A MATERNAL-EFFECT SEX-TRANSFORMATION MUTANT OF THE HOUSEFLY, *MUSCA DOMESTICA* L.

HIROAKI INOUE AND TOSHIKI HIROYOSHI

Department of Genetics, Osaka University Medical School, 3-57 Nakanoshima 4-chome, Kita-ku, Osaka 530, Japan

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ABSTRACT

A maternal-effect sex-transformation mutant, *transformer* (*tra*), of the housefly is described. It is located on autosome 4 in close linkage with the *Ba* locus. Normally, the sex of *Musca domestica* is determined by the presence or absence of an epistatic factor, *M*. When produced by *tra/tra* mothers, a large fraction of the *tra/tra* genotypic female progeny carrying no *M* factors are transformed to develop into intersexes or fertile phenotypic males. The *tra/+* progeny are also transformed, but less frequently. Aging of the mothers increases the frequency of sex-transformed flies. When produced by *tra/+* mothers, *tra/tra* progeny (but not *+/tra*) occasionally undergo sex transformation. Thus, tra^+ is active both maternally and zygotically. Genotypic males carrying the *M* factor are not affected by the *tra* mutant. It is concluded that the tra^+ gene product is required for female determination and/or differentiation. A model is proposed to explain actions of all the known sex-determination genes in *M. domestica*, and it is discussed in relation to sex-determination mechanisms in several other insect species.

SEX determination involves a series of integrated developmental events which direct individual eggs to choose between alternative pathways of sexual differentiation. In many bisexual species, sex is determined either by the chromosome constitution or by the action of a single gene. The former mechanism includes the "balance system" reported in Drosophila (BRIDGES 1925) and in the nematode *Caenorhabditis elegans* (MADL and HERMAN 1979), as well as the "diplohaploidy system" reported in many Hymenopteran species (reviewed by ROTHENBUHLER 1975; CASSIDY 1975). The latter mechanism, termed "homoheterozygosity or epistatic system," has proved to be applicable to most species of Lepidoptera (*e.g.*, TAZIMA 1964; CASPARI and GOTTLIEB 1975) and many species of Diptera (ENGELMANN 1970), as well as to several vertebrates including mammals (OHNO 1979). In numerous species, single gene mutations have been discovered which cause partial or complete transformation of sexual phenotypes. In *Drosophila melanogaster*, such sex-transformation mutants fall into at least six loci, *Sxl*, *tra*, *tra-2*, *ix*, *dsx* and *her* (reviewed by BAKER and BELOTE 1983). Similar mutants, *tra-1*, *tra-2*, *tra-3*, *fem-1* (=*isx-1*), *fem-2*, *fem-3* and *her-1*, have also been described in *C. elegans* (HODGKIN and BRENNER

1977; NELSON, LEW and WARD 1978; HODGKIN 1980, 1985). In the mammalian system, *P* in goats causes *X/X*; *P/P* genotypic females to develop into intersexes (HAMERTON *et al.* 1969), and *Tfm* in mice causes somatic cells of *XY* genotypic males to undergo female sexual differentiation (LYON and HAWKES 1970). These sex-transformation mutants have been used to elucidate the genetic control of sex determination and/or differentiation.

In the housefly, *Musca domestica* L., several different systems of sex determination have been reported. In the standard strains, flies have five pairs of autosomes and a pair of heterochromatic sex chromosomes, *XX* in the female and *XY* in the male (STEVENS 1908; PERJE 1948). The sex is determined by the presence or absence of the *Y* chromosome, which carries an epistatic male-determining factor, *M*, whereas the *X* chromosome plays no important role in sex determination (HIROYOSHI 1964; RUBINI and PALENZONA 1967). In some laboratory and natural strains, however, both females and males have the *XX* chromosome complement, and the presence or absence of a special autosome that carries an autosomal male-determining factor, A^M, determines sex (A^M system). The occurrence of A^M chromosomes have so far been demonstrated in at least four linkage groups (SULLIVAN 1958; WAGONER 1969; HIROYOSHI and INOUE 1979), and the locations of A^M factors have recently been determined (INOUE, FUKUMORI and HIROYOSHI 1983; INOUE and HIROYOSHI 1984). The *Y* chromosomal and the autosomal *M* factors can be regarded as a polymorphism. Therefore, the presence or absence of the *M* factor appears to be the primary signal for sex determination in these male-heterogametic strains. Furthermore, in some other strains, both females and males have the *M* factor(s) in the homozygous state, and the presence or absence of a hologynically inherited female-determining factor, *F*, determines sex (female-heterogametic or *F* system) (RUBINI 1967; WAGONER 1969; MCDONALD *et al.* 1978; INOUE and HIROYOSHI 1982). Besides these sex factors, a dominant maternal-effect mutant, *Arrhenogenous* (*Ag*), found in a laboratory strain, causes the genotypic female progeny carrying neither *M* nor *F* to develop into fertile phenotypic males (VANOSSI-ESTE 1971; VANOSSI-ESTE and ROVATI 1982). The use of these phenotypic males allowed the establishment of a unique strain in which the sex is determined under the control of maternal genotype (sex predetermination system). Coexistence of multiple sex-determining systems within a species is of particular interest, because it may be an evolutionary link between a variety of sex-determining systems found in related species. The dominant genes, *M*, *F* and *Ag*, are valuable because they may shed light on the mechanism of sex determination in *M. domestica*.

Maternal-effect mutations identify genes in which wild-type products are synthesized during oogenesis and are utilized by the developing zygote. They have been used as valuable tools for elucidating how and when their wild-type gene products regulate the expression of zygotic genes in development. In spite of the abundance of maternal-effect mutations so far identified in a variety of species, only a few examples are known which cause the alteration of sexual phenotypes. In *C. elegans*, a maternal-effect mutant, *tra-3*, causes the genotypic hermaphrodite progeny to develop into sterile phenotypic males

(HODGKIN and BRENNER 1977). Dominant maternal-effect genes, female sex-realizer (*F'*) in the monogenic blowfly, *Chrysomya rufifacies* (ULLERICH 1973, 1980) and *Ag* in *M. domestica* (VANOSSI-ESTE 1971), are of particular interest because they produce fertile sex-transformed progeny.

We describe here a new maternal-effect sex-transformation mutant of *M. domestica*. The mutant, transformer (*tra*), is similar to *Ag* in its action, but it is a recessive mutant on autosome 4. A possible role of the wild-type gene product of the *tra* locus is discussed in relation to other sex-determining genes.

MATERIALS AND METHODS

The mutant to be described was first noted as an occasional appearance of "*sonless*" males in a laboratory III^M strain, + *bwb ge* +/*Bx*^{2} + + *M* (Nagai) (HIROYOSHI, FUKUMORI and INOUE 1982). Because recombination usually does not take place in males of the housefly, the *Bx*^{2} + + *M* chromosome carrying the sole *M* factor in this strain should be inherited holandrically, segregating *bwb ge* females and *Bx*^{2} males in every generation. However, various types of recombinant flies which resulted from exceptional male crossing over were occasionally recovered from this strain. Moreover, *bwb ge* males were also recovered at a frequency of 0.22% (40 of 18,300). These flies, surprisingly, produced no male progeny when outcrossed to females from the conventional *XX-XY* strain and, thus, were denoted *sonless*. When individually mated with the *bwb ge* sib females, all but one of these *bwb ge* males produced only a few, if any, male progeny. This exceptional male produced 16% (54 of 339) males in the F_1, and the subsequent sib-matings using these F_1 flies produced approximately 10–30% males and 5–10% intersexes in later generations. The *bwb ge* male progeny still showed the *sonless* character in the outcross matings. Most likely the *sonless* males were phenotypic males carrying no *M* factor, which resulted from a female-to-male sex transformation. This unique strain, used in the present study, was named Sonless-81 (*Snl*^{81}).

Examinations of external morphology of flies were done with a dissecting microscope. External morphology of terminal segments including the fifth sternite, genitalia and analia was used for sexing, because distinct sexual dimorphisms are seen in these segments. Flies were classified as females or males if they had normally developed terminalia characteristic to each sex, or as intersexes if their terminalia consisted of both female and male parts. Terminology of terminal segments was based on the nomenclature described by WEST (1951). All photographs were taken under a dissecting microscope equipped with a ring-shaped flash lamp.

Genetic markers used were *Rl* (*Rolled wings*, autosome 1), *Mk* (*Masked eyes*, 2), *Bx*^{2} (an allele of *Bx*, *Beadex wings*, 3; hereafter denoted simply as *Bx*), *bwb* (*brown body*, 3), *ge* (*green eyes*, 3), *Ba* (*Bald abdomen*, 4) and *Lp* (*Loop wing veins*, 5). Detailed descriptions of these markers and their linkage relationships are given by HIROYOSHI (1977). Flies were reared at 25 ± 1° under uncrowded conditions, according to the method described by HIROYOSHI (1977), unless otherwise noted.

RESULTS

Inheritance of the sex-transformation gene, *tra*: As described above, males of the *Snl*^{81} strain never produced male progeny when outcrossed to females from the conventional *XX-XY* strains, although they produced approximately 10–30% males and 5–10% intersexes in the intrastrain matings. To examine what sex ratios the *Snl*^{81} females produce, 15 *bwb ge/bwb ge* females from the *Snl*^{81} strain were mated with + *bwb* +/*Bx* + *M* males from a laboratory III^M strain, *bwb/Bx M* (Nagai) (hereafter denoted *tester*), and then eggs laid by individual females were collected at 3-day intervals for 9 days. As a control,

TABLE 1

Segregations from individual crosses between + *bwb ge* +/+ *bwb ge* + females from the *Snl*^{81} strain and + *bwb* + +/*Bx* + + *M* males from the *tester* strain

Females	Cross (no.)	*Bx*$^+$ *bwb*			*Bx bwb*$^+$			*Bx*:*Bx*$^+$	TR (%)[b]
		♀	♀	♂[a]	♀	♀	♂		
Type 1	1	77	0	0	0	0	43	0.56	0
	2	105	0	0	0	0	60	0.57	0
	3	81	0	0	0	0	56	0.69	0
	4	158	0	0	0	0	116	0.73	0
	5	51	0	0	0	0	39	0.76	0
	6	78	0	0	0	0	60	0.77	0
	7	151	0	0	0	0	134	0.88	0
Type 2	1	43	1	14	0	0	32	0.55	25.9
	2	81	4	27	0	0	86	0.76	27.7
	3	95	6	43	0	0	123	0.85	34.0
	4	19	5	9	0	0	26	0.79	42.4
	5	25	2	17	0	0	24	0.55	43.4
	6	76	8	65	0	0	135	0.91	49.0
	7	70	5	64	0	0	126	0.91	49.6
	8	66	7	88	0	0	114	0.71	59.0

[a] Phenotypic males carrying no *M* factor.

[b] TR(%) represents a percentage of sex-transformed flies (intersexes and phenotypic males) in the *Bx*$^+$ *bwb* genotypic female progeny.

23 *bwb*/*bwb* females taken from the *tester* strain were similarly examined. The advantage of using *tester* males instead of the conventional *XY* males is that the male-determining factor, *M*, of the *tester* males is marked with a dominant mutation, *Bx*, so that we can easily distinguish the genotypic female and male progeny. From these crosses, therefore, *Bx*$^+$ *bwb* flies would be recovered as females, whereas *Bx bwb*$^+$ flies are recovered as males.

The control females all produced *bwb* females and *Bx* males, as expected. In contrast, two types of segregation data were obtained from the *Snl*^{81} females (Table 1). Seven females (type 1) produced *bwb* females and *Bx* males, as expected, whereas the remaining 8 females (type 2) produced not only the expected *bwb* females and *Bx* males but also *bwb* males and some *bwb* intersexes. The ratio of the number of *Bx* genotypic males to the number of *Bx*$^+$ genotypic females was similar between type 1 females and type 2 females (0.56–0.88 *vs.* 0.55–0.91). Apart from the effect of *Bx* mutant on viability, there was no selective death with respect to one genotypic sex when comparing type 1 and type 2 females. The *bwb* males produced by the type 2 females proved to be *sonless* in subsequent outcross matings. These combined results suggest that some of the genotypic female progeny produced by the type 2 females did not develop into females but were transformed into intersexes or phenotypic males. It is probable that this sex transformation is caused by a maternal-effect mutant carried by the *Snl*^{81} females, and hereafter, we refer to *transformation rate* [TR(%)] to express the proportion of genotypic female progeny of an individual female which undergo sex transformation.

Inheritance of this hypothetical sex-transformation gene was examined as follows. First, *bwb/bwb* females taken from the *tester* strain were mated with *bwb ge/bwb ge* males from the *Snl*^{81} strain. Some of the F_1 females were backcrossed to *bwb ge/bwb ge* males from the *Snl*^{81} strain and were individually allowed to lay eggs in order to determine whether they produced sex-transformed progeny. Three of the 11 F_1 females produced sex-transformed progeny, but at extremely low frequencies, 0.02–3.6% (see below, *Zygotic expression of the* tra *locus*). Second, the remaining F_1 females were mated with the *tester* males, + *bwb* +/*Bx* + *M*, and were individually allowed to lay eggs in order to check whether they produced sex-transformed flies. None of the F_1 females (0 of 23) produced sex-transformed progeny. Third, the F_2 females obtained from the backcross were mated with the *tester* males, and their offspring were similarly examined. Some of the F_2 females (10 of 29) did produce sex-transformed flies at frequencies ranging from 18.5 to 98.7%. It is concluded that the sex transformation is caused by a recessive maternal-effect mutant, here named *transformer* (*tra*), the effect of which is extremely variable, causing genotypic female progeny to develop into normal females, intersexes or completely transformed fertile males.

Linkage analysis of *tra*: The linkage group of *tra* was determined by using five dominant mutations, *Rl* (autosome 1), *Mk* (2), *Bx* (3), *Ba* (4) and *Lp* (5). For this purpose, it is necessary to use *tra/tra* females. The following matings were made to identify such females. In a linkage test for autosome 1, for example, +/+; + *bwb ge* +/+ *bwb ge* + females from the *Snl*^{81} strain were mated with +/*Rl*; + *bwb* + +/*Bx* + + *M* males. The progeny +/*Rl*; + *bwb ge* +/+ *bwb* + + phenotypic males must be *tra*/+, because such flies are recovered only from *tra/tra* mothers. These males were backcrossed to females from the *Snl*^{81} strain. These females were individually allowed to lay eggs and were checked to see if they produced both female and male progeny, or only female progeny. In the F_2, females were taken from cultures in which many male sibs were simultaneously recovered, ensuring that their mother was *tra/tra*. These females, Rl$^+$ or Rl, were mated with + *bwb* +/*Bx* + *M* males and were individually allowed to lay eggs in order to check whether they produced sex-transformed flies. If *tra* is located on autosome 1, the F_2 females, Rl$^+$ and Rl, obtained from this scheme should have the *tra/tra* and *tra/Rl* chromosomes, respectively. Similar procedures were used in other linkage tests, except that in the linkage test for autosome 3, + +/*Mk* *M*(II^M); +/*Bx* males were used instead of the +/*Rl*; + *bwb* +/*Bx* + *M*(III^M) males. Results, given in Table 2, clearly demonstrate that *tra* is located on autosome 4.

Next, we carried out experiments to determine the map distance between *Ba* and *tra*. For this purpose, we established a new strain, *Snl*^{82}, in which *tra* is homozygously fixed. This was accomplished by crossing + *tra*/+ *tra* females and + *tra/Ba* + phenotypic males obtained from the linkage experiments described above. Then, + *tra*/+ *tra* phenotypic males taken from the *Snl*^{82} strain were mated with *Ba* +/+ *tra* females, and the resulting *Ba* +/+ *tra* females backcrossed to the + *tra*/+ *tra* males. The F_2 females, *Ba*$^+$ or *Ba*, were mated with *tester* males and were individually allowed to lay eggs to check whether

TABLE 2

Linkage analyses of *tra*: F_2 females carrying a *Snl*^{81} strain-derived chromosome(s), designated *tra*, in either heterozygous or homozygous state were mated with *tester* males to check whether they produce sex-transformed flies

Linkage group	Presumptive genotype of F_2 females	No. of F_2 females that produced sex-transformed flies	No. of F_2 females that produced no sex-transformed flies
I	*tra/Rl*	6	12
	tra/tra	8	8
II	*tra/Mk*	3	4
	tra/tra	3	7
III	*tra/Bx*	8	2
	tra/tra	6	7
IV	*tra/Ba*	0	19
	tra/tra	20	0
V	*tra/Lp*	5	8
	tra/tra	9	11

For mating procedures, see text.

TABLE 3

Zygotic effect of *tra*

Cross (♀ × ♂)	Phenotype of progeny	♀	⚥	♂	♀ : ⚥ + ♂	TR (%)[a]
1. *tra/tra* × *tra/Ba*	+	105	18	20	105 : 38	26.6 } *
	Ba	138	13	12	138 : 25	15.3 }
2. *Ba/tra* × *tra/tra*	+	1599	31	4	1599 : 35	2.1
	Ba	1575	0	0	1575 : 0	0

[a] TR(%) represents the frequency of sex-transformed flies.
* Significant at the 5% level ($\chi^2 = 5.882$).

they produced sex-transformed flies. Of the 227 Ba^+ and 205 *Ba* females examined, only one recombinant *Ba* female which produced sex-transformed progeny was identified. The recombination frequency between *Ba* and *tra* is thus ~0.2%.

Zygotic expression of the *tra* locus: The zygotic effect of *tra* was examined by using + *tra/Ba* + and + *tra/+ tra* phenotypic males (Table 3). In these crosses, only female progeny would be expected to appear unless sex-transformation takes place. When produced by + *tra/+ tra* mothers (cross 1), + *tra/+ tra* progeny were sex transformed much more frequently than + *tra/Ba* + (26.6% *vs.* 15.3%, $\chi^2 = 5.822$, $P < 0.05$), demonstrating the partial rescue of *tra* maternal effect by the tra^+ sperm. When produced by *Ba* +/+ *tra* mothers (cross 2), sex transformation took place, but only in + *tra/+ tra* progeny and at an extremely low frequency. These results demonstrate that the *tra* locus is expressed not only during oogenesis but also zygotically.

Effect of female age on expressivity of *tra*: In all of the experiments

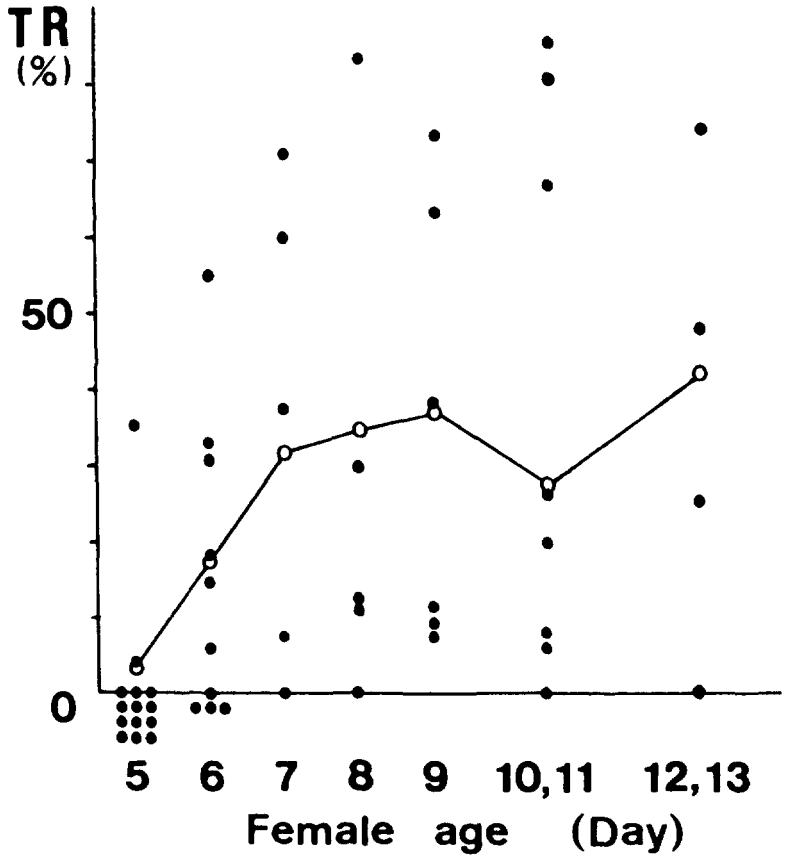

FIGURE 1.—Effect of aging on expressivity of *tra* maternal effect. Frequencies of sex-transformed flies [TR(%)] in daily broods of individual *bwb ge/bwb ge*; *tra/tra* females were examined by mating with the *tester* males. A closed circle represents TR(%) of each female, and an open circle represents that of the pooled progeny.

described above, the frequency of sex-transformed progeny from *tra/tra* females was always lower in the first 3-day brood as compared with those recovered from the later broods, suggesting that the maternal age affects the expression of the mutant. To confirm this fact, the frequency of sex-transformed flies was examined by daily collection of eggs produced by each of 14 *tra/tra* females, although they did not lay eggs every day. Due to a lowered fecundity in aged females, eggs laid by the 10- to 13-day-old females were pooled for every 2 days. Results are shown in Figure 1. All females produced the first egg masses on the fifth day after emergence. Among them only two produced sex-transformed flies. The overall frequency of sex-transformed flies in the pooled progeny of the 6-day-old mothers was still lower than the frequency of those produced by the same mothers during 7 to 13 days (17.7% *vs.* 27.7–42.6%). These results suggest that the expression of *tra* is weak when the carrier females are young.

External morphology of *tra*-transformed flies: The expression of the ma-

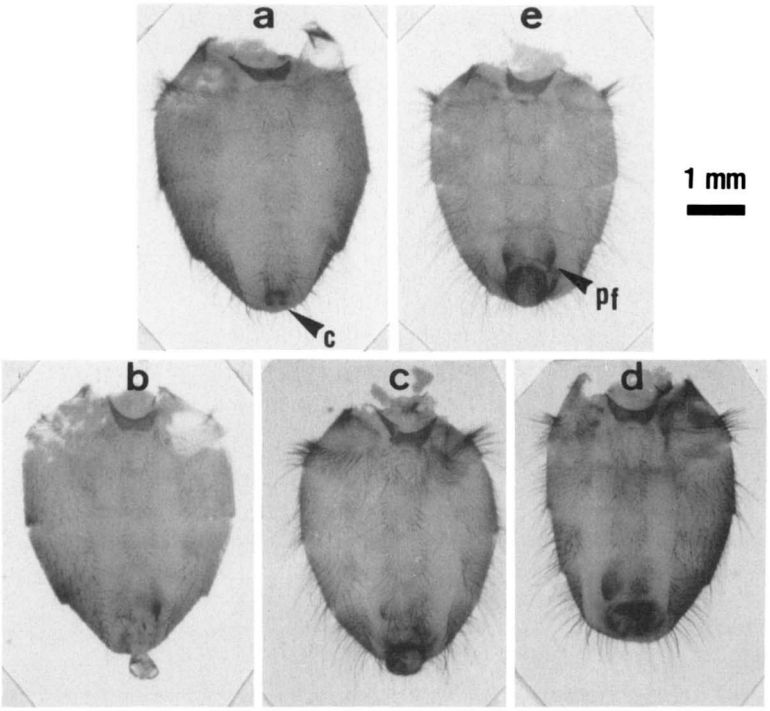

FIGURE 2.—Ventral views of female (a), intersex (b, c, d) and male (e) phenotypes of genotypic female progeny produced by a single *tra/tra* female. The genotype of these flies was *tra/+*. Abbreviations: p_f, primary forceps on the fifth sternite; c, cerci. See text for explanations.

ternal sex-transforming effect of *tra* is extremely variable in that nontransformed fertile females, intersexes and phenotypic fertile males are usually recovered from the same *tra/tra* mother. In the *tester* strain, the head-frons (the width of head/the width of frons) ratio in the females is 3.2 ± 0.1 (mean ± SE, $N = 20$) and that of the male is 6.9 ± 0.3 ($N = 26$). Similar values are observed in the Snl^{82} strain; *i.e.*, 3.2 ± 0.1 ($N = 21$) for the nontransformed females and 6.5 ± 0.4 ($N = 15$) for the sex-transformed phenotypic males. Sex-transformed phenotypic males also resembled genotypic males in other quantitative characters, such as body size, body pigmentation and bristle length. Intersexes showed a series of intermediate phenotypes in these characters.

Remarkable sexual dimorphisms are seen in the terminalia, including both genitalia and analia. Figure 2 shows ventral views of abdomens of a nontransformed female, intersexes and a sex-transformed phenotypic male. No morphological differences are observed either between nontransformed females and normal *XX* females or between phenotypic males and normal *XY* males. The nontransformed female (Figure 2a) has long bristles on the nonsclerotized fifth sternite. The segments 6–11 form a membraneous ovipositor with nonsclerotized tergites and sternites denominated dorsal and ventral chitinous rods, respectively. These segments are usually intruded into the body cavity,

and only a pair of button-shaped cerci, appendages of the segment 11, can be seen. The phenotypic male (Figure 2e) has short bristles on the fifth sternite, which bears a pair of heavily sclerotized primary forceps. The segments 6–8 are sclerotized to form male-type genitalia. Intersexes show a series of intermediate sexual phenotypes. Three examples are shown here. A female-like intersex (Figure 2b) has the fifth sternite which bears only a right half of primary forceps. The segments 6 and 7 form a female-type membraneous ovipositor, but the segment 8 is partially sclerotized to form a rudimentary male-type genitalia. The terminal segment bears no cerci. An intermediate-type intersex (Figure 2c) has nearly complete female-type fifth sternite, except for a pigmented patch in the left region. The segment 6 is membraneous, but the segments 7 and 8 are heavily sclerotized to form male-type genitalia. A male-like intersex (Figure 2d) has the partially sclerotized fifth sternite. The segments 6 to 8 form male-type genitalia, although the rotation of these segments is incomplete.

DISCUSSION

The *tra* mutant is a recessive maternal-effect mutant that causes the genotypic female progeny carrying no *M* factors to follow the male pathway of sexual differentiation to varying degrees. The mutant is located on autosome 4 near the *Ba* locus. The expressivity of *tra* is not complete, and the phenotypes of the genotypic female progeny range from the normal female, through various degrees of intersexuality, to the completely transformed fertile male. The mutant also has a zygotic effect, such that the genotypic female progeny produced by *tra/tra* mothers are transformed much more frequently when they are *tra/tra* than when they are *tra/+*. Moreover, the *tra/tra* progeny of *+/tra* mothers can undergo sex transformation, although at very low frequencies. A similar mutant, *Arrhenogenous* (*Ag*), has been identified on autosome 1 (VANOSSI-ESTE 1971; VANOSSI-ESTE and ROVATI 1982).

The present results suggest that (1) the tra^+ gene product is necessary for female determination and/or differentiation and that (2) the *tra* locus is expressed during oogenesis and also in zygotes. It is noted also that the *tra* effect is more pronounced in aged mothers (Figure 1). A similar aging effect has been reported for *Ag* (VANOSSI-ESTE and ROVATI 1982). This suggests that changes in the physiological condition of mothers, the activity of the reproductive system for example, may affect the expressivity of these maternal-effect mutations. Production and/or storage of the tra^+ gene product may not proceed constantly but, rather, in a sporadic manner in time and space, so that individual eggs may receive various amounts of the product. The fact that intersexes with a male head and a female genitalia, or vice versa, occasionally were observed suggests that the tra^+ product may not always be distributed evenly in the egg cytoplasm. It is known that sex in *M. domestica* is determined in a cell autonomous manner (MILANI 1975). The expressivity of *tra* is not affected by temperature, because the frequency of sex-transformed flies is similar at three different temperatures, 17, 25 and 31° (data not shown).

It has been said that Musca and Drosophila, although they are both Dipter-

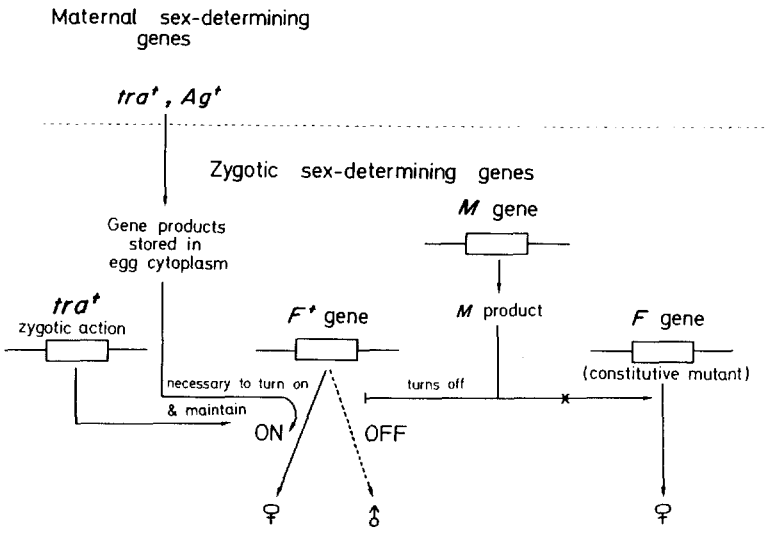

FIGURE 3.—A model for the control of sex determination in *Musca domestica*.

ans, have completely different sex-determination mechanisms: the epistatic system in the former and the balance system in the latter (ENGELMANN 1970). We should like to emphasize, however, that they may, in fact, share a common genetic system for the control of sex determination. In *M. domestica*, the presence or absence of the *M* factor instructs the male or female pathway, respectively, of sexual differentiation. Since maternal-effect mutants, *Ag* (VANOSSI-ESTE 1971) and *tra* (this study), are able to produce fertile phenotypic males carrying no *M* factors, the *M* factor itself is not essential for male differentiation, although it determines maleness. This indicates that the *M* factor controls the expression of another gene(s) involved in sex determination and/or differentiation. The female-determining factor *F*, a fourth chromosomal dominant, causes genotypic males carrying one or more *M* factors to develop as fertile females (RUBINI 1967; MCDONALD *et al.* 1978). The maternal effect of *Ag* is completely rescued by the *F* gene (ROVATI and VANOSSI-ESTES 1978). The epistatic relationship of *F* and *tra* could not be examined because *tra* is closely linked with the *F* locus.

Here, we present a model explaining the actions of the known sex-determining genes in *M. domestica* (Figure 3). The tra^+ gene is active both maternally and zygotically. The maternal tra^+ product may be necessary to turn on the F^+ gene, and the zygotic tra^+ product may be required to maintain the turned-on state of the F^+ gene. The F^+ gene, when turned on, leads both somatic and germ cells to follow the female differentiation pathway. Various *M* loci may simply represent polymorphic variations of the *M* factor, the role of which is to turn off the F^+ gene. Thus, the *F* mutation may be simply a constitutive mutant. Genotypic female progeny of *tra/tra* mothers may have various amounts of the maternally synthesized tra^+ product, sometimes sufficient and sometimes insufficient, to turn on the F^+ gene: flies then develop as females, intersexes or phenotypic males, depending on the amount of F^+ gene product

or the number of cells expressing F^+. When these flies carry the *M* factor, F^+ gene is turned off irrespective of the amount of tra^+ product, and then flies develop as males. The reason that the mutant of *M* factor has not been reported is probably because the loss-of-function would simply lead flies to differentiate as normal females and, hence, be unnoticed. Loss-of-function mutants of the F^+ gene have not been reported.

The model described above may be regarded as similar to the sex determination mechanism envisaged in *D. melanogaster* (CLINE 1978, 1983a,b, 1984; BAKER and BELOTE 1983). When the *X:A* ratio is 1.0, the Sxl^+ gene is activated in the presence of maternally synthesized da^+ gene product. The Sxl^+ product, in turn, activates the tra^+ and $tra\text{-}2^+$ genes, and their products then cause the dsx^+ gene to take the female mode of expression and female differentiation ensues. When the *X:A* ratio is 0.5, the Sxl^+ gene is not activated, hence tra^+ and $tra\text{-}2^+$ are not activated, and dsx^+ remains in the basal male mode of expression, which leads to male differentiation. The counterparts of tra^+, F^+ and *M* in *M. domestica* may be found in *D. melanogaster* as da^+, Sxl^+ and the *X:A* ratio (=0.5), respectively. We note that, whereas the *tra* maternal-effect phenotype in *M. domestica* is sex transformation, the *da* maternal-effect phenotype in *D. melanogaster* is sex-specific lethality (*i.e.*, no sex-transformed progeny are observed). This is probably because with *da* the sex-transformed females are also "transformed" with respect to their levels of *X*-linked gene activities (*i.e.*, they exhibit male-like dosage compensation), and this is lethal to diplo-*X* flies. This explanation is consistent with the observation that some partially transformed flies from *da/da* mothers can be recovered if the flies carry male-specific lethal mutations (e.g. *mle/mle*), which would prevent *X* chromosome hypertranscription (CLINE 1984). At present, equivalents for the control genes, tra^+, $tra\text{-}2^+$ and dsx^+, in *D. melanogaster* have not been identified in *M. domestica*. The above similarities may represent not mere coincidences but the real relatedness of the two Dipteran species.

It should be mentioned here that, in *M. domestica*, there is no dosage compensation. The heterochromatic sex chromosomes, *X* and *Y*, have no major genes except for the *M* factor on the *Y*. In *D. melanogaster*, genes responsible for dosage compensation are known also to play important roles in sex determination (SKRIPSKY and LUCCHESI 1982; BAKER and BELOTE 1983; UENOYAMA 1984; CLINE 1984).

It is important to note that all the sex transformation mutants so far identified in *D. melanogaster* do not cause sex transformation in germ-line cells, thus producing sterile sex-transformed flies (BAKER and BELOTE 1983). Thus, these mutations are detrimental mutations that cannot be used directly as materials for the evolution of sex-determining systems. In contrast, all of the sex-transformation mutants so far known in *M. domestica* are capable of producing fertile sex-transformed flies. These mutations apparently have led to the establishment of multiple sex-determining systems in this species. The *F* factor has established the female heterogamety, *i.e.*, *F*/+; *M*/*M* females and +/+; *M*/*M* males, in some natural populations (WAGONER 1969; RUBINI, HEEMART and FRANCO 1977; INOUE and HIROYOSHI 1982). A similar female het-

erogametic strain has been reported in a local race of *Chironomus tentans* (THOMPSON 1971), in which the presence or absence of *M* determines sex in normal strains, as in *M. domestica*. It is tempting to speculate that the same basic genetic mechanism is involved in the female heterogamety reported in many species of Lepidoptera (TAZIMA 1964; CASPARI and GOTTLIEB 1975).

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