Odorant Receptor Polymorphisms and Natural Variation in Olfactory Behavior in *Drosophila melanogaster*

Stephanie M. Rollmann,^{*,1} Ping Wang,^{†,‡,2} Priya Date,^{*} Steven A. West,^{‡,§} Trudy F. C. Mackay^{†,‡}
and Robert R. H. Anholt^{†,‡,§}

^{*}Department of Biological Sciences, University of Cincinnati, Cincinnati, Ohio 45221 and [†]Department of Genetics, [‡]W. M. Keck Center for Behavioral Biology and [§]Department of Biology, North Carolina State University, Raleigh, North Carolina 27695

Manuscript received May 30, 2010
Accepted for publication July 10, 2010

ABSTRACT

Animals perceive and discriminate among a vast array of sensory cues in their environment. Both genetic and environmental factors contribute to individual variation in behavioral responses to these cues. Here, we asked to what extent sequence variants in six *Drosophila melanogaster* odorant receptor (*Or*) genes are associated with variation in behavioral responses to benzaldehyde by sequencing alleles from a natural population. Sequence analyses showed signatures of deviations from neutrality for *Or42b* and *Or85f*, and linkage disequilibrium analyses showed a history of extensive recombination between polymorphic markers for all six *Or* genes. We identified polymorphisms in *Or10a*, *Or43a*, and *Or67b* that were significantly associated with variation in response to benzaldehyde. To verify these associations, we repeated the analyses with an independent set of behavioral measurements of responses to a structurally similar odorant, acetophenone. Association profiles for both odorants were similar with many polymorphisms and haplotypes associated with variation in responsiveness to both odorants. Some polymorphisms, however, were associated with one, but not the other odorant. We also observed a correspondence between behavioral response to benzaldehyde and differences in *Or10a* and *Or43a* expression. These results illustrate that sequence variants that arise during the evolution of odorant receptor genes can contribute to individual variation in olfactory behavior and give rise to subtle shifts in olfactory perception.

RESEARCHERS in many scientific fields have long appreciated that different animal species perceive the world differently. In fact, these differences are so striking that new disciplines have arisen to study the adaptations of sense organs to the environment (*e.g.*, ALI 1978; LYTHGOE 1979; DUSENBERY 1992). Differences in sensory perception exist not only between species, but also between populations of a single species and between individuals within a population. What is the underlying genetic architecture for individual variation in sensory perception?

Olfaction provides an excellent model for examining the underlying genetic mechanisms that result in variation in behavior. In both vertebrates and invertebrates, odorants are detected by families of odorant receptors expressed in populations of olfactory receptor

neurons (ORNs), whose activation elicits a distinct spatial pattern of glomerular activity in the brain (BUCK and AXEL 1991; VASSAR *et al.* 1994; MOMBAERTS *et al.* 1996; LAISSUE *et al.* 1999; GAO *et al.* 2000; VOSSHALL *et al.* 2000; BHALERAO *et al.* 2003; WANG *et al.* 2003). This combinatorial code allows for discrimination of a diverse repertoire of odorants.

Drosophila melanogaster has a relatively simple olfactory system with only 60 odorant receptor (*Or*) genes (VOSSHALL and STOCKER 2007) compared to ~1000 in the mouse (ZHANG and FIRESTEIN 2002; ZHANG *et al.* 2004). The 60 genes are located throughout the genome, and 2 of these genes are alternatively spliced for a total of 62 identified proteins (CLYNE *et al.* 1999; GAO and CHES 1999; VOSSHALL *et al.* 1999; ROBERTSON *et al.* 2003). Furthermore, clusters of *Ors* throughout the genome suggest several recent gene duplication events (ROBERTSON *et al.* 2003).

The response spectra of individual ORNs have been extensively characterized using extracellular electrophysiological recordings from single sensilla on the antennae and maxillary palps. Recordings from basiconic sensilla on the antenna identified classes of neurons with distinct olfactory response profiles organized as two to four neurons in each sensillum with specific neuronal combinations occurring in distinct spatial regions of the antenna (DE BRUYNE *et al.* 1999, 2001).

Supporting information is available online at <http://www.genetics.org/cgi/content/full/genetics.110.119446/DC1>.

Sequence data from this article have been deposited with the EMBL/GenBank Data Libraries under the following accession nos.: *Or7a*, GU445973–GU446022; *Or10a*, GU446073–GU446269; *Or42b*, GU445928–GU445972; *Or43a*, GU446270–GU446436; *Or67b*, GU446437–GU446587; and *Or85f*, GU446023–GU446072.

¹Corresponding author: Department of Biological Sciences, University of Cincinnati, Cincinnati, OH 45221-0006.
E-mail: stephanie.rollmann@uc.edu

²Present address: Department of Neurobiology, Duke University Medical Center, Durham, NC 27710.

The majority of ORNs express a unique odorant receptor in addition to the highly conserved coreceptor, *Or83b* (JONES *et al.* 2005). Studies of a null mutant of *Or83b* implicated this receptor in positioning odorant receptor proteins in the sensory dendrites (LARSSON *et al.* 2004; BENTON *et al.* 2006). Odorant receptors in *Drosophila* have an atypical membrane topology with a cytoplasmic N terminus and an extracellular C terminus (BENTON *et al.* 2006). Specific domains in the third cytoplasmic loops of two odorant receptors, *Or22a* and *Or43a*, have been implicated to interact with the third loop of *Or83b* (BENTON *et al.* 2006). *Drosophila* odorant receptors act as ligand-gated nonselective cation channels formed by a dimeric complex between a unique Or and the *Or83b* coreceptor (SATO *et al.* 2008; WICHER *et al.* 2008).

Several studies have examined ligand specificities of individual odorant receptor proteins and demonstrated that they respond to diverse and overlapping suites of ligands. Response profiles for many receptors have been characterized using the Gal4/UAS system to drive expression of individual odorant receptors in a mutant ORN lacking expression of its endogenous receptor, followed by electrophysiological recording (DOBRITSA *et al.* 2003; HALLEM *et al.* 2004; HALLEM and CARLSON 2006). In addition, misexpression studies of *Or43a* resulted in a reduction of behavioral avoidance responses to benzaldehyde (STORTKUHL *et al.* 2005). This result combined with electrophysiological recordings from ORNs and heterologous expression in *Xenopus* oocytes further functionally characterized the odorant response profiles of this receptor (WETZEL *et al.* 2001) and identified several *Or43a* ligands, such as fruit-derived odorants benzaldehyde, cyclohexanone, cyclohexanol, and benzyl alcohol (STORTKUHL and KETTLER 2001; HALLEM *et al.* 2004).

Despite advances in our understanding of odor coding, the molecular mechanisms responsible for variation in olfactory perception remain poorly understood. *D. melanogaster* is especially amenable to conducting such studies given its quantitatively simple olfactory system and since large numbers of genetically identical individuals can be reared in a common environment and these individuals can be subjected to simple, rapid, and highly reproducible quantitative behavioral assays (ANHOLT and MACKAY 2004). Here, we examine how molecular variation in odorant receptors contributes to variation in olfactory behavior in inbred lines derived from a natural population of *D. melanogaster*. We focused our analyses on six odorant receptors, *Or7a*, *Or10a*, *Or42b*, *Or43a*, *Or67b*, and *Or85f*, which have been shown by electrophysiology (STORTKUHL and KETTLER 2001; HALLEM *et al.* 2004; STORTKUHL *et al.* 2005; HALLEM and CARLSON 2006), through heterologous expression systems (WETZEL *et al.* 2001), or by calcium imaging studies (WANG *et al.* 2003) to respond to benzaldehyde. Significant variation in behavioral responses to benzaldehyde

has been observed previously in this population and was normally distributed as is typical for a quantitative trait influenced by multiple genes (WANG *et al.* 2007). Here, we report associations between olfactory behavior and sequence variants in three *Or* genes. To validate the reliability of these associations we measured responses to a structurally similar odorant, acetophenone, in the same population, and showed that the associations with variation in responses to both odorants are largely similar with occasional molecular polymorphisms associated with variation in response to only one, but not the other odorant. These observations illustrate how sequence variants that arise during the evolution of *Or* genes can contribute to individual variation in olfactory behavior, how polymorphisms can give rise to subtle shifts in olfactory perception, and how naturally arising mutations within a population can combine to generate broad individual variation in sensory perception.

MATERIALS AND METHODS

Drosophila stocks: *D. melanogaster* isofemale lines were established from flies collected from a natural population in Raleigh, North Carolina. Each isofemale line was subsequently inbred by 20 generations of full-sib mating to generate wild-derived inbred lines (AYROLES *et al.* 2009). All flies were reared on standard agar–yeast–molasses medium at 25° under a 12-h light/dark cycle. These are the same lines used to examine phenotypic variation in odor-mediated behavior by (WANG *et al.* 2007, 2010).

Behavioral assay: Variation in odor-mediated behavioral responses to benzaldehyde and acetophenone was measured by WANG *et al.* (2007) and WANG *et al.* (2010), respectively, using the behavioral assay of ANHOLT *et al.* (1996). Dose-response experiments were conducted to determine the optimal concentration of benzaldehyde and acetophenone needed to maximally resolve variation among the wild-derived lines (WANG *et al.* 2007, 2010). Briefly, five flies of a single sex were placed in a vial demarcated into two equal compartments. The odorant, 3.5% (v/v) benzaldehyde or 3.5% (v/v) acetophenone, was then introduced on a cotton swab and the number of flies in the distal-most compartment of the vial were scored every 5 sec for 1 min. An average score was recorded and the assay was repeated 10 times for each sex for each genotype. The score for each line was the average of these 10 replicates, with a score of 0 indicating maximal attraction to the odorant and a score of 5 indicating maximum repulsion.

Identification of odorant receptor polymorphisms: Genomic DNA was extracted using Gentra DNA isolation kits (Gentra Systems, Minneapolis, MN). Primers were designed approximately every 500 bp from the published DNA sequence (DRYSDALE *et al.* 2005) for each odorant receptor gene (*Or7a*, *Or10a*, *Or42b*, *Or43a*, *Or67b*, and *Or85f*) to obtain overlapping fragments resulting in full-length sequencing of the coding and non coding regions, as well as 5'- and 3'-untranslated regions. PCR products were amplified and sequenced directly using ABI big dye terminator cycle sequencing chemistry [Applied Biosystems (ABI), Foster City, CA] with the original PCR primers as well as internal sequencing primers. Sequences were aligned using Vector NTI Suite 11 software (Informax, Frederick, MD) to identify polymorphic sites.

Population genetic analyses: Population genetic analyses were conducted for each of the six odorant receptor loci using DnaSP 5.10.00 (LIBRADO and ROZAS 2009; <http://www.ub.es/>

dnasp). Estimates of population genetic parameters π and θ , and Tajima's D (Tajima 1989), were calculated. Significant departures from neutrality were examined using the McDonald–Kreitman (MK) test (McDonald and Kreitman 1991). For the MK test, *D. melanogaster* population sequences were compared to *D. simulans* sequence (Release 1.3, Apr 2005, droSim1), with the exception of *Or85f* in which *D. sechellia* (Release 1.3, Oct 2005, droSec1) was used for comparison. A single allele of *Or67b* contained a premature stop codon and was not included in the MK analysis (interpretation of analyses did not differ with its inclusion). Linkage disequilibrium (LD) between markers was determined using Tassel 2.1 software (<http://www.maizogenetics.net/tassel>) and significant differences between polymorphic markers were examined using Fisher's exact test. Haplotypes were determined using SNAP software (Price and Carbone 2005).

Genotype–phenotype associations: To assess the extent to which individual polymorphisms are associated with olfactory behavior, associations between molecular polymorphisms and behavior were determined using ANOVA according to the two-way factorial model $y = \mu + M + S + M \times S + E$, with the two main fixed effects being molecular marker (M) and sex (S), and where E indicates error. Differences in trait values among haplotypes were assessed by two-way factorial ANOVA with the model $y = \mu + H + S + H \times S + E$, with the two main fixed effects of haplotype (H) and sex (S), and where E indicates error. We accounted for multiple testing using permutation tests (Churchill and Doerge 1994). Phenotypic line means were randomly permuted with respect to marker data. A thousand permuted data sets were generated and the lowest P -value for each of the 1000 permuted data sets was recorded. A distribution of P -values was generated and significant genotype–phenotype associations were determined if the P -value in the nonpermuted data was in the lower 5% of the distribution of permuted values. Singletons were excluded from LD and genotype–phenotype association analyses. The numbers of sequenced alleles for association studies for benzaldehyde are as follows: *Or10a*, $N = 177$; *Or43a*, $N = 152$; *Or67b*, $N = 136$. For acetophenone the numbers of sequenced alleles are as follows: *Or10a*, $N = 174$; *Or43a*, $N = 149$; *Or67b*, $N = 135$.

Quantitative reverse transcriptase PCR: Total RNA was isolated from wild-derived inbred lines that corresponded to either the haplotype with the highest response or the haplotype with the lowest response to benzaldehyde for each of the three genes, *Or10a*, *Or43a*, and *Or67b*. Four independent RNA samples were extracted for each line and sex using the RNeasy kit (ABI). RNA samples were treated with DNase (Ambion, Austin, TX) and reverse transcribed using the High Capacity cDNA Reverse Transcription kit (ABI). Real-time quantitative PCR was performed using TaqMan reagents on an ABI PRISM 7500 system. Primer–probe sets were custom designed as follows: *Or10a*, forward (For) CCAACTGCTGGTTTATTGCTATGG, reverse (Rev) CGAGTCGACGTTGTTTGGCTTAA, probe FAM-CACAGACCAGTGCTACTTT; *Or43a*, For CCTACTACAATCGGGCCAATGAAAT, Rev GTACCAGG GCATTGTAAACAG, probe FAM-ATGCCTCGAGAACAAAC; *Or67b*, For GTGGAGTACAGTGCTATGCA, Rev AGGCGAC GAGACTGTAGATTATACT, probe FAM-CAAAATGCGAGT TAATCG. Odorant receptor expression was normalized to the expression of ribosomal protein, *rpl32* (ABI Assay ID: Dm02151827_g1). Relative expression was determined by comparison of dT values relative to *rpl32* expression using the $2^{-\Delta\Delta CT}$ method (Livak and Schmittgen 2001). Three technical replicates were performed for each extract of each line and sex. Expression differences between the high and low responder haplotypes for each sex were determined by ANOVA.

RESULTS

Identification of polymorphisms in Odorant receptor genes: We sequenced six odorant receptor genes (*Or7a*, *Or10a*, *Or42b*, *Or43a*, *Or67b*, and *Or85f*) previously shown to respond to benzaldehyde (Stortkuhl and Kettler 2001; Wetzel *et al.* 2001; Wang *et al.* 2003; Hallem *et al.* 2004; Stortkuhl *et al.* 2005) in 50 wild-derived inbred lines from a single natural population (Ayroles *et al.* 2009). For each *Or* gene we identified single nucleotide polymorphisms (SNPs) and insertion/deletion polymorphisms (indels) that were present in more than one line (Figure 1; supporting information, Table S1). Polymorphisms in these receptors result in five amino acid substitutions in *Or7a* (A2T, R47Q, P245H, I282V, and A311G), five amino acid substitutions in *Or10a* (F18L, I264V, M347V, C350S, and G396D), two amino acid substitutions in *Or42b* (S159P, S206T), four amino acid substitutions in *Or43a* (I170M, V254A, L287M, and F333I), six amino acid substitutions in *Or67b* (Q4E, Y25H, G121D, Q124K, L221I, and R369K), and seven amino acid substitutions in *Or85f* (L47V, R96G, T164I, G186S, A228E, H253R, and V331A). There were also several amino acid substitutions that were represented in only one inbred line: *Or10a* (R109S and E228K), *Or42b* (L132Q, I276V, and Q352H), *Or67b* (K131N and L237F), and *Or85f* (H167Q).

To assess the extent of nonrandom associations among polymorphic markers we conducted an LD analysis. For each odorant receptor, significant LD was commonly observed only between polymorphisms in close physical proximity (Figure 2). However, *Or* loci differed in the level and pattern of LD. Little LD was observed between polymorphisms in the ~2-kb region of *Or42b*, while LD was observed among markers throughout the ~2.5-kb region encompassing *Or43a*. In addition, we detected long-range linkage disequilibrium in *Or10a* among polymorphic nucleotide marker C-375T and eight markers (T857A, C867T, T1346C, T1372A, G1373T, G1377A, G1550C, and 1606Del) spanning an ~2-kb region. Also, in *Or67b* long-range LD was observed among nucleotide marker T1922A and five markers (G1506T, C1316T, 784Del, C662T, and C523T) (an ~1.4-kb region).

Population genetic analyses: Population genetic analyses of the sequences of these six *Or* genes revealed overall estimates of nucleotide diversity (π) between 0.0014 and 0.0122 and θ_w between 0.0015 and 0.0082 (Table 1). For all six loci, approximately the same sized (~2.3-kb) genomic region was examined. Overall, higher nucleotide variation was observed in introns compared to coding regions, as is typical for coding sequences that are under selective constraint. Estimates of nucleotide diversity in *Or67b* may be influenced by the putative upstream regulatory region of a closely neighboring gene of unknown function (*CG8336*, ~500 bp

FIGURE 1.—Molecular variation in six *Or* genes. The gene structure for each receptor is schematically represented with a horizontal line denoting genomic DNA and exons represented by shaded boxes. The number of SNPs in the coding and noncoding regions is shown within each exon or within hexagons, respectively. Open boxes denote 5'-UTRs and 3'-UTRs. The number of indels is indicated by numbers within inverted triangles.

away). These population genetic parameters are consistent overall with average diversity values for *D. melanogaster* (MORIYAMA and POWELL 1996; ANDOLFATTO 2001) and similar to what has been observed for odorant-binding proteins (WANG *et al.* 2007). An exception is *Or42b* in which estimates of π were reduced ($\pi = 0.0014$) relative to other *D. melanogaster Or* loci.

Next, we tested for deviations from neutrality using the MK test (McDONALD and KREITMAN 1991). The MK test compares within-species variation with between-species divergence. This method takes advantage of the expectation under the neutral mutation hypothesis that the ratio of nonsynonymous to synonymous (silent) differences between species should be the same as the ratio of nonsynonymous to silent polymorphisms within species. A significant deviation in ratios between species *vs.* within species rejects the neutral mutation hypothesis. We observed no departure from neutrality in the coding regions of four of six odorant receptor loci (Table 2). However, we detected significant deviations from neutrality for *Or42b* and *Or85f* that appear to be driven by a reduction in silent-site variation within species (Table 2). Calculations of Tajima's *D* revealed estimates for *Or42b* and *Or85f* to be -0.3185 and 0.2067 , respectively. These estimates do not differ significantly from expectations under the neutral mutation model (Tajima 1989).

Associations between polymorphisms in *Or* genes and olfactory behavior: To assess to what extent sequence variation in these odorant receptors may

contribute to natural variation in olfactory behavior, we conducted first a preliminary analysis on ~ 50 alleles from this wild-derived population for each of these six *Or* genes. This analysis was designed to identify loci in which more polymorphisms than expected by chance were associated with variation in odor-mediated behavior in response to benzaldehyde (WANG *et al.* 2007). On the basis of results of these analyses, we focused our investigation of genotype–phenotype relationships on three *Or* genes (*Or10a*, *Or43a*, and *Or67b*) and we obtained additional sequences of alleles for each of these loci.

For each polymorphic marker, the statistical association between the marker and the phenotypic means for olfactory behavior to benzaldehyde was determined using two-way factorial analysis of variance. We identified multiple polymorphisms in each of these three *Or* genes (*Or10a*, *Or43a*, and *Or67b*) that significantly contributed to variation in responsiveness to benzaldehyde in this population (Figure 3). Thirteen polymorphisms in *Or10a* contributed to variation in behavioral responses, exceeding the permutation threshold. It is of note that of those markers, C-375T and eight markers, T857A, C867T, T1346C, T1372A, G1373T, G1377A, G1550C, and 1606Del, were in complete LD (Figure 2). In the case of *Or43a*, 13 polymorphisms were significantly associated with variation in response to benzaldehyde (Figure 3). Markers C-167A and C-152T are in complete LD as well as marker group C1158T, T1167A, G1169A, and T1175A. Substantial LD ($r^2 > 0.87$) was also observed between T576C, C685G and C1158T, T1167A, G1169A, and T1175A (Figure 2). Furthermore, of the significantly associated SNPs, one (C685G) resulted in an isoleucine to methionine (I170M) substitution that is located extracellularly. Finally, 12 polymorphisms in *Or67b* contributed to variation in behavior (Figure 3). Of those markers, there were three groups whose members were in complete LD: C523T, C662T, 784Del, C1316T, G1506T, and T1922A; G932A, G943A, and A944C; and T1475A and G1524T (Figure 2).

As similar large collections of wild-derived inbred lines from other populations of *D. melanogaster* do not exist, we devised an alternative strategy to confirm the validity of the observed associations. To independently verify the observed associations while at the same time gaining additional insights into the relationship between variation in olfactory behavior and variation in *Or* gene polymorphisms, we repeated our association analyses with an independent set of behavioral measurements of responses to a structurally similar odorant, acetophenone, which differs from benzaldehyde only in the presence of a methyl group (Figure 3). Previous studies showed substantial correlation between behavioral responses to benzaldehyde and acetophenone (WANG *et al.* 2010). Thus, the prediction is that the association profiles from the analyses for both odorants would show global similarity, while sporadically uncov-

FIGURE 2.—Linkage disequilibrium between polymorphisms in each of six *Or* genes. Segments below and above diagonals reflect r^2 values and P -values, respectively, for all possible marker pair combinations. P -values are determined by Fisher's exact test (not corrected for multiple tests).

ering individual polymorphisms that are associated with only one, but not the other odorant. Indeed, we found overlapping sets of SNPs contributing to variation in behavioral responses to both odorants. Fifteen polymorphisms in *Or10a*, 2 polymorphisms in *Or43a*, and 14 polymorphisms in *Or67b* were associated with variation in responses to acetophenone (Figure 3). Of those markers, many were identical to those contributing to variation in responses to benzaldehyde and the association profiles shown in Figure 3 show remarkable similarities.

As expected, there were also polymorphic markers associated with variation in behavior that were distinct for each odorant. For *Or10a*, C1636T was exclusively associated with variation in responsiveness to benzaldehyde and C-465A, C757T, and G806T with responsiveness to acetophenone. For *Or43a*, all but marker 12 were solely associated with variation in response to benzaldehyde. However, it should be noted that C-356T, C-167A, and C1158T were just below the permutation threshold of significance for responsiveness to acetophenone. Furthermore, one SNP (C670T) contributed only to

variation in responses to acetophenone. Finally, in the case of *Or67b*, only G932A, G943A, and A944C were significantly associated with behavioral responses to both odorants. Eleven distinct SNPs contributed to variation in responses to acetophenone. Of those markers, we observed three discrete groups of markers (C695T and C728T, A799T and 803Del, and A1330C and T1335A) whose members were in complete LD. Thus, one might expect that among these three odorant receptors *Or67b* might be particularly well positioned to discriminate these two odorants.

We estimated the expected genetic variance contributed by each molecular polymorphism associated with behavioral responses to benzaldehyde and acetophenone in an outbred population with the same allele frequencies found in the inbred lines and assuming random mating (Table S2). Under an additive model, the genetic variance (V_A) contributed by each biallelic locus is $2pq\alpha^2$, where p and q are the allele frequencies and α is one-half the difference in the mean of the trait between the two homozygous marker genotype classes (FALCONER and MACKAY 1996). The estimate of the

TABLE 1
Population genetic parameters

Genes	No. sequences	Length (bp)	No. segregating sites	π	θ_w
<i>Or7a</i>					
CDS	50	1254	26 (4) ^a	0.0044	0.0047
Intronic regions	50	203	5 (0)	0.0033	0.0055
5'-UTR	50	426	4 (1)	0.0023	0.0022
3'-UTR	50	454	15 (7)	0.0046	0.0074
Combined	50	2337	50 (12)	0.0040	0.0048
<i>Or10a</i>					
CDS	197	1221	26 (7)	0.0022	0.0036
Intronic regions	197	319	24 (3)	0.0159	0.0139
5'-UTR	197	488	12 (1)	0.0021	0.0043
3'-UTR	197	267	19 (6)	0.0160	0.0123
Combined	197	2295		0.0056	0.0061
<i>Or42b</i>					
CDS	41	1200	8 (3)	0.0012	0.0016
Intronic regions	41	124	1 (0)	0.0022	0.0020
5'-UTR	41	359	2 (1)	0.0015	0.0013
3'-UTR	41	370	2 (0)	0.0016	0.0014
Combined	41	2053	13 (4)	0.0014	0.0015
<i>Or43a</i>					
CDS	145	1131	13 (2)	0.0031	0.0021
Intronic regions	145	545	18 (3)	0.0072	0.0060
5'-UTR	145	435	13 (1)	0.0066	0.0055
3'-UTR	145	473	10 (3)	0.0031	0.0038
Combined	145	2584	54 (9)	0.0045	0.0038
<i>Or67b</i>					
CDS	148	1266	36 (8)	0.0060	0.0051
Intronic regions	148	531	57 (5)	0.0308	0.0194
5'-UTR	148	188	3 (1)	0.0051	0.0029
3'-UTR	148	401	13 (1)	0.0109	0.0058
Combined	148	2386	109 (15)	0.0122	0.0082
<i>Or85f</i>					
CDS	36	1179	13 (1)	0.0037	0.0027
Intronic regions	36	185	3 (0)	0.0042	0.0039
5'-UTR	36	290	8 (4)	0.0057	0.0067
3'-UTR	36	474	8 (2)	0.0030	0.0041
Combined	36	2128	32 (7)	0.0038	0.0036

^a Singletons are given in parentheses.

heritability attributable to each marker is V_A/V_P , where V_P is the estimated phenotypic variance of the trait in this population (WANG *et al.* 2007, 2010). Each marker is expected to account for ~1.6–3.2% of the total phenotypic variation in olfactory behavior. However, LD among the markers precludes summing the contribution from each marker to infer the total variance explained by each locus. These effect sizes are consistent with other studies in which polymorphisms in odorant-binding proteins contributed 3–6% to the total phenotypic variance in olfactory behavior (WANG *et al.* 2007, 2010).

As each *Or* gene contained multiple SNPs that were associated with variation in olfactory behavior, we examined their combined effects by conducting haplotype analyses. Analyses of the polymorphisms significantly associated with variation in responses to benzaldehyde identified 6 haplotypes for *Or10a*, 17 for *Or43a*, and 9 for *Or67b* (Figure 4; Table S3). For

polymorphisms associated with variation in responsiveness to acetophenone, haplotype analyses identified 9 haplotypes for *Or10a*, 3 for *Or43a*, and 12 for *Or67b* (Figure 4; Table S3). The majority of haplotypes were present at low frequency. A notable exception was observed for *Or43a* in which there were two SNPs associated with variation in behavioral responses to acetophenone that formed 3 haplotypes (AT, CT, and CC) at individual frequencies of 0.207, 0.271, and 0.521, respectively. Of those haplotypes significant phenotypic differences were observed between the AT and CC haplotypes. The CC haplotype had a significant reduction in least-squares mean responses to acetophenone relative to the AT haplotype. In addition, we observed for all three *Or* genes significant differences among haplotypes in their responsiveness to both odorants (Figure 4; Table S4a). We estimated the contribution of each locus to variation in olfactory

Differences in odorant receptor gene expression among high- and low-responder haplotypes: SNPs can influence protein structure and function (HOEKSTRA *et al.* 2006), regulate gene expression levels (CAMPBELL *et al.* 2006; WANG *et al.* 2010), or affect the structure and stability of mRNA (NACKLEY *et al.* 2006; WANG *et al.* 2007). Nonsynonymous polymorphisms are likely to result in changes in protein structure/function, while SNPs in noncoding regions can result in changes in gene expression levels or mRNA stability. Although all may contribute to behavioral variation, the majority of SNPs associated with variation in behavioral responses in our study were in noncoding regions. To test if there was a correspondence between variation in behavioral response to benzaldehyde and expression differences in *Or10a*, *Or43a*, and *Or67b*, we selected wild-derived lines that corresponded to the haplotype with either the highest response or the lowest response to benzaldehyde for each gene and measured transcript abundance using quantitative PCR (Figure 5; Table S5). We observed that haplotypes associated with the highest

*0.001 < *P* < 0.01.

behavior in the population of inbred lines on the basis of haplotype analysis. Variation in olfactory behavior among haplotypes accounted for 10.1–16.3% of the total variance in response to benzaldehyde and 3.9–11.8% of the total variance in response to acetophenone (Table S4b).

FIGURE 3.—Associations between polymorphisms in each of three *Or* genes and variation in olfactory behavioral responses. Polymorphic markers are depicted on the x-axis, and $\log(1/P)$ values for marker-trait associations on the y-axis. Marker-trait associations for benzaldehyde and acetophenone are indicated by the red and gray lines, respectively. The dashed red and gray horizontal lines indicate the corresponding permutation thresholds.

FIGURE 4.—Haplotype analysis of SNPs/indels significantly associated with variation in behavioral responses to either benzaldehyde (red bars) or acetophenone (gray bars) for *Or* genes *Or10a*, *Or43a*, and *Or67b*. Significant phenotypic differences among haplotypes were determined by ANOVA and haplotypes that differed significantly in olfactory behavior were determined by *post hoc* Tukey's tests at $P < 0.05$ and are indicated by different letters above the bars. Specific haplotype sequences for each of the odorant receptors and their frequencies are presented in Table S3.

response scores were associated with a decrease in *Or10a* expression in both sexes ($P < 0.0001$ for both males and females), with *Or10a* expression lower in high-responder males and females by ~ 54 and 37% , respectively. We analyzed the sexes separately since we observed a significant haplotype-by-sex interaction. We observed a significant male-specific increase in *Or43a* expression ($P < 0.0168$) in which *Or43a* expression was $\sim 37\%$ higher in males of the high-responder haplotype. We did not see a significant difference in *Or67b* gene expression between the high-responder and low-responder haplotypes.

DISCUSSION

Chemical cues play a major role in environmental adaptation during evolution. Consequently, individual *Or* loci are likely subject to different evolutionary pressures. Indeed, our molecular evolutionary analysis of nucleotide variation in six *Or* genes revealed differences in their evolutionary dynamics, even though these odorant receptors have at least partially overlapping ligand specificities (HALLEM *et al.* 2004; FISHLEVICH and VOSSHALL 2005; HALLEM and CARLSON 2006). This is suggested from differences in estimates of nucleotide diversity, especially for *Or42b* where $\pi = 0.0014$ (Table 1), and is evident from

the results of our McDonald–Kreitman tests, which showed no departure from neutrality for the coding region of four of the six odorant receptors, but a

FIGURE 5.—Correspondence between behavioral response to benzaldehyde and differences in *Or10a* and *Or43a* expression. Expression of (a) *Or10a* and (b) *Or43a* in male and female high- and low-responder haplotypes is shown. Odorant receptor expression is shown relative to the expression in the low responder. Data are shown as mean $\pm$ SEM, * $P < 0.05$; **** $P < 0.0001$.

significant deviation from neutral expectations for *Or42b* and *Or85f* (Table 2). The observed reduction in nucleotide variability of *Or42b* may be due to its location near the centromeric region of chromosome arm 2R, which shows reduced recombination (ASHBURNER 1989; KLIMAN and HEY 1993; CHARLESWORTH 1996). Reduction in nucleotide diversity in this region suggests either a selective sweep or background selection (BEGUN and AQUADRO 1992; CHARLESWORTH *et al.* 1993). Three of the five replacement polymorphisms were represented in only one inbred line, but Tajima's *D* estimates suggest the frequency spectrum of polymorphisms is only slightly negatively skewed and not significantly different from neutral expectations (TAJIMA 1989). These observations suggest a model of background selection contributing to the observed reduction in synonymous polymorphism in the *Or42b* region (BRAVERMAN *et al.* 1995). Interestingly, *Or42b* has also been identified as one of the most conserved *Or* genes among *Drosophila* species (McBRIDE *et al.* 2007). *Or85f*, however, is located more distally on chromosome arm 3R. Here, possible explanations for the reduction in synonymous polymorphisms include balancing selection in addition to background selection or a selective sweep. The lack of a significant Tajima's *D*, however, is not reflective of expectations under either a model of selective sweep or balancing selection in which we might predict to see a significant negative or positive Tajima's *D*, respectively. Thus, the selective pressures that give rise to deviations from neutrality at this locus remain unclear.

We identified molecular polymorphisms in *Or10a*, *Or43a*, and *Or67b* that are associated with behavioral variation in responses to both benzaldehyde and acetophenone. Our results are consistent with the combinatorial nature of odor coding in which odorant receptors overlap in their ligand response profiles and there is functional redundancy among receptors (HALLEM *et al.* 2004; HALLEM and CARLSON 2006; VOSSHALL and STOCKER 2007). It should be noted that we examined only six genes from the multigene family of odorant receptors and that additional *Or* genes may also contribute to variation in behavioral responses to these odorants. Furthermore, the statistical power for detecting associations between SNPs and variation in olfactory behavior increases as sample size increases. Increasing sample sizes within a population and surveying additional populations may identify more SNPs associated with variation in behavioral responses to both benzaldehyde and acetophenone.

Within each gene, multiple SNPs or indels were associated with variation in behavioral responses to both odorants. The majority of these polymorphisms were in noncoding regions. As postulated previously (WANG *et al.* 2007), mutations in noncoding regions that do not directly influence protein structure can be primary agents of behavioral variation by regulating gene expression or affecting the structure and stability of mRNA.

Indeed, misexpression of *Or43a* resulted in changes in avoidance behavior to benzaldehyde (STORTKUHL and KETTLER 2001; STORTKUHL *et al.* 2005).

We found that high- and low-responder haplotypes differed in the expression of *Or10a* and *Or43a*, with differences in direction and sex specificity. Closely related odorants, like benzaldehyde and acetophenone, are expected to interact with multiple, albeit overlapping, odorant receptors with different affinities. Reduction in the expression level of one receptor may have differential effects on recognition of these two odorants depending on their relative affinities for the receptor and for other cognate receptors. For example, in a case in which a receptor binds one odorant with high affinity and a structurally similar odorant with lower affinity, a small reduction in the expression level of the receptor could potentially affect the behavioral response to one odorant, but not the other. The contribution to odorant perception of these odorants hence is dependent on the relative weight of interactions with the specific receptor in the context of the entire combinatorial set of cognate odorant receptors. It is also possible that additional regulatory polymorphisms may be present in upstream or downstream flanking regions that extend beyond the sequenced regions. Such polymorphisms are not detected in our current study. Finally, it is possible that potential regulatory sites in introns might change the structure or stability of the mRNA, as previously suggested for intronic polymorphisms in odorant-binding proteins associated with olfactory behavior (WANG *et al.* 2007). Extensive phenotypic plasticity in the expression of chemosensory genes in *D. melanogaster* in response to different developmental, physiological, and social conditions has been documented (ZHOU *et al.* 2009). A detailed analysis of the role of chemosensory gene regulation in modulating behavior awaits further study, but results from this study illustrate the complex relationship between the chemosensory receptor transcriptome and olfactory behavior.

Protein-coding changes can also influence variation in behavior by directly influencing protein structure and function. Amino acid substitutions in vertebrate chemosensory receptors can result in changes in ligand-binding affinity and confer differences in odorant sensitivity (KRAUTWURST *et al.* 1998; FEINSTEIN and MOMBAERTS 2004; REED *et al.* 2004; NIE *et al.* 2005; ABAFFY *et al.* 2007; KELLER *et al.* 2007). Thus, it is of particular interest that *Or43a* harbors an isoleucine to methionine substitution that contributes to variation in responsiveness to benzaldehyde. This amino acid residue is predicted to be located extracellularly and hence could influence ligand binding.

We observed that many of the SNPs and indels associated with variation in behavioral responses to benzaldehyde also contributed to variation in behavioral responses to acetophenone. These results are in line with previous electrophysiological studies in which *Or10a* was

shown to respond strongly to aromatic compounds that have both a benzene ring and a carbonyl group (FISHILEVICH and VOSSHALL 2005; HALLEM and CARLSON 2006). These two odorants also act as ligands for *Or67b* (FISHILEVICH and VOSSHALL 2005). Furthermore, mis-expression studies of *Or43a* identified benzaldehyde as a strong ligand (STORTKUHL *et al.* 2005) and in a screen of structurally similar chemicals suggest that ligands for *Or43a* should contain a benzene ring and a polar group (STORTKUHL and KETTLER 2001; WETZEL *et al.* 2001). Finally, behavioral responses to acetophenone were strongly correlated with responses to benzaldehyde for this population (WANG *et al.* 2010). The overlapping set of SNPs contributing to variation in behavioral responses to both odorants provides independent validation for the reliability of our association tests. However, the fact that certain SNPs are associated with variation in response to one odorant but not the other, especially in the case of *Or67b*, provides a glimpse into the manner in which sequence variants that arise during evolution can potentially generate subtle shifts in odorant response profiles.

In addition to polymorphisms in *Or* genes that are associated with variation in responses to benzaldehyde and acetophenone, studies on *Odorant binding protein* (*Obp*) genes identified SNPs within the *Obp99* gene cluster associated with differences in responses to these odorants (WANG *et al.* 2010). The functional relationships between sequence variants in *Obp* genes and in *Or* genes associated with responses to similar odorants, however, remain to be elucidated. Here, we have shown that sequence variants that arise during the evolution of *Or* genes can contribute to individual variation in olfactory behavior and give rise to subtle shifts in olfactory perception. It seems reasonable to predict that the combined effects of polymorphisms throughout the entire *Or* gene repertoire would generate broad individual variation in chemosensory perception.

We thank Peter Andolfatto, Mary Anna Carbone, Richard Kirby, John Layne, and Ken Petren for helpful discussions or technical assistance and Allison Weber for help with the permutation tests. This research was supported by grants from the National Institutes of Health to S.M.R. (GM080592), R.R.H.A. (GM059469), and T.F.C.M. (GM045146).

LITERATURE CITED

- ABAFFY, T., A. MALHOTRA and C. W. LEUTJE, 2007 The molecular basis for ligand specificity in a mouse olfactory receptor. *J. Biol. Chem.* **282**: 1216–1224.
- ANDOLFATTO, P., 2001 Contrasting patterns of Xlinked and autosomal nucleotide variation in *Drosophila melanogaster* and *Drosophila simulans*. *Mol. Biol. Evol.* **18**: 279–290.
- ANHOLT, R. R. H., and T. F. C. MACKAY, 2004 Quantitative genetic analyses of complex behaviours in *Drosophila*. *Nat. Rev. Genet.* **5**: 838–849.
- ANHOLT, R. R. H., R. F. LYMAN and T. F. C. MACKAY, 1996 Effects of single *P*-element insertions on olfactory behavior in *Drosophila melanogaster*. *Genetics* **143**: 293–301.
- ALI, M. A., 1978 *Sensory Ecology: Review and Perspectives*. Plenum Press, New York.
- AYROLES, J. F., M. A. CARBONE, E. A. STONE, K. W. JORDAN, R. F. LYMAN *et al.*, 2009 Systems genetics of complex traits in *Drosophila melanogaster*. *Nat. Genet.* **41**: 299–307.
- ASHBURNER, M., 1989 *Handbook on Drosophila*. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.
- BEGUN, D. J., and C. F. AQUADRO, 1992 Levels of naturally occurring DNA polymorphism correlate with recombination rates in *D. melanogaster*. *Nature* **356**: 519–520.
- BENTON, R., S. SACHSE, S. W. MICHNICK and L. B. VOSSHALL, 2006 Atypical membrane topology and heteromeric function of *Drosophila* odorant receptors in vivo. *PLoS Biol.* **4**: 240–257.
- BHALERAO, S., A. SEN, R. STOCKER and V. RODRIGUES, 2003 Olfactory neurons expressing identified receptor genes project to subsets of glomeruli within the antennal lobe of *Drosophila melanogaster*. *J. Neurobiol.* **54**: 577–592.
- BRAVERMAN, J. M., R. R. HUDSON, N. L. KAPLAN, C. H. LANGLEY and W. STEPHAN, 1995 The hitchhiking effect on the site frequency spectrum of DNA polymorphism. *Genetics* **140**: 783–796.
- BUCK, L., and R. AXEL, 1991 A novel multigene family may encode odorant receptors: a molecular basis for odor recognition. *Cell* **65**: 175–187.
- CAMPBELL, D. B., J. S. SUTCLIFFE, P. J. EBERT, R. MILITERNI, C. BRAVACCIO *et al.*, 2006 A genetic variant that disrupts *MET* transcription is associated with autism. *Proc. Natl. Acad. Sci. USA* **103**: 16834–16839.
- CHARLESWORTH, B., 1996 Background selection and patterns of genetic diversity in *Drosophila melanogaster*. *Genet. Res.* **68**: 131–149.
- CHARLESWORTH, B., M. T. MORGAN and D. CHARLESWORTH, 1993 The effects of deleterious mutations on neutral molecular variation. *Genetics* **134**: 1289–1303.
- CHURCHILL, G. A., and R. W. DOERGE, 1994 Empirical threshold values for quantitative trait mapping. *Genetics* **138**: 963–971.
- CLYNE, P. J., C. G. WARR, M. R. FREEMAN, D. LESSING, J. H. KIM *et al.*, 1999 A novel family of divergent seven-transmembrane proteins: candidate odorant receptors in *Drosophila*. *Neuron* **22**: 327–338.
- DE BRUYNE, M., P. J. CLYNE and J. R. CARLSON, 1999 Odor coding in a model olfactory organ: the *Drosophila* maxillary palp. *J. Neurosci.* **19**: 4520–4532.
- DE BRUYNE, M., K. FOSTER and J. R. CARLSON, 2001 Odor coding in the *Drosophila* antenna. *Neuron* **30**: 537–552.
- DOBRTSA, A. A., W. VAN DER GOES VAN NATERS, C. G. WARR, R. A. STEINBRECHT and J. R. CARLSON, 2003 Integrating the molecular and cellular basis of odor coding in the *Drosophila* antenna. *Neuron* **37**: 827–841.
- DRYSDALE, R. A., M. A. CROSBY and THE FLYBASE CONSORTIUM, 2005 Flybase genes and gene models. *Nucleic Acids Res.* **33**: D390–D395.
- DUSENBERY, D. B., 1992 *Sensory Ecology: How Organisms Acquire and Respond to Information*. W. H. Freeman, New York.
- FALCONER, D. S., and T. F. C. MACKAY, 1996 *Introduction to Quantitative Genetics*, Ed. 4. Longmans Green, Harlow, Essex, UK.
- FEINSTEIN, P., and P. MOMBAERTS, 2004 A contextual model for axonal sorting into glomeruli in the mouse olfactory system. *Cell* **117**: 817–831.
- FISHILEVICH, E., and L. B. VOSSHALL, 2005 Genetic and functional subdivision of the *Drosophila* antennal lobe. *Curr. Biol.* **15**: 1548–1553.
- GAO, Q., and A. CHES, 1999 Identification of candidate *Drosophila* olfactory receptors from genomic DNA sequence. *Genomics* **60**: 31–39.
- GAO, Q., B. YUAN and A. CHES, 2000 Convergent projections of *Drosophila* olfactory neurons to specific glomeruli in the antennal lobe. *Nat. Neurosci.* **3**: 780–785.
- HALLEM, E. A., and J. R. CARLSON, 2006 Coding of odors by a receptor repertoire. *Cell* **125**: 143–160.
- HALLEM, E. A., M. G. HO and J. R. CARLSON, 2004 The molecular basis of odor coding in the *Drosophila* antenna. *Cell* **117**: 965–979.
- HOEKSTRA, H. E., R. J. HIRSCHMANN, R. A. BUNDEY, P. A. INSEL and J. P. CROSSLAND, 2006 A single amino acid mutation contributes to adaptive beach mouse color pattern. *Science* **313**: 101–104.
- KLIMAN, R. M., and J. HEY, 1993 Reduced natural selection associated with low recombination in *Drosophila melanogaster*. *Mol. Biol. Evol.* **10**: 1239–1258.
- JONES, W. D., T. A. NGUYEN, B. KLOSS, K. J. LEE and L. B. VOSSHALL, 2005 Functional conservation of an insect odorant receptor gene across 250 million years of evolution. *Curr. Biol.* **15**: R119–R121.

- KELLER, A., H. ZHUANG, Q. CHI, L. B. VOSSHALL and H. MATSUNAMI, 2007 Genetic variation in a human odorant receptor alters odour perception. *Nature* **449**: 468–472.
- KRAUTWURST, D., K.-W. YAU and R. R. REED, 1998 Identification of ligands for olfactory receptors by functional expression of a receptor library. *Cell* **95**: 917–926.
- LAISSUE, P. P., C. REITER, P. R. HIESINGER, S. HALTER, K. F. FISCHBACH *et al.*, 1999 Three-dimensional reconstruction of the antennal lobe in *Drosophila melanogaster*. *J. Comp. Neurol.* **405**: 543–552.
- LARSSON, M. C., A. I. DOMINGOS, W. D. JONES, M. E. CHIAPPE, H. AMREIN *et al.*, 2004 *Or83b* encodes a broadly expressed odorant receptor essential for *Drosophila* olfaction. *Neuron* **43**: 703–714.
- LIBRADO, P., and J. ROZAS, 2009 DnaSP v5: a software for comprehensive analysis of DNA polymorphism data. *Bioinformatics* **25**: 1451–1452.
- LIVAK, K. J., and T. D. SCHMITTGEN, 2001 Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\Delta\Delta CT}$ method. *Methods* **25**: 402–408.
- LYTHGOE, J. N., 1979 *The Ecology of Vision*. Clarendon Press, Oxford.
- MCBRIDE, C. S., J. R. ARGUELLO and B. C. O'MEARA, 2007 Five *Drosophila* genomes reveal nonneutral evolution and the signature of host specialization in the chemoreceptor superfamily. *Genetics* **177**: 1395–1416.
- MCDONALD, J. H., and M. KREITMAN, 1991 Adaptive protein evolution at the *Adh* locus in *Drosophila*. *Nature* **351**: 652–654.
- MOMBAERTS, P., F. WANG, C. DULAC, S. K. CHAO, A. NEMES *et al.*, 1996 Visualizing an olfactory sensory map. *Cell* **87**: 675–686.
- MORIYAMA, E. N., and J. R. POWELL, 1996 Intraspecific nuclear DNA variation in *Drosophila*. *Mol. Biol. Evol.* **13**: 261–277.
- NACKLEY, A. G., S. A. SHABALINA, I. E. TCHIVILEVA, K. SATTERFIELD, O. KORCHYNSKYI *et al.*, 2006 Human catechol-O-methyltransferase haplotypes modulate protein expression by altering mRNA secondary structure. *Science* **314**: 1930–1933.
- NIE, Y., S. VIGUES, J. R. HOBBS, G. L. CONN and S. D. MUNGER, 2005 Distinct contributions of T1R2 and T1R3 taste receptor subunits to the detection of sweet stimuli. *Curr. Biol.* **15**: 1948–1952.
- PRICE, E. W., and I. CARBONE, 2005 SNAP: workbench management tool for evolutionary population genetic analysis. *Bioinformatics* **21**: 402–404.
- REED, D. R., S. LI, X. LI, L. HUANG, M. G. TORDOFF *et al.*, 2004 Polymorphisms in the taste receptor gene (*Tas1r3*) region are associated with saccharin preference in 30 mouse strains. *J. Neurosci.* **24**: 938–946.
- ROBERTSON, H. M., C. G. WARR and J. R. CARLSON, 2003 Molecular evolution of the insect chemoreceptor gene superfamily in *Drosophila melanogaster*. *Proc. Natl. Acad. Sci. USA* **100**: 14537–14542.
- SATO, K., M. PELLEGRINO, T. NAKAGAWA, T. NAKAGAWA, L. B. VOSSHALL *et al.*, 2008 Insect olfactory receptors are heteromeric ligand-gated ion channels. *Nature* **452**: 1002–1006.
- STORTKUHL, K. F., and R. KETTLER, 2001 Functional analysis of an olfactory receptor in *Drosophila melanogaster*. *Proc. Natl. Acad. Sci. USA* **98**: 9381–9385.
- STORTKUHL, K. F., R. KETTLER, S. FISCHER and B. T. HOVEMANN, 2005 An increase receptive field of olfactory receptor *Or43a* in the antennal lobe of *Drosophila* reduces benzaldehyde-driven avoidance behavior. *Chem. Senses* **30**: 81–87.
- TAJIMA, F., 1989 Statistical method for testing the neutral mutation hypothesis by DNA polymorphism. *Genetics* **123**: 585–595.
- VASSAR, R., S. K. CHAO, J. M. SITCHERAN, L. NUNEZ, L. B. VOSSHALL *et al.*, 1994 Topographic organization of sensory projections to the olfactory bulb. *Cell* **79**: 981–992.
- 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., H. AMREIN, P. S. MOROZOV, A. RZHETSKY and R. AXEL, 1999 A spatial map of olfactory receptor expression in the *Drosophila* antenna. *Cell* **96**: 725–736.
- VOSSHALL, L. B., A. M. WONG and R. AXEL, 2000 An olfactory sensory map in the fly brain. *Cell* **102**: 147–159.
- WANG, J. W., A. M. WONG, J. FLORES, L. B. VOSSHALL and R. AXEL, 2003 Two-photon calcium imaging reveals an odor-evoked map of activity in the fly brain. *Cell* **112**: 271–282.
- WANG, P., R. F. LYMAN, S. A. SHABALINA, T. F. C. MACKAY and R. R. H. ANHOLT, 2007 Association of polymorphisms in odorant-binding protein genes with variation in olfactory response to benzaldehyde in *Drosophila*. *Genetics* **177**: 1655–1665.
- WANG, P., R. F. LYMAN, T. F. C. MACKAY and R. R. H. ANHOLT, 2010 Natural variation in odorant recognition among odorant binding proteins in *Drosophila melanogaster*. *Genetics* **184**: 759–767.
- WETZEL, C. H., H. J. BEHRENDT, G. GISSELMANN, K. F. STORTKUHL, B. HOVEMANN *et al.*, 2001 Functional expression and characterization of a *Drosophila* odorant receptor in a heterologous cell system. *Proc. Natl. Acad. Sci. USA* **98**: 9377–9380.
- WICHER, D., R. SCHAFER, R. BAERNFEIND, M. C. STENSMYR, R. HELLER, *et al.*, 2008 *Drosophila* odorant receptors are both ligand-gated and cyclic-nucleotide-activated cation channels. *Nature* **452**: 1007–1011.
- ZHANG, X., and S. FIRESTEIN, 2002 The olfactory receptor gene superfamily of the mouse. *Nat. Neurosci.* **5**: 124–133.
- ZHANG, X., I. RODRIGUEZ, P. MOMBAERTS and S. FIRESTEIN, 2004 Odorant and vomeronasal receptor genes in two mouse genome assemblies. *Genomics* **83**: 802–811.
- ZHOU, S., E. A. STONE, T. F. C. MACKAY and R. R. H. ANHOLT, 2009 Plasticity of the chemoreceptor repertoire in *Drosophila melanogaster*. *PLoS Genet.* **5**: e1000681.

Communicating editor: M. LONG

GENETICS

Supporting Information

<http://www.genetics.org/cgi/content/full/genetics.110.119446/DC1>

Odorant Receptor Polymorphisms and Natural Variation in Olfactory Behavior in *Drosophila melanogaster*

Stephanie M. Rollmann, Ping Wang, Priya Date, Steven A. West, Trudy F. C. Mackay and Robert R. H. Anholt

Copyright © 2010 by the Genetics Society of America
DOI: 10.1534/genetics.110.119446

TABLE S1
Molecular polymorphisms in odorant receptor loci^a

<i>Locus</i>	Location	Nucleotide change	Amino acid change	Allele frequency ^b
<i>Or7a</i>	Upstream genomic	-277In (1 bp)	-	0.449 (22)
<i>Or7a</i>	Upstream genomic	T-275C	-	0.449 (22)
<i>Or7a</i>	Upstream genomic	-96Del (1 bp)	-	0.041 (2)
<i>Or7a</i>	Upstream genomic	G-18C	-	0.102 (5)
<i>Or7a</i>	Upstream genomic	T-6C	-	0.102 (5)
<i>Or7a</i>	Exon 1	G4A	A2T	0.082 (4)
<i>Or7a</i>	Exon 1	G27A	-	0.122 (6)
<i>Or7a</i>	Exon 1	T51G	-	0.163 (8)
<i>Or7a</i>	Exon 1	C75T	-	0.122 (6)
<i>Or7a</i>	Exon 1	G90T	-	0.082 (4)
<i>Or7a</i>	Exon 1	A117C	-	0.082 (4)
<i>Or7a</i>	Exon 1	G120C	-	0.082 (4)
<i>Or7a</i>	Exon 1	G140A	R47Q	0.082 (4)
<i>Or7a</i>	Exon 1	A381G	-	0.143 (7)
<i>Or7a</i>	Exon 1	C402T	-	0.102 (5)
<i>Or7a</i>	Exon 1	T420C	-	0.122 (6)
<i>Or7a</i>	Exon 1	A435G	-	0.102 (5)
<i>Or7a</i>	Exon 1	G552A	-	0.061 (3)
<i>Or7a</i>	Exon 1	C588T	-	0.245 (12)
<i>Or7a</i>	Exon 1	T609G	-	0.245 (12)
<i>Or7a</i>	Exon 1	C633T	-	0.204 (10)
<i>Or7a</i>	Exon 1	C734A	P245H	0.041 (2)
<i>Or7a</i>	Exon 1	A844G	I282V	0.041 (2)
<i>Or7a</i>	Exon 1	C932G	A311G	0.041 (2)
<i>Or7a</i>	Exon 1	G966A	-	0.286 (14)
<i>Or7a</i>	Exon 1	T1092G	-	0.306 (15)
<i>Or7a</i>	Exon 1	G1116A	-	0.225 (11)
<i>Or7a</i>	Intron 1	1200Del (2 bp)	-	0.184 (9)
<i>Or7a</i>	Intron 1	T1280A	-	0.082 (4)
<i>Or7a</i>	Intron 1	C1293G	-	0.082 (4)

<i>Or7a</i>	Intron 1	C1307T	-	0.082 (4)
<i>Or7a</i>	Intron 1	A1341T	-	0.041 (2)
<i>Or7a</i>	Intron 1	C1355G	-	0.041 (2)
<i>Or7a</i>	Downstream genomic	T1660A	-	0.265 (13)
<i>Or7a</i>	Downstream genomic	A1695C	-	0.122 (6)
<i>Or7a</i>	Downstream genomic	C1699T	-	0.122 (6)
<i>Or7a</i>	Downstream genomic	T1770C	-	0.143 (7)
<i>Or7a</i>	Downstream genomic	G1798A	-	0.122 (6)
<i>Or7a</i>	Downstream genomic	G1804C	-	0.122 (6)
<i>Or7a</i>	Downstream genomic	A1809C	-	0.122 (6)
<i>Or7a</i>	Downstream genomic	C1862T	-	0.041 (2)
<i>Or10a</i>	Upstream genomic	C-475T	-	0.025 (4)
<i>Or10a</i>	Upstream genomic	T-473C	-	0.025 (4)
<i>Or10a</i>	Upstream genomic	C-465A	-	0.160 (26)
<i>Or10a</i>	Upstream genomic	G-463A	-	0.018 (3)
<i>Or10a</i>	Upstream genomic	A-450T	-	0.024 (4)
<i>Or10a</i>	Upstream genomic	C-446T	-	0.053 (9)
<i>Or10a</i>	Upstream genomic	C-375T	-	0.029 (5)
<i>Or10a</i>	Upstream genomic	C-246G	-	0.011 (2)
<i>Or10a</i>	Upstream genomic	G-81A	-	0.085 (15)
<i>Or10a</i>	Upstream genomic	T-71A	-	0.096 (17)
<i>Or10a</i>	Upstream genomic	A-15G	-	0.011 (2)
<i>Or10a</i>	Exon 1	T52C	F18L	0.102 (18)
<i>Or10a</i>	Exon 1	G69C	-	0.119 (21)
<i>Or10a</i>	Exon 1	C156T	-	0.068 (12)
<i>Or10a</i>	Exon 1	T159A	-	0.051 (9)
<i>Or10a</i>	Exon 1	A222C	-	0.023 (4)
<i>Or10a</i>	Exon 1	C438T	-	0.051 (9)
<i>Or10a</i>	Exon 1	C457T	-	0.062 (11)
<i>Or10a</i>	Exon 1	A468C	-	0.074 (13)
<i>Or10a</i>	Intron 1	545Del (13 bp)	-	0.017 (3)
<i>Or10a</i>	Intron 1	G572C	-	0.198 (35)
<i>Or10a</i>	Intron 1	In579Del (9 bp); In582/585 (A/C), (T/T)	-	0.023 (4)

<i>Or10a</i>	Intron 1	A597C	-	0.141 (25)
<i>Or10a</i>	Intron 1	599Del (2 bp)	-	0.141 (25)
<i>Or10a</i>	Intron 1	T609A	-	0.299 (53)
<i>Or10a</i>	Exon 2	C653T	-	0.130 (23)
<i>Or10a</i>	Exon 2	C757T	-	0.085 (15)
<i>Or10a</i>	Intron 2	G806T	-	0.107 (19)
<i>Or10a</i>	Intron 2	G834T	-	0.040 (7)
<i>Or10a</i>	Intron 2	A843T	-	0.040 (7)
<i>Or10a</i>	Intron 2	T850A	-	0.074 (13)
<i>Or10a</i>	Intron 2	T857A	-	0.028 (5)
<i>Or10a</i>	Exon 3	C867T	-	0.028 (5)
<i>Or10a</i>	Exon 3	A925G	I264V	0.017 (3)
<i>Or10a</i>	Exon 3	C1131T	-	0.017 (3)
<i>Or10a</i>	Intron 3	C1163T	-	0.130 (23)
<i>Or10a</i>	Intron 3	A1193G	-	0.107 (19)
<i>Or10a</i>	Exon 4	A1232G	M347V	0.011 (2)
<i>Or10a</i>	Exon 4	T1241A	C350S	0.124 (22)
<i>Or10a</i>	Exon 4	G1303C	-	0.311 (55)
<i>Or10a</i>	Exon 4	T1346C	-	0.028 (5)
<i>Or10a</i>	Intron 4	T1372A	-	0.028 (5)
<i>Or10a</i>	Intron 4	G1373T	-	0.028 (5)
<i>Or10a</i>	Intron 4	G1377A	-	0.028 (5)
<i>Or10a</i>	Intron 4	T1388C	-	0.164 (29)
<i>Or10a</i>	Intron 4	1398In (1 bp)	-	0.164 (29)
<i>Or10a</i>	Intron 4	T1436G	-	0.051 (9)
<i>Or10a</i>	Intron 4	T1438A	-	0.288 (51)
<i>Or10a</i>	Intron 4	C1439T	-	0.339 (60)
<i>Or10a</i>	Intron 4	G1447C	-	0.288 (51)
<i>Or10a</i>	Intron 4	C1452A	-	0.339 (60)
<i>Or10a</i>	Exon 5	G1481A	G396D	0.017 (3)
<i>Or10a</i>	Exon 5	T1482C	-	0.203 (36)
<i>Or10a</i>	Downstream genomic	G1550C	-	0.028 (5)
<i>Or10a</i>	Downstream genomic	T1566A	-	0.362 (64)
<i>Or10a</i>	Downstream genomic	T1584A	-	0.316 (56)

<i>Or10a</i>	Downstream genomic	1593Del (1 bp)	-	0.316 (56)
<i>Or10a</i>	Downstream genomic	G1595A	-	0.316 (56)
<i>Or10a</i>	Downstream genomic	1606Del (1 bp)	-	0.028 (5)
<i>Or10a</i>	Downstream genomic	T1615G	-	0.367 (65)
<i>Or10a</i>	Downstream genomic	T1625C	-	0.333 (59)
<i>Or10a</i>	Downstream genomic	C1636T	-	0.068 (12)
<i>Or10a</i>	Downstream genomic	T1668A	-	0.486 (85)
<i>Or10a</i>	Downstream genomic	T1753A	-	0.124 (21)
<i>Or10a</i>	Downstream genomic	G1755A	-	0.126 (21)
<i>Or10a</i>	Downstream genomic	T1762C	-	0.380 (62)
<i>Or42b</i>	Upstream genomic	G-57A	-	0.429 (18)
<i>Or42b</i>	Exon 1	G351A	-	0.093 (4)
<i>Or42b</i>	Exon 1	T475C	S159P	0.046 (2)
<i>Or42b</i>	Exon 1	T616A	S206T	0.465 (20)
<i>Or42b</i>	Exon 1	C714T	-	0.300 (12)
<i>Or42b</i>	Intron 2	G1205T	-	0.140 (6)
<i>Or42b</i>	Downstream genomic	G1498A	-	0.279 (12)
<i>Or42b</i>	Downstream genomic	A1538G	-	0.046 (2)
<i>Or42b</i>	Downstream genomic	1585In (19 bp)	-	0.256 (11)
<i>Or43a</i>	Upstream genomic	C-356T	-	0.278 (40)
<i>Or43a</i>	Upstream genomic	G-325A	-	0.040 (6)
<i>Or43a</i>	Upstream genomic	T-324C	-	0.497 (71)
<i>Or43a</i>	Upstream genomic	C-259A	-	0.040 (6)
<i>Or43a</i>	Upstream genomic	-258Del (1 bp)	-	0.040 (6)
<i>Or43a</i>	Upstream genomic	C-230T	-	0.013 (2)
<i>Or43a</i>	Upstream genomic	C-194T	-	0.228 (34)
<i>Or43a</i>	Upstream genomic	T-190C	-	0.027 (4)
<i>Or43a</i>	Upstream genomic	-179In (7 bp)	-	0.322 (49)
<i>Or43a</i>	Upstream genomic	C-167A	-	0.367 (55)
<i>Or43a</i>	Upstream genomic	C-152T	-	0.362 (54)
<i>Or43a</i>	Upstream genomic	C-120A	-	0.208 (31)
<i>Or43a</i>	Upstream genomic	A-113C	-	0.013 (2)
<i>Or43a</i>	Upstream genomic	-22In (1 bp)	-	0.040 (6)
<i>Or43a</i>	Exon 1	G66T	-	0.033 (5)

<i>Or43a</i>	Exon 1	C261T	-	0.186 (27)
<i>Or43a</i>	Intron 1	T576C	-	0.176 (26)
<i>Or43a</i>	Intron 1	C590G	-	0.013 (2)
<i>Or43a</i>	Intron 1	T607A	-	0.013 (2)
<i>Or43a</i>	Intron 1	C611T	-	0.476 (68)
<i>Or43a</i>	Exon 2	A667G	-	0.479 (69)
<i>Or43a</i>	Exon 2	C670T	-	0.483 (69)
<i>Or43a</i>	Exon 2	C685G	I170M	0.196 (29)
<i>Or43a</i>	Intron 2	C920T	-	0.315 (47)
<i>Or43a</i>	Intron 2	A922G	-	0.309 (47)
<i>Or43a</i>	Exon 3	T999C	V254A	0.027 (4)
<i>Or43a</i>	Intron 3	C1158T	-	0.193 (29)
<i>Or43a</i>	Intron 3	T1167A	-	0.199 (30)
<i>Or43a</i>	Intron 3	G1169A	-	0.199 (30)
<i>Or43a</i>	Intron 3	T1175A	-	0.199 (30)
<i>Or43a</i>	Exon 4	T1237C	-	0.449 (66)
<i>Or43a</i>	Exon 4	1240 (T/A/C)	-	0.463 (68); 0.299 (44); 0.238 (35)
<i>Or43a</i>	Exon 4	C1241A	L287M	0.303 (44)
<i>Or43a</i>	Intron 4	C1306A	-	0.247 (37)
<i>Or43a</i>	Intron 4	C1336T	-	0.061 (9)
<i>Or43a</i>	Exon 5	T1445A	F333I	0.013 (2)
<i>Or43a</i>	Intron 5	T1548C	-	0.238 (35)
<i>Or43a</i>	3' UTR	C1722G	-	0.020 (3)
<i>Or43a</i>	3' UTR	G1908A	-	0.128 (19)
<i>Or43a</i>	3' UTR	G1909T	-	0.129 (19)
<i>Or43a</i>	3' UTR	1909In (1 bp)	-	0.125 (19)
<i>Or43a</i>	3' UTR	T1921C	-	0.020 (3)
<i>Or43a</i>	3' UTR	C2044T	-	0.020 (3)
<i>Or43a</i>	Downstream genomic	C2075T	-	0.154 (23)
<i>Or43a</i>	Downstream genomic	G2090A	-	0.158 (23)
<i>Or43a</i>	Downstream genomic	T2104A	-	0.167 (24)
<i>Or67b</i>	Upstream genomic	T-154C	-	0.324 (44)
<i>Or67b</i>	Upstream genomic	A-128T	-	0.404 (55)
<i>Or67b</i>	Exon 1	C10G	Q4E	0.022 (3)

<i>Or67b</i>	Exon 1	T73C	Y25H	0.015 (2)
<i>Or67b</i>	Exon 1	G90A	-	0.412 (56)
<i>Or67b</i>	Exon 1	G123C	-	0.059 (8)
<i>Or67b</i>	Intron 1	A140G	-	0.051 (7)
<i>Or67b</i>	Intron 1	T141C	-	0.037 (5)
<i>Or67b</i>	Intron 1	C144T	-	0.434 (59)
<i>Or67b</i>	Intron 1	A148C	-	0.103 (14)
<i>Or67b</i>	Intron 1	T173C	-	0.434 (59)
<i>Or67b</i>	Intron 1	G183C	-	0.419 (57)
<i>Or67b</i>	Exon 2	A234C	-	0.015 (2)
<i>Or67b</i>	Intron 2	G313C	-	0.301 (41)
<i>Or67b</i>	Intron 2	C331A	-	0.228 (31)
<i>Or67b</i>	Intron 2	T336C	-	0.294 (40)
<i>Or67b</i>	Exon 3	C480T	-	0.059 (8)
<i>Or67b</i>	Exon 3	G484A	G121D	0.022 (3)
<i>Or67b</i>	Exon 3	C492A	Q124K	0.331 (45)
<i>Or67b</i>	Exon 3	A494G	-	0.096 (13)
<i>Or67b</i>	Intron 3	C523T	-	0.022 (3)
<i>Or67b</i>	Exon 4	A623C	-	0.015 (2)
<i>Or67b</i>	Exon 4	C662T	-	0.022 (3)
<i>Or67b</i>	Exon 4	C695T	-	0.419 (57)
<i>Or67b</i>	Exon 4	C728T	-	0.419 (57)
<i>Or67b</i>	Exon 4	C737A	-	0.272 (37)
<i>Or67b</i>	Exon 4	G752A	-	0.272 (37)
<i>Or67b</i>	Intron 4	G757A	-	0.272 (37)
<i>Or67b</i>	Intron 4	C759T	-	0.272 (37)
<i>Or67b</i>	Intron 4	A760G	-	0.272 (37)
<i>Or67b</i>	Intron 4	761In (4 bp)	-	0.272 (37)
<i>Or67b</i>	Intron 4	A766C	-	0.272 (37)
<i>Or67b</i>	Intron 4	G767T	-	0.272 (37)
<i>Or67b</i>	Intron 4	G769C	-	0.272 (37)
<i>Or67b</i>	Intron 4	770In (6 bp)	-	0.272 (37)
<i>Or67b</i>	Intron 4	C773T	-	0.404 (55)
<i>Or67b</i>	Intron 4	C775T	-	0.272 (37)

<i>Or67b</i>	Intron 4	C778G	-	0.272 (37)
<i>Or67b</i>	Intron 4	A782G	-	0.272 (37)
<i>Or67b</i>	Intron 4	784Del (1 bp)	-	0.022 (3)
<i>Or67b</i>	Intron 4	T789C	-	0.272 (37)
<i>Or67b</i>	Intron 4	T795A	-	0.272 (37)
<i>Or67b</i>	Intron 4	A797T	-	0.272 (37)
<i>Or67b</i>	Intron 4	A799T	-	0.404 (55)
<i>Or67b</i>	Intron 4	803Del (4 bp)	-	0.404 (55)
<i>Or67b</i>	Intron 4	C811T	-	0.022 (3)
<i>Or67b</i>	Exon 5	T863C	-	0.029 (4)
<i>Or67b</i>	Exon 5	C866A	-	0.029 (4)
<i>Or67b</i>	Exon 5	C903A	L221I	0.022 (3)
<i>Or67b</i>	Intron 5	T929A	-	0.382 (52)
<i>Or67b</i>	Intron 5	G932A	-	0.125 (17)
<i>Or67b</i>	Intron 5	G943A	-	0.125 (17)
<i>Or67b</i>	Intron 5	A944C	-	0.125 (17)
<i>Or67b</i>	Intron 5	A957C	-	0.434 (59)
<i>Or67b</i>	Intron 6	C1158T	-	0.015 (2)
<i>Or67b</i>	Intron 6	A1175T	-	0.441 (60)
<i>Or67b</i>	Intron 6	A1176G	-	0.434 (59)
<i>Or67b</i>	Exon 7	T1199C	-	0.441 (60)
<i>Or67b</i>	Exon 7	C1220T	-	0.147 (20)
<i>Or67b</i>	Exon 7	T1250A	-	0.382 (52)
<i>Or67b</i>	Exon 7	G1283A	-	0.404 (55)
<i>Or67b</i>	Intron 7	G1310A	-	0.316 (43)
<i>Or67b</i>	Intron 7	C1316T	-	0.022 (3)
<i>Or67b</i>	Intron 7	A1323G	-	0.316 (43)
<i>Or67b</i>	Intron 7	T1326G	-	0.316 (43)
<i>Or67b</i>	Intron 7	A1330C	-	0.404 (55)
<i>Or67b</i>	Intron 7	T1335A	-	0.404 (55)
<i>Or67b</i>	Intron 7	T1342A	-	0.088 (12)
<i>Or67b</i>	Intron 7	A1345C	-	0.081 (11)
<i>Or67b</i>	Intron 7	1347In (1 bp)	-	0.088 (12)
<i>Or67b</i>	Exon 8	G1365T	-	0.404 (55)

<i>Or67b</i>	Exon 8	1374 (G/A/T)	-	0.669 (91); 0.243 (33); 0.088 (12)
<i>Or67b</i>	Exon 8	C1426T	-	0.154 (21)
<i>Or67b</i>	Intron 8	T1475A	-	0.125 (17)
<i>Or67b</i>	Intron 8	A1478T	-	0.441 (60)
<i>Or67b</i>	Intron 8	C1483T	-	0.441 (60)
<i>Or67b</i>	Intron 8	T1489G	-	0.037 (5)
<i>Or67b</i>	Intron 8	G1506T	-	0.022 (3)
<i>Or67b</i>	Intron 8	T1509C	-	0.419 (57)
<i>Or67b</i>	Exon 9	G1524T	-	0.125 (17)
<i>Or67b</i>	Exon 9	G1568A	R369K	0.426 (58)
<i>Or67b</i>	Intron 9	T1709A	-	0.412 (56)
<i>Or67b</i>	Intron 9	C1716T	-	0.022 (3)
<i>Or67b</i>	Exon 10	C1759T	-	0.338 (46)
<i>Or67b</i>	3' UTR	G1783T	-	0.338 (46)
<i>Or67b</i>	3' UTR	G1801T	-	0.412 (56)
<i>Or67b</i>	3' UTR	A1821G	-	0.235 (32)
<i>Or67b</i>	3' UTR	A1823T	-	0.235 (32)
<i>Or67b</i>	3' UTR	Del1825 (1 bp)	-	0.235 (32)
<i>Or67b</i>	3' UTR	C1829T	-	0.235 (32)
<i>Or67b</i>	3' UTR	T1860G	-	0.237 (32)
<i>Or67b</i>	Downstream genomic	T1913A	-	0.082 (11)
<i>Or67b</i>	Downstream genomic	C1921T	-	0.336 (45)
<i>Or67b</i>	Downstream genomic	T1922A	-	0.022 (3)
<i>Or67b</i>	Downstream genomic	G2101A	-	0.299 (40)
<i>Or67b</i>	Downstream genomic	A2116G	-	0.299 (40)
<i>Or67b</i>	Downstream genomic	A2128T	-	0.301 (40)
<i>Or85f</i>	Upstream genomic	G-254T	-	0.140 (7)
<i>Or85f</i>	Upstream genomic	T-191A	-	0.160 (8)
<i>Or85f</i>	Upstream genomic	G-181A	-	0.261 (12)
<i>Or85f</i>	Upstream genomic	C-4T	-	0.333 (16)
<i>Or85f</i>	Exon 1	C139G	L47V	0.041 (2)
<i>Or85f</i>	Exon 1	C204T	-	0.477 (21)
<i>Or85f</i>	Exon 1	T207C	-	0.477 (21)
<i>Or85f</i>	Exon 1	T210G	-	0.477 (21)

<i>Or85f</i>	Exon 1	A286G	R96G	0.378 (17)
<i>Or85f</i>	Exon 1	C491T	T164I	0.044 (2)
<i>Or85f</i>	Exon 1	G556A	G186S	0.227 (10)
<i>Or85f</i>	Exon 1	A683C	A228E	0.429 (21)
<i>Or85f</i>	Exon 1	A758G	H253R	0.044 (2)
<i>Or85f</i>	Intron 1	A774G	-	0.040 (2)
<i>Or85f</i>	Intron 1	G807T	-	0.060 (3)
<i>Or85f</i>	Exon 3	T1113C	V331A	0.040 (2)
<i>Or85f</i>	Intron 3	T1282C	-	0.396 (19)
<i>Or85f</i>	Exon 4	G1329C	-	0.360 (18)
<i>Or85f</i>	Exon 4	G1362A	Stop	0.367 (18)
<i>Or85f</i>	Downstream genomic	C1592A	-	0.060 (3)
<i>Or85f</i>	Downstream genomic	T1622C	-	0.102 (5)
<i>Or85f</i>	Downstream genomic	A1711G	-	0.044 (2)
<i>Or85f</i>	Downstream genomic	T1766A	-	0.120 (6)
<i>Or85f</i>	Downstream genomic	T1824A	-	0.160 (8)
<i>Or85f</i>	Downstream genomic	A1828G	-	0.125 (6)

^aThe first base of the initiation codon for each locus corresponds to a position of +1. Insertion (In) position is indicated by the base pair position prior to the In and the deletion (Del) position is the first base pair position of the Del. For nucleotide changes the most common allele is indicated first.

^bAllele frequencies are the frequency of the rare allele, with the number of lines with the rare allele in parenthesis.

TABLE S2

Variance components for each marker associated with behavioral responses to benzaldehyde and acetophenone

Gene	Odorant	SNP	Parameter estimate		
			q^a	a^b	V_a/V_p^c
<i>Or10a</i>	Benzaldehyde	C-375T	0.029	0.441	0.019
<i>Or10a</i>	Benzaldehyde	G834T	0.04	0.365	0.018
<i>Or10a</i>	Benzaldehyde	A843T	0.04	0.365	0.018
<i>Or10a</i>	Benzaldehyde	T857A	0.028	0.429	0.017
<i>Or10a</i>	Benzaldehyde	C867T	0.028	0.429	0.017
<i>Or10a</i>	Benzaldehyde	C1163T	0.13	0.255	0.025
<i>Or10a</i>	Benzaldehyde	T1346C	0.028	0.429	0.017
<i>Or10a</i>	Benzaldehyde	T1372A	0.028	0.429	0.017
<i>Or10a</i>	Benzaldehyde	G1373T	0.028	0.429	0.017
<i>Or10a</i>	Benzaldehyde	G1377A	0.028	0.429	0.017
<i>Or10a</i>	Benzaldehyde	G1550C	0.028	0.429	0.017
<i>Or10a</i>	Benzaldehyde	1606Del	0.028	0.429	0.017
<i>Or10a</i>	Benzaldehyde	C1636T	0.068	0.293	0.019
<i>Or10a</i>	Acetophenone	C-465A	0.16	0.181	0.020
<i>Or10a</i>	Acetophenone	C-375T	0.029	0.380	0.019
<i>Or10a</i>	Acetophenone	C757T	0.085	0.218	0.017
<i>Or10a</i>	Acetophenone	G806T	0.107	0.210	0.019
<i>Or10a</i>	Acetophenone	G834T	0.04	0.329	0.019
<i>Or10a</i>	Acetophenone	A843T	0.04	0.329	0.019
<i>Or10a</i>	Acetophenone	T857A	0.028	0.383	0.018
<i>Or10a</i>	Acetophenone	C867T	0.028	0.383	0.018
<i>Or10a</i>	Acetophenone	C1163T	0.13	0.210	0.023
<i>Or10a</i>	Acetophenone	T1346C	0.028	0.383	0.018
<i>Or10a</i>	Acetophenone	T1372A	0.028	0.383	0.018
<i>Or10a</i>	Acetophenone	G1373T	0.028	0.383	0.018
<i>Or10a</i>	Acetophenone	G1377A	0.028	0.383	0.018
<i>Or10a</i>	Acetophenone	G1550C	0.028	0.383	0.018
<i>Or10a</i>	Acetophenone	1606Del	0.028	0.383	0.018
<i>Or43a</i>	Benzaldehyde	C-356T	0.278	0.189	0.025
<i>Or43a</i>	Benzaldehyde	C-194T	0.228	0.196	0.023
<i>Or43a</i>	Benzaldehyde	C-167A	0.367	0.151	0.018
<i>Or43a</i>	Benzaldehyde	C-152T	0.362	0.142	0.016
<i>Or43a</i>	Benzaldehyde	C-120A	0.208	0.236	0.032
<i>Or43a</i>	Benzaldehyde	T576C	0.176	0.190	0.018
<i>Or43a</i>	Benzaldehyde	C685G	0.196	0.179	0.017
<i>Or43a</i>	Benzaldehyde	C1158T	0.193	0.198	0.021
<i>Or43a</i>	Benzaldehyde	T1167A	0.199	0.182	0.018
<i>Or43a</i>	Benzaldehyde	G1169A	0.199	0.182	0.018
<i>Or43a</i>	Benzaldehyde	T1175A	0.199	0.182	0.018

<i>Or43a</i>	Benzaldehyde	1240 (T/A/C)	nd ^d	nd	nd
<i>Or43a</i>	Benzaldehyde	T1921C	0.02	0.545	0.020
<i>Or43a</i>	Acetophenone	C-120A	0.208	0.169	0.022
<i>Or43a</i>	Acetophenone	C670T	0.483	0.119	0.016
<i>Or67b</i>	Benzaldehyde	A494G	0.096	0.269	0.022
<i>Or67b</i>	Benzaldehyde	C523T	0.022	0.496	0.018
<i>Or67b</i>	Benzaldehyde	C662T	0.022	0.496	0.018
<i>Or67b</i>	Benzaldehyde	784Del	0.022	0.496	0.018
<i>Or67b</i>	Benzaldehyde	G932A	0.125	0.239	0.022
<i>Or67b</i>	Benzaldehyde	G943A	0.125	0.239	0.022
<i>Or67b</i>	Benzaldehyde	A944C	0.125	0.239	0.022
<i>Or67b</i>	Benzaldehyde	C1316T	0.022	0.496	0.018
<i>Or67b</i>	Benzaldehyde	T1475A	0.125	0.247	0.023
<i>Or67b</i>	Benzaldehyde	G1506T	0.022	0.496	0.018
<i>Or67b</i>	Benzaldehyde	G1524T	0.125	0.247	0.023
<i>Or67b</i>	Benzaldehyde	T1922A	0.022	0.494	0.018
<i>Or67b</i>	Acetophenone	T-154C	0.324	0.135	0.020
<i>Or67b</i>	Acetophenone	C695T	0.419	0.161	0.032
<i>Or67b</i>	Acetophenone	C728T	0.419	0.161	0.032
<i>Or67b</i>	Acetophenone	C773T	0.404	0.160	0.031
<i>Or67b</i>	Acetophenone	A799T	0.404	0.160	0.031
<i>Or67b</i>	Acetophenone	803Del	0.404	0.160	0.031
<i>Or67b</i>	Acetophenone	G932A	0.125	0.194	0.021
<i>Or67b</i>	Acetophenone	G943A	0.125	0.194	0.021
<i>Or67b</i>	Acetophenone	A944C	0.125	0.194	0.021
<i>Or67b</i>	Acetophenone	A957C	0.434	0.146	0.026
<i>Or67b</i>	Acetophenone	G1283A	0.404	0.139	0.023
<i>Or67b</i>	Acetophenone	A1330C	0.404	0.139	0.023
<i>Or67b</i>	Acetophenone	T1335A	0.404	0.139	0.023
<i>Or67b</i>	Acetophenone	G1365T	0.404	0.139	0.023

^aFrequency of rare allele. ^bHomozygous effect of marker represented as one half the average difference in olfactory behavior between the homozygous genotypes.

^cVariance attributable to the marker. ^dNot done.

TABLE S3

Haplotype analysis of polymorphisms associated with either variation in responsiveness to benzaldehyde or acetophenone for each of three odorant receptor loci.

Gene	Odorant	Haplotype No. ^a	Haplotype	Frequency
<i>Or10a</i>	Benzaldehyde	1	CGATCCTTGGGGC	0.794
<i>Or10a</i>	Benzaldehyde	2	TTTATTCATAC(D) ^b	0.029
<i>Or10a</i>	Benzaldehyde	3	CTTTCCTTGGGGC	0.012
<i>Or10a</i>	Benzaldehyde	4	CGATCTTTGGGGT	0.006
<i>Or10a</i>	Benzaldehyde	5	CGATCTTTGGGGC	0.094
<i>Or10a</i>	Benzaldehyde	6	CGATCCTTGGGGT	0.065
<i>Or10a</i>	Acetophenone	1	CCCGGATCCTTGGGG	0.677
<i>Or10a</i>	Acetophenone	2	ACCGGATCCTTGGGG	0.093
<i>Or10a</i>	Acetophenone	3	ACCGGATCTTTGGGG	0.068
<i>Or10a</i>	Acetophenone	4	CTCGTTATTCATAC(D) ^b	0.025
<i>Or10a</i>	Acetophenone	5	CCTTGATCCTTGGGG	0.081
<i>Or10a</i>	Acetophenone	6	CCCTTTTCCTTGGGG	0.012
<i>Or10a</i>	Acetophenone	7	CCCTGATCTTTGGGG	0.006
<i>Or10a</i>	Acetophenone	8	CCCTGATCCTTGGGG	0.006
<i>Or10a</i>	Acetophenone	9	CCCGGATCTTTGGGG	0.031
<i>Or43a</i>	Benzaldehyde	1	CTATACGTAAACT	0.014
<i>Or43a</i>	Benzaldehyde	2	TTATACGTAAACT	0.170
<i>Or43a</i>	Benzaldehyde	3	TCCCCCTCCTGTTT	0.014
<i>Or43a</i>	Benzaldehyde	4	TCATCTCCTGTTT	0.014
<i>Or43a</i>	Benzaldehyde	5	TCATCTGTAAACT	0.014
<i>Or43a</i>	Benzaldehyde	6	TCCCCCTCCTGTCT	0.007
<i>Or43a</i>	Benzaldehyde	7	TTATCTCCTGTCT	0.014
<i>Or43a</i>	Benzaldehyde	8	TTATCTCCTGTTT	0.014
<i>Or43a</i>	Benzaldehyde	9	TTATATGCTGTTT	0.007
<i>Or43a</i>	Benzaldehyde	10	TTATATCCTGTAT	0.007
<i>Or43a</i>	Benzaldehyde	11	TTATATCCTGTCT	0.007
<i>Or43a</i>	Benzaldehyde	12	CCATCTCCTGTTT	0.064
<i>Or43a</i>	Benzaldehyde	13	CCCCCTCCTGTTT	0.333
<i>Or43a</i>	Benzaldehyde	14	CCCCCTCCTGTAT	0.255
<i>Or43a</i>	Benzaldehyde	15	CCCCCTCCTGTAC	0.014
<i>Or43a</i>	Benzaldehyde	16	CCCCCTCCTGTCT	0.014
<i>Or43a</i>	Benzaldehyde	17	CCATCTCCTGTAT	0.035

<i>Or43a</i>	Acetophenone	1	AT	0.207
<i>Or43a</i>	Acetophenone	2	CT	0.271
<i>Or43a</i>	Acetophenone	3	CC	0.521
<i>Or67b</i>	Benzaldehyde	1	ACCTGGACTGGT	0.813
<i>Or67b</i>	Benzaldehyde	2	GTT(D)AACTTTGA ^c	0.022
<i>Or67b</i>	Benzaldehyde	3	GCCTAACCAGTT	0.022
<i>Or67b</i>	Benzaldehyde	4	GCCTGGACAGTT	0.022
<i>Or67b</i>	Benzaldehyde	5	GCCTGGACTGGT	0.007
<i>Or67b</i>	Benzaldehyde	6	GCCTAACCTGGT	0.022
<i>Or67b</i>	Benzaldehyde	7	ACCTAACCAGTT	0.037
<i>Or67b</i>	Benzaldehyde	8	ACCTAACCTGGT	0.015
<i>Or67b</i>	Benzaldehyde	9	ACCTGGACAGTT	0.037
<i>Or67b</i>	Acetophenone	1	CCCCAGGGAAGATG	0.319
<i>Or67b</i>	Acetophenone	2	TCCCAGGGAAGATG	0.215
<i>Or67b</i>	Acetophenone	3	TTTTT(D)GGACACAT ^d	0.267
<i>Or67b</i>	Acetophenone	4	TTTCAGGGAAGATG	0.007
<i>Or67b</i>	Acetophenone	5	TTTCAGGGACACAT	0.007
<i>Or67b</i>	Acetophenone	6	TTTTTT(D)AACCACAT ^d	0.074
<i>Or67b</i>	Acetophenone	7	TTTTTT(D)AACCGATG ^d	0.022
<i>Or67b</i>	Acetophenone	8	TTTTTT(D)GGAAGATG ^d	0.007
<i>Or67b</i>	Acetophenone	9	TTTTTT(D)GGACGATG ^d	0.022
<i>Or67b</i>	Acetophenone	10	TCCCAGAACAACAT	0.022
<i>Or67b</i>	Acetophenone	11	TCCCAGGGACACAT	0.030
<i>Or67b</i>	Acetophenone	12	CTTTT(D)AACCGATG ^d	0.007

^aThe Haplotype No. corresponds to the haplotypes given in Figure 4.

^{b,c,d}The *Or10a* deletion (1606Del) and the *Or67b* deletions (784Del and 803Del) are indicated by a (D), respectively

TABLE S4
ANOVA of haplotypes associated with olfactory behavior
for each of three odorant receptor loci.

A.

Odorant	Gene	Source	df	Type III SS	MS	F	<i>P</i>
Benzaldehyde	<i>Or10a</i>	Haplotype	5	19.907	3.981	7.49	<0.0001
		Sex	1	0.232	0.232	0.44	0.51
		Haplotype x Sex	5	0.878	0.176	0.33	0.89
		Error	328	174.327	0.531		
		Total	339	197.115			
	<i>Or43a</i>	Haplotype	16	26.282	1.643	3.10	<0.0001
		Sex	1	0.517	0.517	0.98	0.32
		Haplotype x Sex	16	1.228	0.077	0.15	1.00
		Error	248	131.240	0.529		
		Total	281	160.855			
	<i>Or67b</i>	Haplotype	8	16.811	2.101	3.84	0.0003
		Sex	1	0.000	0.000	0.00	0.99
		Haplotype x Sex	8	1.305	0.163	0.30	0.97
		Error	250	136.758	0.547		
		Total	267	157.394			
Acetophenone	<i>Or10a</i>	Haplotype	8	17.341	2.168	5.32	<0.0001
		Sex	1	0.674	0.674	1.66	0.20
		Haplotype x Sex	8	1.258	0.157	0.39	0.93
		Error	304	123.808	0.407		
		Total	321	147.023			
	<i>Or43a</i>	Haplotype	2	4.779	2.390	5.76	0.0035
		Sex	1	2.725	2.725	6.57	0.02
		Haplotype x Sex	2	0.003	0.002	0.00	1.00

	Error	274	113.596	0.415		
	Total	279	121.457			
<i>Or67b</i>	Haplotype	11	10.359	0.942	2.43	0.0068
	Sex	1	0.472	0.472	1.22	0.27
	Haplotype x Sex	11	1.216	0.111	0.29	0.99
	Error	246	95.276	0.387		
	Total	269	111.362			

B.

Odorant	Gene	% V_P^*
Benzaldehyde	<i>Or10a</i>	10.1
	<i>Or43a</i>	16.3
	<i>Or67b</i>	10.7
Acetophenone	<i>Or10a</i>	11.8
	<i>Or43a</i>	3.9
	<i>Or67b</i>	9.3

*% total variance due to haplotype

TABLE S5**ANOVA of expression differences for each of three odorant receptor loci.**

Gene	Sex	Source	df	Sum of Squares	F ratio	<i>P</i>
<i>Or10a</i>	Females	Haplotype	1	2.350	31.437	<0.0001
		Line(Haplotype)	4	1.636	5.470	0.0046
		Error				
<i>Or10a</i>	Males	Haplotype	1	42.795	108.249	<0.0001
		Line(Haplotype)	4	66.208	41.869	<0.0001
		Error				
<i>Or43a</i>	Females	Haplotype	1	0.006	0.024	0.8798
		Line(Haplotype)	1	2.932	12.643	0.0062
		Error				
<i>Or43a</i>	Males	Haplotype	1	1.789	8.581	0.0168
		Line(Haplotype)	1	1.483	7.113	0.0258
		Error				