Differentiation of a Male-Specific Muscle in *Drosophila melanogaster* Does Not Require the Sex-Determining Genes *doublesex* or *intersex*

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ABSTRACT

A pair of muscles span the fifth abdominal segment of male but not female *Drosophila melanogaster* adults. To establish whether genes involved in the development of other sexually dimorphic tissues controlled the differentiation of sex-specific muscles, flies mutant for five known sex-determining genes were examined for the occurrence of male-specific abdominal muscles. Female flies mutant for alleles of *Sex-lethal*, defective in sex determination, or null alleles of *transformer* or *transformer-2* are converted into phenotypic males that formed male-specific abdominal muscles. Both male and female flies, when mutant for null alleles of *doublesex*, develop as nearly identical intersexes in other somatic characteristics. Male *doublesex* flies produced the male-specific muscles, whereas female *doublesex* flies lacked them. Female flies, even when they inappropriately expressed the male-specific form of *doublesex* mRNA, failed to produce the male-specific muscles. Therefore, the wild-type products of the genes *Sex-lethal*, *transformer* and *transformer-2* act to prevent the differentiation of male-specific muscles in female flies. However, there is no role for the genes *doublesex* or *intersex* in either the generation of the male-specific muscles in males or their suppression in females.

TO accommodate the change in form, locomotion and behavior of the adult holometabolous insect, the musculature of the vermiform larva is histolyzed and replaced (for review, CROSSLEY 1978). During metamorphosis, the adult abdominal musculature is constructed *de novo* from several clusters of mesodermal precursors, which found particular muscles in each hemisegment (BATE, RUSHTON and CURRIE 1991; BROADIE and BATE 1991). These precursors, in association with nerves innervating the hemisegment, attach to sites on the newly formed pupal cuticle to create individual abdominal muscles (CURRIE and BATE 1991). Fusion of nearby myoblasts with the developing muscle contribute to the final syncytial cell (CURRIE and BATE 1991). A similar pattern of abdominal muscles is present in both sexes except for a pair of large dorsal muscles in the fifth abdominal segment of male flies that are absent in female flies (also called the muscle of Lawrence) (LAWRENCE and JOHNSTON 1984; COURCHESNE-SMITH and TOBIN 1989; GAILEY, TAYLOR and HALL 1991).

While the developmental processes involved in adult myogenesis are becoming clearer, the genetic and molecular mechanisms that specify the identity of muscles is still poorly understood. Experimental studies on muscle development in other insects have suggested that the presence of attachment sites in the cuticle and innervation by motorneurons are important components in the normal development of muscles (for review, NÜESCH 1985). In Drosophila, examination of larvae homeotically transformed by *Ultrabithorax* mutations have shown that the influence of the cuticle on muscle pattern is not a simple induction, since the musculature transformations are not in register with the cuticular transformations (HOOPER 1986). Homeotic mutations can affect both the muscle pattern and the overlying ectoderm in concert, such as the formation of the male-specific muscle in other abdominal segments transformed to the fifth abdominal segment by mutations in the *Abdominal B* portion of the bithorax complex (LAWRENCE and JOHNSTON 1984). The source of muscle patterning information, in at least one case, clearly derives from the central nervous system. From elegant experiments using sexually mosaic flies, the formation of the male-specific muscle was found to be dependent on the innervation of muscles by male-specific motorneurons and not a cell autonomous decision by the muscle-forming cells themselves or dependent on the sexual or segmental identity of the cuticle (LAWRENCE and JOHNSTON 1984, 1986). These sexually dimorphic muscles are a tractable system in which to study the mechanisms responsible for determining muscle identity in Drosophila.

In Drosophila, the initial chromosomal assignment of sexual identity, the *X* chromosome to autosome ratio, is translated into the proper sex-specific differentiative signal within cells of sexually dimorphic somatic tissues by the activity of a small number of genes. Five genes have been identified as belonging

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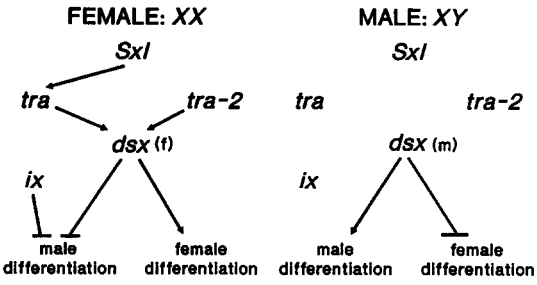

FIGURE 1.—Model of the transfer of information from the chromosomal assessment of sexual identity through the genes of the somatic arm of the sex-determining hierarchy (see also BAKER and BELOTE 1983; CLINE 1985; NÖTHIGER and STEINMANN-ZWICKY 1985; WOLFNER 1988; BAKER 1989; SLEE and BOWNES 1990; STEINMANN-ZWICKY, AMREIN and NÖTHIGER 1990). The first gene shown in this hierarchy is *Sxl*, the maternal and zygotic genes that transmit the chromosomal signal to *Sxl* are not shown. In chromosomally female flies, the action of *Sxl* on the primary *transformer* transcript results in the production of a female-specific *tra* protein which in combination with the *tra-2* protein directs the female-specific splicing of the *dsx* primary transcript and the generation of a female-specific *dsx* protein. In chromosomally male flies, the only functional product is derived from the default splicing of the *dsx* primary transcript and results in the formation of a male-specific *dsx* protein. The *dsx* proteins are transcriptional regulators of downstream genes (BURTIS *et al.* 1991) and have the effect of blocking the expression of sexual characteristics of the opposite sex (BAKER and RIDGE 1980; BAKER and BELOTE 1983) or activating the proper sexual pathway (TAYLOR and TRUMAN 1992).

to the sex-determining cascade that regulates sexual development: *Sex-lethal* (*Sxl*), *transformer* (*tra*), *transformer-2* (*tra-2*), *doublesex* (*dsx*) and *intersex* (*ix*). The organization of these genes into a genetic hierarchy, as shown in Figure 1, has been established by genetic and molecular experiments (for reviews, BAKER and BELOTE 1983; CLINE 1985; NÖTHIGER and STEINMANN-ZWICKY 1985; TOMPKINS 1986; WOLFNER 1988; BAKER 1989; SLEE and BOWNES 1990; STEINMANN-ZWICKY, AMREIN and NÖTHIGER 1990).

The current state of our understanding of the genetic and molecular aspects of the sex-determining hierarchy is summarized below. In female zygotes, the *X* chromosome to autosome ratio is interpreted by the products of several maternal and zygotic genes (CLINE 1978, 1986, 1988; OLIVER, PERRIMON and MAHOWALD 1988) leading to the transcriptional activation of *Sxl* with subsequent maintenance by autoregulation (CLINE 1984). Expression of a functional *Sxl* protein causes the production of a female-specific *tra* product by the promotion of a sex-specific splice of *tra* pre-mRNA (NAGOSHI *et al.* 1988; INOUE *et al.* 1990). Female differentiation depends on the combined activity of the female-specific *tra* product and the *tra-2* protein. Chromosomally female flies (*X/X*) expressing *Sxl* alleles, mutant for sex determination functions, or null alleles of *tra* or *tra-2* develop somatically as phenotypic males. In these mutants, all somatic tissues that have been examined are affected including fat body cells (BOWNES and NÖTHIGER 1981; OTA *et al.* 1981; BELOTE *et al.* 1985; BOWNES, SCOTT and BLAIR 1987; BOWNES, STEINMANN-ZWICKY and NÖTHIGER 1990), external cuticle and genital disc (STURTEVANT 1945; WATANABE 1975; BAKER and RIDGE 1980; WIESCHAUS and NÖTHIGER 1982; BELOTE and BAKER 1982; EPPER and BRYANT 1983; CLINE 1984; DIBENEDETTO *et al.* 1987; MONSMA and WOLFNER 1988; CHAPMAN and WOLFNER 1988; TAYLOR 1989; FENG, SCHIFF and CAVENER 1991), nervous system and male-specific courtship behaviors (MCROBERT and TOMPKINS 1985; TOMPKINS 1986; TOMPKINS and MCROBERT 1989; TAYLOR and TRUMAN 1992). Thus, the wild-type differentiative function of the *Sxl*, *tra* and *tra-2* genes in female somatic tissue is to suppress masculine development. In contrast, the somatic development of chromosomally male flies (*X/Y*) is unaffected by null mutations in these three genes.

The dsx^+ gene plays a pivotal role in the cascade since it is the only gene that provides functional proteins in both sexes, which act as transcription regulators of target genes involved in terminal differentiation processes (BAKER and RIDGE 1980; BAKER and WOLFNER 1988; BURTIS and BAKER 1989; BURTIS *et al.* 1991). In females, under the direction of the female-specific *tra* product and the *tra-2* protein, the primary *dsx* transcript is spliced into a female-specific *dsx* mRNA (BAKER and WOLFNER 1988; NAGOSHI *et al.* 1988; BURTIS and BAKER 1989; RYNER and BAKER 1991; HEDLEY and MANIATIS 1991; HOSHIJIMA *et al.* 1991). The resulting female *dsx* product has been proposed to cause female sexual differentiation in most sexually dimorphic tissues by repressing male differentiation (BAKER and RIDGE 1980; BAKER and BELOTE 1983) and in a portion of the nervous system by activation of the female pathway (TAYLOR and TRUMAN 1992). In males or in females missing *tra* or *tra-2* function, the *dsx* pre-mRNA is spliced into the male-specific form of *dsx* mRNA (NAGOSHI *et al.* 1988). The male-specific *dsx* product, then, mediates somatic male differentiation in most tissues by the suppression of female differentiation (BAKER and RIDGE 1980; BAKER and BELOTE 1983) or in a part of the nervous system by an activation of the male pathway (TAYLOR and TRUMAN 1992). In the absence of *dsx* function, both chromosomally male and female flies develop into phenotypic intersexes (HILDRETH 1965; BAKER and RIDGE 1980; POSTLETHWAIT, BOWNES and JOWETT 1980; OTA *et al.* 1981; BOWNES and NÖTHIGER 1981; NÖTHIGER, ROOST and SCHÜPBACH 1980; CHAPMAN and WOLFNER 1988; FENG, SCHIFF and CAVENER 1991). In addition, suppression of male sexual development in *X/X* flies requires ix^+ gene function (KROEGER 1959; BAKER and RIDGE 1980; CHAPMAN and WOLFNER 1988).

This paper examines the role of the known sex-determining genes in directing the development of

TABLE 1

Number and genotype of abdomens examined for the presence of the male specific muscle

Genotype	X/X	X/Y
Control		
CS	45 (0%)	34 (100%)
Single loss-of-function mutations		
Sex-lethal		
Sxl^{fm7M1}/Sxl^{M1fm3}	21 (y^+ w^+ cm^+ v; 100)	
Sxl^{fm7M1}		12 (y^+ cm v; 100)
Sxl^{M1fm3}		3 (y w; 100)
transformer		
tra^1/tra^1	19 (y; 100)	5 (y^+ Y; 100)
$tra^1/DF(3L)st^{j7}$	20 (100)	13 (B^s Y; 100)
$tra^4/Df(3L)st^{j7}$	19 (100)	18 (B^s Y; 100)
$tra^5/Df(3L)st^{j7}$	15 (100)	8 (B^s Y; 100)
transformer-2		
$tra\text{-}2^1/tra\text{-}2^1$	7 (y; 100)	NT
$tra\text{-}2^B/tra\text{-}2^B$	23 (100)	5 (B^s Y; 100)
$tra\text{-}2^B/Df(2R)trix$	15 (y^+; 100)	18 (y; 100)
$tra\text{-}2^{ts1}/tra\text{-}2^{ts1}$ (25°)	7 (y; 100)	5 (y^+ Y; 100)
$tra\text{-}2^{ts1}/tra\text{-}2^{ts2}$ (16, 18, 25, 29°)	74 (y; 100)	11 (y^+ Y; 100)
$tra\text{-}2^{ts2}/tra\text{-}2^{ts2}$ (16, 25°)	29 (y; 100)	NT
doublesex		
dsx^1/dsx^1	17 (y; 0)	7 (y^+ Y; 100)
dsx^1/dsx^{19}	13 (y; 0)	12 (y^+ Y; 100)
dsx^1/dsx^{15}	4 (y^+; 0)	2 (y; 100)
dsx^1/dsx^{16}	8 (y^+; 0)	13 (y; 100)
dsx^{15}/dsx^{19}	24 (y^+; 0)	14 (y; 100)
dsx^{16}/dsx^{19}	25 (y^+; 0)	6 (y; 100)
$Df(3R)dsx^{15}/dsx^{23}$	40 (0)	14 (B^s Y; 100)
intersex		
ix^1/ix^1*	22 (0)	8 (100)
ix^2/ix^2*	21 (0)	27 (100)
$ix^{D10036a}/ix^{D10036a}$*	18 (0)	17 (100)
$ix/Df(2R)en^B$	36 (w^+; 0)	15 (w; 100)
$ix^2/Df(2R)en^B$	7 (w^+; 0)	5 (w; 100)
$ix^{D10036a}/Df(2R)en^B$	9 (w^+; 0)	8 (w; 100)
Double loss-of-function mutations		
tra^1 dsx^1/tra^1 dsx^1	10 (y; 100)	10 (y^+ Y; 100)
$ix^1/ix^1;Df(3R)dsx^{15}/dsx^{23}$	21 (0)	15 (B^s Y; 100)
Single gain-of-function mutations		
Sex-lethal		
$Sxl^{M1fPa\text{-}ra}$ (25°)	5 (0)	21 (100)
transformer		
$hstra^+$ $Df(3L)st^7/tra^1$	12 (100)	10 (B^s Y; 100)
transformer-2		
$tra\text{-}2^B/tra\text{-}2^B$; $tra\text{-}2^+$ $TxL3/tra\text{-}2^+$ $TxL3$	25 (0)	10 (B^s Y; 100)
$tra\text{-}2^+$ $TxF4/tra\text{-}2^+$ $TxF4$; $tra\text{-}2^B/tra\text{-}2^B$	22 (0)	11 (B^s Y; 100)
doublesex		
dsx^D/dsx^+	53 (0)	10 (B^s Y; 100)
dsx^M/dsx^+	25 (0)	10 (B^s Y; 100)
$dsx^D/Df(3R)dsx^{15}$	56 (0)	38 (B^s Y; 100)
dsx^D/dsx^1	24 (0)	9 (B^s Y; 100)
Double gain-of-function mutations		
ix^1/ix^1; dsx^D/dsx^+	14 (0)	7 (B^s Y; 100)
$ix^1/Df(2R)en^B$; dsx^D/dsx^+	11 (0)	4 (B^s Y; 100)

The percentage of preparations with segment spanning muscles is given in parentheses after the designation of the visible markers used to score the chromosomal sex of the adults. The complete genotype of the flies listed below is provided in MATERIALS AND METHODS. Abdominal muscles were stained with the anti-myosin antibody and viewed under fluorescent optics or visualized under polarized light. Female and/or male flies, when mutant for some of the sex-determining genes, express sexual phenotypes that differ from their chromosomal sexual identity. The chromosomal constitution of individual flies was established with the following *Y* chromosome markers *Bar-stone* (B^s), which causes small eyes, or y^+, in a *yellow* (*y*) background, or the *X* chromosome markers in crosses between *y* homozygous females and y^+ males or between *white* (*w*) females and w^+ males.

* Flies were sexed by phenotype rather than with genotypic markers.

sexually dimorphic skeletal muscles. The presence or absence of the male-specific muscle was assayed in abdominal cuticle preparations from flies whose phenotypic sex had been altered by mutations in these genes. Due to the nonautonomous nature of the male-specific muscle, the presence or absence of the muscle is a reflection of sex determination decisions made in another tissue, the central nervous system. The proper sexual expression of sex-specific muscles is regulated by the activity of *Sxl*, *tra* and *tra-2* genes but not by the *dsx* or *ix* genes. This genetic analysis suggests that additional genes in the sex-determining hierarchy, as yet unidentified, occupy a position analogous to that of *dsx* in the suppression of the appearance of the male-specific muscle in female flies.

MATERIALS AND METHODS

Muscle visualization: Dorsal abdomens were dissected from 1–4-day-old adults. The abdominal half-shells, composed of the dorsal cuticle and underlying muscles, were fixed in 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4) for 0.5–1 hr. Tissues were incubated in anti-myosin antisera (a gift from D. KIEHART) (KIEHART and FEGHALI 1986) at a dilution of 1:200 in 0.1 M phosphate buffer, containing 0.5% Triton-X and 1% heat-inactivated normal goat serum. An anti-rabbit secondary antibody conjugated to rhodamine (Cappel) was used for visualization of the muscles. Incubation times ranged from overnight to 2 days, depending on the quantity of subcutaneous fat body. Muscle lengths were measured using camera lucida tracings made under either polarized light or fluorescent optics.

Fly stocks: Animals were raised on a standard diet of molasses, cornmeal and agar supplemented with live yeast. All flies were maintained at room temperature except for the temperature-sensitive mutants of *tra-2*. Flies mutant for one or more of the sex-determining genes were generated from standard crosses. The mutations of the sex-determining genes used in this study are described below. Descriptions of all other mutations are found in LINDSLEY and ZIMM (1992). Wherever applicable, new allele designations conform to those used in LINSDLEY and ZIMM (1992).

Since many of the mutations used alter the phenotypic sexual differentiation with respect to the chromosomal sexual identity, chromosomally female and male flies, in most cases, were distinguished through the use of genetic markers on the *X* or *Y* chromosome as listed in Table 1. Chromosomally male flies were distinguished from female flies in four ways. Male flies that carry a *Bar* (B^s) *Y* chromosome have small eyes compared to their wildtype sisters. Females that are *yellow* (*y*) or *white* (*w*) homozygotes when crossed to males with normal *X* and *Y* chromosomes have *y* or *w* sons

and daughters wild type for these markers. When homozygous *y* females are crossed to $y/y^+ Y$ males, the daughters are *y* and the sons are wild type in color.

Three *Sxl* alleles (provided by T. CLINE) were used. In transheterozygotes, two alleles, *cm* Sxl^{fm7M1} ct^6 *v*, and *y w* Sxl^{Mlfm3} ct^6 *v*, have sufficient *X* chromosome dosage compensation to permit the survival of chromosomally female flies but transform them into phenotypic males (CLINE 1984). A temperature-sensitive allele *cm* $Sxl^{Ml_1fPa\text{-}ra}$ ct^6 *v* causes male flies to develop as external intersexes at 25° (MAINE *et al.* 1985).

Three *tra* loss of function alleles (provided by J. M. BELOTE, F. M. HOFFMAN and B. S. BAKER) were used: *th st* tra^1 *in ri* p^p (STURTEVANT 1945), tra^4 *kar ry red*, and tra^5 *kar ry red* (BELOTE *et al.* 1990). The tra^1 allele is a small deficiency of the coding region (MCKEOWN, BELOTE and BAKER 1987). The deficiency $Df(3L)st^{j7}$ (73A1-2; 73B1-2), *Ki roe* p^p (MCKEOWN, BELOTE and BAKER 1987; BELOTE *et al.* 1990) uncovers the *tra* locus. The *hstra*$^-$ female strain used in this study (a gift from M. MCKEOWN) converts chromosomally male flies and *X/X tra* null females into externally female flies at room temperature (BOGGS *et al.* 1987; MCKEOWN, BELOTE and BOGGS 1988). This strain carries a *P* element-induced transposition of the female-specific tra^+ cDNA under a heat shock promoter which has been recombined onto the $Df(3R)st^{j7}$ chromosome (BOGGS *et al.* 1987).

Four loss of function *tra-2* alleles were used (gifts of J. M. BELOTE and B. S. BAKER): *tra-2* (WATANABE 1975), *cn* $tra\text{-}2^B$ *bw* (BELOTE and LUCCHESI 1980; MATTOX and BAKER 1991), *cn* $tra\text{-}2^{ts1}$ *bw* (BELOTE and LUCCHESI 1980; AMREIN, MANIATIS and NÖTHIGER 1990) and *cn* $tra\text{-}2^{ts2}$ *bw* (BELOTE and BAKER 1982; AMREIN, MANIATIS and NÖTHIGER 1990). A small deficiency *Df(2R)trix*; 51A1-2;51B6 (GORALSKI, EDSTROM and BAKER 1989) uncovers the *tra-2* locus. Two lines carrying an insert of $tra\text{-}2^+$ cDNA, TxF4M8F8 (TxF4) and TxL3M3M2 (TxL3) (gifts from T. GORALSKI and B. S. BAKER) in a $tra\text{-}2^B$ mutant background were used. In the $TxF4tra\text{-}2^+$ line, the $tra\text{-}2^B$ masculinization of the genitalia of female flies or male sterility was not fully rescued (T. GORALSKI, unpublished results), both phenotypes were completely rescued in $TxL3tra\text{-}2^+$ (GORALSKI, EDSTROM and BAKER 1989).

Four point mutations of *dsx* were used (provided by B. S. BAKER): dsx^1 p^p (HILDRETH 1965), dsx^{15} ($dsx^{D100.41}$) and dsx^{16} ($dsx^{D100.42}$, both isolated by I. DUNCAN), and dsx^{198} (dsx^{EFH48}, isolated by T. HAZELRIGG). The heteroallelic combination of chromosomal rearrangements dsx^{23} ($In(3R)dsx^{D+R3}$, *bx sr e*)/$Df(3R)dsx^{15}$ ($Df(3R)dsx^{M+R15}$) (DUNCAN and KAUFMAN 1975; BAKER and WOLFNER 1988; BAKER *et al.* 1991) eliminates all *dsx* sex-specific transcripts on Northern blots (R. NAGOSHI, personal communication). Two dominant mutations (provided by B. S. BAKER and R. NAGOSHI), dsx^D *Sb* e^s (FUNG and GOWEN 1957; GOWEN and FUNG 1957; DENELL and JACKSON 1972) and dsx^M (MISCHAIKOW 1959; NÖTHIGER, ROOST and SCHÜPBACH 1980) were tested.

Three loss of function alleles at the *ix* locus were examined (provided by B. CHASE and B. S. BAKER): *pr cn* ix^1 (MORGAN, REDFIELD and MORGAN 1943), ix^2 (MEYER and EDMONDSON 1951), $ix^{D100.36a}$ (isolated by I. DUNCAN). The deficiency $Df(2R)en^B$ (47E3-6;48A4) (EBERLEIN and RUSSELL 1983) (provided by B. CHASE), uncovers the *ix* gene (B. CHASE, personal communication).

The following double mutant combinations were constructed using standard genetic crosses from single mutant stocks: (1) B^sY; *pr cn* ix^1/*SM1*; $Df(3R)dsx^{15}$/*TM2*, (2) *pr cn* ix^1/*SM1*; $In(3R)dsx^{23}$/*TM2* and (3) *pr cn* ix^1/*SM1*; dsx^D *Sb e*/*TM2*. The tra^1 dsx^1 p^p/*TM6B* stock was a gift from B. S. BAKER.

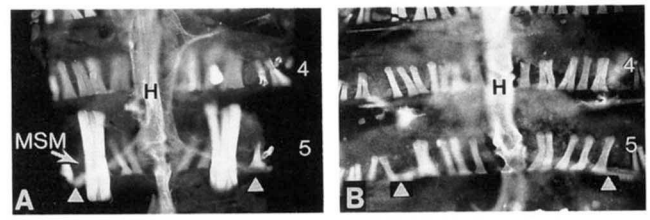

FIGURE 2.—Photomicrographs of the fourth and fifth dorsal abdominal segments of Canton-S flies that were stained with the anti-myosin antibody. The heart demarcates the dorsal midline and is tethered to the tergites by alary muscles which insert laterally (arrowheads). (A) In the male abdomen, the large male specific muscles (MSM, arrow) are located medial to the alary insertions. More lateral portions of the abdomen have been dissected away. (B) In the female abdomen, muscle fibers medial to the alary insertions were the same size as those positioned closer to the dorsal midline. 4, Fourth abdominal tergite; 5, fifth abdominal tergite; H, heart. Anterior is to the top in this and all subsequent figures.

RESULTS

Sexually dimorphic abdominal muscles in control flies: In the fifth abdominal segment of male flies, the dorsal midline is flanked by a pair of large multifiber muscles (LAWRENCE and JOHNSTON 1984; COURCHESNE-SMITH and SMITH 1989; GAILEY, TAYLOR and HALL 1991; Figure 2A; Table 1). These male-specific muscles were always found medial to the alary muscle insertions that anchor the heart to the dorsal cuticle (Figure 2A). Each muscle is composed of around five to six closely associated fibers (Table 2), that insert into the cuticle as a coherent bundle. For a more detailed physical characterization of the male-specific muscle, the insertion sites of this muscle were compared to those of the fifth abdominal internal oblique muscle, a retained larval muscle present transiently in newly eclosed flies (MILLER 1950; CROSSLEY 1978; KIMURA and TRUMAN 1990). The posterior insertion site of the male-specific muscle is apposed to the attachment site of the interior oblique muscle, which marks the fifth/sixth segmental boundary. The anterior insertion point of the male-specific muscle was close to, but not coincident with, the insertion site of the interior oblique muscle, which demarcates the fourth/fifth segmental boundary (data not shown). Thus, the male-specific muscle spans nearly the entire dorsal width of the fifth abdominal segment.

In the fifth abdominal segment of female flies, small dorsal longitudinal muscles occupied a position comparable to that of the male-specific muscle in male flies (MILLER 1950; LAWRENCE and JOHNSTON 1984; CURRIE and BATE 1991; GAILEY, TAYLOR and HALL 1991; Figure 2B). Two to four individual muscle fibers were located medially near the alary insertions; one or more of these fibers may represent the female replacements of the male-specific muscle (Figure 2B). Each of these muscle fibers in a female had a posterior insertion within the intersegmental cuticle near the margin of the fifth abdominal tergite and an anterior

TABLE 2

Average number of muscle fibers in male specific muscles

Genotype	No. of muscle fibers in the MSM	
	X/X	*X/Y*
Control		
CS	0.0	5.7 ± 0.2
Single loss-of-function mutations		
Sex-lethal		
Sxl^{fm7M1}/Sxl^{M1fm3}	5.6 ± 0.2[a]	NT
transformer		
$tra/Df(3L)st^{j7}$	5.4 ± 0.2[a]	5.4 ± 0.2[a]
$tra^{4}/Df(3L)st^{j7}$	5.8 ± 0.2[a]	5.8 ± 0.2[a]
$tra^{5}/Df(3L)st^{j7}$	5.5 ± 0.3[a]	4.9 ± 0.2
transformer-2		
$tra\text{-}2^{B}/tra\text{-}2^{B}$	2.9 ± 0.2[c]	NT
$tra\text{-}2^{B}/Df(2R)trix$	4.5 ± 0.2[b]	4.5 ± 0.3[b]
$tra\text{-}2^{ts1}/tra\text{-}2^{ts2}$ (18°)	5.0 ± 0.1[a]	4.9 ± 0.1[b]
$tra\text{-}2^{ts1}/tra\text{-}2^{ts2}$ (29°)	5.6 ± 0.2[a]	4.8 ± 0.3[b]
doublesex		
dsx^{1}/dsx^{19}	0.0	5.4 ± 0.3[a]
dsx^{1}/dsx^{16}	0.0	4.8 ± 0.1[b]
dsx^{15}/dsx^{19}	0.0	4.9 ± 0.2[b]
dsx^{16}/dsx^{19}	0.0	5.0 ± 0.3[a]
$dsx^{23}/Df(3R)dsx^{15}$	0.0	5.6 ± 0.2[a]
intersex		
ix^{1}/ix^{1}	0.0	5.4 ± 0.2[a]
Double loss-of-function mutations		
$tra^{1}\ dsx^{1}/tra^{1}\ dsx^{1}$	5.5 ± 0.2[a]	5.5 ± 0.2[a]
$ix/ix;\ dsx^{23}/DF(3R)dsx^{15}$	0.0	4.6 ± 0.2[b]
Single gain-of-function mutations		
transformer		
$hstra,\ Df(3L)st^{j7}/tra^{1}$	4.6 ± 0.1[b]	4.2 ± 0.1[b]
doublesex		
$dsx^{D}/Df(3R)dsx^{15}$	0.0	5.3 ± 0.2[a]

[a] Not significantly different from CS control male value (paired two-tailed *t*-test, STATVIEW: $p < 0.05$).

[b] Significantly different from CS control male value (paired two-tailed *t*-test: $p > 0.05$), but not from the $B^{s}Y$; $tra^{5}/Df(3L)st^{7}$ value (paired two-tailed *t*-test: $p < 0.05$). These mutant *tra* males were able to copulate efficiently even though they have fewer muscle fibers (B. TAYLOR, unpublished results).

[c] Significantly different from both the CS control male and the $B^{s}Y$; $tra^{5}/Df(3L)st^{7}$ values ($p > 0.05$).

insertion about halfway into the fifth tergite, aligned with the attachments of other sex-nonspecific muscle fibers located next to the midline in both sexes.

To establish the normal size range for control male and female muscles in the cuticle preparations used in this study, the length of sex-specific muscles found in male abdomens and the comparable muscles in female abdomens were determined. The ratio of the length of the male-specific muscle, or the analogous muscles in females, to the length of a more medial nonsex-specific longitudinal muscle was computed from measurements of *camera lucida* drawings of the muscles (see Figure 7 for details). In control males, the sex-specific muscles ranged from 1.6 to 2.8 times as long as neighboring nonsex-specific muscles (Figures 2A and 7). This range of values for the relative length of male-specific muscles in different preparations probably reflects variations in stretching incurred during mounting and/or unequal degrees of post-fixation contraction. In control females, the muscles found at the same medial location as the male-specific muscle were identical in size to the neighboring nonsex-specific dorsal muscles, with a proportional length between 0.9 and 1.0 (Figures 2B and 7).

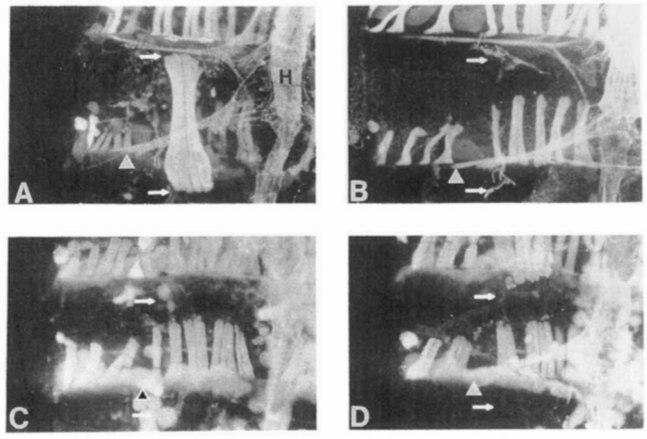

FIGURE 3.—Photomicrographs of the fifth abdominal dorsal hemisegment in mutant *X/X* flies stained with the anti-myosin antibody. The dorsal midline is to the right in each photograph. White arrows mark the expected position of the anterior and posterior insertion sites of the male-specific muscle. As in Figure 2, a white arrowhead indicates the lateral attachment site of the alary muscle. Only in the *X/X*; tra^{-} mutant abdomen was there a muscle that reached the anterior or posterior insertion sites expected for the male-specific muscle. (A) *X/X*; $tra^{5}/Df(3L)st^{j7}$; (B) *X/X*; $dsx^{1}/Df(3R)dsx^{15}$; (C) *X/X*; $ix^{1}/Df(2R)en^{B}$; (D) *X/X*;ix^{1}/ix^{1};dsx^{D}/dsx^{+}.

Sexually dimorphic abdominal muscles in *Sxl*, *tra* and *tra-2* loss-of-function mutant flies: Female flies mutant for *Sxl* alleles, defective in sex-determination, *tra* or *tra-2* loss-of-function alleles differentiate into flies expressing male characteristics in their somatic tissues (STURTEVANT 1945; WATANABE 1975; BAKER and RIDGE 1980; POSTLETHWAIT, BOWNES and JOWETT 1980; BOWNES and NÖTHIGER 1981; OTA *et al.* 1981; BELOTE and BAKER 1982; CLINE 1984; BELOTE *et al.* 1985; MCROBERT and TOMPKINS 1985; SCHÄFER 1986; DIBENEDETTO *et al.* 1987; CHAPMAN and WOLFNER 1988; MONSMA and WOLFNER 1988; FENG, SCHIFF and CAVENER 1991). To determine whether sex-specific muscles were masculinized in these transformed females, the dorsal abdominal musculature was examined in the transheterozygote *X/X*; Sxl^{fm7M1}/Sxl^{M1fm3} (Sxl^{-}) flies as well as four *tra* (tra^{-}) and six *tra-2* ($tra\text{-}2^{-}$) null allele combinations. In every case, segment-spanning muscles were present in the fifth abdominal segment indicative of a transformation to the male-specific muscle phenotype (Table 1). *Camera lucida* drawings of the muscles in the fifth abdominal dorsal hemisegment of representative *X/X* Sxl^{-} (Figure 4A), tra^{-} (Figures 3A and 4B) and $tra\text{-}2^{-}$ (Figure 4C) mutant adults show that the male-specific muscles produced in these phenotypic males were similar to

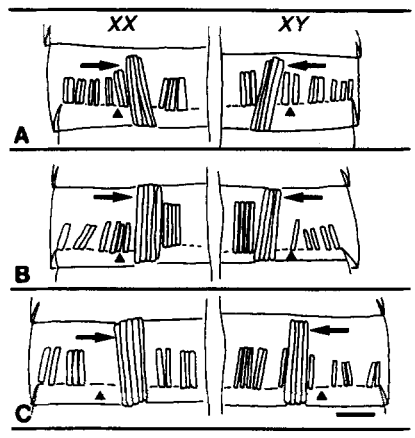

FIGURE 4.—*Camera lucida* drawings of the fifth abdominal dorsal hemisegment from *X/X* and *X/Y Sxl*$^-$, *tra*$^-$ or *tra-2*$^-$ mutant animals. Drawings of the fifth hemitergite of chromosomally male flies are on the right and those from chromosomally female flies are on the left of the dorsal midline. The horizontal lines mark the anterior and posterior boundary of the fifth segment. The arrowhead indicates the position of the lateral alary insertion site in a hemisegment. Arrows point to the male-specific muscle in each of the mutant male and female hemisegments. (A) *X/X*; Sxl^{M1fm3}/Sxl^{fm7M1} and *X/Y*; Sxl^{fm7M1}. (B) *X/X* and *X/Y*; $tra^1/Df(3L)st^{j7}$. (C) *X/X* and *X/Y*; $tra\text{-}2^B/Df(2R)trix$.

the male-specific muscles produced by their *X/Y* siblings (Figure 4, A–C).

In order to demonstrate that the sex-specific musculature was completely masculinized in female flies that developed as phenotypic males, the length and fiber number of male-specific muscles from chromosomally female *Sxl*$^-$, *tra*$^-$ and *tra-2*$^-$ flies were compared to those in their *X/Y* siblings and control flies. Segment-spanning muscles of the fifth abdominal segment in $X/X\ Sxl^{fm7M1}Sxl^{M1fm3}$, $tra^1/Df(3L)st^{j7}$ or $tra\text{-}2^B/Df(2R)trix$ abdomens had the same range of muscle lengths as those in *X/Y* control, *Sxl*$^-$, *tra*$^-$ or *tra-2*$^-$ mutant abdomens (Figures 4 and 7). As judged by the composition of the male-specific muscle, the sex-specific abdominal muscles in *X/X Sxl*$^-$ and *tra*$^-$ mutants were completely transformed, since muscles in these mutants had the same number of fibers as in control or *X/Y tra*$^-$ males (Table 2). The fiber composition of male-specific muscles in *X/X tra-2*$^-$ mutants was more variable. However, in all of the *tra-2* mutant genotypes examined except one, $tra\text{-}2^B$, the number of fibers present in the male-specific muscle was within the range found in *X/Y* flies that exhibit normal courtship and copulatory behaviors (BAKER and RIDGE 1980; MCROBERT and TOMPKINS 1985; B. TAYLOR, unpublished observations).

In chromosomally female flies mutant for three different *tra-2* alleles, the sex-specific differentiation of the male-specific muscle did not parallel the development of other sexually dimorphic somatic characteristics. Female flies homozygous for the $tra\text{-}2^B$ allele did not produce a completely masculinized male-specific muscle since these muscles were composed of only 2.9 muscle fibers (Table 2). Furthermore, some of these fibers had an appropriate posterior insertion site but fail to reach the appropriate anterior insertion site, attaching only slightly farther anteriorly than the insertion sites of neighboring nonsex-specific muscles (data not shown). By other criteria, such as external morphology, these mutant $tra\text{-}2^B$ flies appeared to be completely male-like (BELOTE and BAKER 1983; TAYLOR 1989). The characterization of the $tra\text{-}2^B$ allele as a hypomorphic allele for the sexual transformation of abdominal muscles is supported by the strengthening of the mutant phenotype when the $tra\text{-}2^B$ allele is placed over a deficiency. In $X/X\ tra\text{-}2^B/Df(2R)trix$, the number of fibers found in the male-specific muscle increased to the lower range of muscle fibers found in males (Table 2) and the proper male-specific muscle anterior insertion sites and muscle length were restored (Figure 7). In contrast, the temperature sensitive *tra-2* alleles, $tra\text{-}2^{ts1}$ and $tra\text{-}2^{ts2}$, behaved as *tra-2* null alleles for the expression of the male-specific muscle. Male-specific muscles were present in chromosomally female flies bearing any combination of $tra\text{-}2^{ts}$ alleles reared under constant temperatures regimes that varied from the female-specifying temperature, 16°, to the male-specifying temperature, 29° (Tables 1 and 2). This finding differs from the well documented temperature dependence for the masculinization or feminization of sexually dimorphic tissues in these *X/X* mutant flies (BELOTE and BAKER 1982, 1987; EPPER and BRYANT 1983; BELOTE *et al.* 1985; BOWNES, SCOTT and BLAIR 1987; CHAPMAN and WOLFNER 1988; FENG, SCHIFF and CAVENER 1991).

Loss-of-function mutations of *Sxl*, *tra* and *tra-2* appear to have no effect on the sexual development of somatic tissues in male flies (STURTEVANT 1945; WATANABE 1975; BAKER and RIDGE 1980; POSTLETHWAIT, BOWNES and JOWETT 1980; BOWNES and NÖTHIGER 1981; OTA *et al.* 1981; BELOTE and BAKER 1982; CLINE 1984; BELOTE *et al.* 1985; MCROBERT and TOMPKINS 1985). The male-specific muscles present in Sxl^{fm7M1} or Sxl^{M1fm3} male abdomens have the same insertion sites (Figure 4A) and muscle length (Figure 7) as found for male-specific muscles from Canton-S males. Similar results, including the number of fibers present in the male-specific muscle, were obtained for males flies mutant for *tra*$^-$ (Figures 4B and 7; Table 2) and *tra-2*$^-$ (Figures 4C and 7; Table 2). In one of the *tra*$^-$ genotypes, $tra^5/Df(3L)st^{j7}$, the *X/Y* flies had fewer fibers per muscle than *X/Y* control or the other *X/Y tra*$^-$ males. This difference in fiber number did not seem to have any functional consequences, since males of this genotype exhibited normal courtship and copulatory behaviors with females (B. TAYLOR, unpublished observations).

Sexually dimorphic abdominal muscles in *Sxl*, *tra* and *tra-2* gain-of-function mutant flies: If null mu-

tations of *tra* and *tra-2* in chromosomally female flies result in the differentiation of a male-specific muscle, then the expression of the female-specific tra^+ products as well as the $tra\text{-}2^+$ product in the appropriate null mutant background should restore normal female abdominal muscles. To test for this possibility, *X/X* flies carrying a molecularly generated dominant allele of the tra^+ gene ($hstra^+$) in a tra^- mutant background (BOGGS *et al.* 1987) were examined for the presence or absence of the male-specific muscle. These mutant females exhibit female external morphology when raised at 25° without exposure to any heat pulses (MCKEOWN, BELOTE and BOGGS 1988). However, in these same flies the sex-specific muscle phenotype remained male-like (Table 1) with the number of muscle fibers within the normal range for male-specific muscles (Table 2). The appearance of the male-specific muscle could not be prevented by any of several heat pulse regimes conducted throughout development (data not shown). This failure to feminize the male-specific muscle in X/X tra^- flies by the presence of the $hstra^+$ construct may be due to insufficient levels or inappropriate expression in the necessary cells as has been postulated for the failure to make functional germ cells (MCKEOWN, BELOTE and BOGGS 1988).

Similar experiments were conducted using $tra\text{-}2^+$ insertions in a $tra\text{-}2^B$ mutant background. The expression of $tra\text{-}2^+$ in this, admittedly hypomorphic, mutant background completely feminized the abdominal musculature. For one of these genotypes, *X/X*; $tra\text{-}2^+TxF4/tra\text{-}2^+TxF4$; $tra\text{-}2^B/tra\text{-}2^B$, the differentiation of the external genitalia was intersexual (T. GORALSKI, personal communication) suggesting that there may be a tissue-specific regulation of this insertion or different levels of $tra\text{-}2^+$ gene product are needed to suppress the development of male tissue in other somatic tissue like the genitalia compared to sex-specific muscles.

Chromosomally male flies are transformed into phenotypic females by gain-of-function mutations of *Sxl* and *tra* (MAINE *et al.* 1985; MCKEOWN, BELOTE and BOGGS 1988). A complete transformation to female morphology should also include the replacement of the male-specific muscle by muscles with female morphology. Male flies bearing the $Sxl^{M1fPa\text{-}ra}$ mutation have a temperature-sensitive transformation from male to female external cuticle phenotype (MAINE *et al.* 1985). *X/Y*; $Sxl^{M1fPa\text{-}ra}$ flies raised at 25° contained a normal male-specific muscle (Table 1; data not shown) although the genitalia showed intersexual differentiation (MAINE *et al.* 1985). Expression of the female specific $hstra^+$ product even in the absence of any heat pulses causes the external, sexually dimorphic cuticle of male flies to develop with a female morphology (MCKEOWN, BELOTE and BOGGS 1988). However, in these chromosomally male flies, the muscle phenotype remained male-like (Table 1). As was noted before, the failure to feminize the sex-specific abdominal musculature in *X/Y* tra^- flies may also be due to inadequate quantity or improper spatial and/or temporal expression of the $hstra^+$ product.

In the case of *tra-2*, somatic cells in male flies normally express $tra\text{-}2^+$ mRNA so that production of ectopic $tra\text{-}2^+$ in a null background would not sexually transform male flies to phenotypic females (AMREIN, GORMAN and NÖTHIGER 1988; GORALSKI, EDSTROM and BAKER 1989; MATTOX, PALMER and BAKER 1990). As expected, male flies with a $tra\text{-}2^+$ insert in a $tra\text{-}2^B$ mutant background produced a normal male-specific muscle (Table 1).

Sexually dimorphic abdominal muscles in *dsx* and *ix* loss-of-function mutant flies: Previous genetic analyses showed that null mutations of *dsx* (dsx^-) caused similar intersexual transformations of sexually dimorphic tissues in both chromosomally male and female flies (HILDRETH 1965; BAKER and RIDGE 1980; NÖTHIGER, ROOST and SCHÜPBACH 1980; POSTLETHWAIT, BOWNES and JOWETT 1980; OTA *et al.* 1981; BOWNES and NÖTHIGER 1981; NÖTHIGER *et al.* 1987). Unexpectedly, no sexual transformation of the dorsal musculature occurred in either chromosomally male or female dsx^- flies. In *X/X* dsx^- flies, the dorsal musculature of seven different homozygous or *trans*-heterozygous *dsx* null allele combinations was characteristically female in morphology (Figure 3B; Table 1). As depicted in the *camera lucida* drawings of dorsal muscles from a *dsx trans*-heterozygote $dsx^{23}/Df(3R)dsx^{15}$, which produces no sex-specific transcripts as judged by Northern blot analysis (R. NAGOSHI, personal communication), only fibers with the insertion sites of dorsal longitudinal muscles were present in the fifth abdominal segment (Figure 5A). These fibers were same size as those of control female muscles (Figure 7).

In *X/Y* flies, the male-specific muscle was present in all flies mutant for any of the seven dsx^- homozygous or heterozygous combinations examined (Table 1; Figure 5A). When muscles from several different dsx^- mutants were examined, the average number of fibers per male-specific muscle did not differ from the number found in muscles of other male genotypes competent for courtship and copulation (Table 2). The range of muscle lengths in X/Y $dsx^{23}/Df(3R)dsx^{15}$ abdomens was the same as found in Canton-S males or those males expressing *dsx* male-specific mRNA, generated from a dominant *dsx* allele, with or without a normal dsx^+ allele (Figure 7). Although there appears to be no difference between the male-specific muscles in *X/Y*; dsx^- and other males, it was not possible to test directly whether the male-specific muscle in *X/Y*; dsx^- mutants performed normally in copulation, since

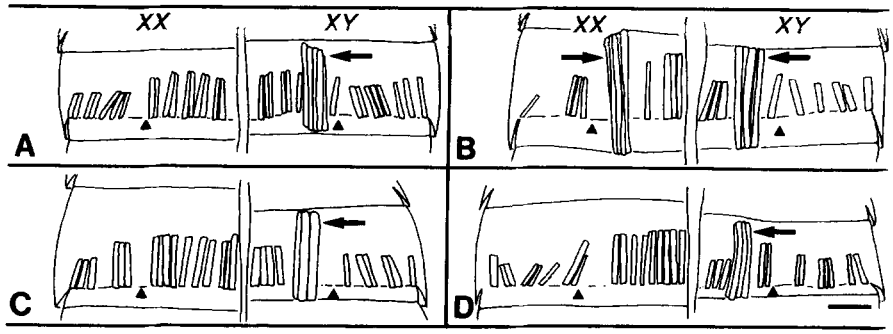

FIGURE 5.—*Camera lucida* drawings of the fifth abdominal dorsal hemisegment from *dsx*⁻ and/or *ix*⁻ mutant flies as in Figure 4. A male-specific muscle is not found in chromosomally female flies that are mutant for null alleles of *dsx* or *ix* alone or in combination. (A) *X/X* and *X/Y*; $dsx^{23}/Df(3R)dsx^{R15}$. (B) *X/X* and *X/Y*; $tra^1\ dsx^1/tra^1\ dsx^1$. (C) *X/X* and *X/Y*; $ix^1/Df(2R)en^B$. (D) *X/X* and *X/Y*; ix^1/ix^1; $dsx^{23}/Df(3R)dsx^{15}$.

these flies are unable to copulate because of the abnormal differentiation of their genitalia (HILDRETH 1965).

As a further test of the role of the *dsx* gene in muscle development, the relationship between *tra* and *dsx* was examined using double mutants. Both male and female $tra^1\ dsx^1$ ($tra^-\ dsx^-$) homozygotes exhibit intersexual characteristics, such as the simultaneous expression of both male and female genitalia, similar to flies mutant for *dsx* alone, suggesting that *dsx* is epistatic to *tra* (OTA *et al.* 1981; BAKER and RIDGE 1980; BAKER and BELOTE 1983). In contrast to the expression of other sexually dimorphic characteristics, female $tra^1\ dsx^1$ homozygotes have male-specific muscles that conformed to those of Canton-S males when judged by the location of insertion sites (Figure 5B), relative muscle length (Figure 7) and average number of fibers making up the male-specific muscle (Table 2). Thus, the loss of *dsx* function in female flies does not circumvent the masculinization of the male-specific muscle caused by loss of *tra* function.

Although the dsx^+ gene, through the expression of an active dsx^+ female specific product, was the most likely candidate for the tra^+ and $tra\text{-}2^+$ dependent regulation of sex-specific muscle development, several other possibilities are implicit in the genetic model presented in Figure 1. The first possibility tested was that the wild-type product of the *ix* gene might act to suppress the male specific muscle in female flies. Indeed, *ix*, has been shown to be needed for the suppression of male sexual differentiation in sexually dimorphic tissues in females (MORGAN, REDFIELD and MORGAN 1943; MEYER and EDMONDSON 1951; KROEGER 1959; BAKER and RIDGE 1980; OTA *et al.* 1981; SCHÄFER 1986; CHAPMAN and WOLFNER 1988). The position of *ix* in the genetic hierarchy is compatible with a potential role in controlling the sexual differentiation of abdominal muscles in females, although there is no indication from previous genetic analysis that *ix* is a target for tra^+ and $tra\text{-}2^+$ activity (BAKER and RIDGE 1980). Female flies lacking *ix* function (ix^-) have dorsal musculature appropriate for female flies (Table 1). The muscles in the fifth abdominal segment have insertion sites (Figures 3C and 5C) and muscle lengths (Figure 7) that completely correspond to the female pattern of muscles. This finding is inconsistent with the hypothesis that the *ix* gene functions to prevent the development of the male-specific muscle in females. The loss of *ix* gene function also appeared to have no effect on the production of the male-specific muscle in *X/Y*; ix^- flies (Figure 5C and 7; Tables 1 and 2).

A second possibility for the genetic control of sex-specific muscles in females was that the wild-type *dsx* and *ix* gene products had redundant functions in females; thus, either gene product acting alone would be capable of suppressing male-specific muscle development. In the double mutant combination constructed to test this hypothesis, ix^1/ix^1; $dsx^{23}/Df(3R)dsx^{15}$, only muscles similar to female muscles formed in the fifth abdominal segment (Table 1, Figures 5D and 7). Loss-of-function of either or both loci appears to have no effect on the ability of female flies to prevent the male-specific muscle from forming.

Sexually dimorphic abdominal muscles in *dsx* gain-of-function mutant flies: Male-specific muscles appeared in chromosomally female flies mutant for tra^- or $tra\text{-}2^-$ alleles; these flies inappropriately express male-specific *dsx* mRNA (NAGOSHI *et al.* 1988). The formation of this sex-specific muscle in females, then, could depend on the presence of the male-specific *dsx* protein (BURTIS and BAKER 1989). Dominant *dsx* alleles, such as dsx^D and dsx^M, have *dsx* pre-mRNA which can only be spliced into male-specific *dsx* mRNA (BAKER and WOLFNER 1988; NAGOSHI *et al.* 1988; NAGOSHI and BAKER 1990). Several genotypes of chromosomally female flies making only male-specific *dsx* mRNA, $dsx^D/Df(3R)dsx^{15}$, dsx^D/dsx^1 or dsx^M/dsx^1, were examined. The ectopic expression of male-specific *dsx* mRNA is insufficient to cause any segment-spanning muscles to be formed in these mutant females (Table 1). The dorsal muscles present in the fifth abdominal segment of *X/X*; $dsx^D/Df(3R)dsx^{15}$ have the female pattern of attachment sites (Figure 6A) and the length of the muscles conformed to the size of muscles in normal females (Figure 7). Furthermore, there was no change in the appearance of female dorsal musculature in mutant females that had

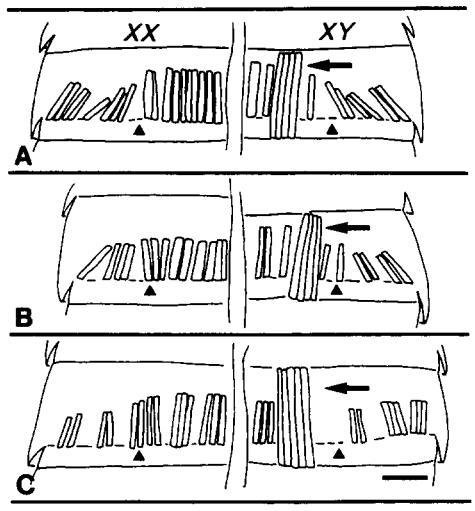

FIGURE 6.—*Camera lucida* drawings of the fifth abdominal dorsal hemisegment from *dsx* gain-of-function mutants as in Figure 4. Chromosomally female flies that ectopically express the male *dsx* product do not produce the male-specific muscle. (A) *X/X* and *X/Y*; $dsx^{D}/Df(3R)dsx^{15}$. (B) *X/X* and *X/Y*; dsx^{D}/dsx^{+}. (C) *X/X* and *X/Y*; ix^{1}/ix^{1}; dsx^{D}/dsx^{+}.

the capacity to make both male and female-specific *dsx* mRNAs, dsx^{D}/dsx^{+} and dsx^{M}/dsx^{+} (Figures 6B and 7). Even under conditions where the suppression of male differentiation is reduced by loss-of-function at the *ix* locus, as in *X/X*; ix^{1}/ix^{1}; dsx^{D}/dsx^{+}, the abdominal muscles developed with female morphology and size (Table 1; Figures 6C and 7). Thus, the ectopic expression of the male-specific *dsx* product in females does not cause the male-specific abdominal muscle to be produced.

DISCUSSION

The primary finding of this paper is that the genetic cascade responsible for translating the initial chromosomal signal of sexual identity into the differentiation of sex-specific abdominal muscles is composed of only a subset of the previously identified sex-determining genes operating in other sexually dimorphic tissues. As summarized in Figure 8, the male-specific abdominal muscle developed in chromosomally female flies mutant for *Sxl* alleles, defective in sex-determining gene functions, or null alleles of *tra* or *tra-2*. Thus, the wild-type activity of these three genes is instrumental in preventing the development of the male-specific muscle in female abdomens, similar to their role in the suppression of masculine traits in other sexually dimorphic tissues (STURTEVANT 1945; WATANABE 1975; BAKER and RIDGE 1980; BOWNES and NÖTHIGER 1981; OTA *et al.* 1981; WIESCHAUS and NÖTHIGER 1982; BELOTE and BAKER 1982; EPPER and BRYANT 1983; CLINE 1984; BELOTE *et al.* 1985; MCROBERT and TOMPKINS 1985; SCHÄFER 1986; TOMPKINS 1986; BOWNES, SCOTT and BLAIR 1987; DIBENEDETTO *et al.* 1987; MONSMA and WOLFNER 1988; CHAPMAN and WOLFNER 1988; TOMPKINS and MCROBERT 1989; BOWNES, STEINMANN-ZWICKY and NÖTHIGER 1990; TAYLOR 1989; FENG, SCHIFF and CAVENER 1991; TAYLOR and TRUMAN 1992).

Downstream of *tra* and *tra-2*, at the next tier of the regulatory hierarchy, *dsx*, in males, and both *dsx* and *ix*, in females, function to transfer the signal of sexual identity into the appropriate terminal differentiation of sexually dimorphic tissues (Figure 1). The phenotype of the abdominal musculature in *dsx* and *ix* mutants was not consistent with a role for these two genes in the development of sex-specific abdominal muscles. In chromosomally female flies, neither the absence of *dsx* gene activity, the ectopic expression of the male-specific *dsx* product or the concomitant loss of *ix* function caused the male-specific muscle to form. Indeed, the abdominal muscle phenotype in all *dsx* and/or *ix* mutant females tested had female morphology (Figure 8). The inability of the dominant *dsx* alleles to masculinize female abdominal musculature could be ascribed to inadequate levels or improper temporal or spatial expression of the dsx male protein, similar to the argument already presented for the failure of heat shock in males and females carrying a *hstra* allele to feminize the male-specific muscles in male and female tra^{-} mutants or the $Sxl^{M1fPa\text{-}ra}$ to feminize the abominal musculature of male flies. However, this explanation for the results of the *dsx* dominant alleles in females seems unlikely due to the normal male-specific muscle phenotypes of male flies with either null or dominant alleles of *dsx*. In chromosomally male flies, the loss of *dsx* function did not eliminate or alter the size or number of fibers of the male-specific muscle (Figure 8). Male and female *dsx* mutants expressed two muscle phenotypes which corresponded to their chromosomal constitution. Mutations in *dsx* did not hinder the proper transmission of the chromosomal signal of sexual identity into the development of the expected sexual phenotype of male or female abdominal muscles in stark contrast to their effect on other sexually dimorphic tissues causing identical intersexual development in both males and females (HILDRETH 1965; BAKER and RIDGE 1980; POSTLETHWAIT, BOWNES and JOWETT 1980; NÖTHIGER, ROOST and SCHÜPBACH 1980; POSTLETHWAIT, BOWNES and JOWETT 1980;OTA *et al.* 1981; BOWNES and NÖTHIGER 1981; SCHÄFER 1986; NÖTHIGER *et al.* 1987; CHAPMAN and WOLFNER 1988; FENG, SCHIFF and CAVENER 1991).

No muscle phenotypes were observed in any of the *dsx* or *ix* mutants examined that were intermediate in the number of fibers or locations of the anterior and/or posterior insertion site. Muscles that display intermediate attachment sites, potentially intersexual muscles, have been observed in the male-specific muscles of male flies with certain mutant combinations in the

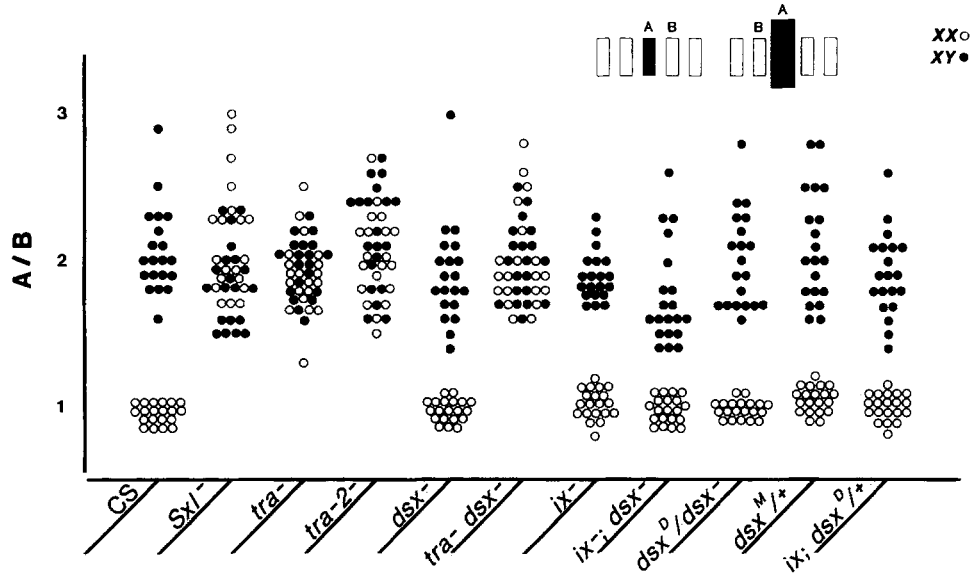

FIGURE 7.—Anterior-posterior length of the male-specific muscle or its homolog in different mutants. To correct for differences in muscle contraction in individual preparations, the relative length of the male-specific muscle or its homolog was calculated as a ratio of its length to that of nonsex-specific muscles closer to the midline. For each genotype, the lengths of each muscle were measured from *camera lucida* drawings of the muscles present at positions A and B, as illustrated in the cartoon inset for 20 *X/X* and *X/Y* hemisegments. The dorsal midline passes between the two cartoon illustrations. Sxl^- (*X/X*; Sxl^{fm7M1}/Sxl^{M1fm3} and *X/Y*; Sxl^{fm7M1} or Sxl^{M1fm3}), tra^- (*X/X* and *X/Y*; $tra^1/Df(3L)st^{J7}$), *tra-2*$^-$ (*X/X* and *X/Y*; $tra\text{-}2^B/Df(2R)trix$), dsx^-, (*X/X* and *X/Y*; $dsx^{23}/Df(3R)dsx^{15}$), $tra^-\ dsx^-$ (*X/X* and *X/Y*; $tra^1\ dsx^1/tra^1\ dsx^1$), ix^- (*X/X* and *X/Y*; $ix^1/Df(2R)en^B$), ix^-; dsx^- (*X/X* and *X/Y*; ix^1/ix^1; $dsx^{23}/Df(3R)dsx^{15}$), dsx^D/dsx^- (*X/X* and *X/Y*; $dsx^D/Df(3R)dsx^{15}$), $dsx^M/+$ (*X/X* and *X/Y*; dsx^M/dsx^+), *ix* $dsx^D/+$ (*X/X* and *X/Y*; ix^1/ix^1; dsx^D/dsx^+).

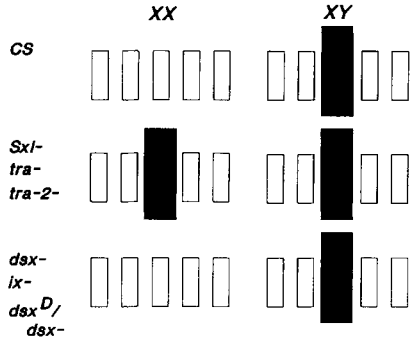

FIGURE 8.—Summary of the sex-specific muscle phenotypes in the various sex-determining mutants. Null mutations at either the *Sxl*, *tra* or *tra-2* loci resulted in the appearance of a male-specific muscle in chromosomally female flies. However, *X/X* flies that were mutant for either null alleles of *dsx* and/or *ix*, or dominant alleles of *dsx* had only the female form of the muscles in the fifth abdominal segment and did not produce the male-specific muscle. The dorsal midline passes between the cartoons depicting the dorsal abdominal hemisegments of *X/X* and *X/Y* flies.

fruitless locus and in females mutant for the $tra\text{-}2^B$ allele.

From the results presented in this paper, the genetic model of the sex-determining cascade controlling sex-specific muscle development diverges from the model presented in Figure 1. This divergence appears at the level where information about sexual identity is transmitted to genes involved in the terminal differentiation of tissues (BAKER and BELOTE 1983; BURTIS *et al.* 1991). The genetic pathway leading to sexually dimorphic muscle development could take either of two forms: shortened or branched. If the cascade for muscle development is truncated at the level of the *dsx* gene, then the female-specific *tra* protein with the *tra-2* protein would be expected to interact directly with downstream genes involved in terminal differentiation. The only molecular mechanism ascribed to these genes is the control of differential splicing of primary transcripts of several different genes. Both *tra* and *tra-2* proteins are needed to mediate the splicing of the *dsx* pre-mRNA into the female-specific *dsx* mRNA, whereas, *tra-2* protein in the absence of the *tra* protein is able to regulate the splicing of its own primary transcript and that of another gene, *exuperantia*, in the male germline (NAGOSHI *et al.* 1988; HEDLEY and MANIATIS 1991; HOSHIJIMA *et al.* 1991; RYNER and BAKER 1991; MATTOX and BAKER 1991). For the genetic hierarchy controlling the development of sex-specific muscles to be truncated, then, the role of these two genes would be postulated to cause the sex-specific splicing of a bank of primary transcripts from downstream differentiation genes. The alternative hypothesis, a branched pathway, intercalates at least one intermediary regulatory gene between the *tra* and *tra-2* level and the terminal differentiation genes leading to sex-specific muscle development. This postulated regulatory gene would be analogous to *dsx* in the hierarchy that regulates muscle development and expected to exhibit some form of sex-specific slicing of its primary transcript. Although the presence of an unidentified sex-deter-

mining gene is the more likely of the two alternatives, only further genetic and molecular identification and characterization will distinquish between them.

The male-specific muscle in the fifth abdominal segment of male flies appears to replace one or more of the dorsal longitudinal muscles normally found in the same segment of females. The differentiation of this male-specific muscle has been shown not to depend on the sex of the cuticle where the muscle attaches or on the sex of the myoblasts that coalesce into the mature muscle but on the sex of the innervating motorneuron (LAWRENCE and JOHNSTON 1984, 1986). These male-specific motorneurons may be respecified larval neurons that are retained from the larval cohort of motor neurons or newly generated postembryonic neurons; sex-specific motor neurons in the moth, *Manduca sexta*, are generated by both mechanisms (GIEBULTOWITZ and TRUMAN 1984; THORN and TRUMAN 1989; R. THORN personal communication). If the sex-determining pathway branches, then the site of action of the sex-determining hierarchy may then be in these male-specific motor neurons or their counterparts in females.

Other potential sites of action in the central nervous system for non-*dsx*-dependent sexual differentiation are the courtship centers of the brain and thorax (HALL 1977, 1979; TOMPKINS and HALL 1983). Female flies mutant for loss of sex-determining function alleles of *Sxl*, or null alleles of *tra* or *tra-2* courted and copulated like normal male flies (STURTEVANT 1945; WATANABE 1975; BAKER and RIDGE 1980; MCROBERT and TOMPKINS 1985; BELOTE and BAKER 1987; TOMPKINS and MCROBERT 1989; B. J. TAYLOR, unpublished results). However, females mutant for null alleles of *dsx* or *ix* did not exhibit any male courtship behaviors (MCROBERT and TOMPKINS 1985; B. J. TAYLOR, unpublished results). Thus, the transmission of sexual identity into the proper sexual differentiation of the central nervous system may be more diverse compared to other sexually dimorphic tissues, since some neurons or their precursors depend on the activity of *dsx* (TAYLOR and TRUMAN 1992) and other neurons, such as the male-specific motor neurons, may require another sex-determining gene.

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LITERATURE CITED

AMREIN, H., M. GORMAN and R. NÖTHIGER, 1988 The sex-determining gene *tra-2* of Drosophila encodes a putative RNA binding protein. Cell **55:** 1025–1035.

AMREIN, H., T. MANIATIS and R. NÖTHIGER, 1990 Alternatively spliced transcripts of the sex-determining gene *tra-2* of Drosophila encode functional proteins of different size. EMBO J. **9:** 3619–3629.

BAKER, B. S., 1989 Sex in flies: the splice of life. Nature **340:** 521–524.

BAKER, B. S., and J. M. BELOTE, 1983 Sex determination and dosage compensation in *Drosophila melanogaster*. Ann. Rev. Genet. **17:** 345–93.

BAKER, B. S., and K. A. RIDGE, 1980 Sex and the single cell. I. On the action of major loci affecting sex determination in *Drosophila melanogaster*. Genetics **94:** 383–423.

BAKER, B. S., and M. W. WOLFNER, 1988 A molecular analysis of *doublesex*, a bifunctional gene that controls both male and female sexual differentiation in *Drosophila melanogaster*. Genes Dev. **2:** 477–489.

BAKER, B. S., G. HOFF, T. C. KAUFMAN, M. W. WOLFNER and T. HAZELRIGG, 1991 The *doublesex* locus of *Drosophila melanogaster* and its flanking region: a cytogenetic study. Genetics **127:** 125–138.

BATE, M., E. RUSHTON and D. A. CURRIE, 1991 Cells with persistent twist expression are the embryonic precursors of adult muscles in Drosophila. Development **113:** 79–90.

BELOTE, J. M., and B. S. BAKER, 1982 Sex determination in *Drosophila melanogaster*: Analysis of *transformer-2*, a sex-transforming locus. Proc. Natl. Acad. Sci. USA **79:** 1568–1572.

BELOTE, J. M., and B. S. BAKER, 1983 The dual functions of a sex determination genes in *Drosophila melanogaster*. Dev. Biol. **95:** 512–517.

BELOTE, J. M., and B. S. BAKER, 1987 Sexual behavior: Its genetic control during development and adulthood in *Drosophila melanogaster*. Proc. Natl. Acad. Sci. USA **84:** 8026–8030.

BELOTE, J. M., and J. C. LUCCHESI, 1980 Male-specific lethal mutations of *Drosophila melanogaster*. Genetics **96:** 165–186.

BELOTE, J. M., A. M. HANDLER, M. W. WOLFNER, K. J. LIVAK and B. S. BAKER, 1985 Sex-specific regulation of yolk protein gene expression in Drosophila. Cell **40:** 339- 348.

BELOTE, J. M., F. M. HOFFMAN, M. MCKEOWN, R. L. CHORSKY and B. S. BAKER, 1990 Cytogenetic analysis of chromosome region 73AD of *Drosophila melanogaster*. Genetics **125:** 783–793.

BOGGS, R. T., P. GREGOR, S. IDRISS, J. M. BELOTE and M. MCKEOWN, 1987 Regulation of sexual differentiation in *D. melanogaster* via alternative splicing of RNA from the *transformer* gene. Cell **50:** 739–747.

BOWNES, M., and R. NÖTHIGER, 1981 Sex determining genes and vitellogenin synthesis in *Drosophila melanogaster*. Mol. Gen. Genet. **182:** 222–228.

BOWNES, M., A. SCOTT and M. BLAIR, 1987 The use of an inhibitor of protein synthesis to investigate the roles of ecdysteroids and sex-determination genes on the expression of the genes encoding the Drosophila yolk proteins. Development **101:** 931–941.

BOWNES, M. A., M. STEINMANN-ZWICKY and R. NÖTHIGER, 1990 Differential control of yolk protein gene expression in fat bodies and gonads by the sex-determining gene *tra-2* of Drosophila. EMBO J. **9:** 3975–3980.

BROADIE, K. S., and M. BATE, 1991 The development of adult muscles in Drosophila: ablation of identified muscle precursor cells. Development **113:** 103–118.

BURTIS, K. C., and B. S. BAKER, 1989 Drosophila *doublesex* gene controls somatic sexual differentiation by producing alternatively spliced mRNAs encoding related sex-specific polypeptides. Cell **56:** 997–1010.

BURTIS, K. C., K. T. COSCHIGANO, B. S. BAKER and P. C. WENSINK, 1991 The *doublsex* proteins of *Drosophila melanogaster* bind directly to a sex-specific yolk protein gene enhancer. EMBO J. **10:** 2577–2582.

CHAPMAN, K. B., and M. F. WOLFNER, 1988 Determination of

male-specific expression in Drosophila accessory glands. Dev. Biol. **126**: 195–202.

CLINE, T. W., 1978 Two closely linked mutations in *Drosophila melanogaster* that are lethal to opposite sexes and interact with daughterless. Genetics **90:** 683–698.

CLINE, T. W., 1984 Autoregulatory functioning of a Drosophila gene product that establishes and maintains the sexually determined state. Genetics **107:** 231–277.

CLINE, T. W., 1985 Primary events in the determination of sex in *Drosophila melanogaster*, pp. 301–327 in *Origin and Evolution of Sex*, edited by H. O. HALVORSON and A. MONROY. Alan R. Liss, New York.

CLINE, T. W., 1986 A female-specific lesion in an X-linked positive regulator of the Drosophila sex determination gene, *Sex-lethal*. Genetics **113:** 641–663 (corrigendum **114:** 345).

CLINE, T. W., 1988 Evidence that *sisterless-a* and *sisterless-b* are two of several discrete "numerator elements" of the X/A sex determination signal in Drosophila that switch *Sxl* between two alternative stable expression states. Genetics **119:** 829–862.

COURCHESNE-SMITH, C. L., and S. TOBIN, 1989 Tissue-specific expression of the 79B actin gene during development. Dev. Biol. **133:** 313–321.

CROSSLEY, C. A., 1978 The morphology and development of the Drosophila muscle system. pp. 499–560 in *The Genetics and Biology of Drosophila*, Vol. 2b, edited by M. ASHBURNER and T. R. F. WRIGHT. Academic Press, New York.

CURRIE, D. A., and M. BATE, 1991 The development of adult abdominal muscles in Drosophila: myoblasts express twist and are associated with nerves. Development **113:** 91–102.

DENELL, R. E., and R. JACKSON, 1972 A genetic analysis of *transformer-dominant*. Drosophila Inform. Serv. **48:** 44–45.

DIBENEDETTO, A. J., D. M. LAKICH, W. D. KRUGER, J. M. BELOTE, B. S. BAKER and M. F. WOLFNER, 1987 Sequences expressed sex-specifically in *Drosophila melanogaster* adults. Dev. Biol. **119:** 242–251.

DUNCAN, I. W., and T. C. KAUFMAN, 1975 Cytogenetic analysis of chromosome 3 in *Drosophila melanogaster*: mapping of the proximal portion of the right arm. Genetics **80:** 733–752.

EBERLEIN, S., and M. A. RUSSELL, 1983 Effects of deficiencies in the *engrailed* region of Drosophila. Dev. Biol. **100:** 227–237.

EPPER, F., and P. J. BRYANT, 1983 Sex-specific control of growth and differentiation in the Drosophila genital disc, studied using a temperature-sensitive *transformer-2* mutation. Dev. Biol. **100:** 294–307.

FENG, Y., N. M. SCHIFF and D. R. CAVENER, 1991 Organ-specific patterns of gene expression in the reproductive tract of Drosophila are regulated by the sex-determining genes. Dev. Biol. **146:** 451–460

FUNG, S. C., and J. W. GOWEN, 1957 The developmental effect of a sex-limited gene in *Drosophila melanogaster*. J. Exp. Zool. **34:** 515–532.

GAILEY, D. A., B. J. TAYLOR and J. C. HALL, 1991 Elements of the *fruitless* locus regulate the development of the muscle of Lawrence, a male-specific structure in the abdomen of *Drosophila melanogaster* adults. Development **113:** 879–890.

GIEBULTOWICZ, J. M., and J. W. TRUMAN, 1984 Sexual differentiation in the terminal ganglion of the moth *Manduca sexta*: role of sex-specific neuronal death. J. Comp. Neurol. **226:** 87–95.

GORALSKI, T. J., J.-E. EDSTROM and B. S. BAKER, 1989 The sex determination locus *transformer-2* of Drosophila encodes a polypeptide with similarity to RNA binding proteins. Cell **56:** 1011–1018.

GOWEN, J. W., and S. C. FUNG, 1957 Determination of sex through genes in a major sex locus in *Drosophila melanogaster*. Heredity **11:** 395–402.

HALL, J. C., 1977 Portions of the central nervous system controlling reproductive behaivor in *Drosophila melanogaster*. Behav. Genet. **7:** 291–312.

HALL, J. C., 1979 Control of male repreoductive behavior by the central nervous system of Drosophila: dissection of a courtship pathway by genetic mosaics. Genetics **92:** 437–457.

HEDLEY, M. L., and T. MANIATIS, 1991 Sex-specific splicing and polyadeylation of *dsx* pre-mRNA requires a sequence that binds specifically to *tra-2* proteins *in vitro*. Cell **65:** 579–586.

HILDRETH, P. E., 1965 *Doublesex*, a recessive gene that transforms both males and females of Drosophila into intersexes. Genetics **51:** 659–678.

HOOPER, J. E., 1986 Homeotic gene function in the muscles of Drosophila larvae. EMBO J. **5:** 2321–2329.

HOSHIJIMA, K., K. INOUE, I. HIGUCHI, H. SAKAMOTO and Y. SHIMURA, 1991 Control of *doublesex* alternative splicing by *transformer* and *transformer-2* in Drosophila. Science **252:** 833–836.

INOUE, K., K. HOSHIJIMA, H. SAKAMOTO and Y. SHIMURA, 1990 Binding of the Drosophila *Sex-lethal* gene product to the alternative splice site of *transformer* primary transcript. Nature **344:** 461–463.

KIEHART, D., and R. FEGHALI, 1986 Cytoplasmic myosin from *Drosophila melanogaster*. J. Cell Biol. **103:** 1517–1525.

KIMURA, K.-I., and J. W. TRUMAN, 1990 Postmetamorphic cell death in the nervous and muscular systems of Drosophila. J. Neurosci. **10:** 403–411.

KROEGER, H., 1959 The genetic control of genital morphology in Drosophila. Wilhelm Roux's Arch. Entwicklungsmech. Org. **151:** 301–322.

LAWRENCE, P. A., and P. JOHNSTON, 1984 The genetic specification of pattern in a Drosophila muscle. Cell **36:** 775–782.

LAWRENCE, P. A., and P. JOHNSTON, 1986 The muscle pattern of a segment of Drosophila may be determined by neurons and not by contributing myoblasts. Cell **45:** 505–513.

LINDSLEY, D. L., and G. ZIMM, 1992 *The Genome of Drosophila melanogaster*. Academic Press, San Diego, Calif.

MAINE, E. M., H. K. SALZ, P. SCHEDL and T. W. CLINE, 1985 *Sex-lethal*, a link between sex determination and sexual differentiation in *Drosophila melanogaster*. Cold Spring Harbor Symp. Quant. Biol. **50:** 595–604.

MATTOX, W., and B. S. BAKER, 1991 Autoregulation of the splicing of transcripts from the *transformer-2* gene of Drosophila. Genes Dev. **5:** 786–96.

MATTOX, W., M. J. PALMER and B. S. BAKER, 1990 Alternative splicing of the sex- determination gene *transformer-2* is sex-specific in the germ line but not in the soma. Genes Dev. **4:** 789–805.

MCKEOWN, M., J. M. BELOTE and B. S. BAKER, 1987 A molecular analysis of *transformer*, a gene in *Drosophila melanogaster* that controls female sexual differentiation. Cell **48:** 489–499.

MCKEOWN, M., J. M. BELOTE and R. T. BOGGS, 1988 Ectopic expression of the female *transformer* gene product leads to female differentiation of chromosomally male Drosophila. Cell **53:** 887–895.

MCROBERT, S. P., and L. TOMPKINS, 1985 The effect of *transformer*, *doublesex* and *intersex* mutations on the sexual behavior of *Drosophila melanogaster*. Genetics **111:** 89–96.

MEYER, H. U., and M. EDMONDSON, 1951 New mutants. Drosophila Inform. Serv. **25:** 73

MILLER, A., 1950 The internal anatomy and histology of the imago of *Drosophila melanogaster*, pp. 420–534 in *Biology of Drosophila*, edited by M. DEMEREC. Wiley, New York.

MISCHAIKOW, E., 1959 New mutants. Drosophila Inform. Serv. **33:** 98.

MONSMA, S. A., and M. F. WOLFNER, 1988 Structure and expression of a Drosophila male accessory gland gene whose product resembles a peptide pheromone precursor. Genes Dev. **2:** 1063–1073.

MORGAN, T., H. REDFIELD and L. V. MORGAN, 1943 Maintenance

of a Drosophila stock center in connection with investigations of the constitution of germinal material in relation to heredity. Carnegie Inst. Washington Year Book **42:** 171–174.

NAGOSHI, R. N., and B. S. BAKER, 1990 The regulation of sex-specific RNA splicing at the Drosophila *doublesex* gene: *cis*-acting mutations in exon sequences alter sex-specific RNA splicing patterns. Genes Dev. **4:** 89–97.

NAGOSHI, R. N., M. MCKEOWN, K. C. BURTIS, J. M. BELOTE and B. S. BAKER, 1988 The control of alternative splicing at genes regulating sexual differentiation in *D. melanogaster*. Cell **53:** 229–236.

NÖTHIGER, R., M. ROOST and T. SCHÜPBACH, 1980 *Masculinizer* is an allele of *doublesex*. Drosophila Inform. Serv. **55:** 118.

NÖTHIGER, R., and M. STEINMANN-ZWICKY, 1985 Sex determination in Drosophila. Trends Genet. **1:** 209–215.

NÖTHIGER, R., M. LEUTHOLD, N. ANDERSEN, P. GERSCHWILER, A. GRÜTER, W. KELLER, C. LEIST, M. ROOST AND H. SCHMID, 1987 Genetic and developmental analysis of the sex-determining gene 'double sex' (*dsx*) of *Drosophila melanogaster*. Genet. Res. **50:** 113–123.

NÜESCH, H., 1985 Control of muscle development, pp. 425–452 in *Comprehensive Insect Physiology Biochemistry and Pharmacology*, Vol. 11, edited by G. A. KERKUT and L. I. GILBERT. Pergamon Press, New York.

OLIVER, B., N. PERRIMON and A. P. MAHOWALD, 1988 Genetic evidence that the *sans fille* locus is involved in Drosophila sex determination. Genetics **120:** 159–171.

OTA, T., A. FUKUNAGA, M. KAWABE and K. OISHI, 1981 Interactions between sex- transformation mutants of Drosophila. I. Hemolymph vitellogenins and gonad morphology. Genetics **99:** 429–441.

POSTLETHWAIT, J. H., M. BOWNES and T. JOWETT, 1980 Sexual phenotype and vitellogenin synthesis in *Drosophila melanogaster*. Dev. Biol. **79:** 379–387.

RYNER, L. C., and B. S. BAKER, 1991 Regulation of *doublesex* pre-mRNA processing occurs by 3′-splice site activation. Genes Dev. **5:** 2071–2085.

SCHÄFER, U., 1986 The regulation of male-specific transcripts by sex determining genes in *Drosophila melanogaster*. EMBO J. **5:** 3579–3582.

SLEE, R., and M. BOWNES, 1990 Sex determination in *Drosophila melanogaster*. Q. Rev. Biol. **65:** 175–204.

STEINMANN-ZWICKY, M., H. AMREIN and R. NÖTHIGER, 1990 Genetic control of sex determination in Drosophila. Adv. Genet. **27:** 189–237.

STURTEVANT, A. H., 1945 A gene in *Drosophila melanogaster* that transforms females into males. Genetics **30:** 297–299.

TAYLOR, B. J., 1989 Sexually dimorphic neurons of the terminalia of *Drosophila melanogaster*. II. Sex-specific axonal arborizations in the central nervous system. J. Neurogenet. **5:** 193–213.

TAYLOR, B. J., and J. W. TRUMAN, 1992 Commitment of abdominal neuroblasts in Drosophila to a male or female fate is dependent on genes of the sex-determining hierarchy. Development **114:** 625–642.

THORN, R. S., and J. W. TRUMAN, 1989 Sex-specific neuronal respecification during the metamorphosis of the genital segments of the tobacco hornworm moth *Manduca sexta*. J. Comp. Neurol. **284:** 489–503.

Genet. **4:** 333–337.

TOMPKINS, L., 1986 GENETIC CONTROL OF SEXUAL BEHAVIOR IN *Drosophila melanogaster*. Trends Genet. **6:** 14–17.

TOMPKINS, L., and J. C. HALL, 1983 Identification of brain sites controlling female receptivity in mosaics of *Drosophila melanogaster*. Genetics **103:** 179–195.

TOMPKINS, L., and S. P. MCROBERT, 1989 Regulation of behavioral and pheromonal aspects of sex determination in *Drosophila melanogaster* by the *Sex-lethal* gene. Genetics **123:** 535–541.

WATANABE, T. K., 1975 A new sex-transforming gene on the second chromosome of *Drosophila melanogaster*. Jpn. J. Genet. **50:** 269–271.

WEISCHAUS, E., and R. NÖTHIGER, 1982 The role of the "*transformer*" genes in the development of genitalia and analia of *Drosophila melanogaster*. Dev. Biol. **90:** 320- 334.

WOLFNER, M. F., 1988 Sex-specific gene expression in somatic tissues of *Drosophila melanogaster*. Trends Genet. **4:** 333–337.

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