A Genetic Analysis of *hermaphrodite*, a Pleiotropic Sex Determination Gene in *Drosophila melanogaster*

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ABSTRACT

Sex determination in Drosophila is controlled by a cascade of regulatory genes. Here we describe *hermaphrodite* (*her*), a new component of this regulatory cascade with pleiotropic zygotic and maternal functions. Zygotically, her^+ function is required for female sexual differentiation: when zygotic her^+ function is lacking, females are transformed to intersexes. Zygotic her^+ function may also play a role in male sexual differentiation. Maternally, her^+ function is needed to ensure the viability of female progeny: a partial loss of her^+ function preferentially kills daughters. In addition, *her* has both zygotic and maternal functions required for viability in both sexes. Temperature sensitivity prevails for all known *her* alleles and for all of the *her* phenotypes described above, suggesting that *her* may participate in an intrinsically temperature-sensitive process. This analysis of four *her* alleles also indicates that the zygotic and maternal components of of *her* function are differentially mutable. We have localized *her* cytologically to 36A3-36A11.

IN most animals, sexual identity is first determined by the inheritance of sex chromosomes. The interpretation of this signal and the coordination of sex-specific development is then carried out by a system of regulatory genes. In Drosophila, many of the key components of this regulatory system are well understood (for reviews, see BAKER 1989; CLINE 1989; SLEE and BOWNES 1990; STEINMANN-ZWICKY, AMREIN and NÖTHIGER 1990; BELOTE 1992; BURTIS and WOLFNER 1992).

The primary sex-specific signal in Drosophila is the ratio of *X* chromosomes to autosomes (*X:A*): cells with an *X:A* ratio of 0.5 differentiate as male; cells with an *X:A* ratio of 1 become female (BRIDGES 1925; BAKER and BELOTE 1983; CLINE 1985, 1988b). This difference demands a way of equalizing *X*-linked gene expression, a process generally known as dosage compensation. Equalization is achieved in Drosophila by hypertranscribing the single *X* chromosome in males–each cell in females transcribes both *X* chromosomes at a basal level (reviewed in BAKER and BELOTE 1983; LUCCHESI and MANNING 1987).

Many of the genes regulating dosage compensation have been identified by their sex-specific or sex-preferential lethal phenotypes (CLINE 1976, 1978, 1986, 1988a; BELOTE and LUCCHESI 1980; UCHIDA, UENOYAMA and OISHI 1981; GRANADINO, CAMPUZANO and SÁNCHEZ 1990). Genes controlling most aspects of somatic sexual differentiation have been identified by their effects on the sex-specific development of sexually dimorphic cuticular structures (MORGAN, REDFIELD and MORGAN 1943; STURTEVANT 1945; HILDRETH 1965; WATANABE 1975).

The earliest sex-specific regulatory events affect both sexual differentiation and dosage compensation, because *Sex-lethal* (*Sxl*) regulates both processes (reviewed in CLINE 1988b). *Sxl* is activated in females and remains inactive in males (CLINE 1980, 1984; BELL *et al.* 1988; SALZ *et al.* 1989; BOPP *et al.* 1991; KEYES, CLINE and SCHEDL 1992). *Sxl* function in females is needed to prevent *X*-chromosome hypertranscription during female development (LUCCHESI and SKRIPSKI 1981; GERGEN 1987; GORMAN, KURODA and BAKER 1993). In somatic tissues, activation of *Sxl* transmits a female-determining signal through *transformer (tra)*, via a cascade of regulated splicing, producing a female-specific product of the *doublesex* (*dsx*) gene (BOGGS *et al.* 1987; NAGOSHI *et al.* 1988; BELL *et al.* 1988; BURTIS and BAKER 1989; INOUE *et al.* 1990). In males, lacking such regulation, *dsx* transcripts are spliced into an alternative male-specific mRNA encoding a male-specific form of the *dsx* protein. The sex-specific *dsx* gene products control external morphological differences between males and females, as well as many sex-specific differences in internal tissues (reviewed in BURTIS and WOLFNER 1992); at least one additional regulatory gene must function downstream of *tra* but independently of *dsx* to control the development of a sex-specific muscle (TAYLOR 1992). *Sxl*, *tra* and *dsx* constitute a core group of control genes that are expressed sex specifi-

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cally. Misexpression of these genes leads to sexually inappropriate developmental choices (CLINE 1979; BAKER and RIDGE 1980; MCKEOWN, BELOTE and BOGGS 1988; BURTIS and BAKER 1989; BELL *et al.* 1991). Not surprisingly, loss-of-function mutations in all of these genes have phenotypes that affect only sex-specific developmental processes.

In contrast, other components of this system have multiple developmental functions. For example, *transformer-2* (*tra-2*) encodes an RNA-binding protein that acts with *tra* to regulate the correct splicing of *dsx* in the female soma (NAGOSHI *et al.* 1988; AMREIN, GORMAN and NÖTHIGER 1988; GORALSKI, EDSTRÖM and BAKER 1989; HEDLEY and MANIATIS 1991; HOSHIJIMA *et al.* 1991; RYNER and BAKER 1991). However, mRNAs encoding *tra-2* proteins are expressed in both sexes, in somatic and germ-line tissues (MATTOX, PALMER and BAKER 1990). In addition to its role in the female soma, *tra-2* is also needed in the male germ line for fertility (BELOTE and BAKER 1983), and *tra-2* directs a male germ-line specific splice choice in its own transcripts (MATTOX and BAKER 1991).

Pleiotropy is also a common theme for regulators of *Sxl*. For example, *daughterless* (*da*) has a maternal function specifically required for viability of female progeny and necessary for *Sxl* activation (reviewed in CLINE 1989). In addition, *da* has maternal and zygotic functions essential for viability in both sexes. The sex-specific maternal *da* function is germ-line dependent, whereas the sex-nonspecific maternal *da* function depends only on the soma (CRONMILLER and CLINE 1987). Zygotic *da* function is essential for peripheral nervous system development (CAUDY *et al.* 1988). The relationship between *Sxl* regulation and neurogenesis continues to be elaborated as additional genes with roles in both processes are characterized (CLINE 1989; MURRE *et al.* 1989a, 1989b; TORRES and SANCHEZ 1989; PARKHURST *et al.* 1990; ERICKSON and CLINE 1991; YOUNGER-SHEPHERD *et al.* 1992). Additional zygotic positive regulators of *Sxl* with essential functions in both sexes include *fl(2)d* and the segmentation gene *runt* (GRANADINO, CAMPUZANO and SÁNCHEZ 1990; DUFFY and GERGEN 1991; GRANADINO *et al.* 1992).

Here we introduce *hermaphrodite* (*her*), another pleiotropic gene that functions in Drosophila sex determination. Zygotic *her* function is required for sexual differentiation–in females, and possibly also in males. In addition, zygotic *her* function is needed in both sexes for viability. Function of *her* is also needed maternally: under semirestrictive conditions, the viability of females is preferentially compromised; but under extreme conditions, lack of maternal *her* function is lethal to all progeny. To establish whether all of the observed *her* phenotypes are attributable to a single gene, and whether they can be mutated differentially, we have analyzed four different *her* alleles. These alleles all behave as loss-of-function temperature-sensitive alleles, revealing the complex consequences of losing wild-type *her* function.

MATERIALS AND METHODS

Flies were raised on corn meal, yeast, agar, sucrose, dextrose, propionic acid medium. Experiments were performed at the temperatures indicated; stocks were maintained at approximately 21°. All mutations not referenced in the text can be found in LINDSLEY and ZIMM (1992).

The *her* alleles: The her^{1} allele was identified from a collection of temperature-sensitive mutations induced with EMS (kindly provided by JACK LEVY). The her^{2} allele is a spontaneous mutation that was identified on a specific SM1 balancer chromosome. The $her^{l(2)mat}$ allele is a spontaneous allele identified by REDFIELD (1924, 1926). Isolation of her^{3} is described below. The her^{1} and $her^{l(2)mat}$ alleles are cytologically normal; her^{2} has the cytological aberrations of the SM1 chromosome on which it arose; her^{3} is associated with a complex cytological rearrangement with one breakpoint in 36A.

Isolation of her^{3}: We isolated her^{3} in an F_1 screen for X-ray induced mutations that fail to complement her^{1} for effects on bristle number of the sixth sternite in males. In this screen, *b pr* males were exposed to 4000R and mated to females from a *her^{1} pr mle cn tra-2/CyO* tester stock at 21.5° ± 0.5°. A control cross using *Df(2L)TE116(R)GW23(her^{-})/CyO* established that her^{null} alleles could be recovered; 3200 males bearing mutagenized *b pr*/tester chromosomes were scored; 20 males with >2 sixth sternite bristles were recovered and retested for their ability to complement her^{1} zygotic functions; six were fertile, yielding one new *her* allele.

Zygotic effects: Morphology of live or preserved adults was examined under the dissecting microscope or in mounted preparations, prepared as described in BAKER, CARPENTER and RIPOLL (1978), and viewed with phase contrast optics on a Zeiss Axiophot microscope.

Maternal effects: To test for sex-specific maternal effects, *her* experimental mothers and sibling her^{+} control mothers were obtained by crossing *her*/balancer females to *her*/balancer; *Dp(2;3)osp^{3}, Ki her^{+}*/+ males. Thus the *her/her* mothers and the sibling control mothers (*her/her*; *Dp(2;3)osp^{3}, Ki her^{+}*) differ only by the absence of the duplication-bearing chromosome. Mothers were raised at 18°, held at 18° for 7–15 days after eclosion and then mated at 25° for at least 48 hr before progeny were collected, at 25°, for experiments.

Sex-specific viability effects can be highly sensitive to genetic background (CLINE 1988a). The experimental design described here probably helps to minimize such effects in several ways. First, experimental mothers are siblings of control mothers–they differ only by the presence or absence of the duplication-bearing chromosome. Second, one *her* allele is always introduced from a stock that has the her^{+} duplication. This may reduce the accumulation of suppressing modifiers in that stock. Third, the outcrossing of cultures may help to reduce the effects of recessive modifiers. We found results to be much more consistent when the procedures above were followed than when mutant flies were tested directly from stock cultures.

For analysis of sex-nonspecific maternal effects, mothers were collected as above (for sex-specific maternal effects). After maturing for >7 days at 18°, they were brooded overnight (on agar/apple juice plates) at 18°, shifted to 29°

for 48 hr, brooded overnight again, shifted back to 18° for 72 hr, and brooded once again. Embryos were allowed >48 hr to hatch at 18° and >24 hr to hatch at 29°. For analysis of embryonic defects, mutant and control mothers were brooded at 29°. To examine early defects, embryos were collected and prepared as if for antibody staining (dechorionated with bleach, fixed in 4% paraformaldehyde/PBS/heptane, vitelline membranes removed by shaking in methanol). After rehydration in PBS, embryos were stained with Hoechst 33258, destained, and examined with epifluorescent optics. To examine cuticle defects, unhatched embryos were dechorionated, then mounted and cleared with polyvinyl lactophenol and viewed with phase contrast optics.

Deficiency mapping: For these experiments, *her^1 or/SM1* mothers were crossed to *Df*/balancer fathers (balancer = *SM1* or *CyO*) at 29°. Deficiencies were considered to complement *her^1* for viability if survival of *her^1/Df* individuals was >70% relative to expected numbers at 29°. In addition, the *her^1/Df* females and males were examined for intersexual characteristics. The *her^1/Df* females were assayed for sex-nonspecific or sex-specific maternal effects. To assay for sex-nonspecific maternal effects, mothers were crossed to *Canton S* fathers at 29° and the hatching frequencies for the embryos were observed. Deficiencies were considered to complement *her* for this function if >60% of the embryos hatched in this assay. To assay for sex-specific maternal effects, mothers were crossed to *Sxl^{fm1} oc v ptg* fathers and the ratio of female to male progeny surviving (eclosing) was noted. Deficiencies were considered to complement *her* for this function if the ratio of female:male progeny was > 0.7. Of the deficiencies reported here, only *Df(2L)TE116(R)GW23* failed to complement *her* for any function, and this deficiency was defective for all *her* functions (see RESULTS). In this case, the analysis was more complex, because failure to complement *her* for one function (such as viability or sexual development) can preclude the analysis of other functions. In addition to the deficiency mapping experiments reported in Figure 2, and the results reported for *Df(2L)TE116(R)GW23*, a set of preliminary deficiency mapping experiments were performed utilizing synthetic deficiencies constructed from the T(Y;2) collection of LINDSLEY *et al.* (1972). These experiments localized zygotic *her* functions to a region consistent with the results reported in Figure 2. However in these preliminary experiments–using genetically marked *Y*-linked deficiencies that were either introduced into females with attached-*X* chromosomes or maintained in *XXY* stocks with free *X* chromosomes–*her^1/Df* zygotic phenotypes were similar to (or weaker than) *her^1/her^1* phenotypes, and *her^1* maternal effects (sex-nonspecific and sex-specific) were rescued. In light of the similarities of maternal *her* function to maternal *da* function (M. A. PULTZ and B. S. BAKER, submitted) and in light of the report of SANDLER (1972) that leaky zygotic defects of *da^1* can be suppressed by introduction of extra heterochromatin, we felt that crosses that introduce variable quantities of additional heterochromatin with deficiencies are not clearly interpretable for analyzing the nature of *her* alleles or for deficiency mapping. We therefore chose to concentrate on the analysis of the simpler *her$^-$* deficiency—the only one currently available for this region—*Df(2L)TE116(R)GW23*.

Duplication mapping: *Dp(2;3)osp^3, Ki* was tested for its ability to complement *her* alleles for zygotic effects on viability and on sexual differentiation of males and females, as well as maternal effects (sex-nonspecific and sex-specific) according to the criteria described for deficiency mapping above. All phenotypes were assayed for the genotype *her^{1}or/her^1 or; Dp(2;3)osp^3, Ki*. In addition, the ability of this duplication to rescue *her* maternal effects in numerous allelic combinations is illustrated in the controls throughout this report (see RESULTS). The male-fertile *Dp(2;Y)B108* was also tested for its ability to rescue the zygotic effects of *her* on viability and sexual differentiation, as summarized in Figure 2. *X/Dp(2;Y)B108; her^1 or/SM1* males were crossed to *X/X; her^1 or/SM1* mothers to assay *XY her* duplication-bearing males. Males of the same genotype were crossed to [$\overline{XX}$] *XX/Y; her^1 or/SM1* mothers (to assay $\overline{XX}$*/Y her* duplication-bearing females).

TABLE 1

Zygotic effects of *her* alleles on female sexual differentiation: complementation at 18° *vs.* 29°

		her^3	*her^1*	*her^2*	*her$^{l(2)mat}$*
Df(her$^-$)	18°	Lethal	Weak intersex	Female	Female
	29°	Lethal	Lethal	Intersex	Female
her^3	18°	Intersex	Weak intersex	Female	Female
	29°	Intersex	Intersex	Intersex	Female
her^1	18°		Female	Female	Female
	29°		Intersex	Intersex	Female
her^2	18°			—	Female
	29°			—	Female
her$^{l(2)mat}$	18°				Female
	29°				Female

Full genotypes: Female flies were examined from all crosses in Table 3 in which the mutant females survived (for full genotypes, see Table 3); flies were also examined from additional crosses, listed below; >25 females were examined for each surviving genotype–except for four genotypes, at 29°, where females survived very rarely (see Table 3): *her^1/her^3* (10); *her^2/her^3* (2); *her^3/her^3* (8); *her^2/Df(her$^-$)* (1). *Df(her$^-$) = Df(2L)TE116(R)GW23, cn* (see also Table 6 and Figure 2). *Df(her$^-$)*/balancer diplo-*X*-individuals develop as normal females (18° and 29°).

Additional crosses: *her^1 × her^3 = b her^1/Gla × b her^3 pr/CyO pr cn. her^3 × her^3 = b her^3 pr*/balancer × *b her^3 pr*/balancer, where the balancer is *CyO* or *T(2;3) CyO; TM6B* (described in Table 2). *her^2/Df(her$^-$) = SM1, her^2 cn^2 Cy/b pr × Df(2L)TE116(R)GW23, cn/CyO pr cn^2*.

RESULTS

Zygotic effects on sexual differentiation: A zygotic role for *hermaphrodite* in sexual differentiation was first indicated by the observation that *her^1* homozygous females are transformed to intersexes under semirestrictive and restrictive conditions (25° and 29°). The *her^2* and *her^3* alleles are also defective for this zygotic *her* function. In contrast, *her$^{l(2)mat}$* is not defective for zygotic *her* sex determination functions, though it is defective for *her* maternal functions (see below). Table 1 presents an overview of the effects of *her* alleles on female sexual differentiation: the relative severity of the defects is *her^3 > her^1 > her^2 > her$^{l(2)mat}$*, and the zygotic effects of *her^1* and *her^2* are temperature sensitive. In addition, the effects of *her^1* are enhanced by a *her$^-$* deficiency. Thus, the effects of *her^1* on female sexual development are attributable to a partial loss of *her$^+$* function, thereby revealing a wild-type function of *her* and establishing that *her* is a new sex determination gene in *Drosophila melanogaster*.

The *her* female sexual differentiation phenotype (Figure 1) is a "true intersex" phenotype, similar to that of *dsx* and *ix* (BAKER and RIDGE 1980). In "true intersex" individuals, each cell is intersexual; in contrast, "mosaic intersex" individuals have a mixture of cells, some with male-like and others with female-like morphologies (BAKER and BELOTE 1983; MAINE *et al.* 1985). One indicator of a "true intersex" phenotype is seen in the female counterparts of the male sex comb bristles. Wild-type males have a row of about 9–14 enlarged, blunt bristles in a row that is rotated to an orientation approximately perpendicular to bristle rows on the metatarsus (TOKUNAGA 1962; HILDRETH 1965; BAKER and RIDGE 1980). In contrast, wild-type females have a row of about 3–8 tapered bristles that is approximately parallel to the other bristle rows (Figure 1a). In "true intersexes" the row is partially oriented toward the male orientation, as is seen in chromosomal females with impaired *her* function (Figure 1b). The number of bristles in the rotated row is also increased. For example, when raised at 29°, the mean number of bristles for $her^1/+$ control females is 4.4 ± 0.6; for her^1/her^1 chromosomal females the mean is 6.9 ± 1.0, and for her^1/her^2 chromosomal females the mean is 6.8 ± 0.6. These results are similar to those obtained for *dsx* by HILDRETH (1965).

The pigmentation of the abdomen and the morphology of genitalia and analia are also intersexual in *her* females (Figure 1). In wild-type females (Figure 1c), the fifth abdominal segment is pigmented only along the posterior margin, whereas in wild-type males (Figure 1, d and e), this segment is completely pigmented. In strongly transformed females lacking normal *her* function (Figure 1, f and g), pigmentation extends through the anterior of the fifth abdominal segment. Female and male genitalia and the sexually dimorphic analia are derived from three separate primordia of the genital disc (NÖTHIGER, DÜBENDORFER and EPPER 1977; SCHÜPBACH, WIESCHAUS and NÖTHIGER 1978; EPPER and NÖTHIGER 1982; EPPER and BRYANT 1983). In wild-type females, the female genital primordium develops, the male genital primordium is repressed and the analia develop with a characteristically female orientation and morphology as a pair of upper and lower plates (Figure 1c). In wild-type males, the male genital primordium develops, the female genital primordium is repressed (Figure 1, d and e) and the analia develop with a characteristically male morphology as a pair of left and right plates. In diplo-*X* her^- individuals, elements of both the female and the male genital primordia develop, and the analia are transformed toward a male-like morphology (Figure 1, f to i)–as is also typical for intersexes caused by a loss of *dsx* or *ix* function (HILDRETH 1965; BAKER and RIDGE 1980). In contrast, diplo-*X* her^1 homozygotes raised under permissive conditions (18°) or diplo-*X* $her^{l(2)mat}/her^-$ individuals develop as normal females (Figure 1, j and k).

Lack of *her* function also has effects on males that may indicate a weak transformation to intersexuality. For example, males with a partial loss of *her* function have bristles on the sixth sternite (Table 2). In wild-type adults, the sixth sternite is sexually dimorphic: males usually have no bristles and females have about 19–24 (BAKER and RIDGE 1980). *XY* flies strongly transformed to intersexuality by complete loss of *dsx* function have approximately 14–20 such bristles (BAKER and RIDGE 1980). *XY* flies slightly transformed to intersexuality by partial loss of *dsx* function have a reduced number of such bristles (BAKER *et al.* 1991; Table 2D). Table 2A shows that the sixth sternite bristle phenotype in *her* males parallels the profile of allelic strength for the sexual transformations of *her* in females ($her^3 > her^1$, $her^2 > her^{l(2)mat}$). This male *her* phenotype is temperature-sensitive in her^1/her^1 individuals (Table 2B) and is due to a lack of *her* function (not shown). Bristles on the male sixth sternite are highly reliable as an indicator of impaired *her* function, such that this phenotype can be used for the efficient isolation new *her* alleles (see MATERIALS AND METHODS).

To ask whether effects on male development would be enhanced by a more severe loss of *her* function, we examined her^3/her^3 males that had eclosed and compared them with those that had died as pharate adults (Table 2C)–the latter having failed to survive due to the deleterious effects on viability associated with a loss of zygotic *her* function (see below). Males that had died as pharate adults (without eclosing from their pupal cases) had more sixth sternite bristles. In addition, more than one-third of these males had rotated genitalia, a phenotype not usually seen in *her* males that have managed to eclose. This combination of sixth sternite bristles and rotated genitalia is similar to the phenotype of males with a partial loss *dsx* function (for example, males hemizygous for the hypomorphic dsx^{H55} allele, Table 2D). Together, these experiments suggest that males with insufficient *her* function for survival to adulthood are more abnormal and perhaps more perturbed in their sexual differentiation than their more viable brothers. Moreover, stronger morphological effects of *her* on male development may be masked by the effects of *her* on viability.

In summary, we find that three loss-of-function temperature-sensitive *her* alleles have pronounced zygotic effects on female sexual development, as well as weaker zygotic effects on male development. The her^3 allele has the strongest effects in both sexes; her^1 and her^2 have weaker effects, and $her^{l(2)mat}$ provides

TABLE 2

Zygotic effects of *her* on male development

A. Allelic series, 25°.
Bristles on the sixth sternite in males:[a]

Experimental	her^3/her^1	her^1/her^1	her^2/her^1	$her^{l(2)mat}/her^1$
Mean:	6.0	2.7	1.9	0.0
Range:	(3–12)	(0–7)	(0–7)	(0)
Control				
Mean:	0.15	0.10	0.05	0.0
Range:	(0–1)	(0–1)	(0–1)	(0)

B. Temperature sensitivity, her^1/her^1.
Bristles on the sixth sternite in males:[a]

Experimental	18°	25°	29°
Mean:	0.6	2.7	4.8
Range:	(0–3)	(0–7)	(1–13)
Control			
Mean:	0.0	0.1	0.0
Range:	(0)	(0–1)	(0)

C. Extreme phenotypes (her^3/her^3):

Experimental	25°, eclosed	25°, dead	29°, dead
Mean (6th st. bristles):	5.7	9.8	10.0
Range (6th st. bristles):	(2–11)	(3–15)	(5–14)
Rotated genitalia:	0/20 (0%)	8/22 (36%)	9/20 (45%)
Control	25°, eclosed		29°, eclosed
Mean (6th st. bristles):	0.0		0.0
Range (6th st. bristles):	(0)		(0)
Rotated genitalia:	0/20 (0%)		0/20 (0%)

D. Effects of dsx^{H55}, for comparison to effects of *her*:

Experimental	dsx^{H55}/dsx^{H55}	dsx^{H55}/dsx^{null}
Mean (6th st. bristles):	1.2	8.2
Range (6th st. bristles):	(0–3)	(4–15)
Rotated genitalia:	0/20 (0%)	20/20 (100%)
Control		
Mean (6th st. bristles):	0.1	0.1
Range (6th st. bristles):	(0–1)	(0–2)
Rotated genitalia:	0/20 (0%)	0/40 (0%)

[a] For each genotype and for each corresponding control, 20–40 males were scored.

Full genotypes and crosses: General form of the crosses: mutant/Balancer × mutant/Balancer. In each case, dominant markers on the balancer chromosomes were used to distinguish experimental (mutant/mutant) from control (mutant/Balancer) genotypes, except as noted (2C).

2A, 2B: her^2 = *SM1* her^2 *Cy*, and her^3 = her^3 *pr*. $her^{l(2)mat}$ is unmarked. Balancer = *Gla*. $her^1 \times her^1$ = her^1 *pr/Gla* × *b* her^1/*Gla*. $her^1 \times her^3$ = *b* her^1 *pr/Gla* × her^3 *pr/Gla*. For other crosses, her^1 = her^1 *pr*. *Gla* individuals served as controls.

2C: *b* her^3 *pr*/Balancer × *b* her^3 *pr*/Balancer. Balancer = *T(2;3)CyO;TM6B,Tb*. This balancer makes it possible to score her^3/her^3 individuals as Tb^+ pupae (sibling *Tb* pupae served as controls). Flies which had eclosed were scored as *b* (mutant) or b^+ (control).

2D: dsx^{H55}/Balancer = dsx^{H55}*red e/TM6*, *Ubx*. dsx^{null}/Balancer = *Df(3R)dsx*$^{M+R3}$*/TM3*, *Sb*. For the cross dsx^{H55}*red e/TM6*, *Ubx* × *Df(3R)dsx*$^{M+R3}$*/TM3*, *Sb*, 20 males were scored for each control class (dsx^{H55}*/TM3* and dsx^{null}*/TM6*) and these control data were pooled.

sufficient her^+ function for normal morphology in both sexes.

Zygotic effects on viability: Zygotic her^+ function is also needed for viability in both sexes (Table 3). The effects of her^3, her^1 and her^2 are enhanced over *Df(her*$^-$*)*, consistent with the interpretation that these are partial-loss-of-function alleles with respect to this zygotic function. The her^3, her^1 and her^2 alleles are all temperature-sensitive for zygotic viability. However, homozygotes for $her^{l(2)mat}$ are completely viable at all temperatures and $her^{l(2)mat}$ is completely viable over other *her* alleles at 25° and 18°. At 29°, the viability of $her^{l(2)mat}$ over other *her* alleles and over a her$^-$ deficiency was reduced in this experiment; though in several other experiments of this type (not shown) these $her^{l(2)mat}/her^-$ genotypes appeared fully viable. This variability may be due to slight differences in genetic background or temperature in different experiments, and the low values may indicate a slight failure of $her^{l(2)mat}$ to complement other *her* alleles for zygotic effects on viability. Zygotic effects of *her* on viability are manifested not only in the reduced numbers of survivors but also in their phenotypes: her$^-$ individuals tend to be smaller in size than their *her*/+ siblings, and in some genetic backgrounds they have reduced, roughened eyes and/or incised wings. Taken together, these results for zygotic viability suggest an order for the strengths of *her* alleles ($her^3 > her^1$, $her^2 > her^{l(2)mat}$) consistent with the analysis of *her* effects on sexual differentiation.

Maternal effects on viability of daughters: Our study of sex-specific *her* maternal effects was guided by the finding that her^1 is allelic to a mutation that was isolated in the 1920s on the basis of its deleterious maternal effects on female viability (REDFIELD 1924, 1926). The original analysis by REDFIELD reported an average recovery of 5.5 sons for every daughter at "room temperature." Further analysis by SANDLER (1977) yielded 4.2 males for every surviving female at 25°. This maternal effect mutation was not named by its discoverer and has been known as *lethal(2)maternal* (SANDLER 1977); we have designated it here as $her^{l(2)mat}$. In the analysis of *her* zygotic phenotypes, described above, $her^{l(2)mat}$ does not appear to be a *her* allele. In contrast, $her^{l(2)mat}$ clearly fails to complement other *her* alleles under highly restrictive conditions (29°) for a sex-nonspecific maternal effect lethal phenotype (see the next section below). This raised an additional question: would the other *her* alleles have effects like those of $her^{l(2)mat}$–reduced viability of daughters relative to sons–under semipermissive conditions?

To test for sex-specific maternal effects, mothers were raised at 18°–a temperature that yields sexually normal and fertile females for most combinations of *her* alleles (Table 1)–then mated to wild-type *Canton S* fathers and brooded at 25° (see MATERIALS AND METHODS). All fertile combinations of *her* alleles yielded reduced ratios of daughters to sons in this

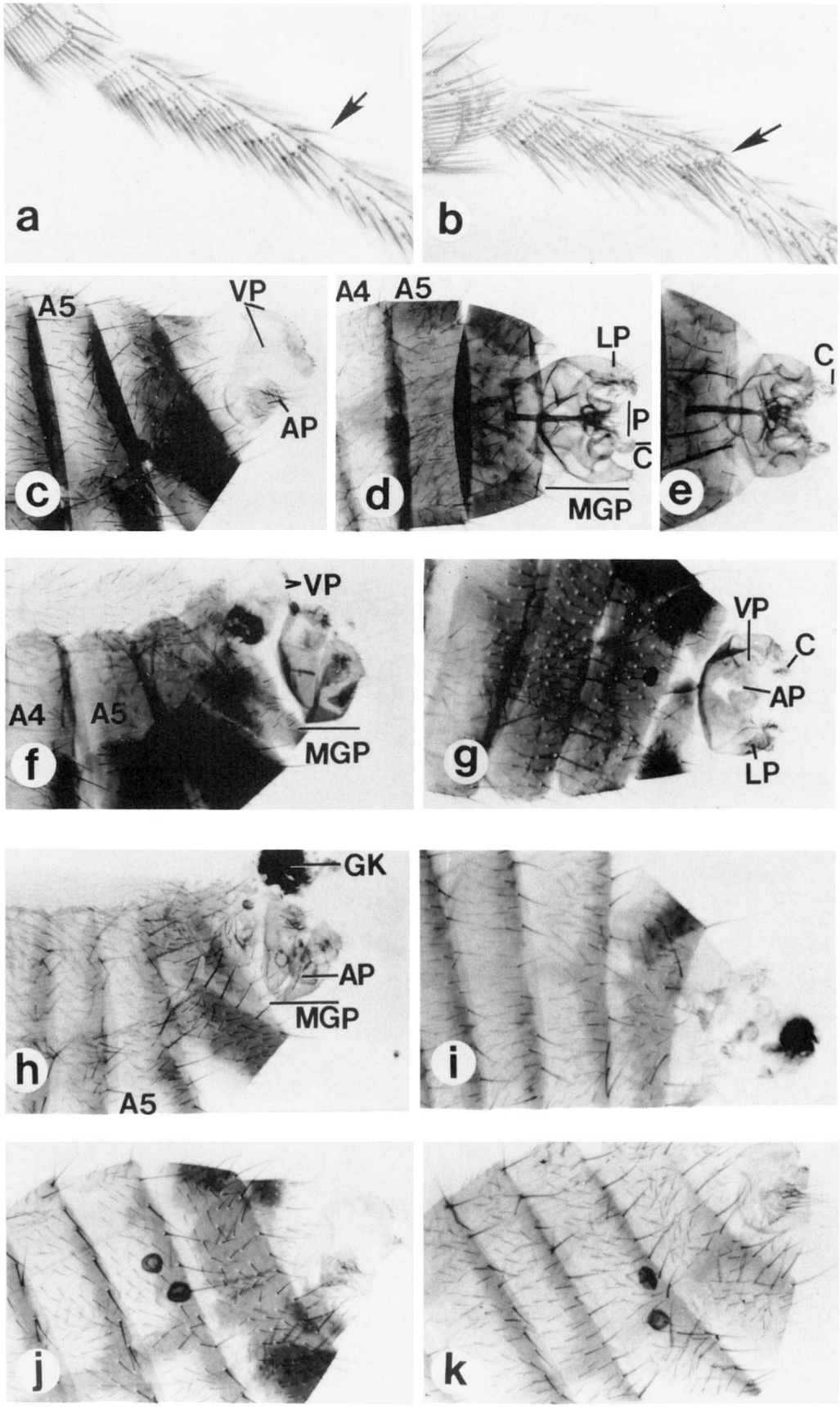

FIGURE 1.—Zygotic effects of *her* on sexual differentiation in females. Development of sexually dimorphic structures on the foreleg (a,b) and the abdomen (c–k). The female counterparts of the male sex comb bristles are indicated with arrows for (a) Canton S, 25° and (b) her^1/her^2, 29°. Partial rotation of this row and increased bristle number indicate a "true intersex" phenotype. A wild-type female abdomen (c) shows normal female abdominal pigmentation, genitalia and analia (Canton S, 25°); A5: fifth abdominal segment, pigmented only posteriorly;

TABLE 3

Zygotic effects of *her* on viability in both sexes

	Proportion of *her* individuals surviving[a] (number surviving/number expected): Females / Males			
	her^3	her^1	her^2	$her^{l(2)mat}$
18°				
$Df(her^-)$	<0.01 (0/151)	<0.01 (0/102)	0.25 (34/135)	0.55 (99/181)
	<0.01 (0/124)	0.07 (7/102)	0.20 (23/115)	0.52 (108/207)
her^3	0.41 (53/130)	0.58 (131/227)	1.15 (129/113)	1.26 (149/118)
	0.39 (46/118)	0.84 (175/209)	0.92 (96/104)	0.94 (89/95)
her^1		0.98 (196/199)	1.14 (191/168)	1.02 (140/137)
		0.72 (145/201)	1.13 (190/168)	0.94 (126/135)
her^2			—	1.02 (196/193)
				0.99 (142/144)
$her^{l(2)mat}$				1.23 (129/105)
				1.13 (117/104)
25°				
$Df(her^-)$	<0.01 (0/189)	<0.01 (0/100)	<0.01 (0/131)	0.57 (116/202)
	<0.01 (0/192)	<0.01 (0/129)	<0.01 (0/152)	0.67 (108/162)
her^3	0.02 (3/136)	0.23 (34/150)	0.08 (12/157)	1.02 (95/93)
	0.05 (8/154)	0.24 (43/230)	0.19 (35/184)	1.22 (98/81)
her^1		0.43 (92/213)	0.28 (44/160)	1.05 (243/231)
		0.53 (119/224)	0.33 (59/177)	1.12 (160/143)
her^2			—	1.22 (226/187)
				0.96 (137/142)
$her^{l(2)mat}$				1.15 (156/136)
				1.09 (139/128)
29°				
$Df(her^-)$	<0.01 (0/109)	<0.01 (0/163)	<0.01 (0/110)	0.20 (41/201)
	<0.01 (0/121)	<0.01 (0/153)	<0.01 (0/117)	0.23 (41/182)
her^3	<0.01 (0/138)	0.01 (1/92)	0.02 (2/105)	0.23 (59/130)
	<0.01 (0/130)	0.07 (7/95)	0.15 (15/98)	0.23 (46/100)
her^1		0.17 (12/70)	0.09 (15/161)	0.28 (29/104)
		0.18 (15/86)	0.32 (45/143)	0.38 (35/92)
her^2			—	0.25 (29/116)
				0.26 (27/101)
$her^{l(2)mat}$				1.20 (85/71)
				0.92 (64/70)

The general form of crosses is *her/Gla* × *her/Gla* (except for crosses with $Df(her^-)$, see below). For crosses of this form, the number of expected *her* individuals is calculated as one-half of the number of surviving *Gla* individuals. For all crosses with $Df(her^-)$, the deficiency was introduced from the father. For all other crosses in columns 1–3, the allele on the X axis was introduced from the mother, except for her^1 × her^2. For crosses of *her* alleles × $her^{l(2)mat}$ (column 4) $her^{l(2)mat}$ was introduced from the father.

Viability and fertility of $Df(2L)TE116(R)GW23(her^-)/Gla$ flies is low, so the $Df(her^-)$ crosses included a segregating duplication, $Dp(2;3)osp^3$, *Ki* her^+, which covers the material deleted by the deficiency (also see Table 6, Figure 2). Here, the cross is *her/Gla* mothers × *her/Gla*; $Dp(her^+)$, *Ki*/+ fathers. The $her/Df(her^-)$; $Dp(her^+)$/+ flies were used as the control class for estimating expected numbers of $her/Df(her^-)$ individuals.

Full genotypes: her^2 = *SM1* her^2 *Cy*, and her^3 = her^3 *pr*. her^1 × her^1 = her^1 *pr/Gla* × *b* her^1*/Gla*. her^1 × her^3 = *b* her^1 *pr/Gla* × her^3 *pr/Gla*. For other crosses, her^1 = her^1 *pr*. For crosses to $Df(her^-)$, $her^{l(2)mat}$ = *b* $her^{l(2)mat}$. For heteroallelic crosses, $her^{1(2)mat}$ is unmarked. $her^{l(2)mat}$ × $her^{l(2)mat}$ = $her^{l(2)mat}$*/Gla* × *b* $her^{l(2)mat}$*/Gla*. $Df(her^-)$ × *her* = $Df(2L)TE116(R)GW23$, *cn/Gla*; $Dp(2;3)osp^3$, *Ki* her^+/+ fathers × *her/Gla* mothers.

[a] Eclosion is the criterion for survival.

VP: vaginal plates, derivatives of the female genital primordium, bearing characteristic vaginal teeth; AP: anal plates, upper and lower. Wild-type male abdomens, (d, e) show normal male abdominal pigmentation and male genital structures, analia are out of the plane of focus (Canton S, 25°); A4, A5: fourth and fifth abdominal segments, fifth segment fully pigmented; MGP: male genital plates, derivatives of the male genital primordium, which include LP: lateral plates and C: claspers; P: phallus apparatus. The *her* intersexual phenotype (f, g) is represented by her^3/her^3 chromosomal females, 18°: both female and male genital structures are present; pigmentation extends almost as far anteriorly in A5 as in a wild-type male; anal plates, left and right, are in a male-like orientation. A weaker intersexual transformation is seen in (h), her^1/her^1 chromosomal female, 29°; GK: genital knob, a mass of poorly differentiated sclerotized tissue seen in place of female genital structures; A5 pigmentation is female-like, anal plates are fused. An even weaker intersexual transformation is seen in (i), her^1/her^2 chromosomal female, 29°: female genitalia are represented by a genital knob, which usually contains spermathecae, male genital structures are poorly developed and disorganized. At 18°, her^1/her^1 females are morphologically normal (j). The $her^{l(2)mat}$ allele complements zygotic *her* effects on female sexual differentiation (k): $her^1/her^{l(2)mat}$ female, 29°.

TABLE 4

Sex-specific maternal effects of *her*

Experimental crosses: *her/her* mothers × wild type fathers (or Sxl^{fm1} fathers)
Control crosses: *her/her*; *Dp(her⁺)/+* mothers × wild type fathers (or Sxl^{fm1} fathers)
Results are given as: ratio (daughters:sons)

Maternal genotype	Experimental mothers		Control mothers	
	Canton S fathers	Sxl^{fm1} fathers	*Canton S* fathers	Sxl^{fm1} fathers
A. Brooded at 25°				
$her^{l(2)mat}/her^{l(2)mat}$	0.07 (70:1012)	<0.003 (0:348)	1.02 (849:836)	0.81 (438:532)
$her^{l(2)mat}/her^{1}$	0.06 (14:223)	<0.003 (0:319)	0.93 (290:311)	0.98 (450:457)
$her^{l(2)mat}/her^{2}$	0.19 (93:484)	0.012 (4:308)	0.84 (162:193)	1.02 (110:107)
$her^{l(2)mat}/her^{3}$	0.22 (377:1683)	0.005 (9:2009)	1.01 (857:850)	0.92 (960:1042)
her^{1}/her^{1}	0.13 (76:608)	0.006 (7:1191)	1.00 (602:601)	0.87 (200:229)
her^{1}/her^{2}	0.14 (57:421)	0.009 (12:1373)	0.93 (341:368)	1.11 (188:169)
her^{2}/her^{3}	0.54 (561:1045)	0.003 (1:397)	1.04 (632:609)	1.06 (281:265)
B. Brooded at 18°				
$her^{l(2)mat}/her^{l(2)mat}$	0.97 (288:297)	0.66 (445:671)	1.06 (225:212)	1.03 (258:251)
$her^{l(2)mat}/Df(her^{-})$	0.36 (115:317)	0.06 (28:479)	0.96 (285:298)	1.03 (817:789)

Full genotypes: $Dp(her^{+}) = Dp(2;3)osp^{3}$, *Ki*, her^{+}. $Sxl^{fm1} = Sxl^{fm1}$ *oc ptg v*. 2A: $her^{l(2)mat}/her^{l(2)mat} = b\ her^{l(2)mat}/her^{l(2)mat}$. $her^{l(2)mat}/her^{1} = b\ her^{l(2)mat}/her^{1}$ *or*. $her^{l(2)mat}/her^{2} = SM1$, her^{2}, *Cy/b* $her^{l(2)mat}$. $her^{l(2)mat}/her^{3} = b\ her^{3}\ pr/her^{l(2)mat}$. $her^{1}/her^{1} = her^{1}\ pr/her^{1}\ px\ sp$. $her^{1}/her^{2} = her^{1}$ *or/SM1*, her^{2}, *Cy*. $her^{2}/her^{3} = SM1$, her^{2}, *Cy/b* her^{3} *pr*. 2B: $Df(her^{-}) = Df(2L)TE116(R)GW23$, *cn*. $her^{l(2)mat} = b\ her^{l(2)mat}$. $her^{1}/her^{2} = her^{1}$ *or/SM1*, her^{2}, *Cy*.

experiment (Table 4), ranging from 0.06–0.54. In addition, surviving daughters had a high frequency of morphological defects not seen in sons. Some daughters had deformations of the head or thorax; others, most frequently, had defective or undeveloped sixth and/or seventh abdominal segments.

When daughters of her^{-} mothers are made heterozygous for a null allele of the *X*-linked *Sxl* gene (Sxl^{fm1}, introduced from the fathers), lethality is enhanced. In this case, the ratio of daughters to sons is <0.02 for all combinations of *her* alleles. We have included these results here because they broaden the description of *her* maternal effects seen with different allelic combinations, and because this genetic interaction has provided a useful, highly stringent assay for the sex-specific aspect of maternal *her* function (for example, see Table 1B and Figure 2, below). Further studies of the relationship between *her* and the other components of the sex determination regulatory hierarchy will be presented elsewhere (M. A. PULTZ and B. S. BAKER, submitted).

The sex-specific maternal effects of *her* are temperature-sensitive, as demonstrated in Table 4B. At 18°, $her^{l(2)mat}$ homozygotes have sufficient her^{+} function to support an even sex ratio among the progeny of *Canton S* fathers; the relative survival of daughters heterozygous for Sxl^{fm1} is also dramatically improved at 18°, although these daughters still do not survive as well as their brothers. Other combinations of *her* alleles also showed temperature sensitivity in such assays.

To test whether the sex-specific maternal effects of *her* are hypomorphic, it was necessary to test the genotype $her^{l(2)mat}/Df(her^{-})$, as the other *her* alleles are lethal or sterile over the her^{-} deficiency. It was also necessary to test the maternal phenotypes at 18°, because both male and female progeny of $her^{l(2)mat}/Df(her^{-})$ mothers die at 25°, due to the hypomorphic nature of the sex-nonspecific *her* maternal effects (see next section, below). However, a clear difference was apparent when $her^{l(2)mat}$ homozygous mothers and $her^{l(2)mat}/Df\ (her^{-})$ mothers were brooded at 18°, as shown in Table 4B: residual *her* function in homozygous mothers is greater than in hemizygous mothers. Therefore, $her^{l(2)mat}$ behaves as a partial loss-of-function mutation in this assay, indicating that the sex-specific maternal-effect phenotype is revealing a wild-type function of *her*.

Taken together, data from Table 4 show that the different *her* alleles cannot be as easily ranked for the strength of their sex-specific maternal effects as for their zygotic effects (above), though $her^{l(2)mat}$ and her^{1} generally appear to have stronger effects than her^{3} and her^{2} in these experiments. In fact, $her^{l(2)mat}$ is a

TABLE 5

Sex-nonspecific maternal effects of *her*

Experimental crosses: *her/her* mothers × *Canton S* fathers
Control crosses: *her/her; Dp(her⁺)* mothers × *Canton S* fathers
Mothers were brooded at 18°, shifted to 29° for 48 hr, brooded at 29°, returned to 18° for 72 hr, and brooded again at 18°.
(Data are shown as proportion of embryos hatching, and number hatching/total)

	Maternal genotype			
	$her^1/her^{l(2)mat}$	her^1/her^2	$her^3/her^{l(2)mat}$	her^3/her^2
I. Brooded at 18°				
Experimental	0.93 (100/107)	0.90 (100/111)	0.98 (101/103)	0.96 (100/104)
Control	0.92 (100/109)	0.89 (100/112)	0.97 (91/93)	0.93 (100/108)
II. Brooded at 29°				
Experimental	0.01 (1/101)	0.01 (1/101)	0.10 (11/109)	0.61 (100/163)
Control	0.81 (100/123)	0.67 (100/149)	0.75 (91/121)	0.64 (100/156)
III. Returned to 18°				
Experimental	0.85 (104/122)	0.82 (121/148)	0.66 (133/203)	0.86 (100/116)
Control	0.77 (102/133)	0.74 (103/139)	0.62 (82/132)	0.85 (108/127)

Full genotypes: $her^1/her^{l(2)mat}$ = her^1 *pr*/$her^{l(2)mat}$.
$her^3/her^{l(2)mat}$ = her^3 *pr*/$her^{l(2)mat}$. her^2/her^1 = *SM1*, her^2 *Cy*/her^1 *pr*.
her^3/her^2 = her^3 *pr*/*SM1*, her^2 *Cy*. *Dp*(her^+) = Dp(2;3)osp^3, *Ki* her^+.
Df(her^-) = *Df(2L)TE116(R)GW23*, *cn*.

reasonable candidate for the allele with the strongest sex-specific maternal effects, although this allele is not defective for *her* zygotic functions (also see sex-nonspecific maternal effects, below).

In summary, all *her* alleles have a temperature-sensitive maternal effect that preferentially impairs the viability of daughters under semirestrictive conditions (25°), and this phenotype appears to be caused by a partial loss of *her* function. Next, we examine the maternal effects of *her* mutations under more severely restrictive conditions.

Maternal sex-nonspecific lethality: In addition to sex-specific maternal effects, revealed under semipermissive conditions (25°), there is also a maternal *her* function required for embryos of both sexes, revealed under severely restrictive conditions (29°). After *her* mothers have been held at 29° for 2 days, progeny of both sexes die as embryos, and the lethal mutant phenotype of embryos becomes progressively more severe as mothers continue to be brooded at 29°. The first obvious defect, detected in the majority of dead progeny, is a differentiated but twisted cuticle, with improperly formed mouthparts due to failure of head involution, and with the posterior abdominal segments wrapped around toward the dorsal side, as though having failed in germ band retraction. As mothers are brooded continuously at 29° for more than 3 days, the cuticle phenotype of the embryos degenerates such that denticles and sclerotized mouthparts are not differentiated and the cuticle appears to contain holes. The eggshells (chorions) of the embryos also become transparent.

Examination of early embryos also revealed severe gastrulation defects, including irregular buckling of the germband, unusually deep indentation of the cephalic furrow, and failure to complete the normal

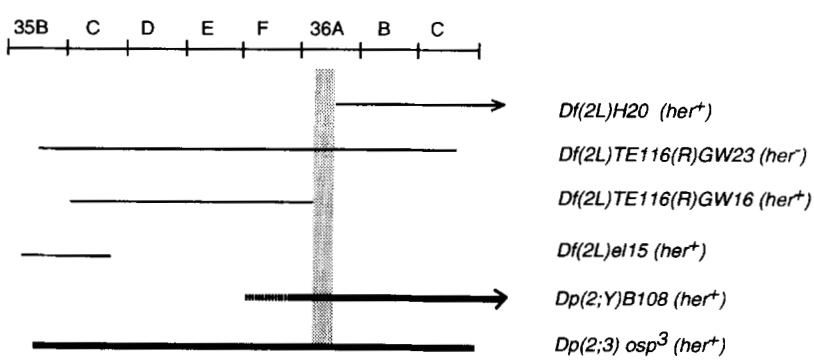

FIGURE 2.—Cytogenetic localization of *her*. Thin lines indicate material deleted, and thick lines indicate material duplicated. All of the deficiencies and *Dp(2;3)osp³* were tested for sex-specific and sex-nonspecific maternal and zygotic *her* phenotypes (see MATERIALS AND METHODS). *Dp(2;Y)B108* was tested only for zygotic effects on sex determination and viability. *Dp(2;Y)B108* rescues zygotic phenotypes in her^1 homozygotes at 29°, but its breakpoint is close enough to exhibit a variegating position effect on the *her* locus at lower temperatures (which normally enhance variegation): partial failure to complement can be detected when *Dp(2;Y)B108* is tested with her^1/*Df TE116(R)GW23*(her^-) at 18°. This analysis places *her* between 36A3 and 35A6-11.

TABLE 6

Rearrangements used for cytogenetic analysis

Rearrangement	Cytology	Source
Df(2L)H20	*Df(2L)36A6-11;36E3-F1.2*	ASHBURNER *et al.* 1990
Df(2L)TE116(R)GW23	*Df(2L)35B4.5;36C7*	ASHBURNER *et al.* 1990
Df(2L)TE116(R)GW16	*Df(2L)35C1;36A3*	ASHBURNER *et al.* 1990
Df(2L) el 15	*Df(2L)35B1.2;35C5*	ASHBURNER *et al.* 1990
Dp(2;Y)B108	*Dp(2;Y)35F-36DE*	LYTTLE 1981
Dp(2;3) osp^3	*Dp(2;3)35B3.4;36C11;98E1.2-F1.2*	ASHBURNER *et al.* 1990

process of germband extension and retraction. Ventral furrow formation and differentiation of the amnioserosa often appeared normal in a collection of embryos with uniformly impaired gastrulation, suggesting that the gastrulation defect is not primarily caused by failure to differentiate mesoderm or by misspecification of cell fate in the dorsal region.

All *her* alleles failed to complement in at least one heteroallelic combination for the maternal sex-nonspecific lethal phenotype at 29° (Table 5). This temperature-sensitive phenotype is reversible: after mothers were returned to 18° for 3 days, the progeny of mutant mothers hatched as frequently as the progeny of controls. For one allelic combination, *her*^{3}/*her*^{2}, progeny of mutant mothers hatched at 29° at the same frequency as the progeny of controls, indicating that these alleles complement one another with respect to the sex-nonspecific maternal effect phenotype. The *her*^{3}/*her*^{2} genotype also appeared to be a relatively weak allelic combination when assayed for the sex-specific maternal effect phenotype (Table 4, column 1, *Canton S* fathers).

To determine whether the sex-nonspecific maternal effect phenotype is due to a partial loss of *her* function, we compared progeny of *her*$^{l(2)mat}$/*Df (her*$^-$*)* mothers to progeny of *her*$^{l(2)mat}$/*her*$^{l(2)mat}$ mothers at the semipermissive temperature of 25°. Under these conditions, the proportion of hatching progeny was only 0.13 (15/117) for *her*$^{l(2)mat}$/*Df (her*$^-$*)* mothers, compared with 0.93 (141/152) for *her*$^{l(2)mat}$/*her*$^{l(2)mat}$ mothers and 0.96 (105/109) for *her*$^{l(2)mat}$/*Df(her*$^-$*);Dp(her*$^+$*)* control mothers. Progeny of *her*$^{l(2)mat}$/*Df (her*$^-$*)* mothers that failed to hatch at 25° also showed cuticular defects similar to those seen in progeny of other *her* mothers at 29°. This indicates that the sex-nonspecific maternal effect phenotype of *her* is due to a partial loss of *her* function and therefore reveals a wild-type function of *her*.

Cytogenetic localization: The *her* locus was originally localized by recombination to 52 ± 1, by mapping the zygotic effects of *her*^{1} on sex determination and viability. The *her*$^{l(2)mat}$ mutation had also been mapped by recombination to this region (SANDLER 1977). The *her* locus was then placed cytogenetically within this region, by complementation with deficiencies and duplications, as shown in Table 6 and Figure 2. All of the deficiencies and *Dp(2;3)osp*^{3} were tested for zygotic effects on sexual differentiation in both sexes and for zygotic viability effects. They were also tested for maternal effects, both sex-specific and sex-nonspecific (see MATERIALS AND METHODS). Ability to complement *her* alleles for sex-specific maternal functions was tested using our most stringent assay, the survival of female progeny heterozygous for *Sxl*$^{f\#1}$ (see above). This analysis places *her* between 36A3 and 36A11, a cytogenetic interval where no genetic functions have previously been mapped (ASHBURNER *et al.* 1990).

DISCUSSION

We have described *hermaphrodite* (*her*) as a Drosophila sex-determination regulatory gene based on its effects on female sexual differentiation; *her*$^+$ function may also be required for sexual differentiation in males. This analysis also shows that *her* is pleiotropic: *her* has a zygotic function needed for viability in both sexes, as well as maternal functions. Analysis under semirestrictive conditions reveals a maternal role for *her* that is critical for the survival of female progeny; analysis under highly restrictive conditions reveals that maternal *her* function is important for the survival of progeny of both sexes.

To begin to ask to what extent the different *her* phenotypes are separable, we have characterized four different *her* alleles. We have found that maternal functions are to some degree separable from zygotic functions; but there was no apparent separation of female-specific functions from male-specific functions or of sex-specific functions from sex-nonspecific functions. All of the alleles that are defective for zygotic functions are also defective for maternal functions–these include *her*^{1}, *her*^{2} and *her*^{3}. With respect to zygotic effects (both sex-specific and sex-nonspecific), the strength of the alleles appears to be *her*^{3} > *her*^{1}, *her*^{2} > *her*$^{l(2)mat}$. The *her*$^{l(2)mat}$ allele is not appreciably defective for zygotic effects; but the maternal effects of this allele are as strong as those of the other *her* alleles (if not stronger). Therefore, *her*$^{l(2)mat}$ can be considered to be preferentially impaired in maternal *her* functions, rather than being merely the weakest

allele in the series. We conclude from this analysis that all of the *her* phenotypes are attributable to the same gene, but that the maternal and zygotic aspects of *her* function are differentially mutable.

All four *her* alleles showed some degree of temperature-sensitivity, and all of the *her* phenotypes described here can be influenced by temperature. One apparent exception to this tendency is that the effects on female sexual differentiation in her^3 homozygotes do not appear to be significantly ameliorated even at the lowest temperature assayed (18°). This is probably because the her^3 allele is so strong that it has insufficient function to affect sexual differentiation even at 18°. Note, however, that her^3 homozygotes still exhibit marked temperature-sensitivity with respect to zygotic effects on viability. The temperature-sensitivity of all *her* alleles suggests that *her* may be participating in a physiological process that is intrinsically temperature-sensitive.

To ask whether the *her* phenotypes are due to a loss of *her* function, we examined the phenotype of the *her* alleles in combination with *Df(2L)TE116(R)GW23*, designated here as $Df(her^-)$. This deficiency completely deletes the *her* locus–it extends from 35B to 36C and is $Minute^+$ (Ashburner *et al.* 1990). For all four *her* alleles, phenotypes were more severe when heterozygous with the deficiency than when homozygous, so these are all formally partial loss-of-function alleles. Furthermore, all of the phenotypes analyzed here are formally due to insufficient *her* function. We note that the large size of the her^- deficiency introduces a caveat to the interpretation of all experiments assaying viability: viability could also be impaired by nonspecific synergistic lethal effects. Bearing this caveat in mind, these results are consistent with the interpretation that wild-type function of *her* is required both maternally and zygotically, for sex-specific as well as sex-nonspecific developmental processes.

Our focus in this study is a description of *her* functions as they can be understood by examining the properties of *her* mutations–an analysis of *her* in relationship to other components of the sex determination regulatory hierarchy will be presented elsewhere (M. A. PULTZ and B. S. BAKER, submitted). However, there are some parallels between *her* and two other components of the sex determination regulatory hierarchy, *da* and *dsx*, that we would like to consider briefly here.

The maternal effects of *her* bear formal comparison to those of *da*. Maternal function of both genes is required for the survival of female embryos under semirestrictive conditions, at 25°, and maternal function of both genes is absolutely required for the survival of all progeny under highly restrictive conditions, at 29° (CLINE 1976; CRONMILLER and CLINE 1986). Function of both genes must also be supplied zygotically to ensure viability in both sexes. Furthermore, both *her* and *da* have phenotypes that suggest participation in an intrinsically temperature-sensitive process. For *her*, this interpretation is supported by the temperature-sensitivity of all *her* alleles. For *da*, participation in an intrinsically temperature-sensitive process was first implied by the observation that reduced da^+ dosage yields a temperature-sensitive pattern of interaction with *Sxl* (CLINE 1980). In addition, the temperature-sensitive da^1 mutation is associated with an insertion located 5′ to the translated portion of the *da* gene (CRONMILLER, SCHEDL and CLINE 1988). It is not yet clear whether any sex-nonspecific functions of *her* and *da* will prove to be comparable–further answers to this question await the analysis of a definitive null *her* allele.

The zygotic effects of *her* on sexual differentiation suggest a different comparison. With a partial loss of zygotic *her* function, females are strongly transformed to intersexes while males have more subtle morphological defects. The phenotype seen in her^- males (bristles on the sixth sternite and rotated genitalia) could be interpreted as segmental transformations. However, these effects are similar to those observed when function of *dsx* in males is partially compromised, suggesting that such defects may be caused by weak sexual transformation. Complete loss of *dsx* function in both sexes transforms both females and males to intersexes; but partial loss of *dsx* function (seen with the six existing hypomorphic alleles) strongly transforms females to intersexes while only marginally affecting male sexual development (NÖTHIGER *et al.* 1987; BAKER *et al.* 1991). Thus, we propose that with partially reduced function of either *her* or *dsx*, females succumb more easily than males to intersexual development.

In conclusion, the study of sex determination in Drosophila has revealed that this process depends on two types of regulatory genes. At the core of the regulatory hierarchy, genes like *Sxl*, *tra* and *dsx* are expressed sex-specifically and impose sex-specific developmental decrees. Carrying out these instructions relies on auxiliary genes that are sex-nonspecific in their expression and pleiotropic in their functions. These auxiliary ranks are joined here by *hermaphrodite*, providing another perspective on the complex responsibilities that can be assumed by developmental regulatory genes.

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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 domain. Cell **55:** 1025–1035.

ASHBURNER, M., P. THOMPSON, J. ROOTE, P. F. LASKO, Y. GRAU, *et al.*, 1990 The genetics of a small autosomal region of *Drosophila melanogaster* containing the structural gene for alcohol dehydrogenase. VII. Characterization of the region around the *snail* and *cactus* loci. Genetics **126:** 679–694.

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*. Annu. Rev. Genet. **17:** 345–393.

BAKER, B. S., A. T. C. CARPENTER and P. RIPOLL, 1978 The utilization during mitotic cell division of loci controlling meiotic recombination and disjunction in *Drosophila melanogaster*. Genetics **90:** 531–578.

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. Genetics **94:** 383–423.

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

BELL, L. R., E. M. MAINE, P. SCHEDL and T. W. CLINE, 1988 *Sex-lethal*, a Drosophila sex determination switch gene, exhibits sex-specific RNA splicing and sequence similarity to RNA binding proteins. Cell **55:** 1037–1046.

BELL, L. R., J. I. HORABIN, P. SCHEDL and T. W. CLINE, 1991 Positive autoregulation of *Sex-lethal* by alternative splicing maintains the female determined state in Drosophila. Cell **65:** 229–239.

BELOTE, J. M., 1992 Sex determination in Drosophila melanogaster: from the *X:A* ratio to *doublesex*. Semin. Dev. Biol. **3:** 319–330.

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

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

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.

BOPP, D., L. R. BELL, T. W. CLINE and P. SCHEDL, 1991 Developmental distribution of female specific *Sex-lethal* proteins in *Drosophila melanogaster*. Genes Dev. **5:** 403–415.

BRIDGES, C. B., 1925 Sex in relation to chromosomes and genes. Am. Nat. **59:** 127–137.

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., and M. F. WOLFNER, 1992 The view from the bottom: sex-specific traits and their control in Drosophila. Semin. Dev. Biol. **3:** 331–340.

CAUDY M., E. H. GRELL, C. DAMBLY-CHAUDIERE, A. GHYSEN, L. Y. JAN,*et al.*, 1988 The maternal sex determination gene *daughterless* has zygotic activity necessary for the formation of peripheral neurons in Drosophila. Genes Dev. **2:** 843–852.

CLINE, T. W., 1976 A sex-specific, temperature-sensitive maternal effect of the *daughterless* mutation of *Drosophila melanogaster*. Genetics **84:** 723–742.

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., 1979 A male specific lethal mutation in *Drosophila melanogaster* that transforms sex. Dev. Biol. **72:** 266–275.

CLINE, T. W., 1980 Maternal and zygotic sex-specific gene interactions in *Drosophila melanogaster*. Genetics **96:** 903–926.

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. Liss, New York.

CLINE, T. W., 1986 A female specific lethal 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., 1988a 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.

CLINE, T. W., 1988b Exploring the role of the gene, *Sex-lethal*, in the genetic programing of Drosophila sexual dimorphism, pp. 23–36 in *Evolutionary Mechanisms in Sex Determination* (CRC Uniscience Series), edited by S. S. WACHTEL. CRC Press, Cleveland.

CLINE, T. W., 1989 The affairs of *daughterless* and the promiscuity of developmental regulators. Cell **59:** 231–234.

CRONMILLER, C., and T. W. CLINE, 1986 The relationship of relative gene dose to the complex phenotype of *daughterless* locus in Drosophila. Dev. Genet. **7:** 205–221.

CRONMILLER, C., and T. W. CLINE, 1987 The Drosophila sex determination gene *daughterless* has different functions in the germ line *versus* the soma. Cell **48:** 479–487.

CRONMILLER C., P. SCHEDL and T. W. CLINE, 1988 Molecular characterization of *daughterless*, a Drosophila sex determination gene with multiple roles in development. Genes Dev. **2:** 1666–1676.

DUFFY, J. B., and J. P. GERGEN, 1991 The Drosophila segmentation gene *runt* acts as a position-specific numerator element necessary for the uniform expression of the sex-determining gene *Sex-lethal*. Genes Dev. **5:** 2176–2187.

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.

EPPER, F., and R. NÖTHIGER, 1982 Genetic and developmental evidence for a repressed genital primordium in *Drosophila melanogaster*. Dev. Biol. **94:** 163–175.

ERICKSON, J. W., and T. W. CLINE, 1991 Molecular nature of the Drosophila sex determination signal and its link to neurogenesis. Science **251:** 1071–1074.

GERGEN, J. P., 1987 Dosage compensation in Drosophila: evidence that *daughterless* and *Sex-lethal* control chromosome activity at the blastoderm stage of embryogenesis. Genetics **117:** 477–485.

GORALSKI, T., J. EDSTRÖM 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.

GORMAN M., M. I. KURODA and B. S. BAKER, 1993 Regulation of the sex-specific binding of the *maleless* dosage compensation protein to the male *X* chromosome in Drosophila. Cell **72:** 39–50.

GRANADINO, B., S. CAMPUZANO and L. SÁNCHEZ, 1990 The gene *fl(2)d* is needed for various *Sxl*-controlled processes in Drosophila females. EMBO J. **9:** 2597–2602.

GRANADINO, B., A. SAN JUÁN, P. SANTAMARIA and L. SÁNCHEZ,

1992 Evidence of a dual function in *fl(2)d*, a gene needed for *Sex-lethal* expression in *Drosophila melanogaster*. Genetics **130:** 597–612.

HEDLEY, M. L., and T. MANIATIS, 1991 Sex-specific splicing and polyadenylation of *dsx* requires a sequence that binds specifically to *tra-2* protein *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:** 659–679.

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.

KEYES, L. N., T. W. CLINE and P. SCHEDL, 1992 The primary sex determination signal of Drosophila acts at the level of transcription. Cell **68:** 933–943.

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

LINDSLEY, D. L., L. SANDLER, B. S. BAKER, A. T. C. CARPENTER, R. E. DENNELL, *et al.*, 1972 Segmental aneuploidy and the genetic gross structure of the Drosophila genome. Genetics **71:** 157–184.

LUCCHESI, E. B., and T. SKRIPSKY, 1981 The link between dosage compensation and sex determination in *Drosophila melanogaster*. Chromosoma **82:** 217–227.

LUCCHESI, J. C., and J. E. MANNING, 1987 Gene dosage compensation in *Drosophila melanogaster*. Adv. Genet. **24:** 371–429.

LYTTLE, T. W., 1981 High efficiency production of site-specific *Y*-autosome insertional translocations in *D. melanogaster*. Genetics **97:** s67.

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:** 786–796.

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

MORGAN, T., H. REDFIELD and L. V. MORGAN, 1943 Maintenance of a Drosophila stock center in connection with investigations on the constitution of germinal material in relation to heredity. Carnegie Inst. Wash. Year Book **42:** 171–174.

MURRE, C., P. S. MCCAW and D. BALTIMORE, 1989a A new DNA binding and dimerization motif in immunoglobulin enhancer binding, *daughterless*, *MyoD* and *myc* proteins. Cell **56:** 777–783.

MURRE, C., P. S. MCCAW, H. VAESSIN, M. CAUDY, L. Y. JAN, *et al.*, 1989b Interactions between heterologous helix-loop-helix proteins generate complexes that bind specifically to a common DNA sequence. Cell **58:** 537–544.

NAGOSHI, R. N., M. MCKEOWN, K. 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., A. DÜBENDORFER and F. EPPER, 1977 Gynandromorphs reveal two separate primordia for male and female genitalia in *Drosophila melanogaster*. Wilhelm Roux's Arch. Dev. Biol. **181:** 367–373.

NÖTHIGER, R., M. LEUTHHOLD, N. ANDERSON, P. GERSCHWILLER, A. GRUTER, *et al.*, 1987 Genetic and developmental analysis of sex-determination gene *doublesex* (*dsx*) of *Drosophila melanogaster*. Genet. Res. Camb. **50:** 113–123.

PARKHURST, S. M., D. BOPP and D. ISH-HOROWICZ, 1990 *X:A* ratio, the primary sex determining signal in Drosophila, is transduced by helix-loop-helix proteins. Cell **63:** 1179–1191.

REDFIELD, H., 1924 A case of maternal inheritance in Drosophila. Am. Nat. **58:** 566–569.

REDFIELD, H., 1926 The maternal inheritance of a sex-limited lethal effect in *Drosophila melanogaster*. Genetics **11:** 482–502.

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.

SALZ, H. K., E. M. MAINE, L. N. KEYES, M. E. SAMUELS, T. W. CLINE, *et al.*, 1989 The Drosophila female-specific sex determination gene, *Sex-lethal*, has stage, tissue and sex specific RNAs suggesting multiple modes of regulation. Genes Dev. **3:** 708–719.

SANDLER, L., 1972 On the genetic control of genes located in the sex chromosome heterochromatin of *Drosophila melanogaster*. Genetics **70:** 261–274.

SANDLER L., 1977 Evidence for a set of closely linked autosomal genes that interact with sex chromosome heterochromatin in *Drosophila melanogaster*. Genetics **86:** 567–582.

SCHÜPBACH, T., E. WIESCHAUS and R. NÖTHIGER, 1978 The embryonic organization of the genital disc studied in genetic mosaics of *Drosophila melanogaster*. Wilhelm Roux's Arch. Dev. Biol. **185:** 249–270.

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., 1992 Differentiation of a male-specific muscle in *Drosophila melanogaster* does not require the sex-determining genes *doublesex* or *intersex*. Genetics **132:** 179–191.

TOKUNAGA, C., 1962 Cell lineage and differentiation on the male foreleg of *Drosophila melanogaster*. Dev. Biol. **4:** 489–516.

TORRES, M., and L. SÁNCHEZ, 1989 The *scute* (T4) gene acts as a numerator element of the *X:A* signal that determines the state of activity of *Sex-lethal* in Drosophila. EMBO J. **8:** 3079–3086.

UCHIDA, S., T. UENOYAMA and K. OISHI, 1981 Studies on the sex-specific lethals of *Drosophila melanogaster*. III. A third chromosome male-specific lethal mutant. Jpn. J. Genet. **56:** 523–527.

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

YOUNGER-SHEPHERD, S., H. VAESSIN, E. BIER, L. Y. JAN and Y. N. JAN, 1992 *deadpan*, an essential pan-neural gene encoding an HLH protein, acts as a denominator in Drosophila sex determination. Cell **70:** 911–922.

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