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Review

Sex in flies: What 'body–mind' dichotomy?

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Abstract

Sexual behavior in *Drosophila* results from interactions of multiple neural and genetic pathways. Male-specific *fruitless* (*fru*M) is a major component inducing male behaviors, but recent work indicates key roles for other sex-specific and sex-non-specific components. Notably, male-like courtship by *retained* (*retn*) mutant females reveals an intrinsic pathway for male behavior independent of *fru*M, while behavioral differences between males and females with equal levels of *fru*M expression indicate involvement of another sex-specific component. Indeed, sex-specific products of *doublesex* (*dsx*F and *dsx*M), that control sexual differentiation of the body, also contribute to sexual behavior and neural development of both sexes. In addition, the single product of the *dissatisfaction* (*dsf*) gene is needed for appropriate behavior in both sexes, implying additional complexities and levels of control. The genetic mechanisms controlling sexual behavior are similar to those controlling body sexual development, suggesting biological advantages of modifying an intermediate intrinsic pathway in generation of two substantially different behavioral or morphological states.

Keywords: *Drosophila*; Courtship behavior; *Fruitless*; *Doublesex*; *Retained*; *Dissatisfaction*; Sexual differentiation

Introduction

Homologous patterns of innate behaviors of different species suggest that behavior, like body morphology, is specified by developmental and genetic information within the genome (Lorenz, 1981). Advances in neurobiology, development, genetics and molecular biology allow us to ask not only if genes can control a behavior, but also, how those genes pattern the nervous system to specify the behavior. The interactive reproductive behaviors of male and female *Drosophila* provide an opportunity to genetically dissect the molecular and neural mechanisms of a tractable behavior (Baker et al., 2001; Billeter et al., 2002; Greenspan, 1995; Sokolowski, 2001; Yamamoto et al., 1998).

Drosophila melanogaster males perform a series of courtship steps involving the interplay of multiple sensory inputs (Greenspan and Ferveur, 2000; Hall, 1994; Spieth, 1974). Although male courtship in *Drosophila* is hard-wired, experience can modify a male's ability to discriminate between, for example, mated and unmated females as courtship targets. Females do not display male courtship activity, but perform relatively inconspicuous behaviors associated with mate choice; a virgin female has the ability to be unreceptive to and resist the courtship of a *Drosophila* male by, for example, extruding her ovipositor toward the male, and continually avoiding the male. In principle, females may also have seductive behaviors beyond walking away from a male, but these have not been documented.

Sexual behavior in *Drosophila* is under genetic control (Figs. 1A, B). The presence of the functional female-specific form of the splicing activator, *transformer* (*tra*F), is entirely sufficient to confer a switch from male to female body morphology and behavioral repertoire (McKeown et al., 1988; Arthur et al., 1998). TraF activates female splicing of transcripts from the *doublesex* gene (*dsx*) (Fig. 1C), and of transcripts from the most upstream promoter (P1) of the *fruitless* gene (*fru*) (Fig. 1D) (Burtis and Baker, 1989; Demir and Dickson, 2005; Heinrichs et al., 1998; Ito et al., 1996; Nagoshi and Baker, 1990; Ryner and Baker, 1991; Ryner et al., 1996). In the absence of TraF (i.e., in males), transcripts from *dsx* and *fru* are spliced into their male-specific, default forms. Both male and female forms of *dsx* RNA encode transcription factors that are necessary and sufficient for sex-specific cuticular

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development (Christiansen et al., 2002). Male-specific *fru* RNA (*fru*M) (Fig. 1D) encodes a set of BTB-Zn-Finger class apparent transcription factors important for male courtship activity (Billeter et al., 2006a; Manoli et al., 2006). *fru*F RNA has a stop after codon 94, and its product is inferred to be non-functional. The encoded peptide is not detected by antibodies that detect the same epitope in FruM (Lee et al., 2000), and is inferred to be inhibited in translation (Usui-Aoki et al., 2000), turned over rapidly, or have an unrecognized configuration. The *fru*M promoter is active in the nervous system in central and peripheral regions known to be foci for male behavior (Billeter and Goodwin, 2004; Lee et al., 2000; Manoli et al., 2005; Stockinger et al., 2005).

As *dsx* and *fru* are the only identified targets of *tra*-regulated splicing, the obvious and distinctive mutant phenotypes from the loss of *dsx* and *fru* suggest that they are master regulators of separate developmental pathways governing the sexual identity

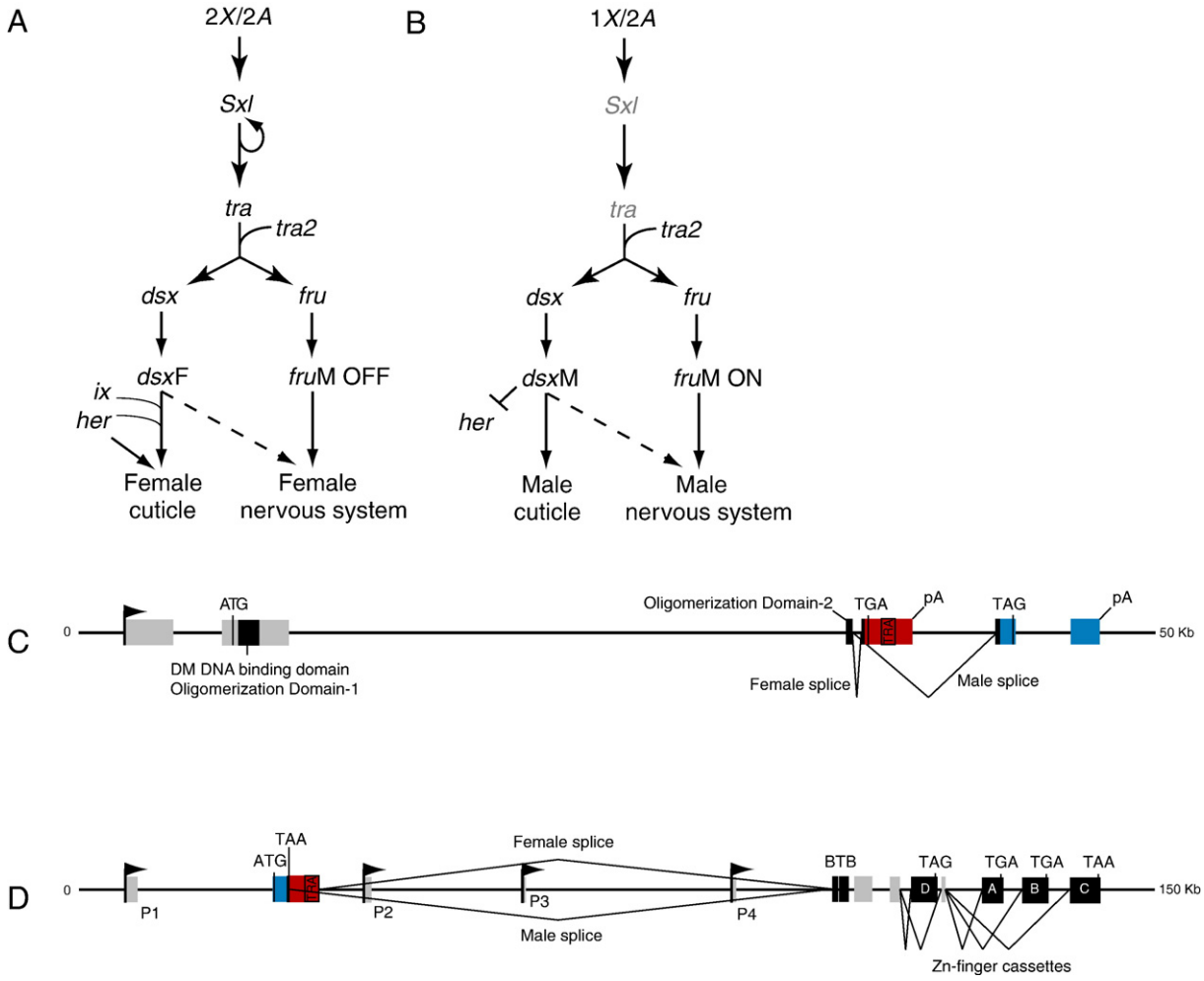

Fig. 1. Sex determination in *Drosophila* (Cline and Meyer, 1996) is governed by the *X*-chromosome-to-autosome (*X*/*A*) ratio in which two *X*-chromosomes, as in females (A), activates *Sex lethal* (*Sxl*) which in turn activates female-specific splicing of *transformer* RNA and production of traF protein. *Sxl* also perpetuates its own splicing-activated female state. TraF along with sex-non-specific Tra2 activates the female-specific splicing of transcripts from *dsx* and *fru*-P1. *dsx*F functions with *intersex* (*ix*) (Garrett-Engele et al., 2002) and *hermaphrodite* (*her*) (Li and Baker, 1998b) to induce female differentiation of the dimorphic cuticle. *her* also functions independently of, but in the same direction as *dsx*F to specify particular aspects of the female cuticle. Female-specific transcripts from *fru*-P1 are apparently not translated (Usui-Aoki et al., 2000). The absence of *fru*M is positive for female behavior (Demir and Dickson, 2005). Dotted arrows indicate the role of *dsx*F in female behavior and neural differentiation as discussed in the text. (B) In males, a single *X*-chromosome does not lead to production of *Sxl* RNA or functional *tra* RNA. Transcripts from *fru*-P1 and *dsx* are spliced into default, male-specific forms. *dsx*M induces male differentiation of the dimorphic cuticle in part by functionally countering *her* activity (Li and Baker, 1998b). Dotted arrows indicate the role of *dsx*M in male behavior and neural differentiation as discussed in the text. *fru*M is important for induction of male-specific differentiation of the nervous system (Billeter et al., 2006b; Demir and Dickson, 2005; Usui-Aoki et al., 2000). (C) *dsx* and its sex-specific splicing pattern. Exon two contains the Zn-finger-related DM DNA binding domain and the first oligomerization domain (in black). Exon three contains the second oligomerization domain (in black) that extends into sex-specific exons (red, female; blue, male). Tra/Tra2-responsive sites are within the female-specific exon (designated as "TRA"). Male-specific and female-specific splice patterns, stop codons, and poly-adenylation signals (pA) are shown. (D) *fru* and its sex-specific splicing pattern. The *fru* locus uses four distinct promoters (P1–4) that give rise to multiple sex-specific and sex-non-specific alternatively spliced transcripts. Transcripts from the P1 promoter are under Tra/Tra2 regulation. Male-specific (blue) and female-specific (red) regions of exon two and their splicing patterns are noted. Tra/Tra2-responsive sites (designated as "TRA") are within the female-specific exonic region. The female-specific splice introduces an early stop codon. All *fru* isoforms contain a BTB dimerization domain, and one of four potential Zn-finger cassettes (A–D) (noted in black). Stop codons within each Zn-finger cassette are noted.

of the 'body' and 'mind', respectively (Baker et al., 2001). The extent at which *dsx* and *fru* interact to pattern sexual behavior has been elusive. Recent work demonstrates that *dsx* significantly contributes to sexual behavior. Here, we discuss the work demonstrating the role of *fru* as a courtship behavior switch gene, and follow with a review of the evidence that other genes and pathways contribute to full male behavior. We continue with a review of recent work revealing *dsx* as a component of this switch system and finish with a discussion of other genes that may function with or through the sexual state of *dsx* and *fru* for complete control of male and female behavior.

fruitless controls sexual behavior in Drosophila

fruM is necessary for male-specific behavioral and neuronal differentiation

The quintessential *fru*M phenotype is male-directed courtship leading to chains of courting mutant males (Gailey and Hall, 1989; Gill, 1963; Hall, 1978), but *fru*M is important for almost all aspects of male courtship behavior, including both amount and type of behavior produced (Villella et al., 1997). *fru*M mutant males have decreased courtship activity, court bisexually or homosexually, and lack parts of the courtship ritual (Anand et al., 2001; Gailey and Hall, 1989; Hall, 1978; Ito et al., 1996; Ryner et al., 1996; Villella et al., 1997).

Behavioral abnormalities suggest *fru*M functions in patterning the neural underpinnings required for courtship behavior (Baker et al., 2001). *fru*M neural function was originally shown by its effects on the muscles of Lawrence (moL), a pair of male-specific muscles of the dorsal fifth abdominal segment (Lawrence and Johnston, 1986). MoL development depends on innervation by a *fru*M+ motorneuron (Billeter and Goodwin, 2004; Currie and Bate, 1995; Gailey et al., 1991; Usui-Aoki et al., 2000). *fru*M mutant males, like females, lack the moL (Gailey et al., 1991), and the innervating neuron switches from the male neurite pattern to the female pattern (Billeter and Goodwin, 2004).

fruM is expressed male-specifically in neurons dedicated to sexual behavior

Immunohistochemistry and *in situ* hybridization show that *fru*M is expressed in approximately 2% of neurons in the male central nervous system (CNS) (Goodwin et al., 2000; Ito et al., 1996; Lee et al., 2000; Ryner et al., 1996). Production of Gal4 in the *fru*-P1 pattern also reveals *fru* expression in behaviorally important parts of the peripheral nervous system such as neurons of the antennal segments, forelegs, mouthparts, and genitalia (Billeter and Goodwin, 2004; Manoli et al., 2005; Stockinger et al., 2005). Inhibition of synaptic transmission by *fru*-P1 neurons ablates all male courtship activity without any observable effects in other general behaviors, suggesting that *fru*-P1 neurons are dedicated to sexual behavior (Manoli et al., 2005, 2006; Stockinger et al., 2005). These data suggest that *fru*M-expressing neurons detect courtship-relevant sensory cues and further centrally process those cues to produce specific aspects of the behavioral repertoire by interactions with pathways for motor output (Baker et al., 2001; Manoli et al., 2006).

*fru*M may regulate courtship behavior by inducing subtle or major sex-specific changes in wiring and development of neurons critical for sexual behavior (Manoli et al., 2005; Stockinger et al., 2005). *fru*-P1-Gal4-driven expression of a membrane-targeted Green Fluorescent Protein (GFP) reveals that the *fru*-P1-defined neural network is essentially monomorphic between males and females, both in number and location of cell bodies, and in their gross projection patterns (Manoli et al., 2005; Stockinger et al., 2005), consistent with *in situ* analyses (Goodwin et al., 2000; Lee et al., 2000). Thus, either subtle changes in neural connectivity, or changes in neurophysiology account for the behavioral effects of *fru*M. Some exceptions are known, including the neurons that innervate the moLs (Currie and Bate, 1995), and a small group of *fru*M+ inter-neurons near the antennal lobe that are sexually dimorphic both in number and projection pattern (Kimura et al., 2005). In males, these neurons survive because of *fru*M-dependent inhibition of apoptosis (Kimura et al., 2005). Although it remains to be determined if the latter kind of sex-specific neuroanatomical differences have behavioral significance (Yu and Dickson, 2006), it is conceivable that the presence of these neurons is required for aspects of male courtship behavior, or that their presence inhibits aspects of female behavior.

fruM is a behavioral 'switch' gene

It has been postulated that *fru*M is the behavioral switch gene in *Drosophila* (Baker et al., 2001). Deletion of the sites required for Tra-regulated splicing generated dominant *Fru*M-expressing mutations (Fru^{M} and $Fru^{\Delta Tra}$) (Demir and Dickson, 2005). Fru^{M} hemizygous males behave as normal wild-type males. Fru^{M} females have notable male courtship activity, although they do not court as actively as Fru^{M} or wild-type males, nor do they carry out advanced steps of courtship such as attempted copulation (Demir and Dickson, 2005). In addition, Fru^{M} females are unreceptive to courting males, and make, but fail to lay eggs, suggesting that *fru*M suppresses female behavior and neural differentiation (Demir and Dickson, 2005). These data support previous inferences (Baker et al., 2001) that *fru*M is a significant component in switching between male and female behaviors.

These findings potentially suggest a model for the genetic control of sexual differentiation in *Drosophila* in which *dsx* governs the sex specificity of the soma, and *fru* controls the sex-specific nervous system (Fig. 1) (Baker et al., 2001; Demir and Dickson, 2005). In this model, the on/off state of *fru*M is the genetic switch between male and female behavior: when *fru*M is off, female behavior occurs by default; when *fru*M is on, male behavior is fully induced. Mutant phenotypes of *fru* and other genes suggest, however, that *fru*M does not act alone, and the switch between male and female behavior occurs as a result of neural and genetic interactions among multiple genes.

fruM does not act alone to control sexual behavior

Maleness retained despite being fruitless

As wild-type females lack both *fru*M and male courtship behavior, it follows, under the single-switch system described

above, that *fru*M null males would lack courtship behavior. This prediction is not met. In single-paired courtship tests, *fru*M null males are void of courtship toward both males or females, but when grouped on food, *fru*M null males generate relatively substantial pair-wise courtship or chaining behavior (Anand et al., 2001; Demir and Dickson, 2005; Shirangi et al., 2006). Thus, males have a neural potential for courtship behavior even in the complete absence of *fru*M. If this neural potential is present in females, it is suppressed by some mechanism.

Mutations in the *retained* (*retn*) gene, an ARID-box transcription factor (Gregory et al., 1996) expressed in a small subset of neurons in the CNS, lead to reduced receptivity to courting males and production of male-like courtship by females, indicating that *retn* represses male courtship behavior in females (Ditch et al., 2005). This courtship occurs in females expressing only the female, presumably non-functional, form of *fru*-P1 transcripts, and in females that completely lack P1 transcripts (Ditch et al., 2005). Thus, *Drosophila* females also have a neural potential for male courtship activity, even in the complete absence of *fru*M. In addition, *fru*M null males also increase courtship with loss of *retn* (Shirangi et al., 2006). Taken together, these data indicate that male and female *Drosophila* have an intrinsic *fru*-independent neural pathway for male courtship behavior that is repressed by *retn*.

Contradictions to the single-switch for behavior

Multiple lines of evidence suggest that additional sex-specific factors are required for complete regulation of male and female sexual behavior. In particular, males and females that are comparable in their state of *fru*M (either fully on or fully off), have different levels and kinds of male courtship. *fru*M null males court, although this courtship is entirely male-directed and notably reduced relative to wild type, but wild-type females, also lacking *fru*M, do not (Anand et al., 2001; Shirangi et al., 2006). Furthermore, *retn* mutant females (*fru*M off) generate less courtship behavior than *retn*;*fru*M null males (Shirangi et al., 2006). Fru^{M} females spend less time courting than do brothers of the same genotype (or wild-type males) (Demir and Dickson, 2005), and are significantly bisexual, courting both sexes (Fig. 2). In addition, Fru^{M} females are qualitatively dissimilar from their brothers in, for example, not attempting copulation (Demir and Dickson, 2005). This is likely not due to egg production or a female body, since, as noted by Hall (1979), gynandromorphs with a male nervous system and a female cuticle, even with an abdomen full of eggs, are often able to bend their abdomen sufficiently to achieve genital contact (Billeter et al., 2006a; Hall, 1979). These data suggest that a male-specific factor, a female-specific factor, or both function in the nervous system, in addition to *fru*M, to regulate male- and female-specific behaviors and neural differentiation. Sex-specific *dsx* is a candidate.

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dsx and the control of courtship behavior

During the period when *dsx* was the only known target of Tra/Tra2 regulation, experiments cast doubt on the sufficiency

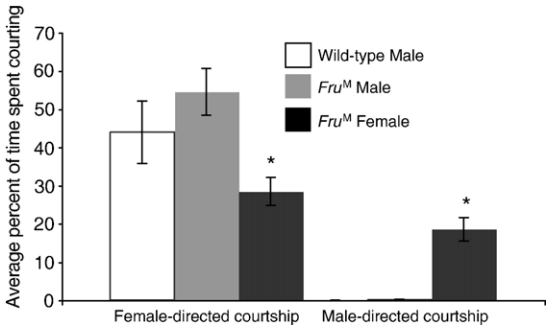

Fig. 2. *fru*M is not fully sufficient to induce normal levels of male courtship behavior directed towards females or males. Pair-wise courtship activity from wild-type males or *fru*M-dominant males or females (Fru^{M}/fru^{4-40}) was measured in a small Plexiglas chamber with wild-type female (left) or *fru*M null male (fru^{sat15}/fru^{4-40}) (right) as courtship targets. The average percent of time spent courting (±SEM) was determined in 10 min of observation for each pair. As published (Demir and Dickson, 2005), Fru^{M}/fru^{4-40} females spend less time courting females than do brothers of the same genotype or wild-type males (left). In addition, Fru^{M}/fru^{4-40} females display significant male-directed courtship, unlike control males (right). *fru*M null males were used as courtship objects to prevent female-directed courtship from the target male, which would complicate behavioral analyses. $*P<0.05$ versus *X*/*Y*; Fru^{M}/fru^{4-40} (Kruskal–Wallis ANOVA); for all genotypes, $N>12$.

of *dsx* for sexual behavior. McRobert and Tompkins (1985) showed that dsx^{-} males generate some male courtship while in pair-wise tests dsx^{-} females do not, indicating that sexual dimorphic behavior was maintained even as males and females had identical intersexual bodies. Taylor et al. (1994) further showed that females expressing *dsx*M but not *dsx*F have male morphology but do not court when tested pair-wise in chambers, while their male brothers of the same genotype are fully male in behavior. Taylor also showed that *dsx*M-expressing males (or wild-type males) have moLs while *dsx*M-expressing females do not (Taylor, 1992). Thus, the loss of *dsx*F or gain of *dsx*M does not induce robust male courtship or neural development in a *diplo-X* female, nor does the loss of *dsx*M fully ablate male courtship behavior.

Some evidence suggests that *dsx* functions in sexual behavior. dsx^{-} Chromosomal males display courtship behavior, but at reduced levels with defects in song production (Taylor et al., 1994; Villella and Hall, 1996). dsx^{-} Males are also mildly bisexual, independent of their intersexual pheromone profile (Villella and Hall, 1996). In addition, expression of *dsx*F in males by a heterologous heat-shock promoter suppresses courtship activity (Waterbury et al., 1999), suggesting that *dsx*F represses male behavior. Thus, although *dsx* is not the dominant switch for sexual behavior as it is for the cuticle, these data suggest a possible role.

Interpretation of these results is complicated by difficulties associated with an intersexual cuticle or with expression from heterologous promoters. The complete loss of *dsx* produces flies of both sexes that are sickly and abnormal in sexually dimorphic cuticle structures, including analia. Interpreting the role of *dsx* in sexual behavior is thus confounded by the use of such intersexual flies (Villella and Hall, 1996). As pointed out by Villella and Hall (1996), "it is possible that a hypothetically intersexual, or perhaps more generically defective, quality of

sensory structures is responsible for subnormal courtships exhibited by *XY dsx* mutants. The reception and initial inputting of reproductively relevant sensory stimuli could therefore be mediocre in these mutants." In addition, it is impossible to address the receptivity of dsx^- females since they cannot copulate using intersexual genitalia, and are less attractive to courting males due to a masculinized pheromone profile and non-female appearance. Thus, the appropriate test for *dsx* function in mating behavior in both sexes requires the use of flies with normally sexually differentiated external morphology.

dsx is a component of the sexual behavior 'switch' in dsx^+ animals

dsx and *fru* are the only known Tra targets. Thus, *dsx* is a prime candidate for the inferred additional sex-specific regulator of behavior. This is consistent with altered behavior of *dsx* mutants. Testing this hypothesis requires assaying the behavioral functions of *dsx* in animals with normal male or female morphology.

Use of a sensitized genetic background allows assays of normally recessive but deleterious mutations in a heterozygous condition. In males, behavioral sensitization has been achieved by lowering the level of *fru*M. Three different *fru* allelic combinations, weak to strong, in males with normal body morphology all show decreased courtship with loss of one copy of *dsx* (Shirangi et al., 2006). This clearly implicates *dsx*M as a positive factor for male behavior.

In females, sensitization has been done in two ways: loss of *retn* function, leading to male-like courtship activity and resistance to courting males, or gain of *fru*M. In *retn* mutant backgrounds, loss of one copy of *dsx*F increases male courtship activity and increases female resistance to mating (Shirangi et al., 2006). Similarly, heterozygosity for *dsx* increases courtship by *fru*M-expressing females (Shirangi et al., 2006). These data clearly implicate *dsx*F as a positive factor for female receptivity and a negative factor for male-like courtship, both in the presence or absence of *fru*M. The data from both sexes show that *dsx* is a true component in switching behavior between male and female states.

In addition to the behavioral experiments above, *dsx* has recently been shown to function with *fru*M in male-specific neural differentiation. *fru*M-positive serotonergic neurons in the abdominal ganglion of males (called SAbg or "serotonergic abdominal *g*iant neurons" (Billeter et al., 2006b)) innervate parts of the male reproductive tract (Billeter et al., 2006b; Lee and Hall, 2001; Lee et al., 2001) and are required for ejaculate transfer during copulation (Lee et al., 2001). *fru*M null males, like wild-type females, lack SAbg neurons (Billeter et al., 2006b; Lee and Hall, 2001), indicating that *fru*M is required for their development. However, Fru^M females do not display full male-like numbers of SAbg neurons (Billeter et al., 2006b), just as *fru*M is not sufficient to induce full male behavior in females (Demir and Dickson, 2005; Manoli et al., 2005) (Fig. 2). Expression of single isoforms of *fru*M only partially induced SAbg neurons in *fru*M null males and wild-type females (Billeter et al., 2006b), but, induction of SAbg neurons was more efficient in *fru*M null males than in wild-type (*fru*M off) females. These data suggest, as with male courtship behavior, that an additional sex-specific component is required for complete male-specific development of SAbg neurons. *dsx* is an obvious candidate. dsx^- Males have fewer SAbg neurons than dsx^+ males while dsx^- females gain some SAbg neurons, but have fewer than dsx^- males (Billeter et al., 2006b). The loss of these neurons in males indicates a positive role for *dsx*M, while the gain of these neurons in females indicates a negative role for *dsx*F, and the difference between mutant males and females is presumably due to *fru*M function. Thus, *dsx*M is necessary to induce male-specific differentiation of some SAbg neurons, and *dsx*F represses *fru*-independent SAbg development. The presence of SAbg neurons in dsx^- females is an example of male-like neural differentiation in the absence of *fru*M. Taken together, the data on sexual behavior and SAbg neurons strongly support the idea that both *dsx* and *fru* are sex-specific components of the sexual behavioral and neural switch.

Beyond fru and dsx: what other genes are required to pattern behavior?

Sex-specific neural and behavioral phenotypes from the loss of dissatisfaction

*fru*M encodes a set of transcription-factor-like proteins with a BTB putative dimerization domain and one of four pairs of Zn-fingers (Ito et al., 1996; Ryner et al., 1996), but no targets of, or factors altered in function by *fru*M are known. As an alternative to molecular searches for *fru*M targets, a way to identify functional activities downstream of known sex-cascade components is to search for mutations that have differential phenotypes in behavior of males and females. *Dissatisfaction* (*dsf*) is such a gene.

The loss of the *dissatisfaction* gene (*dsf*) induces sex-specific neural and behavioral phenotypes in both sexes. As noted in Table 1, like *dsx*, *dsf* acts as a pro-female/anti-male factor in females and a pro-male/presumably anti-female factor in males. dsf^- Males are bisexual and copulate inefficiently. *dsf* Mutant females are unreceptive to courting males and make but fail to lay normal eggs (Finley et al., 1997). Some of these phenotypes have neural correlates. dsf^- Males, but not females, have abnormal neuromuscular junctions (NMJ) in the fifth segment of the ventral abdomen (vA5) where synaptic swellings or boutons are notably larger and fewer in number relative to wild-type males and females (Finley et al., 1997). Furthermore, dsf^- females lack innervation at the uterine wall (Finley et al., 1997). These neural phenotypes correlate with sex-specific behavioral phenotypes in males (deficient abdominal bending during attempted copulation) and females (egg retention) and thus link neural defects with abnormal behaviors. *dsf* encodes a *tailless*-like nuclear receptor (Finley et al., 1998) that functions as a transcriptional repressor (Pitman et al., 2002), and is expressed in the nervous system of both sexes (Finley et al., 1998).

dsf is downstream of tra but independent of dsxM

Since males and females have synapses on vA5 muscles, the male-specific bouton phenotype allows tests of the position of *dsf* in the sex hierarchy. When masculinized by mutations in

Table 1
Summary of genes, and molecular and inferred genetic functions

Gene	Role of the gene product in a particular sex		Molecular function
	Male	Female	
fruitless	Pro-male Anti-female [a]	No obvious function	Set of BTB-Zn finger transcription-factor-like proteins in males only. No published direct targets.
doublesex	Pro-male Anti-female[a]	Pro-female Anti-male	Sex-specific proteins with identical DNA binding domains and sex-specific C-termini. Yolk protein only known direct target. Transcription activated by dsxF and repressed by dsxM.
retained	Pro-female Anti-male[a]	Pro-female Anti-male	ARID box transcription factor. *huckebein* and *zerknullt* known direct targets in embryos. *zerknullt* is repressed by *retn* in regulating behavior.
dissatisfaction	Pro-male Anti-female[a]	Pro-female Anti-male	NR2e class nuclear receptor. Known DNA-binding factor with transcriptional repressor activity.

Pro-male and pro-female refer to enhancement of any aspect of male or female sexual behavior.
Anti-male and anti-female refer to suppression of any aspect of male or female sexual behavior.
[a] Assays of female behavior in morphological males are not possible. Anti-female function of *fru*M is inferred from expression of *fru*M in morphological and chromosomal females. Anti-female aspect of *dsx*M is inferred from its role in repressing both yolk protein expression and female differentiation. Anti-female or pro-female functions of other genes in males are inferred by the observed correlation in females of anti-male and pro-female activities, or of anti-female and pro-male activities (as with *fru*M).

tra, *dsf* mutant females have defective synapses at vA5, like *dsf*$^{-}$ males (Finley et al., 1997). This reveals that the male-specific need for *dsf* depends on the state of *tra*. In the absence of *tra* function, *dsf* is required for proper neural differentiation at the NMJ of vA5. In the presence of *tra*, as in females, *dsf* is not needed for apparently normal development of the synapse, although subtle, female-specific abnormalities are not ruled out.

Regulation of *dsf* by *tra* is not mediated by *dsx*. *dsf* Mutant chromosomal females with *tra* active but expressing *dsx*M and not *dsx*F, have normal synapses at vA5 while their congeneric, *tra* off *X*/*Y* brothers have abnormal synapses (Finley et al., 1997). Thus, whereas the state of *tra* is relevant for *dsf* function, the state of *dsx* is not. This suggests that *dsf* acts downstream of *fru*, downstream of an unknown *tra* target gene, or is itself a *tra* target. The latter is unlikely as searches reveal neither alternative splice products of *dsf* nor clusters of *tra* regulatory sites as in *dsx* and *fru* (Finley et al., 1998).

The genetic relationship between dsf and upstream factors

Based on the data discussed above, we suggest that the need for *dsf* in vA5 neural development is determined by either *fru*M or by some unidentified gene *y* (Fig. 3). In males, *fru*M or gene *y* must independently establish a state making male vA5 neurons sufficiently different from the female state that *dsf* activity is critical for neural function or maintenance. In females, however, a default, *dsf*-independent pathway for apparently normal neural development at vA5 is induced (Fig. 3).

This model can also apply to *dsf* function in regulating male and female behavior. The loss of *dsf* induces sex-specific behavioral abnormalities in both sexes (Table 1) (Finley et al., 1997). We recently found that *dsf* mutant females display substantial male-like courtship behavior when aged (T.R. Shirangi, W. Dewitt, M. McK, manuscript in preparation). This suggests that in females, but not males, *dsf* is pro-female and anti-male, similar to *dsx*F and *retn*. However, given that in males, *dsf* is pro-male (and by analogy to *dsx*M, anti-female), *dsf* activity may be redirected by a sex-specific factor (i.e., *fru*M or gene *y*) to appropriately enhance male courtship behavior (Fig. 3). Epistasis experiments will undoubtedly clarify the genetic relationship between *fru*M and *dsf*.

An intrinsic neural pathway for male and female sexual behavior in Drosophila modulated by a sex-specific switch system

The data described in previous sections show that male behavior is possible in the complete absence of *fru*M (e.g., *fru*M null males), and even in the complete absence of both *fru*M and *dsx*M (e.g., *retn*$^{-}$ females). This strongly suggests an intrinsic genetic and neural pathway for male behavior that is independent of *fru*M, and present in both sexes (Fig. 4). We infer that a sex-non-specific factor (gene *x* in Fig. 4) specifies intrinsic *fru*M/*dsx*M-independent maleness to both sexes and thus accounts for the male-like behaviors observed in *retn* mutant females and *fru*M null males. Given that *retn* functions as a pro-female/anti-male factor in both sexes (Ditch et al., 2005; Shirangi et al., 2006), we infer that males and females also have intrinsic neural femaleness (Fig. 4). This intrinsic bipotential neural pathway is then pushed toward full maleness and away from femaleness by *fru*M and *dsx*M, or toward full femaleness and away from maleness by lack of *fru*M and presence of *dsx*F. *dsf* may function downstream of its sex-specific effector (e.g., *fru*M or lack thereof) to induce aspects of the male or female pathway.

We suggest *fru*-P1 neurons are a major component of the pathway that provides intrinsic capacities for male and female behavior. In this case, the key is not the products derived from *fru*-P1, it is the array of factors that drive *fru*-P1 expression in neurons that generate the complicated neural pathways necessary for sexual behavior. *fru*-P1 neurons are present in females as well as males and have strikingly similar gross projection patterns in both sexes (Manoli et al., 2005; Stockinger et al., 2005). In addition, their function and sex are critical for not only male behaviors, but also female behaviors (Demir and Dickson,

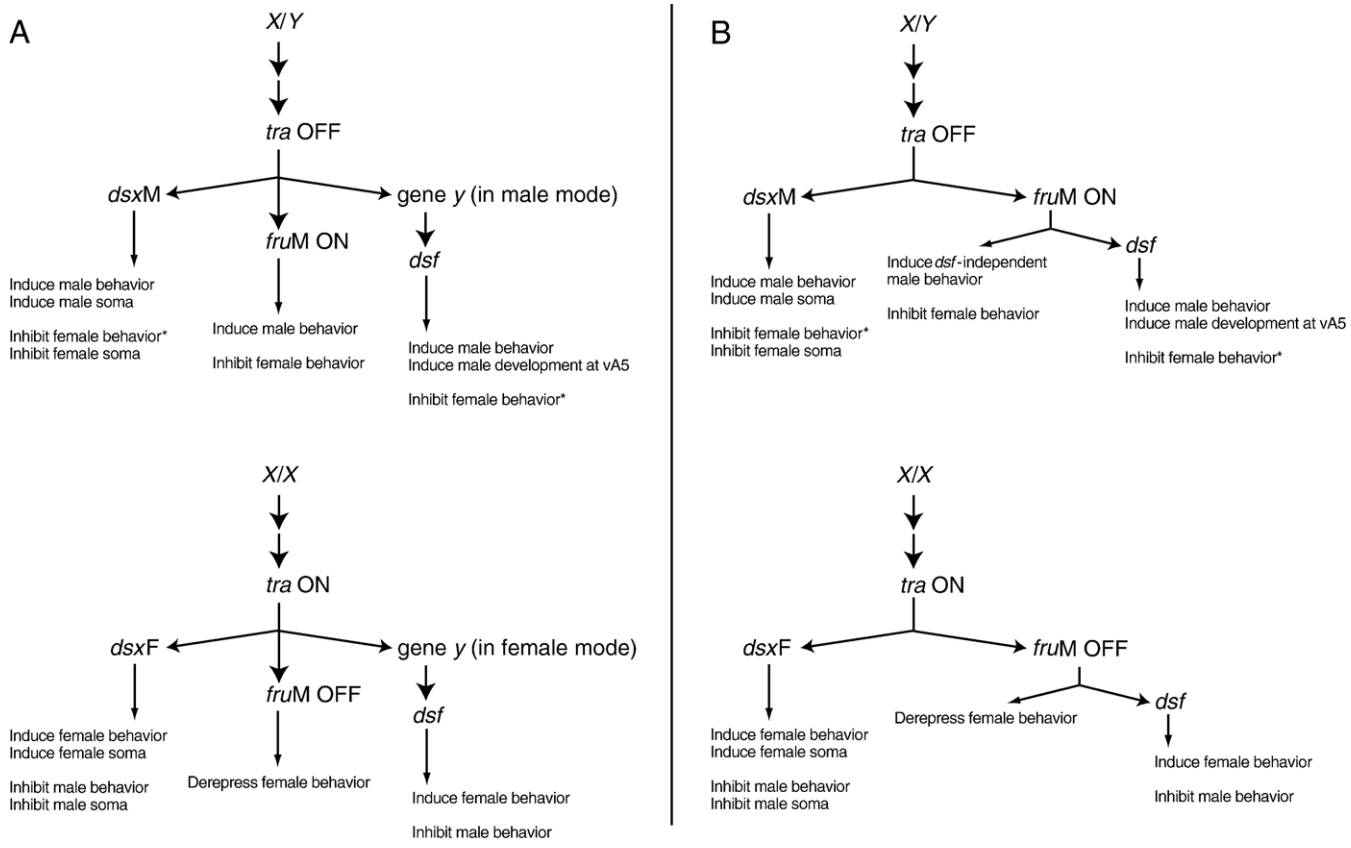

Fig. 3. *dsf* functions downstream of an unidentified sex-specific effector gene *y* (A) or *fru*M (B) to regulate male and female neural and behavioral development. In males (top), the male mode of gene *y* or *fru*M establishes a requirement for *dsf* to induce aspects of male behavior and neural development, and presumably inhibits female differentiation. In females (bottom), the female mode of gene *y* or the absence of *fru*M induces a requirement for *dsf* in female behavior and in inhibiting male behavior. *Given that *dsx*F and *dsx*M are antagonistic in all known functions (Nagoshi and Baker, 1990), and that *dsx*F promotes female behavior in females (Shirangi et al., 2006), we infer that *dsx*M inhibits female behavior. Similarly, we also infer that *dsf* inhibits female behavior in males.

2005; Kvitsiani and Dickson, 2006). *retn* constitutively feminizes the activity of *fru*-P1 neurons in both sexes, likely by action in cells not expressing *fru*-P1 (Shirangi et al., 2006). We suggest that this *retn* function involves direct interaction between *retn* and *fru* neurons, as both *retn* and *fru* neurons are present in the subesophageal ganglion and the abdominal ganglion (e.g., Shirangi et al., 2006). *dsx* also impacts the identity of *fru* neurons, with a significant number of cells expressing both *fru*-P1 and *dsx* (Billeter et al., 2006b). As discussed, *dsf* is a candidate as a downstream effector responding to the presence or absence of *fru*M to reinforce male or female differentiation. Thus, the cells defined by *fru*-P1 are the core of an intrinsic sexual behavior pathway that is shifted to a male or female state by the expression of sex-specific factors such as *fru*M and *dsx*.

The biological and evolutionary advantage of such a system is not conceptually new. A similar system exists in controlling aspects of the sexually dimorphic soma. Notably, *dsx* mutants of both sexes develop as identical intersexes indicating that males and females have intrinsic developmental pathways for the opposite sex. *dsx* Mutant females have rudiments of male-specific sex combs, intermediate male-like abdominal pigmentation, reduced (i.e., less female-like) *yolk protein* expression, and aspects of male genitalia and analia (Hildreth, 1965). Likewise, *dsx* mutant males form bristles on the sixth sternite (a female-specific morphology), have rudimentary (i.e., less-male-like) sex combs, are decreased (i.e., female-like) in abdominal pigmentation, are increased in *yolk protein* expression, and display rudiments of female genitalia and analia (Hildreth, 1965). The potential for *dsx* mutant males and females to develop morphology associated with the opposite sex is, in many cases determined by sex-non-specific genetic determinants. Sex-non-specific *hermaphrodite* (*her*) functions as an independent constitutive pro-female/anti-male factor promoting *yolk protein* expression and aspects of female cuticular development (Li and Baker, 1998a,b; Pultz et al., 1994). *dsx*F independently has similar effects. In males, *her*-dependent feminization is suppressed by *dsx*M (Li and Baker, 1998b). Thus, *her* defines an intrinsic pathway for aspects of female development that is enhanced by *dsx*F and countered by *dsx*M. This is formally similar to the function of *retn* as a constant pro-female factor, with *dsx* and *fru*M modifying the consequences of *retn* action. For other dimorphic morphologies such as sex combs, *her* and *dsx*F must both function, neither alone has an effect, to suppress male-specific development (Li and Baker, 1998b). Of particular interest, sex comb bristles in *her* or *dsx* mutant females are altered in morphology, orientation and number from the bristles at homologous positions on other legs. Thus, rudimentary sex

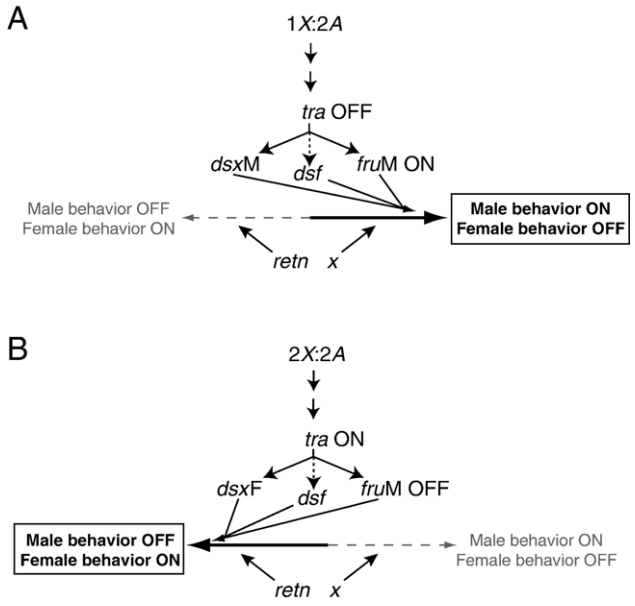

Fig. 4. A sex-specific switch system reciprocally modulates an intrinsic neural pathway for male and female behavior in both sexes. (A) In males, *dsx*M and *fru*M masculinize an intrinsic bipotential neural pathway (double-headed arrow), thus suppressing female behavior and fully activating male behavior. *retn* functions in both sexes as a constitutive pro-female/anti-male factor. An inferred factor (*x*) functions as a constitutive pro-male/anti-female factor that accounts for male-like courtship activity observed among *retn*$^{-}$ females and *fru*M null males. *dsf* functions downstream of *tra*, via *fru* or gene *y*, to promote male behavior in males. (B) In females, the presence of *dsx*F and the absence of both *fru*M and *dsx*M feminize an intrinsic neural pathway to induce full female behavior and suppress male behavior. Males and females have an intrinsic neural capacity for behavior of the opposite sex (dashed arrow), which is specified by factors like *retn* (for female) and "*x*" (for male). *dsf* functions downstream of *tra*, as noted above, to promote female behavior in females. The dotted line from *tra* to *dsf* indicates that the interactions are indirect.

combs in *dsx* mutants point to a basal genetic pathway leading to intermediate sex comb development.

With pressure to generate large phenotypic differences between the sexes, it is genetically economical to have regulatory gene(s) reciprocally regulate an intermediate sex-non-specific state. For example, as noted by Li and Baker (1998b), when considered individually, *dsx*F generates an approximately 30-fold increase in *her*-dependent yolk protein expression while *dsx*M suppresses *her*-dependent activation approximately 180-fold. However, when considered together, *dsx*F and *dsx*M reciprocally modulate *yolk protein* expression approximately 2000-fold (Li and Baker, 1998b). This system generates large sexual differences with minimal genetic expenditure. Thus, "in the presence of selective pressures on both sexes in evolution, one way to increase sexual dimorphism is to have female- and male-specific products of regulatory genes that each have active roles in modifying the effects of pre-existing non-sex-specific regulatory systems in opposite ways, thus generating dramatic sex-specific features" (Li and Baker, 1998b). The same can be applied to sexual behavior. A sex-non-specific basal neural pathway for behavior provides the *fru-dsx*-switch system an efficient means to achieve a large difference between males and females, especially with selective pressures on both sexes.

Conclusion

The convergence of work from a number of laboratories and systems makes it possible to develop an integrated view of the control of sex-specific neural development and behavior in *Drosophila*. We suggest that the core of this pathway is an intrinsic basal pathway with both male and female behavioral potential. This pathway is substantially composed of *fru*-P1-expressing cells, with potential contributions from cells in which *dsx* is expressed. Intrinsic maleness is inferred to result from the activity of some as yet unknown pro-male factor (gene *x* in Fig. 4), while the female pathway appears to be partially defined by the pro-female activities of *retn* neurons acting on the *fru*-P1-defined pathway. This basal pathway is then modified by the actions of both *fru* and *dsx*. *fru*M is a major component of switching from female to male behavior, while the lack of *fru*M is permissive for female behavior. *dsx*, in switching between male and female RNAs and proteins, alters both body differentiation and behavior, including changing the state of known *fru*-P1-expressing neurons.

It is important to note that all factors discussed, when active, are either pro-female and anti-male, or pro-male and anti-female (Table 1). This suggests, consistent with the 'basal pathway model', that changes in sexual behavior result from changing a single pathway between male and female forms, rather than by independent control of separate pathways. This is supported by the highly homologous patterns of *fru*-P1 neurons in males and females (Manoli et al., 2005; Stockinger et al., 2005) and the demonstrable role of *fru*-P1 neurons in both male and female behaviors (Kvitsiani and Dickson, 2006). Although these various pro-male or pro-female activities are inferred ultimately to alter the state and function of *fru*-P1 neurons, at least for *retn* activity on *fru*-P1-mediated pathways, there is evidence of control by cell–cell interaction rather than intracellular interactions (Shirangi et al., 2006).

Some genes, like *fru* and *dsx* switch between male and female forms under direct control of *tra*, while *retn* is pro-female anti-male in both sexes. *dsf*, by contrast, switches between masculinizing and feminizing activities, but does so in response to the state of some factor downstream of *tra* (Finley et al., 1997), either *fru* or some as yet unidentified gene (Fig. 3). If *dsf* is downstream of *fru*, it is the first gene known to contribute as a female regulatory component acting in response to the lack of *fru*M, and the first gene known to mediate the male state of *fru*M. However, this difference is mediated, it is not at the level of transcription (Finley et al., 1998), suggesting the possibility of direct physical interaction between FruM and Dsf proteins in males, and FruM-independent activities of Dsf, potentially at the same genes, in females. Although the original *in situ* analyses indicated that *fru*M and *dsf* do not overlap (Finley et al., 1998), the low level of expression of *dsf* RNA leaves open the possibility that *fru*M and *dsf* overlap in single key cells in, for example, the neurons innervating vA5. Thus, it is not definitive whether *dsf* acts in *fru*-P1 cells.

The existence of a basal, bipotential behavior pathway intermediate between male and female states is relatively new in studies of *Drosophila* sexual behavior. Although a recent

idea, there is certainly a precedent for an intrinsic intermediate basal state in the sexual differentiation of the body. This parallel is made stronger by the role of *dsx* in both pathways, as well as by the example (*yolk proteins*) that an intermediate starting state allows a much larger phenotypic difference between two usually mutually exclusive states than might easily be reached in having two pathways starting from zero (Li and Baker, 1998b).

Molecular and neural connectivity questions abound as more are known and more tools become available. Just considering *dsx* and *fru*, the two known genes of the switching system, we currently know of only one direct molecular target (*yolk proteins*) for either. What other genes do they control and how is that control then integrated with factors like *dsf*?

It is assumed that *fru* and *dsx* are together the only genes directly regulated by *tra* for sexual behavior. This is consistent with inferences from the combined phenotypes and with the lack of any clustered *tra* regulatory sites, as identified in *dsx* and *fru*, associated with any other gene sequenced in *Drosophila*. This inference is still based on lack of evidence. For example, the two-gene model for control of behavior predicts that expressing both DsxM and FruM would be sufficient to fully masculinize female behavior, even with *tra* active. This has not been tested, but should be possible with today's technology. Similarly, the assumption that only *dsx*-repeats as found in *dsx* and *fru* are the only possible sites of regulation by Tra is plausible but only an assumption. For example, Tra2 and RBP1, SR proteins key to binding the *dsx* and *fru* repeats and to regulation of splicing (Heinrichs and Baker, 1995; Lynch and Maniatis, 1995, 1996; Tian and Maniatis, 1993), need not be limited to those sites, nor, *a priori*, is Tra regulation limited to these two sex-non-specific SR proteins. Unlike *tra2*, other key SR proteins may be lethal when absent, thus avoiding most genetic screens for behavioral mutants. This possibility is, in principle, testable by comparing phenotypes of *tra* mutant, *tra2* mutant, and double mutant females for their full array of male courtship behaviors relative to wild-type males.

We have discussed recent works that collectively demonstrate at least some of the components required for full genetic and sex-specific induction of male or female courtship behavior. Male and female behavior in *Drosophila* occurs through neural and regulatory interactions between multiple sex-specific and sex-non-specific pathways. We can now address the molecular and neural mechanisms that account for the genetic interactions observed in the experiments discussed in this review.

Note added in proof

After submission two reports related to this review appeared. Certel et al. (2007) show that particular FruM-positive, octopaminergic neurons within the suboesophageal ganglion regulate a male's decision between male-directed courtship and aggressive behaviors in response to sensory input. Lazareva et al. (2007) infer a role for *dsx*M, but not *fru*M, in the fat body for enhancement of male behavior via a hormone-like activity.

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