AN ABSTRACT OF THE THESIS OF

DeLaine D. Larsen for the degree of Master of Science in Zoology presented on July 24, 1998. Title: The Genetic Regulation of Sex-specific Motorneurons by the *doublesex* Gene in *Drosophila melanogaster* and the Genetic Characterization of an Interaction with the Sex Determination Hierarchy.

Abstract approved: _____

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The remodeling of the central nervous system (CNS) during metamorphosis in *Drosophila melanogaster* is a prime model system in which to study the genetic control of the sexual dimorphisms in the abdominal ganglion of the CNS. I have been using a P[*tau-lacZ*] enhancer trap line, 4.078, to label a segmentally repeated subset of abdominal motorneurons in order to assess the function of the sex determination hierarchy in controlling sex-specific development of the adult nervous system. In both the male and female larva there are 8 sets of these labeled abdominal motorneurons but only six sets in males and five sets in females survive in the adult. When this P[*tau-lacZ*] reporter construct is placed into a *doublesex (dsx)* mutant background, all 8 sets of these labeled abdominal motorneurons survive in both male and female adults. These results strongly suggest that *dsx* plays a role in the sex-specific survival of larval neurons that have functions in the adult.

During the construction of mutant strains containing the sex determining genes *transformer (tra)* and *transformer-2 (tra2)*, a genetic interactor was discovered in the P[*tau*-lacZ] 4.078 line. Female flies heterozygous for either *tra* or *tra-2* alleles and the P[*tau-lacZ*] 4.078 developed with masculinized external and internal sex-specific structures. The external sex-specific structures, such as the genitalia, and ventral muscles are dependent on *dsx* gene function and a dorsal sex-specific muscle is dependent on *fruitless (fru)* gene function. From standard genetic crosses, I have characterized and demonstrated that the genetic interaction is linked to the P-element insertion site, which maps to the 85-87 region on the right arm of the third chromosome. By genetic analysis, this new genetic interactor appears to interfere with the *tra* and *tra2* regulated female-specific functions of both *dsx* and *fru*, potentially by reducing the female-specific splicing of the primary transcripts of the genes *dsx* and *fru*. To test the possibility that this newly

described genetic interactor was allelic to a known gene, *B52*, that maps to the same region of the chromosome and alters *dsx* splicing, complementation tests were conducted which showed that the P[*tau-lacZ*] is not allelic *B52*. Additional phenotypes were observed in the crosses that first detected the interaction, suggesting that this newly described locus may affect other gene functions as well. Among the phenotypes observed were XX intersexes, male-female gynandromorphs (XX//XO mosaics), and non-disjunction events evident as XO males and XXY females. This new locus may represent a new member of the family of genes that influence regulated splicing events.

The Genetic Regulation of Sex-specific Motorneurons by the *doublesex* Gene in
Drosophila melanogaster and the Genetic Characterization of an Interaction with the Sex
Determination Hierarchy

by

DeLaine D. Larsen

A THESIS

submitted to

Oregon State University

in partial fulfillment of
the requirements for the
degree of

Master of Science

Presented July 24, 1998
Commencement June 1999

Master of Science thesis of <u>DeLaine D. Larsen</u> presented on <u>July 24, 1998</u>

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ACKNOWLEDGMENTS

CONTRIBUTION OF AUTHORS

Dr. Barbara Taylor was involved in the design, analysis, and writing of each manuscript. Laura Wilson helped to complete some of the preparations for the work in chapter 2. All work was performed in the laboratory of Dr. Barbara Taylor.

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Chapter 1
Introduction and Background

General Overview of Thesis

An important question in developmental biology is how the enormous variety of neurons are specified. In order to address this general question, I have examined how sex-specific neurons are specified in *Drosophila*. It is well known that the genes of the sex determination hierarchy control the development of all somatic sexually dimorphic tissues in males and females but it is not known how this hierarchy controls the development of sex-specific neurons.

Sexual differentiation is one of the processes that occurs during the metamorphic transition from the vermiform larva to the winged adult and results in the differentiation of male and female external and internal genitalia. During metamorphosis, the central nervous system (CNS) is extensively remodeled to accommodate the requirements of adulthood. Two sources of adult neurons in the CNS are known: pre-existing larval neurons and newly generated adult-specific neurons. In the creation of the adult CNS, larval neurons can follow three fates either remaining the same, 'remodeling' to innervate a new target or dying. Most adult CNS neurons are new, the product of divisions of neuroblasts in the larval stage and, thus, are only part only of the adult nervous system. From studies of the CNS in a variety of insects, larval neurons are the source of virtually all adult motorneurons and a subset of interneurons. Newly produced adult neurons differentiate into additional intraganlionic and interganglionic interneurons. In the body, peripheral neurons, newly produced during metamorphosis, form part of new sensory organs in the adult body and send their axons into the CNS. As part of this transition, many parts of the body develop differently between males and females to subserve reproductive functions. The neuronal control of these sexually dimorphic regions of the

body will involve neurons, which themselves will have sex-specific properties. In order to understand how sex-specific differences in the nervous system are created, it is necessary to examine the effect of mutations in the sex-determination cascade, which controls all somatic sexual differentiation, on the development and function of identified sex-specific neurons.

Since adult motorneurons are derived from selective survival and/or remodeling of larval neurons, I have designed experiments to determine how genes in the sex determination hierarchy control sex-specific abdominal motorneurons. Indeed, in the terminal abdominal segments and the genitalia, the larval motorneuronal pool, which is shared between male and female larvae, becomes two separate adult motorneuronal pools. I have used two methods to approach this problem. The first method used the retrograde transport of a label, lysinated biotin, to the motorneuronal cell body. This method allows the population of motorneurons innervating a segment to be identified in larval and adult wildtype and mutant animals. To examine the fate of a specific subset of neurons found in every abdominal segment of the larva, I used a reporter line that expresses in one subset of motorneurons and examined the staining pattern in control flies and mutants of the sex determination hierarchy. These studies have shown that one of the sex determining genes, *doublesex* (*dsx*), is involved in the refinement of the pattern of sex-specific abdominal motorneurons. In addition, my studies have also uncovered a putative new gene that appears to affect the processing of sex-specific transcripts from the *dsx* and *fruitless* (*fru*) genes.

Metamorphosis and the Remodeling of the Fly

Remodeling of the larval body to create the adult body: As a holometabolous insect, *Drosophila* undergoes metamorphosis to the adult after three larval instars. Most adult tissues are composed of the progeny of imaginal cells, which are initially set aside in the embryo. These imaginal cells do not contribute to larval tissues, but are a proliferative population, which remains undifferentiated as long as the animal is larval. During metamorphosis these cells continue to divide and then differentiate to create adult tissues.

Among the tissues that are replaced during metamorphosis, the change in the musculature is among the most extreme and allows the animal to exhibit a whole new range of behaviors. Larvae differ from adults in their primary means of locomotion, mode of feeding, ability to have sex, and their skeletal support system. In the larva, movement of the body consists of forward, backward, or bending motions caused by a series of peristaltic waves of contractions moving down the abdominal segments. These movements support the restricted larval behavioral repertoire of feeding and escape. The main muscular power for larval motion comes from the contraction of an array of 30 large muscles per hemisegment connected to a flexible hydrostatic skeleton (Bate, 1993; Demerec, 1994). The thoracic-abdominal bands of muscles thus drive all larval movement.

The adult fly, on the other hand, uses an entirely different means of transportation. The fly has legs and wings to provide locomotion and a hard exoskeleton for skeletal support. In general, adult behavior shifts from one mainly focused on feeding towards one focused on reproduction. Braced by the hard exoskeleton, the role of the body wall muscles has shifted to moving limbs for walking, posture, and moving wings for flight. The abdominal musculature functions to stabilize body posture during standing and walking as well as bending during copulation in males and egg laying in females. The abdominal muscles are no longer as large, as in the larval stage, but exist as a series of 17-22 parallel longitudinal fibers for the dorsal musculature, 20 parallel fibers for the lateral musculature, and 5-8 fibers for the ventral musculature (Currie and Bate, 1991).

Both the larval and adult muscles are produced *de novo* in the embryo and pupa respectively. To accommodate the formation of the adult muscles, most of the larval muscles are histolysed by 24 hours after pupal formation (APF) (Currie and Bate, 1991). The adult muscles and epidermis are formed from progenitor cells set aside in the embryo. The muscle progenitors, myoblasts, divide during the larval and early pupal stages, but do not fuse and differentiate until the mid-pupal stage. Histoblasts, precursors for the abdominal epidermis, divide during the prepupal and pupal stages of metamorphosis and then spread to replace the larval epidermis. By 28 hours APF, myoblasts have begun to fuse into myotubes, aligning themselves through contact with the epidermis (Currie and Bate, 1991). The developing epidermis provides signals for the

migration of the nerves and myoblasts. Muscle formation is completed by about 48 hours in the abdomen.

Concurrent with the development of nonsex-specific muscles, the sex-specific muscles form by the same basic developmental processes. The male and female-specific muscles and cuticular structures of the genitalia and posterior abdominal segments (A6 and A7) are regulated by the gene *doublesex* (*dsx*) (Baker and Ridge, 1980; Hildreth, 1965; Merritt, 1994, T.J.S. Merritt and B.J. Taylor, unpublished results). Male and female larvae have eight abdominal segments. After metamorphosis, adult females emerge with abdominal segments A1- A7 and males emerge with abdominal segments A1-A6 anterior to the genitalia. In the sex-specific segments, females have 21 terminal abdominal and genital muscle groups whereas males have 12 muscle groups (Merritt, 1994, T.J.S. Merritt and B.J. Taylor, unpublished results). One of the major sex-specific changes in the abdomen is the loss of the abdominal segment A7 in males, meaning that females have 8 muscle groups in A7 that are of necessity missing in males.

The differences in the number and segmental distribution of abdominal and genital muscles suggests that the number and segmental distribution of their innervating motorneurons will also be sex-specific. For example, the population of motorneurons innervating A7 would be expected to be sex-specific with the males losing their larval motorneurons whereas the females would retain most or all of the motorneurons from this population. Besides this expected set of sex-limited motorneurons, other common abdominal segments have sex-specific muscles and thus should have sex-specific sets of motorneurons (Lawrence and Johnston, 1984; Merritt, 1994). Sex-specific motorneurons likely result from sex-specific survival of subsets of larval motorneurons and/or the sex-specific remodeling of motorneuronal processes in neurons present in both sexes. With the presence of many sex-specific muscles and segments, we anticipate a large population of sex-specific motorneurons involved in their control.

Remodeling of the larval CNS to create the adult CNS: During this transition from the larval to adult stage, the nervous system is extensively restructured to accommodate the requirements of adulthood. Neurons already active in the larva can follow three different fates: remaining the same, respecifying to an adult specific target, or dying (Reviewed in Truman, 1990; Truman *et al.*, 1992; Weeks and Levine, 1992). The addition of new adult

structures and the elimination of larval ones are accompanied by substantial changes in the neuronal circuitry and behavioral repertoire. In addition to the reorganization of larval neurons a large number of adult specific neurons are produced, destined to become incorporated into the adult nervous system (Truman, 1990; Truman and Bate, 1988).

The possible fates that neurons can follow during metamorphosis have been demonstrated in several insect model systems. One of the major systems utilized in understanding the mechanisms involved during metamorphosis is *Manduca sexta*, where much of the work has been done on the hormonal regulation of metamorphosis. There are many advantages for the use of *Manduca sexta* in studies to follow the fate of neurons. One major advantage is that the CNS is composed of unfused ganglia throughout development, which allows for easy segmental identity, and this consistency of structure allows for identification of specific neurons based on location and size at all life stages of the animal. Another advantage of *Manduca* is that the hormonal profile is well established and allows for manipulation of levels for the determination of the role hormones play in different developmental process.

By comparison, *Drosophila melanogaster* is not as well suited for the recognition of individual neurons with the techniques available in *Manduca*. One reason for this is that the ventral nerve cord in *Drosophila* is fused so that segmental identity is difficult to establish in the smaller nervous system. This lack of segmental identity makes it much harder to distinguish the abdominal neurons by simple location within the CNS as found in *Manduca*. Another drawback with the use of *Drosophila* is that the hormonal profile has not been well established, as in *Manduca*, due to the small size of the fly (Bainbridge and Bownes, 1988; Bownes and Rembold, 1987; Riddiford, 1993). It is assumed that the same relative changes for the hormones seen in *Manduca* are also seen in *Drosophila*. The advantage for *Drosophila* is that it is well suited to extensive genetic and molecular analysis to study different developmental processes. New enhancer trap techniques have enabled the identification of specific neurons for different developmental stages. In addition, the introduction of the GAL4::UAS system not only allows for the visualization of specific populations of neurons, but also the expression of specific gene products (Brand and Perrimon, 1993).

Examples of retained or remodeled larval neurons for the adult CNS: From research completed in *Drosophila* and *Manduca* it has been demonstrated that the adult motorneurons and a subset of interneurons are maintained from the larval CNS (Truman, 1990). One of the early papers which explored the fate of larval motorneurons in the abdominal ganglion was done by H.M. Taylor and J.W. Truman (1974) in *Manduca*. Taylor and Truman followed the larval motorneurons through metamorphosis by using cobalt backfills from the cut peripheral nerves at different developmental stages (Taylor and Truman, 1974). From fills in the larval stage it was determined that there were 74 neurons which innervated abdominal segment A4. In the pupal transition only 4 neurons appeared to be lost when the number of motorneurons were counted in diapausing pupa, which arrest at the developmental stage where most of the adult differentiation hasn't occurred (Taylor and Truman, 1974). This number of motorneurons is maintained throughout metamorphosis and can be found in pharate adults, just before eclosion from the pupal case. Of interest was the observation that adult moths, 3 days old in this experiment, have undergone a wave of cell death and only 30 out of the 70 motorneurons can be found by cobalt backfills (Taylor and Truman, 1974). The molecular mechanisms involved in this cell death have been identified using other model systems, such as *C. elegans* and *Drosophila* (See Below).

In addition to simple monitoring of the presence or absence of a larval neuron, the main developmental processes have been well described. The remodeling of neurons involves changing their dendritic and axonal arborizations. The reorganization of the dendritic arbors is significant since these neuronal processes represent the primary targets for synaptic inputs to a cell and directly affect its ability to interact with other neurons in the nervous system. For example, in *Manduca*, the femoral extensor motorneuron innervates the femoral flexor muscle in the larval proleg and is retained to innervate the femoral extensor muscle in the adult leg (Kent and Levine, 1993). During metamorphosis, the dendritic arbors are extensively remodeled to give the larger arborization seen in the adult. When this motorneuron is followed during metamorphosis, its dendrites regress early in the pupal period, followed by a period of growth resulting in the adult pattern (Kent and Levine, 1993). To determine whether these remodeling events were influenced by its peripheral targets, the larval prolegs were

surgically removed before the onset of metamorphosis. This surgical intervention did not alter the pattern of initial regression and the normal basic pattern of outgrowth of adult processes, but these processes extended into different regions than the arbors of control animals (Kent and Levine, 1993). These results illustrate the point that the remodeling of motorneurons is a dynamic process, with some aspects largely independent of the peripheral target, as is the case of dendritic regression and regrowth, but other aspects are target dependent, as is the case of the adult morphology and connectivity of the dendritic arbors.

Not only does the motorneuron remodel, its muscle targets undergo a regression and regrowth which also involves similar changes in the axon terminals of its innervation neuron. This has been illustrated in *Manduca* for the motorneuron that innervates the larval abdominal muscle DEO1. During metamorphosis the DEO1 muscle undergoes degeneration, evident as a decrease in muscle width and the loss of the banding pattern caused by the sarcomeres, and subsequent regrowth of the adult specific muscle DE5 (Hegstrom and Truman, 1996; Truman and Reiss, 1995). The observed degeneration has been shown to be controlled by ecdysteroids while the regrowth depend on axon contact and ecdysteroids together (Hegstrom and Truman, 1996). Corresponding to the degeneration of the DEO1 muscle is the retraction of the innervating motorneuron. By the time the muscle as degenerated its sarcomere structure the corresponding motorneuron has retracted the synaptic end plates to the remnants of the first muscle fiber (Hegstrom and Truman, 1996; Truman and Reiss, 1995). The axon retraction, like the muscle degeneration is controlled by ecdysteroids. The regrowth of the axon into the adult synaptic pattern follows muscle regeneration and is needed for normal muscle growth (Truman and Reiss, 1995).

These are just a few examples of processes involved in remodeling of the larval neurons to accommodate the requirements of adulthood. Many of these studies have been carried out in Manduca because of the reasons previously discussed. In *Drosophila* the thoracic flight muscles and their corresponding motorneurons have been the best characterized system (Fernandes and Keshishian, 1998) with work in the abdominal ganglion being very limited (Currie and Bate, 1995; Taylor and Knittel, 1995).

Examples of sex-specific neuronal remodeling and cell death: Little work has been done to characterize sex-specific changes occurring in the abdominal ganglion in the background of the overall metamorphic transition. It is during metamorphosis when the majority of sexual traits develop in holometabolous insects. Giebultowicz and Truman (1984) have examined the sexual dimorphic population of motorneurons innervating the terminal abdominal segments of *Manduca*. In addition, Thorn and Truman (1989; 1994a; 1994b) have expanded the work on this question by focusing in on specific neurons and muscle targets and exploring the developmental control of their differentiation.

The initial survey done by Giebultowicz and Truman showed marked differences in the numbers of motorneurons filled from the ventral and dorsal nerve roots to abdominal segments A7 and A8 in the adult while equivalent numbers of motorneurons were found in larva (Giebultowicz and Truman, 1984). By following these cells during metamorphosis, the changes in neuronal number and their dendritic arborizations were mapped. These results provide evidence for the remodeling of pre-existing larval motorneurons and sex-specific loss, which was determined to occur on different time frames with males developing the adult pattern later then females (Giebultowicz and Truman, 1984). The sex-specific loss of the motorneurons was shown to be due to cell death by utilizing the method of toulidine blue staining. The number of neurons stained by toluidine blue matched the change in the number of motorneurons, which could be retrogradely labeled through application of cobalt chloride to the nerve (Giebultowicz and Truman, 1984). These studies were the first demonstration of sex-specific loss or remodeling of motorneurons during metamorphosis.

To further characterize the changes in motorneuron number Thorn and Truman (1989) also mapped out the transition from the larval musculature to the sexual dimorphic adult musculature in *Manduca*. All of the larval muscles in the terminal segments degenerate at the same time as the more anterior, nonsex-specific abdominal segments, but the regrowth of the different muscle remnants is sex-specific giving the male- and female-specific compliment of muscles (Thorn and Truman, 1989). By labeling the motorneurons of specific skeletal muscles for both the larva and adult, it was determined that the retained motorneurons correspond to muscles remodeled for an adult function

while motorneurons that are lost have also lost their corresponding target muscle during metamorphosis (Thorn and Truman, 1989).

In addition, Thorn and Truman (1989) also looked at motorneurons that innervate the visceral musculature of the internal reproductive tract. The visceral motorneurons were shown to be different in that they could switch targets during metamorphosis and, in addition, arise *de novo* (Thorn and Truman, 1989). For example, motorneurons that innervate the hindgut in larval animals switch after metamorphosis to innervate the oviducts and spermatheca in females. Other motorneurons are produced by post-embryonic neurogenesis to innervate the ovaries and the male reproductive tract, and thus, are examples of *de novo* production.

After finding evidence for the *de novo* production of sex-specific neurons, the neuroblasts which generate these neurons were identified in developing larvae and pupua. To identify these neuroblasts and the subsequent motorneurons, several techniques were utilized including labeling of dividing cells with either BudR or ^{3}H-Thymidine, since both chemicals will be incorporated into the newly synthesized DNA, and retrograde backfills from the nerve root (Thorn and Truman, 1994a). The population of neuroblasts identified gives rises to the Imaginal Midline Neurons (IMNs) that arise in all segments postembryonically (Thorn and Truman, 1994a). The IMN neurons in the more anterior segments develop as neurosecretory cells, while in the terminal segments they innervate the new visceral muscles of the reproductive tracts in males and females. Both the pattern of labeling the dividing neuroblasts, the appearance of identifiable motorneurons and segment specific loss of motorneurons differed in males and females for these neurons (Thorn and Truman, 1994a).

Following the identification of these postembryonic motorneurons for the terminal segments, Thorn and Truman (1994a; 1994b) also examined the developmental processes controlling their differentiation. To follow the fate of specific neurons, they chose to label the male-specific cells found in A9 for a neuroactive peptide, small cardioactive peptide b (SCPb). By comparison, the IMNs in segments A6-A8 show only weak staining or nonsex-specific staining (Thorn and Truman, 1994a). To examine the effects of the presence or absence of the adult target muscles, they used three methods: nerve transections; the removal of genital primordia; and topical juvenile hormone

application. The nerve transections and the removal of the genital primordia were used to block interactions between the motorneuron and target muscles. The results from the nerve transections were interesting in that some regrowth of axons was observed after the surgical operation, but the number of IMNs was variable and found to corresponded to the amount of innervation present on the sperm ducts (Thorn and Truman, 1994b). When no innervation was present, no motorneurons could be found, but when extensive reinnervation was seen all of the motorneurons could be found in the CNS. When the primordia was removed during the larval stage, to try and prevent the reinnervation problem, all of the motorneurons were found (Thorn and Truman, 1994b). Upon closer inspection it was found that the motorneurons found ectopic targets to innervate during metamorphosis and allowed for their survival into adulthood. Juvenile hormone (JH) is normally present during larval stages to prevent adult differentiation. Before the onset of metamorphosis the levels of JH decrease, which allows the corresponding increase in ecdysteroids to direct adult differentiation (Gilbert *et al.*, 1980; Riddiford, 1980; Riddiford, 1996). By topical application of JH before the onset of metamorphosis, it is possible to delay the adult development of specific structures relative to the rest of the animal. In this case, the purpose was to delay the development of the male reproductive tract, while allowing the motorneurons to continue development along the normal time frame of metamorphosis. The local JH treatment was a means to try and get around the problem of reinnervation and the innervation of abnormal targets. To be sure that the effect of the JH treatment was truly local two morphological aspects were measured. The first measurement was the degree of development found in the male reproductive tract. The second measurement was the degree of fusion found between the A6 and A7 ganglia, which is a normal developmental process during metamorphosis, and would indicate if the motorneurons were directly effected by the JH treatment and not by target interactions (Thorn and Truman, 1994b). From these experiments using ectopic JH treatments, there was a correlation between the amount of development seen in the sperm ducts and the number of IMNs found in the CNS (Thorn and Truman, 1994b). The results from all three experiments suggest that the survival of the IMNs is dependent on their finding a synaptic target for their survival through metamorphosis to adulthood.

Examples of cell death for larval neurons in forming the adult CNS: As discussed above, one important mechanism for forming the adult nervous system is for adult motorneurons to be retained from the larval CNS with neuronal survival dependent on which muscles are maintained during development. The fate of the larval motorneurons that are not maintained is apoptosis (Reviewed byTruman, 1990; Truman *et al.*, 1992; Weeks and Levine, 1992). During metamorphosis there are two main waves of cell death in the CNS, one early in development when the bulk of the body wall musculature is being established and the second wave after eclosion, when neurons only needed for ecdysis behavior are lost (Taylor and Truman, 1974; Truman, 1990; Truman *et al.*, 1992; Weeks and Levine, 1992).

The early wave of cell death has been examined in several model muscle-motorneurons interactions. One example was previously described above for the case of sex-specific motorneurons (Giebultowicz and Truman, 1984; Thorn and Truman, 1989; Thorn and Truman, 1994a; Thorn and Truman, 1994b). Another example that has been extensively studied by Janis Weeks and colleagues are the motorneurons PPR and APR, which innervate muscles in the larval proleg of *Manduca* (Reviewed inWeeks and Levine, 1992). From retrograde fills of these motorneurons, their fates have been followed during the early larval-pupal transition. The PPRs are lost during the larval-pupal transition from the abdominal segments A3-A6 while the APR motorneurons are lost at the larval-pupal transition from abdominal segments A5-A6 and after emergence for abdominal segments A3-A4 (Lubischer and Weeks, 1996; Weeks and Truman, 1985). From these experiments, several important aspects of the developmental control for the loss of these motorneurons have been determined. By endocrine manipulations of the steroids during metamorphosis Weeks (1985) was able to show that the loss of these motorneurons is controlled by the ecdysteroid titres. In addition, she also demonstrated that there are no direct interactions between the muscles, motorneurons, or the sensory inputs that regulate survival, but ecdysteroids independently controlled the loss of both the muscle and motorneuron (Lubischer and Weeks, 1996; Weeks and Truman, 1985). Further proof of independent steroid control on the loss of the APRs has also been demonstrated by the use of *in vitro* cell culture. When the motorneurons were cultured, they responded to the ecdysteroids at the same time during development as had been

observed *in vivo* (Streichert *et al.*, 1997). The PPR and APR motorneurons provide a good example of the control of motorneurons that die in the early stages of metamorphosis by hormones. Few studies have been carried out on *Drosophila*, except for looking at the development of abdominal muscles and the role neurons play in muscle development (Currie and Bate, 1995; Lawrence and Johnston, 1984; Lawrence and Johnston, 1986). Nerve transection of the abdominal nerve at the white prepupal stage did not prevent the development of dorsal longitudinal abdominal muscles showing that these muscles, at least, form independently of innervation though they do not reach their normal adult size (Currie and Bate, 1995). For the adult thoracic indirect flight muscles, innervation ahs been shown to be responsible for some aspects of muscle development in combination with other patterning elements (Fernandes and Keshishian, 1998).

The role of cell death after metamorphosis has been more extensively explored than the initial pupal wave of death. In *Manduca* Taylor and Truman (1974) provided one of the first accounts of the pronounced wave of cell death that occurs after metamorphosis. A general survey was completed by Kimura and Truman (1990) mapping the loss of the larval retained muscles, which participate in ecdysis behavior, and their corresponding motorneurons in *Drosophila*. In addition to mapping the time course of muscle and motorneuronal cell death, experimental ligations between the neck and thorax and between the thorax and abdomen were used to identify the source of signals which might control the loss of the muscles and motorneurons. From these experiments it was found that the muscles were signaled to die by a signal before the onset of ecdysis, while the signal for the motorneurons to die came at the time of wing inflation (Kimura and Truman, 1990). The fate of the muscles and motorneurons were also found to be affected by prolonging the ecdysis behavior, which is done by trapping the animal in another pupal case. If the ecdysis behavior is extended two hours, the death of the muscles is also delayed two hours, but if the ecdysis behavior lasts for more than two hours, then muscle breakdown occurred after a two hour delay. On the other hand, if ecdysis was prolonged and wing inflation wasn't allowed to occur, the motorneurons did not die (Kimura and Truman, 1990). These experiments have provided the initial characterization needed for further studies in the developmental control of these neurons.

In *Drosophila* it is possible to carry out a molecular and genetic analysis unavailable in other systems. Further analysis of the fate of the doomed neurons has been carried out by looking at the expression of the ecdysone receptor (EcR) isoforms. Metamorphosis is hormonally controlled by the ecdysteroids, which bind with the hormone receptors and act as transcription factors. When the expression pattern of two out of the three ecdysone receptor isoforms, EcR-A and EcR-B1, was monitored in the doomed neurons, a high level of EcR-A was present in a group of 284 neurons (Robinow *et al.*, 1993). The distribution of these cells was similar to the distribution that Kimura and Truman (1990) had found previously for the neurons that die after metamorphosis. When the expression pattern of the receptor isoforms were examined during metamorphosis, two general classes of expression were found. Type I expression was defined by the presence of low levels of expressed EcR-A while type II expression was defined as high, sustained levels of EcR-A (Robinow *et al.*, 1993). The type II expression pattern was proposed to correspond to those cells found after adult emergence that were slated to die. Since the levels of ecdysteroids decrease at the end of end of metamorphosis, Robinow et al. also injected 20-hydroxyecdysone to try and alter the fate of these neurons by maintaining a higher level of ecdysteroids. After the injections the identified neurons were maintained past the point at which they would normally degenerate, but they were still able to undergo degeneration at a later time (Robinow *et al.*, 1993). These studies also confirm an important role for ecdysteroids in the control of neuronal cell death after metamorphosis.

Examples of new cell additions to the adult CNS: The last important mechanism for producing the adult nervous system, is the generation of new neurons that only have adult functions. There have been two studies which have identified and followed the development of the postembryonic neuroblasts for *Drosophila* (Truman and Bate, 1988) and *Manduca sexta* (Booker and Truman, 1987). Both studies labeled dividing neuroblasts by either the use of ^{3}H-thymidine or BUdR. In both cases the populations of postembryonic neuroblasts are found in stereotypic locations and can be reproducibly followed in nervous systems throughout development (Booker and Truman, 1987; Truman and Bate, 1988). Similar patterns of neuroblast locations can be found in both *Manduca* and *Drosophila* with the majority of the neuroblasts being found in the brain,

optic lobes, and thoracic ganglia and relatively few neuroblasts in the abdominal ganglia. In *Manduca*, Booker and Truman (1987) found 22-23 pairs of neuroblasts and 1 unpaired neuroblast in each thoracic ganglia, 12 pairs of neuroblasts for the abdominal segment A1 ganglia, and 4 pairs of neuroblasts for the abdominal segments A2-A6 ganglia. The ganglia for abdominal segments A7 and A8 is fused, but the pattern of neuroblasts is similar to the pattern observed in A2-A6 with the exception of an additional 9 pairs of neuroblasts found in the lower ganglia (Booker and Truman, 1987). In *Drosophila*, Truman and Bate (1988) also found 23 paired neuroblasts and 1 unpaired neuroblast in the three thoracic ganglia, 12 paired and 1 unpaired neuroblast in the ganglia that corresponds to A1, 4 paired neuroblasts for the A2 ganglia, and 4 paired neuroblasts for the ganglia corresponding to A3-A7. The terminal pattern of A8 and lower was not reported.

Taylor and Truman (1992) examined a sex-specific population of neuroblasts in *Drosophila*. In both males and females there are 12 neuroblasts found in the terminal abdominal ganglion, which are labeled by *in vitro* incubation with BUdR. In females, all 12 neuroblasts cease mitotic divisions, determined by the inability of the cells to incorporate BUdR into their DNA, during the middle of the third larval instar at the same time as the neuroblasts found in the more anterior abdominal segments (Taylor and Truman, 1992). In contrast, in males, 8 of the 12 neuroblasts found in the terminal abdominal ganglion stop dividing at the same time as the other abdominal neuroblasts (Taylor and Truman, 1992). To look at the genetic control of the development of the 4 neuroblasts, which remain mitotically active into early pupal development, the presence or absence of BUdR labeled cells during late third instar larva or early pupa stages were assayed in several mutations of the sex determination hierarchy. The genes *transformer* (*tra*) and *transformer-2* (*tra2*), both of which cause chromosomal females to develop as phenotypic males, caused both XX and XY nervous systems to have the 4 male-specific neuroblasts (Taylor and Truman, 1992). The other gene examined was *doublesex* (*dsx*), which produces a male- and female-specific DSX protein, which directs the development of sexual features (Burtis and Baker, 1989). In *dsx* null mutant animals only 8 of the 12 neuroblasts could be located at the early stages of development and no neuroblasts were labeled in the late third instar and early pupal stages (Taylor and Truman, 1992). The

dsx^D allele, which is a dominant allele that only expresses the male-specific transcript, was shown to be necessary and sufficient to support the male pattern of neuroblast division. XX and XY animals that carry the dsx^D allele over a deficiency, which removes the coding region of *dsx*, the four male-specific neurons were present (Taylor and Truman, 1992). These results together show that *dsx* has a positive regulatory role in the development of the male-specific neuroblasts. To further explore this question the use of temperature sensitive alleles of *tra2* were used to determine the time frame that *dsx* was needed to commit the neuroblasts to the male pathway. Temperature sensitive alleles are functional when the animal is kept at a low temperature, such as 18°C, but if the animal is kept at a higher temperature, such as 29°C, the protein becomes unstable and thus acts as a null. By moving the animal between different temperatures it is possible to allow for normal female gene expression and development and then changing the gene expression to a male-specific form at different time points. From temperature shift experiments it was determined that the male-specific pathway of development had to be activated by the end of the first instar larval period and be maintained through the period of the production of the neuroblasts (Taylor and Truman, 1992).

Genetics of Sex Determination

The sex determination hierarchy in Drosophila melanogaster: The sex determination hierarchy controls the overall determination of sexual dimorphic differences and segmental differences. The basic framework of the sex determination hierarchy has been worked out at both a genetic and molecular level (Reviewed in Burtis, 1993; Cline and Meyer, 1996; Hall, 1994; MacDougall *et al.*, 1995). In *Drosophila*, a cascade of genes determines sex (Figure 1.1). The primary events are initiated by assessment of the ratio of X chromosomes to autosomal chromosomes (X:A ratio). Female flies have a composition of two Xs to a set of autosomes, which gives a 1:1 ratio, while male flies have only one X to a set of autosomes, which is a 1:2 ratio. The X:A ratio determines if *Sex-lethal* (*Sxl*) will be transcribed and spliced into the functional female form or if the non-functional male form of the protein will be expressed (Bell *et al.*, 1991; Cline, 1984).

Functional SXL protein acts to autoregulate the splicing of its own primary transcript and that of the next gene in the hierarchy, *transformer* (*tra*). TRA and TRA-2 proteins together modulate the splicing of the *doublesex* (*dsx*) primary transcript into the female-specific transcript (Nagoshi *et al.*, 1988). The resulting DSX^F protein suppresses expression of male specific genes and can also activate female specific genes (Burtis *et al.*, 1991; Coschigano and Wensink, 1993). The end result is a phenotypic female. In males, where the non-functional SXL protein is produced, *tra* is not expressed and *dsx* pre-mRNA is spliced into the default male transcript (Nagoshi *et al.*, 1988). The DSX^M protein suppresses the expression of female-specific genes (Burtis, 1993; Burtis *et al.*, 1991; Coschigano and Wensink, 1993; Hildreth, 1965; Jursnich and Burtis, 1993). There also appears to be two other output pathways, identified by the genes *fruitless* (*fru*) and *dissatisfaction* (*dsf*). Both of these genes control male sexual behavior and *dsf* affects female reproductive behaviors (Gailey and Hall, 1989; Hall, 1978; Ito *et al.*, 1996; Ryner *et al.*, 1996; Villella *et al.*, 1997). The role of *fru* is just starting to be revealed on a molecular level, but the exact mechanism and role which *fru* serves in sexual development is still unknown (Ito *et al.*, 1996; Ryner *et al.*, 1996). At this time, there are seven overlapping classes of transcripts known that appear to encode for BTB-Zinc finger family of transcription factors. A subset of these transcripts are sex-specific and spliced under the control of *tra* and *tra-2* (Ryner *et al.*, 1996). The phenotypes observed for *fru* mutants show mainly a male behavioral effect with no effect on the development of external structures such as the genitalia or abdominal segmentation. The expression of sex-specific *fru* appears to be restricted to the central nervous system by *in situ* hybridization. From consideration of the neuronal expression pattern of sex specific *fru* and the behavioral phenotypes, *fru* appears to be a gene responsible for setting up the behavioral circuits responsible for courtship behaviors in the CNS and a single sex-specific muscle, the Muscle of Lawrence (MOL) (Gailey *et al.*, 1991; Ryner *et al.*, 1996; Taylor and Knittel, 1995).

Figure 1.1: The Sex Determination Hierarchy in *Drosophila melanogaster*. The flow of information through the sex determination hierarchy (SDH), which controls somatic sexual differentiation, is illustrated in (A). Non-functional gene products are indicated by outlined text with genetic interactions indicated by arrows. The phenotypes controlled by the output genes *dsx*, *fru*, and *dsf* are indicated below the gene name for both males and females. (B) Diagram of regulated alternative splicing events in the sex determination hierarchy. The genetic functions of the genes are listed as on or off, even though there are non-functional proteins present in males for both *Sxl* and *tra*. In females the functional *Sxl* protein regulates the female-specific splicing of *tra* to give a functional protein. With functional *tra* present, *tra* and *tra2* regulate the female-specific splicing of the output genes *dsx* and *fru* resulting in female-specific proteins. In the absence of the female TRA or TRA2 protein, *dsx* and *fru* primary transcripts are spliced in the default male-specific splice, which results in the production of male-specific proteins in *tra* and *tra2* mutant females.

Figure 1.1

(A) SEX DETERMINATION HIERARCHY

FEMALE PATHWAY

MALE PATHWAY

Sex-lethal

Sex-lethal

transformer
transformer-2

transformer
transformer-2

?

?

doublesex
♀ Genitalia
♀ Abdominal.
Segmentation
♀ NBs

fruitless
?

dissatisfaction
♀ Uterine
innervation
♀ Oviposition

doublesex
♂ Genitalia
♂ Abdominal
Segmentation
♂ NBs

fruitless
♂ MOL
♂ Copulation
♂ Courtship

dissatisfaction
♂ Abdominal
innervation
♂ Abdominal
Bending

(B) SPLICING EVENTS IN THE SEX DETERMINATION HIERARCHY:

♂

	OFF		OFF	ON	DEFAULT SPLICE	dsx^M, fru^M
♂	Sxl	→	tra	tra-2	→	
♀	ON		ON	ON	♀-SPECIFIC SPLICE	dsx^F, fru^F

♀

dsf is a gene recognized as belonging to a third output pathway but its precise role is not understood. *dsf* and *fru* mutant males share a similar male-male courtship phenotype that disrupts courtship (Finley *et al.*, 1997). In addition to the behavioral abnormalities *dsf* mutants lack uterine innervation in females and show abnormal innervation of the ventral longitudinal muscles of abdominal segment A5 in males (Finley *et al.*, 1997). These two pathways do not appear to be primarily involved in the development of sex-specific muscles in the terminal abdominal segments or genitalia, but may be involved in the differentiation of some sex-specific motorneurons.

Caenorhabditis elegans sex determination: Caenorhabditis elegans is another powerful genetic model system used to elucidate developmental processes. There are several similarities and differences that can be found between the two genetic cascades that control sex determination (Recently reviewedCline and Meyer, 1996). Both *C. elegans* and *Drosophila* utilize an X:A ratio to initiate hermaphrodite or female development, respectively (Kuwabara and Kimble, 1992; Meneely, 1994; Meneely, 1997). The first difference that is found between *C. elegans* and *Drosophila* is the role played by the target gene of the X:A ratio. In *Drosophila* the target gene, *Sxl*, is turned on in females and activates the female-specific pathway (Bell *et al.*, 1991; Cline, 1984). For *C. elegans*, on the other hand, the target gene, *XO lethal* (*xol-1*), is active in males to repress female-specific development (Cline and Meyer, 1996; Kuwabara and Kimble, 1992; Meneely, 1997).

The next major difference between flies and worms is the nature of the molecular control of each of the sex determination genetic cascades. As described above, *Drosophila's* genetic cascade for somatic tissues is controlled by alternative splicing of the primary transcripts of the genes *tra*, *dsx*, *fru*, and possibly *dsf*. In *C. elegans* the genetic cascade is controlled at the level of transcriptional regulation. The gene *xol-1* heads the pathway and operates by repressing expression of the downstream targets, the *sex and dosage compensation* (*sdc*) genes, with the most likely target being *sdc-2* (Cline and Meyer, 1996; Kuwabara and Kimble, 1992). This leads to the case of the gene *hermaphroditization-1* (*her-1*) being active in males and inactive in females, due to repression by the *sdc* genes. *her-1* is interesting in that it functions in a non-cell autonomous manner, which is the phenomenon of being influenced by cell-cell

interactions in the developing worm and has been demonstrated by mosaic analysis (Hunter and Wood, 1992). The *her-1* gene is required for male development since loss-of-function mutations cause development of hermaphrodites, due to repression of the genes *transformer-2* (*tra-2*) and *transformer-3* (*tra-3*) (Cline and Meyer, 1996; Kuwabara and Kimble, 1992). The role of *tra-2* and *tra-3* is to repress the *feminization* (*fem*) genes, *fem-1*, *fem-2*, and *fem-3*. The role of the *fem* genes is two-fold. Their first function is to the repress the output gene *tra-1*, which encodes a transcription factor and is responsible for hermaphrodite development. Their second function is to permit spermatogenesis in hermaphrodites (Cline and Meyer, 1996; Kuwabara and Kimble, 1992).

Mammalian sex determination: In contrast to sex determination in *Drosophila* and *C. elegans* mammalian sex is not determined by the number of X-chromosomes but by the presence of the Y-chromosome. The Y-chromosome carries the gene *Sry* (*Sex determining Region Y gene*) that dominantly controls the development of testis (Goodfellow and Lovell-Badge, 1993; Lovell-Badge and Hacker, 1995). The developing testes are then responsible for sending signals via hormones to control the differentiation of the embryo, which otherwise would develop as the default female state. The two major hormones released by the testis are androgens, such as testosterone, and Mullerian-inhibiting substance (MIS), also known as anti-Mullerian hormone (AMH) (Behringer, 1995; Lovell-Badge and Hacker, 1995). The role of MIS is to cause the regression of the Mullerian ducts, which are the primordia for the female reproductive organs. Testosterone acts on the Wolffian ducts to control the differentiation of the male reproductive organs (Behringer, 1995). Without the development of the testes, the default female state is followed and ovaries are formed. The ovaries release female gonadal hormones consisting of estrogen and progesterone.

After the development of either the testes or ovaries, sex steroid hormones control the rest of the somatic development of the embryo. Several sexually dimorphic neurons have been examined in mammalian systems. These studies have demonstrated a role of gonadal steroids in the development of either the male or female specific nervous system or sex-specific structures, such as the spinal nucleus of the bulbocavernosus (SNB) and its target muscles (Reviewed by Breedlove, 1992; Weeks and Levine, 1995).

There is also evidence that some somatic sexual differentiation, in addition to the testis, is genetically controlled and not under the influence of gonadal steroids (Recently reviewedArnold, 1996). The best examples of genetic control of sexual differentiation are in marsupials, but the agents of this genetic control are not known. Several structures, including the pouch and mammary glands in females and the scrotal primordia and gubernaculum in males, differentiate before gonad differentiation and are insensitive to steroid treatments (Arnold, 1996). Although no known genes control the development of these structures, it is suggestive of a genetic mechanism and not a hormonal mechanism. Another observation, that also suggests genetic mechanisms, has been observed in *in vitro* cultures of embryonic mesencephalic neurons, which show sex-specific expression of prolactin and tyrosine hydroxylase. The cells that are cultured are removed from the embryo before any detectable levels of steroids are found, but still give either male or female levels of expression after identical culture conditions (Arnold, 1996). Besides these two examples there are other instances in which gonadal steroids are unable to fully explain the observed phenotypes. Further genetic analysis will need to be completed to determine the genetic pathway below the *Sry* gene and to determine the precise nature of the non-steroidal regulatory genes acting in mammalian sex determination.

Evolution of sex determining genes: The early regulation of sex determination has been shown to be very diverse between all animals studied. In the three examples provided above on the genetics of sex determination very diverse mechanisms have been demonstrated to control sex determination and sexual differentiation. In the case of mammalian sex determination, the *Sry* gene shows very little sequence homology between species, with only the DNA binding domain, the HMG-box, showing significant similarity (Greenfield and Koopman, 1996; Lovell-Badge and Hacker, 1995).

Using the *C. elegans* pathway as an example Adam Wilkins proposed that sex determination pathways would evolve from the lower, output genes in the pathways to the upper levels in the pathways (Wilkins, 1995). If this is the case, then the genes in the upper, regulatory roles could easily be very divergent while the genes which direct the developmental processes will likely be more conserved. This idea was recently supported by the research on the gene *mab-3* in *C. elegans*. *mab-3* acts downstream of the switch gene *tra-1* to control male development for the V rays, which are peripheral

sensory structures in the male tail, and to repress vitellogenin expression (Raymond *et al.*, 1998). When the *mab-3* was isolated the DNA-binding motif was two copies of a domain which is similar to the DSX DNA-binding domain found in *Drosophila* (Raymond *et al.*, 1998). With this finding the group tested if *dsx* could be exchanged for *mab-3* function. When either DSX^M protein or MAB-3 protein was ectopically expressed in a *mab-3* mutant background the V ray phenotype was rescued, while expression of DSX^F protein had no effect on the mutant phenotype (Raymond *et al.*, 1998). This is the first demonstration of a sex-determining gene having function in more than one species. Of interest is that only the DNA-binding domains in these two proteins share any homology and that alone is enough to serve a functional effect.

Possible Roles of *dsx* in Development of Sex-Specific Muscles and Motorneurons

Since sex-specific differences in *Drosophila* are the output of a genetic hierarchy, the role of individual genes can be assessed in the development of neurons. Having found that there are three out pathways, there are potentially several types of interactions that could be involved. The most likely candidate to control the bulk of the sex-specific abdominal muscles and motorneurons is *dsx* because *dsx* mutant males and females have intersexual external development unlike mutants in *fru* or *dsf*. Thus, an important question to consider when examining the role *dsx* plays in the development of sex-specific muscles and motorneurons is its possible 'areas of action'. Since *dsx* has already been shown to play a role in the specification of the genitalia, genital muscles and cuticle, there is the possibility that diverse cellular interactions might determine the fate of the muscles, motorneurons, or both. In particular, there are three tissues in which *dsx* could act: the muscles, the epidermis, and the motorneurons. If *dsx* acts directly, then *dsx* activity would be required in both motorneurons and muscles for proper development. Alternatively, *dsx* may act indirectly by one of two possible mechanisms (Figure 1.2). One mode of indirect action would be the presence or absence of a target muscle determining the fate of the motorneuron. Thus, in wildtype males or females, the appropriate development of sex-specific muscles would allow only the respective sex-

specific motorneuron to survive. If this were the case, the expectation for *dsx* mutants, where both male- and female-specific muscles are present, would be the survival of both the male and female populations of motorneurons. For example in A7, the motorneurons associated with A7 should be present since the segment is present in both chromosomally male and female *dsx* flies. The other mode of indirect action would be the presence or absence of a motorneuron, which determines the fate of the target muscle. If this were the case the same expectations would be true as for the first mode of indirect action. There is evidence from other model systems, which suggests that both, direct and indirect effects can determine the fates of motorneurons and target muscles. Most of the research in this area has concentrated on vertebrate systems and have examined the initial development of the neuromuscular junction and the control over the development of the sexual dimorphisms in the muscles and nervous system by the action of steroid hormones.

Evidence for the target muscle determining the fate of its motorneuron: In mammals it has been shown that neonatal muscles are innervated by multiple axons. These axons through a process of competition and retraction undergo synapse elimination until only a single axon innervates the muscle (Purves and Lichtman, 1980). In other vertebrate systems, such as the chick and rat, the role of trophic factors and synaptic activity in the survival of motorneurons and muscles has been demonstrated (Forger *et al.*, 1993; Milligan *et al.*, 1994). Forger *et al* was able to rescue both motorneurons and muscles from developmental death by exogenous treatments of ciliary neurotrophic factor (Forger *et al.*, 1993).

In the sexually dimorphic muscle systems of vertebrates, steroid hormone action occurs in either the muscle, the neuron, or both. In these systems, the primary role of the muscle in neuronal survival has demonstrated the importance of a target in the development of a sex-specific neuromuscular system. Rand and Breedlove (1995) have

Figure 1.2: Model for independent versus dependent regulation by *dsx* in the development of sex-specific muscles and motorneurons.

(A) Model for how *dsx* would regulate the development of female-specific muscles and motorneurons.

(B) Model for how *dsx* would regulate the development of male-specific muscles and motorneurons.

The arrow indicates the site of action for either the DSX^F or DSX^M protein. Hatched muscles and/or motorneurons indicate the structures that are lost during development. Red structures are found in females (top portion), while blue structures are found in males (bottom portion). The left column gives the expected results in the case of direct control by *dsx* on the development of both the muscles and the motorneurons. The right column gives the expected results in the case of indirect control by *dsx*, resulting in *dsx* determining the fate of the muscle and the muscle determining the fate of the motorneuron.

Figure 1.2

(A) Female Specific Muscles

Direct Effect by the Gene *dsx*

Female Larval

Adult — DSX^F — DSX^F

Male Larval

Adult — DSX^M — DSX^M

Indirect Effect by the Gene *dsx*

Target induced survival — DSX^F

Lack of target induced death — DSX^M

(B) Male Specific Muscles

Direct Effect by the Gene *dsx*

DSX^F — DSX^F

DSX^M — DSX^M

Indirect Effect by the Gene *dsx*

DSX^F

Lack of target induced death

Target induced survival — DSX^M

25

shown an indirect effect for steroid hormones on the motorneurons in the spinal nucleus of the bulbocavernosus (SNB) by local treatment to the two sets of muscles innervated, the bulbocavernosus and levator ani muscles. By implanting capsules with either testosterone or hydroxyflutamide, an anti-androgen, at the muscle it was possible to examine the direct effects of the steroid treatment to the muscle alone but not the neurons. The results showed that the motorneurons in hydroxyflutamide treated muscles had a 44% reduction of dendritic arborization when compared to the testosterone treated muscles even though the motorneurons were not directly influenced by the steroid hormone treatment (Rand and Breedlove, 1995). This illustrates an indirect effect on the motorneuron through the direct effect of the steroid hormone on the muscle.

Target derived regulation is not confined to the vertebrate system. In an insect model system, *Manduca sexta*, Thorn and Truman (1994 b) demonstrated that the survival of the imaginal midline neurons, innervating the sperm duct, are dependent upon an interaction with a developing muscle target during metamorphosis to survive. The evidence for this dependent interaction included localized treatment of juvenile hormone, to maintain the muscle target in the larval stage, nerve transection during the pupal stage, and removal of the reproductive tract primordia to eliminate additional target structures. These treatments resulted in a decrease number of the imaginal midline neurons, suggesting that the interaction with the adult muscle target for these neurons increases the survival of these cells (Thorn and Truman, 1994b).

Evidence for the direct determination of target muscles and motorneurons: In weakly electric fish, the sexually dimorphic electric organ discharge shows independent effects of sex steroids on the differentiation of neurons and their target muscles. With androgen treatments, female weakly electric fish show a change in frequency towards the males range of frequency. This effect is seen in the pacemaker nucleus, which drives the frequency of the discharge, the spinal motorneurons, and the electrocytes, which are modified muscle cells that produce the electric discharge. Not only is the motorneuronal output sensitive to sex-steroids but the sensory electroreceptor cells, that receive the signal and are tuned to its own electric organ discharge frequency, also respond to androgen treatment (Mills and Zakon, 1991; Schaefer and Zakon, 1996; Zakon, 1993; Zakon *et al.*, 1991). Even when the motor and sensory components are isolated from

each other, the masculinizing shift occurs suggesting that the steroid hormone works independently on these two systems (Mills and Zakon, 1991; Schaefer and Zakon, 1996; Zakon, 1993; Zakon *et al.*, 1991). Similar dual effects of steroid hormones was found in invertebrate systems (For example,Weeks and Truman, 1985)

Another vertebrate system that shows direct effects of gonadal steroids on neurons and muscles is the sexually dimorphic larynx and their motorneurons in *Xenopus laevis*. In this system, estrogen causes an increase in quantal content, the amount of neurotransmitter released, at the neuromuscular synapse in the larynx of treated females (Tobias and Kelley, 1995). Steroids independently affect muscle size and the shape of the larynx box (Tobias and Kelley, 1995).

The Genetic Control of Programmed Cell Death

Programmed cell death (PCD) is a major process in the development of organisms. The ability to eliminate extra cells gives developing tissue plasticity during development to adjust to the different conditions and maintain the correct proportions of tissues and cells. All developing systems start out with more cells than are needed and the action of programmed cell death trims these superfluous cells back to match other tissues.

Research on the genetic control of programmed cell death has advanced very quickly. Two model systems, *Caenorhabditis elegans* and *Drosophila melanogaster*, have been used to identify genes regulating this major developmental process (recently reviewed in McCall and Stellar 1997). From mutant analysis of PCD in *C. elegans*, a pathway including about 9 *ced* genes has been proposed to lead from the initiation through the execution of cell death. The nematode, *C. elegans* is an excellent system to explore the genetics behind cell death, since all of the cells that normally undergo cell death are known in the developing worm. Three major *ced* genes, *ced-3*, *ced-4*, and *ced-9*, are involved in the pathway. *ced-3* and *ced-4* have been shown to be responsible for activating the cell death program, while *ced-9* appears to have a protective role by inhibiting the cell death program(Ellis and Horvitz, 1986; Hengartner *et al.*, 1992;

Shaham and Horvitz, 1996) (McCall and Steller, 1997). The exact genetic relationship between *ced-3* and *ced-4* has not been elucidated, they could act in parallel pathways or *ced-4* could act upstream of *ced-3* (Shaham and Horvitz, 1996).

In *Drosophila*, the search for the complete cell death pathway is just beginning. Several candidate genes have already been discovered, which include *reaper (rpr)*, *grim*, and *head-involution defective (hid)*. Already the *Drosophila* pathway appears to be more complex than the simple linear model described for *C. elegans*. Understanding the genetics of programmed cell death has been slower in Drosophila. The mutations currently known were found in a genetic screen of deletions that affected programmed cell death. One deletion, H99, appeared to be responsible for normal embryonic programmed cell death (White *et al.*, 1994). In embryos homozygous for this deletion none of the characteristic PCD that is common in embryogenesis was present. All three genes, *rpr*, *grim*, and *hid*, are located in the DNA defined by the deletion H99. The gene *reaper (rpr)* was the first gene identified in the deletion screen (White *et al.*, 1994). Reaper is a 65 amino acid protein that appears to activate the 'death program' through a ced-3/ICE-like protease, but doesn't appear to be a member of the death machinery itself (Pronk *et al.*, 1996; White *et al.*, 1996). *In situ* hybridization shows that *rpr* transcripts appear in cells one to two hours before they can be shown to be dead using vital dyes such as acridine orange (White *et al.*, 1994). Results also suggest that most PCD in *Drosophila* occurs through a common mechanism, which is activated by *rpr*, and multiple signaling pathways converge on *rpr* for induction of cell death (Pronk *et al.*, 1996; White *et al.*, 1996).

Like *rpr*, *hid* appears to induce PCD independently and by itself is sufficient to induce apoptosis when expressed as a transgene (Grether *et al.*, 1995). Even though PCD can by induced by the ectopic expression of *hid*, the normal expression pattern does not strictly correspond to cells that will undergo PCD, as is the case with *rpr* and *grim*, since some expressing cells survive (Chen *et al.*, 1996; Grether *et al.*, 1995; White *et al.*, 1994). Besides their individual roles, a synergistic effect has been shown for *rpr* and *hid* in a specific set of midline cells, which follow a known pattern of cell death during embryonic development. In animals carrying the H99 deficiency, ectopically supplied *rpr* and *hid* genes were expressed individually or co-expressed in the midline cells using

the Gal4::UAS system. When *rpr* and *hid* were co-expressed in the midline cells, a greater number of cells underwent programmed cell death than when either gene was expressed individually. When two copies each of *rpr* and *hid* were expressed in these cells, all of the midline cells and the VUMs, which normally don't undergo cell death, also underwent programmed cell death (Zhou *et al.*, 1997). These data suggest that expression of these genes is sufficient for driving cells to die.

grim appears to also be an activator of the programmed cell death pathway and is independent of *rpr* and *hid* expression. *grim* expression is similar to that of *rpr*, since its expression predicts cell death and when misexpressed it is sufficient to kill cells (Chen *et al.*, 1996).

The downstream targets of *rpr*, *hid*, and *grim* are currently unknown. Since these genes work independently of each other they could converge on to a common pathway that leads to the cell death 'machinery' or they could work in several parallel death pathways which all result in cell death.

Some other genes have been implicated in regulation or causing cell death but their role is not well understood. One of these genes is the ubiquitin gene, which encodes for a highly conserved 76 amino acid protein in eukaryotic cells and is part of the mechanism that targets proteins for degradation (Schwartz, 1991). Thus ubiquitin has a role in normal cellular homeostastis (Schwartz *et al.*, 1990). Schwartz demonstrated that polyubiquitin gene expression was associated with the PCD of the intersegmental muscles in *Manduca sexta*. He showed that expression levels changed during development and specifically in structures that were slated to die. By contrast, the expression of ubiquitin was not correlated with the death of the motorneurons innervating the intersegmental muscles. The results showed that there was not always the temporal coupling of ubiquitin staining that was seen in the muscles and sometimes the increase in staining intensities were seen days in advance and at not of great an increase (Muller and Schwartz, 1995).

Chapter 2

Genetic Regulation of Sex-specific Motorneurons in the Abdominal Ganglion of
Drosophila melanogaster by the Gene *doublesex*

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Introduction

The central nervous system of holometabolous insects, such as *Drosophila melanogaster*, undergoes extensive 'remodeling' during metamorphosis resulting in the more complex CNS required in adulthood (Reviewed in Truman, 1990; Truman *et al.*, 1992; Weeks and Levine, 1992). During the general restructuring of the nervous system during metamorphosis there is also sex-specific restructuring that must occur. Adult males and females have several sexually dimorphic features that directly interact with the nervous system including reproductive behaviors, sex-specific sensory structures, sex-specific muscles and the genitalia (Hall, 1977; Hall, 1978; Merritt, 1994; Taylor, 1989a; Taylor, 1989b).

In *Drosophila melanogaster* the sex determination hierarchy controls sex determination and differentiation. The sex determination hierarchy is a cascade of genes which has been well characterized both at a molecular and genetic level and is regulated by alternative splicing (Reviewed in Baker, 1989; Baker and Belote, 1983; Burtis, 1993; Cline and Meyer, 1996; Hall, 1994; MacDougall *et al.*, 1995). The gene *Sex-lethal* (*Sxl*), which controls the female-specific splicing of itself and the gene *transformer* (*tra*), heads the hierarchy. The next level of the hierarchy are the genes *tra* and *transformer-2* (*tra*), which act together in females to regulate the female-specific splicing of the output genes *doublesex* (*dsx*) and *fruitless* (*fru*). At the bottom of the hierarchy are the output genes *dsx*, *fru* , and *dissatisfaction* (*dsf*). The output genes encode transcription factors that control the somatic differentiation of the sexual dimorphic features of the fly.

Of interest is how the genetics of the sex determination hierarchy control the development of the sex-specific motorneurons in the terminal abdominal ganglion. From work in *Manduca sexta* it has been previous shown that there are sex-specific populations of motorneurons for the segments associated with the genitalia (Giebultowicz and Truman, 1984; Thorn and Truman, 1989). The sex-specific motorneurons for the skeletal muscles in the *Manduca* have also been shown to be retained larval motorneurons, and sex-specific loss of these motorneurons occur during metamorphosis (Giebultowicz and Truman, 1984; Thorn and Truman, 1989). The genetic control of these motorneurons has yet to be determined.

In order to determine the genetic control of sex-specific motorneurons, these motorneurons need to be identified. The motorneurons we have studied are identified by two different methods. The first method utilized a neuronal retrograde transport technique. By incubating the cut end of a segmental nerve root in Biocytin, a lysinated biotin molecule, which is then transported up the axon to the motorneuronal cell body, it is possible to label motorneurons that have axons projecting into a specific nerve. Using this method groups of motorneurons are identified according to the segment that they innervate. The second method utilized a reporter construct which carried a marker, *tau-lacZ*, that had been inserted into the chromosome with a transposable P-element vector. The reporter construct is designed to enable nearby enhancers in the *Drosophila* genome to drive the expression of the reporter gene. In a fly strain, P[*tau-lacZ*] 4.078 (called 4.078), the β-galactosidase label is found in a segmentally repeated set of neurons, the ventral unpaired median cells (VUMs). Using this method, a smaller more defined group of cells can be followed. After the neurons were identified, it has been possible to look at the genetic regulation of their development during metamorphosis in both wildtype and mutants in the sex determination hierarchy. By these techniques, I have shown that *dsx* regulates the survival of VUMs in both male and female adult flies in a sex-specific pattern.

Materials and Methods

*Fly Stocks:*The 4.078 line was maintained as a homozygous line on standard dextrose, cornmeal, and agar food with Nipagin M or propionic acid as a mold retardant. Wildtype flies were Canton-S strain. Standard recombinational techniques were used to generate 4.078, *dsx* recombinants.

Immunohistochemical Detection of Neurons: In order to detect the labeled neurons in the 4.078 line, immunohistochemistry was carried out on dissected CNSs from 3rd instar larva, pupa, and adult 4.078 flies. Preparations were fixed for 1-2 hours in 4% paraformaldhyde and then could undergo one of 3 treatments to improve penetration. The first treatment used was a graded methanol series with 2 minutes at each step using

the following concentrations of methanol, 50%, 75%, 95%, 100%, 95%, 75%, and 50% methanol. The second treatment used was Proteinase-K digestion at the final concentration of 25 ug/ml in Tris-EDTA buffer for 5 minutes in larva and 10 minutes in adults. The final treatment used was incubation in .1M ethanolamine for 30 minutes. After treatments, preparations were blocked in 10% normal goat serum for 1 hour and run though standard immunohistochemistry techniques. α-β-galactosidase (Cappel) and used at the concentration of 1:10,000. A biotinylated secondary antibody (Vector Laboratories) and 1:100 ABC, from the VectaStain kit (Vector Laboratories), were used according to package directions with detection utilizing 3-3'-Diaminobenzidine (DAB) for the colored product.

CNS preparations for X-gal staining were fixed in 4% paraformaldehyde for 10 minutes or in 0.5% glutaraldehyde for 20 minutes before the X-gal reaction solution was added to the preparations. During staining the preparations were maintained at 37°C for 24-48 hours for fixation with 4% paraformaldehyde and 6-12 hours for fixation with 0.5% glutaraldehyde. After staining reached the desired darkness the CNS was rinsed in PBS and then cleared and mounted in ultrapure glycerol.

Retrograde motorneuronal labeling using Biocytin: Motorneurons were labeled by filling the axons with Biocytin in Canton-S and *dsx* 3rd instar larvae and adults. The CNS was dissected free of the body in Schneider's Drosophila medium (Gibco BRL) leaving the nerve root from one hemisegment longer than the other nerves. The CNS was then transferred to a silicion well approximately 1/4 inch in diameter and about 1/8 inch high. The cut end of the nerve was isolated from the rest of the CNS by a grease wall and then exposed to biocytin (E-biotinoyl-L-lysine from Molecular Probes, Inc.). The CNS is allowed to transport the biocytin for 4-6 hours at 4°C. The CNS was removed from the well, rinsed in 0.1 M phosphate buffer (PBS), and fixed in 4% paraformaldhyde in PBS for 1 hour. After fixation the CNS was rinsed and incubated in 1:100 ABC, as per directions from the VectaStain kit (Vector Laboratories), overnight and then processed for HRP activity using Diaminobenzidine for the color detection system.

Ventral Nerve Cord Branching Pattern: The gross morphology of the ventral nerve cord branching pattern was determined for Canton-S and *dsx* mutant animals using α-22C10 antibody to label the axons. Preparations were made by dissecting the CNS free of the

thorax while leaving it attached to the abdomen in a PBS solution. At this point two different dissections were conducted. One dissection was to make a dorsal midline cut from A1 down to the genitalia. The second dissection done was a butterfly prep in which a dorsal midline and two lateral cuts were made from A1 down to the genitalia. In both cases the abdomen was then pinned out flat and fixed for 1 hour in 4% paraformaldehyde. After fixation, the preps were rinsed in PBS and blocked in 10% normal goat serum. The preps were incubated in α-22C10 (Gift from Dr. N.H. Patel) at a concentration of 1:1000 overnight at room temperature, and then incubated for 8 hours with a biotinylated secondary anti-mouse antibody. Detection was done following the directions from the VectaStain Kit (Vector Laboratories) and using DAB for the color detection system. Camera-lucida tracings were made from the preps and combinations of several preps were used to make the branching pattern diagrams.

Results

Gross morphology of the branching of the main abdominal nerve: In *Drosophila* the ventral nerve cord is located in the thorax and three thoracic ganglia and the eight abdominal ganglia are condensed into a single thoracic-abdominal nervous system. The abdominal ganglion contains all of the motorneurons that innervate the abdominal musculature, arranged in an anterior to posterior order by their segmental identity. The axons of motorneurons travel out to the periphery through a series of nerves; the motorneurons innervating the first and second abdominal segments exit in two separate nerve roots exiting from the abdominal ganglia, A1 and A2 respectively. Motorneurons destined to innervate more posterior segments travel in nerves within the abdominal ganglion. After exiting the central nervous system, individual nerves branch at the appropriate segment into the nerve and subdivide into finer branches that innervate individual muscles. In comparison to the early embryo, the subdivision of motorneurons into the intersegmental and segmental nerves is more difficult to discern in the adult abdominal ganglia (Figure 2.1) (For review Goodman and Doe, 1993).

Given the sex-specific differences in the somatic and visceral musculature, the branching pattern of the main abdominal nerve would be expected to differ between males and females at the posterior end of the abdomen. Males have a different number of muscles in abdominal segment A6, compared to females, and the genital segments are directly apposed to A6. In females, there are seven abdominal segments and the very small eighth tergite directly precedes the genital structures. The sex-specific differences in the innervation to the abdomen have not been described in *Drosophila*. Determining the pattern of innervation of the abdomen in wildtype and *dsx* males and females was necessary in order to begin to understand the segmental distribution of motorneurons in a mutant where additional sex-specific structures are present. Segmental nerves containing multiple axons and individual axons are detected immunohistochemically using the antibody MAB-22C10, which localizes to the cytoplasmic face of axonal membranes.

Presumably, as a consequence of these differences in the reproductive system, the main branching pattern of the main abdominal nerve differs. However, these differences are visible in the branching pattern of more anterior segmental nerves that show sex-specific differences in musculature. In males, the main abdominal nerve follows the ventral midline, branching off the segmental nerves for A3 in the preceding segment, A2. Likewise, the A4 nerve branches from the main abdominal nerve in the A3 segmental region. At the beginning of A4 the main nerve root bifurcates and moves to a more lateral position along the ventral surface. After this split, the segmental nerve for A5 splits off at the bottom of the boundary for A4. A small nerve also branches off the A5 segmental nerve and goes into the A6. At the boundary between A5 and A6 the main nerve root bifurcates again, with some branches heading to the internal genitalia. The branching pattern below this point has been undeterminable with the dissections used. The branching pattern becomes very fine to innervate the internal reproductive tracts and the genital muscles.

Figure 2.1: Diagram of branching pattern of the segmental nerves in the male and female abdomen. Abdominal segments are numbered according to Demerec (Demerec, 1994). The drawings include a schematic representation of the internal genitalia and gonads. The external genitalia are not included. The view is of the abdomen sectioned along the dorsal midline and flattened so that the ventral midline is in the middle of the drawing. The main abdominal nerve exits the ventral nerve cord and projects ventrally through the abdomen.

Figure 2.1

VNC

A2
A3
A4
A5
A6

A9-A10

MALE

VNC

A2
A3
A4
A5
A6
A7

A8-A9
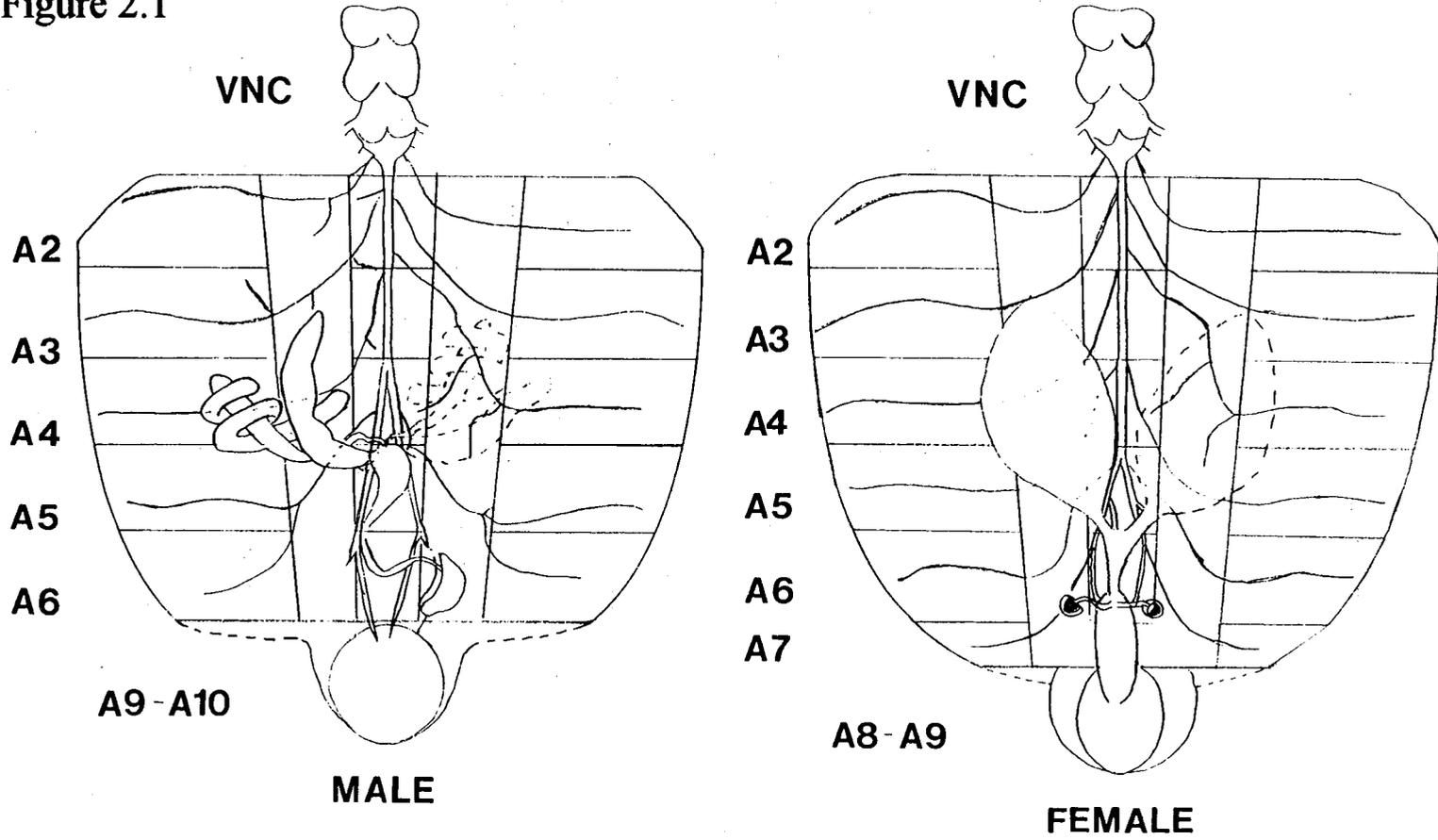
FEMALE

In contrast, the female branching pattern differs by the presence of more posterior branches compared to males. As expected the nonsex-specific segments A3 and A4 have a similar pattern of branching. Indeed, the main difference is that the main nerve root does not bifurcate until the anterior part of the A5 segment at which point the A5 nerve leaves and branches off just before the split in the main nerve root. Also, the next bifurcation of the main nerve root occurs at the boundary between A5 and A6. One of these branches goes to the oviduct and possibly other reproductive organs. As in males the branching pattern below A6 becomes very fine without any clear indication of the segmental nerves to A7 and the reproductive tissues.

In *dsx* males and females, both sets of reproductive structures are formed along with two nearly complete sets of genital muscles. In order to determine the consequence of this mutation on the branching pattern of the main abdominal nerve preparations were also examined for XY and XX *dsx* animals. There are two predictions of the possible effect the *dsx* mutant could have on the branching pattern of the main abdominal nerve. The first possibility is that the pattern of the branching would be the same in *dsx* males and females and be intermediate to the wildtype male and female patterns. Alternatively, additional nerve branching might be necessary to innervate the additional segments and the muscles present in the mutant. Unexpectedly, the branching pattern in the *dsx* mutants was maintained as either the male or female pattern depending on the chromosomal sex of the animal. It is possible that a change in the A7 nerve or the nerves to the genitalia could be affected in the *dsx* mutants, since I was unable to map the branching pattern in the more posterior region of the abdomen.

Expression Pattern of 4.078 P[tau-lacZ w⁺]: Since it proved difficult to map the projections of the whole nerves in *dsx* mutants, I instead used a technique to identify individual sets of neurons. To determine the fate of a specific group of motorneurons and their regulation by *dsx*, a *tau-lacZ* enhancer trap line, 4.078, obtained from John Thomas (Callahan and Thomas, 1994), was used to label the ventral unpaired median cells (VUMs). The larval pattern of staining consists of 11 segmentally repeated groups of three neurons that innervate the eight abdominal segments and the three thoracic segments. These neurons produce a very characteristic axonal projection, which emerges from the ventral surface of the CNS, heads straight up the midline, and splits at the dorsal

surface to leave the CNS in bilaterally symmetric nerves. Examination of control adults from this line show that males and females have a different pattern of VUMs after metamorphosis. In the abdominal ganglion of adults, I found seven groups of VUMs in males and six groups of VUMs in females compared with the eight groups of VUMs found in the larva (Figure 2.2). Because there are still three groups of VUMs in the thoracic segments, innervating identical musculature in males and females, it suggests that the change is due to the sex-specific changes in musculature found in the abdomen. This is further supported by the observation that all eleven sets of VUMs are present in *dsx* mutants (Figure 2.3 and Figure 2.4). This is the first line of evidence that demonstrates a sex-specific regulatory role for *dsx* involving the survival of larval motorneurons in the abdominal ganglion.

Biocytin backfills: The embryonic and larval population of motorneurons has been well established in *Drosophila*. In each hemisegment there are 30 large body wall muscles that are formed in the embryo and maintained through the larval stages (Bate, 1993; Demerec, 1994). Corresponding to these 30 muscles are 31 motorneurons, which have been identified by retrograde fills using a lipophilic dye, DiI, or Lucifer yellow in the embryonic nervous system (Landgraf *et al.*, 1997; Sink and Whitington, 1991). During metamorphosis, the numbers of motorneurons, in each of the non sex-specific segments, are reduced to approximately 15-17 from estimates of retrograde neuronal fills from nerve roots (D.D. Larsen, L.M. Wilson, and B.J. Taylor, unpublished results). Results from the sex-specific segments have given inconclusive results. Several problems have arisen in the data analysis. One of the major problems has been the inability to reproducibly identify individual neurons or groups of neurons. There are inconsistent numbers of neurons found in the backfills looked at for A6 in both wildtype and *dsx* flies.

Figure 2.2: Pattern of β-galactosidase expression in the ventral nerve cord of *4.078* larvae and adults.

(A) Male or female wandering 3rd instar larval CNS. The arrows indicate the 11 groups of VUMs stained at this stage. The top 3 groups of VUMs are located in the thoracic ganglion, while the bottom 8 groups of VUMs are located in the abdominal ganglion. Both males and females (Data not shown) have an identical staining pattern at this stage.

(B) Adult male ventral nerve cord. The arrows indicate the 7 groups of VUMs present in the abdominal ganglion.

(C) Adult female ventral nerve cord. The arrows indicate the 6 groups of VUMs present in the abdominal ganglion.

The VUMS located in the thoracic ganglion are not shown.

Figure 2.3: Pattern of β-galactosidase expression in the ventral nerve cord of *dsx 4.078* adults.

(A) *XY; dsx 4.078/dsx 4.078* CNS (Lateral view).

(B) *XX; dsx 4.078/dsx 4.078* CNS (Lateral view).

In both cases, the CNS is mounted on the side for a view of the cell bodies. The ventral surface is towards the right side of the figure. The arrows indicate that all eight sets of VUMs are present in these animals. The most posterior projections, at the bottom of the abdominal ganglion, are very faint.

Figure 2.4: Summary of the number of VUMs visualized using the *4.078* P[*tau-lacZ*] enhancer trap line. The number of labeled groups of neurons in the abdominal ganglion is noted next to the drawing of the CNS. The drawings are grouped according to genotype with the control *4.078* CNS on the right and the *dsx 4.078* CNS on the left. In the control *4.078* preps females lose one more group of VUMs than males resulting in 6 groups of cells, while in males there are 7 groups of VUMS. In the *dsx 4.078* stock both XX and XY flies have all eight groups of VUMs in the abdominal ganglion. Both genotypes retain the thoracic group of VUMs.

Figure 2.2
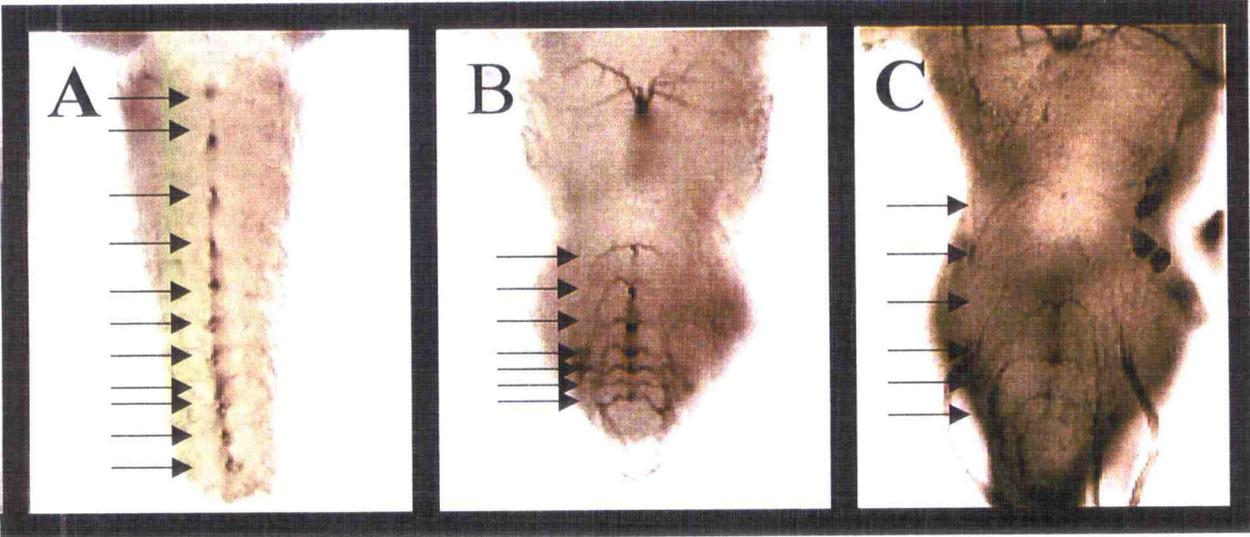
Figure 2.3
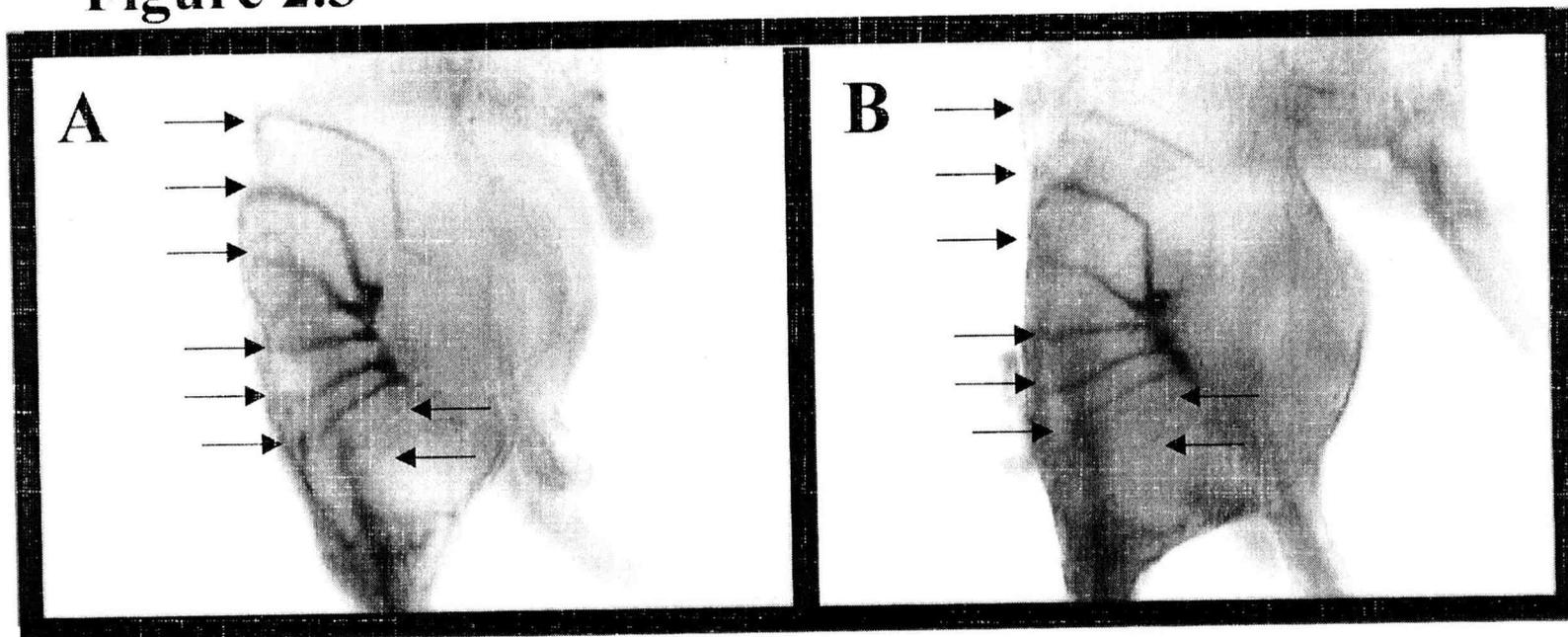
Figure 2.4

EXPRESSION PATTERN OF P[*tau-lacZ*] LINE 4.078
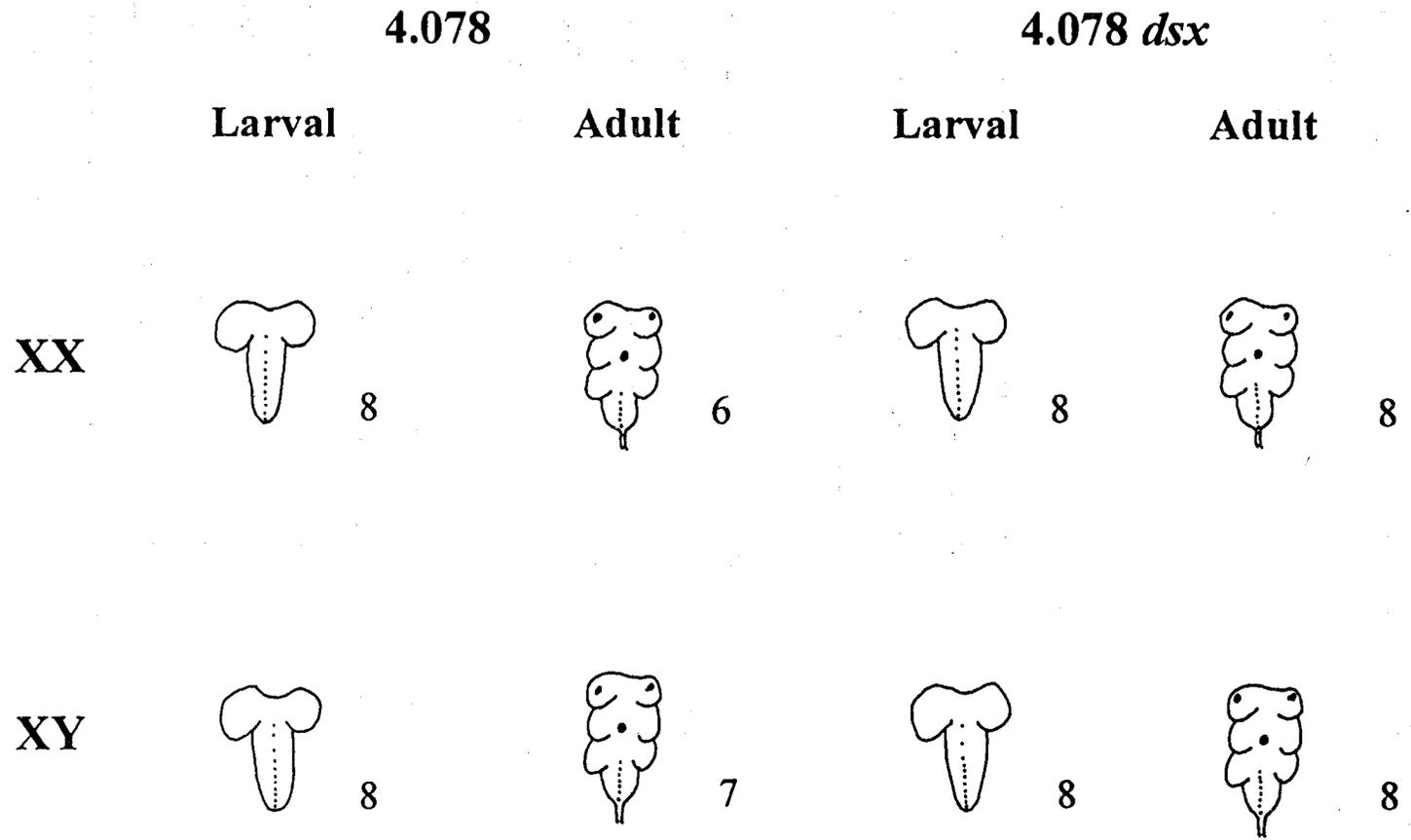
The second problem has been the fine branching of the nerves that innervate the terminal segments. This doesn't allow the isolation of individual segmental nerve roots that innervate a known terminal segment, which is required for completing the biocytin retrograde backfill. A more precise method of labeling individual neurons will have to be utilized or another marker will need to by identified in order to look at the genetic control of the motorneurons innervating the genital muscles.

Discussion

The results from the 4.078 enhancer trap line provides evidence for the regulation of some sex-specific motorneurons in *Drosophila* to be controlled by *dsx*. The results from the retrograde backfills are inconclusive and modification of the method will need to be done for a more complete analysis. A better way to address this problem would be to look at the innervation of specific genital muscles. This would provide a smaller population of motorneurons that could be reproducibly identified. Other methods to consider for future projects would be to identify other reporter lines that label different populations of sex-specific neurons or the use of other markers, such as neuropeptides or processing enzymes, that are located only in a subset of cells.

From the results obtained with the 4.078 enhancer trap line several important implications arise. The first is that with different numbers of cells found in males and females it is likely that there are female and male specific cells that arise from the larval population to give the population of VUMs found in the control adults. This suggests that both DSX^F and DSX^M proteins control the adult sex-specific pattern present in females and males respectively. This is supported by the fact that in *dsx* mutants all eight VUMs found in the larval abdominal ganglia are found in the adult abdominal ganglia of both sexes. If only one of sex-specific proteins were required to produce the female pattern from the male pattern, for example, then you would expect to see a transformation of the female pattern to the male pattern. To further explore this possibility the affect of the dsx^D allele, which only produces the male-specific transcript and masculizes females, on the 4.078 expression pattern could be examined (Denell and Jackson, 1972; Fung and

Gowen, 1957; Gowen and Fung, 1957; Nagoshi and Baker, 1990). By using this allele, it would be possible to see how the expression of only the DSX^M protein would effect the pattern of expression in both chromosomal males and females. This would tell us what part of the pattern is dependent on the DSX^M protein and by inference what part of the pattern is dependent on the DSX^F protein.

The second implication from the results with the 4.078 enhancer trap line is the possible functions that *dsx* could play in controlling the development of sex-specific motorneurons. From preliminary results, the developmental timing of the loss of the 4.078 cells corresponds to the development of the adult muscles (Data not shown). This leads to the question of motorneuron and target muscle interactions. From previous work done on sex-specific motorneurons in *Manduca sexta*, a correspondence between the survival of the target muscle and the survival of the innervating motorneuron has been demonstrated (Thorn and Truman, 1989). In *Drosophila* it is known that innervation plays an important role in the development of the abdominal muscles. In particular the male-specific muscle, the Muscle of Lawrence (MOL), is determined by the sex of the motorneuron and not the sex of the contributing myoblasts (Currie and Bate, 1995; Lawrence and Johnston, 1984; Lawrence and Johnston, 1986). In addition it has been shown that the gene *fruitless* (*fru*) is needed in the innervating motorneuron to induce the extra growth needed for the formation of the MOL muscle (Gailey *et al.*, 1991; Taylor, 1992; Taylor and Knittel, 1995). The question then becomes does *dsx* play a similar role for the formation of the genital muscles? From previous work it is known that *dsx* regulates the formation of the genital muscles and external genitalia (Epper, 1981; Hildreth, 1965; Merritt, 1994). Since the genital muscles and the internal reproductive tract arise from the genital disc, it is unknown if the same developmental process will apply as with the muscles in the more anterior portion of the abdomen. To address this question mosaic animals for *dsx* will need to be examined to determine if the identity of the muscle or the motorneuron is important for the correct development of the genital muscles.

Chapter 3

Identification of a Putative Genetic Interactor with *transformer* and *transformer-2*

DeLaine D. Larsen and Barbara J. Taylor

Introduction

One of the hallmarks of the sex determination hierarchy in *Drosophila* is its means of regulation. Except for the initial reading of the X:A chromosomal ratio, which is set by the relative dosage of certain autosomal and X-chromosomal genes, somatic sex differentiation is regulated via splicing events. Two key splicing events are required for the sexual development of a female. The first key regulated splicing event involves the protein encoded by *Sex-lethal* (*Sxl*). *Sxl* splices its own primary transcript to maintain functional SXL protein (Bell *et al.*, 1991; Cline, 1984) and also directs the female-specific splicing of *transformer* (*tra*) to generate a functional TRA protein (Boggs *et al.*, 1987; Sosnowski *et al.*, 1989). In the absence of female-specific SXL protein, the primary transcripts for *Sxl* and *tra* are spliced to give the non-functional male-specific proteins. The second key regulated splicing event is a female-specific splice by the gene products of the *tra* and *transformer-2* (*tra2*) genes, of the primary transcripts of the genes *doublesex* (*dsx*) and *fruitless* (*fru*), resulting in female-specific proteins (Heinrichs *et al.*, 1998; Nagoshi and Baker, 1990; Nagoshi *et al.*, 1988; Ryner and Baker, 1991; Ryner *et al.*, 1996). When the TRA protein is absent, as is the case in males, a default male-specific splice occurs, resulting in male-specific proteins.

To gain a better understanding of how this sex-specific splicing is regulated and to find other genes involved in this process, a number of heterozygous interaction screens were conducted in several laboratories. These screens typically used female flies that were doubly heterozygous for *tra* and *tra2*. Females of this genotype develop as phenotypic females, but are masculinized if they are also mutant for a single copy of certain other genes. Such phenotypic analyses identified other genes that interact with the known sex determining genes. The primary visible masculinization displayed by these triply heterozygous females, aberrant genitalia and increased abdominal pigmentation, is due to the mis-splicing of the *dsx* primary transcript into the male default pathway and the consequent reduction of female *dsx* mRNA levels. The degree of masculinization in an individual fly appears to depend on the relative proportions of *dsx* male and *dsx* female transcripts in individual cells. The RNA detection assays are

sensitive and *dsx* mutant animals are viable, resulting in several proteins involved in splicing being identified.

Other types of genetic interactor screens have also identified splicing factors, some of which have subsequently been shown to be involved in the splicing of the *dsx* primary transcripts. For example, a screen using a special allele of *white* (*w*), called w^a, has identified two genes involved in regulated splicing, *B52* and *Darkener of apricot* (*Doa*) (Peng and Mount, 1995). Currently, only four genes have been identified as interactors with *tra* and *tra2*. These include *B52*, *rbp1*, *Doa*, and *rox21* and they have been postulated to function as components of the spliceosome (Brand *et al.*, 1995; Kim *et al.*, 1992; Mattox, 1998; Peng and Mount, 1995). Still other possible interactors have been identified using an EST sequence screen and searching for homologues with RNA binding proteins. Two proteins isolated from this screen, 9G8 and SF2, are of particular interest since they can be crosslinked to the *tra* and *tra2* complex, which binds the *dsx* pre-mRNA (Amrein *et al.*, 1994; Mattox, 1998). Although the proteins isolated primarily affect splicing, other proteins might be detected in genetic or molecular interaction screens that may function to stabilize *tra* and/or *tra2* transcripts or activate the TRA and/or TRA2 proteins by post-transcriptional modifications, such as phosphorylation. It is however unlikely that all of the relevant proteins that are involved in the regulated sex-specific splicing and metabolism of *fru* and *dsx* primary or mRNA transcripts have been identified.

While attempting to construct a stock containing the P[*tau-lacZ*] line 4.078, an enhancer trap line that labels the VUM neurons, in a *tra2* mutant background I serendipitously observed a genetic interaction. In this interaction, chromosomally female flies developed as intersexes with masculinized external structures, suggesting the presence of at least one genetic locus in the 4.078 P-element line that behaved as an interactor in the sex determination hierarchy (SDH). Since the initial interaction was very strong, I conducted an extensive characterization of this genetic interaction with the SDH by the following experiments. First, the precise relationship between the putative interacting locus and the genes in the SDH was determined by analyzing flies that were doubly and triply heterozygous for sex determination genes. Second, allelism tests were made between the putative mutation in the 4.078 P-element line and relevant genes in the

sex determination cascade and also other loci that are known to regulate splicing of *dsx* primary transcripts. Finally, subsequent crosses were undertaken to demonstrate that the locus involved in the genetic interaction was tightly linked to the P-element. By genetic analysis, the interaction appears to be at the level of *tra* and *tra2* in the SDH and acts to cause the mis-splicing of the primary transcripts of the output genes *dsx* and *fru* in females.

Materials and Methods

Genetic crosses: Unless otherwise noted all genetic crosses were carried out at 25° C with a 12L:12D light cycle, on standard dextrose, cornmeal, and agar food with Nipagin M or propionic acid as a mold retardant. In table 3.1 the full genetic notation of all the stocks used in the crosses are listed. Descriptions of all chromosomes and mutations can be found in Lindsley and Zimm (1992) and/or on FlyBase. Description of the creation of the P-element construct found in the 4.078 stock and its initial mapping to the 3rd chromosome is reported in Callahan and Thomas (1994; a generous gift of Dr. J. Thomas). Crosses were made to mutations from all three levels of the sex determination hierarchy, including *Sex-lethal* (*Sxl*), *transformer* (*tra*), *transformer-2* (*tra2*), *doublesex* (*dsx*), and *fruitless* (*fru*). Descriptions of the alleles are found below. There is a full description of every cross performed in for this chapter, including the parental genotypes, purpose of the cross, offspring counts and results and conclusions from each cross, in appendix A.

In some of the crosses genetic markers were present on the sex chromosomes to allow the determination of chromosomal sex, either XX or XY, and the identification of nondisjunction events resulting in XXY females and XO males. These markers include *Bar*^{S} (*B*^{S}), *yellow* (*y*), and *white* (*w*). To identify the Y-chromosome, two different marked chromosomes were used, *B*^{S}*Y* and *y*$^+$*Y*. Males that carried the *B*^{S}*Y* chromosome were scored by their small, slit eyes. Males that carried the *y*$^+$*Y* chromosome were scored by their normal body color in crosses in which the X-chromosome carried the mutant allele for *y*, resulting in yellow-bodied females and males of wildtype body color. The

eye color gene *w* is carried on the X-chromosome. In many of my crosses, the females were homozygous for *w⁻* while the males carried a *w⁺* allele. In these crosses, all males should have white eyes and all females should have red eyes. In some cases, these markers allowed us to determine the correct chromosomal sex for sexually abnormal animals.

Genetic interactions were tested using single mutant alleles for four of the five known sex determining genes, *Sxl*, *tra*, *tra2*, and *dsx* (Table 3.1). One *Sxl* allele was tested, Sxl^{fm3} (Gift from Dr. T. Cline). This Sxl^{fm3} allele leads to masculinization of females, but allows for female type dosage compensation (Cline, 1984). Two different *tra* alleles were tested, tra^1 and $Dfst^{J7}$. The tra^1 allele is a small deficiency that takes out the coding region of the gene (Butler *et al.*, 1986; Sturtevant, 1945). $Dfst^{J7}$ (73A1-2; 73B1-2) is a slightly larger deficiency that removes the coding region of *tra* and *dsc73* (*doublesex cognate 73*) (Belote *et al.*, 1990). In addition the stock *w/w; hstra83/+* was used. This stock contains a P-element that allows the leaky *hsp83* promoter to drive the expression of the female-specific transcript of *tra*, thereby converting chromosomal males into phenotypic females at room temperature (McKeown *et al.*, 1988). Three different *tra2* alleles were tested for interaction: $tra2^B$, *tra2*, and *Df(2R)trix*. *Df(2R)trix* (51A1-2; 51B6) is a small deficiency that uncovers the *tra2* locus (Goralski *et al.*, 1989). One *dsx* allele was tested, dsx^1. This allele is the classic mutation reported in Hildreth (1965) and results in intersexual development when homozygous recessive. In addition to testing this allele for a genetic interaction it was also recombined onto the 4.078 P-element chromosome. The gene *fru* was only indirectly tested in crosses in the stocks B^SY; *tra2/CyO; fru^1 P[79B actin-lacZ ry⁺] ry⁻/ TM6C* and B^SY; *trix/CyO; fru^1 P[79B actin-lacZ ry⁺] ry⁻/ TM6C*. In both cases the fru^1 allele, which is a small inversion, was used.

Genetic interactions were tested for two combinations of mutations in the sex determination genes, *tra* plus *tra2* and *tra* plus *dsx*. The double mutations of *tra2*, *tra* and *dsx* were the same alleles tested for interactions as single mutations (Table 3.1).

Table 3.1: Stocks used in the genetic studies of the interactor locus in the 4.078 P-element line. The full genetic notation is given followed by an abbreviation, if used, that would be found in the text. The full description of all of the mutations in this table is found in Lindsley and Zimm (1992) and in an updated version on FlyBase (http:\\www.flybase.bio.indiana.edu:82). The original published references for the isolation of the sex determination or splicing factor mutations is included in the right hand column.

Table 3.1 *Drosophila* Stocks

Stock	Abbreviation used in text	References
Canton-S	CS	
w⁻/w⁻; 4.078w⁺/4.078w⁺	4.078 or 4.078 P-element	(Callahan and Thomas, 1994)
y/y+Y; tra2/CyO; tra/Ubx		
Bˢʸ; cn tra2ᴮ bw/CyO; tra etc/TM2		(Taylor, 1992)
Bˢʸ; tra2/CyO; fruᴵ P[79B actin-lacZ ry⁺] ry⁻/ TM6C	*Bˢʸ; tra2/CyO; fruᴵ 79B/ TM6C*	(Courchesne-Smith and Tobin, 1989; Merritt, 1994)
Bˢʸ; trix/CyO; fruᴵ P[79B actin-lacZ ry⁺] ry⁻/ TM6C	*Bˢʸ; trix/CyO; fruᴵ 79B/ TM6C*	(Courchesne-Smith and Tobin, 1989; Merritt, 1994)
y/y⁺Y; cn trix bw/CyO		(Goralski *et al.*, 1989)
ywf/Bˢʸ; Dfstᴶ⁷/TM6B (Df(3L) stᴶ⁷:73A1-2; 73B1-2)		(Belote *et al.*, 1990)
w/Bˢʸ; traᴵ/TM6B		(Sturtevant, 1945)
y/y⁺Y; traᴵ dsx pᴾ/TM6B		(Sturtevant, 1945) (Hildreth, 1965)
ywf/y⁺Y; dsx pᴾ/TM6B		(Hildreth, 1965)
y/y⁺Y; pᴾ dsx/TM6B		(Hildreth, 1965)
yw Sxlᶠᵐ³ ct v/ y⁺Y; +/+		(Cline, 1984)
wᵃ; B52ᴱᴰ/TM3		(Peng and Mount, 1995)
wᵃ; B52ᴿ²/TM3		(Peng and Mount, 1995)
Df(3R)urd, ru h th st cu ca/TM3	*Df(3R)urd/TM3*	
wᴵᴵᴵ⁸		
w/w; hstra83/+		(McKeown *et al.*, 1988)

Backcrossing of the 4.078 P-element to the w^{1118} stock. To determine whether the locus that was interacting with the sex determination hierarchy was linked to the P-element insert in the 4.078 line, six rounds of backcrossing were conducted after initially outcrossing the P-element line to a wildtype line, marked only with a _white_ mutation, w^{1118}. Females heterozygous for the P-element over a wildtype chromosome produced recombinant progeny, which led to exchanges between the wildtype chromosomes and the chromosomes from the P-element line. Recombinant flies containing the P-element were recovered due to their peach eye color phenotype that indicates the presence of the mini-_white_$^+$ gene, a marker located in the P-element. By assuming that in each generation of backcrossing 50% of the remaining 4.078 genetic material was exchanged; then after six rounds of recombination, approximately 98% of the genetic background from the 4.078 P-element line should have been replaced in the last generation of offspring.

The recombinational crosses began with four different females that were used to generate four independent lines in which most of the original genetic background was eliminated and the P-element retained. Initially, multiple _w/w; 4.078w$^+$/4.078w$^+$_ females were crossed to multiple _w^{1118}/Y; +/+_ males. From this cross five female offspring that carried the P-element were collected and crossed to multiple _w^{1118}/Y; +/+_ males, thus allowing the first round of recombination to occur between the wildtype and 4.078 P-element line chromosomes. Four P-element bearing females were selected from the progeny of the second cross, after one generation of recombination had occurred, and established the base of the four independent lines being established. Each P-element bearing female was mated to three to five _w^{1118}_ males to improve the chances of a productive mating. For subsequent generations, four females carrying the P-element were selected from each of the four independent lines and backcrossed to _w^{1118}_ males, resulting in sixteen crosses being maintained at a time. After six generations of recombination, stocks were established by selecting four single males carrying the P-element from each of the four independent lines and crossing them singly to several _ywf/ywf; dsx p^p/ TM6B_ females. The _ywf/w/Y; 4.078w$^+$/TM6B_ progeny were collected to found the stock. Four single pair crosses were set-up for each of the independent lines because of the uncertainty in single pair crosses. This ensured that enough independent

lines were established to test accurately the linkage between the genetic locus that caused the interaction phenotype and the P-element insertion.

Test of allelism between the splicing factor gene B52 and the 4.078 P-element: Three crosses were carried out to determine whether the 4.078 P-element insertion was an allele of the *B52* gene, which encodes a splicing factor. The three stocks used in the allelism test were the w^a; $B52^{ED}$/TM3 stock, the w^a; $B52^{R2}$/TM3 stock, and the *Df(3R)urd, ru h th st cu ca*/TM3 stock, which uncovers the *B52* locus. These stocks were each crossed to one of the backcross lines, *w/ywf; 4.078/4.078* (Line 2AII-2B ♂2). If the interaction locus were allelic to *B52*, the mutant transheterozygotes would die in the embryonic or larval stage of development. Since embryonic lethality was possible, the crosses were carried out on molasses plates supplemented with yeast paste to facilitate collecting and counting eggs. Eggs were collected twice daily, every morning and evening, by serially transferring the parents to fresh molasses plates, incubated at 25° C. The collected eggs were arranged in a grid on a fresh molasses plate and counted. After 24 hours, the number of embryos that hatched into 1st instar larvae and the number of eggs that failed to develop were counted. Approximately three days after the adults were removed, the third instar larvae on the molasses plates were counted, and transferred to a standard food vial. The numbers of pupae were counted, possibly an underestimate due to pupae that could have been hidden in the food, and the genotype and sex of the adults were scored. The counts from the allelism test crosses were compared to similar counts of wildtype Canton-S flies and the 4.078 stock, Line 2AII-2B ♂2, used in the other crosses for the allelism test.

Cuticle preparations of abnormal flies: To determine the complete external intersexual phenotype of the progeny from various crosses, adult flies were anesthetized with CO_2 for counting and fixed by storing in 95% ethanol in a 15 ml conical tube after collection until processing. Flies were processed to remove soft internal tissues by maceration in hot 10% KOH (Szabad, 1978). After 1-2 minutes a smaller test tube was used to flatten the flies against the side of the test tube. The resulting cuticular preparations were rinsed in distilled water and dehydrated through a graded ethanol series and 100% xylene and mounted between two coverslips in Permount.

In situ hybridization to salivary chromosomes: To determine the cytological location of the P-element in the 4.078 line, salivary polytene chromosomes _in situ_ hybridization, using labeled cDNA probes, was carried out. The pCASPER element, a general P-element vector, was labeled with biotin using the BioNick Labeling System (Gibco BRL). The salivary glands were dissected out of wandering 3rd instar larvae in 0.7% sodium chloride solution. The glands were then transferred to a 3:1 ethanol:acetic acid solution to fix the tissue for 2-3 minutes. After fixing the glands were placed in 10 μl of 45% acetic acid on a silicionized coverslip and left for 1 minute. To spread out the polytene chromosomes the coverslip with the salivary glands were picked up with a gelatin coated slide, tapped with the end of a blunt probe to break open the cells, and spread with a zig-zag motion over the top of the coverslip. To set the chromosomes into place firm pressure was applied with my thumb on the top of the coverslip. The slides were then placed on top of a block of dry ice for one hour. After an hour the coverslip was popped off with a razor blade and the slide was immediately placed in a slide tank with 95% ethanol that was cooled with dry ice to be approximately -20° to -50°C. After the slides had come to room temperature and allowed to air dry they were processed as previously described by Engels et al (1986). The hybridization mixture added to each slide consisted of 2.7 μl formamide (Sigma Chemical), 2.6 μl hybridization buffer (Sigma Chemical), and 0.7 μl of biotinylated DNA probe made above. The hybridization was applied to chromosome squashes from 4.078 larva and hybridization was carried out 37°C overnight. Subsequent detection by streptavidin conjugated to alkaline phosphatase was carried out, using an NBT/BCIP color detection solution (Boerhinger Mannheim) to localize the bound probe, and label the chromosomal location of the P-element insertion site.

Results

Initial identification of genetic interaction: In the process of generating stocks to follow
the role of the sex determining hierarchy and specific neurons labeled by an enhancer trap
tau-lacZ line (see Chapter 2), a variety of unexpected phenotypes were found in a high
percentage of the progeny. These crosses involved males that were mutant for *tra2*
alleles, *tra2/CyO; fru^{1}79B/ TM6C* or *trix/CyO; fru^{1}79B/ TM6C* mated to balanced
females heterozygous for the 4.078 P-element, *+/SM1; 4078w$^+$/ TM6C* (Table 3.2).
Daughters from this cross of the genotype, *tra2/+* or *SM1; 4.078/fru^{1}79B* or *TM6C*,
developed as intersexes. The sexual transformation was extensive and included the
presence of male genitalia, development of partially transformed bristles of the sex-
combs on their foreleg, and the dorsal part of the abdomen in the 5th and 6th segments had
the male pigmentation pattern. In some of these females, the exact structures of the
genitalia could not be determined because the genitalia did not evert and remained in the
abdominal body cavity; this does not occur in wildtype animals. The dorsal/ventral axis
of the genitalia in these transformed females was often displaced with respect to the rest
of the D/V axis of the body due to incomplete rotation of the genitalia during adult
development. More careful analysis of a cross with parents of the same genotype
revealed that 12% of the total flies (n=13 out of 108) were transformed in the *tra2* cross
representing 29% of the XX; *tra2/+; 4.078/+ or fru^1* (n=13 out of 45). From crosses
with the *trix* 8% of the total flies (n=15 out of 184) were transformed, representing 22%
of the XX; *trix/+; 4.078/+ or fru^1* (n=15 out of 69).

In order to determine which 3rd chromosomes were present in the transformed
females, we stained animals for the expression pattern of the two reporter genes present
in the cross, 4.078 and 79B actin-lacZ. A subset of the transformed females was
dissected and the abdomens and CNSs were stained for the presence of β-galactosidase,
since both P-elements had distinct patterns of labeled cells. If the animals had the 79B
actin-lacZ chromosome then specific genital muscles in females and specific genital
muscles and the Muscle of Lawrence (MOL) express β-galactosidase. If the 4.078 P-
element was present then the ventral unpaired median neurons (VUMs) express

Stock crossed to *w/w;+/SM1;4.078w⁺/TM6C*	Total number of offspring	Number of Masculized XXs
BˢY; tra2/CyO; fru¹/TM6C	108	13
BˢY; trix/CyO; fru¹/TM6C	184	15

Table 3.2: The number of abnormal offspring found in the initial crosses that identified the genetic interaction with the 4.078 line and sex determining genes. The genetic notations in red text denote the female parent and in blue text denote the male parent used in the cross. The genotypes of the masculized females were *tra2/SM1 or +; 4.078/TM6B or fru¹79B*.

β-galactosidase. All of the dissected flies carried the 4.078 P-element but were either TM6C or 79B actin-lacZ, indicating that it was the presence of some element associated with the 4.078 chromosome that was responsible for the interaction.

Description of the phenotypes observed in test crosses between the 4.078 P[tau-lacZ] stock and other strains of flies: In the crosses performed to characterize the genetic interaction, several phenotypes were observed in the progeny. These different phenotypes included finding offspring that were: 1) XX//XO chromosomal mosaics, 2) XO males or XXY females, due to chromosomal nondisjunction during meiosis, 3) intersexes, 4) males with abnormal genitalia and/or sex combs, and 5) flies with general developmental defects, such as aberrant abdominal segmentation defects and abnormal development of imaginal disc derivatives, such as eyes and wings. With this large of a number of phenotypes, I developed criteria, described below, to categorize progeny into phenotypic classes.

The first phenotypic class was female intersexes, which represents the strongest interactions observed in these crosses. Animals were scored as intersexes when they had sexually intermediate structures including combinations of male and female genital structures (Figure 3.1 and 3.2), masculinized abdominal pigmentation, and intermediate sex combs (Figure 3.3). In the early crosses, there was a potential problem categorizing animals as intersexes when only the genitalia had an sexually intermediate phenotype. These animals might have been chromosomal mosaics (XX//XO; see below) in which the mosaic dividing line passed through the genitalia, since other cuticle markers were not present in the crosses that would allow for unambiguous identification of mosaicism.

The second phenotypic class was X-chromosome loss mosaics. In these animals, an X-chromosome is lost from a nucleus in a female zygote (XX) at a stage with only a few nuclei; the subsequent divisions of these XO nuclei and XX nuclei result in an XX//XO animal, composed of both male (XO) and female (XX) tisues being present in a single individual fly. Sexual differentiation occurs in a cell autonomous manner dependent on the activity of the sex determining genes. Since the chromosomal loss occurs in the zygote before the establishment of the sex determination hierarchy, XO cells will develop male-specific structures and XX cells will develop female-specific structures if they occur in regions with sexually dimorphic structures. Thus, mosaics

Figure 3.1: Photomicrographs of terminalia in cuticle preparations of wildtype males and females.

(A) Canton-S male genitalia (Lateral View). Dorsal is to the top, anterior is to the left.

(B) Canton-S female genitalia (Lateral View). Dorsal is to the top, anterior is to the right. Abbreviations of genital structures found in the pictures are as follows: A8= tergite of abdominal segment 8 (a female-specific structure). AP= anal plates, C= claspers (a male-specific structure), GA= genital arch (a male-specific structure), and VP= vaginal plates (a female-specific structure).

Figure 3.2: Photomicrograph of intersexual terminalia in cuticle preparations of 4.078 transheterozygotes with mutations in the sex determining genes.

(A) XX; $tra2^B/+$; $tra/4.078$ terminalia from an animal raised at 27°C showing that both male and female genital structures are present. The male genital structures, claspers and genital arch, are dorsal to the female genitalia. Anterior is to the left.

(B) XX; $tra/4.078$ terminalia showing both male and female structures and is similar to that of the intersex in figure 3.1A. Anterior is to the left.

(C) XX; $Dfst^{17}/4.078$ terminalia showing both male and female structures. The anal plate visible in this plane of focus is a lateral plate, like that in wildtype males. Anterior is to the right.

(D) Same preparation as in figure 3.1C but at a different focal plane showing that the anal plate on this side has the dorsal and ventral plates, similar to female anal plates. Abbreviations of genital structures are the same as in figure 3.1.

Figure 3.3: Photomicrographs of the distal forelegs from cuticle preparations.

(A) Canton-S female foreleg. The distal transverse row of bristles at the posterior edge of the first tarsal segment are arranged at 90° to the homologous bristles of the male sex comb.

(B) XX; $tra2/+$; $tra/4.078$ (raised at 27°C) female foreleg. The arrangement of the transverse row of bristles on the first tarsal segment is rotated to an intermediate position (approximately 45°) to either the male or female bristles. These bristles are also intermediate in thickness between those of males and females.

(C) Canton-S male foreleg. The sex comb bristles are rotated 90° to the transverse row of bristles in females.

Proximal is to the top in all figures.

Figure 3.1
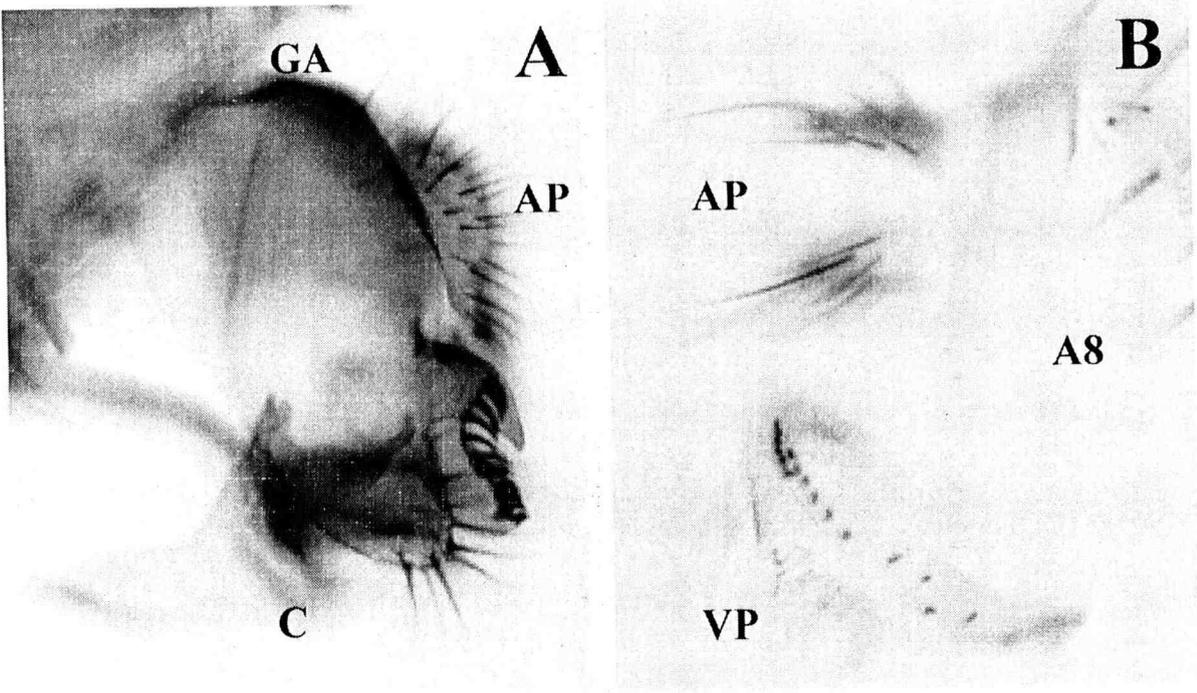
Figure 3.2
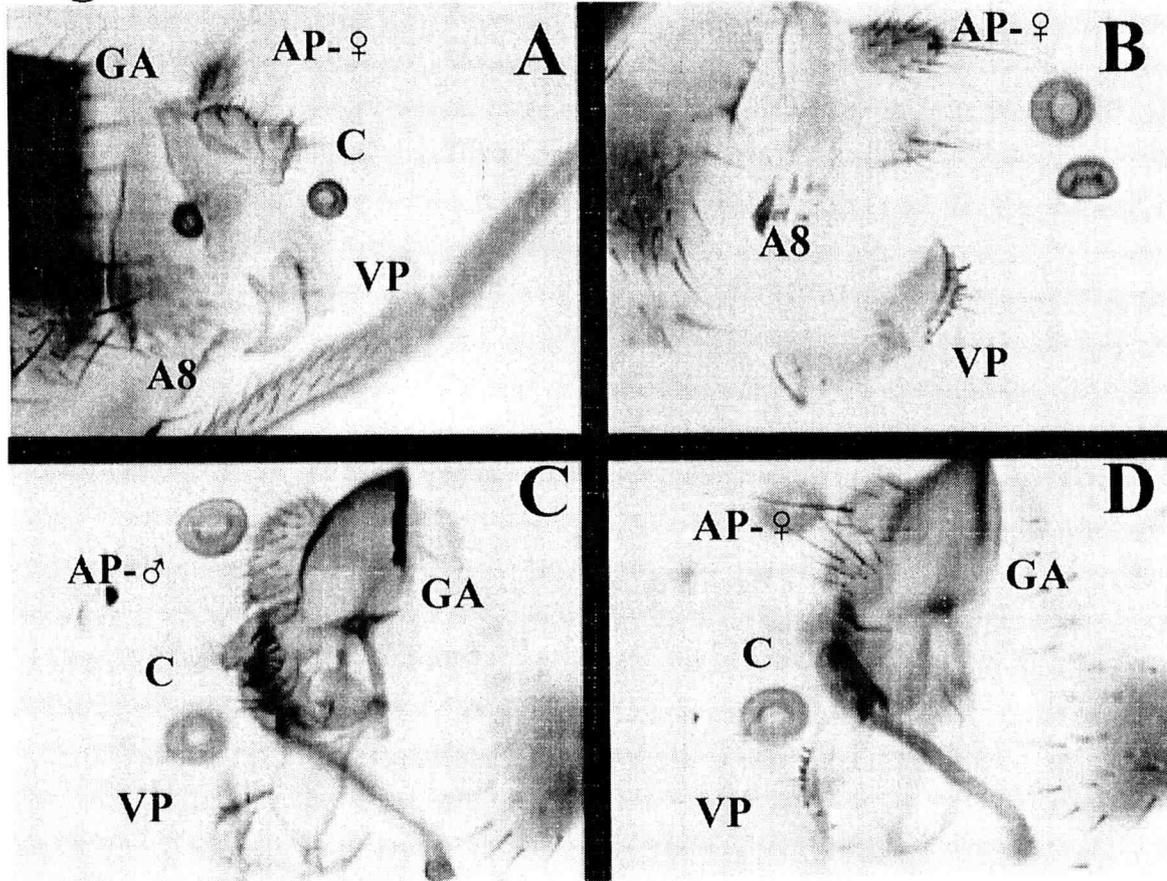
Figure 3.3
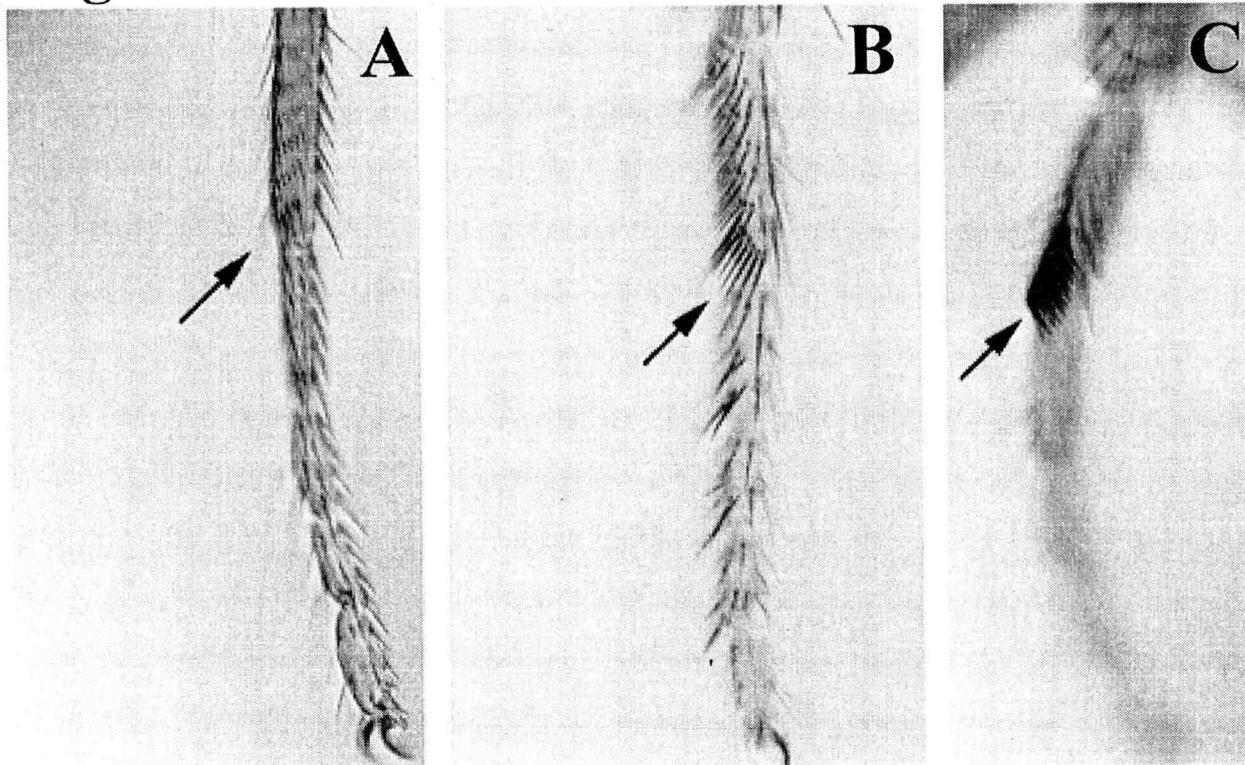
were identified anatomically by one or more of the following criteria: patches of cells that are white due to the loss of the w^+ X-chromosome (XO) in a red, w^+ (XX) eye, presence of sex combs, abdominal pigmentation in A5 and A6, or presence of both male and female genitalia. Individuals would not be identified as mosaic if the XO cells only occupied sexually indifferent external structures. In later crosses, markers for the X- and Y-chromosome were used to permit the identification of XO cells in these non-sexually dimorphic regions of the body.

The third phenotypic class included XO males and XXY females, resulting from sex chromosome nondisjunction events occurring during meiosis. These animals were identified by the loss of Y-chromosome markers in males, indicating a XO male, and the gain of Y-chromosome markers in females, indicating XXY females. Since the chromosomal loss occurs before the activation of the sex determination hierarchy, these animals develop as phenotypically normal males and females.

The fourth phenotypic class included males with abnormal genitalia and sex combs. Several abnormalities were included within this class, with the most severe being defects in the genital structures. These abnormalities were striking and included mirror-image genital structures (i.e. two almost complete complements of structures) and misrotated genitalia (i.e. upside-down or 'crooked' genitalia). The defects in the sex combs consisted of either a second set of teeth below the normal sex combs on the foreleg(s) or missing teeth. In some cases, additional cuticle was observed which caused misalignment of the genitalia and incorrect connection to the abdomen. The identity and source of the additional cuticle is unknown, but may have been due to a small patch of XX tissue, in which case these animals would belong to class II.

The last phenotypic class is diverse and includes general anatomical defects, which occurred at a greater frequency in these crosses than would have been expected in wildtype strains. Even though the frequency is increased it was still low and it is not clear if the phenotype is linked to the interaction observed with the 4.078 line responsible for the other phenotypes or is due to another factor segregating in these crosses. The genotypes observed showed no clear pattern. The anatomical defects include abnormal abdominal segmentation, failure to evert wings, legs, or halters during metamorphosis, additional eye abnormalities associated with the Bar^S marker. The abnormal abdominal

segmentation varied in degrees from having one or two segments not meeting at the dorsal midline or not reaching one side of the abdomen to having all the segments being disorganized with no recognizable pattern. Bar^S eye abnormalities included such phenotypes as additional head bristles and even cuticular tissue growing from the eye.

Determination of the nature of the genetic interaction between genes of the sex determination hierarchy and the 4.078 P[tau-lacZ] stock: There were two likely explanations for the intersexual animals discovered in the *tra2* and 4.078 crosses. The first explanation is there was an allele of one of the sex determining genes in the 4.078 stock. The second explanation is there was a genetic interaction with *tra2* and *Df(2LR)trix* due to the presence of another locus that was not one of the sex determining genes. To distinguish between these two possibilities, crosses were made between the sex determining genes, acting at three different levels of the cascade, and the 4.078 line.

If there was an allele of one of the known sex determining genes in the 4.078 stock, then these crosses would be expected to expose a phenotype by complementation in all progeny of the relevant genotype. Complementation tests were made between the 4.078 strain and *Sxl, dsx,* and *tra.*

If the genetic interactor was an allele of *Sxl* then a cross generating females mutant for the *Sxl^{fm3}* allele and 4.078 would have been expected to develop as males, since this *Sxl* allele in combination with other loss of function *Sxl* alleles masculinizes females (Cline, 1984). In the case where *Sxl* and 4.078 were heterozygous, no abnormal flies were found.

A similar phenotypic masculinization of females would be expected if the 4.078 line carried a mutant allele of *tra.* However, crosses with either the *Df(3R)st^{7}* or *tra* did not lead to masculinization of female transheterozygotes. The *Dfst^{7}* was used since this deficiency also uncovers a locus, *doublesex cognate 73A,* which was identified by screening for *dsx* homologues (D.J. Andrew, Ph.D thesis). In the cross to *tra,* there was one XXY female, one male with upside genitalia, and multiple flies with abnormal *B^S* eyes (Table 3.3).

Stock crossed to $w/w;4.078w^+/4.078w^+$	Total number of offspring	Number of: Mosaics	XO/XXY	Intersexes	Abnormal Genitalia and Sex Combs (XY)	Other
$ywf/B^S Y;Dfst^{77}/TM6B$	396	0	2	1	0	1
$w^-/B^S Y; tra/TM6B$	472	0	1	0	1	28

Table 3.3: The number of abnormal offspring found in the crosses performed to test for allelism of the 4.078 P-element to the gene *tra* and genetic interactions with the gene *tra*. The offspring counts are given according to the cross performed with the female parent in red text and the male parent in blue text by phenotypic class. Descriptions of the phenotypic classes are in the results sections under definitions of phenotypes. The one intersex found was *w/ywf; Dfst^{77}/4.078,* while the male with the abnormal genitalia was *w/B^S Y; tra/4.078.*

In contrast to the masculinization of females caused by mutations in *Sxl*, *tra*, and *tra2*, mutations in *dsx* cause a transformation of both males and females into phenotypic intersexes. If there were a *dsx* allele in the 4.078 line, then an intersexual phenotype would be expected to affect both males and females. In a cross between the 4.078 line and *dsx*, out of 351 offspring only one abnormal offspring was noted, a *w/y⁺Y; 4.078/TM6B* male with abnormal abdominal segmentation. In later crosses, done to put the 4.078 P-element into a *dsx* background and to incorporate the sex chromosome markers *ywf/y⁺Y* into a 4.078 stock, a XX//XO mosaic and a XO male were observed in addition to other flies with abnormal abdominal segmentation and non-everted wings.

The lack of phenotypic effects from the crosses to test complementarity with *Sxl* and *dsx* strongly suggests that there are no alleles of *Sxl* or *dsx* in the 4.078 line. In addition, crosses that test the complementarity of the genetic interactor with *tra* or *tra2*, a high proportion of flies have unusual phenotypes, but do not have the expected phenotypic complete masculininzation expected for an allele of *tra* or *tra2*. These data suggest that the 4.078 line does not have a mutation in *Sxl*, *dsx*, *tra*, or *tra2*. The *fru* gene was only indirectly tested in the crosses with *tra2*, the intersexual phenotypes were independent of the presence of *fru* indicating that there was no relationship between the genetic interaction and *fru*.

After the complementation tests were carried out, the results suggested that there was a genetic interaction between 4.078 and *tra* and *tra2*. Several crosses were carried out to initially determine that there was a genetic interaction between 4.078 and *tra* and *tra2* and later to better define the interaction observed. The design of the crosses was based on previous tests showing that other interacting genetic loci could be detected in animals in which known sex determination genes were also heterozygous. In these tests, the expectation was that in females, the reduction of the levels of products from one or more sex determining genes would sensitize this developmental pathway so that loss of an additional factor operating at the same level of the pathway would lead to intersexual development. The crosses described above also allow the determination of a heterozygous interaction between mutations present in the 4.078 stock and *Sxl*, *dsx*, *tra*, and *tra2*. Only crosses where genetic elements of 4.078 were heterozygous with *tra* or *tra2* showed evidence of abnormal sexual phenotypes suggesting that there was a genetic

interactor and that it operated at the level of *tra* and *tra2* leading to the expression of both *dsx* and *fru* dependent phenotypes in transheterozygous females. The crosses that first showed the interaction phenotype were to *tra2*, in an attempt to introduce the 4.078 P-element into a *tra2* background, as described above. Later crosses that showed a genetic interaction included crosses of the 4.078 stock to *tra2*, *trix*, *Df(3R)st^{j7}*, and *tra* (Table 3.3 and Table 3.4). The numbers of abnormal offspring always come out in higher numbers when crossed to *tra2* alleles than when crossed to *tra* alleles. In addition to the higher numbers, the phenotypes observed are more severe in the crosses to the *tra2* alleles.

After the genetic interaction was determined to be at the level of the *tra* and *tra2* genes in the sex determination hierarchy, the genetic interactor was tested as a transheterozygote with both *tra* and *tra2* by crossing to the double mutant stock B^SY; *tra2^B/CyO; tra/TM2*. Since the temperature at which flies develop can affect the expression of these phenotypes, the cross was carried out at three different temperatures, 16°C, 25°C, and 27°C. At lower temperatures, the time spent in metamorphosis is lengthened. Metamorphosis takes twice as long at 16°C than at 25°C; in other words, the fly spends approximately 200 hours in the pupal case at 16°C and 100 hours at 25°C (Bainbridge and Bownes, 1981). Conversely, at higher temperatures, the duration of metamorphosis is shortened to approximately 78 hours at 29°C from approximately 100 hours at 25°C, since the developmental time is occurring faster (Bainbridge and Bownes, 1981). The incubation temperature has been shown to affect the development of flies heterozygous for *tra* and *tra2* (Peng and Mount, 1995). At higher temperatures, when development is accelerated, intersexual female flies can develop when the genetic dose of *tra* and *tra2* are decreased, as in female flies heterozygous for *tra* and *tra2*. The results from the different temperatures demonstrated a clear temperature effect, with the number of intersexual female flies ranging from 0 at 16°C to 121 at 27°C. At 27°C the majority of the intersexes were of the genotype *w/+; tra2^B/+; 4.078/tra* (n=118, 100% of this genotype), with a few of the genotype *w/+; CyO/+; 4.078/tra* (n=3, 2% of this genotype) (Table 3.5). The cross at 25°C produced five intersexes of the genotype *w/+; tra2^B/+; 4.078/tra* (3.4% of this genotype). There were also a number of mosaic phenotypes seen,

| Stock crossed to _w/w;4.078w+/4.078w+_ | Total number of offspring | Number of: | | | | Other |
		Mosaics	XO/XXY	Intersexes (XX)	Abnormal Genitalia and Sex Combs (XY)	
A: B^SY; tra2/CyO; fru^{1}79B/TM6C	604	1	2	0	0	31
B: B^SY; trix/CyO; fru^{1}79B/TM6C	799	9	6	0	0	18
Stock crossed to _w/w;+/SM1; 4.078w+/4.078w+_						
C: B^SY; tra2/CyO; fru^{1}79B/TM6C	524	1	1	0	1	12
D: B^SY; trix/CyO; fru^{1}79B/TM6C	504	0	1	0	0	17

Table 3.4: The number of abnormal offspring found in the crosses performed to test for allelism of the 4.078 P-element to the gene _tra2_ and genetic interactions with the gene _tra2_. The offspring counts are given according to the cross performed with the female parent in red text and the male parent in blue text by phenotypic class. Descriptions of the phenotypic classes are in the results sections under definitions of phenotypes. The genotypes of the mosaics are as follows. For cross A the genotype observed was w^-/w^+;tra2/+;4.078/fru^{1}79B. For cross B the genotypes observed were w^-/w^+;trix/+;4.078/fru^{1}79B (n=3), w^-/w^+;CyO/+;4.078/fru^{1}79B (n=4), w^-/w^+;trix/+;4.078/TM6C, and w^-/w^+;trix/+;4.078/TM6C. For cross C the genotype observed was w^-/w^+; tra2/+; 4.078/TM6C. The genotype observed for the abnormal genitalia and sex combs class in cross C was w/B^SY; +/CyO; 4.078/fru^{1}79B.

Stock crossed to $w/w;4.078w^+/4.078w^+$	Total number of offspring	Number of: Mosaics	XO/XXY	Intersexes (XX)	Abnormal Genitalia and Sex Combs (XY)	Other
$B^SY; tra2^B/CyO; tra/TM2$ 16°C	794	0	3	0	0	2
$B^SY; tra2^B/CyO; tra/TM2$ 25°C	1247	5	2	5	0	26
$B^SY; tra2^B/CyO; tra/TM2$ 27°C	942	1	4	121	0	36

Table 3.5: The number of abnormal offspring found in the crosses performed to test for a temperature effect with the genetic interaction between the 4.078 P-element and the genes *tra* and *tra2*. The offspring counts are given according to the cross performed with the female parent in red text and the male parent in blue text by phenotypic class. Descriptions of the phenotypic classes are in the results sections under definitions of phenotypes. The genotypes of the mosaics are as follows. For the 25°C cross the genotypes observed were $w^-/w^+;tra2^B/+;4.078/tra$ (n=2), $w^-/w^+;tra2^B/+;4.078/TM2$ (n=2), and $w^-/w^+;CyO/+;4.078/tra$ (n=1). For the 27°C cross the genotype observed was $w^-/w^+;CyO/+;4.078/tra$ (n=1). The genotypes of the intersexes are as follows. For the 25°C cross the genotypes were $w^-/w^+;tra2^B/+;4.078/tra$ (n=4) and $w^-/w^+;tra2^B/+;4.078/tra$ (n=1). For the 27°C cross the genotypes observed were $w^-/w^+;tra2^B/+;4.078/tra$ (n=118) and $w^-/w^+;CyO/+;4.078/tra$ (n=3).

as well as phenotypically inferred XO, and XXY nondisjunction events. It is unlikely that these phenotypes are due to interference with *tra* and *tra2* function in the metabolism of *dsx* and *fru* pre-mRNA but may indicate that there are other genes whose function is also being affected by the locus that has been identified genetically in these experiments. *Determination that the source of the interaction is linked to the 4.078 P-element:* After the identification of a genetic interaction in the 4.078 line, it was important to establish whether the factor was associated with, or nearby, the P-element insertion. Four independent lines were generated by backcrossing to a w^{1118} line for six generations. One stock from each of the four independent lines established from the backcrossing procedure was tested for an interaction with y/y^+Y; *tra2/CyO*; *tra/TM2* when raised at 27°C. All four stocks tested produced intersexes, which were all genotypically *w/y*; *tra2/+*; *tra/4.078* (Table 3.6, Figure 3.4). The y/y^+Y; *tra2/CyO;tra/TM2* stock was also raised at 27°C for a control. In the control we found 4 out of 48 flies with the genotype *y/y*; *tra2/CyO*; *tra/TM2* had a phenotype similar to that seen in the presence of 4.078.

In addition for these transheterozygotes, which were intersexual for external phenotypes controlled by the *dsx* locus, it was important to determine whether the interaction was specific to *dsx* or involved other targets of *tra* and *tra2* action. The other target of TRA/TRA2 splicing regulation is the sex-specific primary transcript of *fru* (Ito *et al.*, 1996; Ryner *et al.*, 1996). TRA/TRA2 proteins mediate the 3' splice choice in *dsx* but the 5' splice choice in *fru* primary transcripts (Heinrichs *et al.*, 1998; Ito *et al.*, 1996; Ryner *et al.*, 1996). The easiest phenotype to assess in these transheterozygotes is the formation of a male specific abdominal muscle, the Muscle of Lawrence (MOL) that is fru dependent (Gailey *et al.*, 1991; Taylor and Knittel, 1995). In *w/y*; *tra2/+*; *tra/4.078* intersexes, their dorsal muscles were masculinized to form MOLs (Figure 3.5). In addition, the position of the ventral longitudinal muscles in the fifth abdominal segment were displaced from their normal position in females into the position found in *dsx* mutants. These data extends the phenotypic intersexuality to include both epidermal and muscular phenotypes suggesting that the

Stock crossed to *y/y⁺Y;tra2/CyO;tra/TM2*	Total number of offspring	Number of: Mosaics	XO/XXY	Intersexes (XX)	Abnormal Genitalia and Sex Combs (XY)	Other
w/w;4.078w⁺/4.078w⁺ (Line 1)	623	0	2	63	1	5
w/w;4.078w⁺/4.078w⁺ (Line 2)	2039	0	2	216	1	10
w/w;4.078w⁺/4.078w⁺ (Line 3)	443	0	1	44	4	17
w/w;4.078w⁺/4.078w⁺ (Line 4)	918	0	3	87	1	6

Table 3.6: The number of abnormal offspring found in the test cross to determine if the genetic interaction with the genes *tra* and *tra2* was linked to the 4.078 P-element. Results are given according to the line used and by phenotypic class. The genetic notations in red text denote the female parent and in blue text denote the male parent used in the cross. Descriptions of the phenotypic classes are in the results sections under definitions of phenotypes. All of XX intersexes are of the genotype *tra2/+; tra/4.078*. The genotypes that correspond to animals found with abnormal genitalia and sex combs are *CyO/+; tra/4.078* and *tra2/+; tra/4.078*.

Figure 3.4: Photomicrograph of intersexual terminalia in cuticle preparations of 4.078 transheterozygotes with mutations in the sex determining genes demonstrating interaction is linked to the P-element.

(A) XX; *tra2/+; tra/4.078* female raised at 27°C from backcross line 1 showing both male and female structures. Anterior is to the left.

(B) XX; *tra2/+; tra/4.078* female raised at 27°C from backcross line 2 showing non-everted genitalia. Only one male clasper can be made out in the middle of the frame. Anterior is to the left.

Figure 3.5: Photomicrograph of the dorsal abdominal segment A5 muscles of a XX; *tra2/+; tra/4.078* (raised at 27°C) female. The long dorsal muscle is in the location of the Muscle of Lawrence (MOL), a male specific abdominal muscle. The posterior insertion site of the MOL is in the tergite of the A6 segment as in true wildtype males. The dashed line indicates the segmental boundary between A5 and A6 with the arrow pointing to the attachment point of the muscle. Notice that the other muscles in A5 have attachment points on the A5 side of the segmental boundary. The dorsal midline is towards the left of the figure. Anterior is to the top.

Figure 3.4
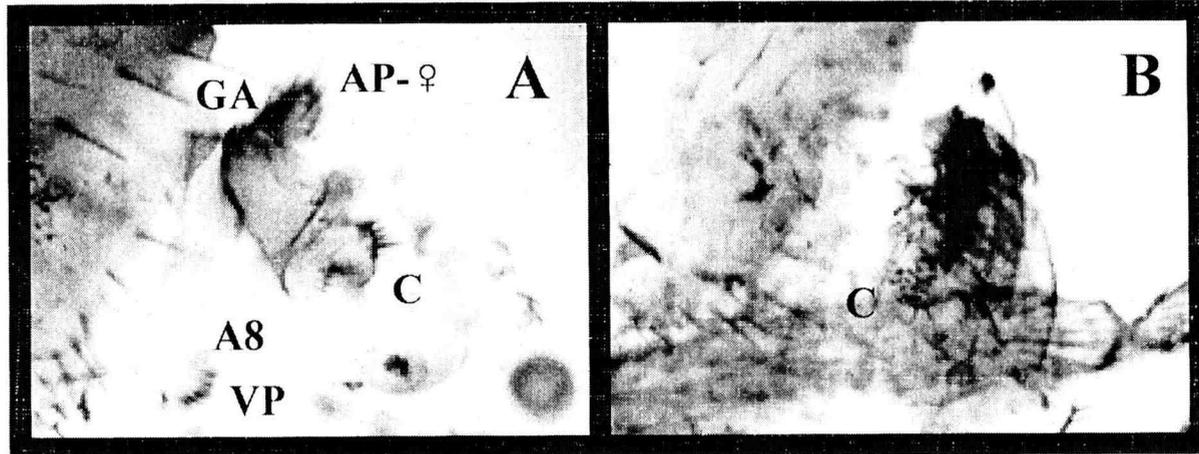
Figure 3.5
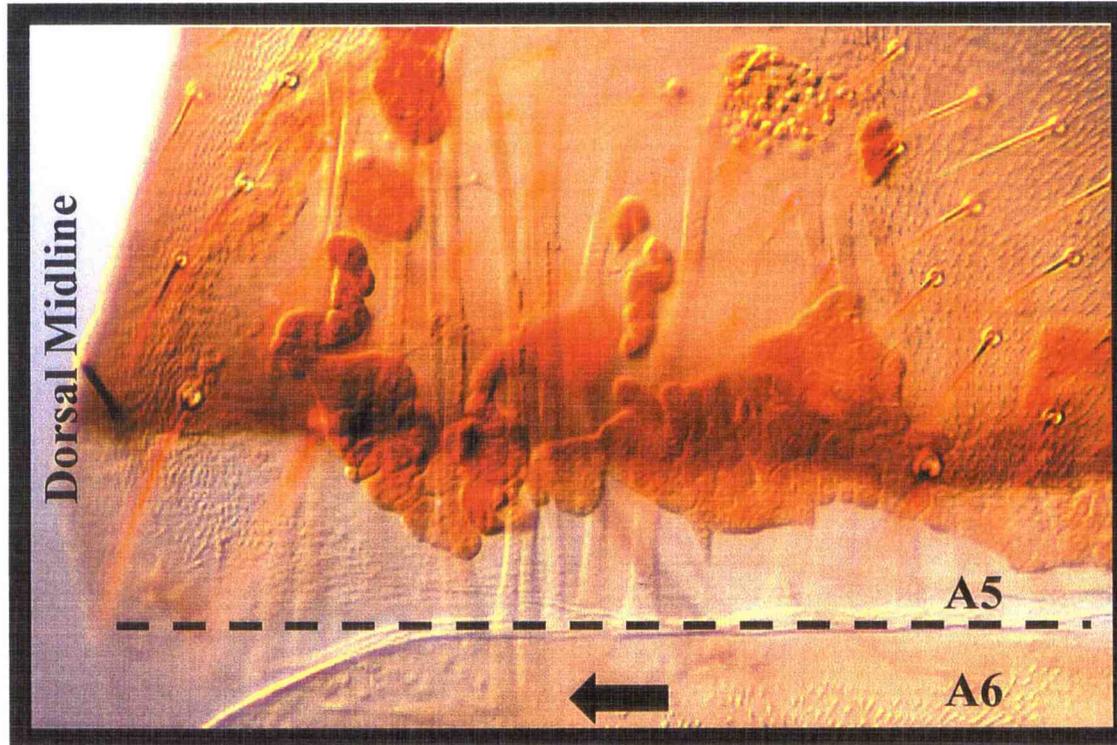
interaction between the 4.078 P-element and the sex determining genes *tra* and *tra2* affects splicing of both *fru* and *dsx* primary transcripts in multiple tissue types.

Determination of no maternal or paternal effect in the genetic interaction: To determine whether the mitotic and/or meiotic nondisjunction events had a maternal component I set up two pairs of reciprocal crosses in which the 4.078 P-element was introduced from either the maternal or the paternal side, and in both homozygous and heterozygous states. One cross utilized the flies from the *hstra83* stock that were not carrying the *hstra83* P-element, so no mutations were present other than the 4.078 P-element and the sex chromosome markers. The second cross utilized the y/y^+Y; *cn trix bw/CyO*; +/+ stock. A 4.078 stock, *ywf/B^SY*; *4.078/TM6B*, that had marked sex chromosomes was used for both crosses to allow unambiguous determination of the chromosomal sex of all the offspring. The outcome of these crosses gave no indication of any maternal effect or homozygous effect from the 4.078 P-element. A similar number of abnormal offspring was observed for all the crosses (Table 3.7 and Table 3.8).

Genetic test for comparison to action of known genetic interactor Doa: Also of interest was seeing whether the 4.078 P-element interacted with the *hstra83* P-element. Males carrying the *hstra83* P-element are feminized by thefemale-specific *tra* gene product under the control of the leaky *hsp83* promoter (McKeown *et al.*, 1988). For one of the known genetic interactors with *tra* and *tra2*, *Darkener of apricot* (*Doa*), an interesting phenotype is observed in the presence of the *hstra83* P-element. When *Doa* and *hstra83* flies are crossed, chromosomally male progeny carrying both alleles, which would normally appear phenotypically as females due to the *hstra83* insert, are masculized (Mattox, 1998). This masculization is postulated to be caused by *Doa* destabilizing the *tra/tra2* female-specific splicing complex. With only a limited supply of female-specific TRA from the P-element, these flies are unable to develop as females (Mattox, 1998). However when the 4.078 P-element was put into the same circumstances with the *hstra83* P-element, chromosomally male progeny carrying the *hstra83* P-element and the 4.078 P-element still develop as phenotypically feminized flies. This result demonstrates that the 4.078 P-element is interacting with *tra* and *tra2* in a different manner from *Doa* and is

		Number of:				
Stock crossed to *ywf/ywf; 4.078w⁺/4.078w⁺*	Total number of offspring	Mosaics	XO/XXY	Intersexes (XX)	Abnormal Genitalia and Sex Combs (XY)	Other
A: *y/y⁺Y; trix/CyO*	116	1	0	0	0	0
Stock crossed to *ywf/ywf; 4.078w⁺/TM6B*						
B: *y/y⁺Y; trix/CyO*	112	0	1	0	0	0
Stock crossed to *ywf/BˢY; 4.078w⁺/4.078w⁺*						
C: *y/y; trix/CyO*	399	1	1	0	0	4
Stock crossed to *ywf/BˢY; 4.078w⁺/TM6B*						
D: *y/y; trix/CyO*	411	0	0	0	0	5

Table 3.7: The number of abnormal offspring found in the crosses performed to test for a possible maternal effect in the identified genetic interaction between the 4.078 P-element and the gene *tra2*. The offspring counts are given according to the cross performed with the female parent in red text and the male parent in blue text by phenotypic class. Descriptions of the phenotypic classes are in the results sections under definitions of phenotypes. The genotypes of the mosaics are as follows. For cross A the genotype observed was *ywf/y⁺Y;+/CyO;4.078/+*, a Y-loss mosaic. For cross C the genotype observed was *ywf/y;trix/+;4.078/+*.

Table 3.8: Data from reciprocal crosses to determine if there was a possible maternal or paternal effect for the observed chromosome loss, which resulted in X-chromosome loss mosaics, XO males, and XXY females. The genetic notations in red text denote the female parent and in blue text denote the male parent used in the cross. Panels A and B show the reciprocal crosses in which one parent was homozygous for the 4.078 P-element. Panels C and D show the reciprocal crosses in which one parent was heterozygous for the 4.078 P-element. No clear maternal or paternal effect was observed given that all 4 crosses resulted in a similarly small number of abnormal offspring.

(A) *ywf/ywf; 4.078/4.078* X *w/B^{S}Y; +/+* (from *hstra83* stock)

	Total # of Offspring	% of Total
Males	387	38.3%
Females	621	61.5%
Mosaics	0	0.0%
XXY	0	0.0%
XO	2	0.2%
Totals:	**1010**	

(B) *w/w; +/+* (from *hstra83* stock) X *ywf /B^{S}Y; 4.078/4.078*

	Total # of Offspring	% of Total
Males	211	49.0%
Females	218	50.6%
Mosaics	1	0.2%
XXY	0	0.0%
XO	1	0.2%
Totals:	**431**	

(C) *ywf/ywf; 4.078/TM6B* X *w/B^{S}Y; +/+* (from *hstra83* stock)

	Total # of Offspring	% of Total
Males; 4.078	216	29.8%
Males; TM6B	124	17.1%
Females; 4.078	219	30.2%
Females; TM6B	163	22.5%
Mosaics	1	0.1%
XXY	0	0.0%
XO	3	0.4%
Totals:	**726**	

(D) *w/w; +/+* (from *hstra83* stock) X *ywf /B^{S}Y; 4.078/TM6B*

	Total # of Offspring	% of Total
Males; 4.078	75	22.4%
Males; TM6B	83	24.8%
Females; 4.078	76	22.7%
Females; TM6B	100	29.9%
Mosaics	0	0.0%
XXY	0	0.0%
XO	1	0.3%
Totals:	**335**	

unlikely to play a similar role in the stability of the TRA/TRA2 complex on the *dsx*
primary transcript. Thus, this result suggests that the genetic interaction observed for the
4.078 P-element is probably part of the splicing machinery and not due to a mRNA
stability role.

Cytological position of the 4.078 insertion site: The cytological map position of the 4.078
P-element is somewhere within the region of 85-87 on the right arm of chromosome 3
(Figure 3.6). The position of the labeled band was verified using landmarks on the
polytene chromosome (Lefevre Jr, 1976). Further information on the localization of the
P-element was obtained by doubly labeling the chromosomes with probes for the P-
element and for *dsx* (map position 84E). In a separate labeling experiment, the position
of the 4.078 P-element was assayed over the *Df(3R)urd* containing chromosome which
removes most or all of 87F.

The 4.078 insertion is not allelic to B52, an essential splicing factor: Although the nature
of the interaction with the sex determination hierarchy is not known directly, it is possible
to generate genetic information about whether the factor interferes upstream or
downstream of the regulated splicing of target pre-mRNAs by *tra* and *tra2*. If the
interactor affected the spicing of *dsx* primary transcripts, then it was possible that the
genetic interactor was allelic to known splicing factors in the region of the P-element
inserted in the 4.078 line (see above). In the region where the 4.078 P-element mapped,
several genes are known to regulate splicing in *dsx* and other primary transcripts. The
most important candidate for testing is the *B52* gene, which interferes with *dsx* splicing
and is positive in a transheterozygous test with *tra* and *tra2* mutations (Peng and Mount,
1995). To test for this possibility, genetic complementation assays were conducted using
a dominant allele of *B52, B52^{ED}*, a recessive revertant of *B52, B52^{R2}*, and a deficiency
that uncovers both *B52* and other suspected splicing factors such as *squid, Df(3R)urd*.
The expectation was that if the genetic interactor were allelic to *B52*, then the animals
that were *4.078/B52* would die at some stage during development. The exact stage at
which the lethality might occur was difficult to predict. Strong loss of function alleles of
B52 are lethal as embryos or in the larval stage, but hypomorphic alleles may only cause
death late in development (Peng and Mount, 1995). To gain the maximum information

Figure 3.6: Photomicrograph of a polytene chromosome *in situ*. The chromosomes were probed with labeled P-element DNA to locate the insertion site of the 4.078 P-element. The arrow marks the band that indicates the presence of the P-element at cytological location 86-87 on the right arm of the third chromosome. The cytological location is determined based on reported landmarks.

Figure 3.6
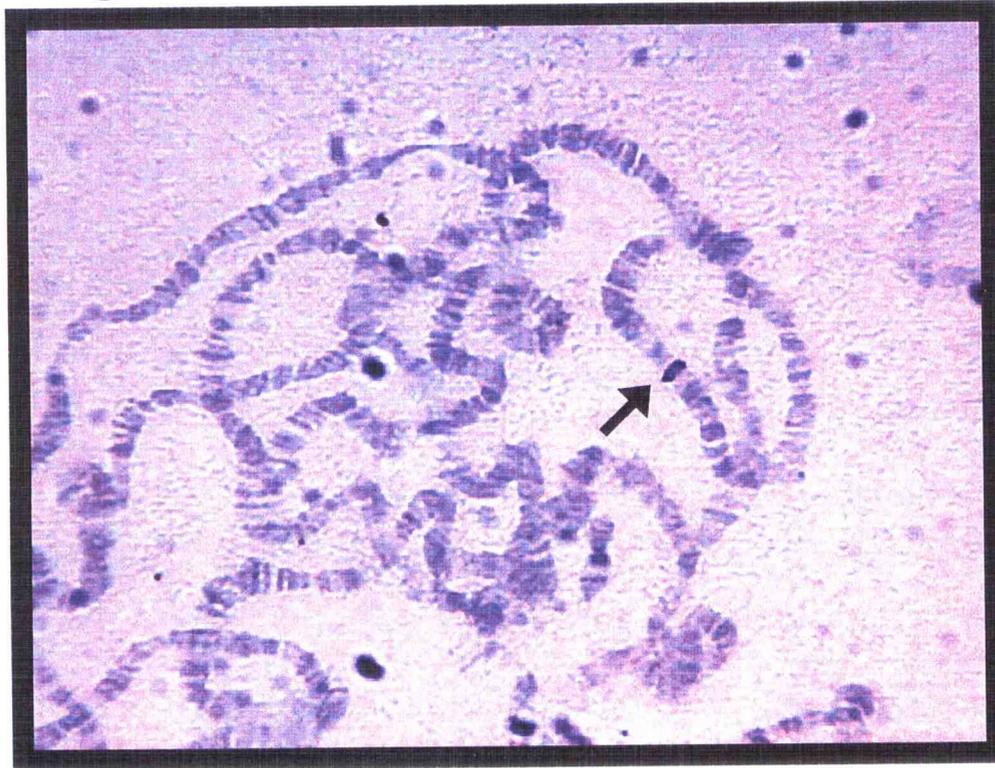
from the complementation tests, the progeny from these crosses were counted at several stages of development in order to be able to assess the lethal phase. In crosses performed between 4.078 and $B52^{ED}$, $B52^{R2}$, and $Df(3R)urd$, the 4.078/mutant adults emerge with the expected Mendelian ratio (Table 3.9A). Egg collections from the test crosses and wildtype stock were followed through the larval and pupal stage and showed that there were no evidence of any loss occurring at these specific stages of development (Table 3.9B). Thus, these tests showed that 4.078 was unlikely to be allelic to $B52$ or to other known splicing factors uncovered by the $Df(3R)urd$.

(A)

Allele being tested	# of eggs laid	# Adults ♂ 4.078/allele tested	# Adults ♀ 4.078/allele tested	# Adults ♂ 4.078/TM3	# Adults ♀ 4.078/TM3	Total Adult Offspring	χ^2 value (p value)
$B52^{ED}$	1154	209 (23%)	219 (24%)	239 (26%)	243 (27%)	910	3.4 (.5>p>.1)
$B52^{R2}$	390	81 (25%)	79 (25%)	92 (29%)	66 (21%)	318	4.3 (.5>p>.1)
$Df(3R)urd$	1156	220 (22%)	239 (24%)	265 (27%)	262 (27%)	986	5.4 (.5>p>.1)
4.078 control	796	324 (46%)	383 (54%)	NA	NA	707	4.9 (.05>p>.025)
Canton-S control	1000	414 (51%)	398 (49%)	NA	NA	812	.32 (.9>p>.5)

(B)

Allele being tested	# of eggs laid	# of eggs hatched to 1st instar larva (% lost)	# of 3rd instar larva transferred to vials (% lost)	# of pupa (% lost)	# of Adults (% lost)
$B52^{ED}$	1154	1024 (11.3%)	965 (5.8%)	923 (4.4%)	910 (1.4%)
$B52^{R2}$	390	346 (11.3%)	332 (4.0%)	328 (1.2%)	318 (3.0%)
$Df(3R)$ urd	1156	1109 (4.1%)	1038 (6.4%)	1012 (2.5%)	986 (2.6%)
4.078 control	796	760 (4.5%)	754 (0.8%)	737 (2.3%)	707 (4.1%)
Canton-S control	1000	910 (9.0%)	883 (3.0%)	859 (2.7%)	812 (5.5%)

Table 3.9: Results of the allelism test between the 4.078 insertion and the essential splicing factor B52. (A) Offspring of the five crosses used in the allelism test were scored, based on genotype and sex. The percentage of the total number of offspring is indicated in parenthesis. The χ^2 value was calculated for four categories: males with 4.078/allele tested, females with 4.078/allele tested, males with 4.078/TM3, and females with 4.078/TM3, with three degrees of freedom for each of the crosses to $B52^{ED}$, $B52^{R2}$, and $Df(3R)urd$. For the control crosses, the χ^2 value is only calculated for two categories, male and female, with one degree of freedom. The bracketed p-values are from a table of critical values (Rohlf, 1981). (B) Counts from the different life stages are given. The percent of animals lost from the previous life stage is also indicated.

Discussion

I have currently demonstrated a genetic interaction between the sex determining genes *tra* and *tra2* with the 4.078 P-element. From genetic crosses, the interaction is at the level of the *tra* and *tra-2* female-specific splice choice resulting in misexpression of the output genes *dsx* and *fru* (Figure 3.7). The resulting intersexes show both *dsx*-dependent and *fru*-dependent phenotypes, likely due the presence of both male- and female-specific mRNAs. In addition, the 4.078 P-element has been shown not to be allelic to another known splicing factor, *B52*, located in the region of the P-element insertion. The identification of this new genetic interactor with *tra* and *tra2* adds a new gene to the short list of known interactors, which also includes *B52*, *rbp1*, *Doa*, and *rox21* (Kim *et al.*, 1992; Mattox, 1998; Peng and Mount, 1995).

One feature shared by all of the previously identified genetic interactors is that they belong to the SR protein family. SR proteins contain a characteristic serine/arginine-rich domain, responsible for protein-protein interactions, and one or more RNA-binding domains (Reviewed in Manley and Tacke, 1996). The current model of SR protein action is recruitment of the splicing complex to the splice-site by protein-protein interactions and by the recognition and binding by proteins of specific RNA sequences, termed splicing enhancers (Reviewed in Hertel *et al.*, 1997). The splicing of *dsx* pre-mRNA has become a model for how alternative splicing is regulated and has provided evidence for the recruitment model of SR protein action (Amrein *et al.*, 1994; Heinrichs and Baker, 1997; Lynch and Maniatis, 1996; Tian and Maniatis, 1994).

The cloning and sequencing of the 4.078 P-element could reveal it to encode one of several known splicing factors that have only been identified by sequence homology. The most promising such candidate is SF2, a splicing factor in *Drosophila* named for the mammalian homology identified in cell culture assays for splicing factors. *In vitro* studies utilizing the yeast two-hybrid system have shown that the *Drosophila* SF2 protein can interact with the TRA2 protein (Amrein *et al.*, 1994). William Mattox recovered the SF2 sequence from an EST screen and mapped it to the region 85-89E on the right arm of the 3rd chromosome (Mattox, 1998). This places it within the region to which the 4.078

Figure 3.7

MODEL FOR SPLICING EVENTS IN THE INTERACTION BETWEEN
4.078 AND THE SEX DETERMINATION HIERARCHY:
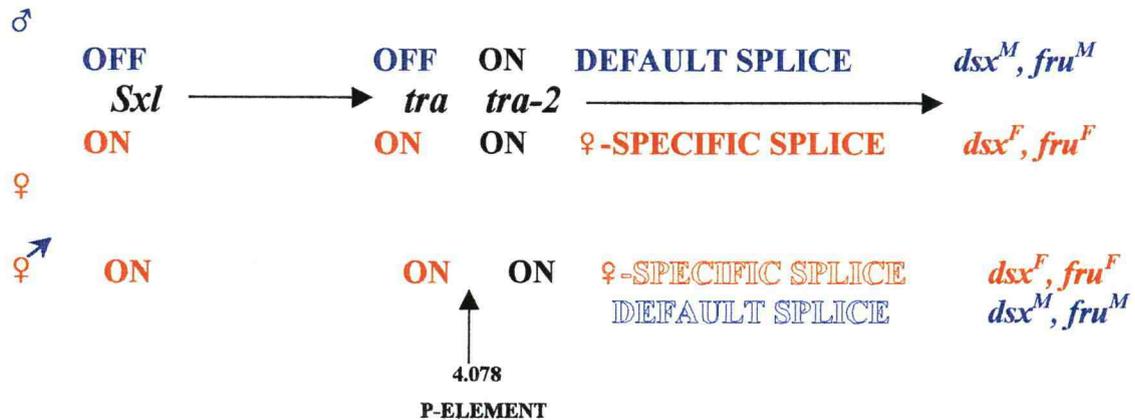
Figure 3.7: Genetic model for the 4.078 genetic interaction with sex determining genes. (A) Diagram of regulated alternative splicing events in the sex determination hierarchy. The genetic functions of the genes are listed as on or off, even though there are non-functional proteins present in males for both *Sxl* and *tra*. In females the functional *Sxl* protein regulates the female-specific splicing of *tra* to give a functional protein. With functional *tra* present, *tra* and *tra2* regulate the female-specific splicing of the output genes *dsx* and *fru* resulting in female-specific proteins. Without the genetic function of *tra* the default male-specific splice occurs for *dsx* and *fru* resulting in male-specific proteins. (B) Diagram of regulated alternative splicing events in the presence of the 4.078 P-element. The normal splicing of *tra* occurs since no abnormal flies are observed in the presence of a reduced dose of functional SXL protein. The regulated female-specific splice choice of both *dsx* and *fru* is disrupted when the genetic dose of *tra* and/or *tra2* is reduced and one copy of the 4.078 P-element is present, resulting in a mixture of both male- and female-specific transcripts for *dsx* and *fru*.

P-element insertion site maps. No known mutations have been found within the SF2 gene, even though function has been assigned to the protein. Should the 4.078 P-element lie within the SF2 gene it will allow us to make small deficiencies in the area by imprecise P-element excision and allow for a genetic characterization of SF2 that isn't currently possible.

To understand better the mechanisms involved in selecting the appropriate splice sites for proper sexual differentiation, we need to identify additional factors that interact with TRA and TRA2 proteins to help regulate the sex-specific splicing of the target genes *dsx* and *fru*. With the emergence of cell specific expression patterns for various alternatively spliced *fru* transcripts the mechanisms that regulate splice choice could help to elucidate gene action in certain cells or tissues. Two observations highlight the importance of identifying additional factors. First, the genetic interactor *Doa* has been shown to affect the sex-specific splicing of the *dsx* primary transcript but not that of the *fru* primary transcript (Mattox, 1998). This distinction was not unexpected, given that the regulated splicing of *fru* occurs at the 5′ end of the intron, where as the regulated splicing of the *dsx* primary transcript occurs at the 3′ end of the intron (Heinrichs *et al.*, 1998; Ryner and Baker, 1991). Second, B52 helps to regulate the splicing of genes including *dsx*, *w*, and *Ultrabithorax* (*Ubx*), but its role, if any, in regulating the sex-specific splicing of *fru* has yet to be explored (Kraus and Lis, 1994; Peng and Mount, 1995). We are interested I whether the regulation of sex-specific splicing for *dsx* and *fru* are controlled by similar mechanisms or whether different protein-protein interactions are important for each gene. Identification of additional genes involved in splicing would allow a genetic dissection of some of the processes. This would help overcome one major drawback to much of the research on splicing by allowing collection of *in vivo* data to supplement the current *in vitro* data.

Chapter 4
Summary and Future Directions

The overall objective of my research is to identify the developmental processes involved in the generation of sexually dimorphic neurons in the central nervous system (CNS) in *Drosophila melanogaster*. Metamorphosis is a time of major developmental change. The larval CNS has to change to be able to perform adult functions. To compensate for the new adult body and behaviors, the larval CNS must be remodeled into the adult CNS. To establish the adult CNS neurons in the larva are remodeled or eliminated and new neurons are added. To examine the genetic control or the restructuring of the CNS, I have used an identified population of neurons to follow development in both control and mutant flies.

From our current understanding of the role of the sex determining genes, *doublesex* (*dsx*) is the most likely candidate for the control of the abdominal motorneurons innervating the terminal sex-specific segments in *Drosophila*. *dsx* has both male and female specific proteins that control sexual differentiation (Burtis and Baker, 1989; Burtis *et al.*, 1991; Coschigano and Wensink, 1993; Jursnich and Burtis, 1993; Nagoshi *et al.*, 1988). Both chromosomal male and female *dsx* mutants have an external intersexual phenotype (Hildreth, 1965). Internally, the body wall muscles include both male- and female-specific muscles in the genitalia (Merritt, 1994; T.J.S. Merritt and B.J. Taylor, unpublished results). The musculature of the genitalia and the abdomen is wildtype in the other two known outputs from the sex determination hierarchy, *fruitless* (*fru*) and *dissatisfaction* (*dsf*). However *fru* and *dsf* regulate a few special motorneurons. *fru* controls the differentiation of the male-specific muscle, the Muscle of Lawrence (MOL) and *dsf* shows a defect in the innervation of the circular uterine muscles in females and the ventral muscles in abdominal segment A5 in males (Finley *et al.*, 1997; Gailey *et al.*, 1991; Taylor, 1992; Taylor and Knittel, 1995). Taken together, *fru* and *dsf* are not expected to have a major role in the development of most of the abdominal motorneurons.

One way to assess the role of *dsx* in the development of the sex-specific nervous system during the metamorphic transition is to use a set of motorneurons that are found

through all developmental stages, but whose characteristics vary sex-specifically. I have identified a P[*tau-lacZ*] line which labels a specific set of motorneurons, the ventral unpaired median cells (VUMs), whose developmental history is known and whose pattern is altered in adults (Callahan and Thomas, 1994). The larval pattern of staining consists of 11 segmentally repeated groups of three neurons that innervate the eight abdominal segments and the three thoracic segments. These neurons produce a very characteristic axonal projection, which emerges from the ventral surface of the CNS, heads straight up the midline, and splits at the dorsal surface to leave the CNS in bilaterally symmetric nerves. Examination of control adults from this line show that males and females have a different pattern of VUMs after metamorphosis. In the abdominal ganglion of adults, I found seven groups of VUMs in males and six groups of VUMs in females compared with the eight groups of VUMs found in the larva. Because there are still three groups of VUMs in the thoracic segments, innervating identical musculature in males and females, it suggest that the change is due to the sex-specific changes in musculature found in the abdomen. This is further supported by the observation that all 11 sets of VUMs are present in *dsx* mutants. This is the first line of evidence that demonstrates a sex-specific regulatory role for *dsx* involving the survival of larval motorneurons in the abdominal ganglion. These studies complement the finding that there are differences in the pattern of nerve root innervation in males and females and suggest that there are more sex-specific differences to be discovered in the CNS.

The role *dsx* fills in determining sex-specific motorneurons is unknown at this time. There are many possible functions that *dsx* could control. One of the most likely and interesting is programmed cell death (PCD). Since the larval population of motorneurons is trimmed down by PCD to give the adult population of motorneurons the genes regulating the determination of that population must converge onto the genes regulating programmed cell death. Since the genes and their interactions involved in the regulation and control of apoptosis are just beginning to be identified all of the convergent signals, which act both positively and negatively, have yet to be identified. With the identification of the role *dsx* plays in determining neuronal identity in the abdominal ganglion it will be possible to determine if *dsx* converges on the genes regulating PCD. The only downstream gene from *dsx* currently known is the *yolk protein*

gene (Burtis *et al.*, 1991; Coschigano and Wensink, 1993). The possibility that *dsx* could also activate or repress the pathway leading to apoptosis will be a large step in finding more downstream targets of the sex determining genes.

In addition to examining the restructuring of the CNS during metamorphosis another project developed from my research. In the process of crossing the 4.078 P-element into the mutant background of other sex determining genes, an interesting genetic interaction was observed. A number of animals from certain crosses had an intersexual phenotype suggestive of an interaction between the 4.078 P-element and the genes in the sex determining hierarchy. After the initial phenotype was observed a series of test crosses were carried out to characterize the interaction genetically. By genetic analysis, the interaction appears to be at the level of *transformer* (*tra*) and *transformer-2* (*tra2*) and acts to cause the mis-splicing of the primary transcripts of the output genes *dsx* and *fru* in females. The production of abnormal male-specific *dsx* and *fru* mRNA is the likely cause of the intersexual phenotype I observed. I used standard genetic crosses to show that the genetic interaction was coupled to the P-element insert. From additional crosses, I know that my mutation is not allelic to one gene in the region of the 4.078 insertion site, *B52*, known to affect *dsx* splicing and that this may be a new gene involved in regulating splicing or maintaining mRNA stability.

The genetic interactor has been genetically characterized and now needs to be molecularly characterized. The structure of P-elements used in *Drosophila* research has made the cloning of the flanking genomic DNA very easy by the use of the plasmid rescue technique (Callahan *et al.*, 1996; Dorn *et al.*, 1993; Hersberger *et al.*, 1996). Plasmid rescue involves isolating and restricting genomic DNA from flies carrying the P-element. Because the DNA used to introduce the P-elements into *Drosophila* is amplified in bacteria, all of the necessary sequences to maintain a plasmid in bacteria are present, including antibiotic resistance and an origin of replication. After the genomic DNA is restricted in smaller pieces and ligated into circular DNA, it is possible to transfect bacteria with the DNA and only the bacteria with the DNA containing the P-element will grow under selection. It is then a manner of sequencing the flanking DNA that was including in the 'plasmid' with the P-element. With this cloned fragment, a variety of techniques, such as finding cDNAs, can be used to clone the gene and a more

precise chromosomal location can be mapped by probing P1 sequences mapped in the *Drosophila* genome.

Our genetic analysis of the 4.078 genetic interactor has not established, with certainty, the nature of the mutant allele. It is quite possible that the phenotypes that I have observed may result from the behavior of an unusual allele and there is no guarantee that other loss-of-function or gain-of-function alleles at the locus will have the same phenotype. Many of the first alleles discovered for genes, such as *engrailed* or *Bithorax*, had visible phenotypes that were not found in other alleles because they were hypomorphs induced by the insertion of a transposable element. It will be necessary then to create new alleles of the 4.078 interactor with the tools that are available. The best way to create new alleles will be by imprecise P-element excision (Dom *et al.*, 1993; Rio, 1991; Tower *et al.*, 1993; Zhang and Spradling, 1993). When an endogenous source of transposase, the enzyme responsible for allowing natural P-element to change position, is supplied to flies containing a P-element it is possible to remove flanking DNA from the insertion site when the P-element 'jumps'. This will produce small deficiencies in the area, which will allow possible genetic nulls to be obtained from the disrupted gene. With a larger disturbance than the P-element insertion, it can be possible to interfere with the function of the gene in a manner that doesn't require a genetic interaction. Many known splicing factors are lethal when null and it will be highly possible that lethal deficiency combinations can be obtained. The strength of the genetic interaction that I have discovered and the nearby localization of the P-element to the interactor locus makes it likely that the P-element excisions will be successful in generating new alleles of the interactor. If these mutations have the same interacting phenotype, then, I will have likely found a new gene involved in the regulation of alternative mRNA splicing. It

is as yet unclear whether the other phenotypes that were also found in the genetic analysis are related to the function that is interacting with the sex-determining cascade. If the gene is involved in regulated splicing then it may also affect the splicing of primary transcripts of genes involved in these processes.

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APPENDIX

GENETIC CROSSES

Appendix- Genetic Crosses

The crosses used to test for a genetic interaction between the 4.078 P-element line and mutations in the sex determination hierarchy are described in detail below. The information is organized as follows:

Cross: The genotype of the parents is indicated with the maternal parent listed first and the paternal parent second. Where different than room temperature the rearing temperature is given in parentheses.

Purpose: A brief narrative describes the purpose of the cross and the information obtained from the cross.

Results: The phenotypes for the progeny are summarized along with the conclusions derived from the cross. The number of offspring by genotype, their percentage of total progeny and the number of abnormal offspring per genotype are tabulated for each cross.

Other details: Where necessary, supplementary information is provided about special feature of a particular cross.

Cross A:

w^-/w^-; $4.078w^+/4.078w^+$ X w; $T(2;3)$ $S9e/SM1$ Cy; $TM6C$ Sb,Tb,e

Purpose:

This cross was used to generate flies with a 4.078 chromosome and balancer chromosomes for the second and third chromosome.

Results:

Virgin females were collected even though one of the dominant markers on the $SM1$ balancer chromosome, $Curly$, was not expressed.

Cross B:

w^-/w^-; $+/SM1$; $4078w^+/TM6C$ X B^SY; $tra2/CyO$; fru^1 $P[79Bactin-lacZ]/TM6C$

Purpose:

This cross was the first step toward generating a 4.078 line that also contained a $tra-2$ mutant 2nd chromosome for determining the role of $tra-2$ on the development of the VUMs, labeled in the 4.078 line. This was the first cross where an interaction was observed.

Results:

From crosses set up at room temperature, chromosomally female flies (non-B^s eye) appeared to be partially transformed to male phenotype since they had male and female genitalia. As this was an unexpected phenotype from this cross, no counts were made from the initial cross. Other phenotypes were also observed (listed below). My preliminary explanation was that I had allowed a $tra2/CyO$

female through and was thus generating *tra2* homozygotes. This was discounted when the same phenotype was seen in the *Df(2L)trix* cross (Cross C), since this deficiency is homozygous lethal. Cuticle preparations were made from the transformed animals from this cross.

In the masculinized females, the phenotypes observed included:
1. Masculinized genitalia were present with all of the expected male structures but the genitalia were in a variety of dorsal/ventral positions reflecting abnormal genital disc rotation.
2. The fifth and sixth abdominal segments had a male pigmentation pattern
3. The sex combs weren't transformed to the male phenotype, but could be intersexual.
4. In some animals, the genitalia did not evert and no external genitalia were visible

To pursue this unexpected outcome, offspring from a second set of parents that had been set up as backups for these crosses the offspring were counted (Cross B,Table 1). These flies had been maintained at 16°C to delay their development enabling them to be used for further crosses if needed. Of the progeny recovered, 12% of the total offspring and 15% of the female population were masculinized in the *tra2* series (Cross B, Table 1) and 8% of the total flies in the *trix* series (Cross C, Table 2). No *tra-2/CyO; 4.078* lines were successfully established.

Other details:

From the progeny of this cross, single pair crosses were used to try to create a line with *tra-2* balanced over *CyO* and homozygous for *4.078*. This was necessary since the *SM1* chromosome could not be distinguished from the + chromosome. In the cross, there was a 50/50 chance of any given fly having the *SM1* balancer.

Another problem was that females had red eyes (w^+) so it was not possible to score them for which of the two P-elements they were carrying. Having found some masculinized flies, some of these were dissected to make adult abdominal muscle and CNS preparations in an attempt to genotype flies by their staining pattern. The dissected tissues were stained with X-gal for the muscles and α-βgal immunohistochemistry for the CNS. All of the transformed animals that were dissected had the 4.078 P-element either in combination with the balancer, *TM6C*, or the *P[79Bactin-lacZ]* suggesting that there was a heterozygous interaction between *4.078* and the *tra-2* or *SM1* chromosome.

Table A.1

Data from w^-/w^-; +/ SM1; 4078w^+/ TM6C X B^SY; tra2/CyO; fru^{1}79B/ TM6C

Genotype	Total # of Offspring	Percentage
Females		
+/CyO;4.078/fru^1 79B	16	15%
+/CyO;4.078 or fru^1 79B/TM6C	6	6%
SM1/CyO;4.078/fru^1 79B	15	14%
SM1/CyO;4.078 or fru^1 79B/TM6C	3	3%
tra2/+ or SM1;4.078/fru^1 79B	22	20%
tra2/+ or SM1;4.078 or fru^{1}79B/TM6C	10	9%
Males		
CyO/+ or SM1;4.078/fru^1 79B	7	6%
CyO/+ or SM1;4.078/TM6C	4	4%
CyO/+ or SM1;fru^1 79B/TM6C	0	0%
tra2/+ or SM1;4.078/fru^1 79B	5	5%
tra2/+ or SM1;4.078/TM6C	5	5%
tra2/+ or SM1;fru^1 79B/TM6C	2	2%
Masculized		
4.078/TM6C	8	7%
4.078/fru^1 79B	5	5%
Totals:	**108**	

Cross C:

w^-/w^-; +/ SM1; 4078w^+/ TM6C X B^SY; trix/CyO; fru^1 [79Bactin-lacZ]/ TM6C

Purpose:

This cross was the first step toward generating the 4.078 line that also contained a Df(2L)trix mutant 2nd chromosome for determining the role of tra-2 on the development of the VUMs labeled in the 4.078 line. Df(2L)trix is a deficiency which uncovers tra2.

Results:

This cross was set up concurrently with the tra-2 cross (Cross B) and a genetic interaction was observed in this cross as well. The results from this cross was simmilar to that of the tra-2 cross (Cross B) except a slightly smaller percentage of total flies (8%) were masculinized (see above for other phenotypes). From these crosses no trix/CyO; 4.078 lines were successfully generated.

Table A.2

Data from w^-/w^-; $+/SM1$; $4078w^+/TM6C$ X B^SY; $trix/CyO$; $fru^l 79B/TM6C$

Genotype	Total # of Offspring	Percentage
Females		
$+/CyO;4.078/fru^l 79B$	14	8%
$+/CyO;4.078$ or $fru^l 79B/TM6C$	10	5%
$SM1/CyO;4.078/fru^l 79B$	9	5%
$SM1/CyO;4.078$ or $fru^l 79B/TM6C$	12	7%
$trix/+;4.078/fru^l 79B$	14	8%
$trix/+;4.078$ or $fru^l 79B/TM6C$	26	14%
$trix/SM1;4.078/fru^l 79B$	13	7%
$trix/SM1;4.078$ or $fru^l 79B/TM6C$	1	1%
Males		
$CyO/+$ or $SM1;4.078/fru^l 79B$	11	6%
$CyO/+$ or $SM1;4.078/TM6C$	6	3%
$CyO/+$ or $SM1;fru^l 79B/TM6C$	10	5%
$trix/+$ or $SM1;4.078/fru^l 79B$	30	16%
$trix/+$ or $SM1;4.078/TM6C$	5	3%
$trix/+$ or $SM1;fru^l 79B/TM6C$	8	4%
Masculized		
$4.078/TM6C$	8	4%
$4.078/fru^l 79B$	7	4%
Totals:	**184**	

Cross D:

w^-/w^-; $+/SM1$; $4.078w^+/4.078w^+$ X B^SY; $tra2/CyO$; $fru^l P[79Bactin-lacZ]/TM6C$
(25°C)

Purpose:

A second attempt to introduce the 4.078 P-element into a *tra-2* background and to try to replicate the previous genetic interaction observed.

Results:

Unlike the previous cross, no female offspring were masculinized. Rather, several sexual mosaic animals were present among the offspring. The male//female mosaicism was very extensive, including many tissues in the body, suggesting that these were caused by early zygotic loss of the X-chromosome in early embryogenesis. In addition several other phenotypes were observed including XO males and some developmental defects such as deformed abdominal segmentation. No lines of *tra-2/CyO; 4.078* were generated.

Table A.3

Data from *w⁻/w⁻; +/SM1; 4.078w⁺/4.078w⁺ X BˢY; tra2/CyO; fru¹ P[79Bactin-lacZ]/TM6C*

GENOTYPE	Total # of Offspring	# of Abnormal	% Abnormal
FEMALES			
w⁻/w⁺; tra2/SM1; 4.078/TM6C	84		
w⁻/w⁺; CyO/+; 4.078/TM6C			
w⁻/w⁺; tra2/SM1; 4.078/fru¹79B	82	1	1.22%
w⁻/w⁺; CyO/+; 4.078/fru¹79B			
w⁻/w⁺; tra2/+; 4.078/TM6C	51	2	3.92%
w⁻/w⁺; tra2/+; 4.078/fru¹79B	70		
w⁻/w⁺; CyO/SM1; 4.078/TM6C			
w⁻/w⁺; CyO/SM1; 4.078/fru¹79B			
w⁻/w⁺; ?; 4.078/TM6C	3		
w⁻/w⁺; ?; 4.078/fru¹79B	2		
MALES			
w⁻/BˢY; tra2/SM1; 4.078/TM6C	66		
w⁻/BˢY; CyO/+; 4.078/TM6C			
w⁻/BˢY; tra2/SM1; 4.078/fru¹79B	71	1	1.41%
w⁻/BˢY; CyO/+; 4.078/fru¹79B			
w⁻/BˢY; tra2/+; 4.078/TM6C	22		
w⁻/BˢY; tra2/+; 4.078/fru¹79B	67		
w⁻/BˢY; CyO/SM1; 4.078/TM6C			
w⁻/BˢY; CyO/SM1; 4.078/fru¹79B			
w⁻/BˢY; ?; 4.078/TM6C	4		
w⁻/BˢY; ?; 4.078/fru¹79B	2		
Totals:	**524**	**4**	**0.76%**

Cross E:

    w⁻/w⁻; +/SM1; 4.078w⁺/4.078w⁺ X BˢY; trix/CyO; fru¹ P[79Bactin-lacZ]/TM6C

    (25°C)

Purpose:

    A second attempt to introduce the 4.078 P-element into a *Df(2L)trix* background and to try to replicate the genetic interaction of Cross C.

Results:

    No intersexual females were found in the offspring from this cross. An XXY female was observed in addition to some males with deformed abdominal segmentation. A male with the genotype *w⁻/BˢY; CyO/+; 4.078/fru¹79B* was found with mirror image male external genitalia, in this animal the anal plates were abnormal since there were four in the central area with the claspers and other structures on the top and bottom of the anal plates. Otherwise the male was normal with respect to other sex-specific cuticular structures, such as sex combs, abdominal pigmentation and eyes.

Table A.4
Data from w^-/w^-; $+/SM1$; $4.078w^+/4.078w^+$ $X B^S Y$; $trix/CyO$; fru^1 $P[79B$ actin-lacZ]/TM6C

GENOTYPE	Total # of Offspring	# of Abnormal	% Abnormal
FEMALES			
w^-/w^+; $trix/SM1$; $4.078/TM6C$	0		
w^-/w^+; $CyO/+$; $4.078/TM6C$	78		
w^-/w^+; $trix/SM1$; $4.078/fru^1 79B$	0		
w^-/w^+; $CyO/+$; $4.078/fru^1 79B$	92	1	1.09%
w^-/w^+; $trix/+$; $4.078/TM6C$	42		
w^-/w^+; $trix/+$; $4.078/fru^1 79B$	76		
w^-/w^+; $CyO/SM1$; $4.078/TM6C$			
w^-/w^+; $CyO/SM1$; $4.078/fru^1 79B$			
w^-/w^+; ?; $4.078/TM6C$	6		
w^-/w^+; ?; $4.078/fru^1 79B$	2		
MALES			
$w^-/B^S Y$; $trix/SM1$; $4.078/TM6C$	51		
$w^-/B^S Y$; $CyO/+$; $4.078/TM6C$			
$w^-/B^S Y$; $trix/SM1$; $4.078/fru^1 79B$	72	1	1.39%
$w^-/B^S Y$; $CyO/+$; $4.078/fru^1 79B$			
$w^-/B^S Y$; $trix/+$; $4.078/TM6C$	31	1	3.23%
$w^-/B^S Y$; $trix/+$; $4.078/fru^1 79B$	50		
$w^-/B^S Y$; $CyO/SM1$; $4.078/TM6C$			
$w^-/B^S Y$; $CyO/SM1$; $4.078/fru^1 79B$			
w^-/w^+; ?; $4.078/TM6C$	1		
w^-/w^+; ?; $4.078/fru^1 79B$	3		
Totals:	**504**	**3**	**0.60%**

Cross F:
w^-/w^-; $+/+$; $4.078w^+/4.078w^+$ $X B^S Y$; $tra2/CyO$; fru^1 $P[79Bactin-lacZ]/TM6C$
(25°C)

Purpose:
To see if the genetic interaction was due to something in the 4.078 stock, a cross was set up using the original 4.078 stock, in which balancer chromosomes are not present. This cross will help pinpoint the source of the genetic interaction, to either the 4.078 stock or the crosses performed to bring in balancer chromosomes.

Results:
No female intersexes were observed in this cross, but a number of mosaic animals were present in this cross. In addition, several other phenotypes were observed including XO males and abnormal abdominal segmentation.

Table A.5

Data from w^-/w^-; +/+; $4.078w^+/4.078w^+$ X B^SY; tra2/CyO; fru^1 P[79Bactin-lacZ]/TM6C

GENOTYPE	Total # of Offspring	# of Abnormal	% Abnormal
FEMALES			
w^-/w^+; tra2/+; 4.078/TM6C	64	1	1.56%
w^-/w^+; tra2/+; 4.078/fru^{1}79B	129	2	1.55%
w^-/w^+; CyO/+; 4.078/TM6C	48		
w^-/w^+; CyO/+; 4.078/fru^{1}79B	93	1	1.08%
w^-/w^+; ?; 4.078/TM6C	9		
w^-/w^+; ?; 4.078/fru^{1}79B	10		
MALES			
w^-/B^SY; tra2/+; 4.078/TM6C	40		
w^-/B^SY; tra2/+; 4.078/fru^{1}79B	99		
w^-/B^SY; CyO/+; 4.078/TM6C	27		
w^-/B^SY; CyO/+; 4.078/fru^{1}79B	70		
w^-/B^SY; ?; 4.078/TM6C	5		
w^-/B^SY; ?; 4.078/fru^{1}79B	10		
Totals:	**604**	**4**	**0.66%**

Cross G:

w^-/w^-; +/+; $4.078w^+/4.078w^+$ X B^SY; trix/CyO; fru^1 P[79Bactin-lacZ]/TM6C

(25°C)

Purpose:

The same rationale is applied here as in Cross F.

Results:

No female intersexes were observed in this cross but a high number of mosaic animals were present in this cross. In addition several other phenotypes were observed including XO males and abnormal abdominal segmentation. Note: A second cross was carried out to try to produce more mosaic animals to examine. The table below is a combination from both of the crosses carried out. The second cross produced fewer abnormal animals with only 2 XO males found.

Table A.6

Data from w^-/w^-; +/+; $4.078w^+/4.078w^+$ X B^SY; trix/CyO; fru^1 P[79Bactin-lacZ]/TM6C

GENOTYPE	Total # of Offspring	# of Abnormal	% Abnormal
FEMALES			
w^-/w^+; trix/+; 4.078/TM6C	136	1	0.74%
w^-/w^+; trix/+; 4.078/$fru^1$79B	209	3	1.44%
w^-/w^+; CyO/+; 4.078/TM6C	111	2	1.80%
w^-/w^+; CyO/+; 4.078/$fru^1$79B	180	7	3.89%
w^-/w^+; ?; 4.078/TM6C	5		
w^-/w^+; ?; 4.078/$fru^1$79B	5		
MALES			
w^-/B^SY; trix/+; 4.078/TM6C	90	1	1.11%
w^-/B^SY; trix/+; 4.078/$fru^1$79B	175	2	1.14%
w^-/B^SY; CyO/+; 4.078/TM6C	100	3	3.00%
w^-/B^SY; CyO/+; 4.078/$fru^1$79B	172	1	0.58%
w^-/B^SY; ?; 4.078/TM6C	2		
w^-/B^SY; ?; 4.078/$fru^1$79B	1		
Totals:	**1186**	**18**	**1.52%**

Cross H:

ywf/ywf; +/+; $4.078w^+/4.078w^+$ X y/y^+Y; cn trix bw/CyO; +/+

ywf/ywf; +/+; $4.078w^+/TM6B$ X y/y^+Y; cn trix bw/CyO; +/+

y/y; cn trix bw/CyO; +/+ X ywf/B^SY ; +/+; $4.078w^+/4.078w^+$

y/y; cn trix bw/CyO; +/+ X ywf/B^SY; +/+; $4.078w^+/TM6B$ (25°C)

Purpose:

To test for a possible maternal effect in the observed genetic interaction with tra2 reciprocal crosses were carried out using the deficiency trix. Body color markers were incorporated to enable X-chromosome loss mosaics to be detected in cuticular tissues that were not sexually dimorphic.

Results:

No clear maternal effect was observed from this series of crosses. One X-loss mosaic was observed from the y/y; cn trix bw/CyO; +/+ X ywf/B^SY; +/+; $4.078w^+/4.078w^+$ cross with the genotype ywf/y; trix/+; 4.078/+. In addition from this cross was an XXY female and flies with abnormal abdominal segmentation and non-everted wings. From the cross y/y; cn trix bw/CyO; +/+ X ywf/B^SY; +/+; $4.078w^+/TM6B$ only with abnormal segmentation and non-everted wings were observed, the majority of which were trix/+; 4.078/+ with one female +/CyO; 4.078/+. From the cross ywf/ywf; +/+; $4.078w^+/4.078w^+$ X y/y^+Y; cn trix bw/CyO; +/+ a Y-loss male was identified from y^+/y^- patches caused by the loss of the y^+Y chromosome. From the cross ywf/ywf; +/+; $4.078w^+/TM6B$ X y/y^+Y; cn trix bw/CyO; +/+ only a XXY female was observed.

Table A.7a

Data from *ywf/ywf; +/+; 4.078/4.078 X y/y⁺Y; cn trix bw/ CyO; +/+*

GENOTYPE	Total # of Offspring	# of Abnormal	% Abnormal
Females			
ywf/y;+/trix;+/4.078	43	0	0.00%
ywf/y;+/CyO;+/4.078	32		
Males			
ywf/y⁺Y;+/trix;+/4.078	29	0	0.00%
ywf/y⁺Y;+/CyO;+/4.078	43	1	2.33%
Totals:	**147**	**1**	**0.68%**

Table A.7b

Data from *ywf/ywf; +/+; 4.078/TM6B X y/y⁺Y; cn trix bw/ CyO; +/+*

GENOTYPE	Total # of Offspring	# of Abnormal	% Abnormal
Females			
ywf/y;+/trix;+/4.078	17		
ywf/y;+/trix;+/TM6B	17		
ywf/y;+/CyO;+/4.078	18		
ywf/y;+/CyO;+/TM6B	18	1	5.56%
Males			
ywf/y⁺Y;+/trix;+/4.078	10		
ywf/y⁺Y;+/trix;+/TM6B	17		
ywf/y⁺Y;+/CyO;+/4.078	6		
ywf/y⁺Y;+/CyO;+/TM6B	9		
Totals:	**112**	**1**	**0.89%**

Table A.7c

Data from *ywf/BˢY; +/+; 4.078/4.078 X y/y; cn trix bw/ CyO; +/+*

GENOTYPE	Total # of Offspring	# of Abnormal	% Abnormal
Females			
ywf/y;+/trix;+/4.078	143	3	2.10%
ywf/y;+/CyO;+/4.078	144		
Males			
y/BˢY;+/trix;+/4.078	134	3	2.24%
y/BˢY;+/CyO;+/4.078	106	1	0.94%
Totals:	**527**	**7**	**1.33%**

Table A.7d

Data from *ywf/B^{S}Y; +/+; 4.078/TM6B X y/y; cn trix bw/ CyO; +/+*

GENOTYPE	Total # of Offspring	# of Abnormal	% Abnormal
Females			
ywf/y;+/trix;+/4.078	56	2	3.57%
ywf/y;+/trix;+/TM6B	71		
ywf/y;+/CyO;+/4.078	58	1	1.72%
ywf/y;+/CyO;+/TM6B	39		
Males			
y/B^{S}Y;+/trix;+/4.078	49	2	4.08%
y/B^{S}Y;+/trix;+/TM6B	46		
y/B^{S}Y;+/CyO;+/4.078	56		
y/B^{S}Y;+/CyO;+/TM6B	36		
Totals:	**411**	**5**	**1.22%**

Cross I:

  w⁻/w⁻; 4.078w⁺/4.078w⁺ X ywf/B^{S}Y; Dfst^{J7}/TM6B (Df(3L) st^{J7}: 73A1-2; 73B1-2)

Purpose:

To test if there was an element in the 4.078 stock which interacted with or was allelic to *tra*.

Results:

In this cross, the *Dfst^{J7}* deficiency was used to uncover the *tra* locus and another potential interactor *dsc73* (*doublesex cognate 73*). One female had an intersexual phenotype with a small piece of male genitalia present in addition to the normal female genitalia. The sex combs were not clearly transformed, but could possibly be intersexual upon closer inspection. This intersexual female suggests that some element in the 4.078 stock interacts with *tra* although this interaction appears to be weaker than the one with *tra-2*.

Two XO males were also found, they were identified because they did not have *Bar* eyes. One was *4.078/TM6B* and the other was *4.078/Dfst^{J7}*. Additionally, a female had an abnormally developed wing.

Table A.8

Data from w⁻/w⁻; 4.078w⁺/4.078w⁺ X ywf/BˢY; Dfst⁷⁷/TM6B

Genotype	Total # of Offspring	Percentage
Females		
4.078/Dfst⁷⁷	120	30%
4.078/TM6B	81	20%
Males		
4.078/Dfst⁷⁷	108	27%
4.078/TM6B	83	21%
Abnormal	4	1%
Totals:	**396**	

Cross J:

   w⁻/w⁻; 4.078w⁺/4.078w⁺ X w⁻/BˢY; tra etc/TM6B   (25°C)

Purpose:

   To see if the interaction of the 4.078 stock with *tra* is still present using a deficiency just in *tra*. There are a number of recessive markers on the *tra* chromosome.

Results:

   Only two abnormal flies were observed. One male was found with upside down genitalia and a XXY female.

Table A.9

Data from *w⁻/w⁻; 4.078w⁺/4.078w⁺ X w⁻/BˢY; tra etc/TM6B*

GENOTYPE	Total # of Offspring	# of Abnormal	% Abnormal
FEMALES			
w-/w-; 4.078/ tra etc	133		
w-/w-; 4.078/TM6B	94	1	1.06%
MALES			
w-/BSY; 4.078/ tra etc	150	2	1.33%
w-/BSY; 4.078/TM6B	95		
Totals:	**472**	**3**	**0.64%**

Cross K:

   w⁻/w⁻; 4.078w⁺/4.078w⁺ X ywf/BˢY; Dfst⁷⁷/TM6B   (25°C)

Purpose:

   To incorporate sex chromosome markers into the 4.078 stock and exchange some of the genetic background.

Results:

   Two sexually abnormal flies were found in this cross, XO male and a *4.078/Dfst⁷⁷* male with genitalia which was not fully rotated, sitting hortizontal instead of vertical on the abdomen, and not connected to the abdomen correctly. In addition

abdominal segmentation and abnormalities in the *Bar* eye phenotype were observed.

Table A.10

Data from w^-/w^-; $4.078w^+/4.078w^+$ X ywf/B^SY; $Dfst^{J7}/TM6B$

GENOTYPE	Total # of Offspring	# of Abnormal	% Abnormal
FEMALES			
w^-/w^+; $4.078w^+/Dfst^{J7}$	145		
w^-/w^+; $4.078w^+/TM6B$	129	2	1.55%
MALES			
w^-/B^SY; $4.078w^+/Dfst^{J7}$	138	1	0.72%
w^-/B^SY; $4.078w^+/TM6B$	76		
Totals:	**488**	**3**	**0.61%**

Cross L:

ywf/w; $4.078w^+/TM6B$ X ywf/B^SY; $Dfst^{J7}/TM6B$ (25°C)

Purpose:

This is the F2 cross to the $Dfst^{J7}$ cross above (Cross K) with the purpose to incorporate sex chromosome markers.

Results:

No abnormal flies were observed. Two separate lines were established with the notation ywf/B^SY; $4.078w^+/TM6B$.

Table A.11

Data from *ywf/w; 4.078w⁺/TM6B* X *ywf/BˢY; Dfst⁷⁷/TM6B*

GENOTYPE	Total # of Offspring	# of Abnormal	% Abnormal
FEMALES			
ywf/ywf; 4.078w⁺/Dfst⁷⁷	71		
ywf/ywf; 4.078w⁺/TM6B	40		
ywf/ywf; Dfst⁷⁷/TM6B	46		
ywf/ywf; TM6B/TM6B	0		
w/ywf; 4.078w⁺/Dfst⁷⁷	62		
w/ywf; 4.078w⁺/TM6B	44		
w/ywf; Dfst⁷⁷/TM6B	47		
w/ywf; TM6B/TM6B	0		
MALES			
ywf/BˢY; 4.078w⁺/Dfst⁷⁷	66		
ywf/BˢY; 4.078w⁺/TM6B	46		
ywf/BˢY; Dfst⁷⁷/TM6B	30		
ywf/BˢY; TM6B/TM6B	0		
w/BˢY; 4.078w⁺/Dfst⁷⁷	73		
w/BˢY; 4.078w⁺/TM6B	37		
w/BˢY; Dfst⁷⁷/TM6B	37		
w/BˢY; TM6B/TM6B	0		
Totals:	**599**	**0**	

Cross M:

$w⁻/w⁻; 4.078w⁺/4.078w⁺$ X $y/y⁺Y; p^p dsx/TM6B$

Purpose:

To see if elements in the 4.078 stock interacted with or were allelic to *dsx*.

Results:

No sexually transformed flies were observed in the *dsx* cross. The only abnormal fly was one male, $w⁻/y⁺Y; 4.078w⁺/TM6B$, that had defective abdominal segmentation evident as one segment not making it all the way across the back. This is potentially a developmental defect.

Table A.11
Data from w^-/w^-; $4.078w^+/4.078w^+$ X y/y^+Y; p^P dsx/TM6B

Genotype	Total # of Offspring	Percentage
Females		
4.078/dsx	146	42%
4.078/TM6B	78	22%
Males		
4.078/dsx	91	26%
4.078/TM6B	35	10%
Abnormal	1	0%
Totals:	**351**	

Cross N:

w^-/w^-; $4.078w^+/4.078w^+$ X ywf/y^+Y; dsx p^P/TM6B   25°C

Purpose:

To incorporate sex chromosome markers into the 4.078 stock and exchange some of the genetic background.

Results:

A male//female mosaic and XO male were found in this cross in addition to other developmental abnormalities including abdominal segmentation and misformed thorax and non-everted wings.

Table A.12
Data from w^-/w^-; $4.078w^+/4.078w^+$ X ywf/y^+Y; dsx p^P/TM6B

GENOTYPE	Total # of Offspring	# of Abnormal	% Abnormal
FEMALES			
w/ywf; 4.078w^+/ dsx p^P	272		
w/ywf; 4.078w^+/TM6B	169	3	1.78%
MALES			
w/y^+Y; 4.078w^+/ dsx p^P	242	1	0.41%
w/y^+Y; 4.078w^+/TM6B	178	2	1.12%
Totals:	**861**	**6**	**0.70%**

Cross O:

> *ywf/w; 4.078w⁺/TM6B X ywf/y⁺Y; dsx pᵖ/TM6B*   25°C

Purpose:

This is the F2 cross to the *dsx* cross above (Cross N) with the purpose to incorporate sex chromosome markers and to produce flies that will be used to generate a stock with both *dsx* and the 4.078 P-element.

Results:

No abnormal flies were observed in this cross.

Table A.13

Data from *ywf/w; 4.078w⁺/TM6B X ywf/y⁺Y; dsx pᵖ/TM6B*

GENOTYPE	Total # of Offspring	# of Abnormal	% Abnormal
FEMALES			
ywf/ywf; 4.078w⁺/ dsx pᵖ	80		
ywf/ywf; 4.078w⁺/TM6B	50		
ywf/ywf; dsx pᵖ/ TM6B	30		
ywf/ywf; TM6B/TM6B	0		
w/ywf; 4.078w⁺/ dsx pᵖ	108		
w/ywf; 4.078w⁺/TM6B	56		
w/ywf; dsx pᵖ/ TM6B	48		
w/ywf; TM6B/TM6B	0		
MALES			
ywf or w/y⁺Y; 4.078/ dsx pᵖ	162		
ywf or w/y⁺Y; 4.078/TM6B	102		
ywf or w/y⁺Y; dsx pᵖ/ TM6B	81		
ywf or w/y⁺Y; TM6B/TM6B	2		
Totals:	719	0	

Cross P:

> *ywf/ywf; 4.078w⁺/ dsx pᵖ X ywf/y⁺Y; dsx pᵖ/TM6B*   (25°C)

Purpose:

To produce a stock with both *dsx* and the 4.078 P-element for genetic analysis of the regulation of the P-element expression pattern in the VUMs. The females were obtained from the offspring of the cross above (Cross O). A recombinational event is required to obtain *dsx* and the *4.078* P-element on one chromosome. Offspring that carry a copy of the 4.078 P-element, seen by the w+ marker, over the balancer TM6B were collected to be used in the single pair crosses described below (Cross Q).

Results:

In addition to the recombinational events observed two XO males were also found within this cross. There were 7 recombinations resulting in the genotype of *4.078 dsx/dsx* and 2 recombinations resulting in the genotype *+/dsx* without the 4.078 P-element or the TM6B balancer present. These 9 events will be used in addition to the positive cases determined in Cross Q.

Table A.14

Data from *ywf/ywf; 4.078w⁺/ dsx pᵖ X ywf/y⁺Y; dsx pᵖ/TM6B*

GENOTYPE	Total # of Offspring	# of Abnormal	% Abnormal
FEMALES			
ywf/ywf; 4.078w⁺ or 4.078w⁺ dsx pᵖ/TM6B	78	1	1.28%
ywf/ywf; 4.078w⁺/dsx pᵖ	93		
ywf/ywf; dsx pᵖ/TM6B	55	1	1.82%
ywf/ywf; 4.078w⁺dsx pᵖ/dsx pᵖ	3		
ywf/ywf; dsx pᵖ/ dsx pᵖ	53		
MALES			
ywf/y⁺Y; 4.078w⁺ or 4.078w⁺ dsx pᵖ/TM6B	61		
ywf/y⁺Y; 4.078w⁺/dsx pᵖ	90		
ywf/y⁺Y; dsx pᵖ/TM6B	63		
ywf/y⁺Y; 4.078w⁺ dsx pᵖ/dsx pᵖ	4		
ywf/y⁺Y; dsx pᵖ/dsx pᵖ	72		
Totals:	**572**	**2**	**0.35%**

Cross Q:

> ywf/y⁺Y; 4.078w⁺ or 4.078w⁺ dsx/TM6B X ywf/ywf; dsx pᵖ/TM6B
> ywf/ywf; 4.078w⁺ or 4.078w⁺ dsx/TM6B X ywf/y⁺Y; dsx pᵖ/TM6B   25°C

Purpose:

Single pair crosses were utilized to set-up stocks that contain *dsx* and 4.078. This requires recombination between the P-element insertion site and the gene *dsx*. Each recombination is unique and requires to be kept as a separate stock. The parents were collected from the above cross (Cross P).

Results:

For the cross *ywf/y⁺Y; 4.078w⁺ or 4.078w⁺ dsx/TM6B X ywf/ywf; dsx pᵖ/TM6B* there were 47 single pair matings set-up. Of these there were 9 crosses which yielded *ywf/y⁺Y; 4.078 dsx/TM6B* stocks, indicating recombination between *dsx* and *4.078* had occurred, and 8 crosses which yielded no offspring. For the cross *ywf/ywf; 4.078w⁺ or 4.078w⁺ dsx/TM6B X ywf/y⁺Y; dsx pᵖ/TM6B* there were 53 single pair matings set-up. Of these there were 2 crosses which yielded *ywf/y⁺Y; 4.078 dsx/TM6B* stocks, indicating recombination between *dsx* and *4.078* had occurred, and 14 crosses which yielded no offspring. Including the number of recombinations that were scored for in Cross P and the single pair crosses the frequency of recombination between *dsx* and *4.078* can be figured to be approximately 3.5%. This value isn't accurate since it was not possible to correctly asses every possible recombination event because only a subset of the flies which could be positive for a recombination event were set up in single pair crosses and not every single pair mating resulted in offspring.

Cross R:

$w/w;$ $4.078w^+/4.078w^+$ X yw Sxl^{fm3} ct $v/y^+Y;$ $+/+$ 25°C

Purpose:

To test if the 4.078 stock interacted with mutations in the Sxl gene.

Results:

No abnormal flies were found and indicates that the 4.078 P-element doesn't interact with Sxl. This Sxl^{fm3} allele leads to masculinization of females, but allows for female type dosage compensation (Cline, 1984).

Table A.15

Data from $w/w;$ $4.078w^+/4.078w^+$ X yw Sxl^{fm3} ct $v/y^+Y;$ $+/+$

GENOTYPE	Total # of Offspring	# of Abnormal	% Abnormal
FEMALES			
w/yw Sxl^{fm3} ct v; 4.078w^+/+	230		
MALES			
w/y^+Y; 4.078w^+/+	216		
Totals:	**446**	**0**	

Cross S:

$w^-/w^-;$ $+/+;$ $4.078w^+/4.078w^+$ X $B^SY;$ cn $tra2^B$ $bw/CyO;$ tra $etc/TM2$ 25°C

$w^-/w^-;$ $+/+;$ $4.078w^+/4.078w^+$ X $B^SY;$ cn $tra2^B$ $bw/CyO;$ tra $etc/TM2$ 27°C

$w^-/w^-;$ $+/+;$ $4.078w^+/4.078w^+$ X $B^SY;$ cn $tra2^B$ $bw/CyO;$ tra $etc/TM2$ 16°C

Purpose:

Removing one copy each of the $tra-2$ and tra genes leads to a sensitized background to observe a heterozygous interaction leading to the production of intersexual females. Additional sensitization is observed at slightly higher rearing temperatures. Thus, these crosses were carried out at three different temperatures to test for such a temperature effect.

Results:

Females that developed with an intersexual phenotype were present in this cross, providing more evidence for the presence of a tra/tra-2 interactor. More intersexual females were found at the higher temperature: 100 % of w^-/w^+; $tra2^B/+;$ $tra/4.078$ females at 27°C were intersexual while only 4.76% and 0 % were intersexual for the 25°C and 16°C crosses respectively. This shows a clear temperature effect for this interaction. In addition sexual mosaics, XO males, XXY females, abnormal abdominal segmentation and abnormal Bar eyes were also observed in this cross. Note: Several crosses were carried out at 27°C and the numbers below are a combination of all the crosses.

Table A.16a

Data from w^-/w^-; $+/+$; $4.078w^+/4.078w^+$ X B^SY; cn $tra2^B$ bw/CyO; tra $etc/TM2$ 25°C

GENOTYPE	Total # of Offspring	# of Abnormal	% Abnormal
FEMALES			
w^-/w^+; tra2B/+; 4.078/TM2	191	2	1.05%
w^-/w^+; tra2B/+; 4.078/ tra etc	147	7	4.76%
w^-/w^+; CyO/+; 4.078/TM2	205		
w^-/w^+; CyO/+; 4.078/ tra etc	197	4	2.03%
w^-/w^+; ?; 4.078/TM2	13		
w^-/w^+; ?; 4.078/ tra etc	10		
MALES			
w^-/B^SY; tra2B/+; 4.078/TM2	99		
w^-/B^SY; tra2B/+; 4.078/ tra etc	133	3	2.26%
w^-/B^SY; CyO/+; 4.078/TM2	117	3	2.56%
w^-/B^SY; CyO/+; 4.078/ tra etc	125		
w^-/B^SY; ?; 4.078/TM2	6		
w^-/B^SY; ?; 4.078/ tra etc	4		
Totals:	**1247**	**19**	**1.52%**

Table A.16b

Data from w^-/w^-; $+/+$; $4.078w^+/4.078w^+$ X B^SY; cn $tra2^B$ bw/CyO; tra $etc/TM2$ 27°C

GENOTYPE	Total # of Offspring	# of Abnormal	% Abnormal
FEMALES			
w^-/w^+; tra2B/+; 4.078/TM2	143	1	0.70%
w^-/w^+; tra2B/+; 4.078/ tra etc	121	121	100%
w^-/w^+; CyO/+; 4.078/TM2	144		
w^-/w^+; CyO/+; 4.078/ tra etc	145	7	4.83%
w^-/w^+; ?; 4.078/TM2	2		
w^-/w^+;?; 4.078/ tra etc	2		
MALES			
w^-/B^SY; tra2B/+; 4.078/TM2	90	1	1.11%
w^-/B^SY; tra2B/+; 4.078/ tra etc	109	1	0.92%
w^-/B^SY; CyO/+; 4.078/TM2	81		
w^-/B^SY; CyO/+; 4.078/ tra etc	104	1	0.96%
w^-/B^SY; ?; 4.078/TM2	0		
w^-/B^SY;?; 4.078/ tra etc	1		
Totals:	**942**	**132**	**14.01%**

Table.16c

Data from w^-/w^-; +/+; $4.078w^+/4.078w^+$ $X\,B^SY$; cn $tra2^B$ bw/CyO; tra etc/TM2 16°

GENOTYPE	Total # of Offspring	# of Abnormal	% Abnormal
FEMALES			
w^-/w^+; tra2B/+; 4.078/TM2	109		
w^-/w^+; tra2B/+; 4.078/ tra etc	103	2	1.94%
w^-/w^+; CyO/+; 4.078/TM2	102		
w^-/w^+; CyO/+; 4.078/ tra etc	123	1	0.81%
w^-/w^+; ?; 4.078/TM2	1		
w^-/w^+; ?; 4.078/ tra etc	1		
MALES			
w^-/B^SY; tra2B/+; 4.078/TM2	83		
w^-/B^SY; tra2B/+; 4.078/ tra etc	99		
w^-/B^SY; CyO/+; 4.078/TM2	54		
w^-/B^SY; CyO/+; 4.078/ tra etc	116		
w^-/B^SY; ?; 4.078/TM2	1		
w^-/B^SY; ?; 4.078/ tra etc	2		
Totals:	**794**	**3**	**0.38%**

Cross T:

w^-/w^-; $4.078w^+/TM6B$ *(from tra etc. cross,* Cross J*)* $X\,B^SY$; cn tra2B bw/CyO; tra etc/TM2 (25°C)

Purpose:

To see if the mosaicism and nondisjunction events were from a maternal effect in the *4.078/4.078* stock.

Results:

The only sexually abnormal fly was a CyO/+; *4.078/TM2* male with two sex combs on the left leg. The only other defect observed was abnormal bar eyes in 11 males.

Table A.17

Data from w^-/w^-; $4.078w^+/TM6B$ X B^SY; cn $tra2B$ bw/CyO; tra $etc/TM2$

GENOTYPE	Total # of Offspring	# of Abnormal	% Abnormal
FEMALES			
w^-/w^+; $tra2B/+$; $4.078/$ tra etc	30		
w^-/w^+; $tra2B/+$; $4.078/TM2$	27		
w^-/w^+; $tra2B/+$; tra $etc/TM6B$	31		
w^-/w^+; $tra2B/+$; $TM6B/TM2$	22		
w^-/w^+; $CyO/+$; $4.078/$ tra etc	21		
w^-/w^+; $CyO/+$; $4.078/TM2$	32		
w^-/w^+; $CyO/+$; tra $etc/TM6B$	24		
w^-/w^+; $CyO/+$; $TM6B/TM2$	11		
MALES			
w^-/B^SY; $tra2B/+$; $4.078/$ tra etc	26		
w^-/B^SY; $tra2B/+$; $4.078/TM2$	17		
w^-/B^SY; $tra2B/+$; tra $etc/TM6B$	10		
w^-/B^SY; $tra2B/+$; $TM6B/TM2$	10		
w^-/B^SY; $CyO/+$; $4.078/$ tra etc	25		
w^-/B^SY; $CyO/+$; $4.078/TM2$	10	1	10.00%
w^-/B^SY; $CyO/+$; tra $etc/TM6B$	17		
w^-/B^SY; $CyO/+$; $TM6B/TM2$	7		
Totals:	**320**	**1**	**0.31%**

Cross U:

ywf/ywf; $+/+$; $4.078/4.078$ X $y/y+Y$; $tra2/CyO$; tra/Ubx

w/ywf; $+/+$; $4.078/4.078$ X $y/y+Y$; $tra2/CyO$; tra/Ubx (27°C)

Purpose:

To test the 4 independent lines established from the backcross procedure (Cross Z) for the intersexual phenotype observed in the original stock. This cross was used because is gives the most extreme phenotype of the genetic interaction.

Results:

One stock from each of the 4 independent lines established was tested for the interaction with y/y^+Y; $tra2/CyO$; $tra/TM2$ when raised at 27°C. All 4 stocks tested have given intersexes for the genotype w/y; $tra2/+$; $tra/4.078$. The percentage of intersexual females varied from as low as 64% to as high as 100% for w/y; $tra2/+$; $tra/4.078$. The y/y^+Y; $tra2/CyO$; $tra/TM2$ stock was also raised at 27°C for a control. In the control we found 4 out of 48 flies with the genotype y/y; $tra2/CyO$; $tra/TM2$ had a phenotype similar to that seen in the presence of 4.078

Other details:

We have also looked at the musculature for the w/y; $tra2/+$; $tra/4.078$ intersexes. From abdominal muscle preps an intermediate MOL (Muscle of Lawrence), which is a fru-dependent phenotype, and ventral muscles, which show a dsx-dependent insertion point are observed. This provides evidence that both dsx and

fru sex-specific splicing is being effected in the genetic interaction observed between *tra-2; tra* and *4.078*.

Table A.18a

Data from *ywf/ywf; +/+; 4.078/4.078 X y/y+Y; tra2/CyO; tra/Ubx* Line: 1BI-2B Male 1

GENOTYPE	Total # of Offspring	# of Abnormal	% Abnormal
Females			
ywf/y; tra2/+; tra/4.078 w⁺	53	34	64%
ywf/y; tra2/+; Ubx/4.078 w⁺	65	2	3%
ywf/y; CyO/+; tra/4.078 w⁺	63	0	0%
ywf/y; CyO/+; Ubx/4.078 w⁺	47	0	0%
Males			
ywf/y⁺Y; tra2/+; tra/4.078 w⁺	70	0	0%
ywf/y⁺Y; tra2/+; Ubx/4.078 w⁺	59	0	0%
ywf/y⁺Y; CyO/+; tra/4.078 w⁺	39	0	0%
ywf/y⁺Y; CyO/+; Ubx/4.078 w⁺	30	1	3%
Totals:	**426**	**37**	**9%**

Table A.18b

Data from *w/ywf; +/+; 4.078/4.078 X y/y+Y; tra2/CyO; tra/Ubx* Line: 1BI-2B Male 1

GENOTYPE	Total # of Offspring	# of Abnormal	% Abnormal
Females			
ywf/y; tra2/+; tra/4.078 w⁺	15	15	100%
ywf/y; tra2/+; Ubx/4.078 w⁺	15	1	7%
ywf/y; CyO/+; tra/4.078 w⁺	12	0	0%
ywf/y; CyO/+; Ubx/4.078 w⁺	11	0	0%
w/y; tra2/+; tra/4.078 w⁺	15	14	93%
w/y; tra2/+; Ubx/4.078 w⁺	10	0	0%
w/y; CyO/+; tra/4.078 w⁺	16	0	0%
w/y; CyO/+; Ubx/4.078 w⁺	7	0	0%
Males			
w or ywf/y⁺Y; tra2/+; tra/4.078 w⁺	15	1	7%
w or ywf/y⁺Y; tra2/+; Ubx/4.078 w⁺	27	0	0%
w or ywf/y⁺Y; CyO/+; tra/4.078 w⁺	27	1	4%
w or ywf/y⁺Y; CyO/+; Ubx/4.078 w⁺	27	0	0%
Totals:	**197**	**32**	**16%**

Table A.18c

Data from *ywf/ywf; +/+; 4.078/4.078 X y/y+Y; tra2/CyO; tra/Ubx* Line: 2AII-2B Male 2

GENOTYPE	Total # of Offspring	# of Abnormal	% Abnormal
Females			
ywf/y; tra2/+; tra/4.078 w⁺	108	90	83%
ywf/y; tra2/+; Ubx/4.078 w⁺	108	0	0%
ywf/y; CyO/+; tra/4.078 w⁺	124	0	0%
ywf/y; CyO/+; Ubx/4.078 w⁺	105	3	3%
Males			
ywf/y⁺Y; tra2/+; tra/4.078 w⁺	117	0	0%
ywf/y⁺Y; tra2/+; Ubx/4.078 w⁺	121	0	0%
ywf/y⁺Y; CyO/+; tra/4.078 w⁺	90	1	1%
ywf/y⁺Y; CyO/+; Ubx/4.078 w⁺	76	1	1%
Totals:	**849**	**95**	**11%**

Table A.18d

Data from *w/ywf; +/+; 4.078/4.078 X y/y+Y; tra2/CyO; tra/Ubx* Line: 2AII-2B Male 2

GENOTYPE	Total # of Offspring	# of Abnormal	% Abnormal
Females			
ywf/y; tra2/+; tra/4.078 w⁺	64	50	78%
ywf/y; tra2/+; Ubx/4.078 w⁺	70	0	0%
ywf/y; CyO/+; tra/4.078 w⁺	74	0	0%
ywf/y; CyO/+; Ubx/4.078 w⁺	83	0	0%
w/y; tra2/+; tra/4.078 w⁺	85	76	89%
w/y; tra2/+; Ubx/4.078 w⁺	92	0	0%
w/y; CyO/+; tra/4.078 w⁺	92	0	0%
w/y; CyO/+; Ubx/4.078 w⁺	64	0	0%
Males			
w or ywf/y⁺Y; tra2/+; tra/4.078 w⁺	166	1	1%
w or ywf/y⁺Y; tra2/+; Ubx/4.078 w⁺	149	0	0%
w or ywf/y⁺Y; CyO/+; tra/4.078 w⁺	135	0	0%
w or ywf/y⁺Y; CyO/+; Ubx/4.078 w⁺	116	0	0%
Totals:	**1190**	**127**	**11%**

Table A.18e

Data from *ywf/ywf; +/+; 4.078/4.078 X y/y+Y; tra2/CyO; tra/Ubx* Line: 3CI-1C Male 2

GENOTYPE	Total # of Offspring	# of Abnormal	% Abnormal
Females			
ywf/y; tra2/+; tra/4.078 w⁺	7	7	100%
ywf/y; tra2/+; Ubx/4.078 w⁺	8	1	13%
ywf/y; CyO/+; tra/4.078 w⁺	8	0	0%
ywf/y; CyO/+; Ubx/4.078 w⁺	11	1	9%
Males			
ywf/y⁺Y; tra2/+; tra/4.078 w⁺	7	3	43%
ywf/y⁺Y; tra2/+; Ubx/4.078 w⁺	15	0	0%
ywf/y⁺Y; CyO/+; tra/4.078 w⁺	7	2	29%
ywf/y⁺Y; CyO/+; Ubx/4.078 w⁺	9	1	11%
Totals:	**72**	**15**	**21%**

Table A.18f

Data from *w/ywf; +/+; 4.078/4.078 X y/y+Y; tra2/CyO; tra/Ubx* Line: 3CI-1C Male 2

GENOTYPE	Total # of Offspring	# of Abnormal	% Abnormal
Females			
ywf/y; tra2/+; tra/4.078 w⁺	15	14	93%
ywf/y; tra2/+; Ubx/4.078 w⁺	25	0	0%
ywf/y; CyO/+; tra/4.078 w⁺	28	0	0%
ywf/y; CyO/+; Ubx/4.078 w⁺	22	0	0%
w/y; tra2/+; tra/4.078 w⁺	22	21	95%
w/y; tra2/+; Ubx/4.078 w⁺	17	0	0%
w/y; CyO/+; tra/4.078 w⁺	25	0	0%
w/y; CyO/+; Ubx/4.078 w⁺	17	3	18%
Males			
w or ywf/y⁺Y; tra2/+; tra/4.078 w⁺	48	1	2%
w or ywf/y⁺Y; tra2/+; Ubx/4.078 w⁺	49	1	2%
w or ywf/y⁺Y; CyO/+; tra/4.078 w⁺	52	2	4%
w or ywf/y⁺Y; CyO/+; Ubx/4.078 w⁺	51	0	0%
Totals:	**371**	**42**	**11%**

Table A.18g
Data from *ywf/ywf; +/+; 4.078/4.078 X y/y+Y; tra2/CyO; tra/Ubx* Line: 4BI-3A Male 1

GENOTYPE	Total # of Offspring	# of Abnormal	% Abnormal
Females			
ywf/y; tra2/+; tra/4.078 w$^+$	39	32	82%
ywf/y; tra2/+; Ubx/4.078 w$^+$	43	0	0%
ywf/y; CyO/+; tra/4.078 w$^+$	46	1	2%
ywf/y; CyO/+; Ubx/4.078 w$^+$	22	0	0%
Males			
ywf/y$^+$Y; tra2/+; tra/4.078 w$^+$	40	0	0%
ywf/y$^+$Y; tra2/+; Ubx/4.078 w$^+$	40	0	0%
ywf/y$^+$Y; CyO/+; tra/4.078 w$^+$	33	1	3%
ywf/y$^+$Y; CyO/+; Ubx/4.078 w$^+$	42	0	0%
Totals:	**305**	**34**	**11%**

Table A.18h
Data from *w/ywf; +/+; 4.078/4.078 X y/y+Y; tra2/CyO; tra/Ubx* Line: 4BI-3A Male 1

GENOTYPE	Total # of Offspring	# of Abnormal	% Abnormal
Females			
ywf/y; tra2/+; tra/4.078 w$^+$	44	30	68%
ywf/y; tra2/+; Ubx/4.078 w$^+$	47	0	0%
ywf/y; CyO/+; tra/4.078 w$^+$	40	0	0%
ywf/y; CyO/+; Ubx/4.078 w$^+$	36	0	0%
w/y; tra2/+; tra/4.078 w$^+$	32	25	78%
w/y; tra2/+; Ubx/4.078 w$^+$	53	0	0%
w/y; CyO/+; tra/4.078 w$^+$	39	0	0%
w/y; CyO/+; Ubx/4.078 w$^+$	41	0	0%
Males			
w or ywf/y$^+$Y; tra2/+; tra/4.078 w$^+$	96	1	1%
w or ywf/y$^+$Y; tra2/+; Ubx/4.078 w$^+$	95	2	2%
w or ywf/y$^+$Y; CyO/+; tra/4.078 w$^+$	48	2	4%
w or ywf/y$^+$Y; CyO/+; Ubx/4.078 w$^+$	42	1	2%
Totals:	**613**	**61**	**10%**

Cross V:

      w^-/w^-; $4.078w^+/4.078w^+$ X y/y^+Y; $tra\ dsx\ p^p/TM6B$ (25°C)

Purpose:

      To see if an interaction is present in a $tra\ dsx\ p^p$ background.

Results:

      No abnormal flies were observed.

Table A.19 Data from w^-/w^-; $4.078w^+/4.078w^+$ X y/y^+Y; $tra\ dsx\ p^p/TM6B$

GENOTYPE	Total # of Offspring	# of Abnormal	% Abnormal
FEMALES			
w-/y; 4.078/ tra dsx pp	176		
w-/y; 4.078/TM6B	95		
MALES			
w-/y+Y; 4.078/ tra dsx pp	125		
w-/y+Y; 4.078/TM6B	98		
Totals:	**494**	**0**	

Cross W:

      ywf/ywf; $4.078w^+/4.078w^+$ X w/B^SY; $+/+$ (from the $hstra83$ stock)

      ywf/ywf; $4.078w^+/TM6B$ X w/B^SY; $+/+$ (from the $hstra83$ stock)

      w/w; $+/+$ (from the $hstra83$ stock) X ywf/B^SY ; $4.078w^+/4.078w^+$

      w/w; $+/+$ (from the $hstra83$ stock) X ywf/B^SY; $4.078w^+/TM6B$

Purpose:

      To test for X-loss mosaics and meiotic nondisjunction events without being in a mutant background and to determine if there is a maternal or paternal effect in the chromosomal loss. All sex chromosomes are marked for clear determination of which parent contributed the chromosomes in the case of meiotic nondisjunction and which parental chromosome is loss in the case of mosaic animals.

Results:

      From this series of crosses seven XO males and 1 male//female mosaic animal was observed. A fly with the genotype w/B^SY; $4.078w^+/+$ was found being phenotypic male for sex combs and abdominal pigmentation but had almost normal female genitalia except A8 was only on one side. This fly can't be put in any category with certainty.

Table A.20a
Data from *ywf/ywf;4.078/4.078* X *w/B^S^Y; +/+* (from *hstra83* stock)

	Total # of Offspring	% of Total
Males	387	38.3%
Females	621	61.5%
Mosaics	0	0.0%
XXY	0	0.0%
XO	2	0.2%
Totals:	**1010**	

Table A.20b
Data from *ywf/ywf; 4.078/TM6B* X *w/B^S^Y; +/+* (from *hstra83* stock)

	Total # of Offspring	% of Total
Males; 4.078	216	29.8%
Males; TM6B	124	17.1%
Females; 4.078	219	30.2%
Females; TM6B	163	22.5%
Mosaics	1	0.1%
XXY	0	0.0%
XO	3	0.4%
Totals:	**726**	

Table A.20c
Data from *w/w; +/+* (from *hstra83* stock) X *ywf /B^S^Y; 4.078/4.078*

	Total # of Offspring	% of Total
Males	211	49.0%
Females	218	50.6%
Mosaics	1	0.2%
XXY	0	0.0%
XO	1	0.2%
Totals:	**431**	

Table A.20d
Data from *w/w; +/+* (from *hstra83* stock) X *ywf /B^S^Y; 4.078/TM6B*

	Total # of Offspring	% of Total
Males; 4.078	75	22.4%
Males; TM6B	83	24.8%
Females; 4.078	76	22.7%
Females; TM6B	100	29.9%
Mosaics	0	0.0%
XXY	0	0.0%
XO	1	0.3%
Totals:	**335**	

Cross X:

w or *ywf/w* or *ywf;* 4.078 *w⁺*/4.078 *w⁺* X *wᵃ; B52ᴱᴰ*/*TM3*
w or *ywf/w* or *ywf;* 4.078 *w⁺*/4.078 *w⁺* X *wᵃ; B52ᴿ²*/*TM3*
w or *ywf/w* or *ywf;* 4.078 *w⁺*/4.078 *w⁺* X *Df(3R)urd, ru h th st cu ca*/*TM3*
w or *ywf/w* or *ywf;* 4.078 *w⁺*/4.078 *w⁺* X *w* or *ywf/w* or *ywf;* 4.078 *w⁺*/4.078 *w⁺*
Canton-S ♀ X *Canton-S* ♂

Note: All 4.078 flies for this test are from Line 2AII-2B ♂2 from the backcross procedure (Cross U and Z).

Purpose:

To test for allelism between the 4.078 P-element and the gene *B52*, which is located at 87F, near the region of the 4.078 insertion site. A similar phenotype is found in the genotype *tra2/+; tra/B52* at 27°C as we observed in *tra2/+; tra/4.078* at 27°C.

Results:

No lethality was detected beyond the controls in the crosses and all genotypes came out in Mendelian ratios. Lethality was used as a measure because *B52* is an essential splicing factor and both alleles, *B52ᴱᴰ* and *B52ᴿ²*, are lethal as homosygotes and when placed over *Df(3R)urd* (Peng and Mount, 1995). If the *4.078* P-element had inserted in a region to disrupt the gene *B52* lethality and/or defects in splicing of genes known to require *B52* function, resulting in *dsx* or *Ubx* phenotypes, would be expected (Peng and Mount). From these crosses we conclude that 4.078 isn't an allele of *B52*.

Table A.21a

Data from *B52* allelism test to *4.078-* Genotypes of Adults to number of eggs laid

Allele being tested	# of eggs laid	# Adults ♂ *4.078*/ *allele tested*	# Adults ♀ *4.078*/ *allele tested*	# Adults ♂ *4.078*/*TM3*	# Adults ♀ *4.078*/*TM3*	Total Adult Offspring
B52ᴱᴰ	1154	209	219	239	243	910
B52ᴿ²	390	81	79	92	66	318
Df(3R) urd	1156	220	239	265	262	986
4.078 control	796	324	383	NA	NA	707
Canton-S control	1000	414	398	NA	NA	812

Table A.21b
Data from *B52* allelism test to *4.078-* Breakdown by life stages

Allele being tested	# of eggs laid	# of eggs hatched to 1st instar larva	# of 3rd instar larva transferred to vials	# of pupa	# of Adults
B52^{ED}	1154	1024	965	923	910
B52^{R2}	390	346	332	328	318
Df(3R) urd	1156	1109	1038	1012	986
4.078 control	796	760	754	737	707
Canton-S control	1000	910	883	859	812

Cross Y:

    w/w; hstra83/+; +/+ X *ywf/B^{S}Y; 4.078/4.078*

    w/w; hstra83/+; +/+ X *w^{+}/Y; Df(3R)urd/TM3*

Purpose:

    To test the possible role of the 4.078 genetic interaction on mRNA and/or the splicing complex stability on the *dsx* primary transcript. Observations by W. Mattox suggests that another interactor, *Darkener of apricot* (*Doa*) plays a role in the stability of the splicing complex on the *dsx* primary transcript, since when *Doa* is crossed into a *hstra83* background XY flies are rescued from the *hstra83* female phenotype. The *hstra83* P-element construct is the female-specific *tra* expressed from the heat-shock promoter *hsp83*. This promoter has enough constitutive expression to cause XY flies to develop as phenotypic females because of the TRA protein being present to splice the downstream genes in the sex determining hierarchy in the female-specific pattern. (McKeown *et al.*, 1988)

Results:

    A role in mRNA stability and/or the splicing complex stability has been genetically ruled out. No rescue of the feminization in males carrying the *hstra83* P-element was observed.

Table A.22a

Data from *w/w; hstra83/+; +/+* X *ywf/B^S Y; +/+; 4.078/4.078*

Genotype	Total # of Offspring	# of Abnormal	% Abnormal
Females			
ywf/w; hstra83/+;4.078/+	35		
ywf/w;+/+;4.078/+	45		
Males			
w/B^S Y; hstra83/+;4.078/+	26		
w/B^S Y; +/+; 4.078/+	29		
Totals:	**135**	**0**	

Table A.22b

Data from *w/w; hstra83/+; +/+* X *w^+/Y; +/+; Df(3R)urd/TM3*

Genotype	Total # of Offspring	# of Abnormal	% Abnormal
Females			
w^+/w;hstra83 or +/+;Df(3R)urd/+	42		
w^+/w;hstra83 or +/+;TM3/+	36		
Males			
w/Y;hstra83/+;Df(3R)urd/+	26		
w/Y; hstra83/+; TM3/+	33		
w/Y; +/+;Df(3R)urd/+	23		
w/Y; +/+; TM3/+	21		
Totals:	**181**	**0**	

Cross Z:
Procedure followed in backcross to w^{1118}:

$w/w;4.078w^+/4.078w^+$ X w^{1118} ♂ ⇨ Collected ♀ ♀ $w/w^{1118};4.078w^+/+$
(50% Exchange)

♀ ♀ $w/w^{1118};4.078w^+/+$ X w^{1118} ♂ ⇨

Collected 4 individual ♀s carrying the P-element to set-up 4 independent lines

4 single ♀ carrying P-element X w^{1118} ♂ ⇨

Collected 3 individual ♀s carrying the P-element from each of the 4 independent lines

12 single ♀ carrying P-element X w^{1118} ♂ ⇨

Collected 3 individual ♀s carrying the P-element from each of the 4 independent lines

The last step was repeated until F7 offspring were obtained. (~98% Exchange)At F7 4 individual ♂s from each of the four independent lines were crossed to $ywf/ywf;$ $dsx/TM6B$. We then collected w/ywf and $w/y^+Y;$ $4.078w^+/TM6B$ offspring to establish stocks.

Purpose:
To exchange the genetic background from the *4.078* P-element stock with the w^{1118} stock. This will determine if the phenotypes that have been observed are genetically linked to the P-element or are from another, unknown genetic lesion found in the *4.078* stock.

Results:
After 6 generations of backcrosses to the w^{1118} stock 4 independent lines were established with 4 individual males that were collected at the end of the procedure.