Genetic Basis of Male Sexual Behavior

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ABSTRACT: **Male sexual behavior is increasingly the focus of genetic study in a variety of animals. Genetic analysis in the soil roundworm *Caenorhabditis elegans* and the fruit fly *Drosophila melanogaster* has lead to identification of genes and circuits that govern behaviors ranging from motivation and mate-searching to courtship and copulation. Some worm and fly genes have counterparts with related functions in higher animals and many more such correspondences can be expected. Analysis of mutations in mammals can potentially lead to insights into such issues as monogamous versus promiscuous sexual behavior and sexual orientation. Genetic analysis of sexual behavior has implications for understanding how the nervous system generates and controls a complex behavior. It can also help us to gain an appreciation of how behavior is encoded by genes and their regulatory sequences.** © 2003 Wiley Periodicals, Inc. J Neurobiol 54: 93–110, 2003

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INTRODUCTION

The primary function of the male sex is to fertilize the female. Often this is accomplished through expression by the male of a special set of behaviors that serve the purpose of bringing the two sexes and their gametes together. In many species, the successful execution of these behaviors is essential for reproduction. Hence, it is not surprising that a significant portion of the nervous system and the behavioral repertoire it generates may be dedicated to the successful accomplishment of this task. Species specificity of mating behavior often contributes to species isolation. Because the process of speciation, and hence of evolution, requires that barriers to cross-fertilization be erected around populations of individuals, understanding how sexual behavior evolves is important for understanding the mechanism of evolution.

Male sexual behavior includes copulation as well as behavioral steps preceding copulation that, among other things, allow the male to locate and detect a mate, assess a potential mating partner's suitability, and stimulate in the partner a receptive response. Expression of these behaviors is integrated and prioritized within the overall behavioral repertoire of the animal, which includes many additional, incompatible behaviors, some essential for individual survival. Analysis of male mating behavior inevitably leads, therefore, to a consideration of the poorly understood neural mechanisms known as drives, which bring about the adaptive integration of different complex sets of behaviors.

Genetics provides a uniquely powerful means to begin to dissect a complex biological phenomenon such as sexual behavior. By leading to the identification of genes required for expression of behavior, genetic analysis helps to determine what circuits are involved and what molecular components give these circuits their functions. Understanding the circuits and molecular components underlying male sexual behavior can lead to an appreciation of how the nervous system directs a complex mechanical task. Evolution has selected for effective mating, and the male nervous system encodes behavioral algorithms that generate a robust outcome that reliably overcomes the inevitable vicissitudes of life. Perhaps lessons can be learned here that will have practical engineering applications in the design of automata. Of course, greater understanding of the function and regulation of the behavioral circuits involved in mating also has potential implications for the medical treatment of sexual dysfunction in man.

Beyond an appreciation of mechanism and all that

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Table 1

Gene	Gene Product	Behavioral or Nervous System Function
C. elegans		
cat-2	tyrosine hydroxylase	dopamine biosynthesis
egl-1	general cell death gene	kill HSN neurons in male
egl-5	Hox family transciption factor	ray differentiation
egl-19	L-type voltage-gated calcium channel	copulation (prolonged spicule protraction)
lin-32	bHLH transcription factor	ray development
lov-1	putative transmembrane protein w/seq. similarity to PKD1	copulation (response, location of vulva)
mab-23	DM-domain family transcription factor	multiple aspects of male differentiation, copulation
mab-3	DM-domain family transcription factor	multiple aspects of male differentiation, copulation
mab-5	Hox family transcription factor	ray development
mab-9	T-box family transcription factor	development of spicules, neurons, etc.
mod-5	serotonin reuptake transporter	sex drive (mate searching)
pkd-2	Ca^{++} channel with similarity to PKD2	copulation (response, location of vulva)
tra-1	Zn-finger transcription factor	sex determination (hermaphrodite development)
unc-29	nicotinic acetylcholine receptor	copulation (rapid spicule prodding)
unc-38	nicotinic acetylcholine receptor	copulation (rapid spicule prodding, prolonged spicule protraction)
unc-68	sarcoplasmic calcium channel	copulation (rapid spicule prodding)
unc-77	unknown	drive (mate searching)
Drosophila		
amnesiac	neuropeptide	experience-dependent modification of drive
courtless	ubiquitin-conjugating enzyme	drive
dissatisfaction	nuclear hormone receptor family transcription factor	aspects of courtship, copulation
don Giovanni	unknown	induction of female rejection signal, response
doublesex	DM-domain family transcription factor	many aspects of male development, minor role in behavior
dunce	cAMP-specific phsophodiesterase	experience-dependent modification of drive
fruitless	BTB Zn finger family transcription factor	male differentiation of CNS
he's not interested	unknown	drive
lingerer	unknown	copulation
period	component of fly circadian oscillator	courtship (singing)
rutabaga	adenylate cyclase	experience-dependent modification of drive
stuck	unknown	copulation
technical knockout	mitochondrial ribosomal protein	courtship
tra	splicing factor	sex determination (female development)
mouse		
DMRT1	DM-domain family transcription factor	sex determination (testes development)
TRP2	TRP family ion channel	discrimination of the sexes
αER, βER	estrogen α and β receptor types	male sexual behavior
prairie vole		
V1aR	arginine vasopressin (AVP) V1a receptor	affiliative sexual behavior (olfactory investigation and grooming)

this implies, understanding of the genes and gene expression patterns leading to expression of sexual behavior raises another broad question of general interest: how is the behavior encoded in the DNA? This issue arises with regard to any behavior, but sexual behavior, because of its irrelevance to individual survival, dimorphism between the sexes, species-specificity, obvious governance by a drive mecha-

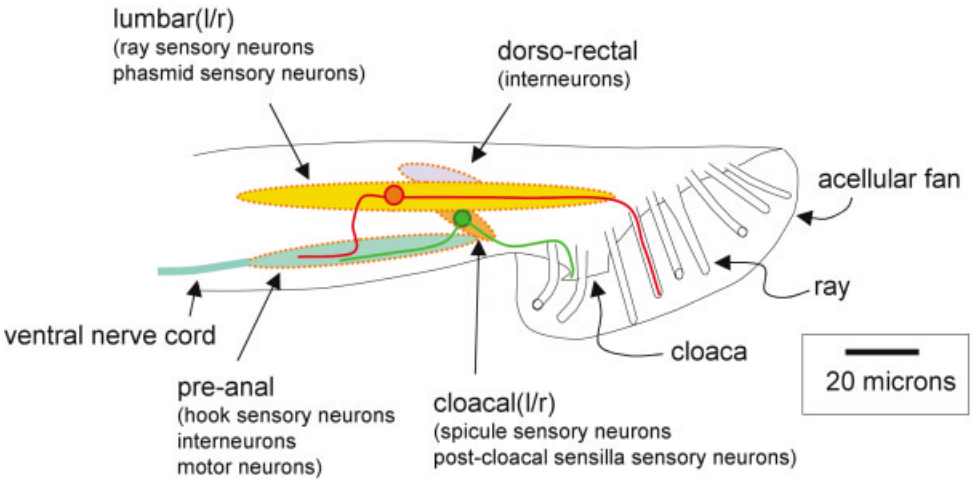
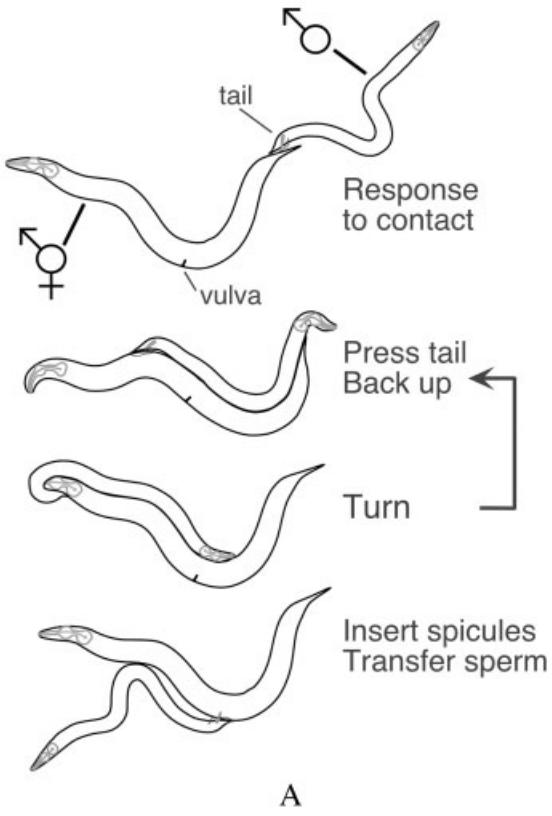


Figure 1 Ganglia of the *C. elegans* male tail. The drawing shows external features in a lateral view of the tail, including the sensory rays on one side. Not shown are the internal spicules; during mating, these are projected through the cloacal opening and inserted into the hermaphrodite vulva. Male-specific neurons are present in six separate ganglia. Two representative sensory neurons are shown: a ray sensory neuron, with its dendrite extending into one of the 18 rays, and a hook sensory neuron, with its dendrite in the hook anterior of the cloaca. Both send axonal processes into the preanal ganglion. There are additional sensory neurons in the spicules, as well as interneurons and motorneurons. Most of the complex circuitry that directs mating behavior is in the preanal ganglion. Some male-specific axons extend through the ventral cord into the nerve ring, where there is additional male-specific circuitry of unknown extent.

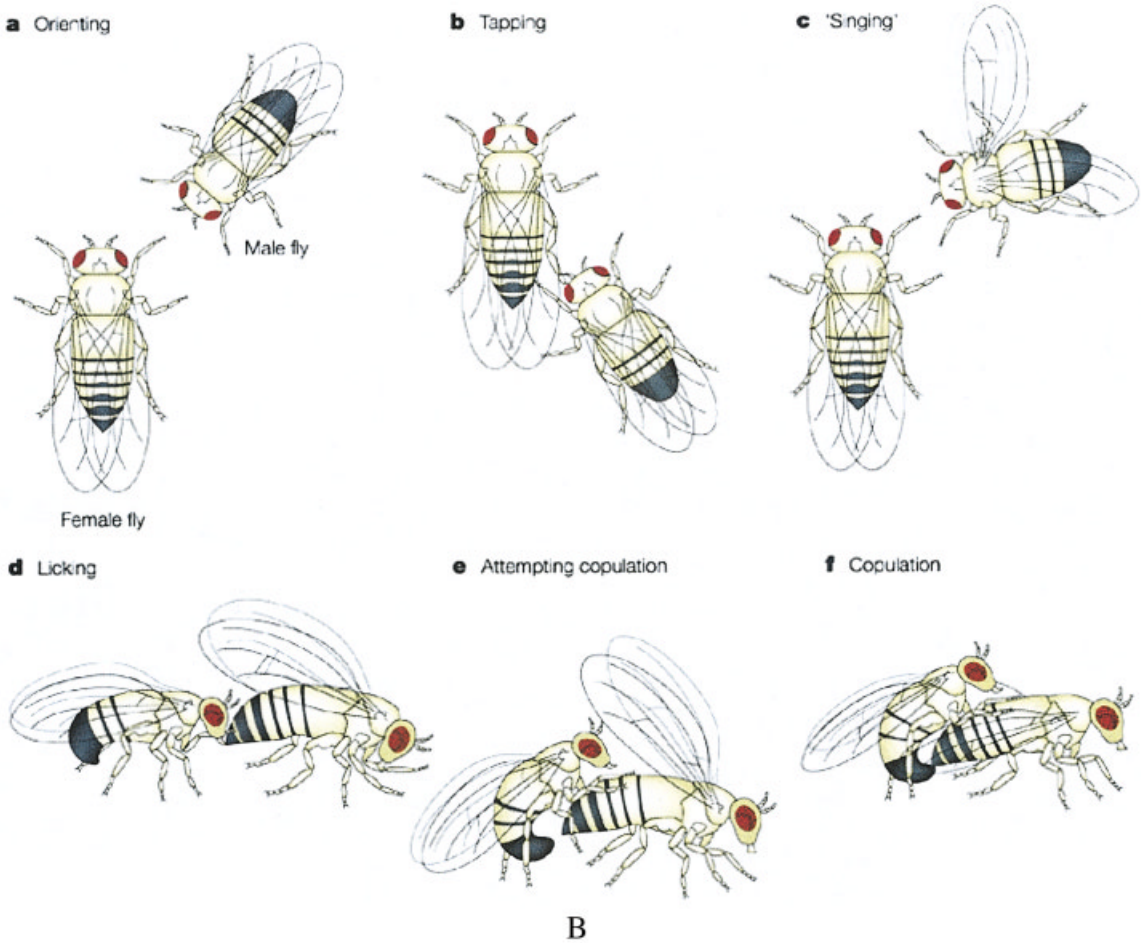
nism, and tight focus on a clear and specific goal, appears to offer a particularly intriguing example. Concerning as it does the process by which a digital code of four base pairs gives rise to a cellular system out of which the four-dimensional phenomenon of a behavioral pattern emerges, while allowing at the same time for modulation of this behavioral pattern by prior experience, the abstract question of coding is less of interest from the practical standpoint than from an intellectual and philosophical one. Are there "behavioral" genes, that is, genes identifiable with and devoted to specific complex behavioral patterns? Or does a complex behavior arise so diffusely from the aggregate activities of the ensemble of developmental and nervous system genes that no one gene may be associated with a particular behavioral pattern? Are natural behavioral variants genetically based or do they arise from stochastic perturbations of development or from environmental influences or experience? Adaptive novelties in behavior must originate from changes in the genetic code, while contained within the genetic programs of present day animals there is likely evidence of their evolutionary history. Some observable behavioral phenomena may indeed be comprehensible only in light of this evolutionary history. Given the vast complexity of the nervous system, both anatomically and in terms of its functional output and plasticity in response to experience, achieving a satisfying understanding of sexual behavior in terms of a four-letter code would appear to be a daunting prospect, but it is likely not an impossible one.

In this review we discuss the genes required for male sexual behavior and insights into behavioral mechanisms that have emerged through the identification and analysis of mutants. The genes mentioned are listed in Table 1. We restrict our focus primarily to sexual behaviors related more-or-less directly to the accomplishment of fertilization. We do not include the many social and sexual behaviors expressed by the males of many species that are related to competition with other males, including display, territoriality, and conflict, or male contributions to care of offspring.

Most of the research reviewed is on the fruit fly *Drosophila* and the nematode *Caenorhabditis elegans*, but we cover the genes by behavioral category and type of function, rather than by organism. Because of its genetic accessibility and long history as a laboratory animal, most studies of the genetics of sexual behavior have been carried out in *D. melanogaster* and its congeneric relatives. A large number of mutations affecting courtship and copulation have been studied, and the reader is referred to recent reviews for a full discussion of the original literature (Hall, 1994; Greenspan, 1995a; Yamamoto et al., 1997; Ferveur, 1997; Greenspan and Ferveur, 2000;


tail
Response
to contact
vulva
Press tail
Back up
Turn
Insert spicules
Transfer sperm

A


a Orienting
Male fly
Female fly
b Tapping
c 'Singing'
d Licking
e Attempting copulation
f Copulation

B

Figure 2

Sokolowski, 2001). *C. elegans*, having two orders of magnitude fewer neurons than *Drosophila,* is a far simpler model for dissecting the elementary steps of a behavioral sequence and identifying their underlying cellular basis. While there are many studies of male sexual behavior in vertebrates, these are largely behavioral and physiological, rather than genetic (see Kelley, 1988; Hull et al., 2002). Naturally occurring variation is observed in virtually all animals in such phenomena as partner-gender choice, mate bonding, copulatory behavior, and rearing of offspring (Bagemihl, 1999). Such variation raises the question of whether it is genetically based or the result of developmental variation or experience. In humans, variation among individuals in sexual development, identity, orientation, and behavior arouses much interest, which has lead to studies providing insight into the mechanism of sex determination and into the possible genetic basis of sexual orientation. Studies of monogamous mating versus promiscuous mating in prairie vole species has demonstrated, counter-intuitively perhaps but encouragingly, that in spite of their apparent subtlety and complexity, even social and sexual behaviors of mammals can be associated with defined sequences in the genetic code.

SEXUAL DIMORPHISM IN THE NERVOUS SYSTEM

C. elegans

The most fully described nervous systems in terms of sexual development are those of *C. elegans* and the related freeliving nematode species *Panagrellus redivivus*. In these species, the male nervous system is some 25% larger than that of the hermaphrodite and has extensive circuitry not found in the other sex (Sulston and Horvitz, 1977; Sulston et al., 1980; Sternberg and Horvitz, 1982). In *C. elegans*, all the neurons in both sexes are known, together with their lineal relationships to one another and to embryonic progenitor cells (Sulston and Horvitz, 1977; Sulston et al., 1980, 1983). In the 302-neuron nervous system of the adult hermaphrodite, connectivity among the neurons has been established from electron micrographs of serial thin sections (White et al., 1986). The male nervous system contains almost all of the same neurons as that of the hermaphrodite plus an additional set of 79 neurons and 36 neuronal support cells (Sulston et al., 1980). Connectivity in the male nervous system remains to be determined.

Circuitry involving most of the male-specific neurons is present in a large ventral ganglion just anterior of the cloaca, the preanal ganglion (Sulston et al., 1980) (Fig. 1). Sensory inputs from a variety of specialized sensory cells provide input to the central circuitry and guide the succession of behavioral responses. Output is in part through muscles common to both sexes, and in part through specialized sex muscles in the posterior region. Some male-specific interneurons send axons from the preanal ganglion through the ventral cord up to the head, where they enter the nerve ring (unpublished observations). These presumably play a role in integrating copulatory behavior within the overall behavioral repertoire of the animal. Thus, in addition to wiring within the preanal ganglion and other tail ganglia, there is male-specific circuitry in the nerve ring, what serves as a CNS in nematodes. The extent to which there are also sex-specific changes in the wiring of CNS and other neurons common to both sexes is unknown. Studies of gene expression have shown that there are sexual differences between neurons found in both sexes (Troemel et al., 1995).

In spite of having such an extraordinarily small number of neurons when compared with other animals, the *C. elegans* male is capable of surprisingly complex and sophisticated sexual behavior (Fig. 2). Copulatory behavior is evoked when sensory neurons in the tail detect contact with the hermaphrodite. Such contact initiates a stereotyped series of behavioral steps that lead up to insertion of the male's spicules into the hermaphrodite vulva followed by ejaculation (Loer and Kenyon, 1993; Liu and Sternberg, 1995; Emmons and Sternberg, 1997). Little or no reciprocal behavior is apparent on the part of the hermaphrodite. The roles played by male-specific tail sensory neurons, interneurons, and motor neurons in each of these steps were determined by examining male behavior after individual cells or subsets of cells were eliminated by laser microbeam ablation (Loer and Kenyon, 1993; Liu and Sternberg, 1995; J. Sulston, personal communication).

Cell ablation and genetic studies verify that the *C.*

Figure 2 Mating behavior of *C. elegans* (A) and *Drosophila* (B). In both species, mating may be analyzed in terms of a series of behavioral steps, as shown. Part (B) reprinted by permission from Nature Reviews Genetics (M. B. Sokolowski, Nature Reviews Genetics 2:879–890) © 2001 Macmillan Magazines Ltd.

elegans mating behavioral program consists of an ordered series of steps, each comprising a set of simple motor actions. Execution of each step is triggered by sensory inputs and tends to lead to acquisition of new inputs that will trigger an advance of the program to the next step. However, the order of execution of steps is not obligatory—the program can jump forward to a later step, skipping over intervening steps, or backup and restart the sequence from an earlier step if required. Sensation of the hermaphrodite body by the rays causes a halt to forward motion, arching of the posterior body to press the tail ventral side against the hermaphrodite, and backwards swimming. If a male lacks rays, this response to the hermaphrodite can also be triggered, albeit inefficiently, by sensory neurons in the hook and postcloacal sensillae. Thus, there is provision for redundancy in sensory input, and although the circumcloacal sensory neurons are primarily involved in later steps of the program (identifying the vulva and triggering spicule insertion), they can have a different effect on behavior if the animal is at a different stage of the program. If the male encounters the end of the hermaphrodite before locating the vulva, his predicament is sensed by the rays, which trigger a deep ventral bend of the tail to bring the male around to the other side. Sensation of the vulva redundantly by hook and postcloacal sensillae halts backwards swimming and initiates an attempt at spicule insertion. Spicule insertion has been subjected to more detailed analysis and itself consists of a series of substeps: a rapid (7 Hz) prodding, followed by, if the tips of the spicules breach the vulval opening, a greater protraction that results in a penetration of the vulva that is sustained while ejaculation takes place (Garcia et al., 2001). Advancement of the program from periodic to sustained contraction of the spicule protractor muscles is triggered by a sensory neuron within the spicules themselves.

In addition to copulatory behavior, *C. elegans* males exhibit specific behaviors that appear to be associated with locating a mating partner. Simon and Sternberg (2002) have shown that males respond to a short-range diffusible signal from the hermaphrodite by more frequently reversing direction of locomotion, a response that tends to keep them in the hermaphrodite's vicinity. The cue is sex-specific, being generated only by hermaphrodites and eliciting a behavioral response only in the male. Lipton and Emmons have observed in the laboratory that when no hermaphrodites are present, adult males exhibit a wandering behavior that may serve the function of locating a distant hermaphrodite. If adult males are isolated on food, they will in time wander away from the food source rather than remaining indefinitely and feeding. This behavior is not exhibited by adult hermaphrodites or by juveniles of either sex. Because this behavior is blocked by the presence of hermaphrodites, it may be a mate-searching behavior. Thus, the male's locomotion is programmed to facilitate mate location and is modifiable by sexual cues.

Drosophila

In contrast to *C. elegans*, studies so far in fruit flies have not identified a significant, exclusively male central neural structure, or an extensive set of male-specific neurons. However, this observation may not be generalizable to all insects; for example, male houseflies have a specialization in their compound eye (Hardie, 1986). In *Drosophila*, much of male behavior may emerge from cells or circuits present in both sexes that are functionally modified to express two forms of behavior. Male mating behavior begins with a series of courtship actions: orienting (the male orients himself to the female), following (the male follows the female if she moves), tapping (the male touches the female with his foreleg), "singing" (the male vibrates his extended wing), licking (the male licks the female genitalia), and finally tail curling and copulation (Hall, 1994; Greenspan, 1995a) (Fig. 2). These steps are induced by visual, odorant, and gustatory cues. Anatomical correlates in the peripheral nervous system include differences in male antennal structure and in the number of chemosensory sensillae on the legs and maxillary palps. In the brain, there are male-female differences in axon morphology of the projections of leg sensory neurons into the thoracic ganglion (Possidente and Murphey, 1989), in the number of cells in the mushroom bodies (Technau, 1984), and in motor neurons that innervate genital muscles (Taylor, 1989a,b). Apart from these differences, however, the brains of male and female flies look remarkably similar.

Although extensive sexual dimorphism may not be apparent in the fly nervous system, the fly brain is nevertheless genetically specified in male and female forms, as males that have never seen another fly are attracted to females and know how to mate. Temperature shift studies with a temperature-sensitive allele of the *tra-2* sex- determination gene have shown that in the absence of the female-determining sex- determination signal, events occur during the late pupal stage that result in male instead of female adult behavioral patterns (Belote and Baker, 1987; Arthur et al., 1998). Specific brain regions or other parts of the nervous system that must develop along the male pathway to give male behavioral patterns have been identified in numerous studies of mosaic animals gen-

erated by a variety of techniques that take advantage of the cell-autonomous nature of the sex-determination system (see Greenspan and Ferveur, 2000). For example, specific glomeruli in the antennal lobe must be male for an animal to discriminate male from female, the posterior dorsal brain must be male for orientation, tapping, following, wing extension, and licking, whereas for attempted copulation the thoracic ganglia must also be male. In another approach, neurons involved in sex-specific pathways have been visualized by analyzing the expression patterns of genes such as *fruitless* (*fru*) and *dissatisfaction* (*dsf*) that encode transcription factors required for male sexual behavior (discussed further below). *fru* and *dsf* are expressed in restricted regions of the brain consistent with the known functional regions identified in mosaic studies. Many of the neurons expressing these proteins are present in both males and females.

Vertebrates

Sexual dimorphism in the brains of vertebrates has been extensively studied and described in many species (see Gorski, 2000). In mammals, including humans, differences may be seen in a number of areas, including in the accessory olfactory bulb, the medial preoptic nucleus of the hypothalamus (MPOA), the sexually dimorphic nucleus of the preoptic area, the interstitial nuclei of the hypothalamus 2 and 3, and the bed nucleus of the stria terminalis (Meisel and Sachs, 1994; Swaab and Hofman, 1995; Gorski, 2000). The MPOA has been implicated in the control of consummatory aspects of male mating behavior in many different vertebrates, including rodents and reptiles. Neurons in the MPOA serve to coordinate genital reflexes and enhance species-specific motor patterns during copulation (Hull et al., 1999). Attempts have been made to establish the significance of sex-specific differences in brain morphology by documenting correlations with sexual orientation in humans (Swaab and Hofman, 1995). The most striking vertebrate brain sexual dimorphisms involve the song nuclei of songbirds (Arnold, 1997; Brenowitz, 1997; Schlinger, 1998; Nealen and Perkel, 2000). In zebra finches and other songbirds where only males sing a courtship song, learned from older male tutors, brain nuclei present in males but not in females are responsible for both the acquisition of the courtship song and its execution. In species where both sexes sing, such conspicuous dimorphism is absent.

With vertebrates, especially mammals, the question becomes acute whether sexual behavior is innate or learned. If sex-specific behavior is innate or genetically specified, does it arise from sex-specific differences in brain anatomy and ultrastructure (organizational effects), or is it the result of influences, particularly hormonal influences, on circuits present in both sexes (activational effects) (Gorski, 2000)? Observations such as those in rats, for example, that males sometimes exhibit lordosis, and females sometimes mount, strongly imply a commonality of circuitry in the two sexes. In adulthood, behavior can be modified by hormone administration. In humans, libido is increased by testosterone and decreased by selective serotonin reuptake inhibitors (SSRIs). All these observations indicate plasticity of behavior and could be consistent with a lack of dedicated wiring. However, plasticity during adulthood need not imply common circuitry. In the song nuclei of male songbirds, annual hormonal changes result in changes in neuronal structure. In female rats, the number of hippocampal synapses changes over the 4 day estrous cycle. Thus the distinction between developmental and functional categories may be blurred. Developmental influences of hormones have been extensively documented (see reviews by Kelley, 1988; Arnold, 1997; Schlinger, 1998; Hull et al., 1999; Pfaus, 1999; Gorski, 2000). Thus it remains an open question how plastic and continuously responsive to hormones the brain is.

DEVELOPMENTAL GENES REQUIRED FOR MALE SEXUAL BEHAVIOR

Given that there are male-specific neurons and brain structures, it is expected that some of the genes required for male sexual behavior are developmental genes involved in generation of male parts of the nervous system. Because it is possible to observe cellular-level development of the nervous system in *C. elegans*, this class of genes can be clearly identified. Most of the male-specific nervous system arises postembryonically from the divisions of a handful of blast cells lying in three epidermal tissues in the tail, the rectal epithelium, the ventral epidermis, and the lateral epidermis (Sulston et al., 1980). The rectal epithelium generates the spicules and their associated neurons, neurons of the postcloacal sensilla, and interneurons in the preanal ganglion; the ventral epidermis generates the hook and its associated sensillae; and the lateral epidermis generates the rays. The male-specific musculature also arises postembryonically from the sex myoblast, which generates different muscles in the two sexes. In addition to male-specific cell lineages, there are also modifications of common cell lineages by sex-specific programmed cell deaths. Four male-specific sensory neurons in the head die in

the hermaphrodite, while cells that differentiate into two neurons that innervate the hermaphrodite vulva die in the male.

One approach towards identification of genes required for male development and sexual behavior has been to identify mutations that have their primary effects in the male and cause infertility. The first such male-specific mutations in *C. elegans* were identified by Hodgkin (1983). Several of the genes defined, termed *mab* genes (for male abnormal), were subsequently found to encode developmental regulatory proteins utilized by the developmental programs that generate the male-specific nervous system and other copulatory structures, as well as by developmental programs in the hermaphrodite. Examples are *mab-5*, which encodes a member of the *C. elegans Hox* homeodomain transcription factor family (necessary for development of the rays) (Costa et al., 1988), and *mab-9*, which encodes a T-box family transcription factor (necessary for cell fate specification in the rectal epithelium and hence for generation of the spicules and many male-specific neurons) (Woollard and Hodgkin, 2000). Neither *mab-5* nor *mab-9* acts exclusively in the male, but their mutant phenotypes are much more pronounced in the male than in the hermaphrodite simply because most of the tissues they affect undergo little or no differentiation in the hermaphrodite.

In animals with larger nervous systems, where many genes are involved in generation of the nervous system, some genes with specific effects on behavior may play a role in the development of sex-specific components. Baker et al. (2001) have argued that in *Drosophila*, the gene *fru* (discussed further below) is of this type, having a role in specifying male circuitry in the CNS. Conditional knock-out or knock-in techniques may be used in vertebrates to examine whether a gene plays primarily a developmental role. For example, genetic ablation of the estrogen α and β receptor types results in the complete loss of expression of male sexual behavior in mouse (Ogawa et al., 2000). Whether this is due to a developmental or functional defect could be examined by eliminating the receptor in the adult.

CONTROL OF MALE SEXUAL BEHAVIOR BY THE SEX-DETERMINATION SYSTEM

One class of genes in which mutations exclusively affect one sex or the other is genes of the sex-determination pathway. Because these genes define behavior along with all other aspects of sexual dimorphism, one approach to understanding the genetic basis of male sexual behavior is to ask how sex-determination pathways define behavior by altering the development and function of the nervous system. Sex-determination pathways have been thoroughly investigated in both *C. elegans* and *Drosophila* (Cline and Meyer, 1996), and they are partially understood in mammals and in other vertebrates as well (Nef and Parada, 2000). They act by directing the course of development in one of two directions, one of their primary functions being to ensure that the same choice is simultaneously made by all the tissues, both somatic and germinal, and that this choice is concordant with the genotype of the individual. Consistent with their role as switch genes, genes of the sex-determination pathways are not themselves necessary for sexual development—in their absence, a default choice can be made and fully executed. In *C. elegans* and *Drosophila*, this choice is male development, including behavior, whereas in mammals, which unify sexual development through elaboration of hormones by the gonads, gonadectomy results in female development and behavior. The three studied sex-determination pathways are not conserved, indicating that they have arisen independently in these three lineages.

It appears that one way that genes of the sex-determination pathway direct sexual choice is by tissue or cell-specific modulation of the activities of genes functional in both sexes. For example, one direct target of the major sex-determining transcription factor of *C. elegans*, TRA-1, is the general cell death gene *egl-1* (Fig. 3). *egl-1* is required for the deaths of hermaphrodite-specific neurons (HSNs) in the male, as well as the deaths of other cells throughout the body in both sexes. TRA-1 is active in hermaphrodites and promotes hermaphrodite development while repressing male development. In the HSNs, TRA-1 binds to the *egl-1* promoter, preventing its expression and allowing cell survival (Conradt and Horvitz, 1999). We may speculate that in the hermaphrodite, the activities of genes like *mab-5* and *mab-9* are subjected to tissue-specific repression by TRA-1 in the same way to prevent their participation in the execution of male-specific developmental pathways. For example, with regard to the posterior lateral epidermis, which requires the activity of *mab-5* for development of the male rays, in the hermaphrodite, development of this tissue is identical to development of the epidermis in the midbody and anterior in both sexes, where it does not express *mab-5* and does not develop rays. Thus, it seems likely (though this has not yet been demonstrated) that by blocking *mab-5* activity in the posterior lateral epidermis in the hermaphrodite, TRA-1 effectively blocks male-specific

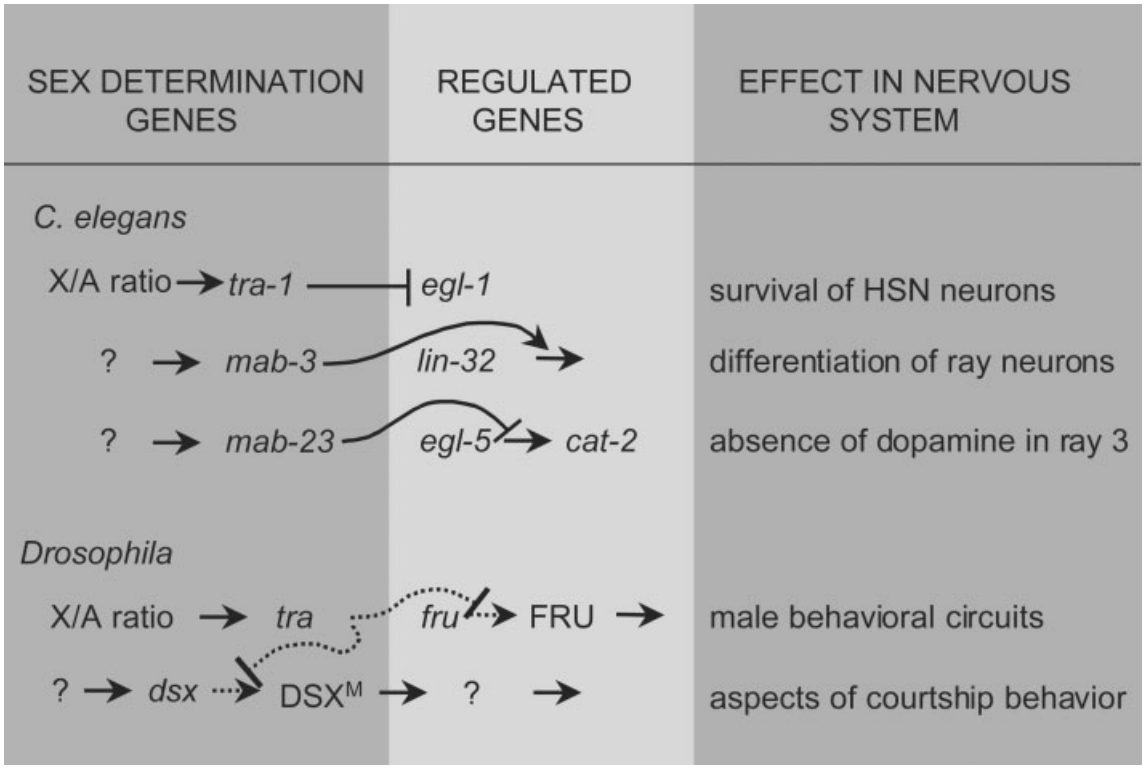


Figure 3 Regulation of behavior by sex-determination genes. In both flies and worms, the primary sex-determination signal is the X/A ratio. In *C. elegans*, the Zn-finger transcription factor gene *tra-1* is activated in hermaphrodites but not in males. By blocking transcription of the cell death gene *egl-1*, TRA-1 allows survival of HSN neurons in hermaphrodites, required for egg laying. *mab-3*, *mab-23*, and *dsx* encode transcription factors of the DM-domain family; their transcriptional regulation has not been determined and may be largely sex nonspecific. *mab-3* and *mab-23* participate in development of the rays by promoting the actions of the general developmental genes *lin-32* (encoding a bHLH transcription factor) and *egl-5* (encoding a Hox transcription factor). Both *mab-3* and *mab-23* have additional functions in male differentiation but no known functions in the hermaphrodite. In *Drosophila, tra* encodes a splicing factor that is activated in females and prevents generation of male splice isoforms of *fru* (encoding a transcription factor of the BTB Zn finger protein family) and *dsx* (dashed lines). *tra* also promotes female development by generating a splice isoform of *dsx* required for female development.

differentiation by causing a spatial transformation (Zarkower, 2001).

Another example of a general factor brought under sex-specific control is the *fru* gene of *Drosophila*, which encodes a transcription factor of the BTB Zn finger protein family (Ito et al., 1996; Ryner et al., 1996; Baker et al., 2001). Several FRU protein isoforms are generated, some of which are expressed in all or nearly all cells of the CNS of both sexes as well as in many other non-neural tissues. Null alleles of the locus are late pupal lethal. Hence the gene has an essential nonsex-specific function in many tissues. However, certain loss-of-function mutations in *fru* result in diverse defects primarily on male sexual behavior, from mild loss of ability to discriminate between the sexes (resulting in *fru* males that court both males and females), to complete loss of courting and copulatory behavior in strong alleles. These behavioral *fru* mutations selectively affect a FRU protein isoform that is present only in the male. This male protein isoform is encoded by a message splice-isoform that is eliminated in the female by splicing activity of the sex-determination pathway (Ryner et al., 1996) (Fig. 3). An alternate female splice isoform yields no protein product, apparently because translation of this splice-isoform is blocked by binding of the TRA protein of the sex-determination pathway (Usui-Aoki et al., 2000). Transcripts leading to the male-specific protein isoform are expressed in a subset of neurons in the CNS found in both sexes, including neurons in regions known to participate in generation of various aspects of male courtship and mating

(Ryner et al., 1996). Because these neurons are also present in females, one can speculate that this FRU isoform is responsible for directing sex-specific aspects of differentiation of the circuits involved, such as their branching, connectivity, neurotransmitter, or neurotransmitter receptor expression patterns, causing them to generate male behavioral patterns. Five additional members of the BTB Zn finger family in *Drosophila* are involved with aspects of nervous system differentiation, including synapse specificity, pathfinding, adult brain morphogenesis, and specificity of neuromuscular connections, lending support to this idea (Baker et al., 2001). *egl-1* and *fru* are the only proven examples of direct targets of sex-determination pathways among general factors, but it is likely that there are many more, and identification of such additional targets is an important research goal.

FAMILY OF MALE-SPECIFIC REGULATORY GENES

In addition to modulating the activities of general developmental genes, sex-determination pathways also target a class of genes whose specific function appears to be to direct aspects of sexual development, particularly male development. These genes are members of the DM-domain transcription factor family and are required for multiple diverse aspects of male development in worms, flies, and vertebrates (Raymond et al., 1998, 2000; Zarkower, 2001; Matsuda et al., 2002). Their conservation across phyla in both structure and function is in contrast to the nonconservation of genes upstream of them in the sex-determination hierarchy (Hodgkin, 1992; Wilkins, 1995; Marín and Baker, 1998). This suggests that they might be descendants of the primordial sex- determination system, one based on a dominant, male-determining sex factor (Hodgkin, 1992; Wilkins, 1995; Zarkower, 2001).

The first gene of this family to be identified was *doublesex* (*dsx*) of *Drosophila*. In the absence of *dsx* function, fly tissues lose coordination of sexual development and produce an intermixture of male and female types. Thus, *dsx*, like genes upstream of *dsx* in the sex-determination pathway, may act as a switch gene at early steps of the developmental hierarchy (Kopp et al., 2000; Keisman and Baker, 2001; Keisman et al., 2001; Sanchez et al., 2001). Expression of *dsx* is targeted by the sex-determination pathway through splicing in the same manner as described for *fru* above (Fig. 3). In the absence of activity of the upstream sex-determination factors (in the male), *dsx* transcripts are spliced in a default mode that generates a male-specific protein isoform, DSX^M. DSX^M directs development into the male pathway. In the female, the sex- determination pathway targets this *dsx* transcript, splicing it into an alternate structure, yielding the protein isoform DSX^F. DSX^F is necessary for female development. If the hypothesis is correct that *dsx* is descended from an ancestral sex-determination system that preceded introduction of the present day sex-determination pathways, then originally the DSX^F isoform was not present; thus the female activities of *dsx* may have arisen more recently.

DSX^M is widely expressed in somatic tissues, including in the nervous system, but it is not responsible for the male choice of all tissues. In particular, DSX^M plays only a minor role in dictating behavior (Villella and Hall, 1996). If DSX^M is expressed in a chromosomal female (that is, in an XX animal in which the male-repressing, female-promoting activity of the sex-determination pathway is functional), an animal male in form develops, but this male fails to court or copulate (Taylor et al., 1994). This indicates that one or more additional genes necessary for male behavior are independently targeted in a negative manner by the sex-determination pathway in parts of the nervous system. We have already seen that one such gene is *fru*, described above. How many additional such genes there may be is unknown.

In *C. elegans*, two proteins related to DSX, MAB-3 and MAB-23, are required for multiple aspects of male development, including behavior (Shen and Hodgkin, 1988; Lints and Emmons, 2002). In *C. elegans* as in *Drosophila*, default sex-determination is male; both *mab-3* and *mab-23* are expressed (through the activities of unknown transcription factors) in the absence of activity of the upstream sex-determination pathway (Fig. 3). DSX^M and MAB-3 have some regulatory targets in common, namely the genes for yolk proteins, which they repress in the male, and DSX^M can substitute for MAB-3 in directing aspects of *C. elegans* male development (Raymond et al., 1998; Yi and Zarkower, 1999). This supports the view that these genes are descended from a common primordial sex-determination gene. Unlike in *Drosophila*, however, *mab-3* and *mab-23* are only required for male development, there being no female isoform corresponding to DSX^F and no apparent effect of null mutations in these genes on the hermaphrodite.

Both *mab-3* and *mab-23* are required for multiple aspects of differentiation or function of male parts of the nervous system (Fig. 3). One function of *mab-3* is in generation of the rays, where it appears to play an accessory role to the broadly acting proneural bHLH transcription factor gene *lin-32* (Zhao and Emmons, 1995; Yi et al., 2000). *lin-32* is required for specifi-

cation of the ray neuroblast fate and expression of the ray cell sublineage, as well as for ray neuron differentiation (Portman and Emmons, 2000). *mab-23* is required later in differentiating ray neurons to regulate their expression of the neurotransmitter dopamine as well as axon pathfinding (Lints and Emmons, 2002). *mab-23* regulates expression of *cat-2*, the gene for the dopamine biosynthetic enzyme tyrosine hydroxylase, in specific ray sensory neurons by modulating the activity of an *AbdominalB*-type Hox gene (*egl-5*), making it dependent on a TGFβ signal. Thus, like *dsx*, and as expected for switch genes, both *mab-3* and *mab-23* appear to direct development into male pathways by modulating the activities of general developmental genes. However, unlike *dsx*, *mab-3* and *mab-23* appear to act closer to or at the end of developmental pathways, possibly even directly on specific differentiation genes.

Consistent with the hypothesis that DM proteins have an ancient role in sexual differentiation, vertebrates also contain these genes, at least some of which have been documented to play a role in development of the male (Raymond et al., 1998). DM family gene expression has been detected in the testes of all vertebrates examined (Zarkower, 2001). Deletion of a mouse member of the family (DMRT1) resulted in abnormal testes development (Raymond et al., 2000), and deletion of a region of chromosome 9 containing a cluster of DM genes resulted in sex-reversal (Ottolenghi et al., 2002). In medaka fish, a DM-domain gene closely related to DMRT1 plays a primary role in sex determination (Matsuda et al., 2002). Given the hormonal mode of vertebrate development, in which development of the soma appears to be entirely dependent on the sex of the gonad, it is unclear whether these genes will be found to play any direct role in differentiation of the vertebrate nervous system.

GENETIC SYSTEMS REQUIRED FOR DETECTION, ATTRACTION, AND DISCRIMINATION

Before he can mate, a male must first locate a female of his species and stimulate in her a receptive response. He attempts to meet these requirements by expressing specific behaviors and by sending signals and assessing signals received. To find a mate, males and many females as well may express specific mate-searching behaviors and strategies; a few of these have been described (e.g., Mendelson and Pfaus, 1989; Uy et al., 2000; Ayasse, et al., 2001). *Drosophila* is highly exploratory and its foraging behavior has been studied (Götz, 1994), but whether there is a specific aspect to this behavior directed towards mate-finding is not known (R. Greenspan, personal communication). Possibly the first mutants that specifically affect a putative male mate-searching behavior are those that have been recently identified in *C. elegans* by J. Lipton and S.W. Emmons.

In many species, the male's task in locating a mate is greatly facilitated by the enlargement of his target due to her broadcasting of a signal of some sort, most commonly chemical, visual, or auditory. Sex pheromones have been extensively studied in insects and other animals. Identification of compounds has been based on sensitive bioassays and many chemical structures have been determined (Roelofs, 1995). Receptivity of male moths to particular blends of pheromonal compounds has been traced to one or more sex-linked genetic loci (Roelofs et al., 1987; Glover et al., 1990). In *Drosophila*, sex pheromone signaling is short-range (less than a few centimeters). Both volatile and contact pheromones guide many of the stages of courtship (Coyne et al., 1994; Ferveur, 1997; Greenspan and Ferveur, 2000). Vocalization is commonly utilized by many species of insects and vertebrates.

Once a male has approached a potential mate closely enough for an interaction to take place, he can undertake an examination of sex, appropriateness, and qualities and attempt to induce a permissive or promoting response. This is often carried out by means of a series of behaviors grouped under the heading of courtship. By and large, both attraction to mates and courtship behavior have been studied in species that do not lend themselves to genetic analysis, and hence most of these behaviors are unexplored by means of genetics. The significant exception to this is *Drosophila*, in which both sexes express specific courtship behaviors, guided by visual, auditory, and chemical cues, and in which many male courtship-defective mutants have been identified and studied. These mutations have been useful in dissection of the behavioral series into independent steps, and in identifying some of the neurological and molecular mechanisms guiding those steps. Thus, known genes are involved in sex discrimination, visual identification, olfactory communication, courtship song generation, plasticity of courtship behavior, and initiation of copulation. The reader is referred to recent reviews for details of these studies (Hall, 1994; Yamamoto et al., 1997; Ferveur, 1997; Greenspan and Ferveur, 2000; Solokowski, 2001). Mutations in most of these genes are pleiotropic, affecting other aspects of behavior in addition to courting and copulation. Given the complex series of cues and actions involved, this is not surprising. Most of the sensory and motor systems uti-

lized are required for many other behaviors as well, and the male requires functioning sensory acuity of all kinds to mate. For example, mutations affecting the eye causing blindness affect mating by preventing the male from detecting visual cues. Mutation in a mitochondrial ribosomal protein gene, *technical knockout*, inhibits courtship because of impaired hearing (Toivonen et al., 2001). However, these many mutations affecting courtship also demonstrate the robustness of the behavior, because few of them prevent copulation entirely.

Exceptions to the rule of pleiotropy are *fru* and *dsf*, mentioned above, which appear to have functions that are required exclusively for sexual behavior. Different *fru* alleles result in blocks at distinct, sometimes multiple, steps of male courtship and copulation, indicating that *fru* appears to function at many diverse steps of the neural pathway (Goodwin et al., 2000). *dsf* encodes a transcription factor of the nuclear hormone receptor family necessary for certain sexual behaviors in both males and females (Finley et al., 1997, 1998). In the male, ability to discriminate the sex of the mating partner is lost in *dsf* mutants, and tail curling and hence copulation is defective, apparently due to abnormal innervation of certain abdominal muscles; females lack receptivity to male advances and are egg-laying defective, apparently due to abnormal innervation of uterine muscles. Both *dsf* and the male-specific form of the *fru* transcript are expressed in limited numbers of neurons, which in the case of *fru* are distributed throughout the brain regions shown by mosaic analysis to generate male mating behavior. Detailed analysis of the functions of these two genes, particularly the functions of *fru* and its diverse mutants, including identification of their target genes, should provide a powerful approach to dissecting the complex central circuitry generating and governing courtship behavior (Baker et al., 2001).

In vertebrates, genetic analysis of complex phenomena is becoming increasingly accessible through ever more powerful reverse genetic techniques. An example, mentioned above, is the genetic ablation in mouse of the estrogen α and β receptor types, which results in the loss of expression of male sexual behavior (Ogawa et al., 2000).

A second example is experiments that utilized PCR-based methods to isolate three families of vomeronasal organ (VNO) receptor genes that are sex-specifically expressed in mammals (Dulac and Axel, 1995; Herrada and Dulac, 1997; Matsunami and Buck, 1997; Ryba and Tirindelli, 1997; Pantages and Dulac, 2000). Each receptor is expressed in a subset of VNO neurons that express only one receptor type, and only one allele of each receptor is expressed in an individual. Interestingly, it has been shown that individual VNO neurons can respond to only one pheromone (Leinders-Zuhall et al., 2000). This importantly suggests that the molecular capacities of VNO neurons may have closely evolved with genetic mechanisms regulating the production of particular pheromones to which these neurons are dedicated. Recently, it has been shown that genetic ablation of a member of the TRP family of ion channels, TRP2, a putative transducer of VNO neuron signals, results in loss of the ability of male mice to discriminate between the sexes (Stowers et al., 2002). TRP2(−/−) males mount both males and females with equal frequency, and do not exhibit the typical aggressive behavior to intruder males. TRP2 mutants resemble animals in which VNO has been ablated, and it has therefore been suggested that other cues are sufficient to elicit male mating behavior but that the choice of mate is specified by the inhibition of male mating by pheromone signals derived from conspecific males.

NATURAL VARIANTS

From the time of Darwin it has been appreciated that sexual systems are particularly sensitive to selective forces and can vary rapidly in evolution. This is especially so for the courtship phase of sexual behavior, and as would be expected, natural variants are an important potential source of mutational changes whose analysis can lead to the genes and molecular mechanisms underlying courtship and other aspects of sexual behavior. Hodgkin and Doniach (1997) examined the males of wild races of *C. elegans* for their mating ability. A male-infertile race lead to the identification of a natural allele of *mab-23*, discussed above, whereas a substantially higher fertility in a race of worms from California was traced to multiple genetic loci. Gems and Riddle (2000) found that males of a *C. elegans* race from Australia appear to generate a signal that induces other males to attempt copulation at their excretory pore.

In *Drosophila*, natural variation in the pheromonal bouquet has been exploited to explore the genetic basis of pheromone production. Volatile and contact pheromones consisting of long-chain hydrocarbons can both promote within-species and inhibit interspecies courtship. The identities and ratios of the various compounds made by males and females vary in a north-south cline within both *D. melanogaster* and *D. simulans*. These as well as differences between hybridizable species of the *melanogaster* species group have been studied to define multiple responsible genetic loci (Ferveur, 1991; Coyne et al., 1994; Ferveur

et al., 1996; Ferveur and Fallon, 1996; Coyne, 1996a,b). Species-specific variation in a second signal contributing to species isolation, the song rhythm periodicity of the male's courtship song, was mapped in interspecies hybrids between *D. simulans* and *D. melanogaster* to the region of the X chromosome containing the *period* (*per*) locus and subsequently traced to specific amino acid changes within the *per* gene product, a major component of the fly circadian oscillator (Wheeler et al., 1991).

In higher animals, where induced mutations are difficult if not impossible to obtain, natural genetic variants play a potentially even more pivotal role in providing useful insights into genetic foundations. Variation in the mating behavior of inbred mouse strains provides a potential route to identify mammalian genes (see Crawley et al., 1997, and references therein). An elegant example where analysis has been pursued concerns the mating behavior of male voles. Comparison of two species of voles revealed that species-specific variation in the expression of the arginine vasopressin (AVP) V1a receptor (VIaR) correlated with species-specific patterns of sexual and social behaviors (Young et al., 1999). Males of the prairie vole (*Microtus ochrogaster*) are social and monogamous while males of the montane vole (*M. montanus*) are asocial and promiscuous. The two species differ in the expression pattern of V1aR in the brain and in response to exogenously supplied ligand, AVP. Prairie vole males respond to AVP injection by increased affiliative behavior (olfactory investigation and grooming) while montane vole males do not. A dimorphic region in the 5′ flanking region of the V1aR promoter accounted for the differences in brain receptor expression patterns. Introduction of the prairie vole version of the gene into transgenic mice resulted in a prairie volelike pattern of V1aR expression and, remarkably, an increase in male affiliative sexual behaviors in response to AVP. This experiment demonstrates that variation in the expression pattern of a single gene can have a profound influence on a web of sexual behaviors in vertebrates. How VIaR stimulation in only some brain regions results in the expression of specific behavioral responses remains unclear. For example, it is unknown whether V1aR is required for the development of particular neural circuits or simply their physiological function in the adult. If the latter is true, then we can assume that the neural machinery required for the execution of affiliative sexual behavior is not dimorphic between species of voles; rather, it is the expression of genes in these neural circuits that is dimorphic.

Another example of a complex set of sexual behaviors in a mammal that is subject to significant polymorphism is partner-gender preference in humans. Family studies have suggested that homosexual orientation may be subject to a degree of heritability in both males and females. Detailed linkage analysis within families with multiple male homosexual members revealed that the male homosexual trait tended to be sex-linked and correlated with inheritance of polymorphic DNA markers from the chromosomal region Xq28 (Hamer et al., 1993; Hu et al., 1995). Female homosexuality showed no such correlation. In a second study, however, linkage of male homosexuality to Xq28 was not confirmed (Rice et al., 1999). It is possible that sexual orientation and many other aspects of human sexuality, including gender identity, which were long thought to be due to environmental factors, that is, subject to learning and molded by experience, may in fact be influenced, if not entirely determined, by genetic makeup. If so, there is no reason to believe, given the power of current genomic techniques, that it will not be possible to identify the underlying genes and alleles involved.

COPULATION

The end of courtship and the beginning of copulation may be defined as the point in a series of mating actions at which essentially continuous contact between the mating partners begins. In *Drosophila,* this would be when the male mounts the female and transfers sperm. Mutations in a number of genes affect this behavioral phase, resulting in males that court normally but fail to attempt copulation, copulate abnormally or incompetently, or terminate copulation attempts prematurely (Yamamoto et al., 1997). Disengagement following copulation has been shown to constitute a separate step with its own genetic requirements by the phenotypes of *stuck* and *lingerer* (Yamamoto et al., 1997).

By the foregoing definition, *C. elegans* does not have a separate courtship phase: direct contact between the male's genital structure and the hermaphrodite appears to trigger the male's copulatory behavioral response. As in *Drosophila*, many mutations decrease the fertility of males because of poor mating ability. Many of these genes affect other aspects of sensory function or behavior (Hodgkin, 1983; Emmons and Sternberg, 1997). For example, consistent with the known role of ciliated neurons in the male genital structure (Liu and Sternberg, 1995), mutations that result in a general sensory defect because of defects in the cilia of sensory neurons are defective in male copulation (Barr and Sternberg, 1999). Several mutations that affect steps of male copulation more

specifically without significant general defects have been isolated (some are termed *cod* genes, for copulation defective). Behavioral analysis reveals that these genes act at one or more steps of the mating program, including response to the hermaphrodite, backing, turning, location of the vulva, spicule insertion, and ejaculation (Emmons and Sternberg, 1997). Most of these genes have not yet been characterized, and this class of genes is far from saturated.

The relative simplicity of *C. elegans* behavioral circuits, whose operation can be dissected by ablating individual neuronal components, combined with analysis of mutants, makes this a powerful system for determining how the functions of individual genes contribute to behavior. Two well-studied examples are *lov-1* (location of vulva) and *pkd-2* (PKD2 homologue). Mutations in these genes, which act in the same pathway, are defective at two behavioral steps: response and location of the vulva (Barr and Sternberg, 1999; Barr et al., 2001). They are expressed in and apparently required for the function of the sensory neurons of the rays, necessary for response, and of the hook, necessary for detection of a signal from the vulva. In addition, they are expressed in the male-specific CEM sensory neurons of the head, suspected of being involved in detection of a hermaphrodite pheromone. *lov-1* and *pkd-2* encode gene products with similarity to the products of the human autosomal dominant polycystic kidney disease genes PKD1 and PKD2, respectively (Barr and Sternberg, 1999). The phenotypes and expression patterns of *lov*-1 and *pkd-2* suggest that they are involved in a sensory signaling pathway, an observation helpful in the formulation of hypotheses regarding the possible function of the human genes in the kidney (Emmons and Somlo, 1999).

Turning (Loer and Kenyon, 1993) and spicule insertion (Garcia et al., 2001) are additional steps of the copulatory program that have been analyzed by means of behavioral assays in conjunction with cell ablation experiments and analysis of mutants. Consistent with the observation that the six male CP ventral cord motor neurons, which innervate the diagonal muscles, contain serotonin, exogenous application of this neurotransmitter stimulates tail curling similar to that expressed during the turning around of the end of the hermaphrodite. Serotonin-deficient mutants are defective in turning. Protraction of the spicules is stimulated by acetylcholine agonists. The first stage of the two-stage spicule motor program, a rapid (7 Hz) prodding, is induced when an unknown signal from the vulva is detected redundantly by sensory neurons in the hook and in the postcloacal sensilla. Acetylcholine secreted by the postcloacal sensory neurons PCB and PCC acts on nicotinic acetylcholine receptors in the spicule protractor muscles (including the products of the genes *unc-38* and *unc-29*), which act primarily to open a sarcoplasmic calcium channel (*unc-68*). When the tips of the spicules penetrate the vulval opening, acetylcholine secreted by the spicule SPC sensory neurons triggers the second stage of protraction: an extensive and sustained contraction of the spicule protractor muscles that causes the spicules to penetrate and remain in the vulva during ejaculation. This greater contraction is activated by *unc-38* and another nicotinic receptor, which open an L-type voltage-gated calcium channel (*egl-19*).

INTEGRATION OF MATING BEHAVIOR WITH OTHER BEHAVIORS: DRIVE, APPETITE, DESIRE

In higher animals, drives play a profound role in regulating behavior, notably sexual behavior, and have been subjected to much study (Pfaus, 1999; Kupferman et al., 2000), but genetic analysis has not been brought to bear. The requirement for adaptive integration and prioritization of complex behavioral patterns arises no less in *C. elegans* or *Drosophila* than in higher mammals. Indeed, expression of sexual behaviors in both animals is plastic, modifiable by physiological needs, environmental signals, and experience. Genetic analysis of these phenomena in worms and flies holds out the promise of shedding light on the neural and molecular mechanisms of drive.

The inclination of the *Drosophila* male to engage in courtship is modified by prior mating experiences (see Yamamoto et al., 1997; Ferveur, 1997; Greenspan and Ferveur, 2000, for references to the original literature). Attempts to court a mated female, which result in a rejection response, decrease courting for a period of several hours afterwards. Unsuccessful attempts to court immature males causes a subsequent decrease in this behavior and an increase in courting virgin females. Mating suppression caused by attempting to court a mated female is induced by as yet unidentified substances produced by the female for several days subsequent to fertilization. The production of these substances, as well as active rejection behavior on the part of the female, is induced in turn by substances transferred in male semen, the production of which is affected by the *don Giovanni* mutant. Not only does the rejected male fail to court other mated females, he also fails to court receptive virgins. This appears to be because of an association of the aversive substances produced by the mated female with other, normally attractive female cues. Mating

suppression can be experimentally induced by pairing receptive females with an aversive substance (quinine). Mutations in genes such as *dunce*, *rutagaba*, and *amnesiac* that disrupt experience-dependent learning and memory also disrupt induction of courtship suppression and its duration, indicating that experience-dependent modification of sexual behavior utilizes the same mechanisms that result in plasticity of other types of behavior (Greenspan, 1995b).

These phenomena, which show that a *Drosophila* male, when presented with identical stimuli may sometimes respond by courting and sometimes not, show that expression of courtship behavior is under some form of neurological control. This control system may be considered the fly equivalent of a drive state. Two mutations that may act on the drive mechanism are *he's not interested* (*hni*) and *courtless* (*col*) (Friedman et al., 1995; Orgad et al., 2000). *hni* and *col* males fail to court or do so only sporadically, yet they lack apparent sensory or motor defects that might account for this failure. *col* encodes a ubiquitin-conjugating enzyme and is an essential gene (Orgad et al., 2000).

In *C. elegans*, the probability of expression of spontaneous mate-searching by the adult male is altered by external cues and physiological status, indicating that here too, expression of a sexual behavior is subject to regulatory mechanisms. Expression of mate-searching is influenced by one or more signals indicating the presence of hermaphrodites, by physiological signals correlated with nutritional status, and by signals from the reproductive system that may indicate reproductive status (J. Lipton and S. W. Emmons, in preparation). Mutations decreasing the level of serotonin but not dopamine, two neurotransmitters involved in motivated and sexual behaviors in mammals, decreased mate-searching. One mutation that abolishes mate-searching falls in the gene *mod-5*, which encodes a serotonin reuptake transporter. A second, in the previously identified gene *unc-77*, also results in increased sensitivity to exogenous serotonin. Thus, serotonergic pathways are implicated in the regulation of *C. elegans* male mate-searching. Remarkably, inhibitors of the serotonin reuptake transporter in humans, such as the fluoxetine antidepressant Prozac, have the unwanted side effect of decreasing libido (Montejo et al., 2001). Thus, *C. elegans* genetics can lead to the identification of genes that are involved in motivational phenomena in mammals. Whether further analysis will reveal that this is because of significant similarity in the underlying neural mechanisms remains to be seen. This is by no means the first example of an unexpected and striking conservation of function of a behavioral gene—for a second example, concerning the role of a neuropeptide Y receptor in *C. elegans* foraging behavior, see the article by deBono, this volume. We may be lucky that certain fundamental aspects of nervous system function and regulation were established early in metazoan evolution and have been maintained in spite of the enormous change in scale that has occurred. If this is true, the experimental power now available in genetic models such as *Drosophila* and *C. elegans*, when combined with genome-based approaches in mammals, may allow for more rapid progress in understanding a complex behavior like male sexual behavior than would have been thought just a few years ago.

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