DROSOPHILA: GENETICS MEETS BEHAVIOUR

Marla B. Sokolowski

Genes are understandably crucial to physiology, morphology and biochemistry, but the idea of genes contributing to individual differences in behaviour once seemed outrageous. Nevertheless, some scientists have aspired to understand the relationship between genes and behaviour, and their research has become increasingly informative and productive over the past several decades. At the forefront of behavioural genetics research is the fruitfly *Drosophila melanogaster*, which has provided us with important insights into the molecular, cellular and evolutionary bases of behaviour.

ACTIVITY–REST CYCLE
This refers to the rhythm of the locomotor activity of a fly during its 24-h activity cycle. It is also called the circadian locomotor activity rhythm.

Department of Zoology, University of Toronto, 3359 Mississauga Road, Mississauga, Ontario, Canada L5L 1C6.
e-mail: msokolow@credit.erin.utoronto.ca

The challenges of behavioural genetics research include: the difficulty in defining and quantifying behaviour (BOX 1); the tremendous within- and between-individual variation in behaviour; the involvement of many genes; and the fact that different genes function in different tissues at different times during the ontogeny of an organism, all of which combine to influence a single pattern of behaviour. The nervous system, the most crucial system in the elicitation of behaviour, is formed during development by networks of interacting genes. Similar networks assemble the physiological structures necessary to generate these behaviour patterns. In addition to these genetic contributions, an organism also experiences environmental conditions throughout its life that can influence its behaviours. Despite these and other sources of complexity, a significant amount of research has been accomplished, most of which has pushed the fruitfly *Drosophila melanogaster* to the forefront of behavioural genetics research.

Model genetic organisms, such as *Drosophila*, have been especially useful for the genetic dissection of developmental and anatomical traits[1]. The fact that many genes found in flies have structural or functional homologues in vertebrates, including humans, means that genetic discoveries in the fruitfly can contribute to our general understanding of evolutionarily conserved developmental and physiological processes[2]. *Drosophila*, however, is much more than just a gene-finding tool for those studying mammalian genes. It is an exceptionally useful genetic model for the study of simple and complex behaviours, and its use as such has given rise to an important body of literature, in which can be found common themes on the molecular, cellular and evolutionary underpinnings of behaviour.

Here, I review the current state of *Drosophila* behavioural genetics by focusing on a set of specific examples and by deriving lessons that might be of general significance to the question of how genes affect complex behaviour. The review centres on a discussion of how genes that are involved in foraging, circadian rhythms, courtship, and learning and memory specifically contribute to their respective behaviours. General principles learned from *Drosophila*, along with a vision for future behavioural research, are discussed towards the end of the review.

Analysing complex behaviour in *Drosophila*

It might come as a surprise to some that *D. melanogaster* shows many exquisitely performed and complex patterns of behaviour. For example, the male fly shows courtship behaviour that is full of sensory stimuli and that requires the female to hear his song, feel his taps and licks, smell his odours and visually evaluate his stature (FIG. 1; discussed in more detail more below). She then chooses whether to copulate with him[3,4]. Flies also show rhythmic behaviours, including ACTIVITY–REST CYCLES that are similar to sleep–wake cycles in mammals[5–7]. Flies show different feeding-related behaviours: some

Box 1 | **Behaviour as a phenotype for genetic analysis**

Behaviour, arguably one of the most complex phenotypes, has been considered to be the action of an animal in response to its internal and external environment. However, this definition of behaviour is vague and not limited to behavioural phenotypes. From an experimental point of view, each behaviour that is under study must be defined in the context of its own paradigm. Practical definitions of behaviour, however, are often challenged by the fact that even the simplest behaviour pattern can be broken down into smaller individual 'behaviours'. For instance, fruitfly courtship behaviour occurs as a sequence of individual behaviours that lead to copulation (FIG. 1). During courtship, flies integrate many olfaction-, mechanosensation- and vision-derived cues, all of which are important components of behaviour. The fact that behaviours seem to be embedded in one another and the fact that they encompass events before their actual performance poses a significant challenge for the behavioural geneticist. In the light of this, how can we determine if a behavioural phenotype is robust enough for genetic analysis?

As with other non-behavioural phenotypes, a relatively simple, easily repeatable and reproducible measure of behaviour is required for genetic screens that often involve thousands of animals[116]. A clear definition of the specific behaviour to be studied must form the basis of any behavioural genetics analysis. Animals of the same age, reproductive condition and experience should be used to minimize developmental contributions to behavioural variation. Furthermore, to maximize differences between strains and to minimize variation within strains, behavioural differences should be rigorously defined in each of the environments under study. Above all, the behaviour being investigated must be defined quantitatively and objectively, and appropriate controls must be carried out to show that it is the behaviour of interest that has been altered by genetic intervention[116].

Undeniably, complex interactions between genes and the environment are often significant in the development and functioning of behaviour. In addition, behavioural phenotypes might be inherently more variable and susceptible to environmental variation than non-behavioural phenotypes because variation in behaviour, unlike that of, for example, the development of an organ, might be subject to fewer developmental constraints. Explaining variation in behaviour as arising from an interdependence between genes and the environment [117], rather than dichotomizing behaviour as innate or learned[38,39], takes much of this complexity into account.

are active when they feed and move about sampling food from various sources, whereas others feed locally[8]. Flies can learn and remember what they have been taught for a significant percentage of their lives, showing all the basic characteristics of mammalian learning and memory[9,10]. Male flies defend their food supply by showing aggressive behaviour[11–13], and females show a simple form of maternal behaviour by choosing an appropriate site to lay their eggs[14]. Flies show sensitivity and tolerance to addictive drugs, such as alcohol[15–17] and cocaine[18,19], and show drug-related behaviours, such as shaking and turning.

During recent years, the molecular mechanisms that underlie some aspects of these behaviours have been revealed by *Drosophila* behavioural geneticists. These discoveries in *Drosophila* have uncovered new genes, proteins and biochemical pathways, and led to discoveries of homologous genes with comparable functions in mammals. Although the mechanisms that underlie mammalian behaviour are more complex than those in the fly, the basic components of such mechanisms are often conserved.

TABLE 1 provides examples of genes that have been identified and cloned for the complex behaviours discussed in this review (that is, foraging, rhythms, courtship, and learning and memory), their expression patterns when known and the nature of their PLEIOTROPIC effects (see supplementary Table 1 online for a more comprehensive version of this table). Other components of *Drosophila* behaviour include olfaction and gustation[20,21] (mediated by olfactory and taste receptors, which have, for example, been discovered using a computer algorithm to find putative receptors in the *Drosophila* genome databases[22–25]), mechanosensation[26,27], optomotor behaviour[28], hearing[29], and sensitivity to ethanol[15,16] and cocaine[18,19]. Normal individual differences in these behaviours have, for the most part, not yet been investigated.

Natural behavioural variants

The natural-variant approach in *Drosophila* behavioural genetics is helping to clarify the nature of the genes and allelic variants that affect normal individual differences in behaviour, how they evolved and how they might differ from laboratory-generated mutants. Why might natural variants be useful tools for behavioural genetics analysis? Because they carry subtle alterations in a gene, such as HYPOMORPHIC MUTATIONS, that probably allow them to survive in nature. By comparison, single-gene mutant studies have shown that when null alleles are generated they often cause pleiotropic effects and so produce unrelated phenotypes (TABLE 1). Instead, natural allelic variants can cause behaviour-specific alterations in an organism, and not other unrelated, pleiotropic phenotypes. If this is true, then it is likely that natural variants will help us to understand how genes affect behavioural processes.

Insights into the genetic and molecular bases of natural variation can be gained from: first, studying naturally occurring behavioural variants[30]; second, studying naturally occurring behavioural variants that carry variations in a gene first identified through mutagenesis[31]; and last, QUANTITATIVE TRAIT LOCUS mapping techniques to analyse strains that differ in their behaviour. This approach is exemplified by the study of natural variation in bristle number in flies[32], which showed that natural variants can be used to find both known, and new, genes and pathways, and that genes identified through mutagenesis can vary in natural populations. These and other data to be discussed below show that the natural variant and single-gene mutant approaches to behavioural genetics are complementary.

Food-related behaviours and the* foraging *gene. The best example of normal variation in behaviour that has been studied genetically and molecularly is that of foraging-related behaviour in *Drosophila*[8,30]. Rover and sitter fruitfly larvae show different patterns of foraging behaviour when searching for food (FIG. 2a). Rovers show longer foraging trails on food and have a greater tendency to leave a patch of food than sitters do. This behavioural difference is shown only in the presence of food.

What lessons can be learned from studies of the rover/sitter variants? Both rovers and sitters should be considered as wild-type phenotypes as they are maintained in nature at appreciable frequencies, with rovers

PLEIOTROPY
The phenomenon in which a single gene is responsible for several distinct and seemingly unrelated phenotypic effects.

HYPOMORPHIC MUTATION
A mutation that does not completely eliminate the wild-type function of a gene and therefore causes a less severe phenotype than a loss-of-function (or null) mutation.

QUANTITATIVE TRAIT LOCUS
A genetic locus that is identified through the statistical analysis of a complex trait. These traits are typically affected by more than one gene and by the environment.

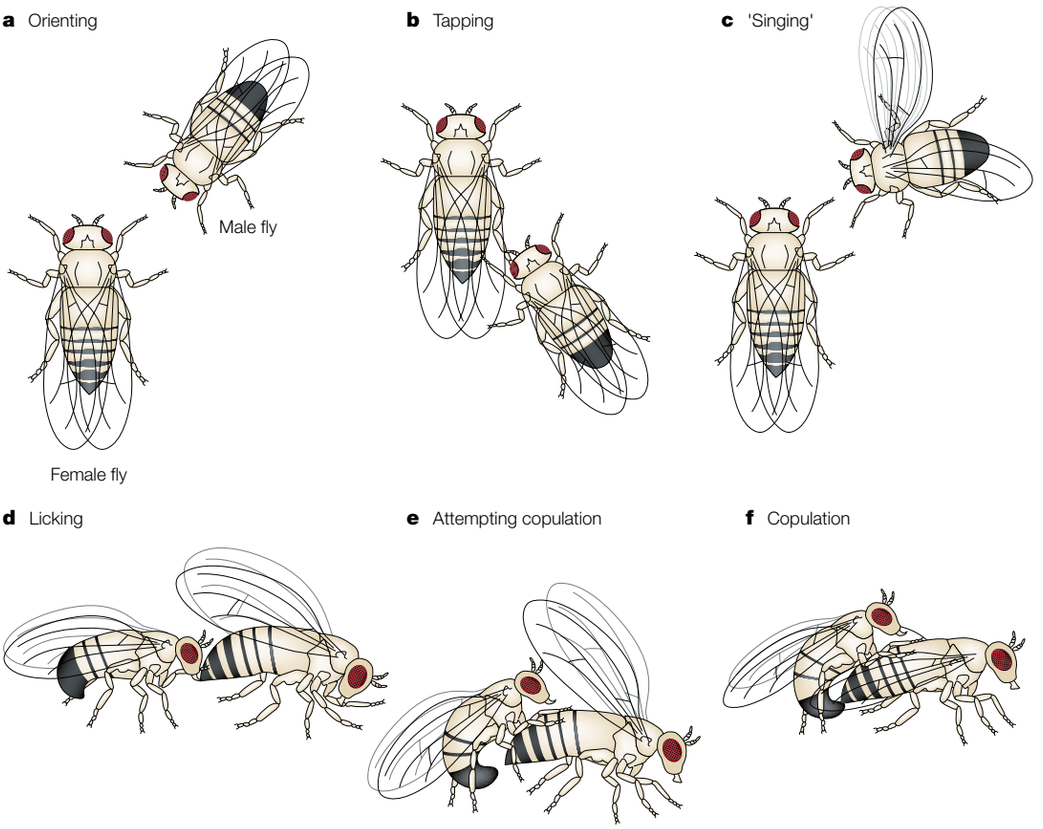

Figure 1 | **Sequence of courtship behaviours shown by *Drosophila melanogaster* males towards females. a** | The male fruitfly orientates towards the female, then follows her, **b** | taps her, and **c** | sings a species-specific courtship song by vibrating one wing. **d** | Finally, he licks the genitalia of the female, and **e** | curls his abdomen in an attempt to copulate with her.

comprising 70% and sitters 30% of the population[33,34]. These normal individual differences in behaviour are explained by variation in a single gene called *foraging* (*for*), the rover (*for*^{R}) allele of which is genetically dominant to the sitter (*for*^{s}) allele[35,36]. The bimodality of the foraging trail lengths (FIG. 2b) indicates that both forager types might be maintained by natural selection, and we have shown that density-dependent selection can shift *for* allelic frequencies such that rovers are selected for in crowded larval environments and sitters in less crowded ones[34].

The rover/sitter forager variants also show the lack of a relationship between HERITABILITY and PHENOTYPIC PLASTICITY. These normal behavioural patterns, although 'genetically based', are plastic, and can be modified by the internal and external environment of the fly. Larval and adult flies with rover alleles can be made to behave as sitters after a short period of food deprivation[8,37]. Conversely, those with sitter alleles can be made to behave like rovers by altering other environmental parameters (M. Suster and M.B.S., unpublished observations). So, both intrinsic and extrinsic factors influence the expression of rover/sitter behaviour. This example shows that it is inappropriate to suggest that genes determine or code for a behaviour, or that innate behaviours are inherently less variable than those that are learned[38,39].

The genetic basis of behaviour is often complex. The naturally occurring rover/sitter trait is inherited as a single major gene (*for*) that is influenced by minor genes, which have smaller effects on the phenotype than *for*[35]. The cloning of *for*[36,40,41] showed that it is an essential gene that has pleiotropic effects, and that it functions in development and behaviour. It encodes a cGMP-dependent protein kinase (PKG), and its cloning provided the first piece of evidence that food-related behaviours involve cGMP signalling in flies[41]. The *for* gene spans more than 40 kb and is alternatively spliced to produce three major transcripts (T1–T3) and several minor ones. T1–T3 are found throughout development, but their abundance is developmentally regulated, and their functions are now under investigation.

Another important finding from this work is that very subtle differences in PKG enzyme activity and in transcript abundance account for the behavioural differences between the foraging variants. Rovers have only a 12% increase in PKG enzyme activity in their heads compared with sitters. If only a few cells are responsible for this difference, then the percentage difference in PKG in these cells could be quite high. Alternatively, a small percentage difference might be sufficient to generate these normal differences in behaviour — for example, if different activity levels were to determine the substrate of the enzyme. These subtle differences at the

HERITABILITY
The fraction of the phenotypic variance that is due to additive genetic variance.

PHENOTYPIC PLASTICITY
The modifiability of the phenotype by the environment.

Table 1 | **Examples of cloned genes that influence complex behaviour**

Behavioural category Gene; synonym	Molecular function	Expression pattern	Behavioural pleiotropy	Developmental pleiotropy	Reference
Circadian rhythm					
period (*per*)	Transcription cofactor	BN and NN	Locomotor rhythms, eclosion rhythms, courtship, cocaine sensitivity, others		7
timeless (*tim*)	Interacts with Per	BN and NN	Locomotor rhythms, eclosion rhythms, sleep		7
double-time (*dbt*); *discs overgrown* (*dco*)	Casein kinase I	BN and NN	Locomotor rhythms, sleep, cocaine sensitivity	Imaginal disc overgrowth, pupal lethality	7
Clock (*Clk*); *jrk*	Transcription factor	U	Locomotor rhythms, eclosion rhythms, cocaine sensitivity		7
cycle (*cyc*); *Mop3*	Transcription factor	N	Locomotor rhythms, eclosion rhythms, cocaine sensitivity		7
cryptochrome (*cry*)	Homology with blue-light-sensitive DNA-repair enzymes	BN and NN	Resetting of behavioural rhythms		7
Pigment-dispersing factor (*Pdf*)	Neuropeptide hormone	N	Locomotor rhythms		7
disconnected (*disco*)	Transcription factor	N and NN	Locomotor rhythms, eclosion rhythms	Visual system defect	7
cAMP-dependent protein kinase II (*pka-RII*)	Protein kinase	N	Locomotor rhythms, cocaine sensitivity, ethanol sensitivity	Ovary development	18
Courtship					
fruitless (*fru*)	Transcription factor	N and NN	All aspects of male courtship	Abnormal muscle of Lawrence	3
doublesex (*dsx*)	Transcription factor	N and NN	Song defect	Sterility, abnormal yolk protein production	3
dissatisfaction (*dsf*)	Steroid hormone receptor	N and NN	Poor sex discrimination, reduced female receptiveness	Slow copulation, no voluntary egg laying	3
courtless (*crl*)	Ubiquitin-conjugating enzyme	N and NN	Failure to court	Male sterile	3
slowpoke	Calcium-activated potassium channel	N and NN	Song defect	Flight defect	3
cacophony (*cac*); *nightblind A* (*nbA*)	Voltage-sensitive calcium channel	N and NN	Song defect, optomotor behaviour, photophobic	Phototransduction	121
dissonance (*diss*); *no on-or-off transient* (*nonA*)	RNA binding	U	Song defect, optomotor behaviour	Phototaxis	121
Learning and memory					
dunce (*dnc*)	cAMP-specific phosphodiesterase	U	Locomotor rhythms, ethanol tolerance	Female sterility, decreased female longevity	10
rutabaga (*rut*)	Adenylate cyclase	U	Courtship learning, ethanol tolerance, grooming		10
amnesiac (*amn*); *cheapdate* (*chpd*)	Neuropeptide	N	Ethanol tolerance	Decreased heart rate	10
latheo (*lat*)	DNA-replication factor	N	Larval feeding	Pupal lethality	10
Shaker (*Sh*)	Voltage-sensitive potassium channel	N	Courtship suppression, gustation defect, ether sensitivity	Decreased longevity	9
G protein s α 60A (*G-s α 60A*)	Heterotrimeric G protein	N and NN	Visual behaviour, cocaine sensitivity	Larval/pupal lethality	10
DCO; *cAMP-dependent protein kinase 1* (*Pka-C1*)	Protein Ser/Thr kinase	N and NN	Locomotor rhythms, ethanol tolerance	Female sterility, wing/eye/leg morphogenesis defect	10
cAMP-response-element-binding protein B at 17A (*CrebB17A*); *dCREB*	Transcription factor	U	Locomotor rhythms	Larval lethality	10
Calcium/calmodulin-dependent protein kinase II (*CaMKII*)	Protein Ser/Thr kinase	N	Courtship suppression	NMJ branching defect	10
Neurofibromatosis 1 (*Nf1*)	Ras GTPase activator	U		Growth defect	10
Feeding/foraging					
foraging (*for*); *dg2*	cGMP-dependent protein kinase	N and NN	Rover and sitter morphs	Pupal lethality, hypoxia recovery	41

BN, broad neural; N, neural; NMJ, neuromuscular junction; NN, non neural; PCD, programmed cell death; U, ubiquitous.

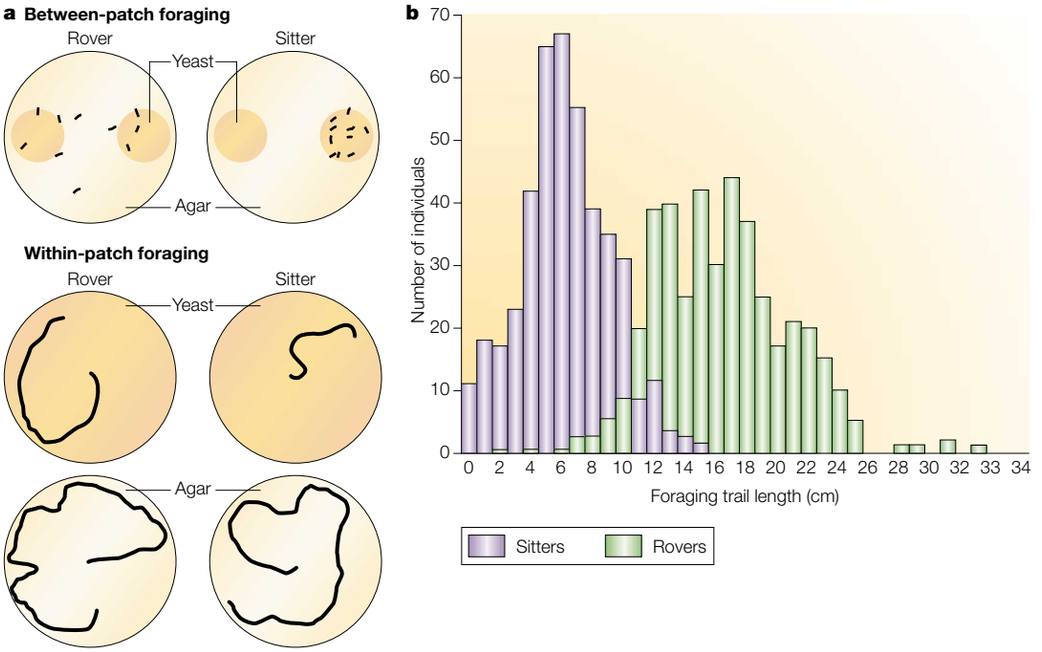

Figure 2 | **Sitter and rover foraging behaviour. a** | A quick assay for rover/sitter behaviour in *Drosophila melanogaster* larvae that uses a food source (a patch of yeast and water paste) on an agar plate. In between-patch foraging, it is highly probable that rover larvae will leave a patch of food, whereas sitter larvae will move to the nearest food patch and remain feeding on it. Within a food patch, it is possible to measure the distance a larva travels (foraging trail length) on a yeast patch in 5 min. Rover/sitter behaviour is conditional on the presence of food in the environment[8], because in its absence (on agar), both rover and sitter larvae move equally rapidly, showing that sitter behaviour does not arise through a general sluggishness in crawling behaviour. **b** | A minimal overlap in foraging trail lengths between the variants show that rover and sitter are discrete categories of foraging behaviour. By measuring this trait, larvae of unknown genotype can be classified as being either rover or sitter.

molecular level might be representative of how behaviour is modulated in natural populations.

Two *Caenorhabditis elegans* variants show many similarities to the rover/sitter behaviours in flies[42]. Some worms forage like rovers, whereas others resemble sitters[30]. Individual differences in *C. elegans* foraging behaviour result from variation in the coding region of the *npr-1* gene, a homologue of a mammalian neuropeptide Y receptor that is known to be involved in the regulation of food intake[43]. These findings indicate that *C. elegans* and *Drosophila* could be developed as genetic models for studying food-related behaviours in mammals. Finally, the finding of rover and sitter natural variants in flies and worms provides us with an ecologically and evolutionarily relevant model with which to ask how natural selection acts on principal signal-transduction pathways to produce related behaviour variants in very different species.

Fly behavioural mutants

In 1961, the first published study on *Drosophila* behavioural genetics used artificial selection on natural populations to alter the upward or downward movement of normal flies walking in a vertical maze[44]. The polygenes involved in this behaviour could not be localized at the time owing to the limited tools available. Seymour Benzer provided the field of *Drosophila* behavioural genetics with the single-gene mutant approach[45]. He reasoned that normal or 'wild-type' versions of the genes that influence specific behaviours could be mutated one gene at a time, and that the resulting behavioural effect would shed light on the function of the mutated gene. Indeed, the single-gene mutant approach has allowed *Drosophila* researchers to significantly advance our understanding of the mechanisms that underlie many neurobiological and behavioural phenotypes, including circadian rhythms, learning and courtship. Overexpression studies in transgenic flies have also provided insight into human neurological disorders, such as Parkinson disease and Huntington disease[46].

Circadian rhythms

Major breakthroughs in our understanding of the molecular basis of biological rhythms have come from studies in *Drosophila*. Genetic screens have isolated flies that show alterations in two outputs of the circadian clock: ECLOSION RHYTHMS and the activity–rest cycle (FIG. 3). Some genes involved in clock function affect both measures of circadian rhythms, whereas others affect only one.

The *period* (*per*) gene, discovered by Ron Konopka and Benzer in 1971 (REF. 47), was the first *bona fide* clock gene found in any organism. Three original mutations in *per* caused the lengthening (per^{l}), shortening (per^{s}) and arrythmicity (per^{0}) of the period of the rhythm. The discovery that mutations in a single gene, *per*, could alter circadian behaviour was the first step towards a

ECLOSION RHYTHM
The timing of the emergence of the adult fly from its pupal case, which usually occurs at dawn.

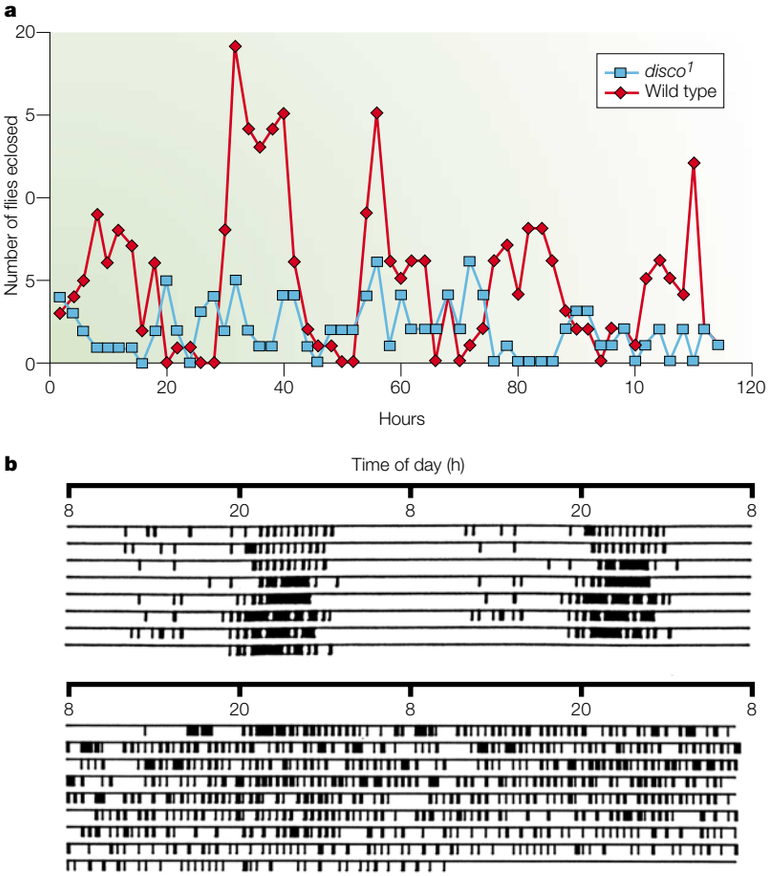

Figure 3 | **Eclosion and circadian rhythms in flies.** The *disconnected* (*disco*) gene encodes a transcription factor, which when mutated affects both eclosion and locomotor activity rhythms, as do mutants alleles of *per* and *tim*. **a** | The eclosion activity rhythms of wild-type and mutant (*disco*^{1}) flies under constant darkness (free-running conditions). Like null *per* mutants, *disco* mutant flies show arrhythmic eclosion patterns compared with wild-type flies, which emerge around every 24 h. **b** | The locomotor activity rhythms of wild-type (upper) and *disco* mutant (lower) flies under constant darkness. The locomotor activity of *disco* mutant flies is arrhythmic compared with the ~24-h free-running rhythm of wild-type flies. Dark bars show periods of activity. (Figures kindly provided by and modified with permission from Jeff Hall, Department of Biology, University of Brandeis, Massachusetts, USA.)

molecular analysis of circadian rhythms. Mutations in a second clock gene, *timeless* (*tim*) were found to produce both a strong behavioural phenotype and an effect on *per* expression[48–50]. The cloning and subsequent sequence analysis of *per* and *tim* did not hint at their biochemical functions at that time as both were new genes. However, the molecular analysis of *per* and *tim* showed that they are regulated in a cyclic manner. Both genes are transcribed early in the day, but the highest levels of their mRNAs are found late in the day and into the beginning of the night[51–53]. During the night, the Per and Tim proteins accumulate and form a heterodimer that moves into the nucleus to bind the transcription factors Clock (Clk) and Cycle (Cyc). This binding to Clk and Cyc prevents Clk and Cyc from binding to the promoters of *per* and *tim*, which results in the transcriptional repression of *per* and *tim*. Late in the night and early in the morning, Tim and Per, respectively, degrade, so facilitating the subsequent rise in the RNA levels of both genes. Mutations in *per* and *tim* affect this feedback loop in a way that is consistent with alterations in behaviour. For example, in the *per*^{l} mutant, the affinity of the Per–Tim interaction is decreased, such that the entry of the heterodimer into the nucleus is delayed, causing an extension of the cycle[54,55]. The *per*^{s} mutant seems to speed up the daily disappearance of Per, perhaps by decreasing its stability, causing a shortening of the cycle[56]. Although *per* and *tim* are expressed widely in the organism, *per* expression alone in the lateral neurons of the brain rescues the behavioural rhythm[57]. MOSAIC ANALYSIS has shown that expression of *per* in these cells results in strong circadian rhythms[58] and that the ablation of the lateral neurons causes a loss of rhythmicity[59].

The molecular mechanisms underlying clock function that were first discovered in *Drosophila* are now known to underlie circadian rhythms in many other species, including vertebrates. Familial advanced sleep phase syndrome (FASPS), an autosomal-dominant circadian rhythm variant in human populations, occurs in individuals who are described as 'morning larks'[60]. These individuals show a 4-h advance of their sleep–wake cycle. Positional mapping of the FASPS phenotype shows that alterations in the human homologue of the fly *per* gene (*PER2*) cause this syndrome in some families. Individuals who are affected with FASPS have a serine-to-glycine mutation in the casein kinase I (CKI)-binding region of *PER2*. CKI is known to be involved in *Drosophila* circadian rhythms; some mutant alleles of *double-time* (*dbt*) (a *Drosophila* CKI) cause a shortened rhythm in flies[61]. Studies of *Drosophila per* have therefore allowed researchers to predict the function of human *PER* homologues, and further analyses have shown that this function is conserved across phyla. So, the genetic analysis of rhythm behaviour in *Drosophila* has provided us with a molecular basis for understanding biorhythms in all organisms.

Courtship and the *fruitless* gene

Drosophila male courtship behaviour is species specific. As described above, *D. melanogaster* show several steps in their courtship behaviour (FIG. 1), and different regions of the nervous system have roles in the manifestation of these steps[62,63].

The *fruitless* (*fru*) gene was originally identified on the basis of the aberrant courtship behaviour shown by *fru* mutant *D. melanogaster* males. These males do not distinguish between male and female flies while courting and, when housed together, form mating chains that result from males following each other while showing courtship behaviour[62,64]. The *fru* gene does not seem to affect female behaviour, general locomotion or wing usage in males[65]. It is a member of the *Drosopila* sex-determination cascade[66–68] and belongs to the BTB-zinc-finger family of transcription factors; however, *fru* transcriptional targets have yet to be identified. The *fru* gene is a complex locus that spans ~130 kb. It has four promoters (P1–P4), but only the pre-mRNA from the P1 *fru* promoter is spliced in a sex-specific manner[67] (FIG. 4), and P2–P4 have no sex-specific functions[64,66,69]. As is the case

MOSAIC ANALYSIS
The process of following the progenitors of a single cell (a clone). Clonal analysis can be used to infer several things, such as when gene action takes place and if lineage has a role in cell-fate determination.

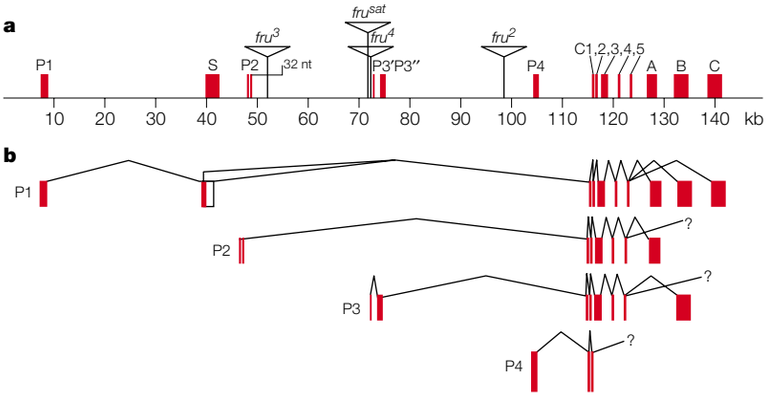

Figure 4 | **Genomic and transcript map of the *fru* locus. a** | The *fru* genomic region, showing *fru* exons, the promoters P1–P4 and *fru* mutations that affect splicing. Insertion sites that give rise to several *fru* mutant alleles (fru^3, fru^2 and fru^{sat}) are shown. These mutant alleles have helped to determine which combinations of transcripts are important for viability. The size of the promoters can be seen from the scale (in kilobases), with P2 being only 32 nucleotides (nt) in length. A, B and C are three alternatively used exons found in P1-derived transcripts. It is not known whether these exons are present in the other promoter-derived transcripts. **b** | Transcripts from the P1 promoter are spliced in a sex-specific manner compared with transcripts from the other promoters, which are non sex specific. The common exons (C1–C5) encode the BTB domain, which is thought to be involved in protein dimerization. Male-specific transcripts from the P1 promoter add 101 amino acids to the amino terminus of the BTB domain, compared with transcripts from the P4 female-specific promoter and to those from the P2, P3 and male-specific P4 promoters. (Reproduced with permission from REF. 63 © (2001) Elsevier Science.)

with *for*, both viable and lethal *fru* mutations exist; the viable alleles alter male-specific courtship functions. The *fru* gene also has pleiotropic effects and is responsible for a range of phenotypes, some of which affect male courtship behaviour. A particular set of Fru proteins that are generated from transcripts associated with the P1 promoter are responsible for the role of *fru* in courtship[63], and *fru* seems to be required for almost all steps in the courtship behaviour[67]. Severe viable alleles of *fru*, generated by transposable element insertions, almost completely abolish courtship behaviour and cause aberrant splicing of the sex-specific transcripts[64]. A weaker insertion allele, fru^2 (FIG. 4), produces a different aberrant splicing pattern and results in problems with song production[65]. Another viable *fru* mutant stops the courtship sequence at an even later step, during sperm transfer[69], and another, fru^1, results from an inversion that alters the spatial pattern of gene expression in the brain[64,69].

The male-specific *fru* gene transcripts are expressed in the central nervous system (CNS)[64,67,69], with the strongest expression found during the pupal period when it is thought that the circuitry important for male sexual behaviour is built into the CNS[70,71]. Approximately 20 groups of cells that express the male-specific *fru* gene transcripts are distributed throughout the CNS, in both the brain and the ventral nerve cord[69]. This expression pattern indicates that the cells that express *fru* transcripts are not localized within one substructure. Targeted expression of the male-specific *fru* transcripts in a *fru* mutant background should uncover where and when wild-type *fru* needs to be expressed for normal male courtship behaviour. On the basis of the studies of *fru*, Bruce Baker *et al.*[63] speculated that male sexual behaviour arises as sensory information is transmitted to the CNS through non-sex-specific sensory systems. This information is then processed by the higher-order neuropil (the cells that express *fru*) in a sex-specific manner using the putative sex-specific circuitry in which *fru* acts. Subsequently, sexual behaviour is possibly initiated through non-sex-specific motor systems. Identification of the circuit that *fru*-expressing neurons might form is one of the next challenges for *fru* researchers.

Although courtship behaviours are stereotyped (*fru* is important in what has been called the 'specification' of courtship behaviour[63]), male courtship behaviour also has plastic components that have been studied from the viewpoint of learning and memory. Males show a suppression in courtship that can last for 3 h after encountering a mated female. Courtship suppression has similar properties to ASSOCIATIVE LEARNING[72]. Mutations in genes, such as *dunce* (*dnc*), *rutabaga* (*rut*) and *amnesiac* (*amn*), that affect cAMP signalling and that disrupt olfactory-based avoidance associative learning[73] also alter courtship suppression in *Drosophila*[74,75]. Modest reductions in calcium/calmodulin-dependent protein kinase II (CaMKII) and protein kinase C (PKC), two kinases known to be involved in neuronal plasticity, alter different aspects of courtship suppression[76–78]. So, different signal-transduction pathways have overlapping roles in the experience-dependent modification of courtship behaviour.

Learning and memory

Drosophila can learn and remember[9,73]. The genetic dissection of learning and memory in the fruitfly was initiated in the Benzer lab[79] using an OLFACTORY-BASED SHOCK-AVOIDANCE LEARNING procedure that was used to isolate *dnc*, the first single-gene mutant for associative learning. *dnc* mutants have abnormally high levels of cAMP because they lack phosphodiesterase, which degrades cAMP. Importantly, there is evidence that cAMP signalling modulates synaptic structural and functional plasticity, which is thought, in part, to underlie learning and memory[80,81]. *dnc*, like *for* and *fru*, is a large, complex locus that has several transcripts and pleiotropic effects during development and adulthood. It is expressed widely in the nervous system during these stages, and some mutant alleles of *dnc* cause alterations in the structure of MUSHROOM BODIES, which are required for olfactory-based shock-avoidance learning[82]. A model for this type of learning in *Drosophila* mushroom body neurons is shown in FIG. 5.

Evidence is accumulating that the mushroom bodies have a crucial role in olfactory-based shock-avoidance learning and memory formation in *Drosophila*. The chemical ablation of adult mushroom bodies abolishes shock-avoidance olfactory learning[82], and mutants with structural abnormalities in the mushroom bodies show olfactory learning defects. Disruption of cAMP signalling in the mushroom bodies abolishes olfactory learning[83], whereas restoration of normal *rut* function in the mushroom bodies of *rut* mutant flies restores learning[84]. Finally, disruption of neurotransmission in *Drosophila* mushroom bodies blocks the retrieval, but not the acquisition, of memory[85].

ASSOCIATIVE LEARNING
A form of learning whereby the subject learns about the relationship between two stimuli, or between a stimulus and a behaviour.

OLFACTORY-BASED SHOCK-AVOIDANCE LEARNING
A learning model whereby a shock is paired with one of two olfactory stimuli offered to the animal, so that the animal learns to avoid the stimulus paired with the shock in a subsequent choice test that does not include a shock.

MUSHROOM BODIES
Two prominent bilaterally symmetrical structures in the fly brain that are crucial for olfactory learning and memory.

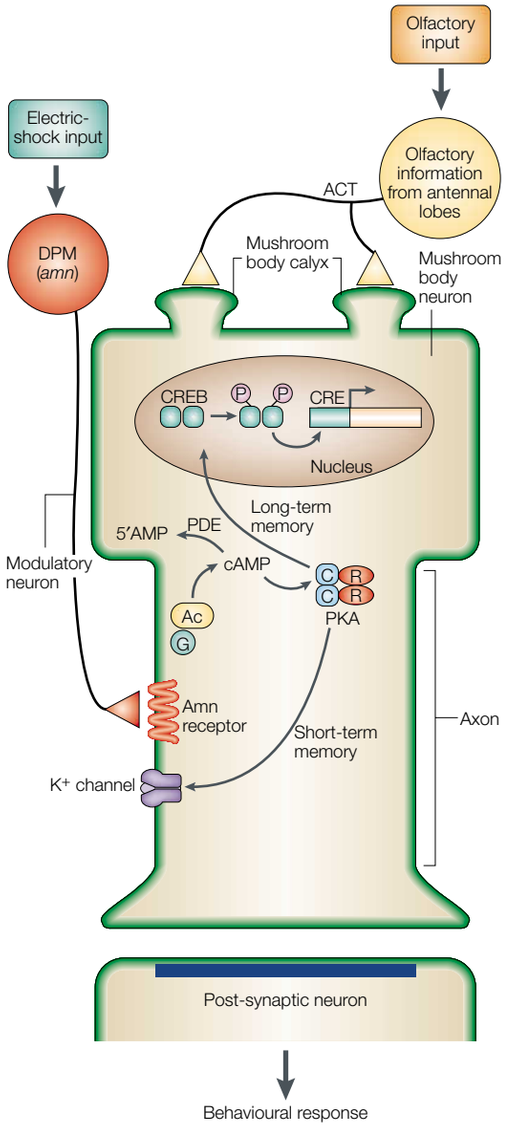

Figure 5 | **A model for olfactory-based shock-avoidance learning in *Drosophila* mushroom body neurons.** A mushroom body neuron gets olfactory information from: first, the antennal lobes through ACT (antennoglomerular tract) interneurons that synapse with the calyx of a mushroom body neuron; and second, from DPM (dorsally paired medial neurons), which release the amnesic (Amn) neuropeptide into modulatory neurons after the delivery of an electric shock to the fly. The axons of the DPM neurons, in which *amn* is expressed, are thought to synapse onto mushroom body axons to cause the release of putative modulatory neuropeptides. The simultaneous activity of these two pathways causes the stimulation of adenylate cyclase (Ac) — encoded by *rutabaga* (*rut*) — which is principally expressed in the axons and axon terminals of mushroom body neurons. The stimulated Ac then activates a G-protein-coupled receptor (G), which causes elevated cAMP levels. The increase in cAMP gives rise to either a short-lived change in the excitability of the mushroom body neuron (short-term memory) or a long-lasting change (long-term memory). The *dunce*-encoded cAMP phosphodiesterase (PDE) and the catalytic (C) and regulatory (R) subunits of protein kinase A (PKA) are among several genes that are preferentially expressed in mushroom body neurons. When PKA is activated for a short period of time, it is thought that downstream changes in the K^+ channels of the axon affect output from the post-synaptic neuron. Post-translational modifications and changes in gene expression thought to be involved in long-term memory occur partly through the phosphorylation of the transcriptional activator CREB by PKA, which then, in turn, binds to cAMP-responsive elements (CRE) that are located in the upstream regions of cAMP-inducible genes. P, phosphorylation. (Modified with permission from REFS 10,120).

Many other genes that affect learning and memory have been identified using diverse genetic approaches (see supplementary Table 1 online). Together, these mutants have been used to dissect the biochemical pathways that are involved in learning and memory phases similar to those that occur in other organisms, including *Aplysia* and mammals[86–88]. Additional FORWARD GENETIC screens in *Drosophila* should identify new genes and pathways that influence learning and memory.

Some flies with genetic alterations can learn but have difficulty remembering. Normal flies can remember what they have been taught for more than a week. A 40% decrease in the level of the catalytic subunit of PKA causes mild effects on learning but not on memory. By comparison, an 80% reduction in activity results in significant learning and memory deficits, indicating that the level of the catalytic subunit of PKA can affect the severity of this defect[89,90]. Mutations in the *amn* gene affect memory retention but not acquisition; *amn* flies can learn but they forget what they have learned after 1 h (REFS 9,91). The predicted gene product of *amn* is a preproneuropeptide that is thought to stimulate cAMP synthesis[16,92]. The *amn* gene is highly expressed in two large neurons that seem to project over the lobes of the mushroom bodies[93,94]. Overexpression of the CREB repressor selectively blocks long-term memory, but leaves learning and short-term memory intact. By contrast, overexpression of the CREB activator produces long-term memory even when the flies are only trained using a short-term memory procedure[95,96].

Starting with the discovery of *dnc* and *rut*, studies of learning and memory in *Drosophila* have uncovered the importance of the cAMP signalling pathway for olfactory-based shock-avoidance learning and memory. The functions of the mammalian homologues of these *Drosophila* genes have also been investigated (for example, see REFS 97,98). The identification, using forward genetic screens, of new genes that influence learning and memory will undoubtedly lead to the identification of new genes and pathways of relevance to learning and memory in many organisms.

Looking to the future

D. melanogaster has been successfully used to identify genes that affect complex behaviours, many of which can be directly related to their mammalian counterparts. But is *Drosophila* more than just a means to identify genes and pathways? What general principles about the genetic control of behaviour have emerged from this field in the past decade? What further questions remain, and how can they be pursued in the future both experimentally and theoretically?

Are there behaviour-specific genes? Some behavioural geneticists use the term 'behavioural gene' as a shortcut for saying "a gene that influences the expression of a behaviour pattern". This term is misleading because it implies that the gene is dedicated to its behavioural function alone. In most cases, this is highly unlikely because genes that have a role in behaviour almost always have pleiotropic effects[28,99,100] (TABLE 1).

Box 2 | **Genetic background and behavioural phenotypes**

The increasing awareness that several genes influence behaviour has prompted a re-examination of what geneticists call wild-type or normal behaviour. As has been reported for the mouse, wild-type flies differ in many developmental, morphological and behavioural characteristics[102]. The complex interactions between the genes that underlie a behavioural phenotype can be shown by the effect of genetic background on the phenotype. Genetic background effects can be gene or phenotype specific. For example, circadian phenotypes associated with the *per*^{s} (short period) allele can be susceptible to genetic background changes[118]. Learning is strongly affected by genetic background, such that some genetic backgrounds can alleviate the defects in learning caused by a mutation[119]. In addition, learning mutants kept in the lab for many years can 'lose' their phenotype, showing relatively normal learning scores. However, the phenotype can reappear once the line is outcrossed to the original genetic background[119]. Presumably, the learning phenotype is lost because of natural selection for genes that modify the phenotype in that genetic background, indicating that a mutation in a learning-associated gene is deleterious, even under laboratory conditions. The existence of such genetic background effects indicates that behavioural phenotypes are highly sensitive to interacting networks of genes and environments throughout development and adulthood.

According to Baker and colleagues[63], an exception to this might be *fru* (specifically, the sex-specific transcripts of *fru* that are associated with the P1 promoter), as they argue that *fru* exemplifies an, as yet undiscovered, group of highly dedicated regulatory genes that specify behaviour in *Drosophila*. However, *fru* might be a highly dedicated regulatory gene because of its role in the sex-determination pathway and not because of its function in behaviour. Corina Schutt and Rolf Nothiger[101] argue that sex-determination systems in Diptera evolve more rapidly than in other gene systems in general, and that the gene system that controls sex determination in *Drosophila* is uncharacteristically rigid. As a result, we might expect components of this system (for example, the Fru proteins that affect courtship behaviour) to be inherently less variable, and thus less modifiable by the environment, than other genes that influence behaviour. If true, this would make *fru* an interesting exception to the idea that there are no behaviour-specific genes, transcripts or promoters.

The 'behavioural genes' terminology should not imply that a gene controls or determines behaviour. Rather, genes influence the development and functioning of normal behaviour patterns by contributing to the development of the parts of the nervous system that are required for the performance of an adult behaviour. Genes can also have a role in the performance of the adult behaviour or they might be involved in both the development and functioning of behaviour. By analysing natural variants and mutants, genes involved in any or all of these processes will be uncovered.

Many of the genes that influence behaviour were discovered in behavioural screens for mutants that show alterations in a behaviour of interest. Further investigations into these genes uncovered that all of them have pleiotropic effects and several functions in both development and behaviour, or in several behaviours (see supplementary Table 1 online). Why is there no gene the sole function of which is behaviour? One rather simple explanation might be that the gene is not the appropriate unit with which to explain the relationship between genes and behaviour, because changes to the regulatory components of a gene, such as to its promoter or splice sites, might be what influence behavioural variation in laboratory[100] and natural populations. According to this idea, selective pressures would act on enhancers or splice variants to modify the development or activity of circuits that underlie behaviour. This idea is supported by examples such as *fru*, where a promoter-specific transcript (P1) makes a unique contribution to the specification of neural circuits for courtship behaviour. The ratio of transcripts might also have a role in generating behaviour. For example, one transcript might be important for the behaviour, but when its abundance is severely reduced — for example, by mutation, a lack of negative feedback might allow the increased expression of another transcript, such that a normal behavioural pattern can still be produced. This compensation might be common in behavioural systems and might explain why knockouts of genes thought to be important in behaviour sometimes have no behavioural effect[102]. (Redundancy from other genes is also sometimes given as an explanation for this finding; however, evidence is lacking as to whether compensation or redundancy adequately explain the results.) These compensatory actions might be a characteristic of the interacting gene networks that are found in behavioural systems, and might also depend on EPISTATIC interactions that occur on different genetic backgrounds[103] (BOX 2). These ideas should be thoroughly tested in the future as new behavioural mutants and the corresponding genes are discovered.

Subtle mutations: tools for analysing behaviour. How can we meaningfully analyse the effect of alterations in a single gene on the performance of a behaviour when we know that most genes have pleiotropic functions? When a gene known to influence behaviour is knocked out or inactivated, disruptions to several biological functions can result that reflect a role for this gene in both development and behaviour. Many genes that alter behaviour are essential genes (such as *for*[41], *scribbler* (*sbb*), which is involved in *Drosophila* larval turning behaviour[104], *fru*[67], and *latheo* (*lat*)[105], which is involved in learning). It is notable that more subtle gene alterations, such as hypomorphic mutations, often show only the behavioural alteration and not other unrelated phenotypes, and are more likely to reflect the subtler genetic influences on behaviour that occur in nature[106]. These milder mutations, and the ability to target the expression of a gene to certain tissues in the organism during development (for example, by using the GAL4/UAS SYSTEM), should allow us to disentangle the role of a gene in development from its role in the behaviour itself. For instance, variants that produce a partial loss of function in kinases, such as PKA and CaMKII (a 10–20% reduction in kinase level), cause changes in fly behaviour that are specific to learning and memory, whereas severe mutations in these genes are lethal[106]. Even though these kinases are involved in many biological processes, a subtle change in kinase activity exerts a potent effect on the behavioural

FORWARD GENETICS
A genetic analysis that proceeds from phenotype to genotype by positional cloning or candidate-gene analysis.

EPISTASIS
An interaction between non-allelic genes, such that one gene masks or interferes with the expression of the other gene.

GAL4/UAS SYSTEM
Used in *Drosophila* to target the expression of specific genes to specific tissues. UAS stands for the upstream-activating system of the yeast *GAL4* gene.

phenotype, presumably by acting in specific cells at specific times. These data indicate that, in the future, subtle hypomorphic mutants, rather than severe alleles, should be used for more detailed studies of gene function in behaviour. The analysis of natural variants and screens for suppressors targeted to specific tissues should reveal more about the genes and pathways that are important to individual differences in behaviour.

Genes act through neuronal networks. To understand how genes contribute to behaviour, we must identify and characterize the units of behavioural function: the neuronal networks that produce movements and organize them into the appropriate temporal and spatial patterns that characterize a given behaviour. So far, physiological studies of behaviour in *Drosophila* have shown the usefulness of the fly as a model system for synapse function, but it is not clear from the results of these studies whether they can be used as a basis for understanding the central neuronal networks that are crucial to many complex behaviours.

Ideally, with respect to complex behaviours, we would like to be able to measure synaptic properties from central nervous systems in mutants with altered behaviour; however, these measurements are very difficult to obtain in *Drosophila*. The *Drosophila* larval neuromuscular junction (NMJ) has been used to investigate the links between synaptic development and synaptic properties, to make inferences about adult behaviour. For example, there is increasing evidence that cAMP signalling modulates the synaptic structural and functional plasticity that is thought to partly underlie learning and memory[80,81,107]. Many *Drosophila* learning mutants show activity-dependent alterations in synaptic function at the NMJ (for example, *dnc* mutants show both increased neuronal activity and synaptic arborizations). In addition, rover and sitter larval variants differ in their synaptic physiology in a manner that seems to be correlated with their behaviour[108,109]. However, these physiological studies at the NMJ are, at best, correlated with the behavioural variation under study.

Only a few neural circuits have been characterized in *Drosophila,* and of these, all reflect relatively simple behaviour patterns, such as the flight-related reflex circuit and the leg resistance reflex circuit[110]. The advantage of these simple neural circuits is that they consist of relatively few neurons that are identifiable. By contrast, the neural substrates involved in more complex behaviours, such as courtship or circadian rhythms, are comparably large and widespread, as discussed above. Consequently, the neural circuits for the complex behaviours discussed in this review have not yet been characterized because this is a difficult task, given the number of overlapping circuits that underlie complex behaviours. The *GAL4*/UAS expression system[111] is a potentially powerful tool for identifying such neural circuits as it allows toxins, such as tetanus toxin[112], to be targeted to specific neurons during development, such that the subsequent effects on synaptic activity, for example, can be measured[110,113]. Furthermore, the advent of new intact or dissociated physiological preparations for physiologically assaying larval[114] or adult[115] neuronal networks should help to address gene function in specific motor circuits, to link ultimately a gene or its subcomponents (for example, transcripts and splice variants) to the actual performance of a behaviour.

Although there are many unknowns in the puzzle, the powerful array of genetic techniques in *Drosophila* and its well-established place as a model organism in developmental genetics, provides researchers with a unique opportunity. That opportunity is to create a comprehensive picture of how continuing interactions between the genome and environment select for naturally occurring mutations that can act on specific neuronal networks to modify the development and/or functioning of behaviour. No doubt, *Drosophila*, with its array of genetic resources and behaviourally based screens to identify new genes involved in conserved pathways, is at the forefront of behavioural genetics analysis. Undeniably, this little fly will continue to provide us with many tantalizing discoveries.

1. Nusslein-Volhard, C., Frohnhofer, H. G. & Lehman, R. Determination of anteroposterior polarity in *Drosophila*. *Science* **238**, 1675–1681 (1987).
2. Adams, M. D. *et al.* The genome sequence of *Drosophila melanogaster*. *Science* **287**, 2185–2195 (2000).
3. Greenspan, R. J. & Ferveur, J.-F. Courtship in *Drosophila*. *Annu. Rev. Genet.* **34**, 205–232 (2000).
4. Yamamoto, D., Jallon, J. M. & Komatsu, A. Genetic dissection of sexual behavior in *Drosophila melanogaster*. *Annu. Rev. Entomol*. **42**, 551–585 (1997).
5. Hendricks, J. C. *et al.* Rest in *Drosophila* is a sleep-like state. *Neuron* **25**, 129–138 (2000).
6. Shaw, P. J., Cirelli, C., Greenspan, R. J. & Tononi, G. Correlates of sleep and waking in *Drosophila melanogaster*. *Science* **287**, 1834–1837 (2000).
References 5 and 6 indicate that *Drosophila* show many of the characteristics of the mammalian states of sleep and waking, allowing the fly to be used as a model for the genetic analysis of activity–rest cycles.
7. Williams, J. A. & Seghal, A. Molecular components of the circadian system in *Drosophila*. *Annu. Rev. Physiol.* **63**, 729–755 (2001).
8. Sokolowski, M. B. & Riedl, C. in *Molecular-Genetic Techniques for Brain and Behaviour* (eds Gerlai, R. & Crusio, W.) 517–532 (Elsevier, Amsterdam, 1999).
9. Dubnau, J. & Tully, T. Gene discovery in *Drosophila*: new insights for learning and memory. *Annu. Rev. Neurosci*. **21**, 407–444 (1998).
10. Waddell, S. & Quinn, W. G. Flies, genes, and learning. *Annu. Rev. Neurosci.* **24**, 1283–1309 (2001).
11. Jacobs, M. E. Influence of β-alanine on mating and territorialism in *Drosophila melanogaster*. *Behav. Genet*. **8**, 487–502 (1978).
12. Hemmat, M. & Eggleston, P. Competitive interactions in *Drosophila melanogaster*: recurrent selection for aggression and response. *Heredity* **60**, 129–137 (1988).
13. Hoffmann, A. A. Geographic variation in the territorial success of *Drosophila melanogaster* males. *Behav. Genet.* **19**, 241–255 (1989).
14. Ruiz-Dubreuil, G., Burnet, B. & Connolly, K. Behavioural correlates of selection for oviposition by *Drosophila melanogaster* females in a patchy environment. *Heredity* **73**, 103–110 (1994).
15. Cheng, Y. *et al. Drosophila* fasciclinII is required for the formation of odor memories and for normal sensitivity to alcohol. *Cell* **105**, 757–776 (2001).
16. Moore, M. S. *et al.* Ethanol intoxication in *Drosophila*: genetic and pharmacological evidence for regulation by the cAMP signaling pathway. *Cell* **93**, 997–1007 (1998).
17. Scholz, H., Ramond, J., Singh, C. M. & Heberlein, U. Functional ethanol tolerance in *Drosophila*. *Neuron* **28**, 261–271 (2000).
18. Park, S. K., Sedore, S. A., Cronmiller, C. & Hirsh, J. Type II cAMP-dependent protein kinase-deficient *Drosophila* are viable but show developmental, circadian, and drug response phenotypes. *J. Biol. Chem.* **275**, 20588–20596 (2000).
19. Andretic, R., Chaney, S. & Hirsh, J. Requirement of circadian genes for cocaine sensitization in *Drosophila*. *Science* **285**, 1066–1068 (1999).
References 15–19 show that many of the same genes are involved in many behavioural phenotypes, such as in learning and sensitivity to ethanol, or circadian rhythms and cocaine response. These findings support the involvement of overlapping pathways and gene networks in behaviour.
20. Ueno, K. *et al.* Trehalose sensitivity in *Drosophila* correlates with mutations in and expression of the gustatory receptor gene *Gr5a*. *Curr. Biol*. **11**, 1451–1455 (2001).

21. de Bruyne, M., Foster, K. & Carlson, J. R. Odor coding in the *Drosophila* antenna. *Neuron* **30**, 537–552 (2001).
22. Clyne, P. J. *et al.* A novel family of divergent seven-transmembrane proteins: candidate odorant receptors in *Drosophila*. *Neuron* **22,** 327–338 (1999).
23. Vosshall, L. B., Amrein, H., Morozov, P. S., Rzhetsky, A. & Axel, R. A spatial map of olfactory receptor expression in the *Drosophila* antenna. *Cell* **96**, 725–736 (1999).
24. Clyne, P. J., Warr, C. G. & Carlson, J. R. Candidate taste receptors in *Drosophila*. *Science* **287**, 1830–1834 (2000).
25. Scott, K. *et al.* A chemosensory gene family encoding candidate gustatory and olfactory receptors in *Drosophila*. *Cell* **104**, 661–673 (2001).
References 21–25 show how the computational analysis of fly genome sequence data allowed the discovery and analysis of taste and olfactory receptor families. These receptors had previously remained elusive.
26. Kernan, M., Cowan, D. & Zuker, C. Genetic dissection of mechanosensory transduction: mechanoreception-defective mutations of *Drosophila*. *Neuron* **12**, 1195–1206 (1994).
27. Eberl, D. F., Hardy, R. W. & Kernan, M. J. Genetically similar transduction mechanisms for touch and hearing in *Drosophila*. *J. Neurosci*. **20**, 5981–5988 (2000).
28. Pflugfelder, G. O. Genetic lesions in *Drosophila* behavioural mutants. *Behav. Brain Res.* **95**, 3–15 (1998).
29. Eberl, D. F., Duyk, G. M. & Perrimon, N. A genetic screen for mutations that disrupt an auditory response in *Drosophila melanogaster*. *Proc. Natl Acad. Sci. USA* **94**, 14837–14842 (1997).
30. Sokolowski, M. B. Genes for normal behavioral variation: recent clues from flies and worms. *Neuron* **21**, 1–4 (1998).
31. Sawyer, L. A. *et al.* Natural variation in the *Drosophila clock* gene and temperature compensation. *Science* **278**, 2117–2120 (1997).
32. Mackay, T. F. C. Quantitative trait loci in *Drosophila*. *Nature Rev. Genet.* **2,** 11–20 (2001).
This review provides a road map for quantitative trait loci analysis, which can be applied to *Drosophila* behavioural traits.
33. Sokolowski, M. B. Foraging strategies of *Drosophila melanogaster*: a chromosomal analysis. *Behav. Genet.* **10**, 291–302 (1980).
Discovery of the rover–sitter behavioural polymorphism.
34. Sokolowski, M. B., Pereira, H. S. & Hughes, K. Evolution of foraging behavior in *Drosophila* by density dependent selection. *Proc. Natl Acad. Sci. USA* **94**, 7373–7377 (1997).
A good example of experimental evolution in the laboratory, in which larvae with rover or sitter alleles were selected for depending on the degree of crowding.
35. de Belle, J. S. & Sokolowski, M. B. Heredity of rover/sitter: alternative foraging strategies of *Drosophila melanogaster*. *Heredity* **59**, 73–83 (1987).
36. de Belle, J. S., Hilliker, A. J. & Sokolowski, M. B. Genetic localization of *foraging* (*for*): a major gene for larval behaviour in *Drosophila melanogaster*. *Genetics* **123**, 157–164 (1989).
This reference reports the genetic localization of the rover–sitter trait by lethal tagging.
37. Graf, S. A. & Sokolowski, M. B. The rover/sitter *Drosophila* foraging polymorphism as a function of larval development, food patch quality and starvation. *J. Insect Behav.* **2**, 301–313 (1989).
38. Wahlsten, D. & Gottlieb, G. in *Intelligence, Heredity, and Environment* (eds Sternberg, R. J. & Grigorenko, E. L.) 163–192 (Cambridge Univ. Press, New York, 1997).
39. Wahlsten, D. in *Theoretical Advances in Behavioral Genetics* (eds Royce, J. R. & Mos, L.) 426–481 (Sijthoff & Nordhoff, Germantown, Maryland, 1979).
40. de Belle, J. S., Sokolowski, M. B. & Hilliker, A. J. Genetic analysis of the *foraging* microregion of *Drosophila melanogaster*. *Genome* **36**, 94–101 (1993).
41. Osborne, K. A. *et al.* Natural behavior polymorphism due to a cGMP-dependent protein kinase of *Drosophila*. *Science* **277**, 834–836 (1997).
Cloning of the rover–sitter trait; the first example of the molecular identification of a naturally occurring behavioural variation.
42. de Bono, M. & Bargmann, C. I. Natural variation in a neuropeptide Y receptor homolog modifies social behavior and food response in *C. elegans*. *Cell* **94**, 679–689 (1998).
43. Kaga, T., Fujimiya, M. & Inui, A. Emerging functions of neuropeptide Y Y(2) receptors in the brain. *Peptides* **22**, 501–506 (2001).
44. Hirsch, J. & Erlenmeyer-Kimling, L. Sign of taxis as a property of the genotype. *Science* **134**, 835–836 (1961).
One of the first papers to show that a fly behavioural phenotype can be artificially selected for.
45. Benzer, S. Genetic dissection of behavior. *Sci. Am.* **229**, 24–37 (1973).
The ideas behind the single-gene mutant approach to fly behaviour genetics are clearly set out in this article and are as valid today as they were almost 30 years ago.
46. Feany, M. B. & Bender, W. W. A *Drosophila* model of Parkinson's disease. *Nature* **404**, 394–398 (2000).
47. Konopka, R. J. & Benzer, S. Clock mutants of *Drosophila melanogaster*. *Proc. Natl Acad. Sci. USA* **68**, 2112–2116 (1971).
This paper reported for the first time a gene (the *period* gene) that is involved in circadian rhythmicity. This discovery enabled the later genetic and molecular analysis of clock function in many organisms.
48. Sehgal, A., Price, J. L., Man, B. & Young, M. W. Loss of circadian behavioral rhythms and *per* RNA oscillations in the *Drosophila* mutant *timeless*. *Science* **263**, 1603–1606 (1994).
49. Vosshall, L. B., Price, J. L., Sehgal, A., Saez, L. & Young, M. W. Block in nuclear localization of period protein by a second clock mutation, *timeless*. *Science* **263**, 1606–1609 (1994).
50. Myers, M. P., Wager, S. K., Wesley, C. S., Young, M. W. & Sehgal, A. Positional cloning and sequence analysis of the *Drosophila* clock gene, *timeless*. *Science* **270**, 805–808 (1995).
51. So, W. V. & Rosbash, M. Post-transcriptional regulation contributes to *Drosophila* clock gene mRNA cycling. *EMBO J.* **16**, 146–155 (1997).
52. Hardin, P. E., Hall, J. C. & Rosbash, M. Feedback of the *Drosophila period* gene on circadian cycling of its messenger RNA levels. *Nature* **343**, 536–540 (1990).
53. Sehgal, A. *et al.* Rhythmic expression of *timeless*: a basis for promoting circadian cycles in *period* gene autoregulation. *Science* **270**, 808–810 (1995).
54. Gekakis, N. *et al.* Isolation of *timeless* by PER protein interaction: defective interaction between *timeless* protein and long-period mutant PERL. *Science* **270**, 811–815 (1995).
55. Curtin, K. D., Huang, Z. J. & Rosbash, M. Temporally regulated nuclear entry of the *Drosophila* period protein contributes to the circadian clock. *Neuron* **14**, 365–372 (1995).
56. Marrus, S. B., Zeng, H. & Rosbash, M. Effect of constant light and circadian entrainment of *perS* flies: evidence for light-mediated delay of the negative feedback loop in *Drosophila*. *EMBO J.* **15**, 6877–6886 (1996).
57. Frisch, B., Hardin, P. E., Hamblen, C. M., Rosbash, M. & Hall, J. C. A promoterless *period* gene mediates behavioral rhythmicity and cyclical *per* expression in a restricted subset of the *Drosophila* nervous system. *Neuron* **12**, 555–570 (1994).
58. Ewer, J., Frish, B., Hamblen, C. M., Rosbash, M. & Hall, J. C. Expression of the *period* clock gene within different cell types in the brain of *Drospohila* adults and mosaic analysis of these cells' influence on circadian behavioral rhythms. *J. Neurosci.* **12**, 3321–3349 (1992).
59. Renn, S. C. P., Park, J. H., Rosbash, M., Hall, J. C. & Taghert, P. H. A pdf neuropeptide gene mutation and ablation of PDF neurons each cause severe abnormalities of behavioral circadian rhythms in *Drosophila*. *Cell* **99**, 791–802 (1999).
The discovery of a neuropeptide that is thought to function in the output of the clock.
60. Toh, K. L. *et al.* An *hPer2* phosphorylation site mutation in familial advanced sleep phase syndrome. *Science* **291**, 1040–1043 (2001).
61. Price, J. L. *et al. double-time* is a novel *Drosophila* clock gene that regulates PERIOD protein accumulation. *Cell* **94**, 83–95 (1998).
62. Greenspan, R. J. Understanding the genetic construction of behavior. *Sci. Am*. **272**, 74–79 (1995).
63. Baker, B. S., Taylor, B. J. & Hall, J. C. Are complex behaviors specified by dedicated regulatory genes? Reasoning from *Drosophila*. *Cell* **105**, 13–24 (2001).
64. Goodwin, S. F. *et al.* Aberrant splicing and altered spatial expression patterns in *fruitless* mutants of *Drosophila melanogaster*. *Genetics* **154**, 7225–7245 (2000).
Details of the *fru* splicing pattern were worked out in this paper.
65. Villella, A. *et al.* Extended reproductive roles of the *fruitless* gene in *Drosophila melanogaster* revealed by behavioral analysis of new *fru* mutants. *Genetics* **147**, 1107–1130 (1997).
66. Anand, A. *et al.* Molecular genetic dissection of the sex-specific and vital functions of the *Drosophila melanogaster* sex determination gene *fruitless*. *Genetics* **158**, 1569–1595 (2001).
67. Ryner, L. C. *et al.* Control of male sexual behavior and sexual orientation in *Drosophila* by the *fruitless* gene. *Cell* **87**, 1079–1089 (1996).
68. Ito, H. *et al.* Sexual orientation in *Drosophila* is altered by the *satori* mutation in the sex-determination gene *fruitless* that encodes a zinc finger protein with a BTB domain. *Proc. Natl Acad. Sci. USA* **93**, 9687–9692 (1996).
References 67 and 68 show that *fru*, which was known to affect sexual behaviour, acts in the sex-determination pathway.
69. Lee, G. *et al.* Spatial, temporal, and sexually dimorphic expression patterns of the *fruitless* gene in the *Drosophila* CNS. *J. Neurobiol*. **43**, 404–426 (2000).
70. Belote, J. M. & Baker, B. S. Sexual behavior: its genetic control during development and adulthood in *Drosophila melanogaster*. *Proc. Natl Acad. Sci. USA* **84**, 8026–8030 (1987).
71. Arthur, B. I., Hauschteck-Jungen, E., Nothiger, R. & Ward, P. I. A female nervous system is necessary for normal sperm storage in *Drosophila melanogaster*: a masculinized nervous system is as good as none. *Proc. R. Soc. Lond.* B **265**, 1749–1753 (1998).
72. Ackerman, S. L. & Siegel, R. W. Chemically reinforced conditioned courtship in *Drosophila*: responses of wild-type and the *dunce, amnesiac* and *don giovanni* mutants. *J. Neurogenet.* **3**, 111–123 (1986).
73. Tully, T. & Quinn, W. G. Classical conditioning and retention in normal and mutant *Drosophila melanogaster*. *J. Comp. Physiol.* A **157**, 263–277 (1985).
74. Gailey, D. A., Jackson, F. R. & Siegel, R. W. Conditioning mutations in *Drosophila melanogaster* affect an experience-dependent behavioral modification in courting males. *Genetics* **106**, 613–623 (1984).
75. Siegel, R. W. & Hall, J. C. Conditioned responses in courtship behavior of normal and mutant *Drosophila*. *Proc. Natl Acad. Sci. USA* **76**, 3430–3434 (1979).
76. Griffith, L. C. *et al.* Inhibition of calcium/calmodulin-dependent protein kinase in *Drosophila* disrupts behavioral plasticity. *Neuron* **10**, 501–509 (1994).
77. Griffith, L. C., Wang, J., Zhong, Y., Wu, C.-F. & Greenspan, R. J. Calcium/calmodulin dependent protein kinase II and potassium channel subunit eag similarly affect plasticity in *Drosophila*. *Proc. Natl Acad. Sci. USA* **91**, 10044–10047 (1994).
78. Kane, N. S., Robichon, A., Dickinson, J. A. & Greenspan, R. J. Learning without performance in PKC-deficient *Drosophila*. *Neuron* **18**, 307–314 (1997).
79. Quinn, W. G., Harris, W. A. & Benzer, S. Conditioned behavior in *Drosophila melanogaster*. *Proc. Natl Acad. Sci. USA* **71**, 707–712 (1974).
80. Zhong, Y., Budnik, V. & Wu, C. F. Synaptic plasticity in *Drosophila* memory and hyper-excitable mutants: role of the cAMP cascade. *J. Neurosci*. **12**, 644–651 (1992).
81. Zhong, Y. & Wu, C. F. Altered synaptic plasticity in *Drosophila* memory mutants with a defective cAMP cascade. *Science* **251**, 198–201 (1991).
82. de Belle, J. S. & Heisenberg, M. Associative odor learning in *Drosophila* abolished by chemical ablation of mushroom bodies. *Science* **263**, 692–695 (1994).
In this study, the mushroom bodies of flies were chemically ablated to show that these structures have a definitive role in olfactory-based shock-avoidance learning.
83. Connolly, J. B. *et al.* Associative learning disrupted by impaired Gs signaling in *Drosophila* mushroom bodies. *Science* **274**, 2104–2106 (1996).
84. Zars, T., Fischer, M., Schulz, R. & Heisenberg, M. Localization of a short-term memory in *Drosophila*. *Science* **288**, 672–675 (2000).
These authors showed that synaptic plasticity in a small region of the mushroom bodies was sufficient for memory formation. They targeted expression of a *rut*$^{+}$ transgene in a *rut*$^{-}$ background to regions of the adult brain.
85. Dubnau, J., Grady, L., Kitamoto, T. & Tully, T. Disruption of neurotransmission in *Drosophila* mushroom body blocks retrieval but not acquisition of memory. *Nature* **411**, 476–480 (2001).
This paper shows that synaptic transmission between mushroom body neurons is required during memory retrieval, but not during its acquisition or storage.
86. Bartsch, D. *et al.* Enhancement of memory-related long-term facilitation by ApAF, a novel transcription factor that acts downstream from both CREB1 and CREB2. *Cell* **103**, 595–608 (2000).
87. Sutton, M. A., Masters, S. E., Bagnall, M. W. & Carew, T. J. Molecular mechanisms underlying a unique intermediate

phase of memory in aplysia. *Neuron* **31**, 143–154 (2001).
88. Chain, D. G., Schwartz, J. H. & Hegde, A. N. Ubiquitin-mediated proteolysis in learning and memory. *Mol. Neurobiol*. **201**, 25–42 (1999).
89. Skoulakis, E. M. C., Kalderon, D. & Davis, R. L. Preferential expression in mushroom bodies of the catalytic subunit of protein kinase *A* and its role in learning and memory. *Neuron* **11**, 197–208 (1993).
90. Li, W., Tully, T. & Kalderon, D. Effects of a conditional *Drosophila* PKA mutant on olfactory learning and memory. *Learn. Mem.* **2**, 320–333 (1976).
91. Quinn, W. G., Sziber, P. P. & Booker, R. The *Drosophila* memory mutant *amnesiac*. *Nature* **277**, 212–214 (1979).
92. Feany, M. B. & Quinn, W. G. A neuropeptide gene defined by the *Drosophila* memory mutant *amnesiac*. *Science* **268**, 869–873 (1995).
93. Waddell, S., Armstrong, J. D., Kitamoto, T., Kaiser, K. & Quinn, W. G. The *amnesiac* gene product is expressed in two neurons in the *Drosophila* brain that are critical for memory. *Cell* **103**, 805–813 (2000).
94. Rosay, P., Armstrong, J. D., Wang, Z. & Kaiser, K. Synchronized neural activity in the *Drosophila* memory centers and its modulation by amnesiac. *Neuron* **30**, 759–770 (2001).
References 91–94 present the identification, cloning and spatial requirements of the putative neuropetide amnesiac and its role in memory.
95. Yin, J. C. P. *et al.* Induction of a dominant-negative CREB transgene blocks long-term memory in *Drosophila*. *Cell* **79**, 49–58 (1994).
96. Yin, J. C. P., Vecchio, M. D., Zhou, H. & Tully, T. CREB as a memory modulator: induced expression of a *dCREB2* isoform enhances long-term memory in *Drosophila. Cell* **81**, 107–115 (1995).
References 95 and 96 define a role for CREB in long-term memory. The dominant-negative CREB transgene had opposing effects on long-term memory to that of the CREB overexpressing transgene.
97. Gass, P. *et al.* Deficits in memory tasks of mice with CREB mutations depend on gene dosage. *Learn. Mem.* **5**, 274–288 (1998).
98. Cherry, J. A. & Davis, R. L. Cyclic AMP phosphodiesterases are localized in regions of the mouse brain associated with reinforcement, movement, and affect. *J. Comp. Neurol.* **407**, 287–301 (1999).
99. Hall, J. C. in *Flexibility and Constraint in Behavioral Systems* (eds Greenspan, R. J. & Kyriacou, C. P.) 15–27 (Wiley, New York, 1994).
100. Heisenberg, M. Genetic approach to neuroethology. *Bioessays* **19**, 1065–1073 (1997).
101. Schutt, C. & Nothiger, R. Structure, function and evolution of sex-determining systems in dipteran insects. *Development* **127**, 667–677 (2000).
102. Gerlai, R. Gene-targeting studies of mammalian behavior: is it the mutation or the background genotype? *Trends Neurosci.* **19**, 177–181 (1996).
103. Greenspan, R. J. The flexible genome. *Nature Rev. Genet*. **2**, 383–387 (2001).
104. Yang, P., Shaver, S. A., Hilliker, A. J. & Sokolowski, M. B. Abnormal turning behavior in *Drosophila* larvae: identification and molecular analysis of *scribbler* (*sbb*). *Genetics* **155**, 1161–1174 (2000).
105. Boynton, S. & Tully, T. *latheo*, a new gene involved in associative learning and memory in *Drosophila melanogaster* identified from P element mutagenesis. *Genetics* **131**, 655–672 (1992).
106. Greenspan, R. J. A kinder, gentler genetic analysis of behavior: dissection gives way to modulation. *Curr. Opin. Neurobiol.* **7**, 805–811 (1997).
107. Yao, W. D. & Wu, C. F. Distinct roles of CaMKII and PKA in regulation of firing patterns and K(+) currents in *Drosophila* neurons. *J. Neurophysiol.* **85**, 1384–1394 (2001).
108. Renger, J. J., Yao, W.-D., Sokolowski, M. B. & Wu, C.-F. Neuronal polymorphism among natural alleles of a cGMP-dependent kinase gene, foraging in *Drosophila*. *J. Neurosci.* **19**, RC28 1–8 (1999).
109. Engel, J. E., Xian-Jin, X. J., Sokolowski, J. B. & Wu. C.-F. A cGMP dependent protein kinase gene, *foraging*, modifies habituation of the giant fiber escape response in *Drosophila. Learn. Mem.* **7**, 341–352 (2000).
110. Trimarchi, J. R., Jin, P. & Murphey, R. K. Controlling the motor neuron. *Int. Rev. Neurobiol.* **43**, 241–264 (1999).
111. Brand, A. H. & Perrimons, N. Targeted gene expression as a means of altering cell fates and generating dominant phenotypes. *Development* **118**, 401–415 (1993).
112. Sweeney, S. T., Broadie, K., Keane, J., Niemann, H. & O'Kane, C. J. Targeted expression of tetanus toxin light chain in *Drosophila* specifically eliminates synaptic transmission and causes behavioral defects. *Neuron* **14**, 341–351 (1995).
113. Baines, R. A., Uhler, J. P., Thompson, A., Sweeney, S. T. & Bate, M. Alterered electrical properties in *Drosophila* neurons developing without synaptic transmission. *J. Neurosci.* **21**, 1523–1531 (2001).
114. Cattaert, D. & Birman, S. Blockade of the central generator of locomotor rhythm by noncompetitive NMDA receptor antagonists in *Drosophila* larvae. *J. Neurobiol.* **48**, 58–73 (2001).
115. Wang, Y. *et al.* Genetic manipulation of the odor-evoked distributed neural activity in the *Drosophila* mushroom body. *Neuron* **29**, 267–276 (2001).
116. Sokolowski, M. B. in *Techniques for the Genetic Analysis of Brain and Behavior* (eds Goldowitz, D., Wahlsten D. & Wimer, R. E.) 497 (Elsevier, Amsterdam, 1992).
117. Sokolowski, M. B. & Wahlsten, D. in *Methods in Genomic Neuroscience* (ed. Moldin, S. O.) 3–27 (CRC Press, New York, 2001).
118. Kaneko, M., Hamblen, M. J. & Hall, J. C. Involvement of the *period* gene in developmental time-memory: effect of the perShort mutation on phase shifts induced by light pulses delivered to *Drosophila* larvae. *J. Biol. Rhythms* **15**, 13–30 (2000).
119. de Belle, J. S. & Heisenberg, M. Expression of *Drosophila* mushroom body mutations in alternative genetic backgrounds: a case study of the mushroom body miniature gene (*mbm*). *Proc. Natl Acad. Sci. USA* **93**, 9875–9880 (1996).
This is the only paper to have systematically tested for genetic background effects on a behaviour, in this case learning.
120. Davis, R. L. Mushroom bodies, Ca(2+) oscillations, and the memory gene *amnesiac*. *Neuron* **30**, 653–656 (2001).
121. Yamamoto, D. & Nakano, Y. Sexual behaviour mutants revisited: molecular and cellular basis of *Drosophila* mating. *Cell Mol. Life Sci*. **56**, 634–646 (1999).

Acknowledgements
Thanks to G. Robinson, M. Suster, M. Fitzpatrick, R. Greenspan, D. Sebastian and C. Riedl for comments on a preliminary version of the manuscript. Thanks to M. Busto who compiled table 1, and J. Hall who commented on it. M. Fitzpatrick and C. Riedl helped prepare drafts of the figures. This research was supported by NSERC and MRC grants to M.B.S. M.B.S. is a Canada Research Chair Holder in Genetics.

Online links

DATABASES
The following items in this article are linked online to:
LocusLink: http://www.ncbi.nlm.nih.gov/LocusLink/
amn | Clk | Cyc | *dbt* | *dnc* | *for* | *fru* | *lat* | *per* | *PER2* | *rut* | *sbb* | *tim*
WormBase: http://www.wormbase.org/
npr-1
OMIM: http://www.ncbi.nlm.nih.gov/Omim/
FASPS | Huntington disease | Parkinson disease
FlyBase: http://flybase.bio.indiana.edu/
CaMKII

FURTHER INFORMATION
Flybrain: http://flybrain.uni-freiburg.de/Flybrain/html/
Marla Sokolowski's homepage:
http://cfly.utm.utoronto.ca/sokolab/
Seymour Benzer: http://www.its.caltech.edu/~biology/brochure/faculty/benzer.html
The Interactive Fly:
http://sdb.bio.purdue.edu/fly/aimain/1aahome.htm
Access to this interactive links box is free online.

SUPPLEMENTARY TABLE

Table 1 | **Examples of cloned genes that influence complex behaviour**

Behavioural category Gene; synonym	Molecular function	Expression pattern	Behavioural pleiotropy	Developmental pleiotropy	Reference
Circadian rhythm					
period (*per*)	Transcription cofactor	BN and NN	Locomotor rhythms, eclosion rhythms, courtship, cocaine sensitivity, others		1
timeless (*tim*)	Interacts with Per	BN and NN	Locomotor rhythms, eclosion rhythms, sleep		1
double-time (*dbt*); *discs overgrown* (*dco*)	Casein kinase I	BN and NN	Locomotor rhythms, sleep, cocaine sensitivity	Imaginal disc overgrowth, pupal lethality	1
Clock (*Clk*); *jrk*	Transcription factor	U	Locomotor rhythms, eclosion rhythms, cocaine sensitivity		1
cycle (*cyc*); *Mop3*	Transcription factor	N	Locomotor rhythms, eclosion rhythms, cocaine sensitivity		1
lark	RNA binding	N	Eclosion rhythms	Embryonic fragility	1
cryptochrome (*cry*)	Homology with blue-light-sensitive DNA-repair enzymes	BN and NN	Resetting of behavioural rhythms		1
Pigment-dispersing factor (*Pdf*)	Neuropeptide hormone	N	Locomotor rhythms		1
vrille (*vri*)	Transcription factor	N and NN	Locomotor rhythms	Embryonic lethality	1
ebony (*e*)	β-alanyl dopamine synthetase	N	Locomotor rhythms		
dusky (*dy*); *Andante* (*And*)	Plasma membrane component	N and NN	Locomotor rhythms, eclosion rhythms	Wing development	1
disconnected (*disco*)	Transcription factor	N and NN	Locomotor rhythms, eclosion rhythms	Visual system defect	1
cAMP-dependent protein kinase type II (*pka-RII*)	Protein kinase	N	Locomotor rhythms, cocaine sensitivity, ethanol sensitivity	Ovary development	2
shaggy (*sgg*)	Protein Ser/Thr kinase	N and NN	Locomotor rhythms	Segment polarity, larval/pupal lethality, bristle development	3
takeout (*to*)	Ligand-binding protein	N and NN	Feeding rhythms	Semi-viable	1
Courtship					
fruitless (*fru*)	Transcription factor	N and NN	All aspects of male courtship	Abnormal muscle of Lawrence	4
doublesex (*dsx*)	Transcription factor	N and NN	Song defect	Sterility, abnormal yolk protein production	4
dissatisfaction (*dsf*)	Steroid hormone receptor	N and NN	Poor sex discrimination, reduced female receptiveness	Slow copulation, no voluntary egg laying	4
courtless (*crl*)	Ubiquitin-conjugating enzyme	N and NN	Failure to court	Male sterile	4
slowpoke	Calcium-activated potassium channel	N and NN	Song defect	Flight defect	4
spinster (*spin*)	Membrane protein	N and NN	Reduced female receptiveness	PCD defect	5
cacophony (*cac*); *nightblind A* (*nbA*)	Voltage-sensitive calcium channel	N and NN	Song defect, optomotor behaviour, photophobic	Phototransduction	5
dissonance (*diss*); *no on-or-off transient* (*nonA*)	RNA binding	U	Song defect, optomotor behaviour	Phototaxis	5

fickle (*fic*); *Btk family kinase at 29A* (*Btk29A*)	Protein tyrosine kinase	N and NN	Male genitalia defect	Reduced longevity, head involution defect	6
Learning and memory					
dunce (*dnc*)	cAMP-specific phosphodiesterase	U	Locomotor rhythms, ethanol tolerance	Female sterility, decreased female longevity	7
rutabaga (*rut*)	Adenylate cyclase	U	Courtship learning, ethanol tolerance, grooming		7
amnesiac (*amn*); *cheapdate* (*chpd*)	Neuropeptide	N	Ethanol tolerance	Decreased heart rate	7
latheo (*lat*)	DNA-replication factor	N	Larval feeding	Pupal lethality	7
linotte (*lio*)/*derailed* (*drl*)	Novel/protein tyrosine kinase	N		CC brain defect, MB defect, axon-guidance defect	7
minibrain (*mnb*)	Protein Ser/Thr kinase	N		Reduced brain volume	8
leonardo (*leo*); *14-3-3ζ*	Protein kinase C inhibitor	N and NN		Embryonic lethality, eye-pattern defect	7
nalyot (*nal*); *Adh transcription factor 1* (*Adf1*)	Transcription factor	N and NN		Embryonic lethality, larval sluggishness	7
Shaker (*Sh*)	Voltage-sensitive potassium channel	N	Courtship suppression, gustation defect, ether sensitivity	Decreased longevity,	8
G protein sα 60A (*G-s α 60A*)	Heterotrimeric G protein	N and NN	Visual behaviour, cocaine sensitivity	Larval/pupal lethality	7
DCO; *cAMP-dependent protein kinase 1* (*Pka-C1*)	Protein Ser/Thr kinase	N and NN	Locomotor rhythms, ethanol tolerance	Female sterility, wing/eye/leg morpho-genesis defect	7
ether a go-go (*eag*)	Voltage-sensitive potassium channel	N	Courtship suppression, olfaction defect, ether sensitivity	Leg shaking, decreased longevity, decreased heart rate	4
cAMP-response-element-binding protein B at 17A (*CrebB-17A*); *dCREB*	Transcription factor	U	Locomotor rhythms,	Larval lethality	7
Calcium/calmodulin-dependent protein kinase II (*CamkII*)	Protein Ser/Thr kinase	N	Courtship suppression	NMJ branching defect	7
Dopa decarboxylase (*Ddc*)	Dopa decarboxylase	N and NN	Courtship suppression, eclosion rhythms	Lethality, female infertility, sclerotinization defect	7
Volado (*Vol*); *scab* (*scb*)	α-integrin	N and NN	Locomotory defect	Larval lethality, embryonic development defects	7
fasciclinII (*fasII*)	Cell adhesion	N and NN	Ethanol sensitivity	Synaptic pattern defect, Bolwig's organ defect, eye defect	9
Protein phosphatase 1 at 87B (*Pp1-87B*)	Protein phosphatase type I catalyst	U	Reduced motility	Reduced flight activity	8
Neurofibromatosis1 (*Nf1*)	Ras GTPase activator	U		Growth defect	7
Feeding/foraging					
foraging (*for*); *dg2*	cGMP-dependent protein kinase	N and NN	Rover and sitter morphs	Pupal lethality, hypoxia recovery	10

BN, broad neural; CC, central complex; MB, mushroom body; N, neural; NMJ, neuromuscular junction; NN, non neural; PCD, programmed cell death; U, ubiquitous.

1. Williams, J. A. & Seghal, A. Molecular components of the circadian system in *Drosophila*. *Annu. Rev. Physiol.* **63**, 729–755 (2001).
2. Park, S. K., Sedore, S. A., Cronmiller, C. & Hirsh, J. Type II cAMP-dependent protein kinase-deficient *Drosophila* are viable but show developmental, circadian, and drug response phenotypes. *J. Biol. Chem.* **275**, 20588–20596 (2000).
3. Martinek, S., Inong, S., Manoukian, A. S. & Young, M. W. A role for the segment polarity gene *shaggy/GSK-3* in the *Drosophila* circadian clock. *Cell* **105**, 769–779 (2001).
4. Greenspan, R. J. & Ferveur, J.-F. Courtship in *Drosophila*. *Annu. Rev. Genet.* **34**, 205–232 (2000).
5. Yamamoto, D. & Nakano, Y. Sexual behaviour mutants revisited: molecular and cellular basis of *Drosophila* mating. *Cell Mol. Life Sci.* **56**, 634–646 (1999).
6. Yamamoto, D. & Nakano, Y. Genes for sexual behaviour. *Biochem. Biophys. Res. Commun.* **246**, 1–6 (1998).
7. Waddell, S. & Quinn, W. G. Flies, genes, and learning. *Annu. Rev. Neurosci.* **24**, 1283–1309 (2001).
8. Dubnau, J. & Tully, T. Gene discovery in *Drosophila*: new insights for learning and memory. *Annu. Rev. Neurosci.* **21**, 407–444 (1998).
9. Cheng, Y. *et al. Drosophila* fasciclinII is required for the formation of odor memories and for normal sensitivity to alcohol. *Cell* **105**, 757–776 (2001).
10. Osborne, K. A. *et al.* Natural behavior polymorphism due to a cGMP-dependent protein kinase of *Drosophila*. *Science* **277**, 834–836 (1997).