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Making scents of behavioural genetics: lessons from *Drosophila*

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Summary

The expression of behaviours is influenced by many segregating genes. Behaviours are, therefore, complex traits. They have, however, unique characteristics that set them apart from physiological and morphological quantitative traits. First, behaviours are the ultimate expression of the nervous system. This means that understanding the genetic underpinnings of behaviours requires a neurobiological context, i.e. an understanding of the genes–brain–behaviour axis. In other words, how do ensembles of genes empower specific neural circuits to drive behaviours? Second, behaviours represent the interface between an organism and its environment. Thus, environmental effects are likely to make substantial contributions to determining behavioural outputs and genotype-by-environment interactions are expected to be prominent. It is important to differentiate between genes that contribute to the manifestation of the behavioural phenotype and genes that contribute to phenotypic variation in behaviour. The former are identified by classical mutagenesis experiments, whereas the latter can be detected through quantitative genetic approaches. Genes that contribute to phenotypic variation in behaviour harbour polymorphisms that provide the substrates for evolution. This review focuses on olfactory behaviour in *Drosophila* with the goal to illustrate how fundamental insights derived from studies on chemosensation can be applied to a wide range of behavioural phenotypes.

1. Introduction: olfaction and *Drosophila*

Behaviours mediate adaptive responses that are essential for survival and reproduction. Thus, they represent the ultimate fitness traits and provide the mechanisms through which evolutionary forces can act. Most behaviours are dominated by sensory-motor processes in which sensory input is integrated, evaluated and acted on through motor output. Chemosensation is an important sensory input for most organisms and is essential for the localization of food, avoidance of toxins or predators and assessment of mating partners and oviposition sites. Chemical signals also drive other behaviours, including aggression, courtship and, in mammals, affiliative behaviours and parental care. Thus, studies on the genetics that underlies behavioural responses to chemical signals can provide biologically relevant information from which general principles can be derived which will be broadly applicable.

Drosophila melanogaster provides an excellent model system for studies on the genetic architecture of olfactory behaviour, since its olfactory system has been well characterized (Su *et al.*, 2009) and flies are readily amenable to genetic, neuroanatomical, electrophysiological and behavioural manipulations. An important advantage of flies from a genetic perspective is the ability to generate a virtually unlimited number of individuals of the same genotype that can be grown under controlled environmental conditions. The ability to control both the genetic background and the environment precisely, together with the many publicly available genetic and genomic resources, makes *Drosophila* the perfect organism for studies aimed at dissecting the genetic underpinnings of behaviours.

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2. Functional organization of the olfactory system of *Drosophila*

The main olfactory organs of *Drosophila* are the third antennal segments and the maxillary palps. The third antennal segment contains about 1200 olfactory receptor neurons housed in three morphologically distinct classes of sensilla: basiconic, coeloconic and trichoid sensilla. The maxillary palps contain about 120 olfactory sensory neurons in basiconic sensilla (Stocker, 1994; Shandbhag *et al*., 1999; Su *et al*., 2009). The sensilla are porous structures that contain two – or sometimes in the third antennal segment four – chemosensory neurons that express odorant receptors. They also contain supporting cells that secrete odorant-binding proteins in the perilymph that surrounds the chemosensory dendrites of the olfactory receptor neurons (Shandbhag *et al*., 2001; Galindo & Smith, 2001; Hekmat-Scafe *et al*., 2002). Odorant-binding proteins are the first components of the olfactory system to encounter odorants and are thought to facilitate transport of odorants to their receptors. Recent evidence suggests that they also play a fundamental role in odorant discrimination (Matsuo *et al*., 2007; Wang *et al*., 2007, 2010).

The morphological classes of sensilla are functionally distinct. Basiconic sensilla are dedicated to general odorant discrimination and each olfactory sensory neuron within the basiconic sensilla expresses one or sometimes two unique odorant receptors from a repertoire of 62 *Or* genes (Clyne *et al*., 1999; Gao & Chess, 1999; Vosshall *et al*., 1999). Each unique odorant receptor dimerizes with a common odorant receptor, the Or83b receptor (Larsson *et al*., 2004; Benton *et al*., 2006), and activation of this complex by odorants results in the opening of a cation channel that depolarizes the olfactory sensory neuron (Sato *et al*., 2008; Wicher *et al*., 2008). Coeloconic sensilla are enriched in a cavity of the third antennal segment, the sacculus, and express odorant receptors that resemble ionotropic glutamate receptors (Benton *et al*., 2009). The functions of this family of receptors, which is encoded by 61 functional *Ir* genes, are not clear, but they respond to odorants that include ammonia and phenyl acetaldehyde (Benton *et al*., 2009). Furthermore, in larvae of the mosquito *Anopheles gambiae* behavioural responses to butylamine are mediated via Ir receptors independent of classical Or receptors (Liu *et al*., 2010). The T1 trichoid sensilla express the Or67d receptor and the Lush odorant-binding protein, which mediate recognition of the courtship pheromone 11-*cis*-vaccenyl acetate (Xu *et al*., 2005; Ha & Smith, 2006; Kurtovic *et al*., 2007). In addition, specialized sensilla in the third antennal segment express receptors that recognize carbon dioxide (Jones *et al*., 2007; Kwon *et al*., 2007). These receptors, Gr21a and Gr63a, belong to a multigene family of gustatory receptors (Scott *et al*., 2001).

The axons of olfactory neurons project bilaterally to spherical structures of neuropil, glomeruli, in the antennal lobe (Vosshall *et al*., 2000). For example, the CO_2 sensing neurons in the third antennal segment project to glomerulus V (Suh *et al*., 2004, 2007). The antennal lobes contain about 43 individually identifiable glomeruli (Laissue *et al*., 1999). Elegant studies in which the expression of green fluorescent protein (GFP) is driven by *Or* promoters have resulted in a detailed projection map from peripheral sensory neurons that express distinct *Or* receptors to specific glomeruli in the antennal lobes (Vosshall *et al*., 2000). Electrophysiological studies revealed a stereotypic organization of olfactory sensory neurons in the maxillary palps (de Bruyne *et al*., 1999) and antenna (de Bruyne *et al*., 2001), in which pairs of neurons with defined molecular response profiles always occur together. Systematic ectopic expression studies in which *Or* genes were expressed in a mutant sensillum that lacks expression of its endogenous odorant receptor (Dobritsa *et al*., 2003) have provided extensive documentation of the molecular response profiles of a large fraction of Or receptors in *Drosophila* (Hallem & Carlson, 2006).

Combinatorial activation of odorant receptors by odorants generates a pattern of peripheral activity that is translated into a temporally and spatially dynamic activation pattern of glomeruli in the antennal lobes (Su *et al*., 2009). This glomerular activation map is processed by local interneurons and transmitted via projection neurons to the mushroom bodies and lateral horn of the protocerebrum in the brain (Wong *et al*., 2002; Marin *et al*., 2002; Jefferis *et al*., 2007; Masse *et al*., 2009; Su *et al*., 2009), where olfactory information is interpreted and coupled to an appropriate motor output.

3. Plasticity of the expression of chemosensory genes

The intimate relationship between an organism and its chemical milieu requires adaptations of its chemosensory gene repertoire both over evolutionary time as habitats change and speciation occurs, and during the lifetime of the individual as it experiences changes in its physical and social environment. It is, therefore, perhaps not surprising that odorant binding protein (*Obp*) and odorant receptor (*Or*) gene families evolve rapidly (Gardiner *et al*., 2008; Kopp *et al*., 2008; Sánchez-Gracia & Rozas, 2008). Duplication of ancestral genes has resulted in the formation of clusters of *Obp* and *Or* genes throughout the genome. Examination of *Or* genes in 12 *Drosophila* species shows frequent gains and losses of genes as well as chromosomal rearrangements (Nozawa & Nei, 2007; Sánchez-Gracia *et al*., 2009). Sequence analyses of

members of the *Obp56a–i* and *Obp99a–d* clusters in a population of inbred wild-derived *Drosophila* lines showed different histories of recombination with different signatures of selection, including balancing selection for *Obp99d*, which showed an unusual degree of polymorphism in this population (Wang *et al*., 2007). Several studies have reported the maintenance of segregating *Obp* null alleles (Takahashi & Takano-Shimizu, 2005; Wang *et al*., 2007). Such alleles can be tolerated if compensated for by functional redundancy within the *Obp* family. The best documented example of evolutionary adaptation of *Obp* genes comes from the host plant specialization of *Drosophila sechellia*. This species feeds and oviposits on fruit of *Morinda citrifolia* in its habitat on the Seychelles islands. The Morinda fruit is toxic and repellant to other *Drosophila* species and avoidance of this fruit appears due to hexanoic acid and octanoic acid, which are sensed by chemosensory neurons on the tarsi. A 4 bp insertion in the *Obp57e* gene in *D. sechellia* prevents expression of this *Obp* and results in loss of avoidance of hexanoic acid and octanoic acid, allowing *D. sechellia* to be attracted to the fruit of *M. citrifolia* (Matsuo *et al*., 2007; Dworkin & Jones, 2009). Co-evolution of detoxification mechanisms might protect against toxicity of the Morinda fruit and further enable host plant specialization (Dworkin & Jones, 2009). The chemosensory system of *D. sechellia* has undergone especially rapid evolution and host specialization has resulted in the accelerated loss of chemoreceptor genes (McBride, 2007).

Gene duplication processes during the evolution of chemosensory genes can allow subfunctionalization or neofunctionalization of daughter genes. The *Or22a* and *Or22b* genes provide an example of a recent duplication (Aguadé, 2009). These genes are located in tandem on the chromosome and expressed together in the same olfactory sensory neurons, the ab3A neurons. However, *Or22a* expression alone is sufficient to account for the molecular response properties of these neurons (Dobritsa *et al*., 2003). Gene duplication may also have led to functional divergence among the family of odorant binding proteins and led to co-option of these proteins for functions other than mediating olfaction (Arya *et al*. 2010). This notion is supported by the observation that the expression of *Obp* genes is altered following mating, exposure to starvation stress (Harbison *et al*., 2005), during the development of alcohol tolerance (Morozova *et al*., 2006), and as a correlated response to artificial selection for divergent levels of copulation latency (Mackay *et al*., 2005), aggression (Edwards *et al*., 2006) and sensitivity to alcohol (Morozova *et al*., 2007). Furthermore, members of the *Obp* gene family are expressed not only in chemosensory tissues but also in non-chemosensory tissues, such as the fat body (Fujii & Amrein, 2002) and the male accessory gland (Ayroles *et al*., 2009), where they may serve roles as carriers for lipophilic compounds.

The chemosensory gene repertoire is plastic and expression levels of *Obp* and *Or* genes change dynamically as individual flies go through different developmental stages and experience different physical and social environments (Zhou *et al*., 2009). Exposure to concentrated vapours of ethanol causes a rapid down-regulation of olfactory gene expression, including the common Or83b receptor (Morozova *et al*., 2006). Transcriptional profiling showed altered expression levels of several *Obp* genes in *D. melanogaster* females following mating (McGraw *et al*., 2004). The expression of chemoreceptor genes, especially the *Obp* genes, is characterized by extensive sexual dimorphism (Figs 1 and 2; Zhou *et al*., 2009). Males and females utilize the chemosensory gene repertoire differently, which reflects the strong influence of sex environment on gene expression and the different functional demands that are placed on chemosensation in each sex, such as mate recognition versus selection of oviposition sites. A striking example of sex-specific functional modulation of chemoreceptor expression is the observation that in the malaria-transmitting mosquito, *Anopheles gambiae*, expression of the AgOR1 odorant receptor, which is expressed specifically in female antennae, is dramatically down-regulated following a blood meal (Fox *et al*., 2001).

Specific members of the *Obp* and *Or* gene families change expression levels in larval stages (Fig. 2) and during senescence, following mating (Fig. 1*a*, *b*), and under conditions of solitary growth or exposure to groups of same sex or opposite sex individuals (Zhou *et al*., 2009). Expression of members of the same gene cluster can be regulated independently and analysis of covariant expression levels of chemosensory genes across different conditions reveals 20 small modules of chemosensory genes that are regulated in a coordinated manner (Fig. 3; Zhou *et al*., 2009). Combinations of transcription factors acting on *cis*-regulatory elements either promote or inhibit the expression of specific *Or* genes (Ray *et al*., 2008), and the POU domain transcription factors *acj6* and *pdm3* have been implicated in modulating expression of odorant receptors in subsets of olfactory neurons (Tichy *et al*., 2008; Bai *et al*., 2009; Arya *et al*., 2010; Bai & Carlson, 2010). However, the molecular mechanisms that regulate altered expression of chemosensory genes in response to changes in the environment remain to be elucidated.

4. Mutagenesis and pleiotropy

Whereas chemoreceptors mediate odorant recognition and discrimination, transmission and interpretation

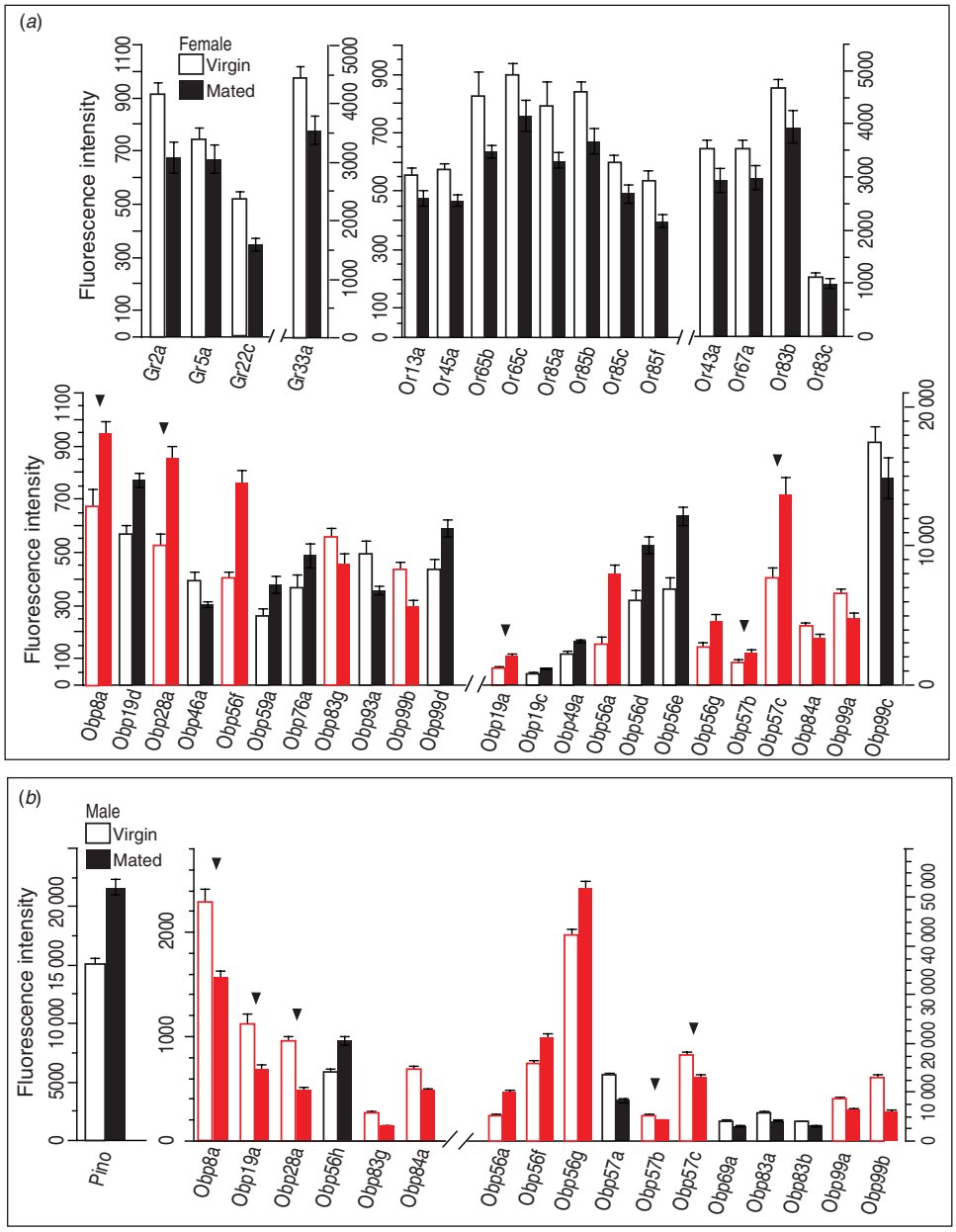

Fig. 1. Phenotypic plasticity in the expression of chemoreceptor genes dependent on the physiological state. Relative expression levels of chemoreceptor genes assessed by hybridization of mRNA extracts to cDNA expression microarrays show differences in the expression of four gustatory receptor (*Gr*), 12 odorant receptor (*Or*) and 23 odorant-binding protein (*Obp*) genes between virgin and mated females (*a*). In contrast, only 17 *Obp* genes along with *Pino*, which encodes Pinocchio that resembles an odorant-binding protein (Rollmann *et al*., 2005), show altered expression in mated compared to virgin males (*b*). Red bars indicate genes that show altered regulation in both sexes and arrowheads indicate antagonistic regulation between males and females (adapted from Zhou *et al*., 2009).

of this chemosensory information and the ensuing behavioural response are influenced by many genes. Early studies on the genetics of olfaction utilized classical mutagenesis approaches and identified, among others, genes essential for neurotransmission, neurodevelopment and regulation of the expression of chemoreceptor genes.

One of the first olfactory mutants identified was *smellblind*, an allele of *paralytic*, which encodes the classical voltage-gated sodium channel (Lilly & Carlson, 1990). Other genes essential for neurotransmission that impact on olfactory behaviour include a second voltage-gated sodium channel (*NaCP60E*; Kulkarni *et al*., 2002) and the synaptic PDZ domain protein *scribble* (Ganguly *et al*., 2003). A quantitative trait locus (QTL) mapping study identified an allele of another PDZ domain protein, *discs lost* (*Vanaso*), as a quantitative trait gene for variation in olfactory behaviour in a mapping population derived from Oregon R and 2b strains (Fanara

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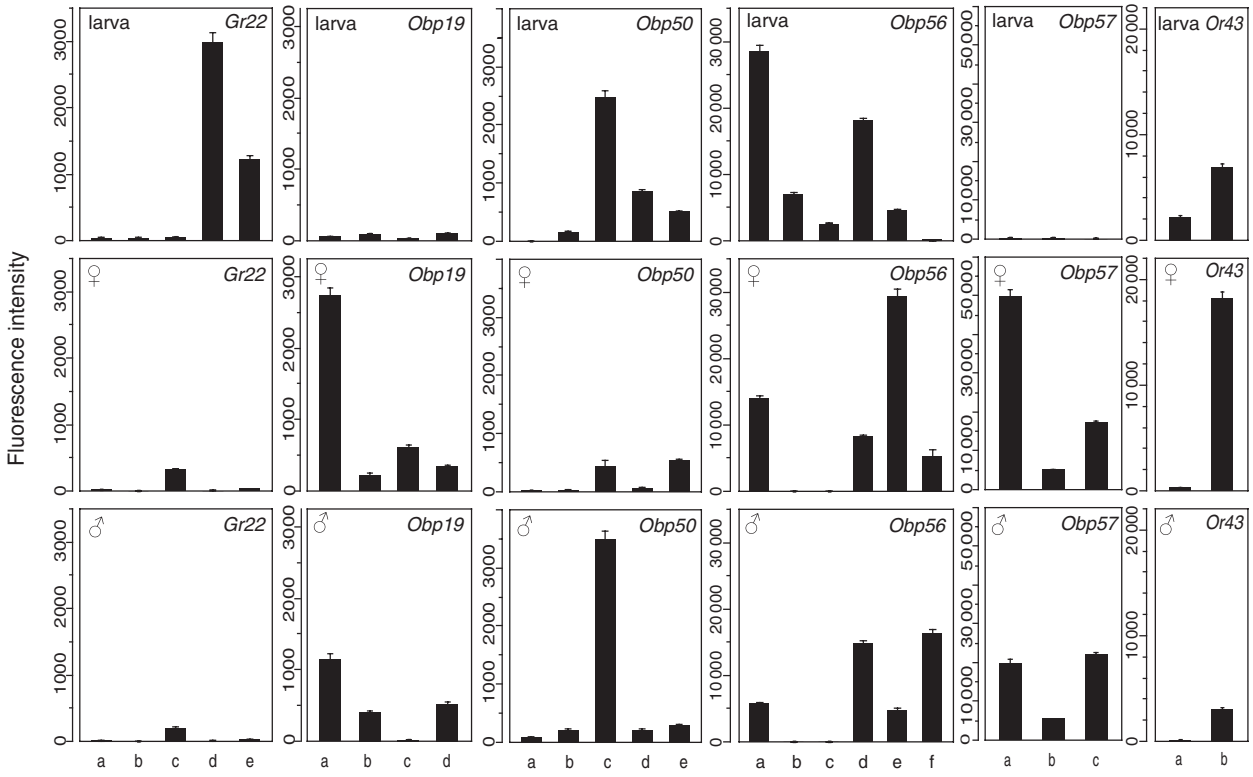

Fig. 2. Phenotypic plasticity in the expression of chemoreceptor genes dependent on the developmental stage and sex. Relative expression levels of chemoreceptor genes assessed by hybridization of mRNA extracts to cDNA expression microarrays show differential gene expression between larval and adult life stages. Sexual dimorphism is prevalent among chemoreceptor genes and members of gene clusters are often regulated independently (adapted from Zhou *et al*., 2009).

et al., 2002). *P*-element insertional mutations in neurodevelopmental genes, such as *Sema-5C* (Rollmann *et al*., 2007) and *neuralized* (Rollmann *et al*., 2008), also affect olfactory behaviour. In addition, a POU-domain transcription factor, *acj6*, that regulates *Or* gene expression in subsets of chemosensory sensilla in the maxillary palps and antennae (Bai *et al*., 2009; Bai & Carlson, 2010) was also initially identified from a mutant screen (Ayer & Carlson, 1991).

Transcriptional profiling in mutant backgrounds identified large numbers of genes with altered transcriptional regulation (Anholt *et al*., 2003). These genes fall into multiple biological function gene ontology categories and are also candidate genes that may contribute to olfactory behaviour. *P*-element insertional mutagenesis screens consistently yielded 4–6% of lines with aberrant olfactory behaviour, reflecting a large mutational target and confirming that a large proportion of the genome contributes to olfactory behaviour (Anholt *et al*., 1996; Sambandan *et al*., 2006). The extreme multigenic nature of behavioural traits is also evident from the hundreds of genes that undergo altered transcriptional regulation as a result of artificial selection, such as selection for low or high aggression (Edwards *et al*., 2006) and sensitivity or resistance to alcohol (Morozova *et al*., 2007). Thus, behavioural phenotypes are emergent properties of ensembles of pleiotropic genes.

5. Genetic networks

Mutagenesis studies are valuable for identifying genes that contribute to a behavioural phenotype and placing such genes in a mechanistic context. A complete understanding of the genetic underpinnings of behaviour, as for any complex trait, requires elucidating the coordinated action of networks of genes which qualitatively and quantitatively determine the expression of the phenotype. Genetic networks can be constructed by regressing phenotypic values of inbred wild-derived lines of *D. melanogaster* on transcript abundance levels across the genome and analysing covariance among the residuals of such regressions (Ayroles *et al*., 2009). This has been done for sleep (Harbison *et al*., 2009) and alcohol sensitivity (Morozova *et al*., 2009). Alternatively, genetic interactions can be inferred directly by measuring additive or non-additive effects on the phenotype of mutant alleles as double heterozygotes. This analysis requires a collection of mutations in the same genetic background. A half-diallel crossing design then allows detection of enhancer or suppressor effects in each double heterozygote by evaluating the difference of

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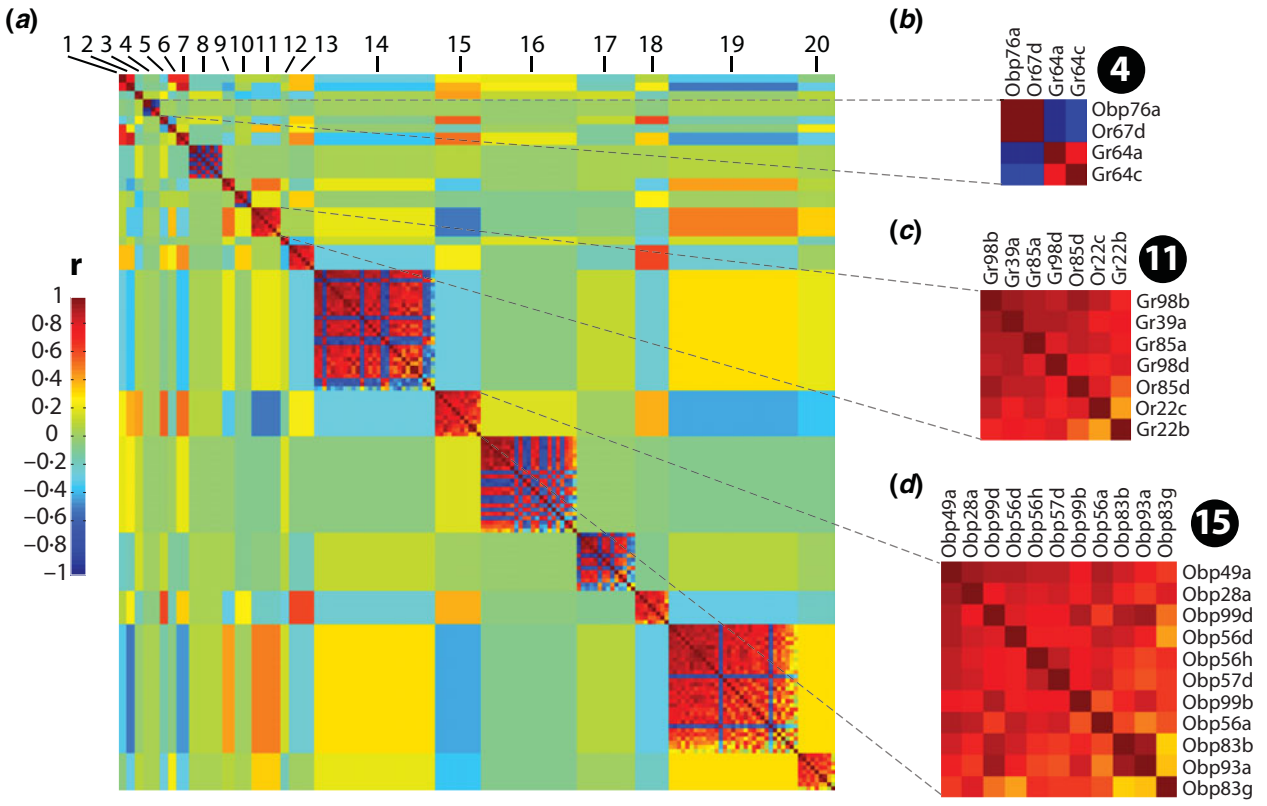

Fig. 3. Cluster analysis of chemoreceptor expression across multiple environmental conditions. The chemosensory gene repertoire clusters in 20 relatively small co-regulated transcriptional modules. Note that *Obp76a*, also known as *Lush*, and its receptor *Or67d* group together in module 4 (adapted from Zhou *et al.*, 2009).

observed phenotypic values from those predicted from the average heterozygous effects of each parent in combination with all other mutations (Fig. 4*a*; Griffing, 1956). Such an analysis has been performed for olfactory behaviour and revealed extensive epistatic interactions among *P*-element alleles with *P*-element insertions that affect the avoidance response to benzaldehyde (Fedorowicz *et al.*, 1998; Sambandan *et al.*, 2006). Extensive epistatic interactions appear to be a general hallmark, at least for behavioural traits. Similar epistatic networks have been identified for startle behaviour (Yamamoto *et al.*, 2008) and climbing behaviour (van Swinderen & Greenspan, 2005).

The vast extent of epistasis among a small group of independently isolated *P*-element-insertion mutants is surprising and reveals an extensive network of enhancer and suppressor effects among genes that contribute to the same phenotype. These epistatic interactions for olfactory behaviour are dynamic and manifested differently at different odorant concentrations, even though the main effects of the mutations are stable (Sambandan *et al.*, 2006). Whereas one might expect epistatic effects to be minor compared to the main mutational effects, this is not always the case. For example, a *P*-element insertion at the *smi21F* locus affects expression of a protein named Pinocchio thought to function in odorant removal from the perilymph (Rollmann *et al.*, 2005). The effect of this *P*-element insertion on olfactory avoidance behaviour to benzaldehyde was small and detected only in females (Anholt *et al.*, 1996). However, the *smi21F P*-element insertion showed extensive epistasis (Fedorowicz *et al.*, 1998). In a recent study, Canton-S chromosomes containing *P*-element insertions that affect startle behaviour along with their co-isogenic *P*-element-free controls were introduced in multiple inbred wild-derived genetic backgrounds. The magnitude of effects of these *P*-element insertional mutations on startle behaviour was attenuated by naturally occurring modifiers and there was a virtual linear relationship between the magnitude of the main mutational effects and the epistatic suppressor effects (Yamamoto *et al.*, 2009). Thus, the dynamics of epistatic interactions can shift the balance of effects on the phenotype of genes within a network during an individual's lifetime, but, perhaps paradoxically, provides robustness to genetic networks over evolutionary time by buffering the effects of newly arising mutations.

The prevalence of both epistasis and pleiotropy implies that the same genes form part of multiple epistatic networks which give rise to different behavioural phenotypes (Fig. 4*b*). For example,

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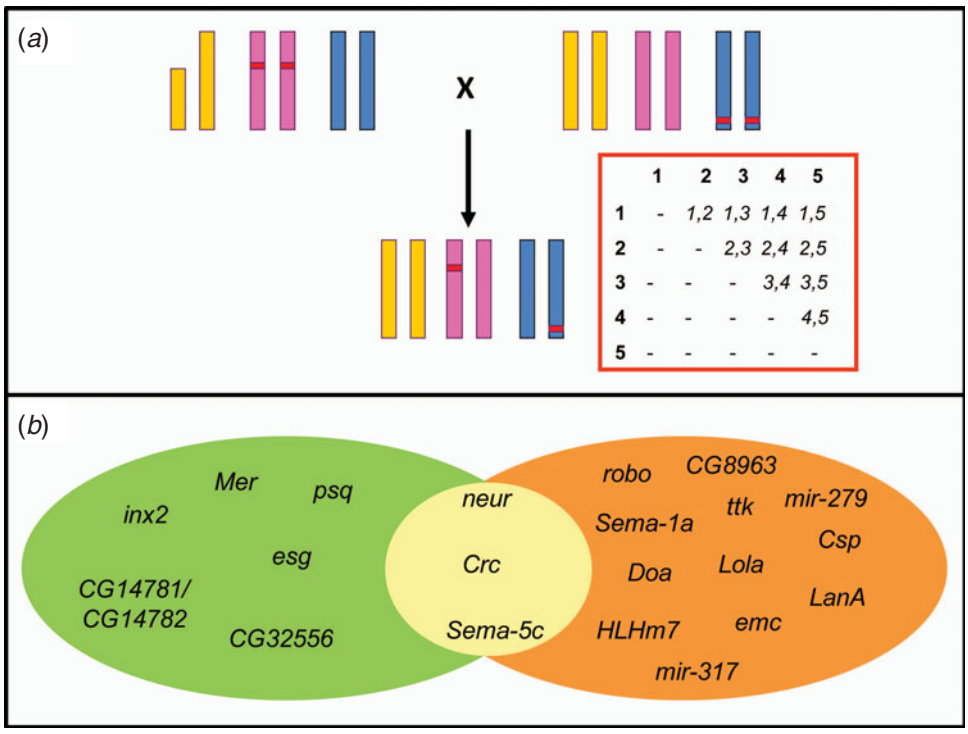

Fig. 4. Identifying epistatic interactions among co-isogenic *P*-element insertion mutants. (*a*) Diagram of the half-diallel crossing principle. The three sets of the major homologous chromosomes of *Drosophila* are colour coded with red bars indicating *P*-element insertion sites. The small fourth chromosome is not depicted. Crosses between a set of homozygous co-isogenic *P*-element insertion lines (five are shown in this example) allow separation of dominance from non-additive enhancer/suppressor effects. (*b*) The analysis depicted in (*a*) can identify ensembles of genes that engage in dynamic epistatic interactions. On the left (green background) is an epistatic ensemble of transposon-tagged genes that affect olfactory behaviour (Sambandan *et al*., 2006); on the right (orange background) is an epistatic ensemble of transposon-tagged genes that affect startle behaviour (Yamamoto *et al*., 2008). Overlap between the networks (yellow background) highlights genes that participate in both networks, illustrating pleiotropy.

P-element-insertions in *neuralized* (*neur*) affect olfactory behaviour, aggression and startle behaviour (Rollmann *et al*., 2008). Epistasis analysis reveals a different context for *neur* in relation to other transposon-tagged genes that affect olfactory behaviour (Sambandan *et al*., 2006) and startle behaviour (Yamamoto *et al*., 2008). Moreover, the consequences of the transposon insertion depend on its precise insertion site and orientation. For example, different *P*-element-insertion alleles for *neur* located only a few base pairs apart differentially affect olfactory behaviour and aggression, and these effects are accompanied by changes in the structure of the mushroom bodies (Rollmann *et al*., 2008).

6. Linking genetic variation to phenotypic variation

Much information on the genetic basis of complex traits comes from associating DNA polymorphisms with phenotypic variation. Unlike the human genome that is characterized by large linkage disequilibrium (LD) blocks (Gabriel *et al*., 2002), LD in *Drosophila* decays rapidly over a few hundred base pairs as a result of the short generation time and, consequently, a more extensive history of recombination. A disadvantage of this limited LD is that it precludes the use of tagging Single Nucleotide Polymorphisms (SNPs) and necessitates obtaining complete sequence information for association studies. An advantage is that once a polymorphism has been identified to be associated with phenotypic variation, this polymorphism is likely to be – or at least to be closely linked to – the causal variant.

Association studies in *Drosophila* have become possible with the generation of a population of 345 lines obtained by 20 generations of inbreeding of isofemale lines from a natural Raleigh, NC, population. Genetic variation within individual lines is minimal, while the collection of inbred wild-derived lines as a whole preserves the genetic diversity present in the original natural population. A subset of 192 of these lines with fully sequenced genomes represents a publicly available resource, the Drosophila Genetic Reference Panel (DGRP), which enables genome-wide association studies in *Drosophila* (Mackay *et al*., 2008).

Prior to the availability of whole genome sequences from the DGRP, sequencing individual candidate genes from among the 345 lines has enabled insights

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in the relationship between variants in the DNA sequence and variation in behavioural phenotypes. Sequencing the *Obp99a–d* group has identified SNPs in *Obp99a*, *Obp99c* and *Obp99d* that were associated with variation in olfactory responses to benzaldehyde (Wang *et al*., 2007). Whereas responses to benzaldehyde in isogenic laboratory strains are always strongly repellent (Anholt *et al*., 1996), phenotypic variation in responses to this odorant in the wild-derived lines is far broader spanning the range from complete attraction to complete repulsion (Wang *et al*., 2007). Such an extensive phenotypic variation is not unique to olfactory behaviour, but has been observed for virtually every trait studied in these lines and often exceeds the phenotypic extremes that can be obtained through artificial selection (Ayroles *et al*., 2009; Morozova *et al*., 2009). When associations between polymorphisms in the *Obp99a–d* cluster and responses to the odorant acetophenone were analysed, SNPs associated with variation in olfactory responses to this odorant were identified in *Obp99a*, *Obp99b* and *Obp99d* (Wang *et al*., 2010). These polymorphic markers, however, were distinct from those associated with variation in olfactory behaviour to benzaldehyde. This is of interest as these two odorants are structurally similar, differing only in a methyl group. These studies showed that odorant-binding proteins recognize odorants in a combinatorial manner, but different SNPs in *Obp* genes generate odorant-specific individual variation in chemosensory behaviour.

It is of interest to note that most of the SNPs associated with variation in olfactory behaviour in the *Obp99a–d* group were SNPs in non-coding regions or synonymous substitutions in the coding region. SNPs in non-coding regulatory regions may affect variation in expression. Synonymous SNPs in the coding region can affect mRNA structure, stability, processing and/or translation efficiency (Wang *et al*., 2007). An even more striking pattern of SNP associations has been found in the *Catsup* gene, which encodes a negative regulator of tyrosine hydroxylase, which is essential for the biosynthesis of dopamine. Here, distinct polymorphisms have been associated with startle-induced locomotion, longevity and sensory bristle phenotypes (Carbone *et al*., 2006) as well as sleep (Harbison *et al*., 2009). Thus, within pleiotropic genes different polymorphisms may be associated with phenotypic variation in different traits.

7. Behavioural genetics: lessons from *Drosophila* and future frontiers

Studies on the genetics of olfactory behaviour in *Drosophila* have yielded several fundamental insights. First, the genetic architecture of behaviour consists of epistatic networks of pleiotropic genes. These networks are plastic and dynamic and modulated by the sex, the social and physical environment and the developmental history of the organism. Second, embedded in these overlapping genetic networks are matrices of polymorphisms that provide the instrument for evolutionary change. A large proportion of these polymorphisms are synonymous or in regulatory regions. Rather than changing the structure of the encoded protein, they may affect expression levels, mRNA structure, stability, processing and/or translation efficiency. A third important insight from studies of whole genome transcriptional profiles of mutants that contain a single *P*-element that affects olfactory behaviour is the discovery that a single mutation or polymorphism can have widespread effects throughout the transcriptome (Anholt *et al*., 2003). This is an important caveat for the interpretation of associations of polymorphisms with disease susceptibility in human population studies. Similarly, the prevalence of epistasis and environment-dependent phenotypic plasticity are likely to apply not only to behavioural phenotypes in model organisms but also to human quantitative traits. Finally, although many genes are pleiotropic and contribute to multiple phenotypes, different alleles harbouring different polymorphisms appear to be associated with different traits.

Thus far, studies on the genetic architecture of behaviour have been mostly descriptive. A daunting challenge for the future will be to predict behavioural outcomes based on analyses of their genetic underpinnings, while taking into account epistatic interactions and environmental effects. A necessary component of this challenge is to link gene expression networks to distinct neuronal populations. Ideally, one would like to monitor the dynamics of whole genome transcriptional profiles in single neurons or small defined neuronal populations in behaving animals. The pursuit of this goal depends on new technological advances in the future.

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