that is shaped through the coordinated expression of ensembles of genes. Relatively little is known, however, about which genes are members of these ensembles and to what extent individual genes contribute to phenotypic variation in behavior. *Drosophila melanogaster* is an ideal model system for studying the genetic basis of complex traits. Because the generation time is short, highly inbred lines can be established readily. Thus, flies with a common genetic background can be generated allowing us to study the effects of a single locus in the absence of other segregating loci that could be contributing to the trait of interest. Furthermore, 'balancer' chromosomes, containing multiple overlapping inversions and a dominant visible marker, have been synthesized for the three major chromosomes. These chromosomes suppress recombinations in their homologues, enabling the cloning of entire chromosomes, construction of chromosome substitution lines and maintenance of

mutant stocks that are not viable as homozygotes. Moreover, the entire *Drosophila* genome has recently been sequenced, which will greatly facilitate the identification of novel genes important for olfaction (Adams *et al.*, 2000; Rubin, 2000).

In addition, the olfactory system of *D. melanogaster*, which consists of the third antennal segments and the maxillary palps, is quantitatively less complex than its vertebrate counterpart. Each third antennal segment contains only ~1000 olfactory receptor cells and each maxillary palp carries 120 chemosensory neurons [reviewed by Stocker (Stocker, 1994)]. These neurons project to 43 morphologically identified glomeruli in the antennal lobe (Laissue *et al.*, 1999; Gao *et al.*, 2000; Vosshall *et al.*, 2000). This represents an ~40-fold reduction in complexity compared with the 1800 glomeruli in the mouse olfactory bulb (Pomeroy *et al.*, 1990). Similarly, whereas the mouse odorant receptor repertoire has been estimated at ~1000 genes (Buck and Axel, 1991), 60 putative odorant receptors have been identified in the *Drosophila* genome (Rubin, 2000). Its suitability for genetic studies and the relative simplicity of its olfactory system make *D. melanogaster* an ideal model organism for studies on the functional genomics of odor-guided behavior.

Quantifying odor-guided behavior

Early studies on olfaction in *Drosophila* identified mutants using a variety of behavioral assays, such as the chemosensory jump assay (Helfand *et al.*, 1989; McKenna *et al.*, 1989), Y- or T-maze assays (Rodrigues and Siddiqi, 1978; Ayyub *et al.*, 1990) and the olfactory trap assay (Woodard *et al.*, 1989). We developed a simple, rapid and highly reproducible 'dipstick' assay to quantify avoidance responses to the repellent odorant, benzaldehyde (Anholt *et al.*, 1996). Single sex groups of five individuals are placed in test vials without food for 2 h. The test vials are divided into three compartments by placing marks on the wall 3 and 6 cm from the bottom of the vial. The odorant is introduced into the top compartment of the vial (mostly occupied by the plug) as an aqueous solution on a cotton wool swab, the tip of which is aligned with the 6 cm mark (Figure 1). The number of flies migrating to the compartment remote from the odor source is measured at 5 s intervals, from 15 to 60 s after introduction of the odor source (Figure 1). The 'avoidance score' of the replicate is the average of these 10 counts, giving a possible range of avoidance scores between 0 (maximal attraction to the odor source) and 5 (all flies are in the compartment away from the odor source for the entire assay period, i.e. a maximal repellent response). The response to distilled water is used as a control. Many replicate assays can be done for each line to obtain average values with reduced standard deviations. The elimination of genetic variation through the use of an isogenic genetic background together with our ability to rapidly accumulate large data sets for each line through repeated measurements provides us with the statistical power to reproducibly resolve not only large phenotypic effects, but also small smell impairments.

The genetic architecture of olfactory behavior in *D. melanogaster*

To assess variation in olfactory behavior in a natural population, 43 X chromosomes and 35 third chromosomes were extracted from a natural population and substituted into a common inbred background (Mackay *et al.*, 1996) (Figure 2). Measurements of avoidance responses to

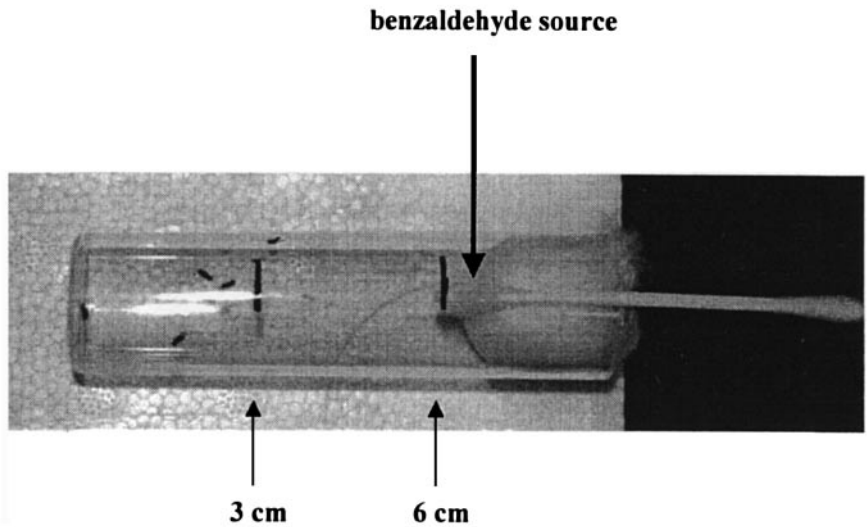

Figure 1 The 'dipstick' assay for measurements of olfactory avoidance responses. The tip of the cotton wool swab is saturated with a benzaldehyde solution. After a 15 s recovery period following introduction of the odor source, the number of flies in the compartment away from the odor source is counted at 5 s intervals and the average of 10 consecutive measurements is recorded as the avoidance score. Details of the assay are described in the text.

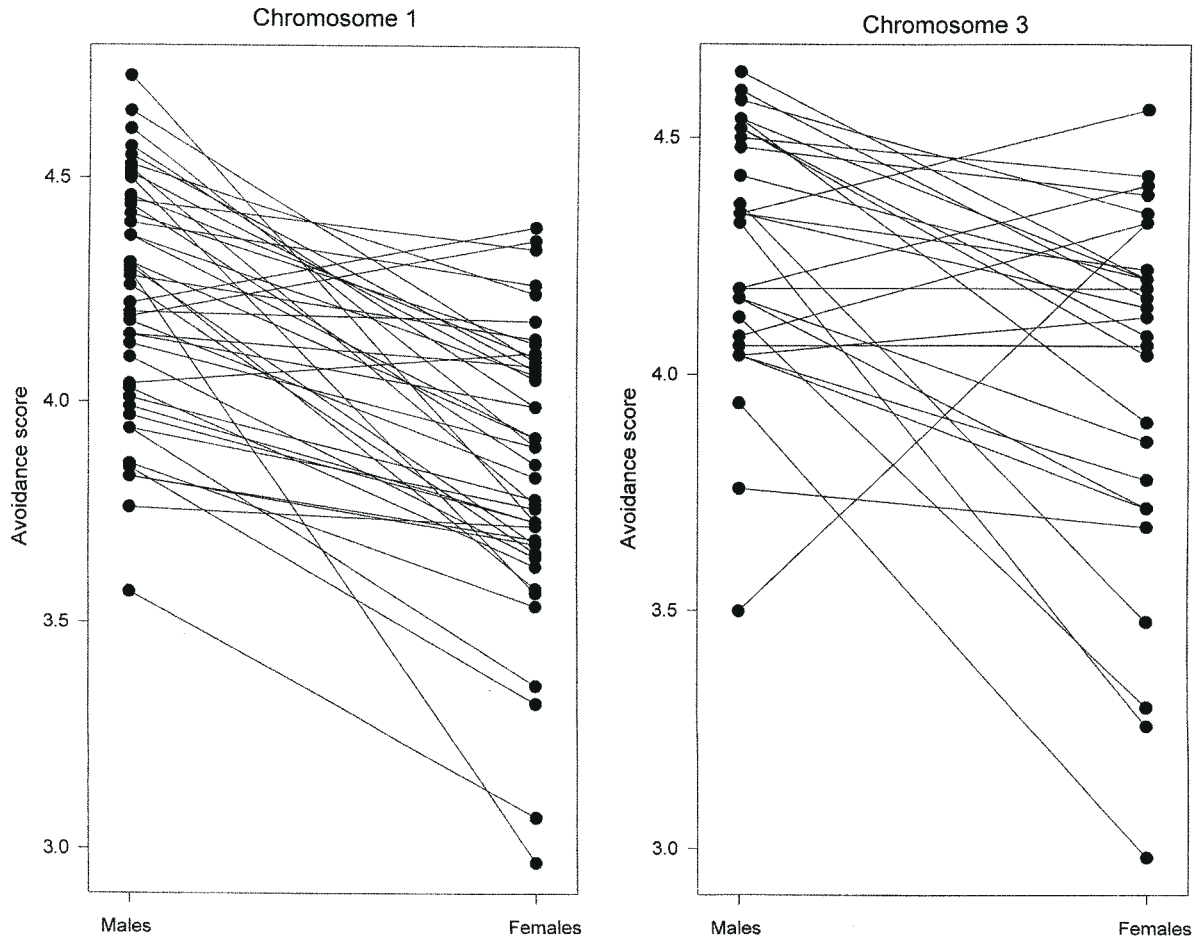

Figure 2 Variation for avoidance response to benzaldehyde among isogenic chromosome 1 (X; left panel) and chromosome 3 (right panel) substitution lines of *Drosophila melanogaster*. The male and female avoidance scores of each line are connected. Adapted from Mackay *et al.* (Mackay *et al.*, 1996).

benzaldehyde demonstrated significant genetic variation in avoidance scores. Surprisingly, the genetic correlations between the sexes for olfactory avoidance behavior were extremely low, suggesting that different genes contribute to variation in avoidance scores in males and females (Mackay *et al.*, 1996) (Figure 2). This may reflect the fact that odor-guided behavior serves different functions in males and females, e.g. only females select or reject oviposition sites. The lack of genetic correlation of avoidance scores between the sexes has profound evolutionary implications. Because the genetic architecture for odor-guided behavior appears sexually dimorphic, it is clear that the olfactory subgenome in males and females evolves along different evolutionary trajectories. This sex × genotype interaction facilitates the maintenance of genetic variation for olfactory behavior, since no single genotype can satisfy optimal fitness requirements for both sexes. Maintaining variation in the trait could ensure survival of the species under the diverse environmental conditions encountered over evolutionary time. Transposon tagging olfactory genes in a controlled genetic background represents one strategy that can be used to systematically dissect the complex genomic architecture that underlies odor-guided behavior.

P-element insertional mutagenesis and epistasis

A comprehensive understanding of the genetic architecture of odor-guided behavior will ultimately require the identification of all the genes that contribute to this trait and characterization of their interactions. One strategy that can, in principle, accomplish this daunting task is the use of *P*-transposable-element insertional mutagenesis, which enables phenotypic effects to be linked directly to gene expression (Cooley *et al.*, 1988; Bellen *et al.*, 1989). The introduction of a transposon in the genome of *D. melanogaster* can result in gene disruption at or near the site of insertion of the transposable element. Introduction of a reporter gene in genetically engineered transposable element constructs (e.g. *P[lArB]*), which can be driven by promoter/enhancer elements near the insertion site, can reveal expression patterns of the affected gene ('enhancer trap'). Furthermore, the use of a cloning vector, such as pBluescript, in the construct can facilitate cloning of flanking sequences adjacent to the site of *P*-element insertion.

We generated 379 isogenic *P[lArB]*-element insert lines (188 are on the second chromosome and 191 on the third chromosome) and screened them for aberrant olfactory responsiveness to benzaldehyde (Anholt *et al.*, 1996). Fourteen *P*-element insert lines were identified that showed a statistically significant reduction in avoidance response to benzaldehyde. Of those lines, four were sexually dimorphic in their responses to benzaldehyde, with females being more severely affected than males (Anholt *et al.*, 1996). *P[lArB]* insertion sites were mapped to specific cytological band positions by *in situ* hybridizations to polytene chromosomes of larval salivary glands. These *P[lArB]*-tagged mutant lines were designated '*smell-impaired*' (*smi*) and each line was named according to its cytological band location (e.g. *smi21F*). Of the 14 *smi* lines originally identified, 12 are amenable to further characterization.

Having available a collection of *smi* lines in a common isogenic background enabled us to investigate whether these genes interact in shaping odor-guided behavior. One method for identifying and ordering genes in functionally interacting groups is to study the enhancer/suppressor effects of one gene on the function or expression of another (García-Bellido, 1981; Botas *et al.*, 1982; Kennison and Russell, 1987; Dambly-Chaudière *et al.*, 1988). We crossed all 12 homozygous *smi* lines in a half-diallel design to generate all 66 possible double heterozygotes and quantified their behavioral responses. The common genetic background of the *smi* lines enabled us to separate average heterozygous effects from epistatic interactions. Significant epistatic interactions were found for nine of the transheterozygous lines, involving 10 of the 12 *smi* loci (Fedorowicz *et al.*, 1998). Interactions among eight of these loci can be represented as an interaction diagram (Figure 3). Thus, these experiments demonstrate that the olfactory subgenome is characterized by a network of genes that display extensive epistasis.

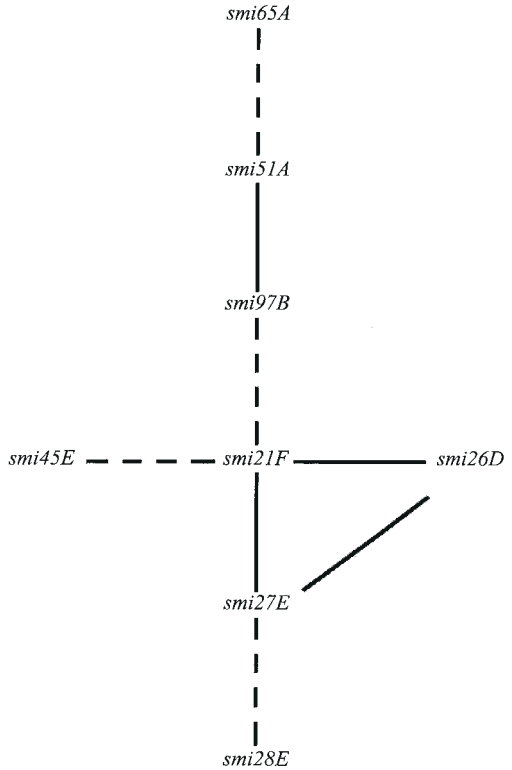

Figure 3 Interaction diagram of *smi* loci. The dotted and uninterrupted lines indicate epistatic effects that enhance and suppress the homozygous mutant phenotype, respectively. Two loci, *smi60E* and *smi61A*, form an independent pair with a positive epistatic effect (not shown). Adapted from Fedorowicz *et al.* (Fedorowicz *et al.*, 1998).

Molecular characterization of *smi* genes

After identifying this set of *smi* lines and demonstrating epistatic interactions among them, the next challenge is to characterize each *smi* locus indicated in Figure 3 at the molecular level. Since the *P[lArB]* construct contains the pBluescript cloning vector, DNA sequences adjacent to the *P*-element insertion site can be rapidly cloned and sequenced. Such sequence information can then be used to identify genes in the vicinity of the *P[lArB]* insertion site that may be responsible for the smell impaired phenotype (Table 1). To be able to conclude that altered expression of a candidate gene is indeed responsible for the observed smell impairment requires extensive further experimentation. First, the *P*-element can be excised and phenotypic revertants can be generated to demonstrate that the *P[lArB]* insertion, rather than an unrelated mutation, is responsible for the smell-impaired phenotype. Secondly, avoidance responses in flies carrying different alleles in the candidate gene must be evaluated, and complementation tests between the original *P[lArB]* insertion line and flies that contain deficiencies or other *P*-element insertions in the region of interest must be performed to provide additional genetic evidence that the candidate gene is indeed associated with the smell-impaired phenotype. Thirdly, it is necessary to demonstrate that expression of the candidate gene is reduced in mutant flies as compared with wild-type or phenotypic revertants. Fourthly, *in situ* hybridization can be used to show that expression patterns in wild-type flies resemble *lacZ* reporter gene expression patterns in the *smi* mutant. Finally, definitive proof that disruption of the candidate *smi* gene is indeed responsible for the smell impaired phenotype can be obtained by demonstrating that introduction of the wild-type gene into the mutant background rescues the mutant phenotype.

Candidate *smi* genes include a wide spectrum of structural and regulatory gene products that may contribute both to the development of the olfactory system and to processes that mediate recognition, processing and integration of olfactory information. Deficits in odorant receptors are likely to generate olfactory impairments specific for certain odorants and the magnitude of such

Table 1 Transposon insertion sites and candidate *smi* genes[a]

P-element insertion	Candidate genes at the cytological location
smi21F	CG5397 (carboxyl esterase); CG4523 (cell adhesion protein); *Acap* (adenyl cyclase-associated protein); CG4887 and CG4896 (RNA binding proteins); CG5001 (heat shock protein); CG5080 (cytoskeletal protein); CG5105 (phospholipase A2 activating protein); **CG4710 (putative odorant binding protein)**; up to 17 unknown gene products
smi26D	CG9493 (protein phosphatase); CG9499 and CG9501 (putative ion channels); CG9507 and CG9505 (endopeptidases); CG9500 (structural protein); *Tig* (Tiggrin; extracellular matrix protein); CG9527 (acyl coenzyme A oxidase homologue); CG9508 (neprilysin); *Cpr* (cytochrome P450 reductase); CG9490
smi27E	CG4496 (zinc finger transcription factor); CG4675 (transport protein); *Wnt4* (Wnt oncogene analogue 4; up to 12 unknown gene products
smi28E	CG7219 (serpin); CG7221 (putative dehydrogenase enzyme); CG7367 (lipase homologue); CG7392 (calmodulin binding protein homologue); CG7424 (ribosomal protein); CG7466 (cell adhesion protein); CG7227 (lysosome membrane protein homologue); CG7356 (γ-glutamyl transferase); *Calo* (calmodulin binding protein); *poe* (transmembrane protein); CG7586 α2-macroglobulin homologue); *Trf* (RNA polymerase II transcription factor); CG8668 and CG8673 (putative galactosyl transferase); up to 17 unknown gene products (including *gel* and *belt*)
smi35A	the alcohol dehydrogenase (*Adh*) region, which has been extensively annotated elsewhere (Ashburner *et al.*, 1999); the *P[lArB]* insertion site is near *wb* (laminin) and ***l(2)34Fa* (dyrk2 kinase homologue)**
smi45E	*Wnt2* (Wnt oncogene analogue 2); *cro* (*croaker*, a courtship impaired and slow mating mutant); CG1931 (cytoskeletal protein); *rdgG* (*retinal degeneration G*; unknown gene product); up to five unknown gene products
smi51A	CG8151 (RNA polymerase II transcription factor); CG8422 (G protein-coupled receptor); CG10104 (endopeptidase); CG17385 and CG17390 (zinc finger transcription factors); *phyl* (*phyllopod*; nuclear protein); *cpsf* (component of the cleavage and adenylation specificity factor complex); *Asx* (*Additional sex combs*; chromatin binding protein); *ttv* (glucuronyl *N*-acetylglucosaminyl transferase homologue); CG10110 (RNA binding protein); up to 23 unknown gene products (including the *oho51*, *auk*, *L* and *xen* loci)
smi60E	*gsb* and *gsb-n* (*gooseberry*; RNA polymerase II transcription factor); *uzip* (integral membrane axon guidance protein); *gol* (*goliath*) and *Tkr* (zinc finger transcription factors); CG2803 (troponin homologue); BcDNA:GH04753 (glutathione *S*-transferase homologue); CG12850 (transcription factor); CG2811 and CG9358 (putative ligand carrier proteins); *RpL19* (ribosomal protein); CG10142, CG9047 and *ESTS:17F2S* (peptidases); *emp* (epithelial membrane protein); *zip* (non-muscle myosin); *ETH* (ecdysis triggering hormone); ***NaCP60E* (sodium channel protein)**; up to 20 unknown gene products
smi61A	CG1201, BcDNA:GH04978 and *Pk61C* (protein kinases); CG1216 and *Gyk* (glycerol kinase); CG11869 (putative microtubule-associated protein); CG13406 (G protein-coupled receptor); *miple2* (midline/pleiotrophin family protein); Lsp1γ (larval serum protein 1 γ-subunit); CG1212 (putative signal transduction protein); CG7051 (dynein-like motor protein); CG7036 (putative transcription factor); *Mtch* (mitochondrial carrier protein); *NitFlit* (nitrilase and fragile histidine triad fusion protein); CG17142 (cytoskeletal structural protein); *Kaz1* (serine protease inhibitor); up to 16 unknown gene products (including *fwd*)
smi65A	CG10541(cytoskeletal structural protein); CG10546 (ligand carrier binding protein); CG17498 (cell cycle regulator); CG5537 (uracil phosphoribosyl transferase); *S6k (*ribosomal protein S-p70-protein kinase); *vn* (neuregulin-like protein); *Bj1* (chromatin binding protein); *33-13* and *Ets65A* (DNA binding proteins); CG10486, CG5592, CG6600 and CG10226 (transport proteins); CG10487 (receptor guanylate cyclase); CG10489 (DNA replication protein); CG13287, CG13296, CG10274 and CG7386 (transcription factors); CG10467 (aldose 1-epimerase homologue); CG10469, CG10472, CG10475, CG10477, CG6457, CG6462, CG6467, CG6483, CG6480 and CG6592 (endopeptidases); CG10163 (phospholipase A1 homologue); *l(3)mbn* [lethal(3) malignant blood neoplasm membrane protein]; CG10533, CG10461, CG10529 and CG12330 (structural proteins); *Lcp20*, *Lcp11*, *Lcp65Aa*, *Lcp65Ac*, *Lcp65Ad*, *Lcp6*, *Lcp65Ab1*, *Lcp65Ab2*, *Lcp65Ae*, *Lcp65Af*, and *Lcp65Ag3* (larval cuticle proteins); *Acp65Aa* (adult cuticle protein); CG13289 (cell adhesion protein); CG6062 and CG6619 (putative signal transduction proteins); CG6610, *l(3)02094* and CG13298 (RNA binding proteins); *Snap25* (synaptosome-associated protein 25kD); CG10160 (lactate dehydrogenase); CG10173 (peptidase); *D19A* and *D19B* (nuclear zinc finger proteins); *lanA* (laminin A); *Mdr65* (multiple drug transporter); *Tm* (transportin); up to 41 unknown gene products (including *Jon65A*, *tantalus* and *prd1*)
smi79E	*Aats-ile* and CG11471(isoleucyl tRNA synthetase); CG7495 (dopamine β-monooxygenase homologue); CG9085 (protein kinase); *Csp* (cysteine string protein); *Ddx1(*ATP-dependent helicase); *Hem* (plasma membrane protein); *Ten-m* (tenascin); up to15 unknown gene products (including *exb*)

Table 1 Continued

P-element insertion	Candidate genes at the cytological location
smi97B	CG6036 (protein phosphatase); CG6162 (transporter protein); *ird15* (*immune response deficient*); CG14239 (putative ion channel); ***scrib* (*scribbled*; an adhesion protein with multiple leucine-rich repeats and PDZ domains)**; CG5443 (hexokinase); *Pdf* (pigment dispersing factor, neuropeptide hormone); *dei* (RNA polymerase II transcription factor); CG5432 (aldolase homologue); CG6490 (cell adhesion protein); up to six unknown gene products
smi98B	CG4849 and CG4980 (RNA binding proteins); CG5540 (olfactory receptor); CG4963 (mitochondrial carrier protein homologue); CG12260, CG12261 and CG4976 (transcription factors); CG5017 and CG5520 (chaperones); CG5527 (endopeptidase); *Acp98AB* (accessory gland specific peptide); *Ets98B (*DNA binding protein); *RpL1* (ribosomal protein); up to 16 unknown gene products

[a]Candidate genes likely to account for smell-impairments induced by *P*-element insertions, as evident from preliminary unpublished experiments, are shown in bold print. Note the large number of predicted transcription units of unknown function, which may harbor genes that contribute to odor-guided behavior. Data were compiled from the *Drosophila* genomic sequence as accessed via Flybase (http://flybase.bio.indiana.edu/). For *smi35A* only the two most likely candidate genes have been indicated. This region has already been annotated extensively (Ashburner *et al.*, 1999). The *P[lArB]* element in *smi79E* has inserted next to a *hoppel* transposon, complicating efforts to identify the affected *smi* gene.

impairment will depend on the redundancy of recognition of the odorant by other receptors. The diversity and restricted size of the *Drosophila* odorant receptor family suggests that this system has less redundancy in odorant recognition than its mammalian counterpart. The best documented example of a specific olfactory deficit due to absence of an odorant receptor has been described for *Caenorhabditis elegans*, where a null mutation in the *odr-10* gene, which encodes an odorant receptor for diacetyl, results in impaired chemotaxis of mutant nematodes to diacetyl (Sengupta *et al.*, 1996).

Whereas disruption of genes encoding odorant binding proteins and odorant receptors will result specifically in chemosensory impairments, gene products that mediate signal transduction, transfer of the signal from the periphery to the central nervous system, signal integration and the generation of a behavioral response are likely to be pleiotropic. For example, mutations in the *retinal degeneration B* (*rdgB*) gene, which encodes a phosphatidyl inositol transfer protein, and in the *norpA* gene, which encodes a phospholipase C, result in both visual and olfactory impairments (Smith *et al.*, 1991; Woodard *et al.*, 1992; Vihtelic *et al.*, 1993; Riesgo-Escovar *et al.*, 1994, 1995).

Candidate genes for previously identified *smi* lines are listed in Table 1. Preliminary characterization of several of these genes implicate a novel putative odorant binding protein (*smi21F*; note its central position in the epistasis diagram in Figure 3); a voltage-gated sodium channel (*smi60E*); *Scribble*, a gene that encodes a protein containing multiple leucine rich repeats and PDZ domains (*smi97B*; Bilder and Perrimon, 2000); and a novel dual-specificity tyrosine-regulated protein kinase, dyrk2 (*smi35A*). Ultimately, a complete understanding of the genetic architecture of odor-guided behavior requires characterization of all the genes involved and epistatic interactions among them, as well as understanding to what extent polymorphisms in each gene contribute to variation in olfactory behavior in nature. Rapid technological advances in functional genomics are bringing the realization of this ambitious goal within reach.

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References

Adams, M., Celniker, S.E., Holt, R.A., Evans, C.A., Gocayne, J.D., Amanatides, P.G. *et al.* (2000) *The genome sequence of* Drosophila melanogaster. Science, 287, 2185–2195.

Anholt, R.R.H., Lyman, F.L. and **Mackay, T.F.C.** (1996) *Effects of single P-element insertions on olfactory behavior in* Drosophila melanogaster. Genetics, 143, 293–301.

Ashburner, M., Misra, S., Roote, J., Lewis, S.E., Blazej, R., Davis, T. *et al.* (1999) *An exploration of the sequence of a 2.9-Mb region of the genome of* Drosophila melanogaster*: the Adh region*. Genetics, 153, 179–219.

Ayyub, C., Paranjape, J., Rodrigues, V. and **Siddiqi, O.** (1990) *Genetics of olfactory behavior in* Drosophila melanogaster. J. Neurogenet., 23, 965–982.

Bellen, H. J., O'Kane, C.J., Wilson, C., Grossniklaus, V., Pearson, R.K. and **Gehring W.J.** (1989) *P-element-mediated enhancer detection: a versatile method to study development in* Drosophila. Genes Dev., 3, 1288–1300.

Bilder, D. and **Perrimon, N.** (2000) *Localization of apical epithelial determinants by the basolateral PDZ protein Scribble*. Nature, 403, 676–680.

Botas, J., del Prado, J.M. and **García-Bellido, A.** (1982) *Gene-dose*

titration analysis in the search of trans-regulatory genes in Drosophila. EMBO J., 1, 307–310.

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

Clyne, P.J., Warr, C.G., Freeman, M.R., Lessing, D., Kim, J.H. and **Carlson, J.R.** *(*1999) *A novel family of divergent seven-transmembrane proteins: candidate odorant receptors in* Drosophila*.* Neuron, 22, 327–338.

Cooley, L., Kelley, R. and **Spradling, A.** (1988) *Insertional mutagenesis of the* Drosophila *genome with single P-elements.* Science, 239, 1121–1128.

Dambly-Chaudière, C., Ghysen, A., Jan, L.Y. and **Jan, Y.N.** (1988) *The determination of sense organs in* Drosophila*: interaction of scute with daughterless.* Roux's Arch. Dev. Biol., 197, 419–423.

Fedorowicz, G.M., Fry, J.D., Anholt, R.R.H., and **Mackay, T.F.C.** (1998) *Epistatic interactions between smell impaired loci in* Drosophila melanogaster. Genetics, 148, 1885–1891.

García-Bellido, A. (1981) *The bithorax syntagma.* In Lakovaara, S. (ed.), Advances in Genetics, Development and Evolution of *Drosophila*. Plenum Press, New York, pp. 135–148.

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

Gao, Q., Yuan, B. and **Chess, A.** (2000) *Convergent projections of* Drosophila *olfactory neurons to specific glomeruli in the antennal lobe*. Nature Neurosci., 3, 780–785.

Helfand, S.L. and **Carlson, J.** (1989) *Isolation and characterization of an olfactory mutant in Drosophila with a chemically specific defect.* Proc. Natl. Acad. Sci. USA, 86, 2908–2912.

Kennison, J.A. and **Russell, M.A.** (1987) *Dosage-dependent modifiers of homeotic mutations in* Drosophila melanogaster. Genetics, 116, 75–86.

Laissue, P.P., Reiter, C., Hiesinger, P.R., Halter, S., Fischbach, K.F. and **Stocker, R.F.** (1999) *Three-dimensional reconstruction of the antennal lobe in* Drosophila melanogaster. J. Comp. Neurol., 405, 543–552.

Mackay, T.F.C., Hackett, J.B., Lyman, R.F., Wayne, M.L. and **Anholt, R.R.H.** (1996) *Quantitative genetic variation of odor-guided behavior in a natural population of* Drosophila melanogaster. Genetics 144, 727–735.

McKenna, M., Monte, P., Helfand, S., Woodard, C. and **Carlson, J.** (1989) *A simple chemosensory response in* Drosophila *and the isolation of acj mutants in which it is affected.* Proc. Natl. Acad. Sci. USA, 86, 8118–8122.

Ngai, J., Dowling, M.M., Buck, L., Axel, R. and **Chess, A.** (1993) *The family of genes encoding odorant receptors in the channel catfish.* Cell, 72, 657–666.

Pomeroy, S.L., Lamantia, A.S. and **Purves, D.** (1990) *Postnatal construction of neural circuitry in the mouse olfactory bulb.* J. Neurosci., 10, 1952–1966.

Riesgo-Escovar, J.R., Woodard, C. and **Carlson, J.** (1994) *Olfactory physiology in the maxillary palp requires the visual system gene rdgB.* J. Comp. Physiol. A, 175, 687–693.

Riesgo-Escovar, J., Raha, D. and **Carlson, J.R.** (1995) *Requirement for a phospholipase C in odor response: overlap between olfaction and vision in* Drosophila*.* Proc. Natl. Acad. Sci. USA, 92, 2864–2868.

Rodrigues, V. and **Siddiqi, O.** (1978) *Genetic analysis of chemosensory pathway.* Proc. Ind. Acad. Sci., 87B, 147–160.

Rubin, G.M. (2000) *Comparative genomics of the eukaryotes.* Science, 287, 2204–2215.

Sengupta, P., Chou, J.H. and **Bargmann, C.I.** (1996) *Odr-10 encodes a seven transmembrane domain olfactory receptor required for responses to the odorant diacetyl*. Cell, 84, 899–909.

Smith, D.P., Stamnes, M.A. and **Zucker, C.S.** (1991) *Signal transduction in the visual system of* Drosophila*.* Annu. Rev. Cell Biol., 7, 161–190.

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

Troemel, E.R., Chou, J.H., Dwyer, N.D., Colbert, H.A. and **Bargmann, C.I.** (1995) *Divergent seven transmembrane receptors are candidate chemosensory receptors in* C. elegans*.* Cell, 83, 207–218.

Vihtelic, T.S, Goebl, M., Milligan, S., O'Tousa, J.E. and **Hyde, D.R.** (1993) *Localization of* Drosophila *retinal degeneration B, a membrane-associated phosphatidylinositol transfer protein.* J. Cell Biol., 122, 1013–1022.

Vosshall, L.B., Amrein, H., Morozov, P.S., Rzhetsky, A. and **Axel, R.** (1999) *A spatial map of olfactory receptor expression in the* Drosophila *antenna.* Cell, 96, 725–736.

Vosshall, L.B., Wong, A.M. and **Axel, R.** (2000) *An olfactory sensory map in the fly brain.* Cell, 102, 147–159.

Woodard, C., Huang, T., Sun, H., Helfand, S.L. and **Carlson, J.** (1989) *Genetic analysis of olfactory behavior in* Drosophila*: a new screen yields the ota mutants.* Genetics, 123, 315–326.

Woodard, C., Alcorta, E. and **Carlson, J.** (1992) *The* rdgB *gene in* Drosophila*: a link between vision and olfaction.* J. Neurogenet., 8, 17–32.

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