Neurogenetics: Singing in the Brain

The *fruitless* gene is well-known to play a key role in determining the sexual identity of the fruitfly's nervous system, but new results show that *doublesex* is also required in thoracic neurons to generate normal male lovesongs.

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In 1973, when the ethologists Lorenz, Tinbergen and von Frisch won their Nobel Prize, they could not have envisaged that within a generation, neurogenetic analysis would be unravelling how gene products mediate animal instincts. A study by Stephen Goodwin's group [1], recently published in *Current Biology,* provides a compelling example of this type of approach. Using the sophisticated genetic and molecular tools available in the fruitfly, they identified sex-specific neurons in the thorax that require both *doublesex (dsx)* and *fruitless (fru)* expression to mediate the courtship song of the male fly. This work adds further support to the view that *fru* alone is not the 'switch' that determines sex-specific behaviour in *Drosophila melanogaster* [2].

Courtship behaviour obviously requires two sexes, and the sex of any individual fruitfly is achieved early in development by counting the number of sex chromosomes and comparing them molecularly to the number of autosomes. In diplo-*X* females, the key gene *Sex-lethal (Sxl)* is activated, and SXL protein then splices its own transcript, as well as that of the downstream gene *transformer (tra)*, in a female mode. Together with non-sex-specific TRA-2 protein, TRA splices the *doublesex* transcript to produce DSX^F, the transcription factor that determines the fly's somatic sexual phenotype. In males, the single X chromosome means an absence of functional SXL and TRA, and *dsx* is spliced in the default male mode, DSX^M [3].

So much for the sex of the fly's 'body', what about its 'mind'? The nervous systems of males and females are engaged differently, as revealed in their courtship, where the male does the running, and the female the running away. The male components of this interaction include tapping the female's bottom with his forelegs, following her as she scampers away, extending one wing nearest her antennae, vibrating it to produce a species-specific lovesong, then licking her derriere, before attempting to copulate [4]. Females do not sing, but fend the male off with their wings or legs, usually kicking him in the head and running away. Fertilised females are rather unreceptive and also have the (disgusting) tendency of extruding their ovipositor in the male's face [5].

If a chromosomally female fly has her *dsx* gene replaced by one that is locked into DSX^M splice mode, we would expect a masculinised female that looks much like a male and behaves like one, even though SXL and TRA are ON. Instead, these flies look, but do not behave like males [6]. In contrast, a chromosomally female fly whose *tra* gene is mutated so TRA is OFF, looks like a male (expected), *and* behaves like a male, including singing a normal lovesong (unexpected) [7]. Consequently, the pathway to the sex of the nervous system depends on TRA, not DSX. The key gene that is regulated by TRA, again by differential splicing, is *fruitless (fru)*, which encodes a BTB domain zinc finger transcription factor [8]. This gene is large and very complex, with sex-specific male transcripts, fru^M, and non sex-specific 'common' transcripts, fru^{COM} [9]. Recent studies have misexpressed FRU^M in females to generate morphological females that court other females, suggesting that *fru* is sufficient to switch this complex innate behavioural sequence into 'male mode' [10,11]. So is Descartes' dualism of body and mind the right way to think about flies in this context, with DSX specifying the sexual identity of body, and FRU the mind?

Not really. First, while these masculinised FRU^M females do appear to go through the full repertoire of male courtship elements, except copulation, their repertoire falls short, both in quantity and quality, of normal male behaviour [1,12]. Furthermore, there is the male-specific abdominal muscle, the MOL, that is determined by FRU^M through the sex of the motor neuron that innervates it, and not by DSX^M: a FRU^M mind–body mix [13]. Conversely, *dsx* is important in determining the sex of some abdominal neuroblasts [14], in producing courtship song hums in males [15], and in activating/repressing male and female courtship behaviour [16,17], so it clearly influences nervous system development.

Goodwin's group [1] have extended these observations to reveal how FRU and DSX cooperate within the mesothoracic ganglion to generate the normal courtship song of the male. They examined the lovesong of chromosomal females that expressed FRU^M, and found that while the females indeed courted other normal females, and extended their wings, their courtship song, which should consist of trains of pulses and hums, was largely non-existent ('singing down the drain', Figure 1). A normal train of song may have up to 30 pulses and half a second of hums [15] but these females produced at most a train of 3–4 pulses, and no hums at all (Figure 1). Thus, FRU^M alone is not sufficient for the production of courtship song, yet the presence of FRU^M and DSX^M in females — as in chromosomal females mutant for TRA — is sufficient (Figure 1), as appreciated more than 25 years ago [6].

The neural basis for song production was demonstrated even earlier using sex mosaics, flies that are part-male, part-female [18]. Male tissue is required in the ventral mesothoracic ganglion to

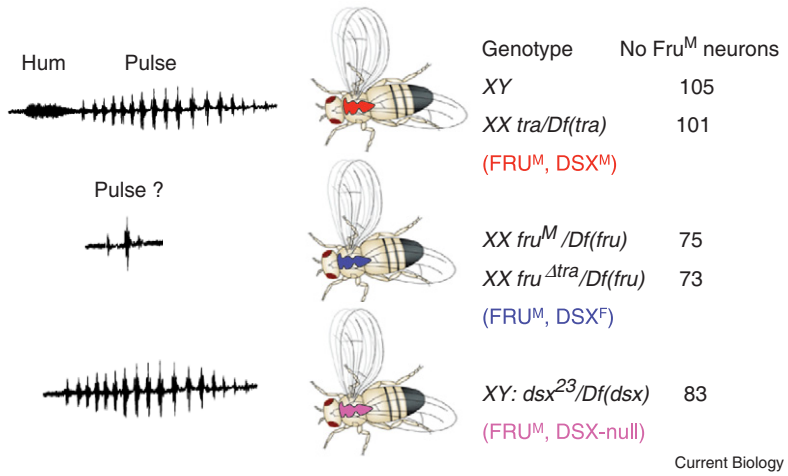

Figure 1. Neurogenetics of *Drosophila* courtship song.

Songs consists of trains of pulses and hums, produced by normal XY chromosomal males, and also by diplo-X females mutant for *tra (tra/Df(tra)).* Both these genotypes express FRU^M and DSX^M (colour coded) in the mesothoracic ganglion (MSG, also colour coded). Females (DSX^F) that express FRU^M or a mutation of *fru* in which the TRA binding sites are deleted, *fru$^{\Delta tra}$* (thereby also producing the default FRU^M isoforms), barely sing in any coherent manner [11]. Males carrying a *dsx-null* mutation, however, produce occasional burst of song containing trains of normal pulses but no hums [15]. The numbers of FRU^M neurons expressed in these genotypes within the MSG are provided [11]. Consequently, those 7–8 neurons present in *dsx-null* males but absent in FRU^M females would be candidates for expressing hum song/extending pulse trains, as well as representing potential targets of repression by DSX^F [17].

generate the basic acoustic parameters of the song, the pulses and hums. So are there any sexual dimorphisms in this region that might explain the absence of courtship song in the normal female? FRU^M is known to prevent programmed cell death in some regions of the CNS [19], and similarly, DSX^M prolongs neuroblast divisions in the male abdominal ganglion [14], both mechanisms leading to higher numbers of male neurons. In fact, males have about 25 more *fru* expressing neurons in the mesothoracic ganglion than females, but this increase in number is not observed in females that express FRU^M (and DSX^F), so FRU^M alone does not specify this dimorphism (Figure 1): nor does DSX^M, because in chromosomal males carrying a *dsx-null* allele, the number of FRU^M neurons in this thoracic region is also reduced, but not quite to the same extent [11]. Interestingly, chromosomal males of this *dsx-null* genotype sing infrequently, but when they do, they produce normal song bursts, but no hum song [15], so there is a correlation between FRU^M mesothoracic ganglion neuron number and the severity of the song defect (Figure 1). Thus, both FRU^M and DSX^M are required to generate this neuronal sexual dimorphism, and by implication, are both required for normal song production. Furthermore, of the 135 or so *fru* expressing neurons in the male mesothoracic ganglion, of which ~100 express FRU^M, there were nearly 20 neurons that co-expressed both DSX^M and FRU^M, thereby representing the candidate neurons for generating normal courtship song.

This study follows another, again by Goodwin's group [13], which revealed a similar co-existence and cooperation between DSX^M and FRU^M in serotonergic abdominal neurons implicated in copulatory behaviour. So the seductive idea of FRU^M alone as dictating nervous system identity, with DSX alone taking on the somatic role, appears to be an oversimplification. One might think of the sexual identity of the male nervous system as being a canvas on which the background is painted by FRU^M while some of the fine detail is added by DSX^M (or *vice versa*).

One of many outstanding questions is how these 20 or so sex-specific thoracic neurons programme the courtship song and whether they do it alone, or form a control network that directs the rest? From an evolutionary perspective, we might expect FRU and DSX sex-specific isoforms and neurons to show interesting patterns of expression in duetting *Drosophila* species where both sexes sing, or for that matter more generally in any sexually charged insect communication [20]. Clearly, neurogenetic analysis of the type discussed above will continue to illuminate the mysteries of complex innate behaviour patterns.

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Self-Fertility: The Genetics of Sex in Lonely Fungi

The genome of the fungus *Aspergillus nidulans* encodes both of the mating-type regulators of sexuality, thus allowing self-fertility. Pheromone signaling genes are induced during sexual development, as found in outcrossing species, but, surprisingly, the regulators do not control expression of these genes.

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Sex is inherently complicated, fascinating and mysterious. Fungi illustrate the full range of sexual lifestyles, with some species displaying typical mating between opposite sexes (outcrossing or heterothallism), while others demonstrate more exotic behaviors, including self-fertility (homothallism), parasexual or mitotic sex, or the presence of multiple sexes (sometimes thousands!). These topics are covered in a new book from ASM Press: *Sex in Fungi: Molecular Determination and Evolutionary Implications* [1], which is timely because the rapidly accumulating fungal genome sequences have triggered a renaissance in the analysis of the sexual lifestyles of fungi. Nowhere is this better illustrated than in filamentous ascomycete species of the genus *Aspergillus*, with a flurry of recent revelations concerning the organization of the master regulatory genes for mating (*MAT* genes), as well as the signaling components such as pheromones, receptors, heterotrimeric G proteins, protein kinases and transcription factors that control mate recognition and sexual development. A study by Paoletti *et al.* [2], reported recently in *Current Biology,* provides an excellent example of the kind of discoveries enabled by the genome sequence information.

Paoletti *et al.* [2] focused on *Aspergillus nidulans*, a species that has all of the options for sexuality, including mating between distinct haploid strains to allow outcrossing, self-fertility in individuals, and parasexual behavior that results in the formation of diploids or heterokaryons (strains with two genetically distinct nuclei). These features and a well-developed suite of techniques for genetic analysis make *A. nidulans* an exceptional model for studying fungal biology [3,4]. The determination of the *A. nidulans* genome sequence [5], and the analysis performed by Paoletti *et al.* [2], has revealed that the genome contains both of the *MAT* sequences encoding the regulatory proteins known to control partner recognition in outcrossing species of filamentous fungi [6]. These proteins are the alpha-box domain protein and the high mobility group (HMG) domain protein that were originally identified in *Neurospora crassa* [6], where they are encoded at the same genomic location, the *MAT* locus. In *N. crassa* and other outcrossing species, each mating partner carries distinct *MAT* sequences that encode either the alpha or the HMG protein, and these proteins specify sexual identity and control the mating events leading to partner fusion. The presence of both functions in the same genome in *A. nidulans* allows the nuclei in one individual to fuse and initiate the meiotic pathway, thus resulting in self-fertility. These observations explain why a colony arising from a single uninucleate spore can respond to appropriate environmental signals to form the specialized nest-like structures called cleistothecia (also called fruit bodies) in which the sexual ascospores are produced (Figure 1). Importantly, Paoletti *et al.* [2] have now demonstrated that the *MAT* sequences are functionally required for fertility, because they found that deletion of the *MAT* genes resulted in diminished production of cleistothecia and the absence of ascospores.

The presence of both regulatory genes in the genome of *A. nidulans* can be considered in the broader context of mechanisms of fertility in the genus *Aspergillus*. About half of the 184 species in the genus are known to have sex and, with a very few exceptions (four to be exact), most of these display self-fertility [7]. The remaining species are thought to lack the capacity for sex although, intriguingly, genome-sequencing projects revealed that some of these fungi do have *MAT* sequences [5,7–9]. For example, the sequenced genome of *A. oryzae* (a species used in the production of soy sauce and miso) contains the gene for the alpha protein, and a survey of other isolates identified some with the