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Developmental Analysis of Two Sex-Determining Genes, *M* and *F*, in the Housefly, *Musca domestica*

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Manuscript received December 17, 1992
Accepted for publication April 2, 1993

ABSTRACT

In the housefly, *Musca domestica*, a single dominant factor, *M*, determines maleness. Animals hemi- or heterozygous for *M* are males, whereas those without *M* develop as females. In certain strains, however, both sexes are homozygous for *M*, and an epistatic dominant factor, F^D, dictates female development. The requirement for these factors was analyzed by producing, with mitotic recombination, mosaic animals consisting of genetically male and female cells. Removal of F^D from an $M/M;F^D/+$ cell at any time of larval development, even in the last larval instar, resulted in sex-reversal, *i.e.*, in the development of a male clone in an otherwise female fly. In contrast, when *M* was removed from *M*/+ cells, the resulting clones remained male despite their female genotype, even when the removal of *M* happened at embryonic stages. The occurrence of spontaneous gynandromorphs, however, shows that the loss of *M* in individual nuclei prior to blastoderm formation causes the affected cells to adopt the female pathway. These results are consistent with the hypothesis that *M* is the primary sex-determining signal which sets the state of activity of the key gene *F* at around the blastoderm stage. Parallels and differences to the sex-determining system of Drosophila are discussed.

IN the order Insecta, a variety of mechanisms for the genetic control of sex determination has evolved. These mechanisms include, sometimes in closely related species, *XY* systems, monofactorial "autosomal" systems without heteromorphic sex chromosomes, maternal effects, relative number of *X* chromosomes to autosomal sets, and haplo-diplo mechanisms. This multitude of control mechanisms for sex determination may in fact represent variations of a single principle commonly used by all insect species (NÖTHIGER and STEINMANN-ZWICKY 1985). The common principle consists of a primary signal that, along with maternal components, acts at around the time of blastoderm formation to regulate a key gene. An active product of this key gene is required throughout development for female differentiation and must be absent if male development is to ensue. The alternative states of this key gene regulate a short cascade of downstream control genes that govern sexual development (NÖTHIGER and STEINMANN-ZWICKY 1985; INOUE and HIROYOSHI 1986).

In most European strains of the common housefly, *Musca domestica*, sex is genetically determined by the presence or absence of a *Y* chromosome with a dominant male-determining factor, *M*. Females are *XX* and males *XY* (STEVENS 1908). Except for *M* on the *Y* chromosome, no marker genes have been found on either the *X* or *Y*. Thus, sex-linked inheritance does not commonly occur (MILANI 1967). Beside these "standard strains," other housefly field strains found in Italy and England have evolved autosomal mechanisms for sex determination, recognized by sex-linked inheritance of traits known to depend on autosomal genes (SULLIVAN 1958; FRANCO, RUBINI and VECCHI 1982; INOUE and HIROYOSHI 1982). In these strains, females and males may both have two *X* chromosomes, but in the male one of the autosomes carries the *M* factor (M strains). This autosomal sex determination mechanism appears further modified in strains that are homozygous for *M* in both sexes. Here, the presence of a dominant epistatic factor, F^D ("$F^{Dominant}$"), promotes female development even in a background of one or several *M* factors ("F^D strains"; MCDONALD *et al.* 1978; for a review see DÜBENDORFER, HILFIKER-KLEINER and NÖTHIGER 1992).

The models proposed by NÖTHIGER and STEINMANN-ZWICKY (1985) and INOUE AND HIROYOSHI (1986) view *M*, whether *Y* chromosomal or autosomal, as the primary signal whose presence or absence determines alternative functional states, "off" or "on," of a key gene *F*, which is assumed to be the wild-type allele of F^D. The factor F^D then could be a constitutive mutation, analogous to the allele Sxl^M of the *Sex-lethal* gene in Drosophila (CLINE 1978, 1979). In standard strains, *M* is assumed to repress *F* which results in male development, whereas absence of *M* allows *F* to become active and initiate female development. The mutation F^D renders the gene insensitive to the repressing action of *M*. The model predicts that *M* represses *F* early and irreversibly in development,

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analogous to the early effect of the X:A ratio on the setting of *Sxl* (*Sex-lethal*) in Drosophila (SÁNCHEZ and NÖTHIGER 1983; BELL *et al.* 1991). If this were correct, M in Musca should only act around blastoderm formation, as does the X:A signal in Drosophila, whereas the F function would be required throughout development, analogous to the *Sxl* function in Drosophila (SÁNCHEZ and NÖTHIGER 1982).

The present investigation attempts to test these predictions. We used X-ray-induced mitotic recombination at various times in embryonic and larval development to eliminate M from heterozygous M cells, or F^D from F^D/F^+ cells homozygous or heterozygous for M. The analysis of genetically female clones in males, and genetically male clones in females, leads us to conclude that M and F of Musca represent the primary signal and a downstream gene, respectively, fulfilling homologous functions to the X:A ratio and to *Sxl* or *tra* in Drosophila.

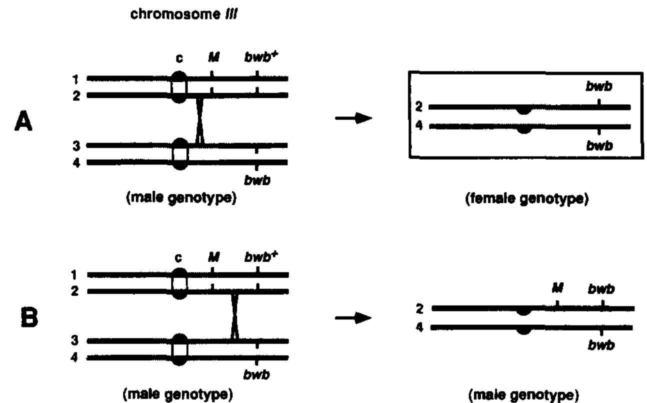

FIGURE 1.—The two possibilities of mitotic recombination events on autosome *III* that produce bwb clones and simultaneously either the loss (**A**) or the retention (**B**) of the male determining factor (M) in the genome of the daughter cell, depending on the site of the recombination event. c, centromeres; 1–4, numbering of the chromatids. All other possible chromatid combinations lead to clones that remain heterozygous for *brown body* (*bwb*) and are therefore not visible on the cuticle of the fly.

MATERIALS AND METHODS

Rearing of flies: Adult *M. domestica* were kept in acrylic glass cages (14 × 14 × 19 cm) and fed on water and sucrose. They were allowed to deposit eggs on cotton wool soaked in milk or on larval rearing medium. Larvae were grown at 25° and 70% relative humidity in this medium (220 g wheat bran, 30 g wheat flour, 250 g fresh milk, 10 g brewer's yeast, 130 ml water, 0.5 g nipagin). In the present paper, the age of embryos and larvae is always expressed in hours after oviposition at 25°.

Genotypes: Gene symbols and mutations are described in MILANI (1967). The autosomal markers used in this study were *bwb* (*brown body*) and *ge* (*green eyes*) on linkage group III, *Ba* (*Bald abdomen*) on linkage group IV, and *ocra* on linkage group V. Since the eye color mutations *ocra* and *ge* are irrelevant to our experiments, they were omitted from the genotypic formulas in RESULTS and DISCUSSION.

For the production of clones without M factor, we used males of genotype bwb^+ *M/bwb;ocra* from a stock in which the females were *bwb;ocra*. The stock was obtained from the Genetics Department of the University of Pavia (Italy). Recombination in males is so rare that there is no exchange between *bwb* and M. Figure 1 shows that two types of bwb clones are expected in irradiated bwb^+ *M/bwb* animals: clones having lost and clones having retained M.

Clones lacking the constitutive allele F^D were generated in female larvae heterozygous for F^D. These animals derived from a stock in which the females were *bwb ge M;* F^D Ba/F^+ Ba^+ (type 1), or from a cross between *bwb ge M;* F^D/F^+ females and XY wild-type males (type 2). With respect to linkage group III, these latter animals had the same genotype as those shown in Figure 1. Both F^D-stocks were obtained from H. INOUE and T. HIROYOSHI of the Osaka University Medical School, Japan.

Type 1 animals were homozygous for *M bwb ge* on chromosome *III* and carried the marker *Bald abdomen* (*Ba*) in *cis* with F^D on chromosome *IV*. *Ba* is a dominant mutation that eliminates most bristles on the abdomen. It maps very close to F^D (0.06% recombination frequency; INOUE and HIROYOSHI 1984). In a F^D Ba/F^+ Ba^+ background, clones that have lost *Ba* by mitotic recombination are recognized by a wild-type bristle pattern. Because of the very close linkage, such cells have almost certainly also lost the F^D allele (see scheme in Figure 2).

Type 2 was heterozygous for *bwb ge M* on chromosome *III* and for F^D on chromosome *IV*, respectively. In this case, cells from which F^D was removed by mitotic recombination were recognized by their male phenotype only, but heterozygosity for *bwb* provided an internal control unrelated to sex, and thus positive proof that mitotic recombination did occur. Unirradiated females of both types and females of a standard strain carrying no autosomal sex-determining factors served as controls.

Gynandromorphic flies appeared at low frequencies (<0.01%) in standard XY strains, but also in strains with autosomal M, and in $M;F^D$ strains.

Irradiation parameters: Clones were generated at various stages of development by X-ray-induced mitotic recombination (NÖTHIGER and DÜBENDORFER 1971). For irradiation, larvae of the first, second and third instar were washed out of the medium. Depending on the sensitivity of the genotype, a dose of 6.0–15.0 Gy was delivered with a Philips MG 160 X-ray machine at 150 kV, 14 mA, 2-mm Al filter, 1-mm acrylic glass, at an irradiation distance of 25.0 cm. The dose rate was 5.0 Gy per minute.

Early embryos from before until just after blastoderm formation were collected at room temperature and irradiated with 1.0–2.0 Gy at 0.5–3 hr after egg deposition. Application of higher doses to whole embryos was lethal. In one experiment, a dose of 5.0 Gy was applied just to the posterior polar region, keeping the rest of the embryos shielded by 5 mm of lead (Pb).

Preparation and analysis of flies: Abdominal carcasses as well as internal and external genitalia were spread on microscope slides and mounted in Euparal. The frequency and the sexual phenotype of the clones in sexually dimorphic regions of the abdomen were assessed with a compound microscope. Since we are only interested in the qualitative aspect of the clones, *i.e.*, their sexual phenotype, we have not estimated clone sizes which we consider irrelevant for our question and which are difficult to measure in the sexually dimorphic regions due to the rareness of bristles. Similarly, we do not want to put much emphasis on clone frequencies since we used variable irradiation doses and genetic backgrounds.

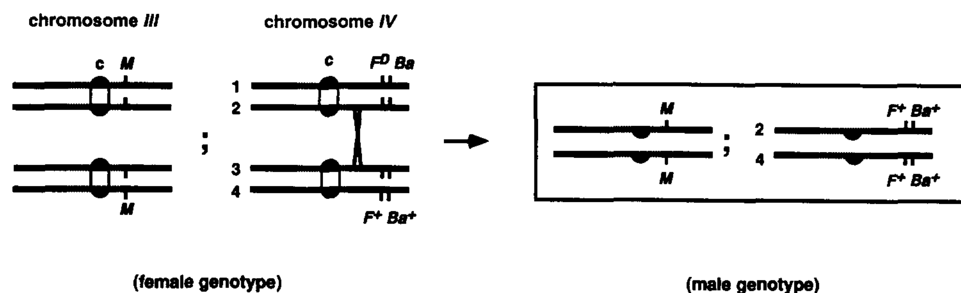

FIGURE 2.—Mitotic recombination in a cell in which $F^{Dominant}$ (F^D) is *cis*-heterozygous with *Bald abdomen* (*Ba*) on autosome *IV* (*cf.* text: "type 1"). The recombination event results in an F^+ clone marked with Ba^+ (framed), whereas the rest of the fly remains phenotypically *Ba* and F^D. Since all cells of this animal also carry *M* on autosome *III*, loss of F^D from individual cells renders these cells genotypically male.

RESULTS

Development of clones without *M* in *M*/+ males: Animals *trans*-heterozygous for *M* and *bwb* on chromosome *III* were irradiated as blastoderm stage embryos (0.5–3 hr), freshly hatched first instar larvae (19 ± 2 hr), second instar larvae (48 ± 2 hr), and early third instar larvae (74 ± 2 hr), respectively (Table 1).

As seen in Figure 1, genotypically female cells are expected as a result of loss of *M*. Among 403 males that eclosed after irradiation at various stages, none showed any female structures. The 50 homozygous bwb clones, recognizable as yellow (bwb) patches in a phenotypically wild-type background, are positive proof that mitotic recombination in the third chromosome did occur and produced marked clones in sexually dimorphic regions. Most of these bwb clones should have lost *M* (see DISCUSSION), but despite their female genotype, none showed female characteristics (Table 1 and Figure 3, A and B). The bwb clones were always confined to the derivatives of only one imaginal disc.

Contrasting results were obtained with the spontaneous gynandromorphs that appeared among unirradiated flies (Figure 3C). In these flies, we found large female areas extending over several body segments in otherwise male flies, indicating that the event producing the female clone must have taken place long before the blastoderm stage when imaginal discs become singled out. The overall appearance of these gynandromorphic flies was strikingly similar to those described by RUBINI, VECCHI and FRANCO (1980) who presented cytological evidence that the cause of gyndromorphism was mitotic recombination during the first nuclear divisions in embryonic development.

Sex reversal in clones from which F^D was removed: The main and important result of this experiment is the appearance of male clones in female flies. Two different genotypes of F^D-females were irradiated for the production of clones without F^D. One genotype (type 1, Figure 2) offered the advantage of a dominant marker (*Ba*) closely linked to F^D, which gave positive proof that in every case of male development F^D had indeed been removed from the genome of the male cells. Unfortunately, this genotype was poorly viable after irradiation of early developmental stages. We obtained only seven small male clones from treatments of third instar larvae (Table 2, footnote *c*). The other genotype (type 2, see MATERIALS AND METHODS) had no marker to monitor absence or presence of F^D, but it had much higher viability after radiation treatment, which allowed the generation of male clones at all larval stages (Table 2, footnote *e*, Figure 3D). Furthermore, its heterozygosity for *bwb* on chromosome *III* provided an internal control for mitotic recombination unrelated to sex.

Table 2 shows that irradiation between the early second larval instar and pupariation produced phenotypically male clones at frequencies ranging from 30 to 55%. All clones were restricted to the derivatives of a single imaginal disc. An example of such a male clone in a female fly is shown in Figure 3D.

In both control series, *i.e.*, nonirradiated females of types 1 and 2, and irradiated females without *M* or F^D of a standard stock, no male patches were observed. This shows that the parameter responsible for the appearance of male clones is the removal of F^D from

TABLE 1

Frequency of bwb clones in a bwb^+ *M/bwb* background

Time of irradiation (hr)	No. of flies	X-ray dose (Gy)	No. of female bwb clones	No. of male bwb clones (%)
0.5–3	148	1.0–5.0	0	4 (2.8)[a]
19 ± 2	114	2.0–5.0	0	7 (6.1)
48 ± 2	75	8.0–10.0	0	18 (24.0)
74 ± 2	66	10.0–13.0	0	21 (31.8)

[a] Of these 4 bwb clones only one was unambiguously identifiable as male, whereas the other 3 were too small for a clear identification of the sex.

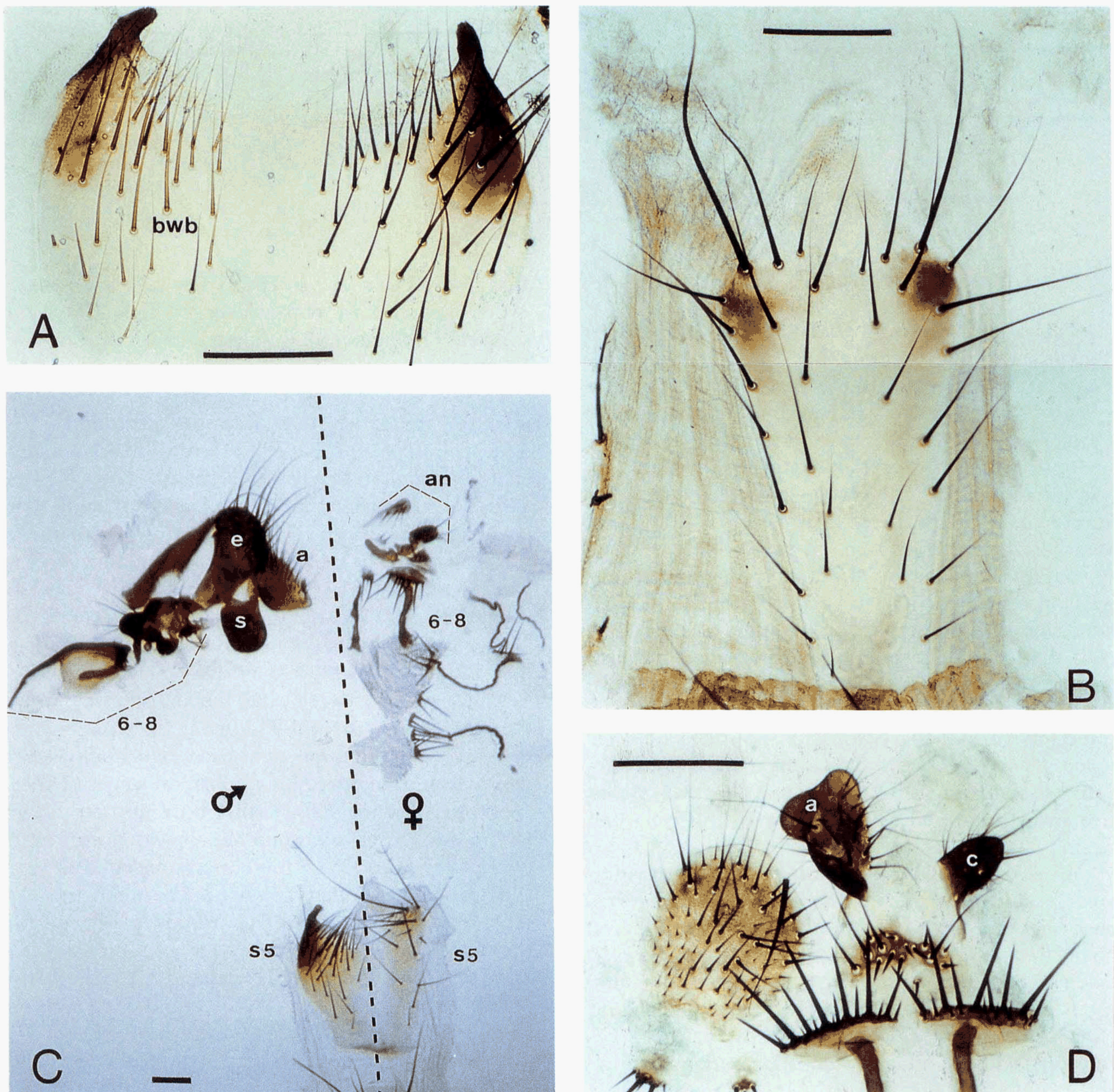

FIGURE 3.—(**A**) A brown body (bwb) clone covering the entire left side of a male sternite 5. Even though the cells in this clone lost their male-determining factor by mitotic recombination induced between 6 and 21 hr of embryonic development, they still metamorphosed into a perfect male pattern. (**B**) A female sternite 5 without clone is shown for comparison. (**C**) Whole-mount preparation of the postabdomen of a gynandromorphic *M. domestica*, spontaneously arisen in our *curly wing* stock. The heavy dashed line separates female and male parts. Abbreviations: (a) male anal plate; (an) female analia; (e) epandrium and (s) surstylus of the male genitalia; (6–8) elements of the abdominal segments 6 to 8. (**D**) A small male clone (a, anal plate) that developed instead of a cercus (c) in the female ovipositor. The animal was a female heterozygous for *M* and F^D, but the cells which formed the isolated male structure represent a clone grown from a cell that had lost F^D by mitotic recombination in the last third of the last larval instar (120 hr of development). The male development of this clone shows that sex determination can still be reversed late in development if the F^D allele is removed from a cell that also harbors the male determiner, *M*. The bars represent 200 μm.

a cell. Over a period of 2 years, five spontaneous gynandromorphic flies appeared in our F^D stocks (estimated frequency around 0 01%). In contrast to the clones generated by mitotic recombination, the male patches of these gynandromorphs extended over the derivatives of several imaginal discs. This indicates that these mosaics arose in a preblastoderm nucleus, most probably by spontaneous mitotic recombination leading to loss of the F^D allele.

As shown in Table 2, bwb clones appeared in the

TABLE 2

Frequency of F^+ clones in an $M;F^D/F^+$ background

Genotype	Time of irradiation (hr)[a]	No. of flies	X-ray dose (Gy)	Male clones (%)	Female clones (%)[b]
M/M; F^D/F^{+}[d]	87–115	17	6–9	7 (41.1)[c]	0
M/+; F^D/F^{+}[e]	48	64	8	19 (29.6)	19 (29.6)
	72	29	15	14 (48.3)	21 (72.4)
	120	71	15	39 (54.9)	50 (70.4)
	144	30	15	14 (46.6)	24 (80.0)
M/M; F^D/F^{+}[f]		177	0	0	0
XX; F^+/F^{+}[g]	72–86	430	7–9	0	0

[a] Hours after egg deposition at 25°.
[b] All female clones showed a *bwb* phenotype due to the loss of bwb^+ by mitotic recombination.
[c] All male clones were of the Ba^+ phenotype.
Full genotypes were:
[d] *bwb ge M/bwb ge M*; F^DBa/F^+Ba^+ (type 1.
[e] *bwb ge M*/bwb^+ ge^+; F^D/F^+ (type 2).
[f] Nonirradiated control: *bwb ge M/bwb ge M*; F^D/F^+.
[g] Irradiated control (standard strain): *XX*; *bwb/bwb*; F^+/F^+.

sexually dimorphic regions with higher frequency than male patches. This is not surprising since even extremely small bwb clones (one bristle) can be seen, whereas a sexually transformed clone must be larger to be recognized as male. Developmental defects, such as reduced survival and defective or missing cuticular elements, also occurred in all genotypes after irradiation, particularly at higher doses. This phenomenon is well known for Drosophila (SCHWEIZER 1972) and will not be discussed any further.

DISCUSSION

The function of the male sex-determiner, *M*: We have used X-ray-induced mitotic recombination to remove *M* from cells of *M* bwb^+/bwb embryos and larvae at various times of development. The resulting bwb clones in the sexually dimorphic regions differentiated perfect male patterns integrated in the surrounding male tissue (Figure 3A). Thus, a clone with a female genotype, *i.e.*, without *M*, apparently differentiates in the male mode if the precursor cells had an active *M* during early embryogenesis. This conclusion, however, is only valid if *M* and *bwb* are located on the same chromosome arm, if most events of mitotic recombination take place proximal to *M* (Figure 1), and if sexual differentiation is a cell-autonomous process. This seems indeed to be the case as we will argue in the following paragraphs.

RUBINI, VECCHI and FRANCO (1980) reported the spontaneous occurrence of gynandromorphs in an *M* bwb^+/bwb strain. In these flies, the male parts were bwb^+ and the female parts were bwb, thus demonstrating cell autonomy of sexual differentiation. This coincidence of sexual phenotype and mutant marker phenotype was also observed in our own gynandromorphs, affecting even individual bristles and thus ruling out hormonal effects or influences by neighboring cells.

In principle, gynandromorphism could be caused by double fertilization, by chromosome loss, or by mitotic recombination. We have not found any description of double fertilization in the literature nor any indication for it in our own crosses with heterozygous animals. Chromosome loss may be involved in the production of gynandromorphs in standard *XX-XY* strains where the *M*-carrying *Y* could be lost without impairing viability of the resulting mosaic animal (RUBINI 1964; BOYES 1967). Monosomy for an autosome, however, should be lethal, as suggested by the phenomenon of pseudolinkage in translocation heterozygotes. These show that hemizygosity even for fragments of an autosomal arm is lethal (WAGONER 1967, 1969; D. HILFIKER-KLEINER, J. SCHÖPFER-BONS and A. DÜBENDORFER, unpublished data). Thus, gynandromorphism in strains with autosomal sex determination is most likely the result of mitotic recombination, and the loss of bwb^+ associated with maleness of the same cells is indicative for *M* and *bwb* being located on the same arm of chromosome *III*. The strong correlation between the bwb phenotype and femaleness in the gynandromorphs also suggests that a large proportion, if not all, of the bwb clones induced in our experiments after the blastoderm stage had indeed lost *M* as a consequence of mitotic recombination which must take place predominantly in the centromeric heterochromatin. In Drosophila, GARCIA-BELLIDO and DAPENA (1974) have shown that mitotic recombination, in contrast to meiotic crossing over, is not depressed in the heterochromatin. These considerations render very unlikely an alternative interpretation, namely that clones from which *M* was removed and which now have a female genotype are poorly viable and may become eliminated. Since there

is no need for, nor any indication of, dosage compensation in genotypes with autosomal M (male) or without M (female), there is no reason to assume any viability problems.

In our gynandromorphic flies and in those described by RUBINI, VECCHI and FRANCO (1980), the bwb areas extended over the derivatives of several imaginal discs. This indicates that the event leading to mosaicism occurred prior to blastoderm formation, before segment-specific disc determination took place. The comparison of the gynandromorphs with our induced clones shows that loss of M during cleavage divisions leads to female development of the affected cells, whereas cells from which M is removed after the blastoderm stage remain male. We interpret this result as indicative of a determinative step by which M, during a critical phase of early embryogenesis, irreversibly inactivates a key gene whose function is needed for female development. This phenomenon finds its perfect analogy in Drosophila where changes in the X:A ratio during cleavage divisions produce gynandromorphs, but no longer do so later in development (SÁNCHEZ and NÖTHIGER 1983; BACHILLER and SÁNCHEZ 1991). This shows that the primary signal of the X:A ratio sets the state of activity of *Sxl* around blastoderm formation, a conclusion that was recently supported by molecular evidence (ERICKSON and CLINE 1991; TORRES and SÁNCHEZ 1991; KEYES, CLINE and SCHEDL 1992).

We tried to induce mitotic recombination during cleavage stages. The small number of prospective imaginal nuclei being present at this stage, and the extreme sensitivity of the Musca embryos to X-rays rendered our attempts unsuccessful. We doubt the preblastoderm origin of the four clones obtained in this series (Table 1, footnote *a*) since these clones were very small and the structures malformed, such that their sex could not be unambiguously determined. This result is consistent with the idea that in Musca, just as in Drosophila (WIESCHAUS and GEHRING 1976), mitotic recombination cannot be induced with X-rays in the nuclear cleavage stages.

The *"female determiner,"* F^D: Irradiation of $F^D/+$ larvae generated clones that differentiated male structures. We conclude that the male clones, which in females of type 1 were marked with Ba^+, are the consequence of losses of F^D in an M background, and that this event led to a sex reversal from female to male. This shows that the female pathway is constantly dependent on the presence of F^D which we view as a constitutively active F gene.

The male phenotype of the clones from which F^D had been removed (Figures 2 and 3D) indicates that the postulated wild-type allele F^+ is inactive in these cells. These results suggest that if M is active at the blastoderm stage, any F^+ allele is irreversibly repressed and does not become *trans*-activated by the constitutive activity of an F^D allele.

Sex determination in Musca: With our experiments, we have characterized two elements, M and F^D, that have opposite effects on sexual development. M appears to be active early in embryonic development to initiate male differentiation, whereas F^D is required throughout larval life to maintain the cells on the female pathway. In standard strains, M is present in males and absent in females, and this difference in genetic information represents the primary signal for the choice of the sexual pathway. Formally, the presence of M in males is analogous to the situation in mammals where the Y chromosome carries the male-determining factor *Sry* (KOOPMAN and GUBBAY 1991). In contrast to mammals, however, where *Sry* is only expressed and active in the prospective testicular cells of the indifferent genital ridge, sex in Musca and Drosophila is an inherent property of individual somatic cells each of which will differentiate according to its own sexual genotype without the involvement of hormones.

Such monofactorial systems of sex determination are known for other dipteran insects, such as Megaselia, Calliphora, Anopheles, Aëdes, Culex, Chironomus (for a survey see NÖTHIGER and STEINMANN-ZWICKY 1985). Musca is unique inasmuch as two factors are known, M and F^D, both of which are dominant. It is formally possible that F^D is a dominant negative, or antimorphic, mutation of a gene that is activated by M and must be active for male development. We favor the simpler view that F^D is a constitutive allele of a gene F whose activity is needed for female sexual differentiation. We are currently testing our hypothesis by trying to produce null alleles of F^D which we predict will cause male development in the absence of M.

A single principle of sex determination in insects: Our experiments show that M acts early in embryogenesis to fix sexual development irreversibly in the male mode, and that in F^D females with an M background, F^D has to be present throughout larval development for female differentiation. These results support the hypothesis (NÖTHIGER and STEINMANN-ZWICKY 1985; INOUE and HIROYOSHI 1986) that M acts as an early signal in sex determination to repress a key gene, perhaps F, whose activity is continuously required for female development.

It has been postulated (NÖTHIGER and STEINMANN-ZWICKY 1985) that the seemingly different mechanisms of sex determination in insects may be based on a common strategy whereby an early acting primary signal irreversibly sets the state of activity of a key gene around blastoderm stage. In *Drosophila melanogaster*, the primary signal is given by the X:A ratio which regulates the key gene *Sxl* (*Sex-lethal*). A ratio

of 2*X*:2A activates an early promoter in the *Sxl* gene leading to functional SXL protein in females. No early transcription occurs in males with an *X*:A ratio of 0.5, and consequently no SXL protein is made. After the blastoderm stage, transcription switches to a late promoter which is active independently of the *X*:A ratio, producing the same pre-mRNAs in both sexes. In females, the preexisting SXL protein achieves splicing of the pre-mRNA in such a way that a functional protein can be made. This trans-activation initiates an autoregulatory loop which maintains a continuous production of SXL protein. In males which lack SXL protein, the pre-mRNA is spliced in the default mode resulting in a nonfunctional product. After the blastoderm stage, the early promoter is no longer responsive and cannot be activated any more even when the *X*:A ratio is experimentally made high. Constitutive mutations in *Sxl* (Sxl^M) lead to the production of functional protein independently of the *X*:A ratio (for key references see BELL *et al.* 1991; KEYES, CLINE and SCHEDL 1992).

Evidence presented here suggests that in Musca, *M* represents the primary signal, and *F* may represent the key gene functionally analogous to *Sxl* in Drosophila. F^D can then be understood as a dominant constitutive mutation of *F*, resembling the mutation Sxl^M of Drosophila, that is insensitive to the repressing action of *M*. Alternatively, F^D might also correspond to a constitutive mutation of *tra*, a gene acting downstream of *Sxl* and being controlled by *Sxl*. For example, the construct *hs-tra-female* expresses the female-specific *tra* product independently of the *X*:A ratio and *Sxl* and is thus able to transform *XY* animals into pseudofemales (MCKEOWN, BELOTE and BOGGS 1988). The demonstrated requirement for *F* activity throughout larval development is compatible with both views.

If the genetic activity of *F* in Musca is analogous to the activity of *Sxl* in Drosophila, one might expect the protein product of F^D to have a *trans*-activating effect on an F^+ allele, or rather its transcripts, present in the same cell. Our experiments suggest that this is not the case for Musca where the F^+ allele is apparently not *trans*-activated by an active F^D allele. This aspect of *F* is more reminiscent of *tra* than of *Sxl*. Thus, whereas a functional analogy exists between *F* and *Sxl* or *tra*, their evolutionary homology may not extend to the level of molecular mechanisms.

We wish to thank MARIA-GABRIELLA FRANCO and PIER-GIORGIO RUBINI of the University of Pavia and TOSHIKI HIROYOSHI of the University of Osaka for kindly supplying us with wild-type and mutant housefly stocks and for practical advice. We are grateful to MARIANA WOLFNER and MONICA STEINMANN-ZWICKY for their critical comments on the manuscript, and we greatly acknowledge the technical assistance by JENNIE SCHÖPFER-BONS, PIA MEIER-GERSCHWILER, ARIANE MINET and MARGRIT SCHMET. This work was supported by the Swiss National Science Foundation grant 31-27953.89 and by the Sandoz-Stiftung zur Förderung der medizinisch-biologischen Wissenschaften.

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Communicating editor: T. SCHÜPBACH