Are Complex Behaviors Specified by Dedicated Regulatory Genes? Review

Reasoning from Drosophila

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A regulatory gene, fruitless, appears to be specifically responsible for building the potential for male sexual behavior into the CNS of Drosophila. We use these and other findings about genes controlling development in model organisms as a basis for a more general discussion of the possibility that the neural circuits underlying other complex behaviors may also be built by the action of specific, dedicated genetic hierarchies.

Do genes control behaviors? This long-standing question at the center of the nature/nurture debate is usually answered by neurobiologists, ethologists, and geneticists by saying: “Well, sort of, but both genes and environment are important in shaping behaviors.” Here we argue, from our perspective as geneticists and biologists, that such answers are flawed in three ways: (1) A significant number of behaviors (e.g., certain fixed action patterns and species-specific innate behaviors) are relatively unaffected by environment and thus appear likely to be largely dictated (in some manner) by genes; (2) They fail to distinguish between different levels at which genes might control behavior; and (3) By placing an emphasis on the genetic and environmental components of the differences between individuals in the expression of behavior, such answers may have obfuscated understanding how the basic potentials for particular behaviors are established. Before we expand on these ideas, we need to define what we mean by (1) “behavior” and (2) “the genetic control of behavior,” as discussions of this topic have frequently suffered from imprecise or no definitions of these terms.

On the Meaning of “Behavior”

We are interested in exploring the degree to which genes may control (as defined below) complex behaviors. “Behavior” has a variety of meanings, and under almost any definition only a subset of behaviors are potential candidates for being controlled by genes. We will therefore briefly examine the various categorizations of behavior to clarify the kinds of behaviors on which we want to focus.

As commonly used, “behavior” encompasses any orderly movement with recognizable and repeatable patterns of activity produced by members of a species (Ridley, 1995). Such a definition includes species-specific behaviors studied extensively by ethologists (e.g., courtship, predation, nurturing, migration, territory marking, web building, nest building, and prey avoidance responses; for review: Lorenz, 1950; Tinbergen, 1951; Ridley, 1995; Alcock, 1998), as well as more general aspects of animal existence such as locomotion, flying, feeding, and drinking (for review: Carlson, 1998). A broader definition of “behavior” might also include homeostatic mechanisms such as maintenance of balance, respiratory rate, and heart rate that are governed by neural circuits. All of the above types of behaviors have obvious biological importance for the survival, reproductive success, or both of an individual and are the kinds of behaviors we want to consider. That is if genes do specify particular behaviors they are most likely to be found for behaviors that play significant roles in the normal life activities of a species, as selection might have led to the evolution of genes controlling the development of the potential for such behaviors.

Behaviors can also be categorized by whether they are primarily innate or significantly modifiable by experience. At one end of this spectrum are fixed action patterns and fairly invariant species-specific behaviors, often termed innate behaviors, which have been documented and characterized in a vast array of ethological and neurobiological studies. Innate behaviors provide some of the most likely cases where genes specifying the circuitry that provides the potential for specific behaviors might, if they exist, be found. Although innate behaviors can be performed by animals raised in isolation, usually some aspects of these behaviors are modifiable by experience. We do not wish to exclude behaviors that are modified by experience from consideration, since the genetic specification of the potential for a basic behavioral modality, and modification of that behavior by experience, need not be mutually exclusive.

The term “behavior” is also used more broadly, particularly by the lay public, to encompass almost any definable set of actions by humans, or other animals. Many of the behaviors that fall under this broader definition are not expected to be under genetic control (e.g., the particular language that a person speaks, the ability to drive a race car well), since it is unlikely there has been selection for these particular behaviors. However, it should be recognized that even for such behaviors there are likely to be genetic components governing how well an individual executes such a behavior. For example, one could imagine studying the ability of humans to skip rope while blowing bubbles with chewing gum. An appropriate study (e.g., tests of individuals after they had practiced this behavior) would likely show both genetic and environmental components to skip/blow bubble behavior. However, for our purposes we exclude from consideration behaviors that are not in a simple sense biologically meaningful—something we can easily envision a role for in the normal biology of a species and on which selection may have directly acted.

We note in closing this section that the aim of this essay is to examine whether any behaviors are con-
trolled by genes. Our point here is that there are behaviors which are clearly important for the survival or reproduction of individuals, and for which selection might have generated genetic systems that specify such behaviors. By contrast, some behaviors have not been directly selected for, and are not expected to be under direct genetic control. Thus, the issue is not whether genes control all behaviors; they do not, but rather whether some/many/most behaviors in the former category might be controlled by genes.

Do Genes Control Behavior?
Textbooks, or reviews that deal with genes and behavior almost always state near the outset of that discussion that no single gene controls a behavior. However, what is meant by "controls" is rarely defined. Yet there are two very different biological meanings of control which reflect two very different levels at which genes can be envisioned as possibly governing behavior. First, a gene might control the actual manifestation of a behavior as it occurs. Second, a gene might function during development to build into the CNS the potential for a behavior: a gene's activity specifically constructs the circuitry that subserves a behavior. We will argue later that neither of these meanings are appropriate for behavioral genetics.

In developmental genetics a gene is said to control (specify) a developmental process if, in an otherwise wild-type organism, the functioning of that gene is necessary and sufficient to direct a particular developmental outcome. Necessity is established by showing that the absence of a gene's function leads to a failure of the developmental process to occur. Sufficiency is established by showing that the expression of a gene in cells where it is not normally expressed, can induce in those cells the pattern of development it normally specifies. For example, the Drosophila eyeless gene is necessary for eye development because the absence of eyeless function results in the absence of eyes, and the eyeless gene is sufficient for eye development because the expression of the eyeless gene in tissues where it is not normally expressed (e.g., the progenitor cells of legs, wings) leads to the formation of eyes at these locations (Halder et al., 1995). A slightly less rigorous level of evidence establishing that a gene specifies a process, which is also used in developmental genetic studies, is to show that a gene is a member of a regulatory hierarchy, other members of which have been shown to specify, by necessity and sufficiency tests, a process. Examples of some developmental regulatory genes that specify particular aspects of fly development are (1) the HOX genes that function to specify segment identity (for review see Gellon and McGinnis, 1998), (2) the eyeless (Halder et al., 1995) and vestigial (Kim et al., 1996) genes that function within developmental fields to specify eye and wing development, respectively, and (3) the Sex-lethal, transformer and doublesex genes that act to specify somatic sexual identity (for review: Cline and Meyer, 1996).

Note that the definition of specify entails demonstrations of necessity and sufficiency in an otherwise wild-type organism for the simple reason that such genes cannot bring about the processes they specify in a vacuum; development is a process. For example, in order for the fly's HOX genes to specify segmental identity, the gap, pair rule, and segment polarity genes must have previously functioned to generate the segmental units in which HOX genes act (for review: Pankratz and Jäckle, 1993). The HOX genes in turn specify the expression of other genes whose products are used to build the differentiated structures of each segment. Similar situations hold for many aspects of development: regulatory genes functioning in hierarchies across time and space are necessary for particular aspects of development to occur. This does not take away from the fact that at particular times and places during development individual regulatory genes act to specify cell fates.

By analogy we will say that a gene specifies a behavior if, in an otherwise wild-type organism, the functioning of that gene is necessary and sufficient to establish the potential for a particular behavior. Note that this definition does not require there be only one regulatory gene specifying a behavior. Indeed, we expect that if there are regulatory genes specifying individual behaviors, they likely function in regulatory hierarchies to build the potential for a specific behavior into the nervous system.

Note also that this definition of specify does not say that it is the gene specifying a behavior that is solely responsible for that behavior. Elementary a priori considerations suggest that the appropriate functioning of many genes is essential for all behaviors. Any behavior requires the functioning of a multicellular circuit beginning with input to the nervous system, propagation and interpretation of that input in the CNS, and output via neurons that directs a response via neuromuscular, or neuroendocrine systems, or both. Impairment of any part of such a circuit is likely to cause decrements in the behavior it subserves. In addition, mutations that affect the general vigor of an organism often impinge on the quality of a variety of behaviors (e.g., Hall, 1994). Thus, many genes must function to set up the nervous system's structure, and subsequently to elicit and manifest neuromuscular/endocrine functions. We take the preceding statement as an obvious, and not a particularly interesting, truth; and it is not germane to the issue we are addressing.

Before proceeding we wish to comment on an alternative view of the role of genes in behavior that is often taken to be antithetical to the notion that genes might specify, or control, behaviors. This view comes from quantitative genetic studies, especially in humans, which seek to characterize the genetic components of behaviors, and, when properly interpreted, to estimate the relative contributions of genes and environment to the variation in a particular behavior within a population. Unfortunately the results of such studies are all too fre-
quently described as showing that there are both significant genetic and environmental components to any behavior, rather than (more correctly) that there are both genetic and environmental components to the degree of individual variation in any behavior within a population. What is important to realize about such studies is that they tell us nothing about whether there is a common genetically specified basal program for a behavior shared by all members of a population. The reason for this is that, by their nature, such quantitative genetic studies (1) can only find effects of genes for which there happen to be allelic differences in a population that affect the behavior, and (2) can only examine the amount and nature of individual-to-individual variation in the behavior within a population. Moreover, the genes detected by this approach bear no necessary relationship to either how important particular genes are developmentally for a behavior, or whether they might control, or specify a behavior. An example may make this point clear.

One classical phenotype that has been subject to substantial quantitative genetic analysis is the number of bristles (peripheral nervous system derived sense organs) in flies (e.g., Thoday, 1959). There is significant intrapopulational variation in bristle number in wild-type fly populations, and strains can be readily selected from wild populations for either increased, or decreased bristle number. When such selected strains are genetically analyzed, it is found that there are many genes that each contribute small amounts to the variation in bristle number. While this tells us something of the origin of the variation of bristle number in wild type flies, it provides no information about whether there is a common program to build bristle sense organs and determine their spacing; yet we know from developmental genetic studies that the formation of bristles and their spacing are dictated by fairly well understood genetic regulatory hierarchies, involving the *achete/scute* genes (for review: Modolell and Campuzano, 1998).

Having made that point, we need to emphasize that at another level the genetic and environmental components to variations in behaviors that are shown to exist by quantitative genetic studies are of fundamental importance. It is these differences between individuals in the alleles they possess at particular genes which, together with individual differences in experience, provides the basis from which the individuality of our behaviors arise.

Do Genes that Specify Behavior Exist?—Other Lessons from Developmental Genetics

If one were to generalize the findings from the past twenty years of developmental genetics, one overriding lesson would be that the construction of each physical part of an organism is controlled by specific regulatory hierarchies. These hierarchies must have acquired their functions as the result of natural selection. It was important—for the survival, or reproductive success, or both, of individuals—that they be constructed in a certain manner; and the route that has been taken time after time after time to insure robust and stable development was to evolve regulatory hierarchies to control the construction of each part. If one accepts this reasoning, then it follows that a whole range of behaviors essential for individual’s survival or reproductive success should also have been subject to similar selective pressures.

These observations raise the following questions: (1) Has evolution solved an organism’s needs for precise behaviors in ways analogous to those evolved to construct each physical part of an organism? (2) If evolution has produced genes that build the potential for specific behaviors into an organism, what phenotypes would be expected for mutations in such genes (that is if a generic behavior is: input—central processing—neural output—mechano/chemical output, which parts of such a circuit are likely to be the domain(s) of a gene specifying a behavior?) and (3) How can genes specifying behaviors, if they exist, be identified?

With regard to the phenotypes one might expect for mutations in genes specifying behaviors, we note the following. The sensory systems providing the inputs that elicit behaviors largely function in a generic fashion. Thus the visual, auditory, tactile, and other sensory inputs that elicit a wide range of behavioral responses are generally received and transmitted by cells that are specific to the type of input, but independent of the specific behavior elicited. However, there are well documented cases in which a sensory system appears to be dedicated to subserving one or a small array of behaviors (e.g., vomeronasal system for pheromones [Halpern, 1987], pressure wave sensors in lacewings [Miller and Olensen, 1979], the auditory system in noctuid moths [Roeder, 1963]). Similarly, the motor and endocrine outputs that bring about the behaviors themselves are also largely generic. For example, legs can be used to run, walk, climb, fight, etc.; and common muscle systems are used for all of these behaviors. Thus, for both the input and output aspects of behaviors there are not, in many cases, the specialized machineries one would expect if these aspects of particular behaviors were specified by dedicated genes.

Thus, if genes specifying behavior exist, they are likely to function in the processing—neural output aspects of specific behaviors. One simple way a gene might function to build the potential for a specific behavior into the nervous system is by constructing the neural circuitry that subserves that behavior. In this regard there are a number of behaviors for which it has been possible to show that the functioning of specific regions in the CNS is important for a given behavior, and even to define something of the roles that particular areas of the CNS have in that behavior (e.g., song and song learning in birds [Bottjer, 1997], and male courtship in *Drosophila* [Greenspan, 1995a]). However, there are very few specific examples of an identified dedicated CNS circuitry for a particular behavior. Among these are the gin trap reflex of pupal *Manduca sexta* (Levine and Truman, 1983), the crustacean stomatogastric system (Miller, 1987), and simple reflex circuits, such as the trap-jaw mechanism in ants (Gronenberg, 1996). On the other hand, available data do not appear to preclude the existence of such specific behavioral circuits in other cases. When we speak of specific dedicated CNS circuitry for a particular behavior we mean to encompass two possibilities. The first is of a dedicated circuitry sufficient to subserve all aspects of a behavior. The second is of a dedicated circuitry that modulates and coordinates the...
activities of more generic neural circuits that provide particular behavioral subroutines which are used in particular aspects of one behavior, but may also be utilized in generating related aspects of other behaviors. Thus if genes specifying behaviors exist, it is not unreasonable to expect that they would function in the behavior-specific parts of the neural circuits they specify.

Finding Candidate Genes Affecting Behaviors

With respect to the third question posed above—how could genes specifying behaviors be identified?—there are currently obstacles to reaching this goal. Most important, candidate genes are needed. With the explosion of neurogenetics during the past 20 years, one might expect a number of such candidate genes would exist, if there were genes which specified behaviors. However, neurogenetics has generally taken a reductionist approach to understanding how nervous systems are built and function, focusing on such topics as synaptogenesis, synapse function, channel genes, pathfinding, etc. (for review: Albright et al., 2000). Such studies have generally not been done in ways that would likely identify genes specifying individual behaviors. Closer to behavior have been studies aimed at dissecting various sensory modalities (e.g., Eberl, 1999; Lessing and Carlson, 1999; Montell, 1999), but again these studies have generally focused on the basic workings of these systems. There are relatively few reports of screens for mutations affecting complex behaviors (e.g., courtship [Yamamoto et al., 1997], learning and memory [Smith and Rubin 1997; Dubnau and Tully, 1998], mechanosensory responses [Kerran and Zuker, 1995], defecation behavior [Thomas, 1990], and circadian rhythms [for review: Dunlap, 1999]). In addition, a number of mutations—identified in studies of various aspects of the nervous system development or function, as well as mutations isolated in studies of other processes—have been assayed for behavioral effects. It is among these mutations that one can currently look for such genes and assess their role in specifying behavior.

Before examining whether any of these genes might specify a behavior, we note the difficulties of directly looking for such genes. Direct screens are difficult, since many mutations will be found that affect any particular behavior, both because of the many cell/tissue types that must function properly for a behavior to occur, and because mutations with nonspecific, general effects on vigor will be recovered. Thus one needs to sort through many mutations affecting a behavior to identify genes whose normal function participates materially in its specification. While a sufficiency test can distinguish a gene specifying a process from one that just plays some role in its execution, sufficiency is not easy to demonstrate; it requires showing that ectopic expression of the gene can lead to the normal process occurring in a novel location. Sufficiency tests are rarely, if ever, used to sort collections of genes into those regulating a process from those just necessary for the process. Rather developmental control genes are identified by detailed characterizations of mutant phenotypes and/or the molecular characteristics of a gene, or its product suggesting that it might have a regulatory function. Then sufficiency tests are done.

Returning to whether any extant mutants might identify genes specifying behaviors, there are a small number of mutations that identify candidates for genes specifying behaviors because of these mutations’ specificities or profound effects on a behavior. Among these are the genes that function in generating circadian rhythms; the for gene, which governs larval foraging behavior in flies (Osborne et al., 1997); a gene encoding a neuropeptide Y receptor homolog important for social behavior and feeding response in C. elegans (de Bon and Bargmann, 1998); and the fruitless gene, which functions in Drosophila male sexual behavior (Ito et al., 1996; Ryner et al., 1996). Moreover, in several cases (clock genes, the neuropeptide Y receptor homolog in C. elegans, and for gene of Drosophila), different alleles of these genes generate different normal behavioral outcomes. These are important findings in behavioral genetics in that they reveal the allelic states of these genes determine the quality of the behavior that is generated. Thus these genes can, and have been, said to control their respective behaviors. However, it is important to note that in such descriptions the word “control” is used to mean controlling the ongoing elaboration of the behavior, as opposed to the developmental meaning of “control”—to specify the development of neural substrates that provide the potential for a behavior. Only in the case of the fruitless (fru) gene can a reasonable argument currently be made for a gene that specifies a behavior.

The Genetic Specification of Male Courtship Behavior in D. melanogaster

Male courtship behavior in Drosophila is governed by a genetic hierarchy that is responsible for all aspects of sexual differentiation. Recently, a previously unrecognized branch of this hierarchy, in which fru is the first (and perhaps only) regulatory gene, was shown to be essential for male sexual behavior (Ito et al., 1996; Ryner et al., 1996). This system provides a strong case for a gene specifying a behavior. Here we focus on the evidence that fru specifies male courtship behaviors and what insights have been gained from studying this gene, as it may provide a model for the identification of genes specifying other behaviors, and what such genes do. Note also that this system highlights how difficult it is, even in a premier model organism, to establish that a gene specifies a behavior.

Drosophila male courtship behavior is a species-specific innate behavior (for review: Hall, 1994; Greenspan, 1995a; Yamamoto et al., 1997; Greenspan and Ferveur, 2000). When a male courts a female he engages in a series of actions including orienting toward and following the female, tapping her with his forelegs, singing a species-specific courtship song by vibrating one wing, licking the female’s genitalia, and finally curling his abdomen to attempt copulation. Male courtship behavior is largely a fixed action pattern; males know how to court without exposure to another animal. One aspect of courtship—discrimination between suitable mates (i.e., virgin females or females who have not recently mated) and unsuitable mates (i.e., recently mated females or young males)—is modifiable by experience (for review: Siegel et al., 1984; Greenspan, 1995b).

Use of male/female mosaics has made it possible to
map some of the regions of the nervous system involved in male sexual behavior. These studies showed that different regions of the CNS are important for individual steps in male courtship behavior. For example, a dorsal posterior region of the brain is essential for early steps in courtship (i.e., orientation, tapping, and wing extension), whereas a region in the ventral nerve cord is necessary for the generation of courtship song (Figures 1A and 1B; for review: Greenspan, 1995a).

A single hierarchy of genetically and molecularly well-characterized regulatory genes (one of which is fru) controls all aspects of somatic sexual differentiation in flies (Figure 2; for review: Cline and Meyer, 1996). The number of X chromosomes initiates the sex-specific expression of the sex determination regulatory genes. There are three levels of regulatory genes in this hierarchy. The activity of genes at each level is controlled by alternative pre-mRNA splicing to produce male- and female-specific mRNAs (Figure 2). Male sexual differentiation is the default state. Males (XY) lack an active signal from the assessment of the number of X chromosomes. Consequently, the pre-mRNAs of genes in the top two levels of the hierarchy are spliced in their default patterns, resulting in mRNAs that do not encode functional proteins. The pre-mRNAs of the two genes in the third level of the hierarchy, doublesex (dsx) and fru, are also spliced in their default patterns and produce mRNAs encoding male-specific DSX and FRU proteins. The male-specific products of dsx and fru are zinc finger transcription factors and specify different aspects of male sexual differentiation.

Wild-type male dsx function is required for all aspects of male somatic sexual differentiation outside of the CNS. dsx function is also required for the male-specific division of a set of neuroblasts found in the abdominal ganglion of both sexes (Taylor and Truman, 1992). Males homozygous for dsx null mutations are able to carry out most aspects of courtship, but do not produce one part of the courtship song (species-specific humming sounds; Villella and Hall, 1996). In addition, their courtship behavior is generally suboptimal. It is suggested that these effects of dsx mutations may result from abnormalities in peripheral sensory structures (Villella and Hall, 1996). Most important, the expression of the wild-type DSX male protein as the only DSX protein in females (i.e., a sufficiency test) showed that while such individuals were transformed to male in nearly all regards, they did not show male sexual behaviors (Baker and Ridge, 1980; Taylor et al., 1994).

Before discussing those aspects of male sexual differentiation controlled by the fru branch of the hierarchy, it is important to point out that the fru locus is complex (Figure 3). The fru transcription unit spans ca. 130 kb and has four promoters, denoted P1—P4. Particularly relevant for our discussion here, it is only the pre-mRNA from the P1 fru promoter that contains the sequences through which the upstream sex determination regulatory proteins, TRA and TRA-2, regulate splicing (Figure 2), and it is only the pre-mRNA from the P1 fru promoter that is spliced sex-specifically (Ryner et al., 1996). Thus, in terms of the functioning of the sex determination hierarchy, only the products of the P1 fru promoter are part of this hierarchy. The products of the other three fru promoters appear to be expressed equivalently in females and males and carry out a sex-nonspecific vital function that is distinct from the role of the P1 promoter products in sexual differentiation (Ryner et al., 1996; Goodwin et al., 2000; Lee et al., 2000; Anand et al., 2001). For the remainder of this article, we consider only the products of the P1 fru promoter.

The fru branch of the sex determination hierarchy is responsible for all, or nearly all, steps of male courtship behavior (Ryner et al., 1996; Villella et al., 1997; Goodwin et al., 2000; Anand et al., submitted). The most severe fru genotypes almost completely abolish all steps of male courtship. As male courtship behaviors are largely a dependent action pattern (i.e., a given step in courtship is dependent on preceding steps having occurred) this result could be either because fru function is required solely for the first step in this sequence, or because it is required for each step. The latter is likely the case since weaker fru alleles and allelic combinations display defects at a number of different stages of courtship. For example, wild-type males choose females rather than males as the appropriate sexual partner, while fru males fail to distinguish between the sexes and as a consequence court females and males roughly equally (Ryner et al., 1996; Villella et al., 1997; Goodwin et al., 2000).

At a slightly later stage of courtship, when wild-type males extend a wing and vibrate it to produce a courtship song, certain fru mutants rarely extend their wing toward females but do not produce courtship pulse song during the short bouts of wing extension they do produce (Ryner et al., 1996; Villella et al., 1997). Finally, it has been recently found by examining very leaky fru mutant combinations that achieve copulation that behaviors during mating, such as transfer of seminal fluids and sperm, and the duration of mating are also governed by fru (Lee et al., 2001). These male courtship effects of fru mutations are not the result of generalized behavioral deficits; fru mutant males—even those that do not produce a courtship song during courtship—performed like wild type in general locomotion and wing usage assays (Villella et al., 1997). These results suggest that the function of the fru branch of the sex determination hierarchy is to specify aspects of sexual differentiation in the CNS responsible for male sexual behavior.

Knowledge of the roles of the sex determination regulatory genes comes from both necessity and sufficiency tests (i.e., showing what the expression of a protein that is normally found in one sex does when expressed in the other sex). For example, in chromosomally female (XX) individuals, null mutations in the tra or tra-2 genes (which are immediately above dsx and fru in the hierarchy and control their expression: Figure 2) result in normal male development in all respects, except for body size, which is female-like. Most important, such transformed females show normal male sexual behavior. That wild-type tra function is sufficient for female development was shown by expressing the female-specific TRA protein in males and showing that they developed as females (McKeown et al., 1988). The female-specific SXL protein (Cline, 1979), as well as both the male-specific and female-specific DSX proteins (Baker and Ridge, 1980; Taylor et al., 1994; Waterbury et al., 1999), have also been shown to be both necessary and sufficient...
Figure 1. CNS of Drosophila with Regions Involved in Male Sexual Behavior and Pattern of Expression of the FRU Male-Specific Proteins

(A) Regions of the Drosophila CNS. SOG, subesophageal ganglion. SP, superior protocerebrum. WNG, wing neuromeres. T1, T2, and T3: first, second, and third thoracic ganglion, respectively. ABD, abdominal ganglion. (B) Regions of the CNS responsible for indicated aspects of male courtship behavior. (C) Anterior view of brain. Yellow dots indicate locations and relative numbers of cells expressing the FRU male-specific proteins. SMPR, superior medial procerebrum. SLPR, superior lateral procerebrum. VLPR, ventral lateral procerebrum. ME, medulla. AL, antennal lobe. (D) Posterior view of brain. Yellow dots as in (C). MB, mushroom body. LHO, lateral horn. ME, medulla. PS, posterior slope. LOP, lobula plate. (E) Ventral view of ventral nerve cord. Yellow dots as in (C). Labels as in (A). (F) Dorsal view of ventral nerve cord. Yellow dots as in (C). Labels as in (A).

The findings that fru functions as a member of the Drosophila sex determination hierarchy and that its role in this hierarchy is necessary for nearly all aspects of male courtship make a strong case for a gene specifying a complex behavior. We note, however, that a sufficiency test has not yet been done to ask, for example, whether expressing the normal complement of male-specific FRU proteins in a female CNS would lead to females displaying male sexual behaviors. Also note the end point of a sufficiency test need not be behavior itself. Any aspect of a gene’s phenotype can be used as an endpoint. For example, particular properties of cells in which a gene is being ectopically expressed (e.g., projection patterns, types of proteins expressed)
spliced in both sexes to one of three alternative exons near the 3'-end of female-specific and female-specific mRNA. The male- and female-specific FRU mRNAs are derived from pre-mRNAs transcribed from the most distal (P1) promoter.

The male-specific DSX protein negatively regulates aspects of female sexual differentiation and positively regulates aspects of male sexual differentiation. The female-specific DSX protein positively regulates aspects of female somatic sexual differentiation and negatively regulates aspects of male somatic sexual differentiation, whereas the male-specific DSX protein negatively regulates aspects of female sexual differentiation and positively regulates aspects of male sexual differentiation. The male- and female-specific DSX mRNAs differ at their N-termini due to sex-specific alternative splicing. In addition, P1 derived transcripts are alternatively spliced in both sexes to one of three alternative exons near the 3'-end of fru transcripts. As a consequence there are three classes of male-specific and female-specific fru mRNAs produced. Conceptual translations of the male- and female-specific fru mRNAs reveal that they all share a common BTB domain, a domain thought to be involved in protein dimerization. The sex-specific alternative splicing at the 5'-end of P1 transcripts produce male mRNAs that encode 101 amino acids N-terminal to the BTB domain, whereas the sequences encoding these 101 amino acids are spliced out of the female mRNAs. The three alternative 3' fru exons found in both male and female mRNAs encode alternative pairs of zinc fingers. Thus fru potentially encodes three BTB zinc finger transcription factors in each sex. Genetic analysis reveals that the male FRU proteins have the functions described in text and antibodies to the 101 amino acid region unique to the male FRU proteins reveals their presence in ca. 1.5% of CNS cells during metamorphosis. Behavioral analysis has revealed no functions for the female-specific FRU proteins. Moreover, immunohistochemistry reveals that these putative female-specific proteins are present at very low levels, if at all, in the CNS. For more details with respect to the depicted genes, as well as other genes in this hierarchy see the review by Cline and Meyer, 1996.

In response to the assessment of the number of X chromosomes the Sex determination regulatory genes are expressed in females by (1) directly regulating the splicing of its own pre-mRNA and (2) directing the splicing of the tra gene’s pre-mRNA in a female-specific pattern. The absence of SXL protein in males allows the housekeeping splicing machinery to direct the default splicing patterns of both Sxl and tra pre-mRNAs. The male-specific Sxl and tra mRNAs do not encode functional proteins due to the presence of stop codons. The female-specific TRA protein, together with sex-nonspecifically expressed TRA-2 protein, directly regulates splicing of the pre-mRNAs of the dsx and fru genes in the third level of the hierarchy to produce female-specific dsx and fru mRNAs. In males the absence of TRA protein leads to the housekeeping splicing machinery directing male-specific patterns of splicing of the dsx and fru pre-mRNAs. The male- and female-specific dsx mRNAs both encode zinc finger transcription factors that have the same DNA binding domain, but different carboxyl termini. The female-specific DSX protein positively regulates aspects of female somatic sexual differentiation and negatively regulates aspects of male somatic sexual differentiation, whereas the male-specific DSX protein negatively regulates aspects of female sexual differentiation and positively regulates aspects of male sexual differentiation. The female- and male-specific fru mRNAs are derived from pre-mRNAs transcribed from the most distal (P1) fru promoter. The sex-specific fru mRNAs differ at their N-termini due to sex-specific alternative splicing. In addition, P1 derived transcripts are alternatively spliced to one of three alternative exons near the 3'-end of fru transcripts. As a consequence there are three classes of male- and female-specific fru mRNAs produced. Conceptual translations of the male- and female-specific fru mRNAs reveal that they all share a common BTB domain, a domain thought to be involved in protein dimerization. The sex-specific alternative splicing at the 5'-end of P1 transcripts produce male mRNAs that encode 101 amino acids N-terminal to the BTB domain, whereas the sequences encoding these 101 amino acids are spliced out of the female mRNAs. The three alternative 3' fru exons found in both male and female mRNAs encode alternative pairs of zinc fingers. Thus fru potentially encodes three BTB zinc finger transcription factors in each sex. Genetic analysis reveals that the male FRU proteins have the functions described in text and antibodies to the 101 amino acid region unique to the male FRU proteins reveals their presence in ca. 1.5% of CNS cells during metamorphosis. Behavioral analysis has revealed no functions for the female-specific fru products. Moreover, immunohistochemistry reveals that these putative female-specific proteins are present at very low levels, if at all, in the CNS. For more details with respect to the depicted genes, as well as other genes in this hierarchy see the review by Cline and Meyer, 1996.

Figure 2. Sex-Specific Patterns of Regulatory Gene Expression in the Drosophila Sex Determination Hierarchy

In response to the assessment of the number of X chromosomes the Sex determination regulatory genes are expressed in females by (1) directly regulating the splicing of its own pre-mRNA and (2) directing the splicing of the tra gene’s pre-mRNA in a female-specific pattern. The absence of SXL protein in males allows the housekeeping splicing machinery to direct the default splicing patterns of both Sxl and tra pre-mRNAs. The male-specific Sxl and tra mRNAs do not encode functional proteins due to the presence of stop codons. The female-specific TRA protein, together with sex-nonspecifically expressed TRA-2 protein, directly regulates splicing of the pre-mRNAs of the dsx and fru genes in the third level of the hierarchy to produce female-specific dsx and fru mRNAs. In males the absence of TRA protein leads to the housekeeping splicing machinery directing male-specific patterns of splicing of the dsx and fru pre-mRNAs. The male- and female-specific dsx mRNAs both encode zinc finger transcription factors that have the same DNA binding domain, but different carboxyl termini. The female-specific DSX protein positively regulates aspects of female somatic sexual differentiation and negatively regulates aspects of male somatic sexual differentiation, whereas the male-specific DSX protein negatively regulates aspects of female sexual differentiation and positively regulates aspects of male sexual differentiation. The male- and female-specific fru mRNAs are derived from pre-mRNAs transcribed from the most distal (P1) fru promoter. The sex-specific fru mRNAs differ at their N-termini due to sex-specific alternative splicing. In addition, P1 derived transcripts are alternatively spliced to one of three alternative exons near the 3'-end of fru transcripts. As a consequence there are three classes of male- and female-specific fru mRNAs produced. Conceptual translations of the male- and female-specific fru mRNAs reveal that they all share a common BTB domain, a domain thought to be involved in protein dimerization. The sex-specific alternative splicing at the 5'-end of P1 transcripts produce male mRNAs that encode 101 amino acids N-terminal to the BTB domain, whereas the sequences encoding these 101 amino acids are spliced out of the female mRNAs. The three alternative 3' fru exons found in both male and female mRNAs encode alternative pairs of zinc fingers. Thus fru potentially encodes three BTB zinc finger transcription factors in each sex. Genetic analysis reveals that the male FRU proteins have the functions described in text and antibodies to the 101 amino acid region unique to the male FRU proteins reveals their presence in ca. 1.5% of CNS cells during metamorphosis. Behavioral analysis has revealed no functions for the female-specific fru products. Moreover, immunohistochemistry reveals that these putative female-specific proteins are present at very low levels, if at all, in the CNS. For more details with respect to the depicted genes, as well as other genes in this hierarchy see the review by Cline and Meyer, 1996.
male-specific fru this time (Belote and Baker, 1987; Arthur et al., 1998) which removes sequences upstream of the fru proposed role in specifying male sexual behavior and groups of cells expressing these tests will confirm that fru.

At the top of the figure, the distribution of fru exons is shown along the genomic region encompassing fru (scale is in kb). There are four fru promoters (P1—P4) spread across ca. 100 kb. Transcripts from all four promoters are spliced to a set of common exon (C1—5) that encode a BTB domain (likely to be involved in protein dimerization). Transcripts from the P1 promoter are sex-specifically spliced (see Figure 1), whereas transcripts from the P2, P3, and P4 fru promoters are identical in the two sexes. Transcripts from the P2, P3, and P4 promoters, as well as the female-specific transcripts from the P1 promoter, introduce only a small number of amino acids N-terminal to the BTB domain, whereas the male-specific transcripts from the P1 promoter introduce 101 amino acids N-terminal to the BTB domain. At the 3’ end of fru, there are three alternatively used exons (A, B, C) that encode alternative pairs of zinc fingers. Exons A, B, and C are all found in male- and female-specific derived P1 mRNAs. Whether these three exons are all found in P2, P3 and P4 mRNAs is unknown; those 3’ exons known to be so used are indicated. Genetic analysis using mutants (some of which are depicted at the top of the figure) and mutant combinations that disrupt subsets of the fru transcript classes, show that some combination of the P2, P3, and P4 transcripts encode a function essential for viability. Transcripts from these promoters are found throughout the CNS, as well as in several other tissues.

Figure 3. Transcription and Splicing Patterns of the fru Gene (Ryner et al., 1996; Goodwin et al., 2000)
At the top of the figure, the distribution of fru exons is shown along the genomic region encompassing fru (scale is in kb). There are four fru promoters (P1—P4) spread across ca. 100 kb. Transcripts from all four promoters are spliced to a set of common exon (C1—5) that encode a BTB domain (likely to be involved in protein dimerization). Transcripts from the P1 promoter are sex-specifically spliced (see Figure 1), whereas transcripts from the P2, P3, and P4 fru promoters are identical in the two sexes. Transcripts from the P2, P3, and P4 promoters, as well as the female-specific transcripts from the P1 promoter, introduce only a small number of amino acids N-terminal to the BTB domain, whereas the male-specific transcripts from the P1 promoter introduce 101 amino acids N-terminal to the BTB domain. At the 3’ end of fru, there are three alternatively used exons (A, B, C) that encode alternative pairs of zinc fingers. Exons A, B, and C are all found in male- and female-specific derived P1 mRNAs. Whether these three exons are all found in P2, P3 and P4 mRNAs is unknown; those 3’ exons known to be so used are indicated. Genetic analysis using mutants (some of which are depicted at the top of the figure) and mutant combinations that disrupt subsets of the fru transcript classes, show that some combination of the P2, P3, and P4 transcripts encode a function essential for viability. Transcripts from these promoters are found throughout the CNS, as well as in several other tissues.

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exactly the latter (Figure 2). Expression of the dsx male protein is both necessary and sufficient for nearly all aspects of male development, except those controlled by fru. Thus, it seems highly likely that further sufficiency tests will confirm that fru does specify male sexual behavior.

The temporal and spatial patterns of expression of the male-specific fru products lend credence to fru’s proposed role in specifying male sexual behavior and provides insights into how fru carries out its function. Both in situ hybridizations to fru transcripts and immunohistochemistry with antibodies that are specific to the male-specific FRU proteins show that the male-specific fru products are expressed almost exclusively in the CNS (Ryner et al., 1996; Goodwin et al., 2000; Lee et al., 2000). Expression of these products can be first detected in a few CNS cells at the very end of the larval period. Expression reaches a maximum at about two days into the pupal period, this time coincides with the period of the major morphogenetic events that shape the adult fly CNS. At the peak of expression ca. 1700 CNS cells (roughly 2% of the CNS) express the male-specific FRU proteins. Temperature shift experiments with temperature-sensitive alleles of the tra-2 gene show that the potential for fru expression of that promoter is abolished in ca. 2/3 of the peak number of cells. The male-specific FRU proteins remain detectable in a higher proportion of the peak number of cells throughout this time period and into young adults.

Cells expressing the male-specific fru products are not localized to one part of the CNS. Instead they are most frequently found in small groups of cells (Figures 1C–1F), and less frequently as single cells, scattered throughout the brain and ventral nerve cord (the fly equivalent of the vertebrate spinal cord). There are ~20 groups of cells expressing these fru products (Lee et al., 2000). Some of these groups correspond to the specific regions of the CNS previously shown to be involved in particular aspects of courtship behavior (Figure 1B). In addition, there are regions containing FRU-expressing cells that had not been previously implicated in male courtship behavior (cf. Greenspan, 1995a).

Classification of the types of cells in which fru is expressed—based on the locations, size and morphologies of their cell bodies—provides insight into fru’s function (Ryner et al., 1996). Few of the cells expressing the male-specific fru products appear to be either sensory or motor neurons. Most cells expressing these products are in higher order neuropils and have morphological characteristics suggesting that they are either local circuit neurons or interganglionic interneurons. That many of these cells are involved in male sexual behavior is strongly suggested by the finding that in the fru’1 mutant, which removes sequences upstream of the fru promoter from which the male-specific products are produced, expression of that promoter is abolished in ca. 2/3 of the cells in which it is normally found (Goodwin et al., 2000; Lee and Hall, 2001). These findings suggest that...
male sexual behavior is the result of sensory information entering the CNS via largely sex-nonspecific sensory systems, which is processed by sex-specific (fru-specified) circuitry in higher order neurons. This information is then used to execute the particular aspects of sexual behavior, which occurs by modulating largely sex-nonspecific motor systems.

Although these developmental, genetic, and behavioral findings with respect to fru are provocative, they also raise a number of neurobiological issues about which little is currently known. For example: Do the fru-expressing neurons comprise a circuit? What are the behavioral roles of individual groups of fru-expressing neurons? Do these neurons bear any lineage relationship to one another? What specific aspects of the development of these neurons are specified by the FRU proteins?

On Finding Other Genes Specifying Behaviors

The finding that fru likely specifies male courtship behavior raises several questions: Do genes specifying other behaviors exist? If so why haven’t they been found and how might they be recognized? With respect to these questions there are some additional relevant findings from developmental genetics.

As to the existence of other genes specifying behaviors, several classic studies using genetic hybridization techniques identified what appear to be single loci affecting specific aspects of animal behavior, such as stridulation pattern in crickets (Bentley and Hoy, 1972), nest provisioning in parrots (Dilger, 1962), and nest type building in mice (Dawson et al., 1988). These genes might be regulatory genes like fru, but it has not been possible to carry out sophisticated genetic and molecular analysis to establish their roles.

With respect to fru itself, it should be noted that the first fru mutant was reported in 1963 (Gill, 1963) over 30 years before fru’s role in controlling male courtship behavior as a member of the sex determination regulatory hierarchy was discovered. The long delay in recognizing fru’s function is not surprising given that the phenotypes of the original partial loss-of-function fru alleles (primarily bisexual courtship and behavioral sterility due to a failure to copulate), while striking, did not really suggest its function. These phenotypes were not obviously qualitatively different from those of other known mutants that affected courtship in flies (e.g., stuck, cactophony; Hall et al., 1980; Hall, 1994). Indeed, it is not fru’s phenotypes per se that single it out from other interesting genes that affect male sexual behaviors (for review: Hall, 1994; Yamamoto et al., 1997; Greenspan anderveur, 2000), but rather the fact that only fru has currently been shown to be a member of a developmental regulatory hierarchy. It is also worth noting that if additional genetic analysis of the fru locus had been done 30–40 years ago (long before the existence of multiple promoters and alternative pre-mRNA splicing were known), such studies could easily have been misleading: since fru also encodes sex-nonspecific products that carry out an essential function (Figure 3), the isolation of other (lethal) alleles could logically have led to the conclusion that fru’s behavioral effects were simply due to reduced expression of a more general vital function.

fruitless is far from unique in terms of a long time lag between the discovery of mutations in a Drosophila gene and a recognition of that gene’s fundamental developmental regulatory function. Mutations in many genes, such as engrailed, eyeless, and vestigial, were all known for many decades prior to the recognition of their central roles in controlling Drosophila development. The major reasons for the delayed recognition of the functions of these regulatory genes are 2-fold. First, a modern genetic view of development did not exist (except perhaps in the mind of Ed Lewis [1996]), and there was thus no conceptual framework to recognize these genes for what they were. Second, many of the molecular and genetic tools whose usage was central to elucidating the functions of these genes either did not exist or were not part of the standard methods used to study development.

With respect to genes specifying behavior one wonders whether here too the mindset of a field has conditioned us against realizing the possibility that such genes might exist. Certainly none of us, despite our many years of having worked on Drosophila sex determination, male courtship behavior, or both, recognized that possibility in any real sense, prior to the recent discoveries with respect to fru. If skepticism regarding the existence of such genes is more general, and we believe it is, we can ask: what is the basis for that skepticism, and most importantly, are there properties of the nervous system that justify such skepticism?

First, most thoughts on the possibility of genes controlling or specifying particular behaviors are colored by a strong cultural reluctance to believe in their existence, because of the implications that the existence of such genes could have for many issues related to free will and responsibility. From a more biological perspective, skepticism about genes controlling or specifying behaviors comes from the awesome properties of learning and memory that are embedded in the nervous system: if nervous systems have such profound properties that are currently beyond our understanding, might not behaviors, many of which are subject to modification via experience, also be special? With respect to the latter issue, there are several points to consider. First, the concepts that a gene might specify the CNS circuitry providing the potential for a behavior, and that that circuitry might be modifiable by experience, do not need to be mutually exclusive. Second, different behaviors within a species, as well as similar behaviors in related species, can be very different in terms of how much they are modifiable by experience (e.g., bird song, for review: Alcock, 1998). These observations raise the possibility that whether there is the potential for experience to modify a behavior may be genetically built into the circuitry subserving individual behaviors. Indeed, building in (or not) such a potential may be part of the construction of such circuitry. Finally, there are many behaviors, probably in all animal species, which, like Drosophila male courtship behavior, are biologically important, action patterns that are more or less fixed and thus largely unaffected by experience. Such behaviors, at the very least, are prime candidates for being constructed by dedicated genetic circuits.

At a developmental level the nervous system might also be viewed as special, in comparison to other deve-
opment systems and thus require qualitatively different solutions for its development. These include the vast number of connections needed in a nervous system; the large distances across which such connections have to be made; and the conceptual problem of trying to conceive how one gene could control the formation of a circuit comprised of many cells with diverse properties and functions. With respect to the first point, we note that coordinate expression of a transcription factor like *fru* within cells comprising a specific circuit in the nervous system might aid in constructing that circuit. This idea has also been recently suggested based on findings with respect to another transcription factor (Arber et al., 2000). With regard to the second point, we note that tracheal and circulatory systems are built across similarly large distances in organisms. The available information on how genes control their construction (for review: Gale and Yancopoulos, 1999; Metzger and Krasnow, 1999) does not reveal processes especially different from how other aspects of development are controlled. With regard to the last issue, we note that the *Drosophila* eyeless and vestigial genes function as part of genetic regulatory hierarchies operating within defined developmental fields to specify the multiple cell types of eyes and wings, respectively. Thus the latter genes provide models for the kinds of functions needed by genes like *fru* that specify behaviors.

We conclude with some remarks about searching for other genes that specify behavior. In this regard we can offer two suggestions, one perhaps obvious, and the other a hopeful speculation.

Other innate species-specific behaviors and fixed action patterns are the obvious places to look for such genes. This should be done in model genetic organisms so that sufficiency tests can be carried out on candidate genes. Of equal importance will be the identification of robust biologically meaningful behaviors. In this regard it is worth noting that there have been relatively few ethological studies of *Drosophila* behaviors in controlled environments, although the techniques are available to do so (for review: Heisenberg, 1997). For such studies it will probably be necessary to give up the laboratory stocks of *D. melanogaster*, which have served geneticists and developmental biologists so well for the past 100 years because, for some 3000 generations, laboratory strains of *Drosophila* have been living in a habitat consisting solely of glass walls, food and potential mates. Under these circumstances the behavioral repertoire of laboratory strains may have become restricted; 3000 generations is almost certainly vastly longer than the time scales that were required to derive domesticated plant or animal species from their wild progenitors. Thus in flies at least, it will likely be wise to rederive from wild populations inbred laboratory strains that live in more complex environments and then subject these strains to observation and genetic analysis.

As a more speculative possibility, we note that one of the more compelling findings to emerge from the molecular characterization of genes is that related proteins are very often deployed to do similar things: For example, HOX genes for specifying segmental identities, NCAMS (fasciclin) for axonal pathfinding, kinesins and other molecular motor gene families for intracellular movement. In this regard it is worth noting that the BTB family of proteins to which *fru* belongs contains about 60 genes in flies, only 11 of which have been studied at all. With regard to the few BTB genes that have been studied, nearly all also encode zinc finger domains and so are likely transcription factors. Several of these genes, like *fru*, encode multiple proteins due to the use of multiple promoters and alternative splicing. Finally, at least 5 of these genes (*longitudinals absent, Broad-Complex, mod(mdg4), fruitless, Tyrosine kinase-related protein*) function in the nervous system (not all have been examined in that regard) where they affect synapse specificity (*mod(mdg4); Gorczyca et al., 1999*), pathfinding (*longitudinals absent; Seeger et al., 1993*), and adult brain morphogenesis (*Broad-Complex; Restifo and Hauglum, 1998*). One of the genes functions in muscle cells and affects the specificity of neuromuscular connections (*abrupt; Hu et al., 1995*). Could other BTB genes be like *fru*, in that a subset of their products are used to build the potential for specific behaviors into the nervous system?

**Closing Remarks**

We make two final points. First, the idea that genes control or specify behaviors is often seen as threatening for cultural reasons such as those touched on above. We do not believe that the ideas set forth here should necessarily generate such concerns. Consider, for example, sexual behavior. An anthropomorphic description of fly courtship behavior can be made that is very similar to the basics of courtship in humans and many other species: recognize a potential mate (distinguish your species, and its sex, from others); get their attention (tapping?); if they don’t run away, and perhaps show some interest, court them (beguile them with a love song?); try to arouse them (licking?); and finally, attempt copulation. Such a basic sequence would seem to be a reasonable strategy for reproductive success. Given the diversity of human sexual behaviors that exists, if there is a human counterpart to *fru*, such a gene’s role might just be to see that the neural circuits were built that coordinate and order such basic steps. The actual events that comprise each step in such a sequence could be shaped by individual-to-individual variations in experience and genotype. This is not dissimilar to what appears to be the basic pattern for a number of behaviors in higher organisms. For example, there is a basic circuitry for song production in certain song birds. In some cases even the rudiments of song appear to be innate, but many aspects of a bird’s song in these species are learned (for review: Bottjer 1997; Alcock, 1998). Generalizing this idea, we also note that many human behaviors are clearly modified by experience. Thus, if the potentials for various biologically meaningful human behaviors are built into the nervous system by genes like *fru*, it is likely that this is done by using such genes to build the basics of the circuit subserving a behavior, as well as building in the potential for experience to shape and mold that circuitry.

Lastly, we note that we view this article as an essay, which comes from the French word “*essai*” meaning “to attempt.” While we have attempted to bring together what we know from a variety of areas to address the question of whether genes specify behavior, we would
be the first to admit that we don’t know everything we should in all the areas we have touched on; but given how narrowly many of us are focused these days, we hope that minor omissions or errors will be forgiven. More importantly, in attempting such an overview we hope that this essay will stimulate others with more knowledge in specific areas to contribute to the discussion of this topic, and, above all, that this essay will stimulate experimentation to address the possibilities raised.

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References