The Male-Determining Activity on the *Y* Chromosome of the Housefly (*Musca domestica* L.) Consists of Separable Elements

Monika Hediger,* Ariane Denise Minet,† Markus Niessen,* Regula Schmidt,* Denise Hilfiker-Kleiner,‡ Şükran Çakır,§ Rolf Nöthiger* and Andreas Dübendorfer*

**Institute of Zoology, University of Zürich-Irchel, CH-8057 Zürich, Switzerland, †Friedrich Miescher-Institute, CH-4058 Basel, Switzerland, ‡Institute of Molecular Cardiology, Medical University of Hannover, D-30625 Hannover, Germany and §Faculty of Veterinary Medicine, University of Ankara, Tr-06110 Ankara, Turkey*

Manuscript received February 24, 1998
Accepted for publication June 8, 1998

ABSTRACT

In the common housefly, the presence or absence of a male-determining factor, *M*, is responsible for sex determination. In different strains, *M* has been found on the *Y*, on the *X*, or on any of the five autosomes. By analyzing a *Y*-autosomal translocation and a ring-shaped, truncated *Y* chromosome, we could show that *M* on the *Y* consists of at least two regions with *M* activity: One of them can be assigned to the short arm of the *Y* chromosome (M^{YS}), which is largely C-banding negative, the other region lies on the C-banding positive long arm of the *Y*, including the centromeric part (M^{YL}). Each region alone behaves as a hypomorphic *M* factor, causing many carriers to develop as intersexes of the mosaic type instead of as males. When introduced into the female germ line by transplantation of progenitor germ cells (pole cells), the M^{YS} shows an almost complete maternal effect that predetermines 96% of the genotypic female (*NoM*) animals to develop as males. In contrast, the M^{YL} has largely lost its maternal effect, and most of the *NoM* animals develop as females. Increasing the amount of product made by either of the two hypomorphic *M* factors (by combining the M^{YS} and M^{YL} or two M^{YS}) leads to complete male development in almost every case. We thus assume that the *Y* chromosome carries at least two copies of *M*, and that these are functionally equivalent.

IN contrast to the well-established genetic hierarchy for somatic sex determination in *Drosophila melanogaster*, only a few components of this pathway are known in the housefly, *Musca domestica*. According to our current model, maleness is determined by a dominant factor *M*, which acts as the primary sex-determining signal to prevent activity of *F*, a gene needed for female sexual differentiation (Nöthiger and Steinmann-Zwicky 1985; Inoue *et al.* 1986; Hilfiker-Kleiner *et al.* 1993). In the absence of *M*, zygotic *F* is activated by maternally provided *F* product, leading to female differentiation (Hilfiker-Kleiner *et al.* 1994; Dübendorfer and Hediger 1998). The action of *F* is continuously required throughout development to maintain the cells on the female pathway (Hilfiker-Kleiner *et al.* 1993; Schmidt *et al.* 1997a). *M* can be overruled by an epistatic factor F^D ($F^{Dominant}$; Rubini 1967; Dübendorfer *et al.* 1992), which dictates female development even in the presence of up to three *M* factors (Rubini *et al.* 1972). F^D is therefore thought to be a constitutive allele of *F* that escapes the repressing action of *M*. The two recessive mutations F^{tra} [described as *transformer* (*tra*) by Inoue and Hiroyoshi 1986] and F^{man} [described as *masculinizer* (*man*) by Schmidt *et al.* 1997a] lead to male development in the absence of *M* and are assumed to be hypomorphic alleles of *F*.

The female determiner F^D was isolated independently from field populations of Japan (Inoue and Hiroyoshi 1982), Australia (McDonald *et al.* 1978), Fiji islands (Inoue and Hiroyoshi 1982), and Turkey (Ş. Çakir, unpublished results). It invariably maps to the same position on autosome *IV*, closely linked to the marker *Bald abdomen* (*Ba*). In contrast, *M* factors are found on the *Y* (M^Y; Hiroyoshi 1964; Rubini and Palenzona 1967), or on the *X* (M^X; Denholm *et al.* 1983), or on any of the five autosomes (M^A; Sullivan 1958; Wagoner 1969; Hiroyoshi and Inoue 1979; Inoue *et al.* 1986; Çakir 1996). The location of the *M* factors on different chromosomes could represent separately evolved sex-controlling elements randomly scattered over the genome, as proposed for *Chironomus thummi* (Kraemer and Schmidt 1993). Alternatively, they could be of common origin and rarely become transposed. This may be the case in *Megaselia scalaris* (Mainx 1966; Traut and Willhöft 1990) and was also suggested for *M. domestica* (Hiroyoshi 1964; Green 1980; Nöthiger and Steinmann-Zwicky 1985).

Corresponding author: A. Dübendorfer, Institute of Zoology, University of Zürich-Irchel, Winterthurerstrasse 190, CH-8057 Zürich, Switzerland. E-mail: andreas@zool.unizh.ch

All *M* factors tested so far (M^I, M^{II}, M^{III}, M^V, and M^Y) perform the same basic function: They prevent the activation of *F*. Moreover, they are able to perform this function not only in the soma, but also in the female germ line (Hilfiker-Kleiner *et al.* 1994; Schmidt *et al.* 1997b). When genetically male ($M/+$) progenitor germ cells (pole cells) are transplanted into female hosts, all donor-derived zygotes develop as males, even those with a female genotype (called *NoM* males because they carry no *M*). This masculinizing maternal effect of the *M* factors is interpreted to be the result of inactivation of *F* in the female germ line leading to oocytes that lack maternal *F* product, which renders the embryos unable to activate their zygotic *F*.

Although the qualitative properties of the *M* factors seem to be equal, there are variations in strength. M^Y, M^{III}, and M^V show strong effects in the soma as well as in the female germ line. M^I, on the other hand, has reduced somatic activity, resulting in some yolk protein production in the fat body of heterozygous fertile $M^I/+$ males, and a weak maternal effect. When M^I is brought into the female germ line, not all *NoM* animals develop as males, but some become intersexes or even functional females. The masculinizing effect of another *M* factor, M^{II}, is complete in the soma, but incomplete in the female germ line (Schmidt *et al.* 1997b). In fact, it seems that the somatic function of *M* and its effect in the female germ line are genetically separable. The mutation *Ag* (*Arrhenogenic*; Vanossi Este and Rovati 1982), presumably an allele of M^I (Schmidt *et al.* 1997b), has lost its somatic function, but retained much of its effect in the female germ line, such that heterozygous animals ($Ag/+$) are females that produce *NoM* males and some intersexes.

In the course of an X-ray mutagenesis designed to find translocations, we recovered three lines with truncated *Y* chromosomes. Two of the lines produced intersexes, indicating disturbance of the function of the M^Y factor. These three truncated chromosomes gave us the opportunity to map *M* on the *Y* chromosome by cytological means. Genetic mapping is not possible, because no mutations are known on the sex chromosomes. On the other hand, by analyzing the various translocation karyotypes and assessing the sexual development of their carriers, we gained insight into the organization of the *Y* chromosomal regions with *M* function.

MATERIALS AND METHODS

Animals and genotypes: Flies were reared as described by Schmidt *et al.* (1997a). Strains and their chromosomal rearrangements are symbolized following the conventions of Drosophila genetics (Ashburner 1989). The chromosomes are listed in the order *X*; *Y*; *I*; *II*; *III*; *IV*; *V*. Reciprocally translocated elements are termed *distal* (D) and *proximal* (P), dependent on whether they exclude or include the centromere.

Gene symbols and mutations were described by Milani (1967) and Hiroyoshi (1977), using the linkage group-karyotype correlation of Wagoner (1967). The autosomal markers in this study are *ac* (*ali curve*) and *Ag* (*Arrhenogenic*) on linkage group I; *ar* (*aristapedia*) on linkage group II; *bwb* (*brown body*) on linkage group III; *ye* (*yellow eyes*), *Ba* (*Bald abdomen*), F^D (dominant female determiner), and F^{man} on linkage group IV; and *snp* (*snip wings*, kindly provided by Dr. J. G. Scott, Cornell University) on linkage group V. A superscript (*X*, *Y*, or roman number) specifies the linkage group of the male determiner *M*, *e.g.*, M^{II} for male determiner on linkage group II.

Two strains with *XX-XY* sex determination (strains 1 and 2), two strains with autosomal sex determination (strains 3 and 4), and two strains with maternal sex determination (strains 5 and 6) were used: (1) *XX-XY*; $+/+$, (2) *XX-XY*; *ac*; *ar*; *bwb*; *ye*; *snp*, (3) *XX*; *ar*; *bwb*; $Ba+/+F^{man}$ (male development occurs by homozygosity of F^{man}, interpreted as a strong hypomorphic allele of *F* (Schmidt *et al.* 1997a), (4) *XX*; $M^{II}+/+$ *ar*; *bwb*; F^D $Ba/++$, (5) *XX*; $Ag/+$; *bwb*, and (6) *XX*; $Ag/+$; *ar*; *bwb*. Females of the genotype *XX*; $Ag/+$ are arrhenogenic, *i.e.*, they produce *NoM* males and intersexes. Daughters are obtained from *XX*; $+/+$ mothers that are also present in the strain (Vanossi Este and Rovati 1982).

Genetic screen for translocations: Male flies, carrying *M* on the *Y* chromosome (strain 1), were kept for 5 days at room temperature, fed with sugar water and milk powder, and then irradiated with 25 Gy using 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 cm. The irradiated males were mated with virgin females of the multimarked strain 2 for 2 days to make sure that only irradiated sperm was used for fertilization. The F_1 males were then individually backcrossed to virgin females of the multimarked strain 2. The F_2 was scored for pseudolinkage between autosomal markers and sex or for production of intersexual flies.

Preparation of mitotic chromosomes recovered from larval brains or adult gonads, and orcein staining were done according to Franco and Rubini (1966) and Rubini *et al.* (1980). The C-banding technique is described by El Agoze *et al.* (1992). Chromosomes were analyzed with phase contrast microscopy. Photographs were taken with a Zeiss axiophot microscope on Agfa 25 film (black/white) or Kodak ektachrome 64T film (color reversal).

Western blotting: The hemolymph of single flies, 4–5 days after emergence, was assayed for yolk proteins as described by Schmidt *et al.* (1997a).

Crosses (for genetic symbols see legend to Figure 1): *Cross 1—Animals with the Y^pII^D, ar^+ chromosome and one copy of zygotic F^+:* To produce animals carrying only the Y^pII^D, ar^+ without the II^PY^D chromosome, we crossed X/Y^pII^D, ar^+; II^PY^D/ar males of strain T(Y;II)2, ar^+ to X/X; ar/ar; $Ba+/+F^{man}$ females (strain 3). Among the offspring (F_1), one very weakly feminized intersex of the phenotype ar^+ Ba^+ was isolated. Analysis of its mitotic chromosomes revealed the absence of the II^PY^D chromosomes showing that this genotype was X/Y^pII^D, ar^+; ar/ar; $++/+F^{man}$. This intersex was backcrossed to virgins of strain 3. The phenotypically ar^+ Ba offspring (F_2) were X/Y^pII^D, ar^+; ar/ar and carried *Ba* in *trans* over F^+ or F^{man}. To determine the presence or absence of F^{man}, the two types of males were individually backcrossed to virgins of strain 3. In the resulting F_3, males of genotype X/Y^pII^D, ar^+; ar/ar; $Ba+/+F^{man}$ could be unambiguously identified. These males were now individually mated with virgin standard females (strain 2; Table 1, line 4). The cross yielded offspring with one copy of F^+, represented by the Ba^+ phenotype (genotype: X/Y^pII^D, ar^+; ar/ar; $++/+F^{man}$; Table 1, line 5), and offspring with two copies of F^+ recognized by their Ba phenotype (genotype: X/Y^pII^D, ar^+; ar/ar; $Ba+/++$; Table 1, line 4. For karyotype X/Y^pII^D, ar^+; ar/ar, see Figure 1E).

Cross 2—Animals with the R(YS) and one copy of zygotic F^+:

Females of the genotype *X/X*; *Ba + ye/+ F^man +* were crossed to *X/R(YS)*; *+ + ye/+ + ye* males. The resulting *X/R(YS)*; *+ F^man +/+ + ye* males were then crossed to virgin standard females (strain 2; Table 1, line 9). The offspring with the ye phenotype had two copies of zygotic F^+ (Table 1, line 9), whereas the ye^+ offspring had only one copy of zygotic F^+ (Table 1, line 10).

Cross 3—Animals with the Y^pII^D, ar^+ or R(YS) and reduced maternal F^+ product: Heterozygous $F^{man}/+$ females produce not only females but also some intersexes and males. Schmidt *et al.* (1997a) proposed that this masculinizing maternal effect is caused by the reduced amount of maternal F^+ product in the eggs. With increasing age of the $F^{man}/+$ mother, the number of intersexes and males increased drastically. We therefore analyzed only the progeny of the first clutch of eggs of every female. Females of the genotype *X/X*; *ar/ar*; *Ba +/+ F^man* were either crossed to *X/Y^pII^D*, *ar^+*; *II^pY^D/ar*; *+ +/+ +* males (Table 1, line 2) or to *X/R(YS)*; *ar/ar*; *+ +/+ +* males (Table 1, line 7). The number of intersexes and males carrying either of the two aberrant *Y* chromosomes was counted.

Cross 4—Animals with two R(YS) chromosomes: X/X; *ar/ar*; *F^D Ba/+ +* females (strain 4) were crossed to *X/R(YS)*; *ar/ar*; *+ +/+ +* males from strain R(YS)1. The F_1 females with the genotype *ar/ar*; *F^D Ba/+ +* carried either two *X* chromosomes or one *X* and the *R(YS)* chromosome. These F_1 females were separated as virgins and backcrossed individually to *X/R(YS)*; *ar/ar*; *+ +/+ +* males from strain R(YS)1 [or to *X/Y^pII^D*, *ar^+*; II^pY^D/ar; *+ +/+ +* males from strain T(Y;II)2, *ar^+*; see cross 5]. F_2 animals with two *R(YS)* chromosomes could arise only when the mother carried a *R(YS)* chromosome. Because we could not distinguish between the two different karyotypes of the mothers, the mitotic chromosomes of all F_2 animals were analyzed, and the number of *R(YS)/R(YS)*; *ar/ar*; *+ +/+ +* males and intersexes was counted (Figure 5, line 7).

Cross 5—Animals with one R(YS) and one Y^pII^D, ar^+ chromosome: The F_1 females of cross 4 were individually crossed to *X/Y^pII^D*, *ar^+*; *II^pY^D/ar*; *+ +/+ +* males from strain T(Y;II)2, *ar^+*. The two different karyotypes of the F_1 females could only be distinguished from their ar offspring. F_1 females with two *X* chromosomes produced only ar females (genotypes: *X/X*; *ar/ar*; *F^D Ba/+ +* or *X/X*; *ar/ar*; *+ +/+ +*), whereas the F_1 females with an *X* and a *R(YS)* chromosome also produced ar males (genotype: *X/R(YS)*; *ar/ar*; *+ +/+ +*). Only the progeny (F_2) of the latter F_1 females was further analyzed. Mitotic chromosome preparations were made of the phenotypically ar^+ offspring (F_2), which consisted of four different karyotypes of females [*X/Y^pII^D*, *ar^+*; *II^pY^D/ar*; *F^D Ba/+ +*, *X/Y^pII^D*, *ar^+*; *ar/ar*; *F^D Ba/+ +*, *R(YS)/Y^pII^D*, *ar^+*; *II^pY^D/ar*; *F^D Ba/+ +*, and *R(YS)/Y^pII^D*, *ar^+*; *ar/ar*; *F^D Ba/+ +*] and four different karyotypes of males [*X/Y^pII^D*, *ar^+*; *II^pY^D/ar*; *+ +/+ +* and *X/Y^pII^D*, *ar^+*; *ar/ar*; *+ +/+ +* (Figure 5, lines 1 and 2) or *R(YS)/Y^pII^D*, *ar^+*; *II^pY^D/ar*; *+ +/+ +* and *R(YS)/Y^pII^D*, *ar^+*; *ar/ar*; *+ +/+ +* (Figure 5, line 4)]. To check for the genotype *X/R(YS)*; II^pY^D/ar; *+ +/+ +*, we dissected some of the F_2 ar males and intersexes (Figure 5, lines 5 and 6).

Cross 6—Animals with two Y^pII^D, ar^+ chromosomes: Some of the ar^+ F_2 offspring of cross 5 were not dissected, but were crossed individually *inter se*. Mitotic chromosome preparations of the F_3 animals were checked for the presence of two *Y^pII^D*, *ar^+* chromosomes, with and without the II^pY^D chromosome (Figure 5, line 3).

Transplantation of pole cells: Using the technique described by Hilfiker-Kleiner *et al.* (1994) and Schmidt *et al.* (1997b), two transplantation series were done:

Series 1: The genotype of the host embryos was *X/X*; *+/+* or *X/Y*; *+/+* (strain 1), and the genotype of the donor embryos was *X/R(YS)*; *bwb/bwb* or *X/X*; *bwb/bwb*. Female hosts were crossed to *X/X*; *bwb/bwb NoM* males (of the *Ag* strain 5), so that donor-derived offspring could be recognized by their bwb phenotype.

Series 2: The genotype of the host embryos was *X/X*; *+/+* or *X/Y*; *+/+* (strain 1), and the genotype of the donor embryos was *X/Y^pII^D*, *ar^+*; *ar/ar*; *bwb/bwb* or *X/X*; *ar/ar*; *bwb/bwb*. Female hosts were crossed to *X/X*; *ar/ar*; *bwb/bwb NoM* males (of the *Ag* strain 6). This allowed us to recognize the donor-derived offspring by their bwb phenotype and to distingush between donor-derived *NoM* animals (ar bwb phenotype) and donor-derived carriers of the *Y^pII^D*, *ar^+* chromosome (ar^+ bwb phenotype).

RESULTS

Localization of M^Y by deletion mapping: In our screen for translocations, we obtained two lines that produced intersexual flies [strains R(YS)1 and T(Y;II)2, *ar^+*]. A third line showed pseudolinkage of the *Y* chromosome and autosome *II*, but did not produce sexually aberrant flies [strain T(Y;II)1]. Karyotype analysis of the males and intersexes from the three lines revealed that in each strain a different part of the *Y* chromosome is deleted (Figure 1). A schematic representation of the various karyotypes that occur in the three strains, together with their effect on sexual differentiation, is given in Figure 2. Animals with a *Y* chromosome from which the distal part of the long arm is missing [a situation that is represented by the aristapedia (ar) males of strain T(Y;II)1 in Figure 2B, line 2] still develop as perfect males. However, a *Y* chromosome that has lost the long arm [ring-*YS* chromosome of strain R(YS)1, Figure 2A] shows reduced *M* activity. This is not only evident from morphologically mosaic animals (Figure 3, A and D) but also from males that produce yolk proteins in their fat body (Figure 2A and Figure 3B, lanes 1, 3, and 4).

With a deletion of the short arm of the *Y*, the remaining long arm and centromeric region can be tested for *M* function. The results show that this chromosomal part also has some *M* activity. Most of the carriers of this *Y^pII^D*, *ar^+* chromosome, however, develop as sexual mosaics with large female parts (Figure 2C, line 2, and Figure 3C), and only 13% (110 out of 845) develop as perfect males (Figure 2C, line 2, ♂[b]). Interestingly, all animals that carried both elements (*Y^pII^D*, *ar^+* and II^pY^D) developed as perfect males (Figure 2C, line 1, ♂[a]). This not only confirms our result that the short arm of the *Y* harbors *M* function, but in addition indicates that the M^Y factor of *M. domestica* can be split into at least two functional parts: one on the short arm (M^{YS}) and one on the long arm (M^{YL}). This situation is shown in Figure 4. When combined, the two parts provide full *M* activity.

Function of M^{YS} and M^{YL} in the soma: The female areas of the sexually mosaic animals in strains R(YS)1 and T(Y;II)2, *ar^+* could either be due to insufficient *M* function of their chromosomes or to loss of the *M*-carrying chromosome. We tried to determine which of the two mechanisms caused mosaicism.

M is assumed to prevent activity of *F* in the zygote,

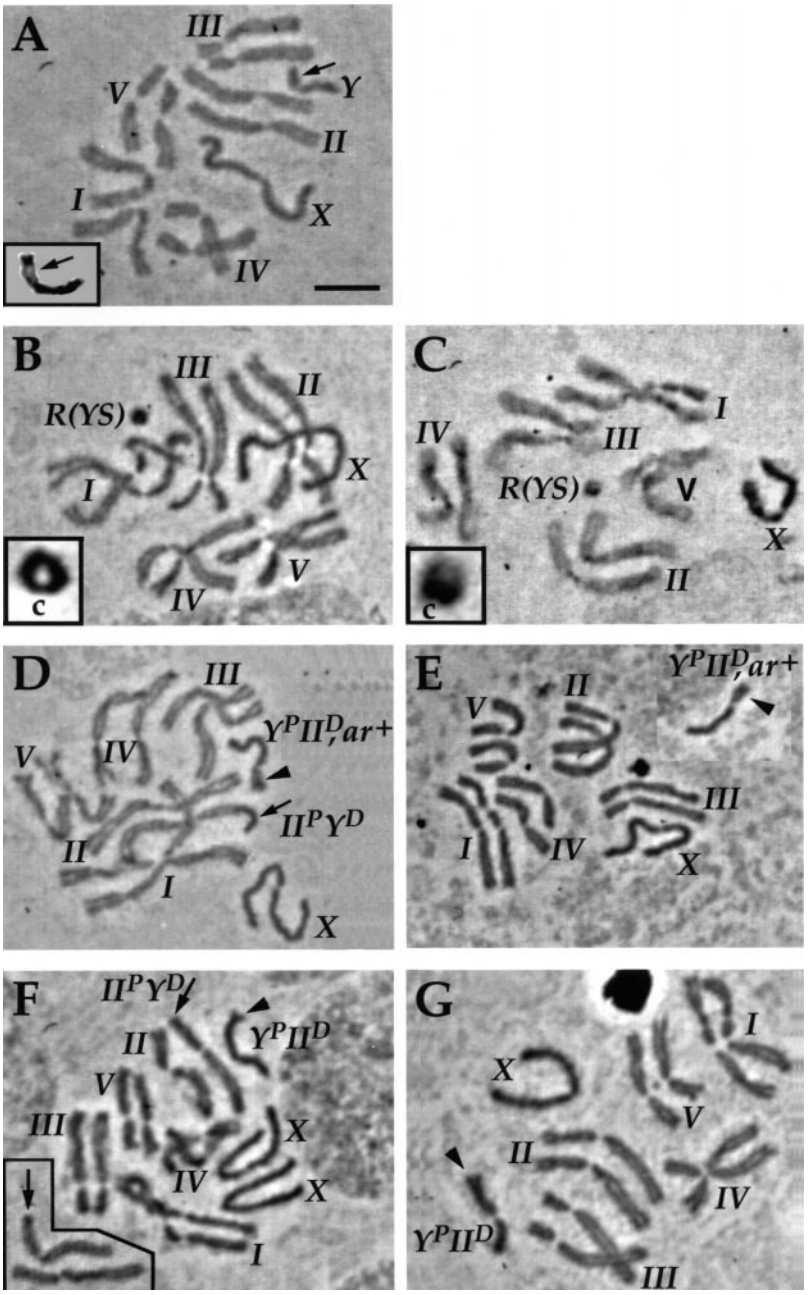

Figure 1.—Mitotic chromosome plates of squashed gonads: A, B, and D–G were orcein stained; C was C-banded. (A) Standard male; the *X* and the *Y* stain more strongly than the autosomes, revealing their heterochromatic character. The *Y* consists of a short (arrow) and a long arm. The two arms of the *Y* chromosome can be distinguished by their different C-banding pattern, shown in the inset: Most of the short arm is C-banding negative (arrow); the long arm and the centromeric region is C-banding positive (dark staining). (B) The truncated *Y* chromosome, present in males and intersexes of strain R(YS)1, forms a ring [*R(YS)* chromosome; see also Figure 2A]. A higher magnification (×4) of the ring-*YS* is given in the inset; c, centromere. (C) C-banding reveals that the ring-*YS* chromosome consists of the short arm (light staining) and the centromeric region (dark staining) of a wild-type *Y* chromosome. The long arm is missing. (D) Male of strain T(Y;II)2, ar^+ carrying a reciprocal translocation involving the *Y* chromosome and autosome *II*. The Y^PII^D, ar^+ chromosome consists of the long arm and the centromeric region of the wild-type *Y* and an attached part of autosome *II*, including the marker *aristapedia*$^+$ (ar^+). This autosomal part can easily be recognized by its light staining and the two spread chromatids (arrowhead). The short arm of the *Y* (arrow) is translocated to the proximal part of autosome *II*, giving rise to chromosome II^PY^D. (E) Intersex of strain T(Y;II)2, ar^+ carrying the Y^PII^D, ar^+ chromosome and two normal autosomes *II* and therefore trisomic for the translocated autosomal piece (arrowhead). (F) ar^+ males of strain T(Y;II)1 are heterozygous for the reciprocal translocation. The autosomal piece attached to the long arm of the *Y* (Y^PII^D chromosome) is indicated by an arrowhead and can be recognized by its v-shape (spread chromatids). The short piece of the long arm of the *Y* translocated to autosome *II* (II^PY^D chromosome; see also inset) can only be identified by the dark-paired tip on one homologue and not on the other (arrow). Shown is a male cell with an aneuploid (*XXY*) chromosomal complement. (G) Phenotypically ar males of strain T(Y;II)1 carry only the Y^PII^D chromosome (arrowhead) and have two normal autosomes *II*, which makes them trisomic for the translocated autosomal piece. Bar, 5 μm.

either by inactivating the maternally provided *F* product, which is an activator of zygotic *F* (Dübendorfer and Hediger 1998), or by repressing the zygotic *F* gene itself, or both. Reducing the amount of maternal *F* product, or the number of *F* alleles in the zygote, or both, should help weak *M* factors exert their function more efficiently. If intersexual development was due to loss of the *M* factor, no correlation between the number of F^+ copies and the incidence of sexual mosaicism should be seen. For these experiments, we used the recently isolated mutation F^{man}, which has properties of a strong hypomorphic allele of *F* (Schmidt *et al.* 1997a).

The results are summarized in Table 1. Reducing the maternally provided F^+ product resulted in a moderate masculinization, as shown by the decrease of intersexes and a corresponding increase of males among the animals that carried the Y^PII^D, ar^+ chromosome (compare lines 1 and 2) or the ring-*YS* chromosome (compare lines 6 and 7). This effect, however, hardly exceeded the expected maternal effect of F^{man} also exerted without any *M* factor in the zygote (Schmidt *et al.* 1997a). This indicates that a reduced amount of maternal F^+ product is not sufficient to intensify significantly the zygotic effect of M^{YL} (Y^PII^D, ar^+ chromosome) or M^{YS} (ring-*YS* chromosome). However, if the dose of F^+ is reduced in the zygote, the reduction of intersexes and

corresponding increase of males is very strong for M^{YL} (compare lines 4 and 5 of Table 1), but not for M^{YS} on the ring-*YS* chromosome (compare lines 9 and 10 of Table 1). This result shows that M^{YL} on the $Y^{P}II^{D}$, ar^{+} chromosome is a hypomorphic *M* factor whose efficiency strongly depends on the copy number of the assumed target gene *F* in the zygote. The absence of such an interaction in the case of M^{YS} on the ring-*YS* chromosome is compatible with loss of the ring-*YS*. Thus, we analyzed the karyotype of larval brain halves where the cells are still mitotically active. Among the adult flies carrying the ring-*YS* chromosome, 8.2% showed a left-right mosaicism in their interocular distance (Figure 3D). Extrapolating from gynandromorphs of *D. melanogaster*, in which the sex of the epidermis correlates with the sex of the underlying tissue, we expected some 8% of the ring-*YS* larvae to be left/right sexual mosaics inside the brain. We analyzed at least 10 metaphase cells from each of 116 brain halves and found that all of them were of the karyotype *X/R(YS)*. This suggests that sexual mosaicism in strain R(YS)1 is not due to chromosome loss, but rather to insufficient activity of *M*.

These results leave us with a puzzle: Why does the weaker of the two hypomorphic *M* factors (M^{YL}) show a dramatic response to a reduced copy number of F^{+} target genes, whereas the stronger M^{YS} does not respond (Table 1)? At present, we do not have enough information about *M* and *F* to offer a plausible and experimentally supported hypothesis.

Interaction of M^{YS} and M^{YL}: We further tested whether there is a qualitative difference between the products of the two M^{Y} activities. If both M^{YS} and M^{YL} were necessary to guarantee male development, only the combination between the chromosomes $II^{P}Y^{D}$ and $Y^{P}II^{D}$, ar^{+} or the chromosomes *R(YS)* and $Y^{P}II^{D}$, ar^{+} should lead to 100% males. The results are summarized in Figure 5. Doubling the dose of the M^{YS} activity on the ring-*YS* chromosome (column B, line 7) has essentially the same

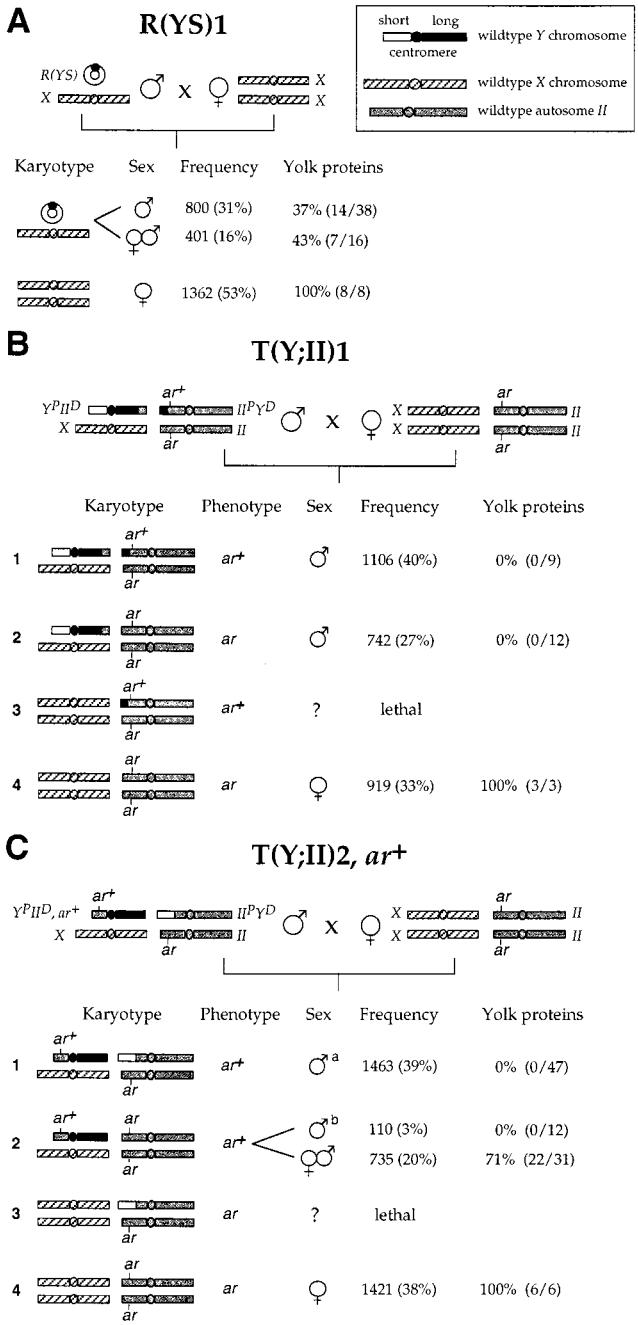

Figure 2.—Karyotypes and their corresponding phenotypes, frequencies, and *M* functions in strains R(YS)1 (A), T(Y;II)1 (B), and T(Y;II)2, ar^{+} (C). Sex was assessed according to morphological criteria. Karyotypes were defined by analyzing mitotic chromosome preparations. The box (upper right in A) shows a wild-type *Y* chromosome with its short arm in white and its centromeric region and the long arm in black. The *X* chromosomes are dashed. Autosomes *II* (gray) that are not involved in any translocation are marked with *aristapedia* (*ar*). The strains R(YS)1 (A) and T(Y;II)2, ar^{+} (C) produce intersexes of the mosaic type (⚥). In strain T(Y;II)2, ar^{+} (C), animals with the karyotypes shown in lines 1 and 2 cannot be distinguished phenotypically. To determine the frequencies of males and intersexes in the two groups, 55 intersexes and 59 males were chosen randomly and their mitotic chromosomes were analyzed. All intersexes carried only the $Y^{P}II^{D}$, ar^{+} chromosome (C, line 2). Among the 59 males, 4 (7%) had the same karyotype as the intersexes (C, line 2, $♂^{b}$), whereas the rest carried both parts of the translocation (C, line 1, $♂^{a}$). The frequencies of males given in lines 1 and 2 were extrapolated from these data. In the two translocation strains, T(Y;II)1 (B) and T(Y;II)2, ar^{+} (C), alternate and adjacent-1 segregation should theoretically lead to four different genotypes (lines 1–4). In both strains, however, there were twice as many males (plus intersexes) as females. Based on this observation and on the fact that all animals carrying two *X* chromosomes had also two normal autosomes *II*, we conclude that *X/X* animals monosomic for the translocated piece of autosome *II* are lethal. In addition, in both strains the animals with a partially trisomic genotype (B and C, line 2) were less frequent than their brothers with a balanced genotype (B and C, line 1). This may indicate that the partially trisomic animals are less viable.

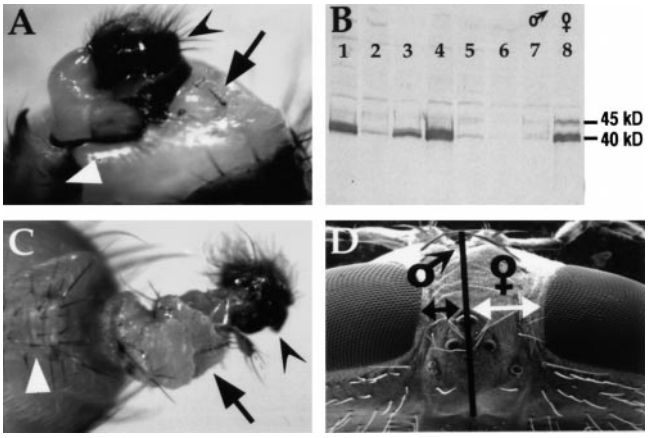

Figure 3.—(A) Weakly feminized sexual mosaic of strain R(YS)1 with a small female structure (black arrow: part of ovipositor) on the abdomen. Sternite 5 (white arrowhead) and genital apparatus (black arrowhead) are male. Weakly feminized mosaics with female areas restricted to one segment were found in strain R(YS)1 in ~90% (378/401) of the cases. (B) Test for yolk proteins in the hemolymph of morphological males of strain R(YS)1 (lanes 1–6) and of a control male (lane 7) and a control female (lane 8) of the multimarked strain 2. All males were fertile. (C) Strongly feminized sexual mosaic of strain T(Y;II)2, ar^+ with large female areas: female sternite 5 (white arrowhead) and an almost complete ovipositor (black arrow), but with a male genital apparatus (black arrowhead). In ~90% (216/243) of the mosaics of strain T(Y;II)2, ar^+, the female parts spanned two or more segments (strongly feminized mosaics). (D) Sexual mosaic of strain R(YS)1 with mosaic interocular distance.

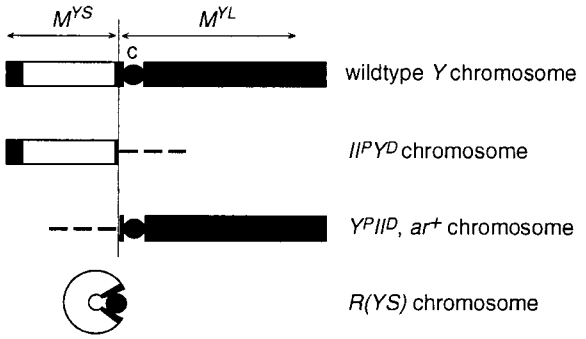

Figure 4.—Schematic drawing of a wild-type *Y* chromosome, the ring-*YS* chromosome of strain R(YS)1, the $Y^{P}II^{D}$, ar^+, and the $II^{P}Y^{D}$ chromosomes of strain T(Y;II)2, ar^+ (c, centromere of *Y*). Only the *Y*-chromosomal parts are shown (the positions of the attached parts of autosome *II* to the $Y^{P}II^{D}$, ar^+ and the $II^{P}Y^{D}$ chromosomes are indicated by broken lines). Both arms of the *Y* have *M* activity, but M^{YS} on the largely euchromatic part (white) has a stronger masculinizing effect than M^{YL}, which is located on the heterochromatic arm (black). The *M* activity of the $II^{P}Y^{D}$ chromosome alone cannot be tested because monosomy for II^{D} is lethal. Its masculinizing effect is only obvious in combination with the $Y^{P}II^{D}$, ar^+ chromosome (see Figure 5).

effect as combining the two original elements of the translocation, *i.e.*, the $Y^{P}II^{D}$, ar^+ and the $II^{P}Y^{D}$ chromosomes (column A, lines 1 and 2), indicating functional equivalence of M^{YS} and M^{YL}. Interestingly, when the ring-*YS* chromosome, which produces 48% males (column B, lines 5 and 6), and the $Y^{P}II^{D}$, ar^+ chromosome, which alone produces only 13% males (column B, line 2), are combined, the proportion of males rises to 95% (column B, line 4). This reveals an interaction between M^{YS} and M^{YL} that exceeds simple additivity.

Unfortunately, the combination of two $II^{P}Y^{D}$ chromosomes or two $Y^{P}II^{D}$, ar^+ chromosomes could not be tested because these karyotypes are lethal. In the first case, lethality is caused by deletion-homozygosity of the translocated part of autosome *II*, whereas in animals carrying two $Y^{P}II^{D}$, ar^+ chromosomes (column B, line 3), lethality is most probably due to the absence of the short arm of the *Y* (see below).

Function of M^{YS} and M^{YL} in the female germ line: All *M* factors tested so far (M^{Y}, M^{I}, M^{II}, M^{III}, and M^{V}; Schmidt *et al.* 1997b) have the same masculinizing maternal effect: They are active in the female germ line where they prevent the activity of *F*. As a result, insufficient amounts of maternal *F* product are deposited in the egg, which predetermines male development of all embryos, including those with a female genotype (*NoM* animals). *Ag*, assumed to be an allele of M^{I} based on its identical map position (Rovati *et al.* 1983; Schmidt *et al.* 1997b), has lost the somatic function, but has largely retained its function in the female germ line. This suggests that the somatic and the germ-line function of an *M* factor are genetically separable.

To analyze whether the spatially separated M^{YS} and M^{YL} also differ in their functions in the female germ line, we transplanted pole cells of the genotype *X/R(YS)* and $X/Y^{P}II^{D}$, ar^+ into wild-type female hosts and crossed these with *NoM* males of an *Ag* stock. The results are summarized in Table 2. The *M* activity of the ring-*YS* chromosome in the female germ line resembles that of the wild-type *Y* chromosome, producing almost purely male offspring. To determine the genotype of these males, we crossed 61 of them individually to standard females (*XX*). Of these, 33 generated sons, intersexes, and daughters, indicating that they were *X/R(YS)* heterozygotes, whereas 28 produced exclusively daughters, which identifies them as *NoM* males. In both types of males, yolk protein synthesis was completely repressed [13 *X/R(YS)* males and 14 *NoM* males tested]. Among the donor-derived offspring of two host females, there were 110 males, but also 11 females and 1 intersex. The presence of males among the offspring indicates that some of the transplanted pole cells were *X/R(YS)*. The exceptional females could have resulted either from an exceptionally weak maternal effect or from transplanted *X/0* pole cells of a donor embryo in which the ring-*YS* chromosome was lost during pole cell formation. If the latter was true, about half of these exceptional females should be *X/0*. The karyotypic analysis of the gonads revealed the presence of two *X* chromosomes in all

TABLE 1

Interactions between the *M* activity of the $Y^{p}II^{D}$, ar^{+} or *R(YS)* chromosome with varying amounts of maternally provided F^{+} products and different copy numbers of zygotic F^{+}

Genotype of mother	Genotype of father	F_1 carrying the $Y^{p}II^{D}$, ar^{+} or the *R(YS)* chromosome					
		Copies of F^{+}					
		Maternal	Zygotic	Genotype	Males (%)	Intersexes (%)	Total no.[a]
1 *XX*; *ar/ar*; + / +	*X/Y*^{p}*II*^{D}, *ar*+; *II*^{p}*Y*^{D}/*ar*; + / +	2	2	*X/Y*^{p}*II*^{D}, *ar*$^{+}$; ?/*ar*; + / +	75.9	24.1	282
2 *XX*; *ar/ar*; F^{man} / +	*X/Y*^{p}*II*^{D}, *ar*$^{+}$; *II*^{p}*Y*^{D}/*ar*; + / +	1	2	*X/Y*^{p}*II*^{D}, *ar*$^{+}$; ?/*ar*; + / +[b]	84.5	15.5	129
3		1	1	*X/Y*^{p}*II*^{D}, *ar*$^{+}$; ?/*ar*; F^{man} / +	97.6	2.4	122
4 *XX*; *ar/ar*; + / +	*X/Y*^{p}*II*^{D}, *ar*$^{+}$; *ar/ar*; F^{man} / +	2	2	*X/Y*^{p}*II*^{D}, *ar*$^{+}$ *ar/ar*; + / +[b]	9.2	90.8	653
5		2	1	*X/Y*^{p}*II*^{D}, *ar*$^{+}$; *ar/ar*; F^{man} / +	95.2	4.8	673
6 *XX*; *ar.ar*; + / +	*X/R(YS)*; *ar/ar*; + / +	2	2	*X/R(YS)*; *ar/ar*; + / +	68.5	31.5	950
7 *XX*; *ar/ar*; F^{man} / +	*X/R(YS)*; *ar/ar*; + / +	1	2	*X/R(YS)*; *ar/ar*; + / +[b]	79.7	20.3	565
8		1	1	*X/R(YS)*; *ar/ar*; F^{man} / +	76.0	24.0	640
9 *XX*; *ar/ar*; + / +	*X/R(YS)*; *ar/ar*; F^{man} / +	2	2	*X/R(YS)*; *ar/ar*; + / +[c]	62.6	37.4	484
10		2	1	*X/R(YS)*; *ar/ar*; F^{man} / +	67.0	33.0	612

For complete genotypes see materials and methods. (?) Represents either the normal autosome *II*, carrying the marker *ar*, or the $II^{p}Y^{D}$ chromosome. The difference in the proportion of F_1 males and F_1 intersexes between lines 1 and 4 is due to the different genotypes of the fathers: In line 1, the father carries—in addition to $Y^{p}II^{D}$, ar^{+}—also the $II^{p}Y^{D}$ chromosome. Most of the male progeny therefore again carry both parts of the *Y* chromosome (see also Figure 2C). In line 4, however, the father has only the $Y^{p}II^{D}$, ar^{+} chromosome and two normal autosomes *II*.

[a] F_1 females were not considered.

[b] Animals with two zygotic F^{+} genes were recognized by their Ba phenotype.

[c] Animals with two zygotic F^{+} genes were recognized by their ye phenotype.

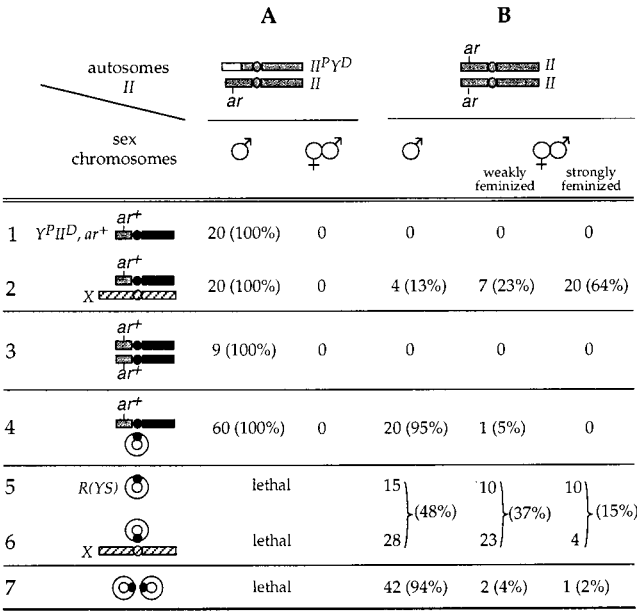

Figure 5.—Numbers (percentages) of animals carrying different combinations of the two aberrant chromosomes ring-*YS* and Y^PII^D, ar^+, either together with autosome II^PY^D (A) or together with two normal autosomes *II* (B). Animals carrying the ring-*YS* chromosome together with the II^PY^D chromosome (A, lines 5–7) are lethal due to their partial monosomy of autosome *II*. The animals were sexed according to their external and internal morphology. The degree of sexual mosaicism is given in the two classes: weakly feminized (female parts are restricted to one segment) and strongly feminized (female parts spanning two or more segments). The karyotype of the animals was determined in chromosome preparations of their germarium. For chromosome symbols see Figure 2.

11 females. This indicates that they resulted from an exceptionally weak maternal effect of the otherwise strongly masculinizing ring-*YS*, rather than from a loss of the ring-*YS* chromosome.

In contrast to the ring-*YS* chromosome, the M^{YL} activity of the Y^PII^D, ar^+ chromosome generally showed a very weak masculinizing effect in the female germ line. When X/Y^PII^D, ar^+; ar/ar germ cells were transplanted into wild-type female hosts, almost all *NoM* animals (recognized by their ar phenotype) developed as fertile females and only very rarely (6 out of 248; Table 2, series 2) into intersexes. This weak maternal effect was clearer in offspring that themselves carried the Y^PII^D, ar^+ chromosome (ar^+ phenotype): In such animals, the maternal effect increased the proportion of males from 13% (Figure 2C) to 65% (174 out of 269; Table 2, series 2). No yolk proteins could be detected in their hemolymph (19 ar^+ males tested).

These results show that the M^{YS} and M^{YL} factors, though at a reduced level, can still execute both functions attributed to a wild-type *M*. They are able to induce male development in the soma and they have a masculinizing maternal effect on the embryos. In both tissues, soma and germline, M^{YL} is much weaker than M^{YS}.

Localization of the viability factors: *M. domestica* requires at least one sex chromosome for viability (Milani 1964; Rubini 1964). In this respect, both heterosomes are equivalent, and *X0* and *Y0* animals are equally viable. This characteristic enabled us not only to localize M^Y by deletion mapping, but also to narrow down the region essential for viability.

With the occurrence of fertile males ($n = 15$), intersexes ($n = 20$), and females ($n = 4$; with F^D) carrying only the ring-*YS* chromosome and no *X* chromosome (Figure 5, column B, line 5; data of females not shown), we can assign the region indispensable for viability to the short arm of the *Y*. Animals carrying only the Y^PII^D, ar^+ chromosome, which represents the long arm of the *Y*, were never found (Figure 5, column B, line 1). This indicates that all vital genes must be located on the short

TABLE 2

***M* activity of the *R(YS)* and Y^PII^D, ar^+ chromosomes in the female germ line**

Series	Genetic sex of donor pole cells	No. of wild-type female hosts (*X/X*)	Adult female hosts were crossed to	No. of host-derived offspring[a]	No. of donor-derived offspring[a]		
				Females	Males	Intersexes	Females
1	*X/X*	8	*NoM* ♂[b]	699	0	2[c]	253
	X/R(YS)	7	*NoM* ♂[b]	1142/4[c]	628[d]	1[e]	11[e]
2	*X/X*	3	*NoM* ♂[f]	263	0	0	176
	X/Y^PII^D, ar^+; *ar/ar*	7	*NoM* ♂[f]	644	174[g]	95[g]/6[h]	242[h]

[a] Donor- and host-derived offspring were distinguished by brown body (bwb) color marker (see materials and methods).
[b] The *NoM* males (males with no *M*) were of the genotype *XX*; *Ag/+*; *bwb* or *XX*; *+/+*; *bwb*.
[c] Very weakly masculinized intersexes (mosaic type), which is due to the rare and weak zygotic effect of the paternally contributed *Ag*.
[d] Outcrosses with standard females showed that 50% of these males had a female genotype (were *NoM* males).
[e] Derived from only two out of the seven host females.
[f] The *NoM* males were of the genotype *XX*; *Ag/+*; *ar*; *bwb* or *XX*; *+/+*; *ar*; *bwb*.
[g] Males and intersexes were ar^+, indicating that they were carrier of the Y^PII^D, ar^+ chromosome.
[h] Animals were ar, indicating that they carried two *X* chromosomes and were therefore the *NoM* animals.

TABLE 3

Summary of the somatic and maternal effects of the various *M* factors

Group	*M* factor	*M* function in soma (carriers of *M*)		*M* function in germ line (*NoM* animals)	
		Phenotype (%)	Yolk proteins (%)	Phenotype (%)	Yolk proteins (%)
i	$M^{Y\,a}$	♂ 100	0	♂ 100	ND
	$M^{III\,b}$	♂ 100	0	♂ 100	ND
	$M^{V\,a}$	♂ 100	0	♂ 100	
ii	$M^{II\,a}$	♂ 100		♂ 98.3	0
		⚥ 0	0	⚥ 1.7	—
		♀ 0		♀ 0	
	$M^{I\,a}$	♂ 100	56	♂ 63.2	ND
		⚥ 0		⚥ 12.6	—
		♀ 0		♀ 24.2	—
	M^{YL}	♂ 6.8	0	♂ 0	
		⚥ 93.2	—	⚥ 2.4	—
		♀ 0		♀ 97.6	—
iii	Ag^{a}	♂ 0		♂ 99.2	0
		⚥ 0.2	—	⚥ 0.8	—
		♀ 99.8	—	♀ 0	
	M^{YS}	♂ 67	37	♂ 96.3	0
		⚥ 33	—	⚥ 0.3	—
		♀ 0		♀ 3.4	—

M function was measured by analyzing the sexual phenotype and yolk protein synthesis. Group i: Wild-type *M* function is shown by exclusively male development. Group ii: *M* factors with stronger activity in soma than in germ line. Group iii: *M* factors with stronger activity in germ line than in soma. —, data not of interest; ND, not determined.

[a] Schmidt *et al.* (1997b).

[b] Hilfiker-Kleiner *et al.* (1994).

arm of the *Y* that appears euchromatic when analyzed by C-banding (Hediger *et al.* 1998). Two similar regions are present on the *X* around the centromere (El Agoze *et al.* 1992) and these might be genetically equivalent to the short arm of the *Y*.

DISCUSSION

The *M* activity on the *Y* consists of two separable elements: The cytogenetic analysis of three aberrant *Y* chromosomes showed that in the housefly at least two regions contribute to the *M* activity of a wild-type *Y* chromosome. One could be assigned to the short arm of the *Y* chromosome (M^{YS}) and is represented by the $II^{P}Y^{D}$ chromosome of the translocation T(Y;II)2, ar^{+} and by the ring-*YS* chromosome of strain R(YS)1. The other is located on the long arm of the *Y* including the centromere (M^{YL}) and corresponds to the *M* activity found on the $Y^{P}II^{D}$, ar^{+} chromosome. When only one of these regions is present, the masculinizing activity is reduced so that many animals become intersexes instead of males. The mosaic type observed in these intersexes is expected when an ambiguous primary sex-determining signal acts transiently at approximately the blastoderm stage to establish the male pathway (Hilfiker-Kleiner *et al.* 1993). Some cells within an embryo respond to this ambiguous signal of M^{YS} or M^{YL} by turning *F* off, others respond by turning *F* on. The situation is similar to that of 2X:3A intersexes of *D. melanogaster* (Dobzhansky and Bridges 1928; Laugé 1968). In these animals, the X:A ratio of 0.67 produces an ambiguous primary signal that activates *Sex-lethal* (*Sxl*) in some cells, leading to female differentiation, and leaves *Sxl* inactive in other cells, directing them into male differentiation (Cline 1983, 1984).

The two factors M^{YS} and M^{YL} seem to be functionally equivalent: Both have male-determining qualities and are active in the soma as well as in the female germ line, though at a reduced level. To restore wild-type *M* activity, two M^{YS} are as efficient as the combination of M^{YS} and M^{YL}. This suggests that M^{YS} and M^{YL} are copies of an ancestral *M* factor. Because, however, M^{YS} and M^{YL} differ in the strength of their masculinizing activity as well as in their interaction with the proposed target gene *F*, the two copies are not identical.

Model of *M* activity in soma and female germ line: A characteristic of all *M* factors tested so far is their ability to prevent the activity of *F* not only in the soma but also in the female germ line. However, the various known *M* factors differ in their strength and tissue-specificity. Three classes can be distinguished (summary in Table 3): (i) *M* factors, such as M^{Y}, M^{III}, and M^{V}, that

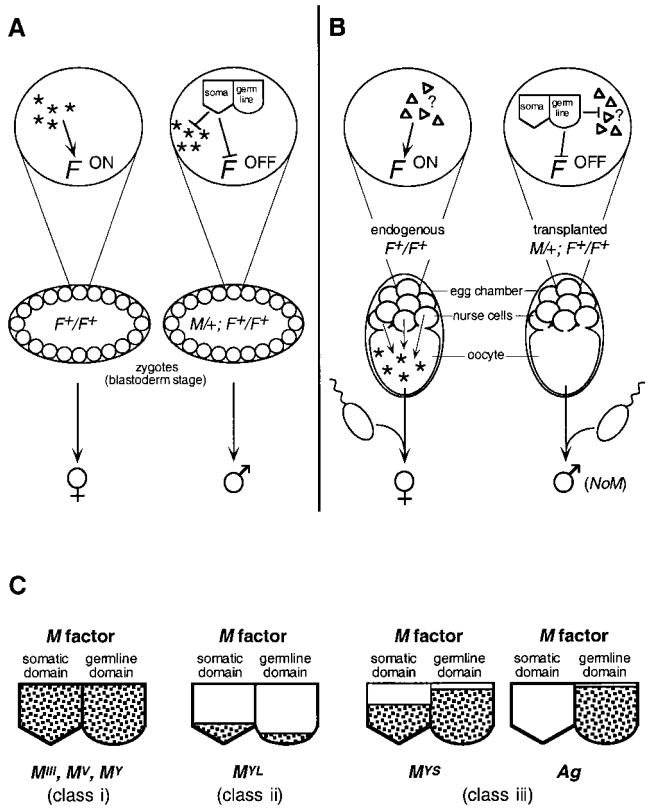

Figure 6.—Model of *M* activity in soma and female germ line. (A) Soma: At around blastoderm stage, sex is cell-autonomously determined. In zygotes without *M*, maternally provided *F* product (★) is necessary to activate the zygotic F^+ alleles, which results in female development. In heterozygous $M/+$ zygotes, *M* prevents the activation of *F*, either directly or by repressing the activating effect of the maternal product, or both. This leads to male development. (B) Female germ line: Endogenous F^+/F^+ and transplanted $M/+$ germ cells are induced by the surrounding soma to undergo oogenesis. In germ cells without *M*, the *F* gene is activated by an unknown mechanism (△), which leads to the deposition of *F* product (★) into the oocyte. This maternal *F* product is a prerequisite for the activation of the zygotic F^+ alleles after fertilization with a sperm without *M*. In $M/+$ germ cells, oogenesis takes place despite an active *M* that prevents the activation of *F*. As a consequence, no maternal *F* product is accumulated, and the oocyte is predetermined for male development, even if the zygote itself does not carry *M* (*NoM* males). (C) Schematic representation of the strength of the *M* effect (shaded area) in soma and germ line. In this model, *M* factors consist of two domains: The somatic domain prevents the activation of *F* in the soma, whereas the germline domain exerts the same function when introduced into the female germ line. In wild-type *M* factors (class i), the two domains have full activity. Other *M* factors show either a stronger reduction of *M* activity in the germ line (class ii) or in the soma (class iii).

exhibit strong *M* activity in both soma and female germ line and that are, therefore, considered functionally wild type (Hilfiker-Kleiner *et al.* 1994; Schmidt *et al.* 1997b); (ii) *M* factors, such as M^I, M^{II}, and M^{YL}, with stronger *M* activity in the soma than in the female germ line (Schmidt *et al.* 1997b; this article); and (iii) *M* factors, such as M^{YS} and *Ag*, that show reduced *M* activity in the soma, but have an almost complete masculinizing effect in the female germ line (Schmidt *et al.* 1997b; this article).

How could *F* be differently controlled by *M* in soma and germ line? In *D. melanogaster*, the key gene for sex determination, *Sxl*, is activated by different mechanisms in soma and germ line (Granadino *et al.* 1993; Steinmann-Zwicky 1993). Musca may well solve the problem similarly, using different mechanisms for the activation of *F* in the two tissues (Figure 6). In contrast to zygotic *F*, which is turned on by maternally provided *F* product (Hilfiker-Kleiner *et al.* 1994; Dübendorfer and Hediger 1998), the activating mechanism in the female germ line is unknown and may actually be different. To prevent the activation of *F* in both tissues, an *M* factor of Musca thus may consist of two domains, a somatic domain to prevent the activating function of the maternal *F* product (Figure 6A) and a germ-line domain to counteract the yet unknown germ-line activators of *F* (Figure 6B). In wild-type *M* factors (class i), both domains would exert full activity. *M* factors of class iii, however, would have a defective somatic domain, leading to reduced (M^{YS}) or absent (*Ag*) activity in the soma (Figure 6C). *M* factors of class ii would be the result of hypomorphic mutations that affect both activities (Figure 6C). Slightly reduced levels of *M* activity may first lead to an effect in the female germ line by the deposition of a reduced amount of *F* product into the oocyte, which will then cause sexually mosaic development of this zygote (as shown by M^{II}; Table 3). *M* factors with further decreased activity also affect the somatic development of their carriers, as manifested by the synthesis of yolk proteins even in morphologically normal and fertile males (M^I; Table 3). *M* factors with very little activity, such as M^{YL}, cause sexually mosaic development of almost all their carriers.

The model of two *M* domains responsible for keeping *F* inactive in the two tissues is compatible with our results. However, alternative models could also apply. The activity of *M* could itself be controlled by regulatory elements specific for soma and germ line, respectively, as discussed earlier by Schmidt *et al.* (1997b). Mutations in these elements could either affect the expression in the soma or in the germ line, or in both tissues. At present, we do not know the mechanisms that lead to different *M* activities in the two tissues. An answer to this question will come from molecular data on *M* and *F*.

We acknowledge the technical assistance of Raymond Grunder, Franziska Rzesnitzek, Gerda Saxer, and Hanna Nägeli. We thank Dr. T. S. Adams for his generous gift of antibodies, Dr. Daniel Bopp for fruitful discussions, and the referees for their comments and suggestions. Our work was supported by the Swiss National Science Foundation grant no. 31-45889.95 and by the Julius Klaus-Stiftung.

LITERATURE CITED

Ashburner, M., 1989 *Drosophila: A Laboratory Handbook.* Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

Çakir, Ş, 1996 The distribution of males having XY and XX chromosomes in housefly populations (Diptera: Muscidae) of Turkey. Genetica **98:** 205–210.

Cline, T. W., 1983 The interaction between *daughterless* and *Sex-lethal* in triploids: a lethal sex-transforming maternal effect linking sex determination and dosage compensation in *Drosophila melanogaster*. Dev. Biol. **95:** 260–274.

Cline, T. W., 1984 Autoregulatory functioning of a *Drosophila* gene product that establishes and maintains the sexually determined state. Genetics **107:** 231–277.

Denholm, I., M. G. Franco, P. G. Rubini and M. Vecchi, 1983 Identification of a male determinant on the X chromosome of housefly (*Musca domestica*) populations in south-east England. Genet. Res. **42:** 311–322.

Dobzhansky, T., and C. B. Bridges, 1928 The reproductive system of triploid intersexes in *Drosophila melanogaster*. Am. Nat. **62:** 425–434.

Dübendorfer, A., and M. Hediger, 1998 The female-determining gene *F* of the housefly, *Musca domestica*, acts maternally to regulate its own zygotic activity. Genetics **150:** 221–226.

Dübendorfer, A., D. Hilfiker-Kleiner and R. Nöthiger, 1992 Sex determination mechanisms in dipteran insects: the case of *Musca domestica*. Dev. Biol. **3:** 349–356.

El Agoze, M., F. Lemeunier and G. Periquet, 1992 Mitotic and salivary gland chromosome analysis in the *Musca domestica* L. (house fly) (Diptera: Muscidae). Heredity **69:** 57–64.

Franco, M. G., and P. G. Rubini, 1966 Genetics of *Musca domestica* L. Mutants, inheritance and cytology. Symp. Genet. Biol. Ital. **13:** 393–453.

Granadino, B., P. Santamaria and L. Sánchez, 1993 Sex determination in the germ line of *Drosophila melanogaster*: activation of the gene *Sex-lethal*. Development **118:** 813–816.

Green, M. M., 1980 Transposable elements in *Drosophila* and other diptera. Annu. Rev. Genet. **14:** 109–120.

Hediger, M., M. Niessen, J. Müller-Navia, R. Nöthiger and A. Dübendorfer 1998 Distribution of heterochromatin on the mitotic chromosomes of *Musca domestica* L. in relation to the activity of male-determining factors. Chromosoma (in press).

Hilfiker-Kleiner, D., A. Dübendorfer, A. Hilfiker and R. Nöthiger, 1993 Developmental analysis of two sex-determining genes, *M* and *F*, in the housefly, *Musca domestica*. Genetics **134:** 1187–1194.

Hilfiker-Kleiner, D., A. Dübendorfer, A. Hilfiker and R. Nöthiger, 1994 Genetic control of sex determination in the germ line and soma of the housefly, *Musca domestica*. Development **120:** 2531–2538.

Hiroyoshi, T., 1964 Sex-limited inheritance and abnormal sex ratio in strains of the housefly. Genetics **50:** 373–385.

Hiroyoshi, T., 1977 Some new mutants and revised linkage maps of the housefly, *Musca domestica* L. Jpn. J. Genet. **52:** 275–288.

Hiroyoshi, T., and H. Inoue, 1979 On the I^M chromosome of the housefly. Jpn. J. Genet. **54:** 434.

Inoue, H., and T. Hiroyoshi, 1982 A male-determining factor of autosome I and occurrence of male-recombination in the housefly, *Musca domestica* L. Jpn. J. Genet. **57:** 221–229.

Inoue, H., and T. Hiroyoshi, 1986 A maternal-effect sex-transformation mutant of the house fly, *Musca domestica* L. Genetics **112:** 469–482.

Inoue, H., T. Tomita and T. Hiroyoshi, 1986 Location of fourth chromosomal male-determining factors of the housefly, *Musca domestica*. Jpn. J. Genet. **61:** 119–126.

Kraemer, C., and E. R. Schmidt, 1993 The sex determining region of *Chironomus thummi* is associated with highly repetitive DNA and transposable elements. Chromosoma **102:** 553–562.

Laugé, G., 1968 Morphologie comparée de la région génitale des intersexués triploïdes de *Drosophila melanogaster*. Ann. Soc. Entomol. Fr. **4:** 481–499.

Mainx, F., 1966 Die Geschlechtsbestimmung bei *Megaselia scalaris* Loew (Phoridae). Z. Vererbungsl. **98:** 49–60.

McDonald, I. C., P. Evenson, C. A. Nickel and O. A. Johnson, 1978 House fly genetics: Isolation of a female determining factor on chromosome 4. Ann. Entomol. Soc. Am. **71:** 692–694.

Milani, R., 1964 Citologia della mosca domestica (*Musca domestica* L.). Quad. Ricerca Scientifica **25:** 111–116.

Milani, R., 1967 The genetics of *Musca domestica* L. and other muscoid flies, pp. 315–369 in *Genetics of Insect Vectors of Disease*, edited by J. W. Wright and R. Pal. Elsevier, Amsterdam.

Nöthiger, R., and M. Steinmann-Zwicky, 1985 A single principle for sex determination in insects. Cold Spring Harbor Symp. Quant. Biol. **50:** 615–621.

Rovati, C., S. Vanossi Este, L. Cima and R. Milani, 1983 Recombination rates of the loci *MI*, *Ag*, *ac*, and *Mdh* (1^{st} CHR.) of *Musca domestica* L. Atti Congr. Naz. It. Entomol. **13:** 50–56.

Rubini, P. G., 1964 Polimorfismo cromosomico in *Musca domestica* L. Boll. Zool. **31:** 679–694.

Rubini, P. G., 1967 Ulteriori osservazioni sui determinanti sessuali di *Musca domestica* L. Genet. Agr. **21:** 363–384.

Rubini, P. G., and D. Palenzona, 1967 Response to selection for high number of heterochromosomes in *Musca domestica* L. L. Genet. Agr. **21:** 101–110.

Rubini, P. G., M. G. Franco and S. Vanossi Este, 1972 Polymorphisms for heterosomes and autosomal sex-determinants in *Musca domestica* L. Atti IX Congr. Naz. It. Entomol. **9:** 341–352.

Rubini, P. G., M. Vecchi and M. G. Franco, 1980 Mitotic recombination in *Musca domestica* L. and its influence on mosaicism, gynandromorphism and recombination in males. Genet. Res. **35:** 121–130.

Schmidt, R., M. Hediger, R. Nöthiger and A. Dübendorfer, 1997a The mutation *masculinizer* (*man*) defines a sex-determining gene with maternal and zygotic functions in *Musca domestica* L. Genetics **145:** 173–183.

Schmidt, R., M. Hediger, S. Roth, R. Nöthiger and A. Dübendorfer, 1997b The *Y*-chromosomal and autosomal male-determining *M* factors of *Musca domestica* are equivalent. Genetics **147:** 271–280.

Steinmann-Zwicky, M., 1993 Sex determination in *Drosophila*: *sis-b*, a major numerator element of the X:A ratio in the soma, does not contribute to the X:A ratio in the germ line. Development **117:** 763–767.

Sullivan, R. L., 1958 Sex limitation of several loci in the housefly. Proc. 10th Int. Congr. Genet. **2:** 282.

Traut, W., and U. Willhöft, 1990 A jumping sex determining factor in the fly *Megaselia scalaris*. Chromosoma **99:** 407–412.

Vanossi Este, S., and C. Rovati, 1982 Inheritance of the arrhenogenic factor *Ag* of *Musca domestica* L. Boll. Zool. **49:** 269–278.

Wagoner, D. E., 1967 Linkage group-karyotype correlation in the house fly determined by cytological analysis of X-ray induced translocations. Genetics **57:** 729–739.

Wagoner, D. E., 1969 Presence of male determining factors found on three autosomes in the housefly, *Musca domestica* L. Nature **223:** 187–188.

Communicating editor: T. Schüpbach