Proc. Natl. Acad. Sci. USA
Vol. 79, pp. 1568–1572, March 1982
Genetics

Sex determination in *Drosophila melanogaster*: Analysis of transformer-2, a sex-transforming locus

(homeotic loci/temperature-sensitive regulatory mutants/sequential gene action/gynandromorphs)

JOHN M. BELOTE AND BRUCE S. BAKER

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

Communicated by Edward B. Lewis, October 23, 1981

ABSTRACT **The transformer-2 (*tra-2*) locus is one of a set of regulatory loci that control sex determination in *Drosophila melanogaster*. Temperature-shift experiments with temperature-sensitive *tra-2* mutants demonstrate that within single cell lineages *tra-2*$^+$ function is required at several times, and probably continuously, during development for the occurrence of a series of determinative decisions necessary for female sexual differentiation. Analysis of the effects of *tra-2* in the genital disc demonstrates that the *tra-2*$^+$ function is necessary in females both to prevent male sexual differentiation and to permit female differentiation. These and other results support the model that the *tra-2*$^+$ and *tra*$^+$ loci act to control the expression of the bifunctional doublesex (*dsx*) locus.**

Recent attempts to elucidate the regulatory programs by which cell and tissue type are determined in *Drosophila melanogaster* have focused on loci identified by homeotic mutants. Because mutations at these loci profoundly alter the developmental fates of whole segments or subsegmental regions it has been suggested that at least some of these loci control key determinative decisions during development (1, 2). Although some homeotic loci are less well characterized than others, it is nonetheless evident that many of them have certain functional features in common. Thus these loci function in a cell-autonomous manner; their wild-type functions are needed late in development for normal differentiation; in at least some cases they begin functioning early and are needed continuously throughout at least much of larval development to maintain a particular determined state. Finally, because loss-of-function mutations at these loci result in the replacement of one structure by another normally found elsewhere, it has been suggested that each of these loci controls a binary decision between alternative levels of differentiation (3). Recent studies of mutants of *D. melanogaster* that affect sex determination show that they identify regulatory loci that share the above features in common with homeotic loci (4). This strengthens the view that there is a common genetical logic used to regulate diverse developmental programs.

Here we focus on how one of these regulatory loci, transformer-2 (*tra-2*), functions in the determination of sex. A null *tra-2* allele, when homozygous in chromosomally female individuals, causes them to develop as males with respect to their external anatomy and internal genital duct system; however, gonads are not transformed into functional testes (4–6). Chromosomally male flies (XY; AA) homozygous for *tra-2* show no obvious morphological effects, although they are sterile. The fact that the presence or absence of a functional *tra-2*$^+$ gene determines whether a chromosomally female individual will develop as a male or female suggests that this locus controls a binary decision as to which program of sexual differentiation will be utilized.

Our studies address the following questions about how *tra-2*$^+$, and by analogy perhaps other homeotic loci, functions to control development. How does *tra-2*$^+$ function during normal female development lead to the absence of male differentiation and the occurrence of female differentiation? Is the *tra-2*$^+$ function concerned solely with preventing male differentiation or solely with causing female differentiation, or is it active in both precluding male and eliciting female development? Are cells determined as male or female by a single temporally distinct event or are different aspects of sex determined at different times within a cell? Is determination truly binary in that across all levels of *tra-2*$^+$ function individual cells can differentiate only as male or female, or can intermediate (intersexual) differentiation also occur? In two of the more broadly studied regulatory systems in *D. melanogaster*—the bithorax complex (1) and sex-determination loci (4)—it is evident that there is a hierarchy of interacting regulatory loci responsible for ensuring normal development. Our final concern here is with how the *tra-2*$^+$ function is integrated with the functions of other sex-determination loci to produce normal sexual development.

The primary determinant of both sex and dosage compensation is the ratio of X chromosomes to autosomes (X:A) (7, 8). One locus, *Sxl*, acts at or shortly after the monitoring of the X:A ratio and appears to be involved in the control of both sex determination and dosage compensation (9, 10). Subsequently, controls of sex determination and dosage compensation diverge. Male-specific lethal mutations at three loci are defective in dosage compensation; these mutations do not affect sex determination in females and a leaky mutation at one of these loci does not affect sex determination in males (11, 12). Mutations are known at four loci [doublesex (*dsx*), intersex (*ix*), transformer (*tra*), and transformer-2 (*tra-2*)] that affect just sex determination (for review see ref. 4). Studies (4) of the latter mutants have led to the proposal (summarized in Fig. 1) that *dsx*$^+$ is a bifunctional locus that can be expressed in either of two alternative ways: in male (1X:2A) individuals *dsx*$^+$ functions to repress female differentiation; in female (2X:2A) flies, *dsx*$^+$ represses male sexual differentiation. The *tra*$^+$ and *tra-2*$^+$ gene products are postulated to act in chromosomally female individuals to maintain the *dsx*$^+$ locus in the female mode of expression.

It is worth noting that at the level of the formal genetics of the systems there are striking similarities between the mechanism by which mating type is controlled in yeast (13) and this proposal for the mechanism of sex determination in *D. melanogaster*: in both cases there is a locus (*dsx* and *MAT*) that is capable of expression in either of two alternative active forms and there are other loci (*tra*, *tra-2*, and *HO*) that act to switch the mode of expression of the bifunctional locus. Moreover, two alleles at *dsx* (*dsx*^{Mas}, *dsx*^{D}) behave as if they are incapable of

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U. S. C. §1734 solely to indicate this fact.

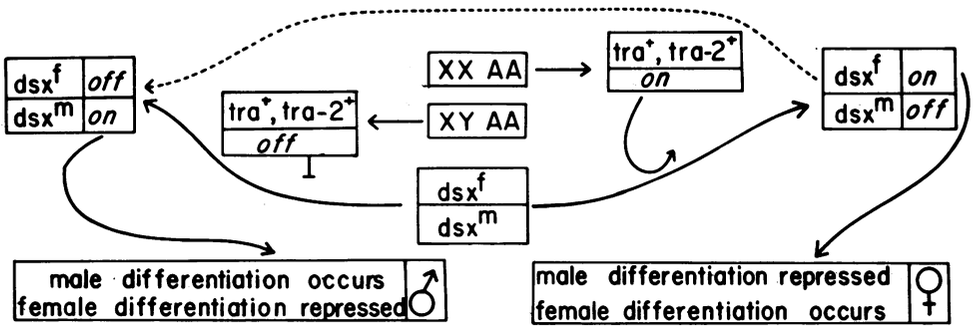

FIG. 1. Model for the roles of the *tra*, *tra-2*, and *dsx* loci in sex determination (4). The bifunctional *dsx* locus can express either of two functions: dsx^f is expressed in females and represses male differentiation; dsx^m is expressed in males and represses female differentiation. The *tra* and *tra-2* loci jointly control the expression of *dsx*. Loss of tra^+ or $tra\text{-}2^+$ function during female development causes *dsx* to switch to the male mode of expression (dashed arrow).

being switched from the male mode of expression to that characteristic of females (4) just as some mutants at *MAT* interfere with mating-type switching. Whether these parallels extend to the molecular level is at present unknown.

MATERIALS AND METHODS

Crosses were performed on standard medium at 25°C (except where noted). For descriptions of markers see ref. 14 or the indicated references.

New Alleles of *tra-2*. The $tra\text{-}2^{ts1}$ mutant was isolated as described (12). $tra\text{-}2^{ts2}$ was induced by ethyl methanesulfonate; details of its isolation will be reported elsewhere.

Females homozygous for either temperature-sensitive allele reared at 29°C are phenotypically male except for the presence of bristles on the sixth ventral abdominal segment (sternite)—a female characteristic—in some $tra\text{-}2^{ts1}$ homozygotes. X/X; $tra\text{-}2^{ts1}/tra\text{-}2^{ts1}$ flies reared at 16°C develop as sterile females with nearly normal morphology, whereas X/X; $tra\text{-}2^{ts2}$ homozygotes reared at 16°C or 18°C are morphologically normal females.

Analysis of *tra-2* Gynandromorphs. Gynandromorphs (XX//X0 mosaics) either homozygous for *tra-2* (experimental) or heterozygous for *tra-2* (control) were produced by crossing *y/y; tra-2/SM1, Cy* females to *R(1)2*, w^{vC}/w^+Y; *Bl tra-2/++* males. XX//X0 progeny were identified on the basis of mosaicism for the marker *y*.

Preparation for Microscopy. Sexual differentiation was scored on flies eviscerated as described in ref. 15 and mounted between coverslips.

RESULTS AND INTERPRETATION

Role of $tra\text{-}2^+$ in the Genital Disc. Because many homeotic loci appear to mediate binary decisions, it is important to understand whether their wild-type function is to either solely repress one developmental pathway or just to elicit the normal developmental pathway. Alternatively, it may be the case that they function both to repress one developmental program and to activate the alternative developmental program. In most systems it is not evident how a genetical distinction between these alternatives can be made. However, in the case of sex determination in the genital primordia such a distinction is possible. This is because by blastoderm there are physically distinct primordia for both male and female genitalia in every embryo (16). There are two alternative hypotheses for the action of $tra\text{-}2^+$ in the genital primordia of females that can account for the observation that, when homozygous in a chromosomally female individual, *tra-2* results in the development of normal male genitalia as well as the absence of any female genital structures.

One possibility is that the chromosomal sex of an individual determines which primordium develops but the pathway of differentiation the primordium follows is governed by the $tra\text{-}2^+$ product. Thus in a diplo-X individual the female primordium would always grow and differentiate, but only in the presence of $tra\text{-}2^+$ function would it differentiate as female; in the absence of $tra\text{-}2^+$ function it would differentiate as male. Alternatively the decision as to which primordium develops may be controlled by $tra\text{-}2^+$, but once signaled to develop by the action (or inaction) of this locus a primordium always differentiates into the genitalia of only one sex. Under this hypothesis, in diplo-X; $tra\text{-}2^+$ individuals $tra\text{-}2^+$ is expressed and signals both the female primordium to develop and male primordium not to develop. In diplo-X; *tra-2/tra-2* individuals the female primordium is not directed to develop and the male primordium is not prevented from developing, and consequently the latter gives rise to a male genital apparatus.

These alternatives can be distinguished by examining XX/X0 mosaics homozygous for *tra-2*. In such mosaics (produced by the loss of an unstable ring-X chromosome during the preblastoderm divisions) the boundary separating XX from X0 tissue occasionally passes through the genital primordia. According to hypothesis I, abnormal male genitalia (i.e., missing or duplicated male structures) would result (Fig. 2). Under the hypothesis II such mosaics would have morphologically normal male genitalia. All 186 XX//X0 mosaics homozygous for *tra-2* examined had normal male genitalia. Of these flies 51 (27%) had $y//y^+$ mosaic genitalia and represent cases in which the mosaic boundary passed through the genital primordia. In XX//X0; $tra\text{-}2^+$ control gynandromorphs the male primordium develops only when it is X0. Cases in which the mosaic boundary passes through the male genital primordium result in the development of partial male genitalia. In 47 (26%) of the 181 control gynandromorphs there was partial development of the male genitalia. This is in good agreement with the frequency (27%) of $y//y^+$ mosaic male genitalia in the experimental series and supports the conclusion that, in *tra-2* homozygotes, the male genitalia

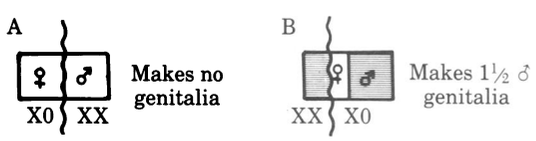

FIG. 2. Examples of abnormal genital development expected in XX//X0; *tra-2/tra-2* gynandromorphs under hypothesis I. Boxes represent the genital primordia. Wavy line represents XX–X0 border. Shaded areas differentiate. Under hypothesis II in all mosaics only the male primordium develops and consequently they always have one set of normal male genitalia.

are derived from the male genital primordium. These observations are consistent only with the second hypothesis, that *tra-2*$^+$ functions both to prevent male development and to cause female development.

Time of Function of the *tra-2* Product. To inquire when *tra-2*$^+$ function determines sex and whether all aspects of a cell's sexual phenotype are determined together, temperature-shift experiments were done with *tra-2*^{ts1} (see Fig. 3 legend). The temperature-sensitive period for *tra-2*^{ts1} begins during the second larval instar and ends during the early-middle pupal period. To focus on the nature of this lengthy temperature-sensitive period the temperature-sensitive periods of *tra-2*^{ts1} in a number of sexually dimorphic tissues were examined.

Qualitative observations on the effects of temperature shifts on the development of the genital disc show that in a single tissue *tra-2*$^+$ function is required throughout a substantial portion of development (from mid-second instar to the early pupal stage). Most strikingly, these temperature shifts suggest that *tra-2*$^+$ function is needed in females not only to initiate development of the female genital primordium and repress development of the male genital primordium (as was shown by the *tra-2* gynandromorph experiment described above) but also subsequently, to determine the pathway of sexual differentiation that will be followed by the developing primordium. Thus, individuals shifted from 16°C to 29°C during mid-second to mid-third instar often developed a second set of rudimentary male genital structures (one or more of the following: penis, postgonites, pregonites, sheath, apodeme, hypandrium) in a region of the adult terminalia distinctly separate from more normal, well-developed male genitalia. The location of this secondary set of male genitalia, which is sometimes found in close association with partially developed female genital structures, suggests that these structures are derived from the female genital primordium. We interpret this observation to mean that in wild-type females *tra-2*$^+$ acts first to allow the female genital primordium to develop but its subsequent action is also required for the female primordium to differentiate into female structures. Thus in *tra-2*^{ts1} homozygotes shifted from 16°C to 29°C during mid-second to mid-third instar the first decision is made at the permissive temperature and so the female primordium is permitted to grow and develop; however, the later decision(s) takes place at the restrictive temperature and in the absence of *tra-2*$^+$ function cells of the female primordium differentiate into male structures. If functional *tra-2* product is absent throughout development, as was the case in the *tra-2* gynandromorph experiment, growth of the female primordium is not initiated and so no duplicate set of male structures develops.

The presence of a second set of female genitalia in flies that underwent the reciprocal (29°C to 16°C) shift could have provided evidence for a similar plasticity in the male genital primordium, but none were observed. However, it is probable that rudimentary female genital structures associated with a set of male genital structures could be easily missed. That this is the case is suggested by the fact that in about 40% of diplo-X; *tra-2*^{ts2}/*tra-2*^{ts2} flies reared at 21°C there are what appear to be a reduced set of elongated vaginal teeth and vaginal plates

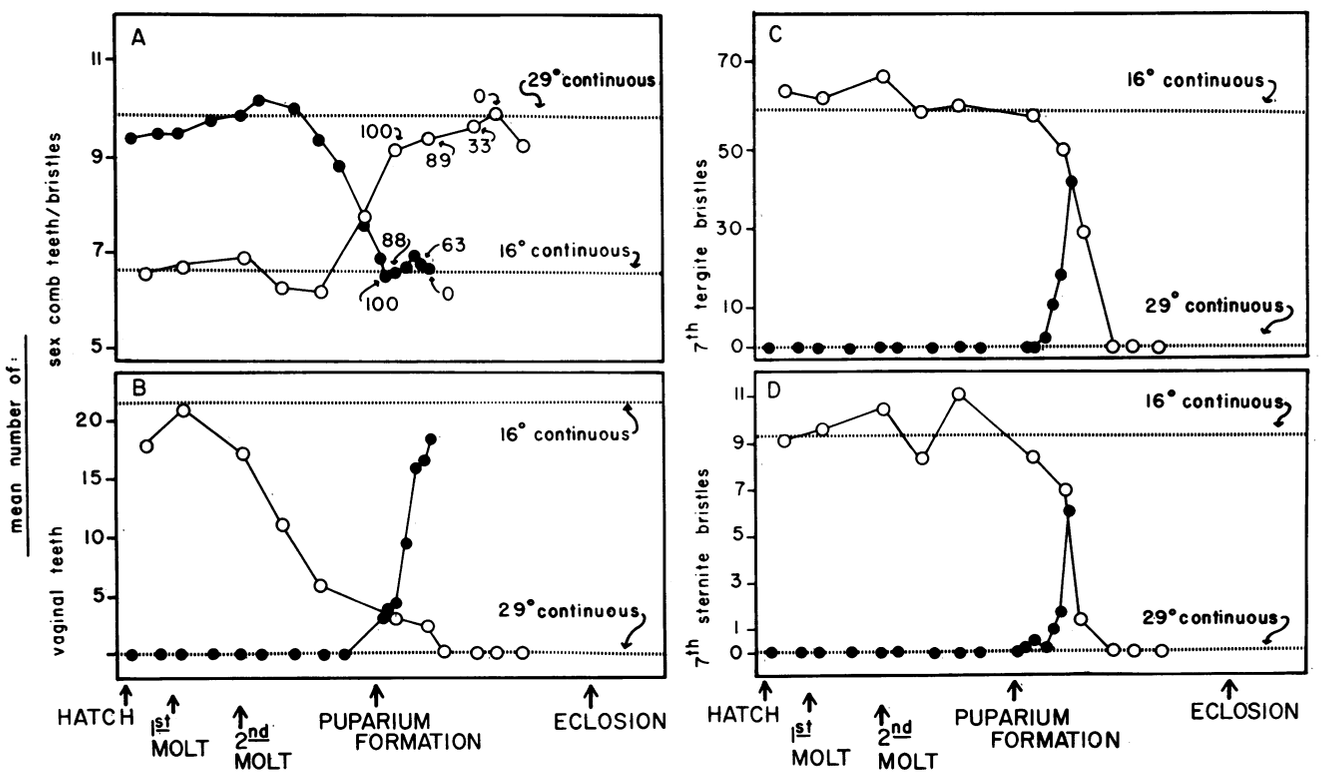

FIG. 3. Temperature-shift experiment. +/B^sY; *cn tra-2*^{ts1} *bw/Cy0* males were mated to +/+; *cn tra-2*^{ts1} *bw/Cy0* females at 16°C or 29°C. For temperature shifts during the larval period bottles were shifted to the other temperature and pupae were collected at 8-hr intervals as described in ref. 17. For temperature shifts postpupariation prepupae and pupae were collected within 8 hr of puparium formation, held at that temperature for appropriate time intervals, and shifted to the other temperature to complete development. (*A*) number of sex comb teeth or corresponding female bristles per foreleg. The numbers with arrows associated with the filled circles indicate the percentage of sex combs that consisted entirely of teeth; the numbers associated with the open circles indicate the percentage of sex combs scored that consisted entirely of bristles. (*B*) number of vaginal teeth per fly. (*C*) number of 7th tergite bristles per fly. (*D*) number of 7th sternite bristles per fly. ●, Samples shifted from 16°C to 29°C; ○, samples shifted from 29°C to 16°C. Abscissae indicate the developmental stage of the flies at the time of the shifts. At 29°C, hatch, 1st molt, 2nd molt, puparium formation, and eclosion occur at 17, 33, 56, 96, and 182 hr after fertilization, respectively. At 16°C, these landmarks occur at 60, 110, 194, 355, and 703 hr after fertilization.

in the region between the normal female genitalia and the anal plates. The location of these secondary female genitalia suggests that they arise from the male genital primordium. Although the morphology of these structures is not distinctive enough for us to be certain they do not represent rudimentary male clasper bristles, the absence of any other male structures supports the conclusion that they are female structures derived from the male genital primordium.

The conclusion that *tra-2*$^+$ function is required to mediate a series of determinative decisions necessary for normal female sexual differentiation is supported by observations on other sexually dimorphic tissues.

Thus functional *tra-2*^{ts1} product is required from just before to just after the time of puparium formation to determine the number of bristles in the last transverse row of the basitarsus of the foreleg (Fig. 3A). Although the number of bristles is established at this stage, their morphology (whether they will form as the thick, blunt male sex comb teeth or as the thin, tapered, female basitarsal bristles) is determined considerably later (Fig. 3A). Thus a shift from 29°C to 16°C at 12 hr after puparium formation results in bristles with female morphology but whose number is similar to that found in a male (Fig. 4*B*). Conversely, a shift from 16°C to 29°C at 24 hr after puparium formation results in bristles with male morphology but whose number is that characteristic of a female (Fig. 4*C*).

Females have a row of short thick blunt bristles (vaginal teeth) on both sides of the vaginal opening. The effects of shifts on vaginal tooth differentiation are plotted in Fig. 3*B*. As shifts from 29°C to 16°C are made progressively later in development, the number of vaginal teeth drops gradually from the normal female number and reaches zero by 6–14 hr after puparium formation. Shifts from 16°C to 29°C before puparium formation do not allow any vaginal teeth to form; progressively later shifts result in a steady increase in the number of vaginal teeth. The effect of *tra-2*^{ts1} on vaginal tooth morphology is temporally separable from its effect on vaginal teeth number. Whereas shifts from 29°C to 16°C always result in morphologically normal vaginal teeth, even when they are reduced in number, shifts from 16°C to 29°C made before 48–64 hr after puparium formation result in the development of vaginal teeth that are longer and more tapered than normal. The long time over which *tra-2*^{ts1} affects vaginal tooth differentiation suggests strongly that the product of this locus acts throughout much of the larval and pupal periods in this tissue.

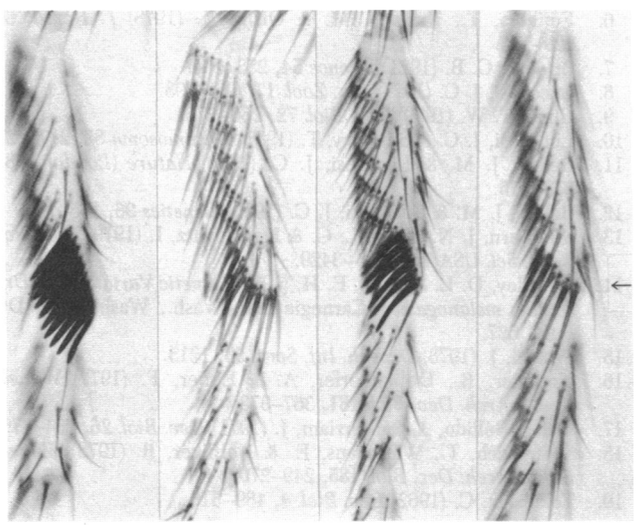

FIG. 4. Forelegs of XX; *tra-2*^{ts1}/*tra-2*^{ts1} flies that have been: (*A*) grown continuously at 29°C (male bristle number and morphology), (*B*) shifted from 29°C to 16°C at approximately 12 hr after puparium formation (male bristle number, female morphology), (*C*) shifted from 16°C to 29°C at approximately 24 hr after puparium formation (female bristle number, male morphology), (*D*) grown continuously at 16°C (female bristle number and morphology). Arrow points to the sex comb region. (×223).

The effect of temperature shifts on 7th tergite and 7th sternite bristle number is plotted in Fig. 3 *C* and *D*. In both tissues there is a short temperature-sensitive period, well after puparium formation.

Effects of Intermediate Levels of *tra-2*$^+$ Function. It is a common perception that as a result of the activity or inactivity of a homeotic locus cells undergo one of two alternative pathways of differentiation. To investigate the effects of the level of *tra-2* activity on sexual differentiation, diplo-X flies homozygous for temperature-sensitive *tra-2* alleles (*tra-2*^{ts1} or *tra-2*^{ts2}) were raised at 16, 18, 21, 25, and 29°C. Examination of these flies supports the conclusion that low, but nonzero, levels of *tra-2* activity result in intersexual differentiation of cells. As examples of such intersexual development we describe below our observations on two sexually dimorphic tissues, the foreleg and the anal plates.

In the foreleg of *tra-2*^{ts1} homozygous females reared at 16, 18, and 21°C the last transverse row of bristles on the basitarsus is partially rotated toward the male position and a central bristle, characteristic of a male foreleg, is present. However, the number and morphology of these bristles is that characteristic of females. In diplo-*X* flies homozygous for *tra-2*^{ts1} or *tra-2*^{ts2} raised at 25°C both the number and the morphology of bristles making up the sex comb are intermediate between those of males and females.

Wild-type females have a pair of chitinous plates (anal plates) located dorsal and ventral to the anal opening, whereas males have left and right anal plates lateral to the anal opening. In *tra-2*^{ts1} homozygous females reared at 16°C the dorsal anal plate is enlarged and often separated into right and left lobes, while the ventral anal plate is reduced. These abnormalities are more extreme in *tra-2*^{ts1} homozygotes reared at 21°C. The dorsal anal plate has the appearance of dorsally fused right and left male anal plates, while the ventral anal plate is greatly reduced or absent. The anal plates also have an intersexual appearance in females homozygous for *tra-2*^{ts1} or *tra-2*^{ts2} raised at 25°C. Although it has been proposed that both anal plates in the two sexes arise from the same primordium (16), our results suggest that the dorsal anal plate in the female is homologus to both anal plates of the male and that the primordium that produces the female ventral anal plate does not differentiate into an anal plate in the male.

Interaction Between *tra-2* and *tra*. Temperature sensitivity of *tra-2*^{ts} alleles is dependent on the number of functional *tra*$^+$ loci present in the genome. Thus in a diplo-X, *tra-2*$^{ts-}$ homozygote, reducing the number of *tra*$^+$ genes from two to one by making the fly heterozygous for *tra*, *tra*^{AC}, or *Df(3L)st*^{SS103} (a deficiency for the *tra* locus) results in a phenotypically male adult even when the fly is allowed to develop entirely at the permissive temperature of 18°C. These results suggest to us that the products of *tra*$^+$ and *tra-2*$^+$ normally interact and that the thermolabile *tra-2*^{ts} product is stabilized by such an association; lowering the amount of *tra*$^+$ gene product results in the *tra-2*^{ts} product becoming nonfunctional even at the permissive temperature.

DISCUSSION

One of the most striking results of these experiments is the demonstration that *tra-2*$^+$ function does not act to determine the sex

of a cell at a single time but instead is required throughout development, perhaps continuously, in single cell lineages to mediate a series of developmental steps necessary for normal sexual differentiation. The sequential action of *tra-2*$^+$ is clearly demonstrated in the foreleg and genital discs, where its role in the determination of female vs. male bristle number is temporally separable from its role in determining whether these bristles will have male or female morphology. Similarly, in the genital primordia *tra-2*$^+$ function appears to be not only necessary to initiate development of the female genital primordium but also required subsequently for its differentiation into female (as opposed to male) structures. Complementary data suggest the absence of functional *tra-2* product is a requisite for both the growth of the male genital primordium and its differentiation into male (as opposed to female) structures. Thus determination of cell type by *tra-2*$^+$ does not occur as an all-or-none event at a discrete developmental stage but rather by mediating a series of determinative decisions.

The *tra-2* locus, like many homeotic genes, controls a decision as to which of two alternative states of differentiation will be achieved. Because the primordia for the male and female genitalia are in physically distinct cell populations it is possible in the case of *tra-2* to independently assess whether the *tra-2*$^+$ function acts as a negative regulator of male sexual differentiation functions and as a positive regulator of female sexual differentiation functions. Our results establish that *tra-2*$^+$ acts in the genital disc of females both to prevent development of the male primordium and to elicit development of the female genital primordium. Thus the loci involved in sexual differentiation in one of the sexes need not be a subset of those used in the other sex. Analyses of homozygous *tra* gynandromorphs suggest a similar mode of action for this gene in the genital disc (4, 18). All other sexually dimorphic structures in the adult derive from the same progenitor cells in the two sexes (16, 19), and thus a dual regulatory role of *tra-2*$^+$ and *tra*$^+$ is not similarly demonstrable in these tissues. Nevertheless we infer that here too the *tra-2*$^+$ and *tra*$^+$ functions are necessary both for the occurrence of female sexual development and the prevention of male sexual development.

The apparent simultaneous positive and negative regulatory functions of the *tra-2*$^+$ product are predicted by the hypothesis that it acts, in conjunction with the *tra*$^+$ product, to regulate the expression of the bifunctional *dsx*$^+$ locus (ref. 4; Fig. 1). In the absence of either *tra*$^+$ or *tra-2*$^+$ function the *dsx* locus represses female sexual differentiation functions. When *tra-2*$^+$ and *tra*$^+$ are expressed they prevent *dsx*$^+$ from repressing female sexual differentiation functions and they cause it to repress male sexual differentiation functions. The finding that the phenotype of *tra-2*^{ts}/*tra-2*^{ts} flies is dramatically influenced by the number of doses of the *tra*$^+$ gene suggests that the products of these two loci may interact. This is consistent with the hypothesis that the products of both loci act, perhaps as subunits of a functional complex, to regulate the expression of *dsx*$^+$. The finding that at intermediate temperatures temperature-sensitive alleles of *tra-2* result in intersexual phenotypes is consistent with previous findings with respect to the *dsx* locus. Null alleles at *dsx* repress neither male nor female sexual differentiation functions and thus both are expressed in each cell and produce intersexual differentiation. Moreover, previous data suggested that when two *dsx*$^+$ loci in a diploid cell are expressed in opposite modes, they function in a mutually antagonistic manner and intersexual development results (4). Thus we imagine that, in *tra-2*^{ts} homozygotes at intermediate temperatures, the level of functional *tra-2* product is not sufficient to maintain *dsx*$^+$ in the female mode of expression and as a consequence *dsx*$^+$ is sometimes expressed in the male mode. Thus cells would be expressing both functions of the *dsx*$^+$ locus, which would result in an intersexual phenotype.

Previous work suggested that by mid-third instar the *tra-2*$^+$ gene has been transcribed sufficiently in the foreleg for normal sexual differentiation (4). These experiments could not distinguish whether sex was determined at this time or at some later stage by the persistence of *tra-2*$^+$ product. The temperature shifts with *tra-2*^{ts1} show that sex determination in the foreleg requires functional *tra-2*$^+$ product 2–4 days after transcription of the *tra-2*$^+$ gene is sufficient for the occurrence of normal sexual differentiation. This suggests, as the simplest interpretation, that the thermolability is in the functioning of the *tra-2*^{ts1} product rather than its synthesis.

These results also have implications for the control of the expression of the *dsx*$^+$ locus. Regardless of the level at which regulation of *dsx*$^+$ expression by the *tra-2*$^+$ product occurs, the temperature-shift experiments strongly suggest that the proper expression of *dsx*$^+$ in females requires the continuous presence of a functional *tra-2*$^+$ product and is readily reversed if functional *tra-2*$^+$ product is removed.

This work was supported by U.S. Public Health Service Grants GM07199 and GM23345.

1. Lewis, E. B. (1978) *Nature (London)* **276,** 565–570.
2. Garcia-Bellido, A. (1977) *Am. Zool.* **17,** 613–629.
3. Kauffman, S. A. (1973) *Science* **181,** 310–318.
4. Baker, B. S. & Ridge, K. (1980) *Genetics* **94,** 383–423.
5. Watanabe, T. K. (1975) *Jpn. J. Genet.* **50,** 269–271.
6. Fujihara, T., Kawabe, M. & Oishi, K. (1978) *J. Hered.* **69,** 229–236.
7. Bridges, C. B. (1921) *Science* **54,** 252–254.
8. Lucchesi, J. C. (1977) *Am. Zool.* **17,** 685–693.
9. Cline, T. W. (1979) *Dev. Biol.* **72,** 266–275.
10. Lucchesi, J. C. & Skripsky, T. (1981) *Chromosoma* **82,** 217–227.
11. Belote, J. M. & Lucchesi, J. C. (1980) *Nature (London)* **285,** 573–575.
12. Belote, J. M. & Lucchesi, J. C. (1980) *Genetics* **96,** 165–186.
13. Strathern, J. N., Blair, L. C. & Herskowitz, I. (1979) *Proc. Natl. Acad. Sci. USA* **76,** 3425–3429.
14. Lindsley, D. L. & Grell, E. H. (1968) *Genetic Variations of Drosophila melanogaster* (Carnegie Inst. Wash., Washington, DC) Publ. 627.
15. Szabad, J. (1978) *Drosph. Inf. Serv.* **53,** 1215.
16. Nothiger, R., Dubendorfer, A. & Epper, F. (1977) *Wilhelm Roux's Arch. Dev. Biol.* **181,** 367–373.
17. Garcia-Bellido, A. & Merriam, J. (1971) *Dev. Biol.* **26,** 264–276.
18. Schupbach, T., Wieschaus, E. & Nothiger, R. (1978) *Wilhelm Roux's Arch. Dev. Biol.* **185,** 249–270.
19. Tokunaga, C. (1962) *Dev. Biol.* **4,** 489–516.