# SEX AND THE SINGLE CELL. I. ON THE ACTION OF MAJOR LOCI AFFECTING SEX DETERMINATION IN *DROSOPHILA MELANOGASTER*[1]

BRUCE S. BAKER AND KIMBERLY A. RIDGE

*Department of Biology, B-022, University of California, San Diego, La Jolla, California 92093*

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ABSTRACT

Sex determination in *Drosophila melanogaster* is under the control of the *X* chromosome:autosome ratio and at least four major regulatory genes: transformer (*tra*), transformer-2 (*tra-2*), doublesex (*dsx*) and intersex (*ix*). Attention is focused here on the roles of these four loci in sex determination. By examining the sexual phenotype of clones of homozygous mutant cells produced by mitotic recombination in flies heterozygous for a given recessive sex-determination mutant, we have shown that the *tra*, *tra-2* and *dsx* loci determine sex in a cell-autonomous manner. The effect of removing the wild-type allele of each locus (by mitotic recombination) at a number of times during development has been used to determine when the wild-type alleles of the *tra*, *tra-2* and *dsx* loci have been transcribed sufficiently to support normal sexual development. The wild-type alleles of all three loci are needed into the early pupal period for normal sex determination in the cells that produce the sexually dimorphic (in pigmentation) cuticle of the fifth and sixth dorsal abdominal segments. *tra*+ and *tra-2*+ cease being needed shortly before the termination of cell division in the abdomen, whereas *dsx*+ is required at least until the end of division. By contrast, in the foreleg, the wild-type alleles of *tra*+ and *tra-2*+ have functioned sufficiently for normal sexual differentiation to occur by about 24 to 48 hours before pupariation, but *dsx*+ is required in the foreleg at least until pupariation.——A comparison of the phenotypes produced in mutant/deficiency and homozygous mutant-bearing flies shows that *dsx*, *tra-2* and *tra* mutants result in a loss of wild-type function and probably represent null alleles at these genes.——All possible homozygous double-mutant combinations of *ix*, *tra-2* and *dsx* have been constructed and reveal a clear pattern of epistasis: *dsx* > *tra*, *tra-2* > *ix*. We conclude that these genes function in a single pathway that determines sex. The data suggest that these mutants are major regulatory loci that control the batteries of genes necessary for the development of many, and perhaps all, secondary sexual characteristics.——The striking similarities between the properties of these loci and those of the homeotic loci that determine segmental and subsegmental specialization during development suggest that the basic mechanisms of regulation are the same in the two situations. The phenotypes and interactions of these sex-determination mutants provide the basis for the model of how the wild-type alleles of these loci act together to effect normal sex determination. Implications of these observations for the function of other homeotic loci are discussed.

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SEX determination in eukaryotes provides a striking example of the differential control of gene expression during development. Since sex determination affects the developmental fate of numerous organ primordia, information as to the nature of the genetic events involved in sex determination should contribute not only to our understanding of sex determination, but also to the elucidation of the mechanisms by which eukaryotes effect the expression of alternative developmental pathways.

Studies of the genetic control of sex determination in a variety of organisms suggest that there are at least two levels at which such control is exerted. In many species, the presence, absence and/or numbers of particular chromosomes play a crucial role in sex determination. In addition, in several of the genetically better-characterized eukaryotes, single gene mutations are known that have profound effects on sexual differentiation. Such mutants have long been known in *Drosophila melanogaster* (STURTEVANT 1945; MORGAN, REDFIELD and MORGAN 1943; FUNG and GOWEN 1957; GOWEN and FUNG 1957; MEYER and EDMONDSON 1951; MEYER 1958; KROEGER 1959; HILDRETH 1965; WATANABE 1975; FUJIHARA, KAWABE and OISHI 1978) as well as other Drosophila species (STURTEVANT 1920, 1921, 1929; LEBEDEFF 1934, 1939; DOBZHANSKY and SPASSKY 1941; NEWBY 1942; SPURWAY and HALDANE 1954; HOLLINGSWORTH 1960, 1964). Recently, such mutations have been found and studied in *Caenorhabditis elegans* (KLASS, WOLF and HIRSH 1976; HODGKIN and BRENNER 1977; NELSON, LEW and WARD 1978), goats (HAMMERTON *et al.* 1969; WACHTEL, BASRUR and KOO 1978), rats (ALLISON, STANLEY and GRUMBRECK 1965), mice (LYON and HAWKES 1970; CATTANACH, POLLARD and HAWKES 1971), dogs (SELDEN *et al.* 1978) and humans (MORRIS 1953). For some recent thoughts on the genetic control of sex determination in mammals, see OHNO (1979).

Sex determination in *Drosophila melanogaster* was one of the first major developmental processes demonstrated to be under genetic control (BRIDGES 1921). Both chromosomal and genic components appear to be involved in this control. At the chromosomal level BRIDGES' (1916, 1921, 1922, 1925, 1932, 1939) studies of individuals with abnormal chromosome complements allowed him to demonstrate: (1) that the *Y* chromosome did not function in sex determination in Drosophila, and (2) that a major determinant of sex was the relative numbers of *X* chromosomes and autosomes. Despite these early successes, our understanding of the process of sex determination in Drosophila has advanced little beyond the balance theory of sex determination put forward by BRIDGES (1921, 1922, 1925, 1932, 1939) to account for the effects of the *X* chromosome:autosome ratio on sex determination. Briefly, the balance theory proposes that the autosomes contain a preponderance of male-determining loci and the *X* chromosome a preponderance of female-determining loci. These loci are presumed to be distributed such that flies with half as many *X* chromosomes as sets of autosomes ($1X2A$) are male, flies with equal numbers of *X* chromosomes and sets of autosomes ($2X2A$, $3X3A$) are female, and individuals in which the *X*:autosome ratio is intermediate ($2X3A$) are intersexes.

BRIDGES' balance theory has focused attention on dosage effects as *the* mechanism of sex determination in Drosophila, and indeed it seems reasonable to suppose that the *X*:autosome ratio plays a major role in the initial determination of sex. However, the fortuitous discovery over the years of a number of point mutants that have profound effects on sexual development identifies a number of additional components necessary for normal sexual differentiation. Mutants at two loci, transformer (*tra*) (STURTEVANT 1945; SEIDEL 1963) and transformer-2 (*tra-2*) (WATANABE 1975), when homozygous in chromosomally female individuals, causes them to develop into males. These transformed females have normal male external cuticle structures, internal genital duct system and courtship behavior. They are sterile, and for *tra* this sterility has been shown to result from a failure to transform the germ line in homozygous *tra* females (MARSH and WIESCHAUS 1978). Indeed, the untransformed germ line in females homozygous for *tra* appears to be the only thing that keeps them from being functional fertile males: transplantation of $X/Y$; $tra^+/tra^+$ testes into developing $X/X$; *tra/tra* individuals gives rise to fertile transformed females (SEIDEL 1963; see also NOVITSKI 1951). The mutant doublesex (*dsx*) (HILDRETH 1965), when homozygous in either chromosomally male or female individuals, causes them to develop into intersexes. Homozygosity for intersex (*ix*) (MORGAN, REDFIELD and MORGAN 1943) transforms females into intersexes, but does not affect males. These mutants thus identify a number of genetic components in addition to the *X*:autosome ratio that are necessary for normal sex determination. The existence of these mutants suggests that sex determination in Drosophila might profitably be approached as a developmental process containing five known (and almost certainly a number of unknown) steps: those controlled by the *X*:autosome ratio and the $ix^+$, $tra\text{-}2^+$, $tra^+$ and $dsx^+$ loci.

In this report, we focus on the roles of these loci in sex determination; experiments dealing with the role of the *X*:autosome ratio in sex determination will be reported elsewhere (BAKER, in preparation). For the *tra-2*, *tra* and *dsx* loci, three questions have been addressed: (1) Does the locus function in sex determination in a cell autonomous manner? (2) When during development has the wild-type allele of the locus functioned (*i.e.*, been transcribed) sufficiently to insure normal sexual development? (3) What are the phenotypes of null alleles at these loci?

In addition, all possible double-mutant combinations of *tra-2*, *ix*, *tra* and *dsx* have been constructed in order to inquire whether these loci can be viewed as specifying sequential steps in a single pathway of sex determination or steps in a set of parallel pathways.

The results of these experiments show that there are strong similarities between the properties of these loci and those of the homeotic loci that determine segmental and subsegmental specializations during development. We suggest that the basic mechanisms of regulation are the same in the two situations. The phenotypes produced by single- and double-mutant combinations of these sex determination mutants provide the basis for a model of how the wild-type alleles

of these loci act together to effect normal sex determination. Implications of these observations for how other homeotic loci may act to specify determination are discussed.

## MATERIALS AND METHODS

All crosses were carried out on corn meal, molasses, yeast, agar, proprionic acid medium at 25°. The chromosomes and markers utilized are listed in Table 1; detailed descriptions can be found in the indicated references and LINDSLEY and GRELL (1968).

*Deficiencies for sex-determination loci:* Deficiencies for the *dsx* locus have been reported by DUNCAN and KAUFMAN (1975). Two deficiencies for *tra* have been used: $Df(3L)st^{SS10^3}$, 73E3; 74A6 (ASHBURNER *et al.* 1979) and *Df(3L)st*, 72F-73A; 74B-C (T. KAUFMAN, personal communication).

*tra-2* is tightly linked to the dominant mutant Lobe (*L*) (WATANABE 1975). Most dominant mutations are neomorphs and revertable by X-ray-induced deletion (LIFSCHYTZ and FALK 1969; LINSDLEY *et al.* 1972). To obtain deficiencies for the *tra-2* locus, *Bl L/SM5* males were irradiated with 3500R (Torrex model 150 X-ray machine, 120 KV, 5 mA, 500 R/min, 1 mm plexiglass filter) and crossed *en masse* to females homozygous for either *ad* or *cv-2*. Males were discarded after five days and the females transferred to fresh food for a second five-day brood. Seven *Bl* $L^+$ progeny were found among 23,741 *Bl L* progeny of irradiated males. Stocks of five of these *L* revertants were obtained, examined in salivary chromosomes and tested for complementation with *tra-2*.

The cytological properties of these revertants are as follows:

| Revertant | Cytology |
|---|---|
| $L^{+R1}$ | Normal |
| $L^{+R3}$ | Inversion or translocation: 51A; heterochromatin |
| $L^{+R4}$ | Df(2R)51A2; 52A12–B1 |
| $L^{+R5}$ | In(2LR)26F; 50F–51A |
| $L^{+R7}$ | Df(2R)50D; 51B5–C2 |

$Df(2R)L^{+R4}$ has a heavily staining doublet just distal to 51A2 that does not appear to match any of the deleted bands. Both $Df(2R)L^{+R4}$ and $Df(2R)L^{+R7}$ fail to complement *tra-2*, placing *tra-2* in salivary region 51A2; 51B5–C2.

*Autonomy and time of gene function*: To determine whether the wild-type alleles of *tra-2*, *tra* and *dsx* are cell autonomous and when during development they are expressed, X-ray-induced mitotic crossing over was used to produce clones of homozygous mutant cells at various times during development. The crosses carried out to generate the individuals to be irradiated were as follows:

(1) for *tra-2*:
   (a) +/+; *pwn tra-2/SM1*; +/+ ♀ × *y*/*y*+*Y*; +/+; $spa^{pol}/spa^{pol}$ ♂ and reciprocal cross.
   (b) +/+; *pwn tra-2/SM1* ♀ × +/*Y*; *M(2)S7/SM1* ♂.

(2) for *tra*:
   (a) *y*/*y*; *jv tra/TM2* ♀ × *y* $f^{36a}$/*Y*; $Dp(1;3)sc^{J4}$, *y*+ *mwh* $M(3)h^{S37}/TM2$ ♂.
   (b) *y*/*y*; *jv tra/TM2* ♀ × *y* $f^{36a}$/*Y*; $Dp(1;3)sc^{J4}$, *y*+ *mwh*/*TM2* ♂.

(3) for *dsx*:
   (a) +/+; *dsx* $p^p$/*TM6* ♀ × *y*/*Y*; *Ki M(3)S31/TM2* ♂.
   (b) +/+; *dsx* $p^p$/*TM6* ♀ × *y*/*Y*; *Ki*/*Ki* ♂.

Developmental age of the progeny of these crosses at the time of irradiation was determined following the procedure of GARCIA-BELLIDO and MERRIAM (1971C). For larval irradiations, asynchronous cultures were irradiated in split bottles and all pupae were collected at about 8-hour intervals. For pupal irradiations, pupae were collected at 8-hour intervals, held the appropriate

time and then irradiated. The irradiation sources utilized were as follows: (1) for *dsx*, GE Maximar 250 X-ray machine (dose rate 100R/min, 250 KV, 15 mA, 0.8 mm Cu HVL); (2) for *tra-2*, a $^{137}Cs$ source (dose rate 1000R/min); (3) for *tra*, a GE orthovoltage X-ray machine (dose rate 149 R/min, 280 KV, 15 mA, 1.5 mm Cu filtration). All doses were 1000R. From these crosses, adults of the required genotypes (Figure 1) were collected and preserved in 3 parts 70% ethanol:1 part glycerol.

In the genotypes represented in Figure 1, the cell markers *y* (absence of $Dp(1;3)sc^{J4}$, $y+$) and *jv* are incorporated to aid in the detection of *tra* clones, and *Ki+* and *pwn* serve similar functions for the detection of *dsx* and *tra-2* clones, respectively. Also included in these individuals are cell markers on other linkage groups ($f^{36a}$ for *tra* and *y* for both *tra-2* and *dsx*) that serve as internal controls. In each of these genotypes, a Minute (*M*) mutant is present in trans to the sex-determination mutant. Thus, clones in which the sex-determination mutant is homozygosed will also be homozygous for the wild-type allele of the *M* locus (Figure 1). Non-Minute cells have a shorter cell cycle time than do *M* cells, and *M+* clones in otherwise *M* flies are much larger than are *M+* clones in *M+* flies (MORATA and RIPOLL 1975). The larger size of these clones is technically advantageous in the foreleg since such clones will be more likely to encompass the 8–12 cells that produce the sex comb in the male or the homologous row of bristles in the female.

*Scoring of clones*: To score clones in the abdominal segments, abdomens were removed from carcasses, eviscerated, mounted and scored as described previously (BAKER, CARPENTER and RIPOLL 1978). In order to detect clones on forelegs, carcasses (less abdomens) were eviscerated by the procedure of SZABAD (1978), mounted in Euparal between coverslips and examined with a Zeiss Universal microscope.

Two criteria have been utilized to recognize the clones of cells in a adult cuticle that are descended from cells in which mitotic exchange homozygosed a sex-determination mutant.

First, such clones can be recognized by the expression of the cell marker linked to the sex-determination mutant. When the cell marker is proximal to the sex determination mutant (as are the markers *pwn* and *Ki+* used with *tra-2* and *dsx*, respectively), then clones homozygous for the

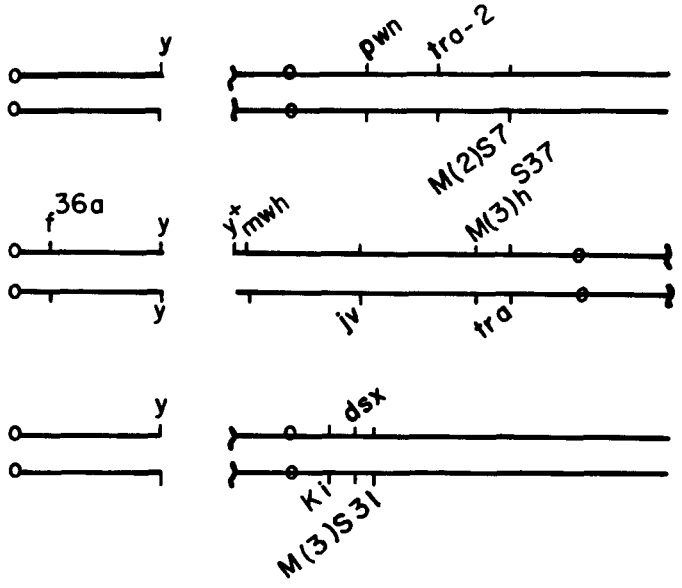


FIGURE 1.—Location and arrangement of markers utilized to study the autonomy and time of expression of the *tra*, *tra-2* and *dsx* loci.

TABLE 1

*Properties of mutants used*

| Locus (symbol) | Chromosome-map position | Relevant properties | Major references† |
|---|---|---|---|
| I. Sex determination mutants: | | | |
| *transformer-2* (*tra-2*) | 2–70 | Transforms females into males; males sterile | WATANABE 1975 |
| (*tra-2$^{OTF}$*) | | Incomplete transformation of females into males; males fertile | FUJIHARA, KAWABE and OISHI 1978 |
| *intersex* (*ix*) | 2–60.5 | Transforms females into intersexes; males normal | KROEGER 1959 |
| (*ix$^{2}$*) | | Like *ix* | |
| *transformer* (*tra*) | 3–45 | Transforms females into males; males normal | STURTEVANT 1945; SEIDEL 1963 |
| (*tra$^{\Delta C}$*) | | Like *tra* | This report |
| *doublesex* (*dsx*) | 3–48.1 | Transforms both males and females into intersexes | HILDRETH 1965 |
| *doublesex-dominant* (*dsx$^{D}$*) | | Dominant, *dsx$^{D}$*/+ transforms females into intersexes; male unaffected | FUNG and GOWEN 1957; DUNCAN and KAUFMAN 1975 |
| *Masculanizer* (*Mas*=*dsx$^{Mas}$*) | | Like *dsx$^{D}$* | |
| II. Deficiencies for sex-determination loci: | | | |
| | Salivary breakpoints | | |
| *Df(2R)L+$^{R4}$* | 51A2; 52A12–B1 | | This report |
| *Df(2R)L+$^{R7}$* | 50D; 51B5–C2 | | This report |
| *Df(3L)st$^{SS103}$* | 73E3; 74A6 | | ASHBURNER *et al.* 1979 |
| *Df(3L)st* | 72F–73A; 74B–C | | KAUFMAN, personal communication |
| *Df(3R)dsx$^{D}$+$^{R5}$* | 84F2–3; 84F16 | | DUNCAN and KAUFMAN 1975 |
| *Df(3R)dsx$^{D}$+$^{R8}$* | 84D11–12; 85A1–2 | | KAUFMAN, personal communication |
| III. Cell marker mutants: | | | |
| | Chromosome—map position | | |
| yellow (*y*) | 1–0.0 | Yellow bristles and cuticle | |
| forked (*f$^{36a}$*) | 1–56.7 | Short twisted bristles | |
| pawn (*pwn*) | 2–58 | Bristles and hairs abnormal | GARCIA-BELLIDO and DAPENA 1974 |
| javelin (*jv*) | 3–19.2 | Abnormal taper to bristles | |
| multiple wing hair (*mwh*) | 3–0.0 | Deranged hair pattern | |
| Kinked (*Ki*) | 3–47.6 | Dominant, abnormal bristle morphology | |
| *Dp(1;3)sc$^{J4}$,y+* | 3–0.0 | Duplication of *y+* carried on tip of *3L* | |

TABLE 1 Continued

| Locus (symbol) | Chromosome-map position | Relevant properties | Major references† |
|---|---|---|---|
| IV. Minute mutants: | | | |
| *M(2)S7* | 2–77.5 | Lengthen mitotic cycles in cell autonomous manner; *M+* clones in *M* flies are larger than in *M+* flies | MORATA and RIPOLL 1975 |
| $M(3)h^{S37}$ | 3–40.2 | | |
| *M(3)S31* | 3–50.0 | | |
| V. Other: | | | |
| *SM1, TM1, TM2, TM6, CxD, SM5* | | Multiply inverted balancer chromosomes carrying dominant markers | |
| $pr, cn, p^p, ab^2, sp^2, ad, cv\text{-}2, spa^{pol}$ | | Recessive visible markers | |
| *Sb, L, Bl* | | Dominant visible markers | |

† LINDSLEY and GRELL (1968) unless noted otherwise.

cell marker will also be homozygous for the more distally located sex-determination mutant. However, in the case of *tra*, the cell markers utilized, *y* [absence of *Dp(1;3)sc*$^{J4}$, *y*+] and *jv*, are located distal to *tra*. In this case, a fraction of the clones homozygous for the cell markers will arise *via* crossovers between the cell markers and *tra*, and thus will not be homozygous for *tra*. That not all *y jv* clones will be homozygous for *tra* does not hinder this analysis, since the proportion of *y jv* clones that are of this type should remain constant throughout development.

The second criterion by which a clone of cells homozygous for a sex determination mutant can be recognized is by the effect of the mutant on the sexual differentiation of the cells in the clone. Although this criterion is clearly practical only if the mutant is autonomous and the clone was generated before the wild-type allele of the locus had completed its functioning, it has proven useful. For *tra-2* and *dsx*, the cell markers utilized are proximal to the sex-determination mutant; exchanges that occur between the cell marker and the sex-determination locus can homozygose the sex-determination mutant, but not the cell marker. Scoring clones on the basis of their sexual phenotype allows such clones to be identified. Moreover, the cell markers utilized with *dsx* and *tra* affect only bristle color or morphology; only a minority of the abdominal cuticle cells produce bristles. Whereas abdominal clones induced during the larval period almost always do encompass bristle-forming cells, such clones induced at later stages of development frequently do not contain bristles because they are smaller. The use of sexual phenotype in addition to cell-marker phenotype to detect clones allows this class of events to be identified.

For the analysis of the action of *tra-2*, *tra* and *dsx* in the portions of the fifth and sixth abdominal tergites that exhibit sexually dimorphic pigmentation patterns, clones were induced and scored in females heterozygous for these mutants. Both cell marker phenotypes and sexual (pigmentation) phenotypes were used to detect clones. Clones detected by cell markers were then scored as to whether they were associated with cuticle that had mutant (male-like) pigmentation or nonmutant (female) pigmentation.

The clonal analysis of *tra-2* and *tra* action in forelegs was also done in females where these mutants lead to the production of sex combs in place of the distal-most row of transverse bristles of the basitarsus. In these experiments, clones were detected exclusively by the presence of sex-comb teeth since the cell markers utilized in these crosses are not readily scorable in this region of the foreleg (STEINER 1976, and personal communication). In experiments involving *tra*, all sex-comb clones resulting from *tra* homozygosis should be *y* and were checked in this regard. For *dsx*, clones were scored in the forelegs of males where homozygosis of *dsx* produces bristles in place of sex-comb teeth. Clones were detected on this basis and then checked to see that they were of the expected cell marker phenotype (*Ki*+).

*Mutant phenotypes and interactions*: The effects of homozygous and hemizygous mutants and combinations of mutants on sexual differentiation of the adult cuticle were scored in whole flies that had been eviscerated in KOH, using the procedure of SZABAD (1978), and mounted in Euparal between coverslips.

For each genotype discussed below, at least 20 flies were scored for the type and degree of sexual differentiation of the following sexually dimorphic parts: sex-comb region of foreleg, sternites 6–7, tergites 5–8, anal plate, genital arch, male genital apparatus (phallus, postgonites, praegonites, sheath, hypandrium, apodeme) and female genital apparatus (vaginal plates, vaginal teeth). Bristles were counted for those parts (foreleg, sternites 6–7, tergites 7–8, vaginal teeth) in which bristle number is strikingly sexually dimorphic.

*Developmental delay*: Minute mutants result in a lengthening of the life cycle, especially the larval period (FERRUS 1975; MORATA and RIPOLL 1975). The severity of this developmental delay differs between *M* mutants. To assess the length of the prepupal periods in the genotypes depicted in Figure 1, the crosses that generated these individuals were repeated simultaneously. Eggs laid during the first 48 hr following mating were discarded and the parents then transferred at two-hour intervals to vials containing fresh, well-yeasted food. From these vials, pupae were collected at eight-hour intervals. For each sample, the mean time from egg laying until pupation of each genotype present was taken as the difference between the midpoints of the times of the egg and pupal collections. A weighted mean egg-to-pupation time was then calculated for each geno-

type from all samples. The results showed that the prepupal period in *y*/+; *pwn tra-2/M(2)S7* females was lengthened by 24 hr, in *y*/+; *dsx* $p^p$/*KiM(3)S31* females by 54 hr and in *y* $f^{s6a}$/*y*; *Dp(1;3)sc*$^{J4}$, *y*+ *mwh M(3)*$h^{S37}$/*jv tra* females by 80 hr compared to their respective non-Minute-bearing sibs, all of which had egg-to-pupal intervals of 108 to 115 hr.

## EXPERIMENTAL RATIONALE AND RESULTS

### *Phenotypes of sex-determination mutants*

Sexual dimorphism is most strikingly evident in three regions of the cuticle of wild-type *D. melanogaster* adults: (1) the fifth and sixth dorsal segments of the abdomen (tergites) that are uniformly darkly pigmented in the male, but yellowish over the anterior portions and darkly pigmented along their posterior margins in the female (Figure 2); (2) the structure of terminal abdominal segments and genitalia; and (3) the most proximal segment of the tarsus of the foreleg (the basitarsus), which, in the male, possesses a sex comb (a row of 9–14 morphologically unique bristles) that is homologous to the most distal transverse row of bristles of the basitarsus in the female, even though this row of bristles and sex comb are oriented 90 degrees apart relative to the long axis of the basitarsus (Figure 3) (TOKUNAGA 1962).

The effects of *tra-2* (WATANABA 1975), *tra* (STURTEVANT 1945; BROWN and KING 1961; SEIDEL 1963), *dsx* (HILDRETH 1965) and *ix* (MORGAN, REDFIELD and MORGAN 1943; KROEGER 1959) on sexual differentiation have been described and are summarized in Table 2.

*tra-2* and *tra* are recessive and, when either is homozygous in chromosomally female individuals, it transforms them into phenotypically normal males with respect to all external sexual characteristics, as well as the internal genital duct system (STURTEVANT 1945; SEIDEL 1963; WATANABE 1975). Moreover, *tra*-transformed females successfully carry out male courtship behavior through to copulation (SEIDEL 1963; HALL 1978). We have observed that *tra-2*-transformed females also court and mate as males. For both mutants, transformed females have the normal body size of females. A second nontemperature-sensitive allele of *tra*, $tra^{AC}$, was found on a *TM1* balancer chromosome by A. T. C. CARPENTER and given to us. *tra*/$tra^{AC}$ females are transformed into males indistinguishable from those produced by homozygosity for *tra*. Neither *tra* nor *tra-2* has any effect on the external or internal gross morphology of chromosomally male individuals. However, homozygous *tra-2* males are sterile, whereas homozygous *tra* males are fertile. A second allele at the *tra-2* locus, $tra\text{-}2^{OTF}$ (FUJIHARA, KAWABE and OISHI 1978), which appears to be a leaky allele at this locus in that it only partially transforms females, is fertile when homozygous or over *tra-2* in males.

When homozygous in either chromosomally male or female individuals, *dsx* transforms these individuals into morphologically identical intersexes (HILDRETH 1965). The pattern of abdominal pigmentation produced by *dsx* is male-like over all but the anterior and lateral margins of the fifth tergite. The fore-leg bristles in all flies are intermediate between those of males and females in both location and morphology. In wild type, the male and female derivatives of the genital disc, with the exception of the anal plates, are derived from separate

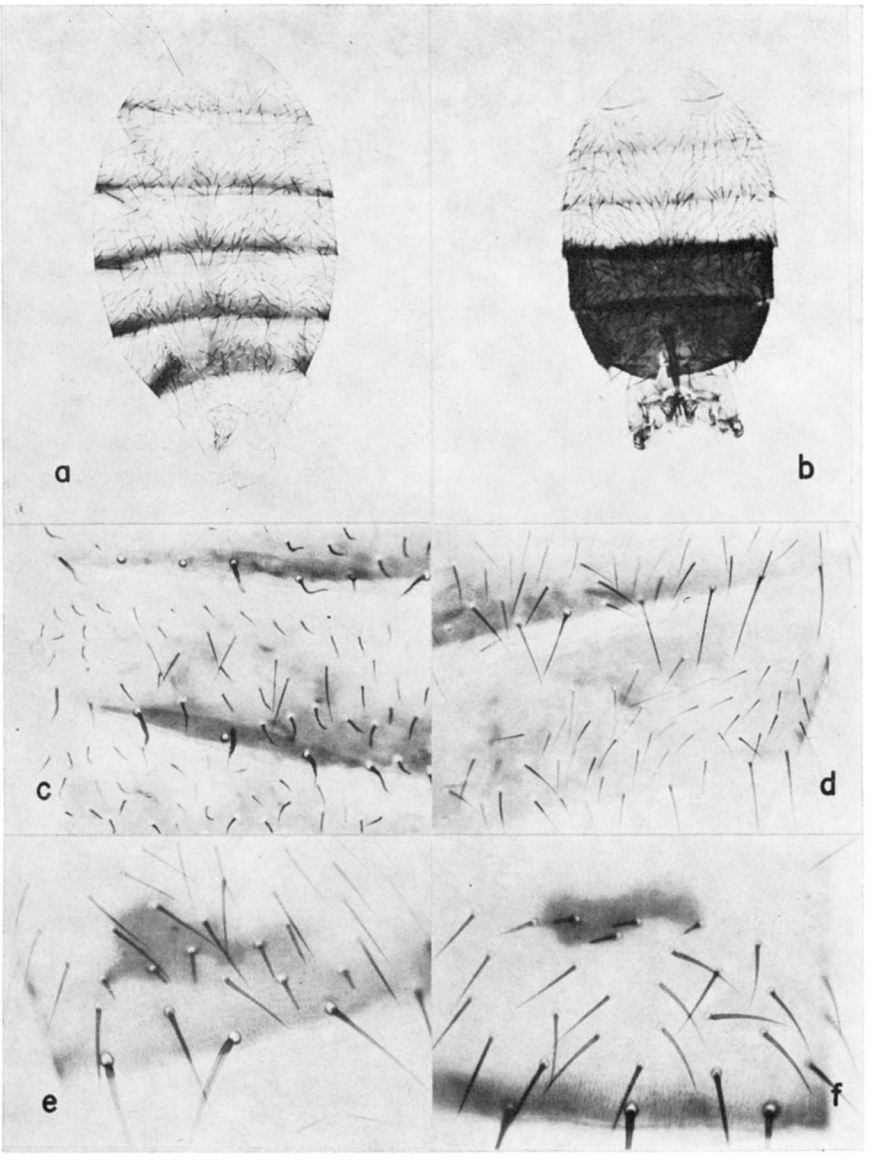


FIGURE 2.—Wild-type male and female abdomens and examples of male pigmentation clones homozygous for sex-determination mutants and linked cell markers. (a) wild-type female abdomen; (b) wild-type male abdomen; (c) homozygous *Ki+ dsx* clone; (d) homozygous *y jv tra* clone; (e–f) homozygous *pwn tra-2* clones.

primordia (NÖTHIGER, DÜBENDORFER and EPPER 1977). In flies homozygous for *dsx*, all but one of the male and female genital disc derivatives (internal as well as external) are found, although they may be reduced or poorly organized. The one structure not found is the apodeme, an internal chitinous extension of the phallus.

TABLE 2

*Phenotypes of sex-determination mutants*

| Genotype | Chromosomal sex | Foreleg† | Abdominal tergites | | | | Abdominal sternites | | Anal plates | Female genitalia¶ | Male genitalia | | | Internal genitalia |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | 5‖ | 6‖ | 7* | 8 | 6* | 7* | | | Genital arch | Phallus apparatus§ | Apodeme | |
| + | ♂ | 9–14 teeth; ♂ loc. | ♂ | ♂ | absent | absent | 0 | absent | ♂ | absent | + | + | + | ♂ |
| + | ♀ | 6–8 bristles; ♀ loc. | ♀ | ♀ | 25–36 | + | 19–24 | 9–12 | ♀ | 22–27 | absent | absent | absent | ♀ |
| *tra/tra* | ♀ | 9–14 teeth; ♂ loc. | ♂ | ♂ | absent | absent | 0.5‡ | absent | ♂ | absent | + | + | + | ♂ |
| *tra-2/tra-2* | ♀ | 10–14 teeth; ♂ loc. | ♂ | ♂ | absent | absent | 0 | absent | ♂ | absent | + | + | + | ♂ |
| *ix/ix* | ♀ | 5–7 lg. bristles; intermediate | ♂ post. 1/3 | ♂ | 11–19 | reduced | 16–19 | 7–11 | fused | 5–15 | + | v. reduced | absent | ♂ + ♀ |
| *dsx/dsx* | ♀ | 5–7 lg. bristles; intermediate | ♂ post. 3/4 | ♂ | 13–20 | reduced | 14–20 | 3–7 | fused | 5–20 | + | reduced | absent | ♂ + ♀ |
| *dsx/dsx* | ♂ | 5–7 lg. bristles; intermediate | ♂ post. 3/4 | ♂ | 13–20 | reduced | 14–20 | 3–7 | fused | 5–20 | + | reduced | absent | ♂ + ♀ |
| $dsx^{D}/+$ | ♀ | 6–8 lg. bristles; intermediate loc. | ♂ post. 1/2 | ♂ | 17–26 | reduced | 18–23 | 4–9 | reduced ♂ | 6–12 | incomplete | v. reduced | absent | ♂ + ♀ |
| $dsx^{D}/Df(3R)dsx^{D+Rs}$ | ♀ | 8–10 teeth§; almost ♂ loc. | ♂ | ♂ | absent | absent | 0–5 | absent | ♂ | absent | + | + | + | —— |
| $dsx^{Mas}/+$ | ♀ | 7–9 v. lg. bristles; ♂ loc. | ♂ post. 3/4 | ♂ | 7–15 | reduced | 11–20 | 1–7 | reduced ♂ | 0–2 | + | + | absent | —— |
| $dsx^{Mas}/Df(3R)dsx^{D+Rs}$ | ♀ | 9–12 teeth; ♂ loc. | ♂ | ♂ | absent | absent | 0 | absent | ♂ | absent | + | + | + | —— |

† Number of sex-comb teeth/bristles; location.
* Number of bristles.
¶ Number of vaginal teeth.
‡ 0.3 bristles on $tra+/tra+$ chromosomally male sibs.
§ Some teeth are pointed but otherwise have morphology of teeth; rotation more than in *dsx/dsx* but less than in wild-type male or $dsx^{Mas}/Df(3R)dsx^{D+Rs}$.
‖ Pigmentation pattern.

Homozygosity for the mutant *ix* transforms females into intersexes quite similar to those produced by *dsx*. The main difference between *ix*- and *dsx*-transformed individuals is that homozygous *ix* intersexes are somewhat less masculinized. Thus, in homozygous *ix* intersexes, the sixth abdominal segment has male-like pigmentation, whereas male-like pigmentation extends over only the proximal third of the fifth abdominal segment. Similarly, although both male and female genital disc derivatives are present, the male genital apparatus is more rudimentary than that produced by *dsx*. Males homozygous for *ix* are morphologically normal and fertile. A second allele at this locus, $ix^2$ (MEYER 1958; MEYER and EDMONDSON 1951), was reported to be sterile when homozygous in males and lethal at high temperatures. By allowing recombination between the $ix^2$-bearing second chromosome and a second chromosome carrying *cn* (a visible marker fairly closely linked to $ix^2$), we have separated the $ix^2$ mutant from the cause(s) of both the male sterility and the temperature-sensitive lethality. The $ix^2$ mutant itself has the same effects as *ix*.

In addition to the above recessive mutants affecting sex determination, three dominant mutants—doublesex Dominant ($dsx^D$) (previously $tra^D$, FUNG and GOWEN 1957; GOWEN and FUNG 1957), Masculinizer (*Mas*) (MISCHAIKOW 1959), Intersex (*Ix*) (KELSTEIN 1938; LINDSLEY *et al.* 1972)—have been reported to affect sex determination.

$dsx^D$ affects only females and, when heterozygous with a wild-type third chromosome, transforms them into intersexes (Table 2). These intersexes are very similar to those produced by homozygosity for *dsx*. However, the $dsx^D/+$ intersexes are more female-like. Thus, in $dsx^D/+$ intersexes there is closer to the female number of bristles on the sixth and seventh sternites and seventh tergite, as well as more vaginal teeth. Similarly, the male derivatives of the genital disc, genital arch and phallus apparatus are more poorly formed than in homozygous *dsx* intersexes. $dsx^D$ was shown by DUNCAN and KAUFMAN (1975) and DENELL and JACKSON (1972) to be allelic to *dsx*. Moreover, DUNCAN and KAUFMAN (1975) and KAUFMAN (personal communication) have induced revertants of $dsx^D$ with X rays and showed that many of these revertants were deletions of the *dsx* locus. Chromosomally female individuals carrying $dsx^D$ over a deficiency for the *dsx* locus were reported to be transformed into phenotypically normal males in all external sexual characteristics (DUNCAN and KAUFMAN 1975). Together with the observation that $dsx^D$-bearing males are normal, this suggests that the function of the $dsx^+$ locus necessary for sex determination in the male is normal in $dsx^D$, but is being expressed in chromosomally female individuals where it is normally not expressed.

The effects of $Mas/+$ are very similar to those of $dsx^D/+$ (Table 2). Moreover, *Mas*, like $dsx^D$, when heterozygous with a deficiency for the *dsx* locus ($Df(3R)dsx^{D+Rs}$), transforms chromosomally female individuals into phenotypically normal males, and has no effect on chromosomally male individuals. Thus *Mas* appears to be a second dominant allele at the *dsx* locus, and we suggest it be renamed $dsx^{Mas}$. Of the intersexual phenotypes produced by alleles at the *dsx* locus, those produced by $dsx^{Mas}/+$, while qualitatively like those pro-

duced by *dsx/dsx* and *dsx*$^{D}$/+, are nevertheless distinctly more male-like. For example, the bristles of the foreleg are almost completely rotated to the position of a sex comb and are larger than the bristles produced by *dsx/dsx* or *dsx*$^{D}$/+, but never develop into sex comb teeth. Similarly, the genital arch and phallus apparatus are more completely developed in *dsx*$^{Mas}$/+ and the female genitalia more poorly developed. Most strikingly, a second set of rudimentary phallus apparatus structures (*i.e.*, penis, postgonite, sheath) are almost always found in the same region as the rudiments of the female genitalia. Even in this latter aspect, the difference between *dsx*$^{Mas}$/+ and *dsx/dsx* or *dsx*$^{D}$/+ is probably merely in the degree to which these intersexes are directed towards male development, since occasionally small portions of a secondary phallus apparatus are also found in this location in the other two genotypes.

The final dominant autosomal mutant reported to affect sex determination, *Ix*, has recently been shown to be a part of the bithorax complex of genes (for review, see LEWIS 1978). Mutations in the centromere-distal portion of this gene complex have been isolated that act in both sexes and lead to the posterior abdominal segments having levels of differentiation that are characteristic of more anterior segments in wild-type (M. CROSBY and E. B. LEWIS, personal communication). Thus, the normally dark-pigmented fifth and sixth tergites of males can be transformed into more anterior segments that superficially resemble the fifth and sixth tergites of females in pigmentation. Similarly, the sixth sternite, which normally lacks bristles in the male, is transformed such that it has bristles—a characteristic of more anterior sternites in the male. These intersegmental transformations probably led to the mistaken suggestion that *Ix* affected sex determination. We suggest that *Ix* no longer be used as a locus designation.

*Phenotypes of null mutants at sex-determination loci*

The identity of the morphological effects of homozygous *dsx* and *dsx*/deficiency-bearing flies led DUNCAN and KAUFMAN (1975) to conclude that *dsx* represented the null condition of this locus. We have repeated their observations (comparing *dsx/dsx* and *dsx/Df(3R)dsx*$^{D+R5}$) and agree with their conclusion: in only one aspect of the sexual phenotype (the number of vaginal teeth) could we detect a difference between *dsx/dsx* (8.6 vaginal teeth) and *dsx/Df(3R)-dsx*$^{D+R5}$ (2.8 vaginal teeth). However, the number of vaginal teeth we observed in *dsx/Df(3R)dsx*$^{D+R5}$ is very close to that reported by HILDRETH (1965) for *dsx/dsx* (3.4 vaginal teeth). Thus, our stock of *dsx* may have modifiers that enhance the development of vaginal teeth. The *dsx*$^{+}$ locus is not required for normal viability (Table 3).

Deficiencies for the *tra*$^{+}$ locus were obtained from M. ASHBURNER [*Df(3L)st*$^{SS103}$, ASHBURNER *et al.* 1979] and T. KAUFMAN [*Df(3L)st*, KAUFMAN, personal communication]. Chromosomally female individuals carrying *tra* over either of these deficiencies are morphologically identical to homozygous *tra* females. Males that are *tra*/deficiency are morphologically normal and fertile, as are homozygous *tra* males. Finally, the viability of *tra*/deficiency individuals

is not substantially different from that of *tra*$^+$/deficiency individuals (Table 3). Thus, *tra* appears to represent the null condition at this locus.

We have induced two deficiencies, *Df(2R)L*$^{+R4}$ and *Df(2R)L*$^{+R7}$ that delete the *tra-2*$^+$ locus (for details, see MATERIALS AND METHODS). Chromosomally female individuals that are *tra-2*/deficiency are transformed externally into morphologically normal males that are indistinguishable from *tra-2/tra-2* transformed females. The external sexual morphology of *tra-2*/deficiency males is normal, as is the morphology of *tra-2/tra-2* males. *tra-2*/deficiency males are sterile, suggesting that the male sterility associated with the *tra-2* chromosome is at the *tra-2* locus. The viability of *tra-2*/deficiency individuals is not significantly less than that of *tra-2*$^+$/deficiency individuals (Table 3). Together, these results suggest that *tra-2* represents the null condition at this locus.

*tra-2*$^{OTF}$, however, most likely is a hypomorphic allele. First, when homozygous, *tra-2*$^{OTF}$ causes an incomplete transformation of females into males (FUJIHARA, KAWABE and OISHI 1978). Second, in *tra-2*$^{OTF}$/*tra-2* the transforma-

TABLE 3

*Effects of hemizygosity for autosomal sex determination mutants on viability*

| | Autosomal genotype of progeny | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Female progeny | | | | Male progeny | | | |
| Cross I. +/*Y*; *Df(3R)dsx*$^{D+R8}$, *Sb/CxD* ♂ × indicated female | | | | | | | | |
| ♀ Parent: | *CxD*/+ or */dsx* | *CxD/TM6* | *Df/TM6* | *Df*/+ or */dsx* | *CxD*/+ or */dsx* | *CxD/TM6* | *Df/TM6* | *Df*/+ or */dsx* |
| *w/w*; *TM6*/+ | 234† | 184 | 192 | 293 | 215 | 146 | 198 | 249 |
| | (1.0)* | (0.79) | (0.82) | (1.25) | (0.92) | (0.62) | (0.85) | (1.06) |
| *w/w*; *TM6/dsx p*$^p$ | 202 | 211 | 166 | 261 | 214 | 176 | 117 | 240 |
| | (1.0) | (1.04) | (0.82) | (1.29) | (1.06) | (0.87) | (0.58) | (1.19) |
| Cross II. +/*Y*; *Df*(3*L*)*st*$^{SS103}$/*TM1* ♂ × indicated female | | | | | | | | |
| | *TM1*/+ or */tra* | *TM1/TM2* | *Df/TM2* | *Df*/+ or */tra* | *TM1*/+ or */tra* | *TM1/TM2* | *Df/TM2* | *Df*/+ or */tra* |
| *y/y*; *TM2*/+ | 271 | 269 | 292 | 285 | 290 | 291 | 291 | 307 |
| | (1.0) | (0.99) | (1.08) | (1.05) | (1.07) | (1.07) | (1.07) | (1.13) |
| *y/y*; *TM2/tra* | 117 | 84 | 90 | 117 | 122 | 95 | 94 | 107 |
| | (1.0) | (0.72) | (0.77) | (1.00) | (1.04) | (0.81) | (0.80) | (0.91) |
| Cross III. +/*Y*; *Df(2R)L*$^{+R4}$, *Bl/SM1* ♂ × indicated female | | | | | | | | |
| | *SM1*/+ or */tra-2* | *Df/SM1* | *Df*/+ or */tra-2* | | *SM1*/+ or */tra-2* | *Df/SM1* | *Df*/+ or */tra-2* | |
| *y/y*; *SM1*/+ | 357 | 216 | 210 | | 386 | 192 | 187 | |
| | (1.0) | (0.61) | (0.59) | | (1.08) | (0.54) | (0.52) | |
| *y/y*; *SM1/tra-2* | 354 | 252 | 231 | | 355 | 254 | 263 | |
| | (1.0) | (0.71) | (0.65) | | (1.00) | (0.72) | (0.74) | |

† Number of progeny of each genotype produced by the indicated crosses.
* Numbers in parentheses are viabilities relative to Balancer/(+ or sex-determination mutant) females (column 1).

tion is more complete (FUJIHARA, KAWABE and OISHI 1978) and is more complete in *tra-2$^{OTF}$/Df(2R)L$^{+R4}$* as well. Males carrying *tra-2$^{OTF}$/Df(2R)L$^{+R4}$* are fertile. This suggests that *tra-2$^{OTF}$* is sufficiently leaky that it does not render males sterile even when hemizygous; however, the alternatives—that the sterility and sexual transformation phenotypes of *tra-2* are due to two separate, but tightly linked, mutants or that the *tra-2* locus is complex—cannot be eliminated.

*Autonomy and time of gene expression of the* dsx, tra-2 *and* tra *loci*

To inquire whether the *tra-2*, *tra* and *dsx* loci each determine sex in cell-autonomous manners, crosses were made in order to generate individuals heterozygous for one of these mutants and linked cell-marker mutants (Figure 1). Cultures containing individuals of these genotypes were irradiated early in development to induce mitotic crossing over. The consequence of the appropriate mitotic exchange and segregation is to generate a daughter cell homozygous for the mutant allele at the sex determination locus and simultaneously homozygous for the recessive allele of the cell-marker locus (Figure 1). Ideally, when such a cell is the progenitor of a clone that produces part of the adult cuticle, the clone is identifiable by its cell-marker phenotype, regardless of its sexual phenotype. Thus, when such cell-marker clones are detected in regions of the adult cuticle that are sexually dimorphic, they can be examined to determine whether they differentiated according to their own (mutant) genome (*i.e.*, autonomously) or according to the wild-type information of the surrounding cells (non-autonomously).

If a locus functions autonomously, as we have shown to be the case for *tra-2*, *tra* and *dsx* (see below), then a simple extension of the experiment designed to inquire about autonomy allows the determination of the developmental time that the wild-type allele of the locus completes its role in sex determination. When homozygous mutant clones induced at progressively later stages of development are examined, it will be found that clones produced before some point in development will differentiate according to their own genotype, whereas homozygous mutant clones generated after that time will differentiate as if they still retained the wild-type allele of that locus. The point at which their differentiation changes from the first pattern to the second will be the last time during development that the transcription of the wild-type allele of the locus is needed for normal sexual differentiation in the tissue being examined.

The autonomy and time of function of the *dsx*, *tra-2* and *tra* loci have been examined in the sexually dimorphic portions of the fifth and sixth abdominal tergites and the forelegs. Since the patterns of cell division that give rise to these two tissues differ substantially, they will be considered separately. Similar experiments examining the function of *tra$^{+}$* in the genital disc have been carried out by WIESCHAUS and NÖTHIGER (personal communication).

*Abdomens:* The abdominal histoblasts, the cells that give rise to each hemitergite of the abdomen, are set aside in the embryo and undergo no cell division during the larval period (GARCIA-BELLIDO and MERRIAM 1971a). At about the

time of pupariation, the abdominal histoblasts begin a rapid series of divisions (cell-cycle times of about 2.7 hours) that lasts for about 24 hours to produce the 3800 cells of each hemitergite (GARCIA-BELLIDO 1973).

We have induced mitotic crossing over during the larval and early pupal periods in flies heterozygous for *tra-2*, *tra* or *dsx* (Figure 1). Since an exchange must be followed by cell division before a marker is rendered homozygous, the earliest time we have homozygosed these sex determination mutants in the abdominal histoblasts is near the time of pupariation. For all three of these loci, homozygous mutant clones arising at this time exhibit the mutant sexual phenotype with respect to abdominal pigmentation (Tables 4 through 6; Figures 3 and 4). For *tra-2*, more than 97% of the *pwn* clones in the portions of the fifth and sixth tergites where sex could be determined were associated with areas of male (*i.e.*, homozygous *tra-2*) pigmentation. Similarly, for *dsx*, more than 80% of the $Ki^+$ clones in these sexually dimorphic abdominal segments were associated with cuticle that had male (*i.e.*, homozygous *dsx*) pigmentation (Table 4; Figures 3, 4b). That about 20% of the $Ki^+$ clones were not associated with darkly pigmented cuticle is almost certainly due to the fact that *dsx*, even when homozygous in entire flies, does not extend the male-like pigmentation pattern to the anterior and lateral edges of the fifth and sixth tergites; in flies irradiated as larvae, all $Ki^+$ clones of more than one bristle that were not associated with regions of male cuticle pigmentation were located along the anterior or lateral margins of the fifth and sixth tergites. Consistent with this interpretation is the fact that these clones were substantially smaller, on the average, than $Ki^+$ clones associated with male pigmentation (Table 5); larger clones did not fall

TABLE 4

*Analysis of autonomy of the* tra-2 *locus and time of* tra-2$^+$ *expression in the abdomen*

| Time Irradiated | Clones: Tergites 5 and 6: Male *pwn* | Tergites 5 and 6: Male no *pwn* | Tergites 5 and 6: Indeterminate *pwn* | Tergites 5 and 6: Female *pwn* | Clones: Tergites 2,3,4 *pwn* | Number of abdomens | Frequency male clones | Frequency *pwn* clones |
|---|---|---|---|---|---|---|---|---|
| No irradiation | 2 | 2 | 1 | 0 | 4 | 295 | 0.014 | 0.024 |
| Pre-pupariation, hrs: | | | | | | | | |
| 120–19 | 59 | 17 | 2 | 2 | 111 | 393 | 0.193 | 0.443 |
| 19–12 | 14 | 9 | 1 | 0 | 24 | 106 | 0.217 | 0.368 |
| 12–4 | 22 | 9 | 2 | 1 | 28 | 142 | 0.218 | 0.373 |
| 4–0 | 5 | 1 | 1 | 1 | 13 | 41 | 0.146 | 0.488 |
| Post-pupariation, hrs: | | | | | | | | |
| 0–5.5 | 8 | 4 | 0 | 1 | 17 | 44 | 0.273 ± 0.067 | 0.591 |
| 5.5–13.5 | 14 | 16 | 1 | 10 | 70 | 107 | 0.280 ± 0.043 | 0.888 |
| 13.5–21.5 | 25 | 34 | 8 | 57 | 174 | 193 | 0.306 ± 0.033 | 1.368 |
| 21.5–29.5 | 6 | 0 | 1 | 96 | 160 | 113 | 0.053 ± 0.019 | 2.33 |

Progeny of +/+; *pwn tra-2/SM1* females crossed to $y/y^+Y$; +/+; $spa^{pol}/spa^{pol}$ males were irradiated at the indicated ages and the resulting $y/+$; *pwn tra-2*/+ female progeny scored for clones as described in MATERIALS AND METHODS.

TABLE 5

*Analysis of autonomy of the* dsx *locus and time of expression of* dsx+ *in the abdomen*

| Time irradiated | Clones: Tergites 5 and 6: Male *Ki+* | Male, no bristle *Ki+* | Indeterminate *Ki+* | Female *Ki+* | Tergites 2,3,4 *Ki+* | Number of abdomens | Frequency male clones | Frequency *Ki+* clones |
|---|---|---|---|---|---|---|---|---|
| No irradiation | 0 | 1 | 4 | 0 | 5 | 197 | 0.005 | 0.051 |
| Pre-pupariation, hrs: | | | | | | | | |
| 122–19 | 42 | 13 | 67 | 14 | 158 | 914 | 0.060 | 0.31 |
| 19–7 | 4 | 1 | 5 | 0 | 11 | 50 | 0.100 | 0.40 |
| 7–0 | 2 | 0 | 3 | 1 | 4 | 29 | 0.069 | 0.35 |
| Post-pupariation, hrs: | | | | | | | | |
| 0–10 | 3 | 2 | 23 | 9 | 48 | 144 | 0.035±0.015 | 0.58 |
| 10–18 | 3 | 7 | 12 | 4 | 40 | 67 | 0.149±0.044 | 0.88 |
| 18–26 | 1 | 5 | 20 | 9 | 25 | 68 | 0.088±0.034 | 0.81 |
| 26–35 | 2 | 3 | 26 | 13 | 51 | 68 | 0.074±0.032 | 1.35 |
| 35–42 | 0 | 0 | 0 | 1 | 3 | 45 | 0.000 | 0.088 |

Clone size in 19–122 hr prepupal irradiation

| | No. bristles | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | >10 |
| *Ki+*, male pigmentation | 1 | 3 | 4 | 6 | 6 | 2 | 6 | 5 | 4 | 3 | 2 |
| *Ki+*, female pigmentation | 5 | 3 | 2 | 1 | 1 | 1 | | | | | |

Progeny of +/+; *dsx* $p^p$/*TM6* females crossed to *y*/*Y*; *Ki M(3)S31/TM2* males were irradiated at the indicated ages and the resulting *y*/+; *Ki M(3)S31/dsx* $p^p$ female progeny scored for clones as described in MATERIALS AND METHODS.

entirely within the region near the anterior and lateral margins and were associated with phenotypically *dsx* cuticle pigmentation.

In the case of *tra*, about 75% of the *y jv* clones in portions of the cuticle where the presence of male pigmentation could be detected were associated with clones of male pigmentation (Table 6; Figures 3, 4c). The 25% of the *y jv* clones that were not associated with male pigmentation are presumably due to exchanges that occurred between *jv* and *tra* and homozygosed *y jv* but not *tra*.

These data, then, establish that the wild-type alleles of all three of these loci function in a cell-autonomous manner in the abdomen and are required in the abdominal histoblasts after the resumption of cell division at pupariation for normal sexual development of the abdominal tergites.

One additional feature of the data from clones induced during the larval period deserves comment. It is usually impossible to ascertain the sex of a cell-marker clone that falls within the portion of the fifth or sixth tergites that is darkly pigmented in females; such clones are listed as "indeterminate" in Tables 4 through 6. There is substantial variation between stocks in the extent of this pigmentation; in the *tra-2*/+ females we used, this area was small compared to that in *tra*/+ and *dsx*/+ females, and the relative frequency of indeterminate clones in the different crosses reflects this difference. Moreover, since the bristle and cuticle portions of a clone are not necessarily coincident, it was not always pos-

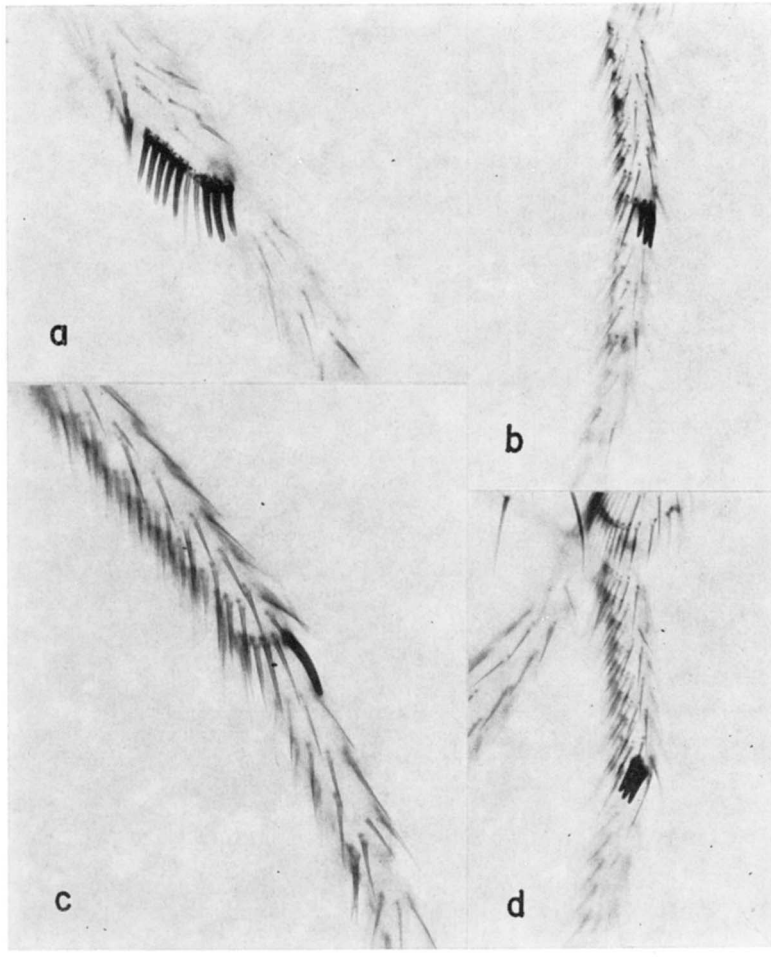


FIGURE 3.—Examples of clones homozygous for sex determination mutants in forelegs. (a) clone of 2 *Ki*+ *dsx* bristles on male foreleg; (b–d) sex-comb teeth produced by homozygous *tra-2* clones. In (b) and (c) note lack of rotation and in (d) partial rotation toward male position.

sible to ascertain the sex of those clones that were near the normally pigmented portion of these tergites in the experiments in which the cell markers affected bristles exclusively (*tra* and *dsx*); since the cell marker *pwn* used with *tra-2* marks hairs as well as bristles, the exact cuticle clonal boundaries, and consequently the sex, could usually be ascertained in these clones. Furthermore, the larger average size of clones in the *tra* experiment relative to the *dsx* experiment (4.3 *y jv* in the 24 to 120 hour pre-pupariation sample *vs.* 3.3 *Ki*+ bristles per clone) contributed to the different proportion of clones of indeterminate sex in the two experiments (29% and 54%, respectively). This difference in mean clone size is probably a reflection of the relative competitive advantages of *M(3)h*+ and *M(3)S31*+ clones in abdomens of flies heterozygous for the respective Minute mutant.

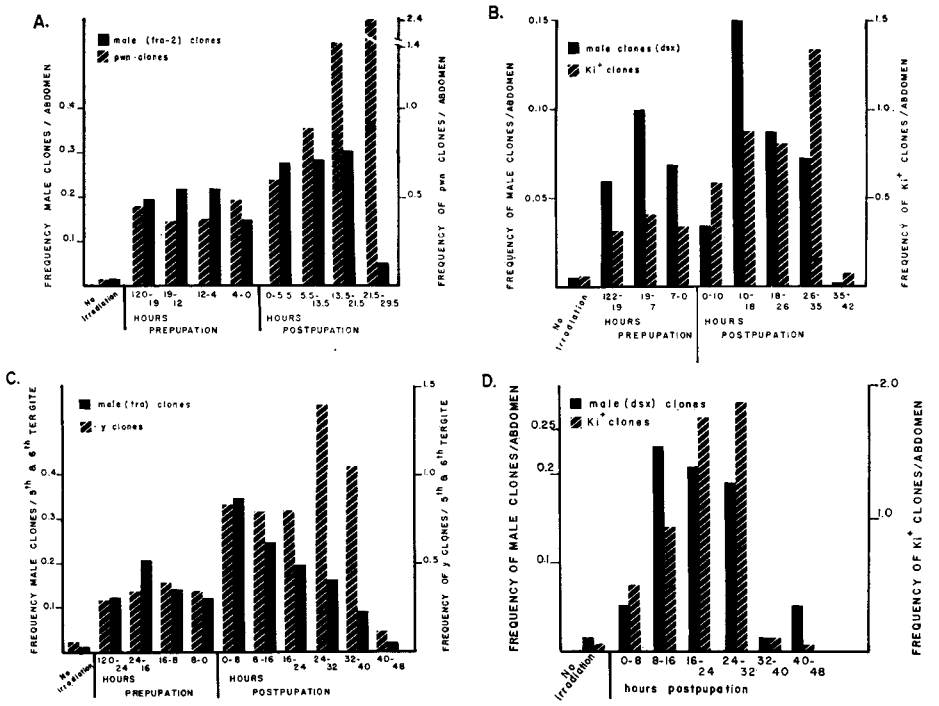


FIGURE 4.—Frequencies of cell-marker and male pigmentation clones produced in the abdomen by irradiation at different times during development. (a) clones in *y*/+; *pwn tra-2*/+ + females; (b) clones in *y*/+; *Ki M(3)S31/dsx* females; (c) clones in *y*/*y* $f^{36a}$; *Dp(1;3)sc*$^{J4}$, *mwh M(3)h*$^{S37}$/ *jv tra* females; (d) clones in *y*/+; *Ki/dsx* females.

To determine when these loci have been transcribed sufficiently to insure normal sexual development in the fifth and sixth tergites, somatic crossing over was induced at a series of times during the early pupal period. Clones expressing the cell markers (*y* for *tra*, *Ki*$^+$ for *dsx* and *pwn* for *tra-2*) on the second through sixth tergites (*dsx* and *tra-2* experiments) or fifth and sixth tergites (*tra* experiment) were scored regardless of their sexual characteristics (Tables 4 through 6) to monitor the time of abdominal histoblast division. The relative frequencies of clones induced at different times exhibit the kinetics expected from the reported proliferation dynamics of the abdominal histoblasts (GARCIA-BELLIDO and MERRIAM 1971a). The frequency of clones is constant during the larval period and begins increasing as cell division commences at pupariation. By 30 to 40 hours post-pupariation, clones are no longer inducible, indicating the cessation of cell division (Figure 4a,b,c).

For all three sex-determination mutants, homozygous mutant clones induced after pupariation can express the mutant sexual characteristics (Figure 4). Thus, transcription of the wild-type alleles of these loci must normally occur into at least the early pupal period. When samples irradiated at progressively later times during the pupal period are compared, we found for all three mutants that:

TABLE 6

*Analysis of autonomy of the* tra *locus and time of expression of* tra$^+$ *in the abdomen*

| Time irradiated | Clones: 5th and 6th tergites | | | | | Number of abdomens | Frequency male clones | Frequency $y(jv)$ clones |
|---|---|---|---|---|---|---|---|---|
| | Male $y\ jv$ | Male no $y\ jv$ | Female $y\ jv$ | Indeterminate $y\ jv$ | $y$ | | | |
| No irradiation | 2 | 1 | 1 | 7 | 3 | 204 | 0.015 | 0.064 |
| Pre-pupariation, hrs: | | | | | | | | |
| 120–24 | 40 | 6 | 14 | 22 | 29 | 362 | 0.127 | 0.290 |
| 24–16 | 29 | 0 | 5 | 6 | 9 | 138 | 0.21 | 0.355 |
| 16–7 | 16 | 3 | 2 | 14 | 20 | 132 | 0.144 | 0.394 |
| 7–0 | 13 | 2 | 1 | 12 | 17 | 123 | 0.122 | 0.350 |
| Post-pupariation, hrs: | | | | | | | | |
| 0–8 | 38 | 9 | 10 | 39 | 26 | 135 | $0.348 \pm 0.041$ | 0.837 |
| 8–16 | 18 | 15 | 12 | 31 | 44 | 133 | $0.248 \pm 0.037$ | 0.789 |
| 16–24 | 5 | 13 | 17 | 24 | 27 | 91 | $0.198 \pm 0.042$ | 0.802 |
| 24–32 | 4 | 10 | 30 | 40 | 47 | 85 | $0.165 \pm 0.040$ | 1.424 |
| 32–40 | 3 | 5 | 20 | 30 | 39 | 87 | $0.092 \pm 0.031$ | 1.057 |
| 40–48 | 1 | 1 | 2 | 7 | 3 | 94 | $0.021 \pm 0.015$ | 0.138 |

Progeny of $y/y$; *jv tra/TM2* females crossed to $y\ f^{36a}/Y$; $Dp(1;3)sc^{J4}$, $y^+$ *mwh* $M(3)h^{S37}$/ *TM2* males were irradiated at the indicated ages and the resulting adults scored for clones as described in MATERIALS AND METHODS.

(1) the proportion of cell-marker clones not associated with sexually mutant cuticle increases, and (2) the proportion of male pigmentation patches not associated with a cell marker clone also increases. These results are expected. Clones induced at later times will be smaller, and because of their small size they will be less likely to encompass a bristle and express the cell marker(s) but will still be recognizable if they produce mutant cuticle pigmentation. Moreover, the determinative event that commits a cell to produce either a bristle or cuticle occurs two cell divisions prior to the termination of division, at the latest (GARCIA-BELLIDO and MERRIAM 1971b); clones induced after this event, therefore, cannot express both cuticle and bristle characteristics. Because of these considerations, we have utilized all male pigmentation clones in determining when these loci have functioned sufficiently to lead to normal sexual differentiation.

A qualitative analysis of the data from the experiment with *Ki M(3)S31/dsx* (Figure 4b) shows that male pigmentation clones are induced at a substantial frequency up until the end of cell division of the abdominal histoblasts. This suggests that the expression of $dsx^+$ is required at least until the termination of cell division for normal sexual development of the abdominal tergites. Indeed, the relative numbers of male pigmentation clones and $Ki^+$ clones in all four post-pupariation samples prior to the termination of cell division are homogeneous by contingency $\chi^2$ test ($\chi^2_3 = 5.96$, $p \cong 0.15$).

It is possible that the presence of a Minute mutant, which was needed for the analysis of the action of this locus in the foreleg, might have altered the experimental outcomes in either of two ways. First, since the cells homozygous for *dsx* are $M^+$ in an otherwise $M$ fly, they undergo more cell divisions than do

other cells present in the tissue at the time of the clone's generation. These extra divisions could dilute out the product of the wild-type allele of the sex determination mutant and thereby make it needed later than it is in $M^+$ individuals. Second, it has been suggested that the Minute loci specify one or another part of the protein synthetic apparatus (RITOSSA, ATWOOD and SPIEGELMAN 1966; HUANG and BAKER 1976); if they do, submaximal protein synthesis could reduce the amount of product of the wild-type allele of the sex determination locus synthesized. This would also cause the wild-type allele to be needed later in development than it is in $M^+$ individuals.

To investigate these possibilities, the time of $dsx^+$ gene function was also determined in *Ki/dsx* (Table 7; Figure 4d). The results are in substantial agreement with those obtained from *Ki M(3)S31/dsx*: a sizable frequency of phenotypically male clones are produced up until the termination of division of the abdominal histoblasts.

Together, these two experiments show that the wild-type allele of *dsx* is needed very late, probably at least until the cessation of cell division, for normal sexual differentiation of the abdominal tergites.

For *tra-2*, the results are very clear (Table 4; Figure 4a); the number of male clones remains high during most of the pupal period and drops precipitously before the cessation of cell division. Consequently, it must be that sufficient transcription of $tra\text{-}2^+$ to allow normal female sexual differentiation occurs prior to the termination of cell division in all cell lineages.

In the experiment with *tra*, there is a gradual decline in the frequency of male pigmentation clones, as well as a gradual decline in the number of male pigmentation clones relative to cell-marker ($y$) clones, throughout the pupal period (Table 6; Figure 4c). The latter decline is significant by a contingency $\chi^2$ test ($\chi^2_4 = 27.5$, $P < 0.01$). This gradual decline in the frequency of male clones could mean that $tra^+$ has functioned sufficiently for normal female differentiation at different times in different cell lineages. However, the interpretation of

TABLE 7

*Analysis of the time of expression of* dsx+ *in the absence of* M(3)S31

| Time irradiated | Clones: Tergites 5 and 6: Male *Ki*+ | Tergites 5 and 6: Male, no *Ki*+ bristle | Tergites 5 and 6: Indeterminate *Ki*+ | Tergites 5 and 6: Female *Ki*+ | Tergites 2,3,4 *Ki*+ | Number of abdomens | Frequency male clones | Frequency *Ki*+ clones |
|---|---|---|---|---|---|---|---|---|
| No irradiation | 0 | 4 | 2 | 3 | 7 | 264 | 0.015 | 0.05 |
| Post-pupariation, hrs: | | | | | | | | |
| 0–8 | 1 | 4 | 21 | 4 | 26 | 97 | 0.052 | 0.54 |
| 8–16 | 6 | 18 | 20 | 8 | 63 | 103 | 0.233±0.042 | 0.94 |
| 16–24 | 2 | 22 | 59 | 25 | 119 | 115 | 0.209±0.038 | 1.78 |
| 24–32 | 3 | 19+1? | 56 | 25 | 133 | 116 | 0.190±0.036 | 1.87 |
| 32–40 | 0 | 1 | 0 | 2 | 5 | 68 | 0.014 | 0.10 |
| 40–48 | 0 | 4 | 0 | 1 | 3 | 69 | 0.058 | 0.06 |

Progeny of +/+; *dsx* $p^p$*/TM6* females crossed to *y/Y*; *Ki/Ki* males were irradiated at the indicated ages and the resulting *y*/+; *Ki/dsx* $p^p$ female progeny scored for clones.

these results is confounded by the fact that the male clones produced are phenotypically yellow and therefore become progressively more difficult to detect at later time points. Thus, while the data suggest that *tra*$^+$ has been transcribed sufficiently for normal female sexual differentiation before the end of cell division, this conclusion is less strong than that for *tra-2*$^+$.

One facet of the *tra-2* data deserves comment. It has been noted that male pigmentation clones not marked with *pwn* can arise *via* exchanges between *pwn* and *tra-2*. If all non-*pwn* male pigmentation clones had this origin, then the proportion of all *tra-2* clones of this type should remain constant throughout development. However, the proportion of non-*pwn* male pigmentation clones is substantially higher in the late time points. The reason for this is that no hairs are produced by the cells that elaborate the cuticle of the posterior portion of the sixth tergite; thus, clones in this region are not capable of producing a *pwn* phenotype unless they encompass a bristle and, as clone size decreases, fewer clones do.

The above data establish that the expressions of the wild-type alleles of *tra-2*, *tra* and *dsx* are required into the pupal period for normal sexual development of the abdominal tergites. *dsx*$^+$ is required throughout the period of proliferation of the abdominal histoblasts. On the other hand, *tra-2*$^+$ and *tra*$^+$ have been transcribed sufficiently for normal sexual differentiation in all or most lineages, respectively, before the termination of cell division. It should be noted that the data for *tra-2*$^+$ and *tra*$^+$ do not say that transcription normally ceases at this time, only that it is sufficient at this time to produce a normal phenotype with respect to the character we have scored. It should also be emphasized that the different times of expression of the loci bear no necessary relationship to the order (if any) in which the products of these loci function.

*Forelegs*: To inquire whether the expressions of the *dsx*$^+$, *tra-2*$^+$ and *tra*$^+$ loci are cell autonomous in other tissues and whether these genes function at the same times in different tissues, we have also induced clones homozygous for each of these mutants at various times during the development of the leg. Specifically, attention has been focused on the action of these loci in the portion of the basitarsus that produces the cells that elaborate either the distal-most transverse row of bristles in the female or the sex comb in the male.

Studies of the proliferative properties of the leg imaginal discs have shown that the disc cells begin dividing shortly after hatching and continue throughout the larval period (BRYANT and SCHNEIDERMAN 1969; STEINER 1976; MADHAVAN and SCHNEIDERMAN 1977). Thus, the homozygosis of mutants *via* mitotic recombination will follow immediately after the induction of mitotic exchange. The probability of inducing a clone that embraces one or more of the approximately ten cells of the basitarsus that produce the sexually dimorphic bristles is quite low at any time during development, on the order of one percent or less for 1000R of X rays (TOKUNAGA 1962; this report). To improve on this frequency, we have made use of the fact that a homozygous $M^+$ cell produced by induced mitotic recombination in a leg disc otherwise composed of phenotypically $M$ cells (genotype: $M^+/M$) has both growth-rate and competitive advantages com-

pared to the Minute cells (MORATA and RIPOLL 1975; STEINER 1976). As a consequence, such a cell will give rise to a clone much larger than those normally induced at that time. How much larger is a function of the severity of the Minute mutant employed. With a strong Minute, a single $M^+$ cell produced in a young $M^+/M$ larva when there are about 30 to 100 cells in a leg disc can give rise to nearly half a leg instead of the small percentage of the leg it would produce if it did not have a growth advantage (STEINER 1976). Thus, by arranging the genotype such that a mitotic exchange that results in homozygosis for a sex-determination mutant simultaneously makes that cell $M^+$ in an otherwise $M$ fly (Figure 1), we greatly increase the number of cells in which exchanges will produce a daughter cell that is a progenitor of the portion of the basitarsus of interest.

The time that $dsx^+$ functions in the foreleg was determined in *dsx/Ki M(3)S31* males that were sibs of the females in which the time of expression of $dsx^+$ in the abdominal tergites was investigated. When homozygous in males, *dsx* leads to the formation of bristles in place of sex-comb teeth; these bristles are intermediate in location between the positions normally occupied by the sex-comb teeth in males and the distal-most transverse row of bristles of the basitarsus in females.

Approximately 2% of the forelegs of *dsx/Ki M(3)S31* irradiated as young larvae contain clones in which $Ki^+$ bristles occur either in the position where sex-comb teeth are normally found or at positions typical of homozygous *dsx* forelegs (Table 8). For several reasons we believe that all of these bristles are produced by clones of homozygous *dsx* cells. First, these bristles are $Ki^+$, and cells that are homozygous $Ki^+$ must also be homozygous for the more distally located *dsx*. Second, in this experiment, as well as the experiments with the *tra* and *tra-2* mutants described below, all of our observations suggest that whether or not the bristles (*dsx*) or sex-comb teeth (*tra-2*, *tra*) produced by a clone homozygous for a sex-determination mutant occupy the same position that they would in a fly homozygous for the mutant is a function of the size of the clone.

TABLE 8

*Frequencies and properties of homozygous* dsx *clones induced in forelegs of* +/Y; dsx $p^p$/Ki M(3)S31 *males at the indicated times*

| Time irradiated | Number clones of bristles (*dsx Ki*$^+$) | Average size clone (No. bristles) | Number forelegs | Frequency clones, % |
|---|---|---|---|---|
| No irradiation | 0 | 0 | 692 | 0 |
| Pre-pupariation, hrs: | | | | |
| 122–98 | 5 | 2.8 | 285 | 1.8 |
| 98–75 | 11 | 2.3 | 372 | 3.0 |
| 75–51 | 8 | 1.8 | 422 | 1.9 |
| 51–27 | 5 | 1.0 | 324 | 1.5 |
| 27–0 | 9 | 1.0 | 272 | 3.3 |

The cross generating these males was: +/+; *dsx* $p^p$/*TM6* females × *y*/*Y*; *Ki M(3)S31/TM2* males.

The more of the distal-most transverse row encompassed by the clone, the more normal is the position of the bristles (or teeth) produced by the clone. The morphology of the individual sex-comb teeth (*tra-2*, *tra*) or bristles (*dsx*) is independent of clone size and indistinguishable from that found in homozygous mutant animals. Finally, to insure that these putative *dsx* clones are not artifacts produced by irradiation, we have X rayed *dsx*$^+$*/Ki M(3)S31* males and looked for the formation of bristles in place of sex-comb teeth. No such transformations were found in 415 forelegs from males irradiated during the larval period. From these observations, we conclude that all *Ki*$^+$ bristles found in place of sex-comb teeth in *dsx/Ki M(3)S31* males are the products of the autonomous action of homozygous *dsx* clones.

The time when *dsx*$^+$ is expressed sufficiently for normal sex-comb development was determined by inducing homozygous *dsx* clones at successive eight-hour intervals throughout the larval period. Because the number of clones per sample was small, samples have been pooled to give a set of 24-hour samples (Table 8). These data show that removal of *dsx*$^+$ from a cell any time during the larval period results in a phenotypically *dsx* clone. As expected, clones induced late in development are smaller than those induced earlier. From these results, we conclude that transcription of *dsx*$^+$ is required in the foreleg disc at least as late as the end of the larval period. Whether it is required as late as the termination of cell division is not clear since, to the best of our knowledge, it is not known whether cell divisions in the foreleg cease at pupariation or shortly thereafter.

The time of *tra* expression in the foreleg was examined by inducing mitotic recombination in *y/y*; *Dp(1;3)sc*$^{J4}$, *y*$^+$ *mwh M(3)h*$^{ss7}$*/jv tra* females (Figure 1). Homozygous *tra* clones were identified by the differentiation of sex-comb teeth. The results of these experiments (Figure 5) show that irradiation at, or prior to, 72 hours before pupariation induced homozygous *tra* clones that expressed the *tra* phenotype (sex-comb teeth) in about 9% of the leg discs irradiated. Between 72 and 40 hours before pupariation, the frequency of such clones drops precipitously and thereafter remains low. The average size of clones, as measured by the number of sex-comb teeth produced, decreases with increasing larval age, as it should (Figure 5). The most straightforward interpretation of these data is that sufficient transcription of *tra*$^+$ has occurred by 72 to 40 hours prepupation to allow for the normal development of the female bristle pattern on the basitarsus.

An alternative interpretation of these data is that during the period of 72 to 40 hours before pupariation the number of cells that, if made *M*$^+$, could serve as progenitor cells for the sex-comb region drops precipitously—perhaps through the occurrence of clonal restrictions. There are two arguments that suggest this possibility is unlikely. Although the numbers are small, our data suggest that the frequency of phenotypically *dsx* clones in the foreleg remains constant throughout the larval period, which is consistent with the notion that restrictions on the developmental potential of cells are not occurring at this time. More directly, we have also induced homozygous *tra* clones in female forelegs in the

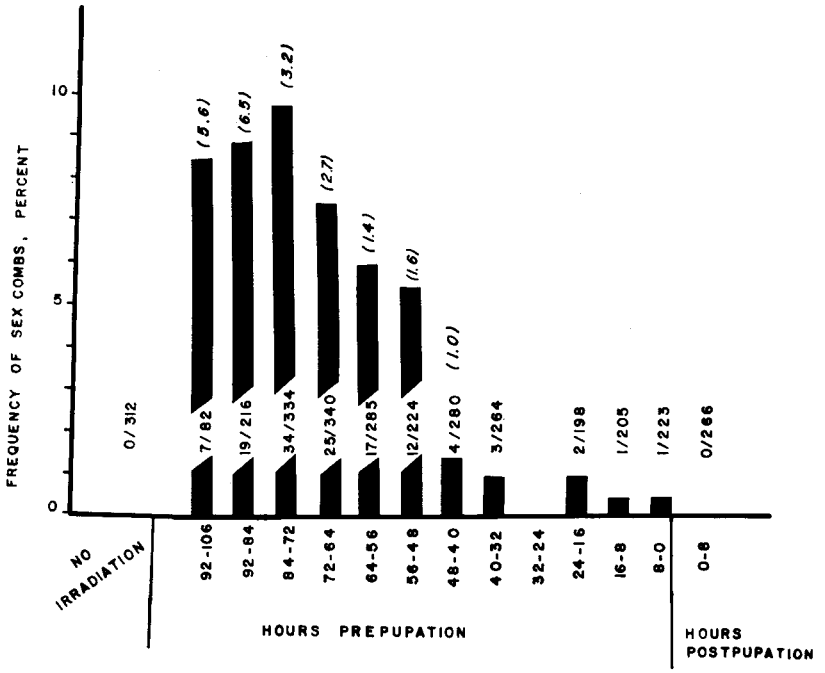


FIGURE 5.—Frequencies and sizes of homozygous *tra* clones giving rise to sex-comb teeth in females irradiated at different times during development. Number of clones of sex-comb teeth and number of forelegs scored at each time point are given by #/#. The average size of clones (number of teeth) at each time point is given in parentheses.

absence of a Minute mutant (Table 9). Although the data are less extensive because of the low frequency of clones, they corroborate the findings from the previous experiment; the frequency of phenotypically *tra* clones declines greatly a day or two before pupariation.

Together, these experiments support the conclusion that by about two days prior to pupariation the wild-type allele of *tra* has been transcribed sufficiently

TABLE 9

*Frequencies and properties of homozygous* tra *clones induced in forelegs of* y/y $f^{36a}$; Dp(1;3)$sc^{J4}$, y+ mwh/jv tra *females at the indicated times*

| Time of irradiation | Number of clones of sex comb teeth | Average clone size (No. teeth) | Number forelegs | Frequency clones, % |
|---|---|---|---|---|
| No irradiation | 0 | 0 | 412 | 0.00 |
| Pre-pupariation, hrs: | | | | |
| 94–70 | 7 | 1.43 | 570 | 1.23 |
| 70–46 | 11 | 1.00 | 747 | 1.47 |
| 46–22 | 4 | 1.00 | 628 | 0.64 |
| 22–0 | 1 | 1.00 | 820 | 0.12 |

The cross generating these females was: *y/y; jv tra/TM2* females × *y* $f^{36a}$/Y; *Dp(1;3)*$sc^{J4}$, *y+ mwh/TM2* males.

to support the differentiation of a normal female pattern on the foreleg.

The time of expression of *tra-2*$^+$ in the foreleg was also determined. This experiment shows that *tra-2*$^+$, like *tra*$^+$, has functioned sufficiently to support normal female foreleg development well before pupariation (Table 10). Irradiations prior to two days before pupariation induce phenotypically *tra-2* clones (*i.e.*, sex-comb teeth) in about 2.5% of forelegs. Between 48 and 24 hours before pupariation, the frequency of such clones decreases dramatically, suggesting that *tra-2*$^+$ is not needed after this time in the female foreleg.

A comparison of the times of function of the wild-type alleles of the *tra-2*, *tra* and *dsx* loci in the abdominal histoblasts and the foreleg imaginal disc reveals similar patterns of gene action in these two tissues. In both tissues, the presence of *dsx*$^+$ is required quite late, up to at least the termination of cell division in the abdomen, the last point at which we could assess a requirement for its expression. The presence of either *tra-2*$^+$ or *tra*$^+$, on the other hand, ceases to be required for the occurrence of normal sexual differentiation in some or all cell lineages before the end of cell division in both tissues.

*Mutant Interactions*

*Double-mutant combinations:* In order to understand how the wild-type alleles of these sex determination loci act to specify normal sexual development, it is necessary to know whether their products function sequentially in a regulatory pathway that controls sexual development or act independently in parallel processes regulating sexual development. One approach that can help to discriminate between these possibilities is to compare the phenotype of flies simultaneously homozygous for mutants at two of these loci to the phenotype produced by the component single mutant. If the mutants being studied are null

TABLE 10

*Frequencies and properties of homozygous* tra-2 *clones induced in forelegs of* +/+; pwn tra-2/M(2)S7 *females at the indicated times*

| Time of irradiation | Number of clones of sex comb teeth | Average clone size (No. teeth) | Number forelegs | Frequency clones, % |
|---|---|---|---|---|
| No irradiation | 2 | 4 | 690 | 0.3 |
| Pre-pupariation, hrs: | | | | |
| 144–120 | 8 | 6.0 | 340 | 2.4 |
| 120–96 | 20 | 4.8 | 525 | 3.8 |
| 96–72 | 18 | 4.3 | 775 | 2.3 |
| 72–48 | 16 | 2.1 | 528 | 3.0 |
| 48–24 | 9 | 1.2 | 433 | 2.1 |
| 24–0 | 1 | (2.0) | 919 | 0.1 |
| Post-pupariation, hrs: | | | | |
| 0–24 | 0 | 0 | 550 | 0.0 |

The cross generating these females was: +/+; *pwn tra-2/SM1* females × +/*Y*; *M(2)S7/SM1* males.

alleles and if the two loci function in sequential steps in a pathway, then the double-mutant combination should exhibit the same phenotype as one of the component single mutants. However, if the two loci specify independent steps in parallel pathways, then the double-mutant combination should exhibit a phenotype that is a composite of the phenotypes produced by the two component single mutants.

There are a number of concerns in making such a double-mutant analysis (for a review, see Pringle 1978). One requirement is that the mutants utilized not be leaky. This criterion is satisfied for *tra*, *dsx* and *tra-2* since we have shown that they behave as null alleles. However, for neither allele at the *ix* locus is there any information on the nature of the mutational lesion other than that they are recessive, which suggests loss or reduced amount of wild-type function. A major weak point in such an analysis is that one cannot be sure that the "unique" phenotype produced by two mutants in independent pathways will be distinguishable from those of the component single mutants.

With such qualifications in mind, we constructed and examined the external morphology of females simultaneously homozygous for all double mutant combinations of *ix*, *tra-2*, *tra* and *dsx*. The results show a clear hierarchy of epistasis. Flies homozygous for *dsx* and any of the other three mutants exhibit the *dsx* phenotype. Thus, flies homozygous for *dsx* and *tra* have the same intersexual phenotype produced by *dsx* alone. This agrees with the finding of MUKHERJEE and HILDRETH (1971) that *dsx* is epistatic to *tra* in the foreleg. Similarly, the intersexual phenotype of chromosomally female individuals that are homozygous for *tra-2* and *dsx* is not distinguishable from that of *tra-2*$^+$; *dsx* individuals. *dsx* and *ix* produce very similar intersexual phenotypes; that produced by *dsx* has better development of the male genital apparatus and more extensive male-like abdominal pigmentation. Females homozygous for *ix* and *dsx* have an intersexual phenotype like that produced by *dsx*. *tra* and *tra-2* produce identical phenotypes in females, and the double mutant produces the same phenotype as the single mutants in agreement with the report of WATANABE (1975). Finally, both *tra* and *tra-2* exhibit complete epistasis to *ix*: chromosomally female individuals that are homozygous for *tra-2* and *ix* or *tra* and *ix* are transformed into phenotypically normal males. Thus, mutants at these four loci exhibit a clear epistatic hierarchy of the form *dsx* > *tra*, *tra-2* > *ix*. This is consistent with the hypothesis that the wild-type alleles of these loci control a series of steps in a single pathway regulating sex determination.

We have also examined the interaction of *tra-2* with *dsx*$^D$, a dominant allele at the *dsx* locus that affects only females and transforms them into intersexes. Chromosomally female individuals that are homozygous for *tra-2* and also carry *dsx*$^D$ over a normal third chromosome are morphologically normal males identical to those produced by homozygous *tra-2* alone. Thus, *tra-2* is epistatic to *dsx*$^D$/*dsx*$^+$.

An allele of *dsx* (*dsx*$^{136}$) that affects only males and transforms them into intersexes quite similar to those produced by *dsx* has recently been isolated by

A. GAREN (personal communication) and kindly supplied to us; $dsx^{136}$ over $dsx$ (or a deficiency for $dsx$) transforms chromosomally male individuals into intersexes, but has no effect in chromosomally female flies. In males, $dsx^{136}$ is fully complemented by the dominant female-specific $dsx$ alleles $dsx^{D}$ and $dsx^{Mas}$: $X/Y$; $dsx^{136}/dsx^{D}$ and $X/Y$; $dsx^{136}/dsx^{Mas}$ individuals are morphologically normal fertile males. Chromosomally female individuals that are $dsx^{136}/dsx^{D}$ or $dsx^{136}/dsx^{Mas}$ are indistinguishable from their chromosomally female sibs that are $dsx^{+}/dsx^{D}$ or $dsx^{+}/dsx^{Mas}$, respectively.

Among the most striking interactions we have studied is that of $dsx^{136}$ with *tra-2*. Since $dsx^{136}$ is male specific, $X/X$; $dsx^{136}/dsx$ individuals that are otherwise wild type are morphologically normal fertile females. However, when $X/X$; $dsx^{136}/dsx$ individuals are made homozygous for *tra-2*, they are transformed into intersexes equivalent to those produced by $dsx^{136}/dsx$ in chromosomally male individuals. Thus, in chromosomally female individuals that are otherwise wild type, $dsx^{136}$ complements $dsx$ but, in the absence of a wild-type allele at *tra-2*, $dsx^{136}$ fails to complement $dsx$ and the combination $dsx^{136}/dsx$ is epistatic to *tra-2/tra-2*.

The results of these studies on mutant interactions are used to construct a model for the mechanism of sex determination in Drosophila, which is described in the DISCUSSION.

## DISCUSSION

The most striking feature of mutants at the *ix*, *tra*, *tra-2* and *dsx* loci is their broad pattern of effects. A single mutant can alter not only the developmental pathway of all external sexual characteristics to that of the other sex, but also that of the internal genital duct system and, at least in the case of *tra* and *tra-2* (the only mutants examined in this regard), courtship behavior as well. The *tra* locus has been shown not to affect sex determination in the germ line (MARSH and WIESCHAUS 1978), and the similarity of the morphology of the gonads in homozygous *tra-2* females to that found in *tra* females suggests that the *tra-2* locus is also inactive in the germ line. These observations suggest that $tra^{+}$, $tra\text{-}2^{+}$, $dsx^{+}$ and $ix^{+}$ act to regulate sexual development in all tissues other than the germ line.

Wild-type males and females differ not only in their internal and external sexual development and behavior patterns but also in: (1) the levels of transcription of *X*-linked genes (*i.e.*, dosage compensation occurs; for review, see LUCCHESI 1977); (2) their susceptibility to killing by sex-specific lethal mutations (BELL 1954; FUKUNAGA, TANAKA and OISHI 1975; GOLUBOVSKY and IVANOV 1972; TANAKA, FUKUNAGA and OISHI 1976; J. BELOTE, personal communication; ZIMMERING and MULLER 1961; SANDLER 1972; CLINE 1976, 1978; MARSHALL and WHITTLE 1978) and sex-specific lethal infections (for review see POULSON 1963); and (3) their overall body size. Neither *tra* nor *dsx* affect dosage compensation (MULLER 1950; SMITH and LUCHESSI 1969) and in no test conducted thus far have any of these mutants altered the susceptibility of

females to a sex-specific lethal agent (SAKAGUCHI and POULSON 1963; COLAIANNE and BELL 1968; WATANABE 1975; MIYAMOTO and OISHI 1975; MARSHALL and WHITTLE 1978) nor do these mutants alter body size (STURTEVANT 1945; BROWN and KING 1961; HILDRETH 1965; WATANABE 1975). These observations suggest that dosage compensation and sex determination in Drosophila are under separate genetic control. This does not preclude the possibility that there are initial events common to the two processes, but, if so, the regulation of these processes diverges sufficiently during development that all secondary sexual characteristics of females can be transformed into those of males without affecting dosage compensation. Similarly, the sex-specific lethality caused by certain mutants and infections would appear to be due to something other than sex (with the possible exception of the sex of the germ line) that differs between *2X2A* and *1X2A* individuals.

That these sex-determination mutants can affect the development of many (all?) secondary sexual characteristics suggests that they identify a set of regulatory loci that control the expression of batteries of structural loci necessary for normal sex determination. Indeed, as has been noted by OUWENEEL (1976), it seems reasonable to consider the functions of these loci as analogous to those of the homeotic loci that specify the development of segmental specializations (for reviews see BAKER 1978; LEWIS 1978; GARCIA-BELLIDO 1977; MORATA and LAWRENCE 1977; GEHRING 1976). In the properties examined thus far, the modes of action of the *tra*, *tra-2* and *dsx* loci are quite analogous to those of other homeotic loci.

We have shown that $tra^+$, $tra\text{-}2^+$ and $dsx^+$ function in a cell-autonomous manner in the foreleg and abdominal histoblasts, and WIESCHAUS and NÖTHIGER (personal communication) have shown that $tra^+$ also acts autonomously in the derivatives of the genital disc. All other homeotic loci examined thus far also act in a cell-autonomous manner. With respect to the mechanism of sex determination in Drosophila, the cell autonomy of *tra*, *tra-2* and *dsx* establishes that the wild-type alleles of these loci do not make diffusable substances (*e.g.*, hormones) that regulate sexual differentiation.

To determine when the wild-type alleles of the *tra*, *tra-2* and *dsx* loci have functioned sufficiently to allow normal sexual differentiation, we have induced mitotic recombination to remove the wild-type alleles of these loci from cells at various times during larval and early pupal development. The results establish that the wild-type alleles of all three loci are needed quite late in development for normal sexual differentiation. In the histoblasts that produce the fifth and sixth abdominal tergites, $tra\text{-}2^+$ and probably $tra^+$ are required until a few cell divisions before the cessation of division at about 24 to 30 hours post-pupariation, whereas the wild-type allele of *dsx* appears to be needed at least up to the termination of cell division. In the foreleg, the wild-type alleles of *tra* and *tra-2* cease being required for normal sexual differentiation about two days before pupariation, whereas $dsx^+$ is needed up to at least pupariation. The substantial temporal difference between the times the wild-type alleles of the transformer loci are required in the foreleg and abdominal histoblasts may simply be a consequence

of the very different proliferation dynamics of these two tissues. Cell-cycle times in foreleg imaginal discs heterozygous for a strong Minute mutant, such as those we employed, are probably on the order of 10 to 12 hours, whereas cell-cycle times in the abdominal histoblasts are about 2.7 hours (GARCIA-BELLIDO 1973; FERRUS 1975). Thus, in both tissues the *tra-2* locus has functioned sufficiently for normal sexual differentiation by about 3 to 5 cell divisions before the cessation of division. That the wild-type alleles of all three of these loci are required into the third larval instar/early pupal period is consistent with the results of analogous experiments on other homeotic loci; in the few instances studied to date, the wild-type alleles of homeotic loci are required into the third larval instar for normal development (for reviews see BAKER 1978; LEWIS 1978; GARCIA-BELLIDO 1977; MORATA and LAWRENCE 1977; GEHRING 1976; OUWENEEL 1976).

Our experiments provide no evidence as to when the $tra^+$, $tra\text{-}2^+$ and $dsx^+$ loci begin being expressed during development. That $tra^+$ begins functioning quite early in at least some tissues is indicated by SEIDEL's (1963) finding that the gonads in homozygous *tra* females are morphologically distinct from normal female gonads as early as the first larval instar. Although it is clearly dangerous to combine data from different tissues in reasoning about gene action, SEIDEL's findings, together with those we report here, are at least consistent with the hypothesis that $tra^+$ expression may begin very early and be required continuously until the late larval/early pupal period to maintain the normal state of sex determination, which is realized when cells differentiate during the pupal period. Such a pattern of gene action has been suggested to hold for many homeotic loci, but in only a few instances (*e.g.*, the bithorax gene complex, LEWIS 1978; CAPDEVILA and GARCIA-BELLIDO 1978) are there clear data indicating that a homeotic locus begins acting early and continues to be required throughout larval development to maintain a particular state of determination.

It should be emphasized that the experiments we have carried out to inquire when the $tra^+$, $tra\text{-}2^+$ and $dsx^+$ loci function identify only the times at which transcription of each of these loci is sufficient for normal sexual differentiation; they do not indicate whether transcription normally ceases then or at some later time. Similarly, with respect to the functioning of the products of the $tra^+$, $tra\text{-}2^+$ and $dsx^+$ loci, these experiments define only the earliest times when their roles in sex determination could be completed. Thus, the finding that $tra^+$ and $tra\text{-}2^+$ are no longer required in some or all cell lineages some time before the end of cell division in both the foreleg and abdominal histoblasts could mean either that sex has been determined at this time or that sufficient wild-type gene product is present in these cells to carry through the last few cell divisions at a high enough concentration to support normal sexual development (*i.e.*, these loci exhibit perdurance, GARCIA-BELLIDO and MERRIAM 1971b). On the other hand, the wild-type allele of *dsx* is required until the end of cell division in both tissues and thus does not exhibit perdurance.

From the observations that *tra*, *tra-2* and *dsx* behave like null alleles at these loci, together with known aspects of the normal development of sexually

dimorphic portions of the wild-type adult, it is possible to make some inferences about the domains and modes of action of the wild-type alleles of these loci. The sexually dimorphic structures of the adult fall into two categories, based on the origin of the structures in the two sexes. On the one hand are the forelegs, abdominal tergites and anal plates where the structures in the two sexes are homologous and represent alternative developmental pathways that can be taken by a single primordium (TOKUNAGA 1962; NÖTHIGER, DÜBENDORFER and EPPER 1977). In contrast, it has recently been shown from an analysis of gynandromorphs that the genitalia of males and females are derived from separate primordia (NÖTHIGER, DÜBENDORFER and EPPER 1977). That mutants at these four sex-determination loci affect all secondary sexual characteristics shows that the regulation of sex determination in both types of tissues is effected by the same signals.

In order to account for the fact that the absence of either *tra*$^+$ or *tra-2*$^+$ function in chromosomally female individuals results in the progenitor cells of the sex-comb region, tergites and anal plates following a male developmental pathway, it is sufficient to postulate that both of these regulatory loci control binary decisions as to which pathway of sexual differentiation will be followed. That in the absence of wild-type function at either locus male differentiation ensues would suggest that maleness is a more primitive (basal) level of development that occurs when a fly is not directed to develop into a female. Again, this pattern of gene action is strikingly similar to that observed with the homeotic loci that regulate segmental specialization; with but one possible exception (KAUFMAN 1978) they appear to control binary decisions (KAUFFMAN 1977; KAUFFMAN, SHYMKO and TRABERT 1978; GARCIA-BELLIDO 1975), and in the absence of wild-type gene function in the target tissue(s) a pattern of normal differentiation characteristic of another segment, or part of a segment, ensues. If attention is restricted to homeotic mutations that cause a loss of wild-type function, a reasonable case can be made that the new pattern of differentiation observed represents a phylogenetically more primitive (basal) level of specialization (LEWIS 1978; GARCIA-BELLIDO 1977).

The effects of the *tra* and *tra-2* mutations on the primordia of the genitalia further define the regulatory functions specified by these loci. The observation that homozygous *tra* gynandromorphs have normal male genitalia (GEHRING, WIESCHAUS and HOLLIGER 1976) suggests that the absence of *tra*$^+$ leads to the activation of the male genital primordium and the suppression of the female genital primordium. This has been most elegantly demonstrated by the clonal analysis of *tra* in the genital disc (WIESCHAUS and NÖTHIGER, personal communication). These results show that in females *tra*$^+$ action is needed both: (1) to prevent the development of the male genital primordium, and (2) to allow the development of the female primordium. Thus, *tra*$^+$ acts *both* to turn on one developmental pathway and to turn off the alternate developmental pathway in the genital disc.

The most parsimonious view of *tra*$^+$-regulated gene expression is that it also has a dual function of turning on one developmental pathway and suppressing

an alternative developmental pathway in tissue where male and female structures are homologous (*i.e.*, foreleg, anal plates, fifth and sixth tergites). If this be so, then the very striking parallels between the regulatory properties of the *tra* locus and those of homeotic loci that control the development of segmental and subsegmental specializations would suggest that such dual regulatory roles may be properties of other homeotic genes as well. This is *contra* to the view that homeotic loci act to bring about specialization by specifying the sequential inactivation (or activation) of batteries of genes (*e.g.*, GARCIA-BELLIDO 1977). Under the latter view, in its simplest form, the transformation observed in the absence of wild-type function at a particular homeotic gene is due simply to the failure to repress the set of differentiation functions that are normally turned off by the wild-type allele of the homeotic locus. The data from *tra* suggest this is not the case at one and, by analogy, perhaps all homeotic loci.

How the wild-type alleles of the transformer loci might act both to prevent the expression of male sexual differentiation functions and cause the expression of female sexual differentiation functions is suggested by a consideration of the *dsx* locus and the interaction of *tra* and *tra-2* with *dsx*.

One of the most salient features of the *dsx* locus is that its absence leads, independent of chromosomal sex, to the differentiation of both male and female sexual characteristics. This is most evident in those derivatives of the genital disc that come from separate primordia in the two sexes. Here, all structures characteristic of both males and females are found in at least some homozygous *dsx* flies (with the exception of the apodeme—an internal chitinous extension of the phallus that serves as a site of muscle attachment), although they may be reduced or poorly organized (HILDRETH 1965). Thus, the information needed for both male and female sexual differentiation is expressed in both sexes in the absence of $dsx^+$.

There are at least two ways of viewing the intersexuality produced by these alleles. On the one hand, this phenotype has been suggested to be the consequence of a defect very early in the process of sex determination that produces or reveals a developmentally primitive intersexual state (HILDRETH 1965; MUKHERJEE and HILDRETH 1971). Alternatively, it may be that the *dsx* phenotype is simply the result of the simultaneous expression of the pathways for both male and female sexual differentiation in each fly. Under the latter view, which we favor, the intersexual nature of those parts that come from the same primordia in the two sexes, such as the foreleg, would be the consequence of the progenitor cells of these structures trying to follow both pathways of sexual differentiation simultaneously and producing intermediate structures. (For consideration of somewhat analogous situations at the *bithorax* gene complex, see MORATA 1975; KIGER 1976.) In either case, the important fact is that the wild-type allele of *dsx* must function in both males and females to prevent the expression of those sexual differentiation functions that do not correspond to their chromosomal sex.

In order to account for opposite functions in the two sexes, we suggest that the $dsx^+$ locus can function in two modes. In females, it acts to specify a product whose function is to preclude expression of the genes that are involved in male

sexual differentiation; in males, it specifies a second product that acts to prevent the expression of female sexual differentiation functions. We make no specification as to whether the product used in one sex is a derivative of that used in the other or whether there are structurally discrete molecules produced in the two sexes. The array of mutational types known at the *dsx* locus lends support to the notion that it is functionally complex. Most alleles are like *dsx*, in that they are recessive and allow both male and female sexual differentiation functions to be expressed in both sexes. [Besides *dsx* and $dsx^{601}$ (PURO 1964), a number of new alleles of this type have been isolated by A. GAREN (personal communication) and T. KAUFMAN and T. HAZELRIGG (personal communication).] In addition, there are two dominant *dsx* alleles, $dsx^{D}$ and $dsx^{Mas}$, neither of which has any effect in males. In females, either dominant mutant over *dsx* (or a deficiency for the locus) transforms females into morphologically normal males (DUNCAN and KAUFMAN 1975; this report). When heterozygous with a $dsx^{+}$-bearing chromosome in females, both $dsx^{D}$ and $dsx^{Mas}$ lead to the production of intersexes very similar in phenotype to those produced by null alleles at the *dsx* locus. The nature of these two dominant alleles will be further considered below. Here, we simply wish to note that the phenotype they produce shows that mutations at the *dsx* locus can impair just female sexual differentiation. Further evidence for a functional complexity at the $dsx^{+}$ locus is provided by the isolation of a recessive allele at this locus that affects only males and transforms them into intersexes similar to those produced by *dsx* (GAREN, personal communication); this mutant has the properties of an allele defective only in the $dsx^{+}$ function normally expressed in males. Moreover, this mutant ($dsx^{136}$) and the dominant *dsx* alleles ($dsx^{D}$ and $dsx^{Mas}$) complement in chromosomally male individuals and $dsx^{136}$ behaves the same as a $dsx^{+}$ chromosome when over either of the dominant mutants in chromosomally female individuals.

Although not all mutant types predicted by our view of the *dsx* locus have yet been reported, the mutants of which we are aware do lend credence to the notion that the locus specifies two alternative functions in a mutually exclusive manner.

Information regarding these alternative expressions of the *dsx* locus comes from the interactions of *dsx* with *tra* and *tra-2*. Because female sexual differentiation does not occur in chromosomally female individuals homozygous for either *tra* or *tra-2* and otherwise wild type, we argued above that the wild-type alleles of these loci are necessary for the expression of female sexual differentiation functions. However, we have also shown that *dsx* is epistatic to both *tra* and *tra-2*: in the absence of $dsx^{+}$ function, female sexual differentiation functions are expressed independently of $tra^{+}$ and $tra\text{-}2^{+}$. Thus, a dependence upon the wild-type alleles of $tra^{+}$ and $tra\text{-}2^{+}$ for the expression of female sexual differentiation functions occurs only in the presence of $dsx^{+}$. Yet, our previous considerations of the $dsx^{+}$ locus led us to suggest that it was bifunctional and in a female was set simply to produce a product that precluded the expression of male sexual differentiation functions.

There are at least two ways of rationalizing these observations. One possibility

is that *dsx*$^+$ acts in females not only to repress male sexual differentiation functions, but also to render female sexual differentiation functions expressable only if *tra*$^+$ and *tra-2*$^+$ function. Under this view, it is necessary to suppose that *tra*$^+$ and *tra-2*$^+$ are also required in conjunction with *dsx*$^+$ in females to prevent the expression of male sexual differentiation functions; loss of function at any of these three loci leads to the expression of male sexual differentiation functions. This model then suggests that all three loci act at the level of the structural loci specifying differentiation functions.

An alternative possibility is that *tra*$^+$ and *tra-2*$^+$ specify functions that are necessary in females either to maintain *dsx*$^+$ (or its product) in the mode of expression proper for females or to set *dsx*$^+$ in this mode of expression. That *tra*$^+$ and *tra-2*$^+$ could act before *dsx*$^+$ was first suggested to us by R. NÖTHIGER, who had reached similar conclusions. Whatever the level of *tra*$^+$ and *tra-2*$^+$ action, this hypothesis requires that the normal mode of expression of the *dsx*$^+$ locus (or its product) is that found in males (*i.e.*, specifying a product that blocks the expression of female sexual differentiation functions) and that both *tra*$^+$ and *tra-2*$^+$ action are required in females to have *dsx*$^+$ expressed in the other mode. Thus, the male phenotype produced by the absence or loss of wild-type function at either transformer locus is viewed as the result of *dsx*$^+$ remaining in (or returning to) the male mode of expression. This hypothesis has the simplicity that it requires the products of *tra*$^+$ and *tra-2*$^+$ to have only one function—regulation of the mode of *dsx*$^+$ expression. By so doing, *tra*$^+$ and *tra-2*$^+$ action is required for both the suppression of male differentiation functions and the expression of female differentiation functions. If this view approximates reality, then the clonal analysis of the time of *tra*$^+$ and *tra-2*$^+$ transcription (this report; WIESCHAUS and NÖTHIGER, personal communication) places constraints on how the transformer loci control *dsx*$^+$ expression. Both the *tra*$^+$ and *tra-2*$^+$ loci are needed relatively late in development, into the second instar in the genital disc, the third instar in the foreleg and the early pupal period in the abdominal tergites. This says that, if they act by irreversibly setting *dsx*$^+$ in the female mode of expression, then sex is determined quite late. Alternatively, if they act by maintaining *dsx*$^+$ in the proper (female) mode of expression, then it is possible that sex is determined quite early and their subsequent action is needed simply to maintain the female state of *dsx*$^+$ expression and hence sex determination.

This view of *dsx*$^+$, *tra*$^+$ and *tra-2*$^+$ function suggests how *ix*$^+$ could act; namely, that *ix*$^+$ specifies a product necessary to prevent the expression of male, but not female, sexual differentiation functions. In otherwise wild-type females, *ix* (which, because it is recessive, we presume to involve a loss of activity) results in the expression of both male and female sexual differentiation functions. Thus, *ix*$^+$ is needed to prevent the expression of male functions in females. That *ix*$^+$ is not involved in the prevention of the expression of female functions is shown by the absence of an effect of *ix* mutants in: (1) normal males and (2) *tra* or *tra-2* transformed females.

A summary of our view of how these four loci interact to bring about sex

determination is presented in Figure 6. The *dsx*+ locus begins development in the mode of expression that represses female sexual differentiation functions. If the chromosomal sex is male (*1X2A*), then *dsx*+ remains in this mode of expression and male development results. If the chromosomal sex is female, then *dsx*+ (or its product) is switched to the opposite (female) mode of function, repressing male sexual differentiation functions. The maintenance of *dsx*+ in the female mode requires the products of *tra*+ and *tra-2*+. The *ix*+ locus has no role in determining the mode of *dsx*+ expression, but, when *dsx*+ is in the female mode, then the *ix*+ product is needed for the successful repression of male sexual differentiation functions.

After the derivation of this model for the action of these loci in sex determination, we constructed an additional multiple mutant genotype that provides a strong test of this hypothesis. The genotype constructed was *X/X*; *tra-2/tra-2*; $dsx^{136}/dsx$. The male-specific *dsx* mutant, $dsx^{136}$, should, according to the model, be unable to carry out the repression of female sexual differentiation functions, but able to function in the other mode (that characteristic of females) and repress male sexual differentiation functions. Thus, *X/X*; $dsx^{136}/dsx$ individuals are normal females because the $dsx^{136}$ locus specifies the repression of male sexual differentiation functions and is maintained in this mode of expression by the wild-type alleles of the *tra* and *tra-2* loci. The model predicts that if *X/X*; $dsx^{136}/dsx$ individuals were made homozygous for *tra-2*, the $dsx^{136}$ gene would be expressed in the other mode (repressing female sexual differentiation functions); since it is defective in this mode, the resulting flies should be *dsx* in phenotype. This is, in fact, the result observed, thus providing strong support for this model.

Although this model for the regulation of sex determination satisfactorily accounts for the phenotypes and interactions of the recessive mutants at the *ix*, *tra-2*, *tra* and *dsx* loci, it does not provide a completely satisfactory view of the two dominant alleles at the *dsx* locus, $dsx^{D}$ and $dsx^{Mas}$. That either $dsx^{D}$ or $dsx^{Mas}$ over a deficiency for the *dsx* locus transforms females into males suggests that

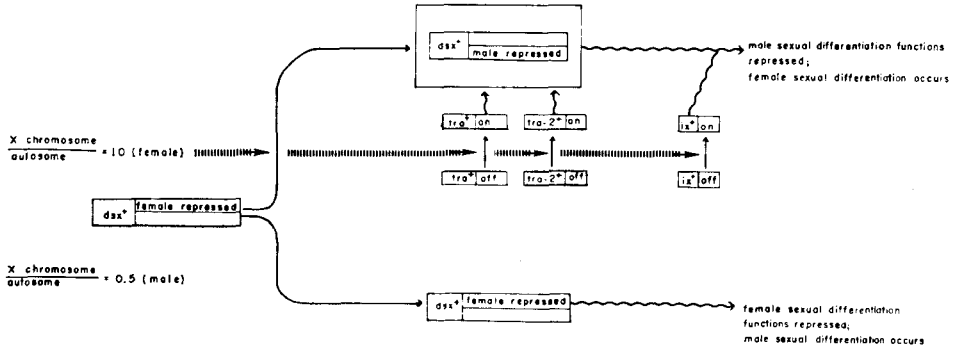


FIGURE 6.—Schematic representation of the model proposed for the roles of the *dsx*+, *tra*+, *tra-2*+ and *ix*+ loci in sex determination. Straight lines indicate changes in the functional status of these genes. Wavy lines indicate functioning of the products of loci. For details see text.

these two alleles result in the *dsx* locus being stuck in its basal mode of expression, preventing the expression of female sexual differentiation functions and allowing the expression of male sexual differentiation functions. Under this view, the intersexual phenotype produced when a dominant *dsx* allele is heterozygous with a $dsx^+$ chromosome in chromosomally female individuals is the consequence of the simultaneous presence in the cell of *dsx* loci in opposite modes: that for preventing the expression of female sexual differentiation functions by the $dsx^D$ (or $dsx^{Mas}$) chromosome and that for preventing the expression of male sexual differentiation functions by $dsx^+$. That the expression of female functions in $dsx^D/dsx^+$ comes from the normal functioning of $dsx^+$ is suggested by the observation that chromosomally female individuals that are *tra-2/tra-2*; $dsx^D/dsx^+$ have a normal male phenotype. If the *dsx* locus specifies diffusible products, it is hard to see how the simultaneous presence of molecules that prevent male and prevent female sexual differentiation would produce the same phenotype as homozygous *dsx*, which we have proposed represents the absence of both molecules. One possibility is that there are alternative pathways by which intersexes can arise, and the intersexes produced by $dsx^D/+$ are the result of such an alternate mode of intersex production. For example, it could be that the products of the *dsx* locus that prevent male and female sexual differentiation are mutually poisonous antagonists and, when both are present, neither is capable of functioning. Alternatively, if the *dsx* locus functions *via* a *cis*-acting control of the expression of (adjacent?) sexual differentiation functions, then the near identity of the phenotypes produced by *dsx/dsx* and $dsx^D/dsx^+$ can be rationalized. Thus, the $dsx^D$ chromosome would be set such that only male sexual differentiation functions were expressed, whereas the $dsx^+$ chromosome would only express female functions.

Although *cis*-action of the *dsx* locus also formally explains these results, we feel uncomfortable with the most simple conceptualization of *cis*-action: that the two batteries (male and female) of sex determination functions are adjacent to the *dsx* locus, which acts to prevent the expression of one or the other battery. The reason for our discomfort is that the overwhelming body of data suggest there is little, if any, clustering of functionally related genes in eukaryotes. However, recent genetic data indicating the involvement of genomic rearrangements in regulation of mating type in yeast (HICKS and HERSKOWITZ 1977; HICKS, STRATHERN and HERSKOWITZ 1977) and molecular data indicating that rearrangements are involved in controlling immunoglobin synthesis (BRACK and TONEGAWA 1977; TONEGAWA *et al.* 1977) and phase variation in Salmonella (ZIEG *et al.* 1977) offer ways by which this discrepancy might be overcome.

Regardless of the way in which these dominant *dsx* alleles produce intersexuality, one aspect of the phenotype of the intersexes produced by $dsx^{Mas}/+$ is worth emphasizing: in addition to a fairly complete set of male genitalia in the normal location, these flies also have a rudimentary set of male genital structures close to the region where the vaginal plates and vaginal teeth are found. This is surprising because there is strong evidence from gynandomorphs (NÖTHIGER, DÜBENDORFER and EPPER 1977) and the analysis of *tra* in the genital disc

(WIESCHAUS and NÖTHIGER, personal communication) that there are separate primordia for the male and female genitalia. If this extra set of male genital structures is derived from the female primordia, as its location would suggest, then it would seem that there are two steps involved in the development of the female primordium. First, it must be given instructions to develop. Once this has occurred, the actual course of differentiation it will undergo is determined by the relative amounts of the substances leading to male *vs.* female sexual differentiation that are present in these cells. This view is consistent with the finding that rudiments of a second set of male genital structures are also found in this location in some homozygous *dsx* flies.

In conclusion, we would like to emphasize that the properties of the loci regulating sex determination in Drosophila are in all aspects studied like those of the homeotic loci that determine segmental and subsegmental specializations. If, indeed, the underlying regulatory processes are of the same type, then a generalization of the model proposed here for the action of the genic components regulating sex determination to other developmental processes would suggest that the specification of determination by other homeotic loci might also be brought about *via* the control of the mode of expression of other bifunctional loci like *dsx*. This predicts at least two classes of homeotic loci: (1) those like $dsx^+$ that act as bifunctional switches and (2) those like $tra^+$, $tra\text{-}2^+$ and $ix^+$ that mediate the expression of these switches. Such a mode of regulation would account for the ubiquity of binary regulatory decisions during Drosophila development (*e.g.*, KAUFFMAN 1977; KAUFFMAN, SHYMKO and TRABET 1978; GARCIA-BELLIDO 1975) without requiring that each successive step of determination involve simply turning off (or on) an additional battery of genes in one subset of a previously homogeneous cell population.

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Corresponding editor: A. CHOVNICK