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

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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\textsuperscript{1Y}), 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\textsuperscript{2Y}). 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\textsuperscript{1Y} shows an almost complete maternal effect that predetermines 96% of the genotypic female (NoM) animals to develop as males. In contrast, the M\textsuperscript{2Y} 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\textsuperscript{1Y} and M\textsuperscript{2Y} or two M\textsuperscript{1Y}) 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\textsuperscript{D} (F\textsuperscript{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\textsuperscript{D} is therefore thought to be a constitutive allele of F that escapes the repressing action of M. The two recessive mutations F\textsuperscript{D} [described as transformer (tra) by Inoue and Hiroyoshi 1986] and F\textsuperscript{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\textsuperscript{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 (Çakir, unpublished results). It invariably maps to the same position on an X chromosome, closely linked to the marker Bald abdomen (Ba). In contrast, M factors are found on the Y (M\textsuperscript{Y}; Hiroyoshi 1964; Rubini and Palenzo 1967), or on the X (M\textsuperscript{1X}; Denholm et al. 1983), or on any of the five autosomes (M\textsuperscript{1A}; Sullivan 1958; Wagner 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 (Mainix 1966; Traut and Willhöft 1990) and was also suggested for M. domestica (Hiroyoshi 1964; Green 1980; Nöthiger and Steinmann-Zwicky 1985).
All M factors tested so far (M\(^{1}\), M\(^{2}\), M\(^{3}\), M\(^{4}\), and M\(^{5}\)) 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 (Hilker-Klier 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\(^{1}\), M\(^{3}\), and M\(^{5}\) show strong effects in the soma as well as in the female germ line. M\(^{1}\), on the other hand, has reduced somatic activity, resulting in some yolk protein production in the fat body of heterozygous fertile M\(^{1}\)/+ males, and a weak maternal effect. When M\(^{1}\) 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\(^{2}\), 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 Est e and Rovati 1982), presumably an allele of M\(^{1}\) (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 disruption of the function of the M\(^{1}\) 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; 1; 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 (all 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\(^{arr}\) on linkage group IV; and snp (snp 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\(^{X}\) 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\(^{arr}\) (male development occurs by homoeogygosi of F\(^{arr}\), interpreted as a strong hypomorphic allele of F (Schmidt et al. 1997a); (4) XX; M\(^{f}\)/+ + 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 Est e 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(II)\(^{ar}\), ar\(^{r}\) chromosome and one copy of zygotic F: To produce animals carrying only the Y(II)\(^{ar}\), ar\(^{r}\) without the Y\(^{ar}\) chromosome, we crossed X(Y;II)\(^{ar}\), ar\(^{r}\); II(Y\(^{ar}\))/ar\(^{r}\) males of strain T(Y;II)\(^{2}\), ar\(^{r}\) to X/X; ar\(^{r}\); Ba +/+ + F\(^{arr}\) females (strain 3). Among the offspring (F\(_{1}\)), one very weakly femi-

**Cross 2—** Animals with the R(YS) and one copy of zygotic F.
Females of the genotype X/X; 8a + ye + F

were crossed to X/R(Y); + + ye + + ye males. The resulting X/R(Y); + F

were crossed to X/Y(Y); ar + + ye + ye males were then crossed to virgin standard (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/Y, ar or R(YS) and reduced maternal F product: Heterozygous F

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

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; 8a + + + F

were crossed either to X/Y(Y); ar/ ar; + + + + + males (Table 1, line 2) or to X/R(Y); 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

Ba/ + + females (strain 4) were crossed to X/R(YS); ar/ ar; + + + + + males from strain R(YS) 1. The F

females with the genotype ar/ ar; F

Ba/ + + carried either two X chromosomes or one X and the R(YS) chromosome. These F

females were separated as virgins and backcrossed individually to X/R(YS); ar/ ar; + + + + + males from strain R(YS) 1 [or to X/Y(Y); ar/ ar; + + + + + males from strain T(Y;II) 2; see cross 5]. F

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

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/Y, ar chromosome: The F

females of cross 4 were individually crossed to X/Y(Y); ar/ ar; + + + + + males from strain T(Y;II) 2, ar/ ar. These F

females could only be distinguished from their ar offspring, F

females with two X chromosomes produced only females (genotypes: X/X; ar/ ar; F

Ba/ + + or X/X; ar/ ar; + + + + + ), whereas the F

females with an X and a R(YS) chromosome also produced males (genotype: X/R(YS); ar/ ar; + + + + + ). Only the progeny (F
)

of the latter F

females was further analyzed. Mitotic chromosome preparations were made of the phenotypically ar offspring (F
), which consisted of four different karyotypes of females [X/Y(Y); ar/ ar; F

Ba/ + +; X/Y(Y); ar/ ar; F

Ba/ + +; R(YS)/Y(Y); ar/ ar; + + + + +; R(YS)/Y(Y); ar/ ar; + + + + +; and R(YS)/Y(Y); ar/ ar; F

Ba/ + +]. Four different karyotypes of males [X/Y(Y); ar/ ar; + + + + +; II/Y(Y); ar/ ar; + + + + +; and R(YS)/Y(Y); ar/ ar; + + + + +] and the F

males from strain R(YS) 1 were tested for the presence of two Y/Y, ar chromosomes, with and without the II/Y(Y) chromosome (Figure 5, line 5).

Cross 6—Animals with two Y/Y, ar chromosome: Some of the ar + F

offspring of cross 5 were not dissected, but were crossed individually to ar females. Mitotic chromosome preparations of the F

animals were checked for the presence of two Y/Y, ar chromosomes, with and without the Y/Y chromosome (Figure 5, line 3).

Transplantation of pole cells: Using the technique described by H. H. Köller-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 embryo was X/X/Y(Y); 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 distinguish between donor-derived NoM animals (ar bwb phenotype) and donor-derived carriers of the Y/Y, ar chromosome (ar bwb phenotype).

RESULTS

Localization of M 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/Y, 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, δ0). Interestingly, all animals that carried both elements (Y/Y, ar and II/Y(Y)) developed as perfect males (Figure 2C, line 1, δ0). This not only confirms our result that the short arm of the Y harbors M function, but in addition indicates that the M factor of M. domestica can be split into at least two functional parts: one on the short arm (M Y) and one on the long arm (M Y).

Function of M* and M‡ 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,
either by inactivating the maternally provided $F$ product, which is an activator of zygotic $F$ (Dübdorfer 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 $F^{\text{mas}}$, 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 $Y^{\text{II}}P$, $ar^{+}$ 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 $F^{+}$ 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 $M^{15}$ ($Y^{\text{II}}P$, $ar^{+}$ chromosome) or $M^{15}$ (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 $M_{Y}$ (compare lines 4 and 5 of Table 1), but not for $M_{Y}$ on the ring-YS chromosome (compare lines 9 and 10 of Table 1). This result shows that $M_{Y}$ on the $Y^{PIID}$, $ar^{+}$ 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 $MY$ activities. If both $M_{YS}$ and $M_{YL}$ were necessary to guarantee male development, only the combination between the chromosomes $IIPYD$ and $YS$, $ar^{+}$ or the chromosomes R(YS) and $Y^{PIID}$, $ar^{+}$ 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
the egg, which predetermines male development of all should be
cient amounts of maternal
where they prevent the activity of
\[F\].
\[X\]ternal effect: They are active in the female germ line
\[MYS\]omes (column A, lines 1 and 2), indicating functional and
umn B, lines 5 and 6), and the
\[YPIID\]i.e. which alone produces only 13% males (column B, line
the wild-type
\[MYS\] (column B, line 4). This reveals an interaction between males, we crossed 61 of them individually to standard
2), are combined, the proportion of males rises to 95% male offspring. To determine the genotype of these
feminized mosaics). (D) Sexual mosaic of strain R(YS)1 with largely retained its function in the female germ line.
mosaic interocular distance. This suggests that the somatic and the germ-line func-

In
\[z\]z
\[3.Ð(A) Weakly feminized sexual mosaic of strain (the positions of the attached parts of autosome
\[X\]short arm of the
\[3), lethality is most probably due to the absence of the were 110 males, but also 11 females and 1 intersex. The
\[YPIID\], a r
\[car\]rying two
\[II\]translocated part of autosome
\[3], lethality is caused by deletion-homozygosity of the males, yolk protein synthesis was completely repressed
\[tested because these karyotypes are lethal. In the ®rst which identi®es them as
\[MYS\]function, but has largely retained its function in the female germ line. This suggests that the somatic and the germ-line func-

Unfortunately, the combination of two \[\Pi Y^0\] chromosomes or two \[\Pi Y^1\], \[\text{ar}^+\] 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 \[\Pi\], whereas in animals carrying two \[\Pi Y^1\], \[\text{ar}^+\] chromosomes (column B, line 3), lethality is most probably due to the absence of the short arm of the \[Y\] (see below).

Function of \[M^{15}\] and \[M^{11}\] in the female germ line: All 
\[M\] factors tested so far (\[M^1\], \[M^3\], \[M^2\], \[M^4\], and \[M^9\];
\[Schmidt\] et al. 1997b) have the same masculinizing ma-
eral 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 \[M^1\] 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 func-

To determine whether the spatially separated \[M^{15}\] and \[M^{11}\] also differ in their functions in the female germ line, we transplanted pole cells of the genotype \[X/ R(YS)\] and \[X/ Y^1\], \[\text{ar}^+\] 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

**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 \(\sim90\%\) (378/ 401) of the cases. (B) Test for yolk proteins in the hemolymph of morphologically 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, \[\text{ar}^+\] 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 \(\sim90\%\) (216/243) of the mosaics of strain T(Y;II)2, \[\text{ar}^+\], 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 \[\Pi Y^1\], \[\text{ar}^+\], and the \[\Pi Y^0\] chromosomes of strain T(Y;II)2, \[\text{ar}^+\] (c, centromere of \[Y\]). Only the \[Y\] chromosomal parts are shown (the positions of the attached parts of autosome \[\Pi\] to the \[\Pi Y^1\], \[\text{ar}^+\] and the \[\Pi Y^0\] chromosomes are indicated by broken lines). Both arms of the \[Y\] have \[M\] activity, but \[M^{15}\] on the largely euchromatic part (white) has a stronger masculinizing effect than \[M^{11}\], which is located on the heterochromatic arm (black). The \[M\] activity of the \[\Pi Y^0\] chromosome alone cannot be tested because monosomy for \[\Pi Y^0\] is lethal. Its masculinizing effect is only obvious in combination with the \[\Pi Y^1\], \[\text{ar}^+\] chromosome (see Figure 3).
TABLE 1

Interactions between the $M$ activity of the $Y^{II^0}$, $ar^+$ or $R(YS)$ chromosome with varying amounts of maternally provided $F^+$ products and different copy numbers of zygotic $F^+$

<table>
<thead>
<tr>
<th>Genotype of mother</th>
<th>Genotype of father</th>
<th>Copies of $F^+$</th>
<th>Genotype</th>
<th>Males (%)</th>
<th>Intersexes (%)</th>
<th>Total no.*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Maternal</td>
<td>Zygotic</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 XX; ar/ar; +/+</td>
<td>X/Y$^{II^0}$, ar+$;$ II$^0$Y$ar$; +/+</td>
<td>2 2</td>
<td>X/Y$^{II^0}$, ar$^+$; ?/ar; +/+</td>
<td>75.9</td>
<td>24.1</td>
<td>282</td>
</tr>
<tr>
<td>2 XX; ar/ar; $F^{mm}/+$</td>
<td>X/Y$^{II^0}$, ar$^+$; II$^0$Y$ar$; +/+</td>
<td>1 2</td>
<td>X/Y$^{II^0}$, ar$^+$; ?/ar; +/+</td>
<td>84.5</td>
<td>15.5</td>
<td>129</td>
</tr>
<tr>
<td>3</td>
<td>X/Y$^{II^0}$, ar$^+$; ar/ar; $F^{mm}/+$</td>
<td>1 1</td>
<td>X/Y$^{II^0}$, ar$^+$; ?/ar; $F^{mm}/+$</td>
<td>97.6</td>
<td>2.4</td>
<td>122</td>
</tr>
<tr>
<td>4 XX; ar/ar; +/+</td>
<td>X/Y$^{II^0}$, ar$^+$; ar/ar; $F^{mm}/+$</td>
<td>2 2</td>
<td>X/Y$^{II^0}$, ar$^+$; ar/ar; +/+</td>
<td>9.2</td>
<td>90.8</td>
<td>653</td>
</tr>
<tr>
<td>5</td>
<td>X/Y$^{II^0}$, ar$^+$; ar/ar; $F^{mm}/+$</td>
<td>2 1</td>
<td>X/Y$^{II^0}$, ar$^+$; ar/ar; +/+</td>
<td>95.2</td>
<td>4.8</td>
<td>673</td>
</tr>
<tr>
<td>6 XX; ar/ar; +/+</td>
<td>X/R(YS); ar/ar; +/+</td>
<td>2 2</td>
<td>X/R(YS); ar/ar; +/+</td>
<td>68.5</td>
<td>31.5</td>
<td>950</td>
</tr>
<tr>
<td>7 XX; ar/ar; $F^{mm}/+$</td>
<td>X/R(YS); ar/ar; +/+</td>
<td>1 2</td>
<td>X/R(YS); ar/ar; +/+</td>
<td>79.7</td>
<td>20.3</td>
<td>565</td>
</tr>
<tr>
<td>8</td>
<td>X/R(YS); ar/ar; $F^{mm}/+$</td>
<td>1 1</td>
<td>X/R(YS); ar/ar; $F^{mm}/+$</td>
<td>76.0</td>
<td>24.0</td>
<td>640</td>
</tr>
<tr>
<td>9 XX; ar/ar; +/+</td>
<td>X/R(YS); ar/ar; $F^{mm}/+$</td>
<td>2 2</td>
<td>X/R(YS); ar/ar; +/+</td>
<td>62.6</td>
<td>37.4</td>
<td>484</td>
</tr>
<tr>
<td>10</td>
<td>X/R(YS); ar/ar; $F^{mm}/+$</td>
<td>2 1</td>
<td>X/R(YS); ar/ar; +/+</td>
<td>67.0</td>
<td>33.0</td>
<td>612</td>
</tr>
</tbody>
</table>

For complete genotypes see materials and methods. (?) Represents either the normal autosome II, carrying the marker ar, or the II$^0$Y$^{II^0}$ chromosome. The difference in the proportion of F1 males and F1 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$^{III}$, $ar^+$—also the II$^0$Y$^{II^0}$ 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$^{III}$, $ar^+$ chromosome and two normal autosomes II.

*a F1 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.

Male—Determined $M$ on the Y of Musca
of the ring-YS chromosome, the M^{\text{YS}} activity of the Y^{\text{II}}^{a}, ar^{+} chromosome generally showed a very weak masculinizing effect in the female germ line. When X/Y^{\text{II}}^{a}, 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^{\text{II}}^{a}, 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^{\text{YS}} and M^{\text{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^{\text{YL}} is much weaker than M^{\text{YS}}.

**Localization of the viability factors:** M. domestica requires at least one sex chromosome for viability (Millani 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^{\text{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 F0) 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^{\text{II}}^{a}, 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 arm of the Y chromosome, the Y0 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^{\text{YL}} activity of the Y^{\text{II}}^{a}, ar^{+} chromosome generally showed a very weak masculinizing effect in the female germ line. When X/Y^{\text{II}}^{a}, 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^{\text{II}}^{a}, 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).

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**TABLE 2**

<table>
<thead>
<tr>
<th>Series</th>
<th>Genetic sex of donor pole cells</th>
<th>No. of wild-type female hosts (X/X)</th>
<th>Adult female hosts were crossed to</th>
<th>No. of host-derived offspring</th>
<th>No. of donor-derived offspring</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Males</td>
<td>Intersexes</td>
</tr>
<tr>
<td>1</td>
<td>X/X</td>
<td>8</td>
<td>NoM</td>
<td>699</td>
<td>2^{a}</td>
</tr>
<tr>
<td>2</td>
<td>X/X</td>
<td>7</td>
<td>NoM</td>
<td>1142/ 4^{b}</td>
<td>1^e</td>
</tr>
<tr>
<td></td>
<td>X/Y^{II}^{0}, ar^{+}; ar/ar</td>
<td>3</td>
<td>NoM</td>
<td>263</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>7</td>
<td>NoM</td>
<td>644</td>
<td>174^{h}</td>
</tr>
</tbody>
</table>

\(^{a}\) 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^{II}^{a}, ar^{+} chromosome.

\(^{h}\) Animals were ar, indicating that they carried two X chromosomes and were therefore the NoM animals.
Male-Determiner M on the Y of Musca

**TABLE 3**

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

<table>
<thead>
<tr>
<th>Group</th>
<th>M factor</th>
<th>M function in soma (carriers of M)</th>
<th>M function in germ line (NoM animals)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Phenotype (%)</td>
<td>Yolk proteins (%)</td>
</tr>
<tr>
<td>i</td>
<td>M \textsuperscript{Y}\textsubscript{a}</td>
<td>♂ 100</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>M \textsuperscript{III}\textsubscript{b}</td>
<td>♂ 100</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>M \textsuperscript{Y}\textsubscript{a}</td>
<td>♂ 100</td>
<td>0</td>
</tr>
<tr>
<td>ii</td>
<td>M \textsuperscript{II}\textsubscript{a}</td>
<td>♂ 100</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>♀ 98.3</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>♀ 0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>M \textsuperscript{I}\textsubscript{a}</td>
<td>♂ 100</td>
<td>56</td>
</tr>
<tr>
<td></td>
<td></td>
<td>♀ 0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>M \textsuperscript{L}\textsubscript{a}</td>
<td>♂ 6.8</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>♀ 93.2</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td></td>
<td>♀ 0</td>
<td>0</td>
</tr>
<tr>
<td>iii</td>
<td>Ag\textsuperscript{a}</td>
<td>♂ 0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>♀ 0.2</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td></td>
<td>♀ 99.8</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>M \textsuperscript{15}</td>
<td>♂ 67</td>
<td>37</td>
</tr>
<tr>
<td></td>
<td></td>
<td>♀ 33</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td></td>
<td>♀ 0</td>
<td>0</td>
</tr>
</tbody>
</table>

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.

\textsuperscript{a} Schmidt et al. (1997b).

\textsuperscript{b} Hilker-Kleiner et al. (1994).

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 \textsuperscript{Y}\textsubscript{a}) and is represented by the \textsuperscript{Y}\textsuperscript{D} chromosome of the translocation T(Y;II)\textsubscript{2}, ar\textsuperscript{+} 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 \textsuperscript{L}\textsubscript{a}) and corresponds to the M activity found on the Y\textsuperscript{II}\textsubscript{D}, ar\textsuperscript{+} 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 (Hilker-Kleiner et al. 1993). Some cells within an embryo respond to this ambiguous signal of M \textsuperscript{15} or M \textsuperscript{L}\textsubscript{a} 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; Lauge 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 \textsuperscript{15} and M \textsuperscript{L}\textsubscript{a} 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 \textsuperscript{15} are as efficient as the combination of M \textsuperscript{15} and M \textsuperscript{L}\textsubscript{a}. This suggests that M \textsuperscript{15} and M \textsuperscript{L}\textsubscript{a} are copies of an ancestral M factor. Because, however, M \textsuperscript{15} and M \textsuperscript{L}\textsubscript{a} 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 \textsuperscript{Y}, M \textsuperscript{III}, and M \textsuperscript{Y}, that
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 (Hilker-Kleiner et al. 1994; Dübdorf 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^15) 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^11; 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^1; Table 3). M factors with very little activity, such as M^14, 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.

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LITERATURE CITED

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