The *doublesex* Locus of *Drosophila melanogaster* and Its Flanking Regions: A Cytogenetic Analysis

Bruce S. Baker,*[,1] Gary Hoff,* Thomas C. Kaufman,[†] Mariana F. Wolfner*[,2] and Tulle Hazelrigg[†,3]

**Biology Department, University of California, San Diego, California 92093 and [†]Department of Biology, Indiana University, Bloomington, Indiana 47405*

Manuscript received September 25, 1989
Accepted for publication September 22, 1990

ABSTRACT

The region of the third chromosome (84D-F) of *Drosophila melanogaster* that contains the *doublesex* (*dsx*) locus has been cytogenetically analyzed. Twenty nine newly induced, and 42 preexisting rearrangements broken in *dsx* and the regions flanking *dsx* have been cytologically and genetically characterized. These studies established that the *dsx* locus is in salivary chromosome band 84E1-2. In addition, these observations provide strong evidence that the *dsx* locus functions only to regulate sexual differentiation and does not encode a vital function. To obtain new alleles at the *dsx* locus and to begin to analyze the genes flanking *dsx*, 59 lethal and visible mutations in a region encompassing *dsx* were induced. These mutations together with preexisting mutations in the region were deficiency mapped and placed into complementation groups. Among the mutations we isolated, four new mutations affecting sexual differentiation were identified. All proved to be alleles of *dsx*, suggesting that *dsx* is the only gene in this region involved in regulating sexual differentiation. All but one of the new *dsx* alleles have equivalent effects in males and females. The exception, dsx^{EFH55}, strongly affects female sexual differentiation, but only weakly affects male sexual differentiation. The interactions of dsx^{EFH55} with mutations in other genes affecting sexual differentiation are described. These results are discussed in terms of the recent molecular findings that the *dsx* locus encodes sex-specific proteins that share in common their amino termini but have different carboxyl termini. The 72 mutations in this region that do not affect sexual differentiation identify 25 complementation groups. A translocation, *T(2;3)Es* that is associated with a lethal allele in one of these complementation groups is also broken at the *engrailed* (*en*) locus on the second chromosome and has a dominant phenotype that may be due to the expression of *en* in the anterior portion of the abdominal tergites where *en* is not normally expressed. The essential genes found in the 84D-F region are not evenly distributed throughout this region; most strikingly the 84D1-11 region appears to be devoid of essential genes. It is suggested that the lack of essential genes in this region is due to the region (1) containing genes with nonessential functions and (2) being duplicated, possibly both internally and elsewhere in the genome.

THE *doublesex* (*dsx*) locus is a pivotal gene in the regulatory hierarchy controlling somatic sexual differentiation in *Drosophila melanogaster* (for reviews see BAKER and BELOTE 1983; CLINE 1985; NOTHIGER and STEINMANN-ZWICKY 1987; BAKER, NAGOSHI and BURTIS 1987). *dsx* is the only gene in the somatic sex determination regulatory hierarchy that functions in the somas of both sexes. It is thought that *dsx* is bifunctional: in males it represses female somatic sexual differentiation whereas in females it represses male somatic sexual differentiation (BAKER and RIDGE 1980). Loss of function mutations at *dsx* result in intersexual development in both chromosomally male and female individuals.

We have carried out a cytogenetic analysis of the *dsx* region (84D-F) in proximal 3R in order to (1) determine the cytological location of *dsx*, (2) identify new *dsx* mutants and (3) determine whether *dsx* was part of a regulatory gene complex controlling sexual differentiation or, alternatively, surrounded by genes of unrelated function.

To determine the cytological location of the *dsx* locus we induced and cytologically characterized a number of inversions, translocations and deletions that inactivate or delete the *dsx* locus. This material has enabled us to molecularly clone the region containing the *dsx* locus and identify the DNA sequences that correspond to *dsx* (BAKER and WOLFNER 1988; BURTIS and BAKER 1989). In addition, we carried out a saturation mutagenesis of the region encompassing the *dsx* locus and report here the isolation of new *dsx* alleles and a collection of mutations that define lethal complementation groups flanking the *dsx* locus.

[1] Current address: Department of Biological Sciences, Stanford University, Stanford, California 94305.
[2] Current address: Section of Genetics and Development, Cornell University, Ithaca, New York 14853.
[3] Current address: Biology Department and Howard Hughes Medical Institute, University of Utah, Salt Lake City, Utah 84112.

MATERIALS AND METHODS

Flies were reared at 25° on a standard cornmeal, yeast, molasses, agar medium supplemented with propionic acid and live yeast. Except for the mutations and rearrangements whose isolation is reported here, and the rearrangements described in Table 1, descriptions of the mutants and chromosomes used in this study can be found in LINDSLEY and GRELL (1968).

Salivary gland chromosome analysis was done on lacto-acetic-orcein stained chromosome preparations from individuals reared at 18°. Cytological analysis was carried out on chromosomes in which the rearrangement under study was heterozygous with a wild type chromosome.

X-ray-induced derivatives of dsx^{Mas}: To induce reversions or inactivations of the dominant *dsx* allele, dsx^{Mas}, males that were $dsx^{Mas}/TM3$ were X-rayed with 4000 R (Torrex X-ray machine, 120 kV, 5 mA, 1-mm plexiglass filter) and crossed to *TM1/TM2* females. The *diplo-X*; $dsx^{Mas}/TM\_$ progeny of this cross were scored for the absence of intersexuality; the dominant dsx^{Mas} allele when heterozygous with a wild-type *dsx* allele in a diplo-*X* individual produces intersexuality. Among 41,689 diplo-*X* $dsx^{Mas}/TM\_$ individuals examined, 41 were normal appearing females and thus represented potential reversions or inactivations of dsx^{Mas}. Thirty one of these females were fertile and transmitted the dsx^{Mas}-derived chromosome of interest. Balanced stocks were made of these chromosomes. All 31 chromosomes were tested genetically for their genotype at *dsx* and all proved to carry loss-of-function *dsx* mutations and thus represent inactivations of dsx^{Mas}. Two of these chromosomes were lost before they could be cytologically analyzed.

Induction of lethal and visible mutations: To induce mutations in *dsx* and the regions flanking it, *red e* males were fed 0.025 M ethyl methanesulfonate (LEWIS and BACHER 1968) and crossed to females bearing the *TM3* balancer chromosome. F_1 *red e/TM3* males were crossed singly to $Df(3R)dsx^{D+R2}/CxD$ females and the presence of lethal or visible mutations ascertained in their *red e*/ $Df(3R)dsx^{D+R2}$ offspring. Balanced stocks of mutants were established from *red e/CxD* sibs. From 2386 fertile F_1 individuals tested, 4 *dsx* mutations and 55 confirmed lethal mutations were recovered.

RESULTS

Analysis of rearrangements in 84D-F: To induce a set of chromosomal rearrangements that would (1) identify the cytological location of *dsx*, (2) generate a set of deletions in the region around *dsx* for use in deletion mapping, and (3) provide breakpoints for identifying the DNA sequences corresponding to *dsx*, a set of inactivations of the dominant *dsx* allele dsx^{Mas} were induced with X-rays (see MATERIALS AND METHODS). These mutations have been named $dsx^{Mas+R\_}$. Salivary gland chromosome squashes from 29 of these revertants were analyzed to determine which were rearrangements and, where rearrangements were detected, the locations of their breakpoints (Table 1, Figure 1). Of the chromosomes bearing $dsx^{Mas+R\_}$, 14 have deficiencies, 5 have translocation breakpoints, and 4 have inversion breakpoints in the region where *dsx* was known to be located (DUNCAN and KAUFMAN 1975; DENELL and JACKSON 1972; BAKER and WOLFNER 1988). The 6 remaining chromosomes are cytologically normal in this region. In addition to the rearrangements just described, 42 rearrangements previously isolated by others in the 84D-F region were also analyzed to determine their breakpoints. These included 10 X-ray-induced revertants of another dominant *dsx* allele, dsx^{D} (these revertants are designated $dsx^{D+R\_}$). Together the results of these analyses (Table 1, Figure 1) served both to provide information about the *dsx* locus and to identify a set of deficiencies that we could use to map other mutations in the 84D-F region.

The *dsx* locus: Previous genetic studies showed that the dsx^{Mas} allele behaved as if it resulted in the constitutive expression of the male-specific function of the *dsx* locus (BAKER and RIDGE 1980). Thus dsx^{Mas} would be expected to be mutated to a recessive allele by mutations that inactivated the *dsx* gene on a dsx^{Mas}-bearing chromosome. Examination of the sexual phenotypes produced by each of the 31 $dsx^{Mas+R\_}$ chromosomes when heterozygous with the original *dsx* point mutant (a null *dsx* allele) showed that these new mutations were all null *dsx* alleles. In addition, the 14 dsx^{Mas} revertants (R1, 4, 5, 12, 13, 18, 23, 30, 35, 39, 40, 41, 46, and 47) that did not have cytologically detectable deficiencies in the *dsx* region were crossed to a deficiency, $Df(3R)dsx^{D+R2}$, that deletes both *dsx* and lethal complementation groups on either side of *dsx* (see below) and the viability and sexual morphology of the $dsx^{Mas+R\_}/Df(3R)dsx^{D+R2}$ progeny examined. In all cases these progeny were viable and displayed the doublesex phenotype. Since the $dsx^{Mas+R\_}$ chromosomes tested included inversions and translocations broken at *dsx*, these results strongly suggest that the *dsx* locus does not encode a vital function.

Our cytological analyses of the $dsx^{Mas+R\_}$ and $dsx^{D+R\_}$ chromosomes showed that the *dsx* gene was located in salivary chromosome band 84E1-2. Thus the 12 inversions and translocations that were isolated as revertants of dominant *dsx* mutants all have breakpoints in salivary band 84E1-2 (Table 1). In addition, the 22 deletions that remove *dsx* have in common only the deletion of all or part of 84E1-2 (Figure 1). This included two deficiencies that have one of their end points in 84E1-2: $Df(3R)dsx^{Mas+R2}$ that begins in 84E1-2 and extends proximally and $Df(3R)dsx^{D+R5}$ that begins in 84E1-2 and extends distally. Transheterozygotes between these two deficiencies are viable and exhibit the doublesex phenotype (data not shown). The end points of these two deletions are about 12 kb apart and the deletions are nonoverlapping (BAKER and WOLFNER 1988). That the *dsx* locus is limited to 84E1-2 is also suggested by the findings that: (1) $Df(3R)Antp^{Ns+R17}$ which begins in 84D13-14 (the bands immediately proximal to 84E1-2) and extends proximally does not remove dsx^{+} function, and (2) *T(2;3)Es*, which is broken immediately distal to 84E1-2 in salivary band 84E3-4, does not affect dsx^{+} function. The latter two rearrangements are about 75 kb apart (BAKER and WOLFNER 1988).

TABLE 1

Cytological limits and origins of chromosomal rearrangements used in this study

Rearrangement	Breakpoints	Reference[a]
I. Deficiencies		
dsx^{D+R1}	84D6-7; 85A1-3	1
dsx^{D+R2}	84D11; 84F16	1
dsx^{D+R5}	84E1-2; 84F11-12	1
dsx^{D+R8}	84D10-11; 85A1-3	6
dsx^{D+R10}	84D11-12; 85A1-3	6
dsx^{M+R2}	84C1-2; 84E1-2	2
dsx^{M+R3}	84D11-14; 84E12-13	2
dsx^{M+R10}	84D3; 84F1-2	2
dsx^{M+R11}	84D8-9; 85A1-3	2
dsx^{M+R15}	84D10-11; 84E8	2
dsx^{M+R21}	84D11-12; 84E8-9	2
dsx^{M+R27}	84D9; 85A1-3	2
dsx^{M+R28}	84D13-E1; 85A4-5	2
dsx^{M+R29}	84C8-D1 interband; 84F6-7	2
dsx^{M+R33}	84D10-11 interband; 85A1-3	2
dsx^{M+R34}	84D3-4; 85B4-5	2
dsx^{M+R37}	84D8; 85B3-5	2
dsx^{M+R43}	84D13-14; 84E6-8	2
dsx^{M+R48}	84D8-10; 85A3-5 + In(3R)83D5-E1; 85A3-5	2
$Antp^{Ns+R17}$	84A6; 84 D13-14	1
$Antp^{+R1P}$	84B3; 84D1-2 + In(3R)84B3; 85C2-3	3
D6	84D2-3; 84F13-16	4
D7	84D5; 84F1-2	4
D8	84D3-5; 85A2-4	4
p^{30}	84F8-10; 85D3-6	5
p^{13}	84F1; 85A3*	5
p^{25}	85A1; 85A3*	5
p^{40}	84E8-9; 85B5-6*	5
Scx^{W+R2}	84A5; 84B6*	7
Scx^{W+R4}	84B3; 84D1-2*	7
II. Inversions		
a. Break at *dsx*		
$(3R)dsx^{D+R3}$	84E1-2; 85D22-E1	1
$(3R)dsx^{D+R7}$	84E1-2; 92C3-6	6
$(3R)dsx^{D+R9}$	84E1-2; 92C3-6	6
$(3R)dsx^{M+R4}$	84D9-10; 84E1-2	2
$(3LR)dsx^{M+R13}$	71F1-2; 84E1-2	2
$(3R)dsx^{M+R18}$	84A; 84E1-2	2
$(3LR)dsx^{M+R23}$	76A5-7; 84E1-2	2
b. Break not at *dsx*		
(3LR)206	65E10-12; 85A1-3	8
(3LR)223	65D5-E1; 84E8-10	8
(3R)Hu	84B3-6|84F1-2; 84B3-6|86C5-6; 84F1-2|86C5-6; tip	9
$(3R)cu^{3J}$	84F6-8; 86D6-10	10
$(3R)Antp^{Ns+R72}$	84B1; 84D13-14	11
III. Translocations		
a. Break at *dsx*		
$T(2;3)dsx^{M+R1}$	61; 84A1-2|28F; 32F|88B1-2; 84E1-2|88B1-2; 100; 21; 28F|84D11-12; 84A1-2|32F; 60	2
$T(2;3)dsx^{M+R5}$	59D1-2; 84E1-2	2
$T(X;3)dsx^{M+R12}$	Complex; 84E1-2; het, 18D3-5; het	2
$T(X;3)dsx^{M+R46}$	het; 84D13-E8	2
$T(2;3)dsx^{M+R47}$	58A1-2; 84E1-2	2
b. Break not at *dsx*		
T(2;3)rn	het; 84D6-9	1
T(2;3)P8	48C1-2; 84C8-D1 interband	12
T(2;3)Es	48A3-4; 84E3-4	13
T(Y;3)D85	84D8-9	14
T(2;3)434.12	21F3-4; 84F13-16	15
T(2;3)434.125	35F; 84E9	15
T(2;3)203	57A5-10; 84C8-D1 interband	16
IV. Cytologically normal dsx^{D} and dsx^{M} revertants		
dsx^{D+R4}	Normal	1
dsx^{D+R6}	Normal	6
dsx^{M+R30}	Normal	2
dsx^{M+R35}	61; 66C|het.; 100-98|het.; ?|81; 98|66C-80C|4th(?) (84 normal)	2
dsx^{M+R39}	Normal	2
dsx^{M+R40}	Normal	2
dsx^{M+R41}	4th|64-67|3-20; 1-3|100A-81; 61-64 in chromocenter (84 normal)	2
dsx^{M+R45}	Normal	2

All breakpoints, except those marked with an asterisk, are based on our own cytology.

[a] DUNCAN and KAUFMAN (1975); 2, this report; 3, LEWIS, KAUFMAN, and DENELL (1980); 4, I. DUNCAN, γ-ray induced; 5, KEMPHUES, RAFF and KAUFMAN (1983); 6, T. KAUFMAN, X-ray induced; 7, HAZELRIGG and KAUFMAN (1983); 8, CRAYMER (1984); 9, KAUFMAN, LEWIS and WAKIMOTO (1980); 10, ASHBURNER *et al.* (1981); 11, DENELL (1983); 12. CRAYMER (1984); 13, The Seattle-La Jolla Drosophila Laboratories (1971); 14, LINDSLEY *et al.* (1972); 15, W. GELBART, X-ray induced; 16, LINDSLEY and ZIMM (1987).

Genetic data suggested that the *dsx* locus is functionally complex and encodes male-specific and female-specific functions (BAKER and RIDGE 1980). It was proposed that in females dsx^{+} functions to repress male sexual differentiation, whereas in males dsx^{+} functions to repress female sexual differentiation.

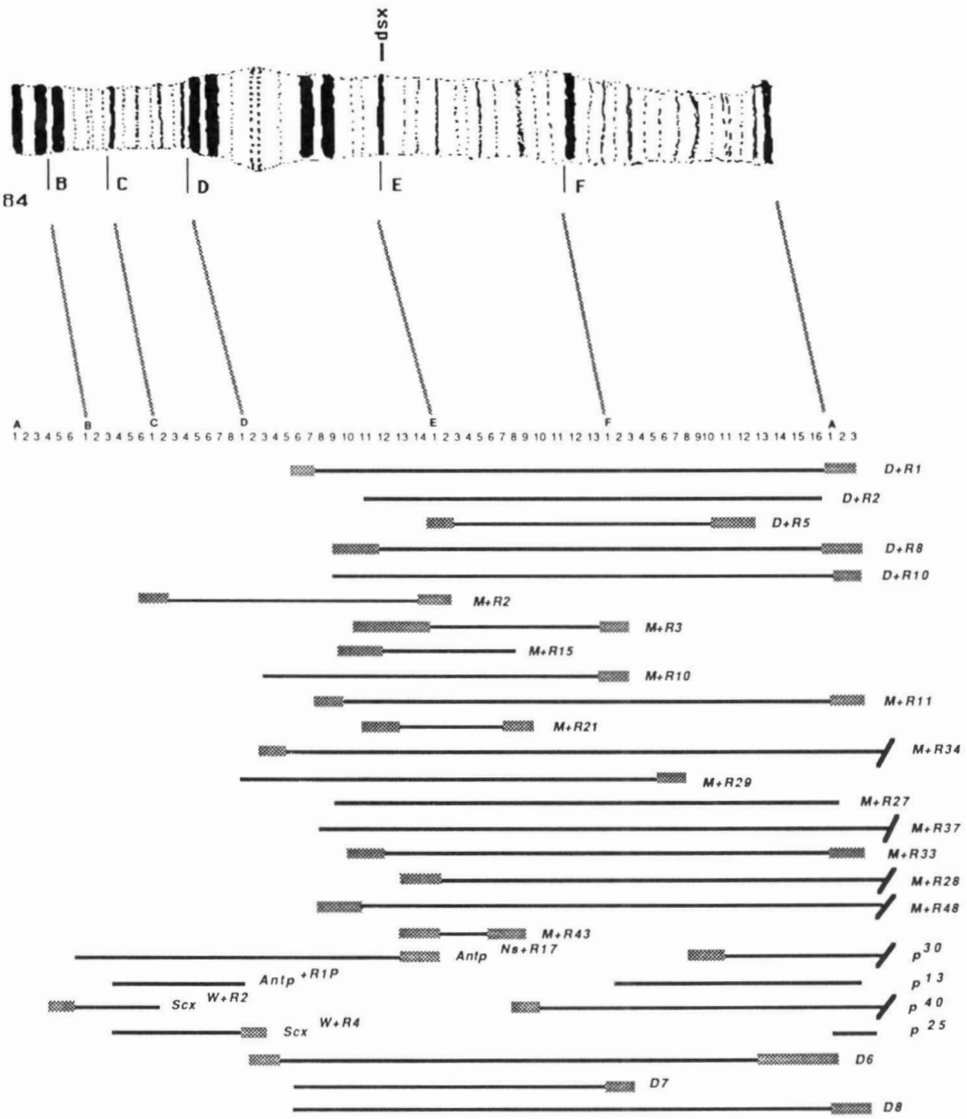

FIGURE 1.—Salivary chromosome region 84 and the extent of the deficiencies used in this study. Shaded areas at the ends of lines represent the uncertainties as to the endpoints of deficiencies.

Consistent with this model was the isolation of dsx^{136}, a recessive male-specific *dsx* mutation (GAREN, described in BAKER and RIDGE 1980), and the existence of dominant *dsx* mutations (*e.g.*, dsx^{D} and dsx^{Mas}) that affected only females. However, no female-specific recessive *dsx* mutation, which would provide more direct support for a separate female-specific function of *dsx*, had been reported at the time we initiated this work; recently such an allele has been identified and characterized (NOTHIGER *et al.*, 1987).

From our screen for mutations exposed by $Df(3R)dsx^{D+R2}$ we recovered four recessive visible mutations that produced doublesex-like phenotypes. Complementation tests with point mutations at, and deletions of, the *dsx* locus established that all four of these mutations are *dsx* alleles. Three of these new *dsx* alleles (dsx^{EFH47}, dsx^{EFH48}, and dsx^{EFK43}) behave, like the original *dsx* allele, as sex-nonspecific loss-of-function *dsx* mutations: both chromosomally male and female individuals homozygous for these alleles are transformed into sexually indistinguishable intersexes (data not shown).

The fourth new *dsx* allele, dsx^{EFH55}, has different effects in males and females. In chromosomally female individuals dsx^{EFH55}, when either homozygous, or heterozygous with a null *dsx* mutation, transforms these individuals into intersexes very similar to those produced by *dsx* null mutations. However, chromosomally male individuals homozygous for dsx^{EFH55} are normal in their sexual development with the exceptions that they are sterile, and have small testes. Males heterozygous for dsx^{EFH55} and a null allele at *dsx*, including $Df(3R)dsx^{D+R2}$, $Df(3R)dsx^{M+R2}$, and $Df(3R)dsx^{M+R15}$, display rotated genitalia in addition to the sterility phenotype. These data suggest that dsx^{EFH55} has a slightly impaired *dsx* male function. The phenotypic effects of dsx^{EFH55} in males are due to a mutation at *dsx* (and not to a mutation at some other

site on this chromosome) since they are expressed when dsx^{EFH55} is heterozygous with either $Df(3R)dsx^{D+R5}$ or $Df(3R)dsx^{M+R2}$. Assuming that dsx^{EFH55} is the result of a single mutation, it would appear to be a lesion in the portion of the *dsx* gene that encodes the common amino termini of the male- and female-specific *dsx* proteins (BURTIS and BAKER 1989), but at a site in this common region that is more important for dsx^{+} function in females.

Further support for the view that the male-specific and female-specific functions of the *dsx* locus are distinct comes from the phenotypes of individuals that are trans-heterozygotes between dsx^{EFH55} and the male-specific *dsx* allele, dsx^{136}. Chromosomally female individuals that are dsx^{EFH55}/dsx^{136} are sexually normal fertile females, consistent with the prior suggestion that dsx^{136} encodes a fully functional *dsx* female function (BAKER and RIDGE 1980). Chromosomally female individuals that are dsx^{EFH55}/dsx^{136} display the rotated genitalia and sterility phenotypes characteristic of dsx^{EFH55} over a null *dsx* allele in males. This result is consistent with the view that the dsx^{136} mutation is defective for the *dsx* male function while the dsx^{EFH55} allele has a nearly normal male function.

We have also examined the phenotype of dsx^{EFH55} when heterozygous with dsx^{D}, one of the dominant female-specific *dsx* alleles. The dsx^{D} mutation, like the other known dominant *dsx* alleles (DENELL and JACKSON 1972; BAKER and RIDGE 1980; NOTHIGER, ROOST and SCHUPBACH 1980; NOTHIGER *et al.* 1987; BAKER and WOLFNER 1988) is a *dsx* allele that results in the constitutive expression of the *dsx* male function. This is because these mutations invariably lead to the primary *dsx* transcripts of these genes being processed into the male-specific *dsx* mRNA (R. N. NAGOSHI and B. S. BAKER, in preparation). Surprisingly, when dsx^{D} is heterozygous with a wild type *dsx* allele in a chromosomally female individual, and thus both the male-specific and female-specific *dsx* mRNAs are being produced (R. N. NAGOSHI and B. S. BAKER, in preparation), that individual develops as an intersex indistinguishable from those produced by null *dsx* mutations. The *dsx*-null phenotype produced by dsx^{D}/dsx^{+} has been suggested to be the result of a mutual antagonism between the male and female products of the *dsx* locus, such that when both are present, neither is able to function (BAKER and RIDGE 1980). Diplo-*X*; dsx^{D}/dsx^{EFH55} individuals are much more male-like (they are phenotypically male except for partially rotated genitalia and, in some cases, having an intermediate number of foreleg bristles, although the latter are in the male position) than are diplo-*X*; dsx^{D}/dsx^{+} individuals suggesting that the female-specific product of dsx^{EFH55} is defective in its ability to compete with the *dsx* male-specific product as well as its ability to direct female sexual differentiation.

As a further test of the nature of dsx^{EFH55} we have examined its interactions with mutations in other sex determination genes. One feature of the current model for the functioning of the hierarchy is that the wild-type functions of the *tra* and *tra-2* loci are necessary in females for the expression of the female-specific *dsx* function, and in the absence of either tra^{+} or $tra\text{-}2^{+}$ function the male-specific *dsx* function is expressed. Consistent with this model is our finding that chromosomally female individuals that are either *tra-2/tra-2*; dsx^{EFH55}/dsx or *tra* dsx^{EFH55}/tra *dsx* are transformed into phenotypic males that display the rotated genitalia and male sterility phenotypes that are characteristic of dsx^{EFH55}/dsx males. Chromosomally female individuals that are either *tra-2/tra-2*; dsx^{EFH55}/dsx^{EFH55} or *tra* dsx^{EFH55}/tra dsx^{EFH55} are transformed into more male-like individuals, similar to *XY*; dsx^{EFH55} homozygotes. However, like *XY*; dsx^{EFH55} homozygotes, *XX* (or *XY*); *tra-2*; dsx^{EFH55} and *tra* dsx^{EFH55} individuals are sterile. These findings are consistent with the recent demonstration (NAGOSHI *et al.* 1988) that the *tra* and *tra-2* products are both needed in females for the female-specific pattern of processing of the *dsx* pre-mRNA and that in the absence of either *tra* or *tra-2* function the alternative pattern of processing produces the *dsx* mRNA that encodes the male-specific *dsx* protein.

Lethal mutations in 84D-F: In order both to inquire whether the *dsx* locus was part of a gene complex that controlled sexual differentiation and to begin to examine the genetic organization of the 84D-F region we screened EMS mutagenized chromosomes over the deficiency $Df(3R)dsx^{D+R2}$ (salivary gland chromosome breakpoints: 84D11; 84F16) for lethal and visible mutations (see MATERIALS AND METHODS). From this screen 55 lethal mutations were recovered. These mutations have been given allele designations of *H*– and *K*–.

A cytogenetic analysis of the region immediately proximal to *dsx* has been previously reported (LEWIS *et al.* 1980; see also CAVENER, OTTESMAN and KAUFMAN 1986). $Df(3R)dsx^{D+R2}$ partially overlaps the deficiency, $Df(3R)Antp^{Ns+R17}$ which LEWIS *et al.* (1980) subjected to saturation mutagenesis. They identified four simple complementation groups and one complex complementation group in the region where $Df(3R)Antp^{Ns+R17}$ and $Df(3R)dsx^{D+R2}$ overlap. We included in our study representative alleles of the simple complementation groups they identified; the first group is represented by *EbR4* and *EbR14*, the second by *EbD10* and *EbR24*, the third by *EbR13* and *EdR1* and the fourth by *EcR6*. All four members of the complex compartmentation group (*EdK13*, *EbR5*, *EcK3* and *EdD10*) were also included in our study.

We also looked for lethal or visible phenotypes among inversions and translocations broken in the 84D-F region by examining the phenotypes of individuals carrying an inversion or translocation over a

deficiency that exposed the rearrangement breakpoint. Among the translocations tested, two [*T(2;3)rn* and *T(2;3)P8*] produced visible phenotypes (rotund and roughened eye, respectively) when heterozygous with $Df(3R)dsx^{M+R2}$. Two translocations [*T(2;3)Es* and *T(2;3)434.12*] had lethals associated with their 84D-F breakpoints, whereas three other translocations [*T(Y;3)D85*, *T(2;3)203* and *T(2;3)434.125*] have no obvious phenotypic effects when heterozygous with deletions that exposed their breakpoints. Similarly three inversions [*In(3LR)223*, $In(3R)Antp^{Ns+R72}$ and $In(3R)Antp^{+R1P}$] had lethals associated with their 84D-F breakpoints, whereas two inversions [*In(3R)Hu*, and $In(3R)cu^{5J}$] had no phenotypic effects when heterozygous with a deletion, $Df(3R)dsx^{D+R2}$, that exposed their 84D-F breakpoint. The inversions and translocations associated with lethal or visible mutations in this region were included with other mutations in the mapping and complementation tests described below.

Deficiency mapping: To examine the genetic structure of the 84D-F region we first deficiency-mapped the lethal mutations in this region. Our initial deficiency mapping of the lethals was with a deficiency, $Df(3R)dsx^{M+R11}$, that extends further both proximally and distally than the screening deficiency, $Df(3R)dsx^{D+R2}$, over which these lethals were isolated. This enabled us to identify lethal mutations that were allelic to extraneous lethals on the chromosome bearing the $Df(3R)dsx^{D+R2}$ screening deficiency; three such lethals were found and discarded. The remaining lethals can be conveniently divided into those mapping proximal to *dsx* (84E1-2) and those mapping distal to *dsx* by use of the deficiency $Df(3R)dsx^{M+R2}$ that has one of its end points in *dsx* and extends proximally past the proximal end of $Df(3R)dsx^{D+R2}$. We describe our first results with respect to the lethals mapping distal to the *dsx* locus and then the results with regard to the lethals mapping proximally.

Deficiency mapping and complementation tests of lethals distal to *dsx* in the 84E1-2 to 84F16 region: The region between the right end of $Df(3R)dsx^{M+R2}$ (84E1-2) and the right end of $Df(3R)dsx^{D+R2}$ (84F16) is potentially subdivided into 14 subregions by 15 overlapping deficiencies (Figure 2). The 53 lethal mutations located in the 84E1-2; 84F16 region were mapped into 11 subregions with these deficiencies. All mutations mapping to a subregion were then crossed *inter se* in order to put them into complementation groups. Our results are described below starting with the region closest to 84E1-2 and proceeding distally.

Region D1: Mutations in this region are defined as those that are not uncovered by $Df(3R)dsx^{M+R2}$ but are exposed by $Df(3R)dsx^{M+R43}$ (84E6-8) and $Df(3R)dsx^{M+R15}$ (84E8). Eleven lethal mutations were identified that mapping into this region. All lethals found were uncovered by both $Df(3R)dsx^{M+R15}$ and $Df(3R)dsx^{M+R43}$. One of the chromosomes (*EdD10*) bearing a lethal in this region has a second lethal mutation proximal to *dsx* in the 84A-B interval (see below). Complementation tests showed that the eleven lethals in region D1 defined 2 complementation groups, *l(3)84Ea* with seven alleles and *l(3)84Eb* with four alleles.

Since these two genes immediately flank the *dsx* locus on its distal side it was of interest to us to determine the order of these genes relative to *dsx*. This was done by combining the elements of two translocations to make a synthetic deficiency that extends proximally from within one of these complementation groups. We then determined whether this deletion removed the other complementation group in region D1. One of the translocations used to make the synthetic deficiency is *T(2;3)Es*, which has a breakpoint in this region (at 84E3-4). *T(2;3)Es* is allelic with one of the lethal complementation groups [*l(3)84Eb*] in this region (Figure 2), from which we infer that its breakpoint inactivates this gene. A third chromosome deficiency extending from the 84E3-4 breakpoint of *T(2;3)Es* proximally (to 84C8-D1) was made by combining the $2^{P}3^{D}$ portion of *T(2;3)Es* with the $2^{D}3^{P}$ portion of a second translocation, *T(2;3)P8*, that is broken in 84C8-D1 (Figure 3). Flies carrying this synthetic deficiency heterozygous with normal second and third chromosomes are viable and fertile. This deficiency fails to complement alleles of the other complementation group in region D1 [*l(3)84Ea*] indicating that the order of genes in this region is *dsx-l(3)Ea-l(3)84Eb*.

The *Erased* (*Es*) mutation associated with the translocation *T(2;3)Es* is the only dominant mutation, other than the dominant *dsx* mutations, that we encountered in the 84D-F region. Both breakpoints of *T(2;3)Es* are in regions for which deficiencies exist (84E and 48A) and deficiencies for neither of these regions produce a dominant phenotype, suggesting that the Erased phenotype is due to a gain-of-function mutation. *Erased* was originally named because its phenotype is a partial removal of the darkly pigmented bands at the posterior margins of the abdominal tergites (LINDSLEY *et al.* 1972). Our examination of the abdomens of *Es*/+ flies showed that both pigmented areas and numbers of bristles at both the posterior and anterior margins of the tergites are substantially reduced and replaced with unpigmented cuticle that contains hairs (Figure 4). In addition, the unpigmented region lacking hairs and bristles that is normally present as a fold between the darkly pigmented posterior region of one tergite and the anterior region of the next tergite appears to be enlarged. This phenotype together with the finding that the second chromosome breakpoint of *T(2;3)Es* is in the same salivary chromosome band (48A1-2) as the *engrailed* (*en*) locus suggested to us the hypothesis that the

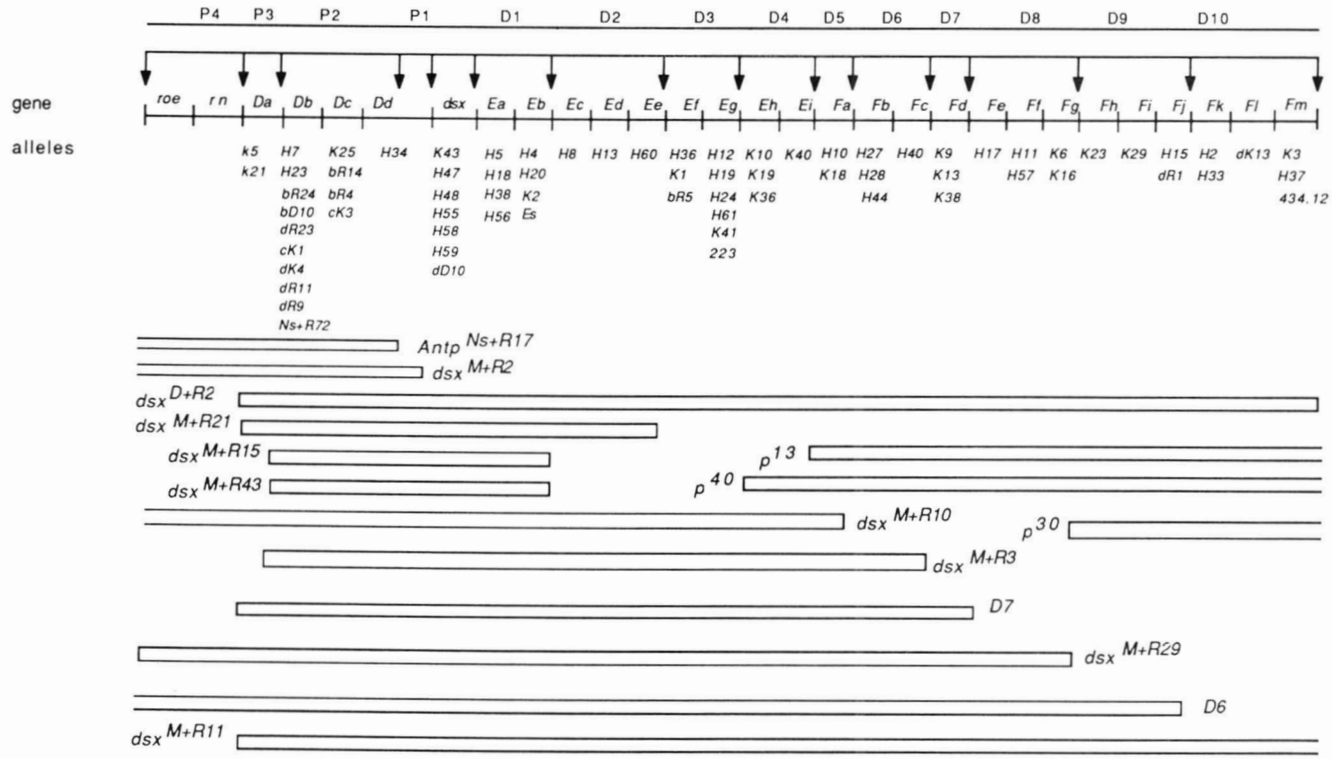

FIGURE 2.—Complementation map of the loci in salivary region 84D-F. Open bars at the bottom of the figure show the extent of deficiencies, the endpoints of which serve to define the boundaries of the segments (P1-4, D1-10) given at the top of the figure. The arrows mark the locations of these breakpoints and separate groups of genes that have been unambiguously ordered. Genes within a segment have not been ordered with the exception of segment D1 (see text). The locus designations are given above the line and the allele designations below the line. *Ns+R72* = *In(3R)Antp*$^{Ns+R72}$, *Es* = *T(2;3)Es*, *223* = *In(3LR)223*, 434.12 = *T(2;3)434.12*. The full names and extents of deficiencies are given in Table 1.

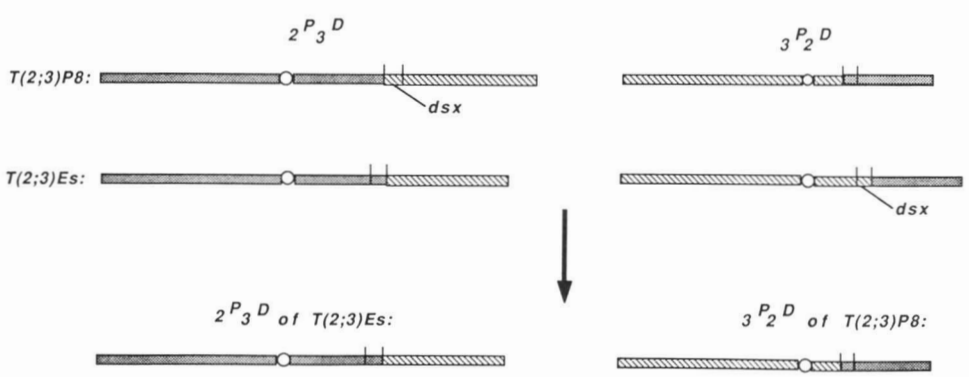

FIGURE 3.—Segmental deficiency produced by combining elements of *T(2;3)Es* and *T(2;3)P8*. Solidly filled regions represent second chromosome material and striped regions represent third chromosome material. A cross between individuals each of whom carries one of the translocations depicted in the top two lines generates progeny with a segmental deficiency for the third chromosome material between the breakpoints of *T(2;3)Es* and *T(2;3)P8*. Each individual also has intact second and third chromosomes that are not depicted.

dominant phenotype of *Es* is due to an abnormal pattern of expression of the *en* locus. It has been shown that the *engrailed* gene normally functions in the posterior portion of each abdominal segment (Hama and Kornberg, personal communication). In the one tergite in which *en* function was genetically examined, *en*$^+$ functions like it does in other parts of the fly, to maintain cells in the posterior compartment as posterior as opposed to anterior (KORNBERG 1981). The posterior compartment in an abdominal segment is the thin band of unpigmented cells that are folded under the darkly pigmented portion of the tergite and connect it to the anterior portion of the next tergite. The cells of the posterior compartment produce neither hairs nor bristles. The dominant phenotype of *Es* suggests the specific hypothesis that this translocation has disrupted the spatial control of the expression of *en*$^+$ in the adult so that it is expressed in some cells of the anterior compartment of the tergite leading to their development as posterior cells.

The limited molecular and genetic data that are available on *T(2;3)Es* are consistent with this hypothesis. The breakpoint of *T(2;3)Es* in the *en* region has been shown to be about 45 kb distal to the *en* structural gene and in a region where other nonlethal breakpoints are found that affect *en* expression (KUNER *et al.* 1985; BAKER and WOLFNER 1988). The separation of the two parts of *T(2;3)Es* in crosses to *T(2;3)P8* described above showed that the Erased phenotype segregated with the $2^{P}3^{D}$ portion of the

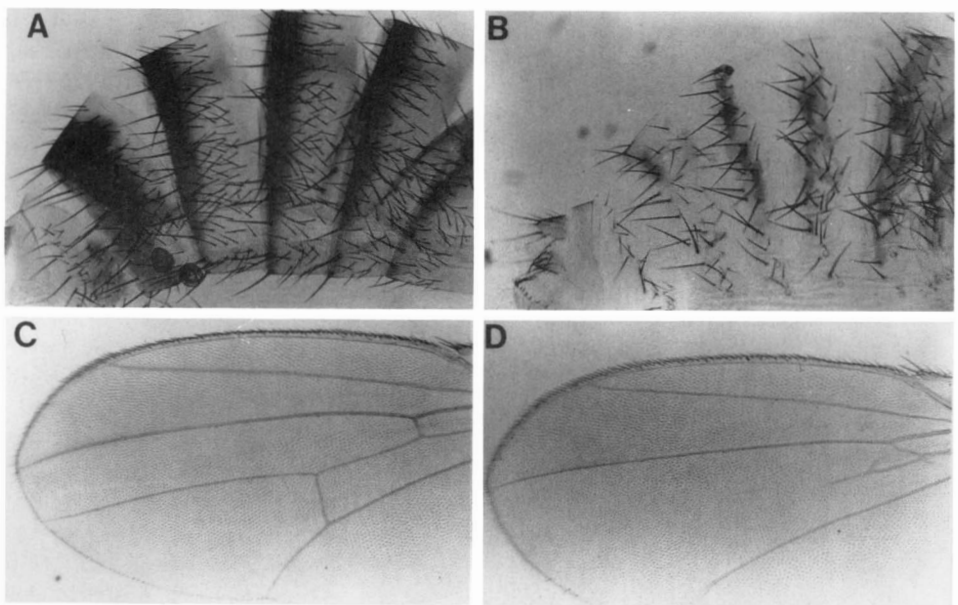

FIGURE 4.—Cuticular patterns on abdomens of *T(2;3)Es/+* and wild type and wings of *T(2;3)Es/en* and wild-type individuals. (a) Wild-type female abdomen, (B) *T(2;3)Es/+* female abdomen, (C) wild-type wing, (D) *T(2;3)Es/en* wing.

translocation, the part that contains the *en* locus. In addition, when *T(2;3)Es* is made heterozygous with en^1 or a deletion for the *en* locus (either $Df(2R)en^A$ or $Df(2R)en^B$ (EBERLEIN and RUSSELL 1983) the abdominal phenotype is not changed but the flies display an engrailed phenotype in the wing similar to that seen in en^1/en^1 individuals (Figure 4). Thus, at least in the wing, the *en* locus on the *T(2;3)Es* chromosome is not being correctly expressed.

Region D2: This region is delimited by the distal breakpoints of $Df(3R)dsx^{M+R15}$ and $Df(3R)dsx^{M+R21}$: mutations in the region fail to complement $Df(3R)dsx^{M+R21}$ but do complement $Df(3R)dsx^{M+R15}$. Three lethals map to this region and identify three complementation groups (Figure 2).

Region D3: Mutations in this region are defined as those that fail to complement $Df(3R)dsx^{M+R10}$ but do complement both $Df(3R)p^{40}$ and $Df(3R)dsx^{M+R21}$. Nine lethal mutations map to this region and define two complementation groups; one group consists of six alleles while the second group is composed of three alleles (Figure 2).

Region D4: Mutations in this region are identified as those that fail to complement $Df(3R)p^{40}$ but do complement $Df(3R)p^{13}$. Four mutations defining 2 complementation groups map into this region; one complementation group has three alleles, while the second group is represented by a single allele (Figure 2).

Region D5: This region is delimited by the proximal breakpoint of $Df(3R)p^{13}$ and the distal breakpoint of $Df(3R)dsx^{M+R10}$. Two lethal mutations identifying a single complementation group map to this region (Figure 2).

Region D6: Mutations in this region are defined as those which fail to complement $Df(3R)dsx^{M+R3}$ but do complement $Df(3R)dsx^{M+R10}$. Four lethal mutations map to this interval. The chromosome bearing one of these lethals, *l(3)EFH44*, also fails to complement $Df(3R)p^{30}$ and thus has a second lethal mutation in the distal portion of salivary region 84F; we have not localized this second lethal any further. The four mutants in region D6 identify two complementation groups; one group consists of three alleles (including *l(3)EFH44*) and the second group is represented by a single allele (Figure 2).

Region D7: This region is delimited by the distal breakpoints of $Df(3R)dsx^{M+R3}$ and *Df(3R)D7*; mutations in this region fail to complement *Df(3R)D7* but do complement $Df(3R)dsx^{M+R3}$. Three lethal mutations map to this region and identify a single complementation group (Figure 2).

Region D8: Mutations in this region are defined as those which fail to complement $Df(3R)dsx^{M+R29}$ but do complement both *Df(3R)D7* and $Df(3R)p^{30}$. Five lethal mutations defining three complementation groups map to this interval; two complementation groups are represented by two alleles each while one group is represented by a single allele (Figure 2).

Region D9: The outside bounds of this region are defined by the proximal breakpoint (84F4-6) of $Df(3R)p^{30}$ and the distal breakpoint (84F13-14) of *Df(3R)D6*. Four lethal mutations map into this region. This region is potentially further subdivisable by the deficiencies $Df(3R)dsx^{M+R29}$ and $Df(3R)dsx^{D+R5}$. All four of the lethals in this region complement $Df(3R)dsx^{M+R29}$ and are thus distal to its endpoint (84F6-7); they were not tested with $Df(3R)dsx^{D+R5}$. These four lethals identify three complementation groups (Figure 2).

Region D10: This region is delimited by the distal breakpoints of *Df(3R)D6* and $Df(3R)dsx^{M+R11}$. Six lethal mutations map to this region. The chromosome bearing one of these lethals, *l(3)EdK13*, has a second

lethal mutation proximal to *dsx* in 84C-D (see below). The mutations in this region fall into three complementation groups; these have three, two, and one alleles, respectively (Figure 2).

The region proximal to *dsx*: The region proximal to *dsx* that is of interest to us is cytogenetically delimited by the proximal breakpoint of the screening deficiency $Df(3R)dsx^{D+R2}$ (84D11) and the distal breakpoint of the deficiency $Df(3R)dsx^{M+R2}$, in *dsx* (84E1-2). Six of the lethal mutations that we induced map into this interval. We have also identified three inversions and translocations that have lethal or visible phenotypes associated with their breakpoints in 84D. In addition, four simple complementation groups and one complex complementation group were placed in this region by LEWIS *et al.* (1980), based on the failures of these mutants to complement the deficiencies $Df(3R)dsx^{D+R2}$ and $Df(3R)Antp^{Ns+R17}$. Before discussing the cytogenetic organization of the region proximal to *dsx* we need to consider the mutations placed in 84D by LEWIS *et al.* (1980) since in several cases we have been unable to confirm their results.

We are in agreement with LEWIS *et al.* (1980) on the locations of two of the lethal complementation groups that they placed in this region; the group identified by the mutations *EbR4* and *EbR14* and the group identified by *EbD10* and *EbR24* (see below). A third complementation group identified by a single allele, *EcR6*, was also placed in this region by LEWIS *et al.* (1980); in our hands this mutant was very leaky and we were unable to deficiency map it.

Two of the other complementation groups LEWIS *et al.* (1980) placed in 84D are not, in fact, located in this region. One of the latter complementation groups was identified by the mutations *EbR13* and *EdR1*. We have confirmed that *EbR13* is lethal when heterozygous with $Df(3R)dsx^{D+R2}$. However, based on the finding that *EbR13* is not exposed by a series of overlapping $Df(3R)dsx^{M+R-}$ deficiencies that *in toto* delete all material between 84C1-2 and 85A3, and thus completely overlap $Df(3R)dsx^{D+R2}$, we conclude that *EbR13* is allelic to an extraneous lethal on the $Df(3R)dsx^{D+R2}$ chromosome. Since *EbR13* is lethal over $Df(3R)Antp^{Ns+R17}$ but not over $Df(3R)dsx^{M+R2}$, one such extraneous lethal is probably located in the 84AB region. The second lethal-bearing chromosome in this complementation group, *EdR1*, carries two lethal mutations. It is not lethal when heterozygous with $Df(3R)dsx^{M+R2}$ or $Df(3R)Scx^{W+R4}$ but is lethal over $Df(3R)Antp^{Ns+R17}$ indicating that it also has a lethal mutation in the 84AB region. This overlaps the region where a lethal on the *EbR13* chromosome maps suggesting that they may be alleles of a lethal complementation group in this region. *EdR1* also fails to complement $Df(3R)dsx^{M+R11}$ and $Df(3R)D6$ but does complement $Df(3R)dsx^{M+R29}$ indicating that it also carries a second lethal mutation distal to *dsx* in section D9. Indeed, it is allelic with one of the point mutations that we induced that maps to section D9 (see above).

LEWIS *et al.* (1980) also placed a complex complementation group consisting of four lethals (*EdK13*, *EbR5*, *Eck3*, *EdD10*) just proximal to *dsx*. Our more extensive deficiency mapping of these lethals showed that they (1) all carried two lethal mutations in the 84A-F region and (2) do not identify a complementation group in 84D. The fact that they are all double mutants probably contributed to their incorrect assignment to a lethal complementation group in the region of overlap between $Df(3R)Antp^{Ns+R17}$ and $Df(3R)dsx^{D+R2}$. Thus the *EdK13* chromosome has one lethal mutation distal to *dsx* in region D10 [*EdK13* is lethal over $Df(3R)dsx^{M+R11}$ but viable over $Df(3R)D6$] and a second lethal mutation proximal to *dsx* in salivary region 84C that is exposed by $Df(3R)Antp^{Ns+R17}$ and by $Df(3R)dsx^{M+R2}$ but not by $Df(3R)dsx^{M+R29}$. The second lethal chromosome in this group, *EbR5*, has one lethal mutation distal to *dsx* in region D3. *EbR5* has a second lethal proximal to *dsx* in 84C-D since it fails to complement $Df(3R)dsx^{M+R2}$, but does complement $Df(3R)dsx^{M+R21}$. Similarly the lethal chromosome *EdD10* has one lethal immediately distal to *dsx* in region D1 and a second lethal proximal to *dsx* between the proximal breakpoints of $Df(3R)Antp^{Ns+R17}$ and $Df(3R)dsx^{M+R2}$. The final lethal chromosome in this group, *EcK3*, does have one lethal mutation in the region immediately proximal to *dsx* but also has a second lethal mutation more proximal that is not complemented by $Df(3R)Antp^{Ns+RIP}$. Our finding that these four chromosomes have lethals in salivary region 84 proximal to where they were located by LEWIS *et al.* (1980) is in agreement with a reexamination of these lethals carried out by CAVENER, OTTESON and KAUFMAN (1986).

We now turn to our results with respect to lethals that map into salivary region 84D immediately proximal to *dsx*. By the use of deficiencies, salivary region 84D can be subdivided into three subregions.

Region P1: This region is delimited by the distal breakpoints of $Df(3R)Antp^{Ns+R17}$ and $Df(3R)dsx^{M+R2}$. No lethal mutations map into this region. The failure to recover mutations falling between *dsx* and the distal break of $Df(3R)Antp^{Ns+R17}$ is probably not attributable to the small size of this region. The DNA corresponding to this region has been cloned and the breakpoints of $Df(3R)Antp^{Ns+R17}$ and $Df(3R)dsx^{M+R2}$ shown to be about 29 kb apart (BAKER and WOLFNER 1988). Moreover, a number of transcripts are produced by this region (see DISCUSSION).

Region P2: Mutations in this region are defined as those that fail to complement both $Df(3R)Antp^{Ns+R17}$ and all of the $Df(3R)dsx^{M+R-}$ and $Df(3R)dsx^{D+R-}$ deficiencies broken in 84D11-14 (*i.e.*, $Df(3R)dsx^{M+R3}$, $Df(3R)dsx^{M+R15}$, $Df(3R)dsx^{M+R21}$, $Df(3R)dsx^{M+R28}$, $Df(3R)dsx^{M+R43}$, $Df(3R)dsx^{D+R2}$, $Df(3R)dsx^{D+R10}$). The

exact cytological localization of breakpoints in this region is difficult in that the interval containing 84D13-14 generally appears as an interband and we have seen the 84D13 and 84D14 bands only rarely in very favorable preparations; we suspect that many of the deficiencies with end points we have located as being within 84D11-14 may actually be broken in 84D11-12.

All but two of the lethals mapping within 84D that we have studied are in the P2 region. Complementation tests between these mutants show that they identify three complementation groups (Figure 2). If we also include the alleles in these complementation groups isolated by LEWIS *et al.* (1980) and not retested by us, these complementation groups are represented by 9, 5, and one alleles, respectively.

Region P3: Unlike all of the above regions whose existence and limits are established by the cytologically distinguishable endpoints of deficiencies, the existence of region P3 is inferred from the results of the complementation tests with two lethals, $l(3)84Da^{K5}$ and $l(3)84Da^{K21}$. These two lethals are allelic and define one complementation group, *l(3)84Da*. Our logic for the localization of this lethal complementation group and the existence of region P3 is as follows.

Lethals on the $l(3)84Da^{K5}$ and $l(3)84Da^{K21}$-bearing chromosomes are exposed by $Df(3R)dsx^{M+R2}$, $Df(3R)Antp^{Ns+R17}$ and the screening deficiency, $Df(3R)dsx^{D+R2}$. Hence, these chromosomes have lethals proximal to *dsx* in region 84D11-14. The proximal end of region P3 is defined by the proximal breakpoint of $Df(3R)dsx^{D+R2}$. By these complementation tests *l(3)84Da* is in the same region as the lethals mapping to region P2. However, unlike the lethals mapping to region P2, $l(3)84Da^{K5}$ and $l(3)84Da^{K21}$ are complemented by only some of the $Df(3R)dsx^{M+R-}$ and $Df(3R)dsx^{D+R-}$ deficiencies that have their left endpoints in the 84D11-14 interval. In particular, $l(3)84Da^{K5}$ and $l(3)84Da^{K21}$ are viable over $Df(3R)dsx^{M+R3}$, $Df(3R)dsx^{M+R15}$, $Df(3R)dsx^{M+R43}$, and $Df(3R)dsx^{D+R10}$, whose proximal endpoints we place at 84D11-14, D11, D13-14, and D11-12 respectively, whereas they are lethal over $Df(3R)dsx^{M+R21}$ and $Df(3R)dsx^{D+R2}$ whose proximal endpoints we place at 84D11-12 and D11, respectively. We interpret these data to mean that $Df(3R)dsx^{M+R3}$, $Df(3R)dsx^{M+R15}$, $Df(3R)dsx^{M+R43}$, and $Df(3R)dsx^{D+R10}$ do not delete far enough into 84D11-12 to expose $l(3)84Da^{K5}$ and $l(3)84Da^{K21}$ whereas $Df(3R)dsx^{M+R21}$ and $Df(3R)dsx^{D+R2}$ delete slightly further into 84D and do expose this lethal complementation group. Thus the proximal breakpoints of these four deficiencies which complement $l(3)84Da^{K5}$ and $l(3)84Da^{K21}$ provide the distal limit of the P3 region.

One complication in the mapping of $l(3)84Da^{K5}$ came from the finding that, whereas it was a complete lethal when heterozygous with some deficiencies, it behaved as a leaky lethal when heterozygous with other deficiencies. One possible explanation for this behavior of $l(3)84Da^{K5}$ was that the chromosome contained two mutants in the 84C-F region; the lethal we have placed in region P3 and a semi-lethal elsewhere. This does not seem to be the case since $l(3)84Da^{K5}$ is fully viable with (1) either $Df(3R)dsx^{D+R5}$ or $Df(3R)p^{40}$ which together delete all of 84E-F and (2) either $Df(3R)Scx^{W+R2}$ or $Df(3R)Scx^{W+R4}$ which together delete all of 84A5-D1,2. Thus lethality or semilethality of $l(3)84Da^{K5}$ is only seen with deletions that remove 84D11-12. In addition, the lethal versus semilethal behavior of $l(3)84Da^{K5}$ with deficiencies that remove 84D11-12 appears to be a function of how far the deficiencies extend distally into 84EF. These data suggest that there is a gene in distal 84F whose dosage affects the viability of $l(3)84Da^{K5}$ hemizygotes. Thus $l(3)84Da^{K5}$ is lethal with all deficiencies tested that remove 84D11-12 and extend distally past 84F6-7 (*i.e.* $Df(3R)dsx^{M+R11}$, $Df(3R)dsx^{M+R27}$, $Df(3R)dsx^{M+R34}$, $Df(3R)dsx^{M+R37}$, $Df(3R)dsx^{M+R48}$, $Df(3R)dsx^{D+R2}$, and *Df(3R)D6* whose distal breakpoints are in 85A1-3, A1-3, B4-5, B3-5, A3-5, 84F16, and F13-16, respectively) but is semilethal with all but one of the deficiencies tested that remove 84D11-12 and extend less far distally (*i.e.*, $Df(3R)dsx^{M+R2}$, $Df(3R)Antp^{Ns+R17}$, $Df(3R)dsx^{M+R29}$, $Df(3R)dsx^{M+R10}$, and $Df(3R)dsx^{M+R21}$ whose distal breakpoints are in 84E1-2, D13-14, F6-7, F1-2, and E8-9, respectively). The one exception to this rule is *Df(3R)D7* (distal break: 84F1-2) which is fully lethal when heterozygous with $l(3)84Da^{K5}$; we suggest that the *Df(3R)D7* chromosome, in addition to the visible deficiency may also have a mutation in the site in 84F that shows the dosage dependent interaction with $l(3)84Da^{K5}$. Finally we should note that tests with $l(3)84Da^{K21}$ have been less extensive and so we don't know whether this interaction is an allele-specific or locus-specific one. However it is intriguing that mutants in two other genes (*rn* and *roe*) that map just proximal to *l(3)84D* in 84D also have their phenotypes substantially enhanced by deletions that extend into 84F (see below).

The 84D1-12 region: The deficiency, $Df(3R)dsx^{D+R2}$, that we used to screen for lethals deletes most of the large salivary doublet 84D11-12, as well as the bands 84D13-14, and thus mutations could have been located anywhere in this interval. The deletion, $Df(3R)Antp^{Ns+R17}$, used by LEWIS *et al.* (1980) to screen for lethals removes all of the bands in 84D and thus lethal mutations mapping anywhere in 84D could have been detected in their screen. Surprisingly, 15 out of the 17 lethals known in region 84D map to the P2 region which probably corresponds to 84D13-14. No lethals have been detected in 84D that map proximal to 84D11-12, even though this region contains some of the most heavily staining salivary chromosome bands in the genome.

While no lethals map to this region it is not devoid of genes: an α-tubulin gene (KALFAYAN and WENSINK 1981; MISCHKE and PARDUE 1982; SANCHEZ *et al.* 1980) and a tRNA gene (HAYASHI *et al.* 1980) have been mapped to proximal 84D by *in situ* hybridization and the structural locus for Esterase C has been mapped to 84D3-5 (CAVENER, OTTESON and KAUFMAN, 1986). Two genes at which mutations to visible, but apparently not lethal, phenotypes occur also map in this region. The first of these is *roughened eye* (*roe*), which was placed in 84D1-2 by CAVENER, OTTESON and KAUFMAN (1986) based on our finding that *roe* was uncovered by *Df(3R)D6* but not uncovered by $Df(3R)dsx^{M+R10}$. However, one of the translations we characterized, *T(2;3)P8*, is allelic with *roe* and is broken in the 84C8-D1 interband suggesting that *roe* may be located slightly more proximally. The second visible mutation mapping to 84D is *rotund* (*rn*) which is associated with the breakpoint of the translocation *T(2;3)rn*. CAVENER, OTTESON and KAUFMAN (1986) place *rn* in 84D3 based on the results of deficiency mapping experiments. This location is consistent with our deficiency mapping and cytological analysis of *T(2;3)rn* (Table 1).

The genes *roe* and *rn* have several unusual features in common. First, they both appear to be genes without lethal alleles. This is suggested most strongly by the finding of pairs of overlapping deletions that are viable over one another and produce either a *rn* or *roe* phenotype (CAVENER, OTTESON and KAUFMAN, 1986). Consistent with these results are our findings that the translocations that appear to be broken in *rn* and *roe* are viable over deletions for these genes. The phenotypes of *rn* and *roe*, while overtly quite different (the reduction and deletion of material in the proximal distal regions of appendages and a roughening of the eye, respectively) have in common the feature that they lead to a reduction in the number of cells in the regions that they affect. That this similarity may not be superficial is suggested by the finding that the phenotypes of both *rn* and *roe* are greatly enhanced when they are heterozygous with deletions that encompass their location and extend into 84F. CAVENER, OTTESON and KAUFMAN (1986) reported that certain deletions of *roe* (*e.g.*, Scx^{W+R4}) when heterozygous with a *roe* allele produced a phenotype similar to homozygous *roe*, but that when the same *roe* allele was heterozygous with a deletion that extended more distally the *roe* phenotype was much more extreme. Our observations are consistent with theirs. Deletions such as $Df(3R)Antp^{Ns+R17}$ and $Df(3R)dsx^{M+R2}$ that, in addition to deleting *roe*, only delete material proximal to 84E, exhibit a typical *roe* phenotype when heterozygous with *roe*. In contrast, the three deletions that we have tested that delete *roe* and extend through 84E into 84F ($Df(3R)dsx^{M+R29}$, $Df(3R)dsx^{M+R33}$, and *Df(3R)D6*) all lead to a greatly reduced eye when heterozygous with *roe*. A strikingly similar phenomenon is observed with *rn*: $T(2;3)rn/Df(3R)dsx^{M+R2}$ individuals display a normal *rn* phenotype, whereas when *T(2;3)rn* is heterozygous with deletions extending through 84E ($Df(3R)dsx^{M+R29}$, or *Df(3R)D6*) there is a much more severe phenotype with the wings being reduced to nubbins [*T(2;3)rn* was not tested with $Df(3R)dsx^{M+R33}$]. The fact that the phenotypes of both *roe* and *rn* are enhanced by deletions that extend into 8E-F suggests the possibility that there is a locus there that is important for the functioning of the wild-type alleles of both *rn* and *roe*.

DISCUSSION

The major impetus for the work reported here was to gain further information about the *dsx* locus. At one level we wished to determine the cytological location of *dsx* in order to carry out a molecular analysis. The cytological characterizations of inversions and translocations broken in *dsx*, and deletions that removed all or part of the *dsx* gene are congruent and place *dsx* in salivary chromosome band 84E1-2. The rearrangements broken in and around *dsx* allowed us to use recombinant DNA technology to molecularly clone the *dsx* locus by chromosomal walking and jumping (BAKER and WOLFNER 1988). The molecular localization of the rearrangements broken in *dsx* reported here showed that the *dsx* locus extends over a region of at least 40 kb (BAKER and WOLFNER 1988). Molecular studies have shown that *dsx* directs the synthesis of male- and female-specific mRNAs (BAKER and WOLFNER, 1988) that arise as the result of differential splicing at the 3′ end of the gene and encode sex-specific proteins having a substantial region in common at their amino termini but different carboxyl termini (BURTIS and BAKER 1989).

The *dsx* rearrangements have also provided strong evidence that the *dsx* locus is concerned solely with sexual differentiation and does not encode a vital function. Thus we found that when any of the inversions and translocations broken at *dsx* provides the sole *dsx* gene in a fly the resulting adult displays the intersexual phenotype expected from an absence of *dsx* function and has normal viability. The conclusion that *dsx* does not encode a vital function is further supported by the finding that all of the EMS-induced lethal mutations that have been isolated in the region surrounding *dsx* (this report; NOTHIGER *et al.* 1987) complement *dsx*.

Our experiments to induce mutations in the *dsx* region were also designed to inquire whether the *dsx* gene was part of a gene complex that regulated sexual differentiation. For this purpose we recovered both visible and lethal mutations over a deficiency that removed *dsx* and extended out from it both distally and proximally. Parallel experiments of NOTHIGER *et al.* (1987) were designed to detect mutations in *dsx*

and the region distal to it. From these two series of experiments 12 visible mutations that affected sexual differentiation were recovered. All of these mutations proved to be alleles at the *dsx* locus, strongly suggesting that *dsx* is the only sex determination regulatory gene in this region.

An additional aim of our work with respect to the *dsx* locus was to gain more information on its genetic structure. Previous genetic analysis had led to the suggestion (BAKER and RIDGE 1980) that the *dsx* locus was functionally complex and had different modes of expression in males and females. This conclusion was based in part on the finding that there were dominant gain-of-function *dsx* alleles that affected only females (DUNCAN and KAUFMAN 1975; DENELL and JACKSON 1972; BAKER and RIDGE 1980; NOTHIGER and STEINMANN-ZWICKY 1980; NOTHIGER *et al.* 1987; BAKER and WOLFNER 1988) as well as a recessive loss-of-function male-specific *dsx* allele (GAREN, described in BAKER and RIDGE 1980). However, no recessive loss-of-function female-specific alleles had been identified; the existence of alleles of the latter type would tend to point towards models in which the different modes of function of *dsx* in males and females was the consequence of the *dsx* locus encoding different products in the two sexes. Thus we wanted to see if recessive female-specific *dsx* alleles could be isolated. Mutagenesis experiments parallel to those we report here have been carried out by NOTHIGER *et al.* (1987). These experiments have resulted in the isolation of recessive female-specific *dsx* alleles. NOTHIGER *et al.* (1987) isolated and characterized one recessive female-specific *dsx* allele (dsx^f) that has no detectable affect in males. We have isolated one *dsx* allele, dsx^{EFH55}, that behaves as a complete loss-of-function mutation in females but only as a very weak *dsx* mutation in males. The analysis of the interactions of dsx^{EFH55} with mutations at the *tra-2* and *tra* loci provide support for our model that the wild type functions of the *tra-2* and *tra* loci act to allow the expression of the *dsx* female-specific function and that in the absence of either *tra-2* or *tra* function the male-specific *dsx* function is expressed. Similar experiments by NOTHIGER *et al.* (1988) with dsx^f have provided additional support for this aspect of the model. These results are consistent with the recent demonstration (NAGOSHI *et al.* 1988) that at the molecular level the *tra* and *tra-2* products are both needed in females for the female-specific pattern of processing of the *dsx* pre-mRNA and that in the absence of either *tra* or *tra-2* function the alternative pattern of processing produces the *dsx* mRNA that encodes the male-specific *dsx* protein.

The array of phenotypes produced by the recessive mutations at *dsx* are consistent with the expectations from the molecular analysis of the *dsx* locus. Most *dsx* mutations have equivalent effects in the two sexes, consistent with the finding that over 3/4 of the *dsx* coding sequence is common to males and females (BURTIS and BAKER 1989). The completely sex-specific *dsx* alleles such as dsx^{136} and dsx^f are likely to be in the sex-specific 3′ exons of *dsx*. The mutation dsx^{EFH55}, which behaves as a strong allele in females and a weak allele in males, has a phenotype that is unexpected. We suggest that this mutation is in the common 5′ end of the *dsx* gene, but in a portion of it that is crucial for the functioning of the *dsx* female-specific protein but of relatively little importance for the functioning of the *dsx* male-specific protein.

We also carried out deficiency mapping and complementation tests with 72 lethal mutations in the region (84D11-12 to 84F16) around *dsx*. These experiments showed that these lethals identified 25 complementation groups. Using an approximation to the Poisson distribution ($n_0 = n_1^2/2n_{\geq 2}$, where n_0 = the number of loci for which there is no extant allele, n_1 = the number of loci for which there is one allele, and $n_{\geq 2}$ = the number of loci with at least two alleles; LEICHT and BONNER, 1988) to estimate the number of genes that have yet to be identified in this interval (assuming all lethal loci are equally mutable) suggests that there are about three genes for which there are not yet mutant alleles. Thus the number of genes estimated to be in this region (84D11-F16) is in fair agreement with the number of salivary gland chromosome bands (31-32) it contains.

Although there is a general correlation between complementation groups and chromosome band number, there are discrepancies between a one-gene/one-band correlation both at the molecular and the finer-scale genetic level in our data. First, the number of transcripts observed does not always correlate with the number of genes detected in these screens. Such discrepancies were first documented by BOSSY, HALL and SPIERER (1984) in the 315-kb region cloned around the *rosy* gene, where there were about three times as many transcripts detected as there were salivary gland chromosome bands. The 107-kb region cloned in the walk at *dsx* encompasses the sites at which *T(2;3)Es* and $Df(3R)Antp^{Ns+R17}$ are broken and thus contains the D1 and P1 regions as defined in this paper. These two regions plus the *dsx* locus lie within a 75-kb stretch of DNA. Since transcripts have been mapped throughout this walk we can examine the relationship between genetically identified genes and transcripts in this interval. In the D1 region—between the *dsx* locus and the breakpoint of *T(2;3)Es*—there is an exact correspondence between complementation groups and transcripts. There are two transcripts produced by the D1 region. One of these transcripts overlaps the site of the breakpoint of *T(2;3)Es* and thus probably identifies the *l(3)84Eb* complementation group, whereas the other transcript derives from DNA between the site of *T(2;3)Es* and *dsx* and thus probably corresponds to the *l(3)84Ea* complementa-

tion group which maps into this interval. In contrast, in the P1 region (between *dsx* and the breakpoint of $Df(3R)Antp^{Ns+R17}$) no genes have been identified. However, there are at least eight size classes of transcripts, probably representing five transcription units, that are produced by the P1 region (BAKER and WOLFNER 1988). Hence in this region there is a gross disparity between the number of genes identified by genetic and molecular criteria.

At the cytogenetic level, we also found some cases where the number of salivary chromosome bands does not correspond to number of genes as identified by complementation groups. Most strikingly a consideration of our data on the distal part of 84D together with the results of LEWIS *et al.* (1980) on 84D, has revealed a markedly asymmetric distribution of mutations along this part of the chromosome. The 84D region contains 14 salivary chromosome bands and eight of these (the 1-2, 3-4, 9-10, and 11-12 doublets) are among the most heavily staining salivary chromosome bands in the genome. Yet all 17 of the lethal mutations that have been isolated in 84D fall into four complementation groups that are located in the very distal part of 84D (D13-14 and possibly the distal part of D11-12). Most (11/17) of these lethal mutations were recovered from a screen that could have detected mutations at any place in 84D (LEWIS *et al.* 1980). The two bands, 84D13 and 14, where most of these lethals map are probably the faintest bands in 84D. We find this result surprising since very few of the chromosomal regions that have been subjected to saturation mutagenesis in Drosophila have shown such a paucity of lethal complementation groups (*e.g.*, LEFEVRE 1981; WRIGHT *et al.* 1981; NICKLAS and CLINE 1983; CROSBY and MEYEROWITZ 1986). Besides the obvious (but we think unlikely) possibility that an apparently genetically empty region like 84D1-11 may be devoid of genes, we can think of two kinds of explanations for the failure to find essential genes in such a region; we believe that both are relevant with respect to the paucity of genes in 84D1-11. One explanation for a region lacking essential genes is that the region is duplicated in the genome and the duplication occurred sufficiently recently that the duplicated genes are still functionally synonymous. A second explanation is that the region does contain genes, but that these genes encode nonessential functions.

There are two lines of evidence that lead us to speculate that the presence of ancestral gene duplications may provide part of the explanation for the failure to detect essential genes in 84D1-11. The first suggestion of gene duplication comes simply from the fact that the 84D1-12 region looks like a reverse repeat with the bands 84D1-6 being mirror images of 84D7-12 (Figure 5). If some, or all of this region is in fact duplicated in this fashion, and the duplicate genes have not functionally diverged very much, then this

FIGURE 5.—Salivary chromosome banding pattern in region 84. Drawing reproduced from BRIDGES (1941).

could account for the failure to identify essential genes in this region. The second reason to think that this region may have been involved in a gene duplication is that one of the genes identified in 84D is an α-tubulin gene. There are known to be four α-tubulin genes in Drosophila genome and these are thought to have arisen from an ancestral gene by gene duplications. If the events that gave rise to the α-tubulin gene family duplicated more than just the α-tubulin gene the existence of synonymous functions in these regions could contribute to the apparent paucity of genes in 84D.

Although the other possible explanation for the failure to recover lethals in this region—that there are no essential genes in this region—seems unlikely given the large size of the region and the relative paucity of documented nonessential genes in Drosophila, this explanation does appear to provide at least part of the reason for the absence of essential genes in salivary region 84D1-11. There are five genes that have been localized to the proximal portion of 84D. Two of these, a tRNA gene (HAYASHI *et al.* 1980) and an α-tubulin gene (SANCHEZ *et al.* 1980; KALFAYAN and WENSINK 1981; MISCHKE and PARDUE 1982), were located there by *in situ* hybridization; no mutations corresponding to either gene are known. The other three genes located in very proximal 84 (in the C8/D1-D3 interval) are *rn*, *roe*, and *Est-C* (CAVENER, OTTESON and KAUFMAN 1986; this report). All three of these genes appear to encode nonessential functions. For both *roe* and *rn* there are overlapping pairs of deletions that display the mutant phenotype and are viable. In addition, there are translocations whose breakpoints are associated with mutations in these genes which are viable over deletions of the genes. For *Est-C*, apparent null alleles are viable over a deletion for the gene (CAVENER, OTTESON and KAUFMAN 1986). Thus some of the material in 84D1-12 appears to encode nonessential functions.

This work was supported by grants from the National Institutes of Health and by a Damon Runyon-Walter Winchell postdoctoral fellowship to M. W. We would like to acknowledge the excellent technical assistance of Karen Chapman.

LITERATURE CITED

ASHBURNER, M., P. ANGEL, C. DETWILER, J. FAITHFUL, D. GUBB, G. HARRINGTON, T. LITTLEWOOD, S. TOSUBOTA, V. VELISSARIOU and V. WALKER, 1981 New mutants. Drosophila Inform. Serv. **56:** 186–191.

BAKER, B. S., and M. J. BELOTE, 1983 Sex determination and dosage compensation in Drosophila melanogaster. Annu. Rev. Genet. **17:** 345–397.

BAKER, B. S., R. N. NAGOSHI and K. C. BURTIS, 1987 Molecular genetic aspects of sex determination of Drosophila. Bioessays **6:** 66–70.

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

BAKER, B. S., and M. F. 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.

BOSSY, B. L., M. C. HALL and P. SPIERER, 1984 Genetic activity along 315 kb of the *Drosophila* chromosome. EMBO J. **3:** 2537–2541.

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.

BRIDGES, P. N., 1941 A revision of salivary gland 3R-chromosome map of *Drosophila melanogaster*. J. Hered. **32:** 299–300.

CAVENER, D., D. C. OTTESON and T. C. KAUFMAN, 1986 A rehabilitation of the genetic map of the 84B-D region in *Drosophila melanogaster*. Genetics **114:** 111–123.

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. HALVERSON and A. MONROY. Alan R. Liss, New York.

CRAYMER, L., 1984 Techniques for manipulating chromosomal rearrangements and their application to *Drosophila melanogaster*. II. Translocations. Genetics **108:** 573–587

CRAYMER, L., 1984 New Mutants. Drosophila Inform. Serv. **60:** 234–236.

CROSBY, M. A., and E. M. MEYEROWITZ, 1986 Lethal mutation flanking the 68C glue gene cluster on chromosome 3 of *Drosophila melanogaster*. Genetics **112:** 785–802.

DENELL, R. E., 1983 Homeosis in Drosophila. L. Complementation studies with revertants of Nasobemia. Genetics **75:** 279–297.

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

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

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

HAYASHI, S., I. C. GILLAM, A. D. DELANEY, R. DUNN, G. M. TENER, T. A. GRIGLIATTI and D. T. SUZUKI, 1980 Hybridization of mRNA *Drosophila melanogaster* to polytene chromosomes. Chromosoma **76:** 65–84.

HAZELRIGG, T., and T. C. KAUFMAN, 1983 Revertants of dominant mutations associated with the antennapedia gene complex of *Drosophila melanogaster*: cytology and genetics. Genetics **105:** 581–600.

KALFAYAN, L., and P. WENSINK, 1981 α-tubulin genes of Drosophila. Cell **24:** 97–106.

KAUFMAN, T. C., R. LEWIS and B. WAKIMOTO, 1980 Cytogenetic analysis of chromosome 3 in *Drosophila melanogaster*: the homeotic gene complex in polytene chromosome interval 84A-B. Genetics **94:** 115–133.

KEMPHUES, K. J., E. C. RAFF and T. C. KAUFMAN, 1983 Genetic analysis of B2t, the structural gene for a testis-specific *B*-tubulin subunit in *Drosophila melanogaster*. Genetics **105:** 345–356.

KORNBERG, T., 1981 Compartments in the abdomen of Drosophila and the role of the *engrailed* gene. Dev. Biol. **86:** 363–372.

KUNER, J. M., M. NAKANISHI, Z. ALI, B. DREES, E. GUSTAVSON, J. THIES, L. KAUVAR, T. KORNBERG, and P. O'FARRELL, 1985 Molecular cloning of *engrailed*: a gene involved in the development of pattern in Drosophila melanogaster. Cell **42:** 309–316.

LEICHT, B. G., and J. J. BONNER, 1988 Genetic analysis of chromosomal region 67A-D of *Drosophila melanogaster*. Genetics **119:** 579–593.

LEFEVRE, G., JR., 1981 The distribution of randomly recovered x-ray-induced sex-linked genetic effects in *Drosophila melanogaster*. Genetics **99:** 461–480.

LEWIS, E. B., and F. BACHER, 1968 Method of feeding ethyl methanesulfonate (EMS) to *Drosophila* males. Drosophila Inform. Serv. **43:** 193.

LEWIS, R. A., T. C. KAUFMAN and R. E. DENELL, 1980 Genetic Analysis of the Antennapedia gene complex (ANT-C): mutant screen of proximal 3R, section 84B-D. Drosophila Inform. Serv. **55:** 85–87.

LEWIS, R. A., T. C. KAUFMAN, R. E. DENELL and P. TALLERICO, 1980 Genetic analysis of the Antennapedia gene complex (ANT-C) and adjacent chromosomal regions of *Drosophila melanogaster*. I. Polytene chromosome segments 84B-D. Genetics **95:** 367–381.

LINDSLEY, D. L., and E. H. GRELL, 1968 *Genetic Variations of Drosophila Melanogaster*. Carnegie Inst. Wash. Publ. 627.

LINDSLEY, D. L., and G. ZIMM, 1987 The Genome of *Drosophila melanogaster*. Part 3: Rearrangements. Drosophila Inform. Serv. **65.**

LINDSLEY, D. L., L. SANDLER, B. S. BAKER, A. T. C. CARPENTER, R. E. DENELL, J. C. HALL, P. A. JACOBS, G. L. G. MIKLOS, B. K. DAVIS, R. C. GETHMANN, R. W. HARDY, A. HESSLER, S. M. MILLER, H. NOZAWA, D. M. PARRY and M. GOULD-SOMERO, 1972 Segmental aneuploidy and the genetic gross structure of the Drosophila genome. Genetics **71:** 157–184.

MISCHKE, D., and M. L. PARDUE, 1982 Organization and expression of α-tubulin genes in *Drosophila melanogaster*. One member of the α-tubulin gene family is transcribed in both oogenesis and later embryonic development. J. Mol. Biol. **150:** 449–466.

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.

NICKLAS, J. A., and T. W. CLINE, 1983 Vital genes that flank Sex-lethal, an X-linked sex-determining gene of *Drosophila melanogaster*. Genetics **103:** 617–631.

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

NÖTHIGER, R., and M. STEINMANN-ZWICKY, 1987 Genetics of sex determination in eukaryotes. Results Prob. Cell Differ. **14:** 271–300.

NOTHIGER, R., M. LEUTHOLD, N. ANDERSON, P. GERSCHWILER, A., GRUTER, 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.

SANCHEZ, F., J. E. NATZLE, D. W. CLEVELAND, M. W. KIRSCHNER and B. J. MCCARTHY, 1980 A dispersed multigene family encoding tubulins in *Drosophila melanogaster*. Cell **22:** 845–854.

The Seattle-La Jolla Drosophila Laboratories, 1971 The use of Y-autosome translocations in the construction of autosomal deficiencies and duplications. Drosophila Inform. Serv. **47:** Suppl.

WRIGHT, T. R. F., W. BEERMAN, J. L. MARSCH, C. P. BISHOP, R. STEWARD, B. C. BLACK, A. D. TOMSETT and E. Y. WRIGHT, 1981 The genetics of dopa decarboxylase in *Drosophila melanogaster*. IV. The genetics and cytology of the 37B10-37D1 region. Chromosoma **83:** 45–58.

Communicating editor: R. E. DENELL