GENETIC DISTORTION OF SEX RATIO IN A MOSQUITO, *AEDES AEGYPTI*[1]

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IN an organism heterozygous for a given pair of alleles, one generally expects equal recovery of each allele in the products of gametogenesis. Genetic systems resulting in nonrandom recovery have stimulated considerable interest in recent years. The sex ratio may be the most obvious character for detection of aberrant segregation. Among the insects, numerous cases of abnormal sex ratios have been investigated and found to have a relatively simple genetic basis.

The mode of sex determination in mosquitos would lead one to expect a 1:1 sex ratio. The culicine genera, including Culex and Aedes, have three pairs of homomorphic chromosomes in both sexes; thus, an XX-XY mechanism cannot be observed cytologically. In *Culex pipiens*, GILCHRIST and HALDANE (1947) postulated that sex is determined by a single gene or a small chromosome segment. They found a recessive gene for white eye color that was approximately 6.3 crossover units from the sex locus; moreover, males were heterogametic. The proposed sex locus was designated *m*, with females *mm* and males *Mm*. MCCLELLAND (1962) demonstrated a similar mechanism in *Aedes aegypti*, using a gene for red eye color. This gene is 6 to 7 units from the sex locus (MCCLELLAND 1966). Additional genes have been mapped on both sides of the sex locus, demonstrating that sex does not occupy a terminal position (MACDONALD 1963; CRAIG and HICKEY 1966). These sex-linked genes have confirmed the conclusion that males are heterogametic for sex (*Mm*). Therefore, the male parent determines the sex ratio in progeny and, given normal segregation, equal numbers of males and females should occur.

Departures from this theoretical expectation often occur in culicine mosquitoes. In *Aedes aegypti*, ratios of about five males to three females are generally reported. CHRISTOPHERS (1960) summarized the records of several workers, stating that males predominate in *A. aegypti* to the extent that frequencies of 35 to 45% female are characteristic. The sex ratio in 19 laboratory strains was determined by CRAIG *et al.* (1961) as part of a broader study of genetic variability in this species. Characteristic and constant differences among strains were reported. Some strains had about 50% females, others had a slight excess of males (about 40% female) and a few showed distinct deviations in sex ratio (below 30% female).

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CRAIG, HICKEY and VANDEHEY (1960) reported that a hereditary factor transmitted by males was responsible for high male ratios in *A. aegypti*. This phenomenon was designated as male-producing or MP. Males from high male-producing families produced a high proportion of males in their own progeny, regardless of the type of female to which they were crossed. This condition was not due to selective mortality, at least in postgametic stages. In 1960, nothing was known about the sex-determining mechanism in *A. aegypti*. In addition, the male-producing lines available for study were highly variable in expression. These factors hampered more precise analysis of the mechanism of inheritance of MP.

The present work was initiated because new crosses with different strains gave more pronounced and predictable distortion of sex ratios. Earlier strains gave about 15 to 30% female, whereas present lines produce about 0 to 15% female. This paper presents an analysis of the mode of inheritance of MP. In addition, data suggesting the mechanism of action are included. Separate reports will be published elsewhere on (1) the distribution and behavior of MP in experimental populations and (2) the effect of environment on expression of MP. A more detailed account of some of this work is given by HICKEY (1965a, b). Nomenclature used in these preliminary reports is superseded by that in the present work.

MATERIALS AND METHODS

The strains used are listed in Table 1. These were selected from the collection of more than 100 strains maintained at the WHO International Reference Centre for Aedes, University of Notre Dame. Most of the work was done with the RED and WART strains. These strains had been maintained as mass-breeding of colonies for several years. Single-pair matings within each of these strains produced progeny of 40 to 60% female, with a mean close to 50%. The MALE-PRODUCING strain was synthesized from matings between these two strains.

Rearing methods used were generally similar to those described by CRAIG and VANDEHEY (1962) for genetic research with *A. aegypti*. Rearing was conducted in an insectary room with a controlled temperature of 26.7 ± 2C and a relative humidity of 80 ± 10%. Larvae were fed on a solution of Liver Powder NF (Nutritional Biochemicals Co.). To insure adequate space for development, larvae were reared in containers which provided at least one cm^2 of surface area per individual. Almost all pupation occurred on days 6–7 after hatching. Pupae were segregated according to sex (females larger than males). Adults emerged two days after pupation, and sex was rechecked at this time.

Single-pair matings were used in all experiments. Adults were fed apple slices or dry sugar cubes. Females were provided with blood meals from an anaesthetized mouse. Oviposition occurred about four days after each feeding; each female produced about 80–90 eggs per blood meal. Eggs were deposited on moist paper in a shell vial. Embryonation was completed in 48–72 hours and egg papers were then dried and stored. Eggs on paper held at a relative humidity of 85% remain viable for up to six months; in this investigation, eggs were generally used within one month. Hatching was accomplished by immersion of eggs in deoxygenated water for 24 hours.

Precautions were taken to avoid selective mortality as a source of bias in collection of sex ratio data. Egg papers were examined for premature hatch, an event which seldom occurred with these techniques. The number of larvae hatched and the number of adults obtained were recorded for most experiments. In those few cases where mortality exceeded 10%, the entire family was discarded. In addition, all families having fewer than 20 offspring from a single oviposition were excluded. Such exceptionally small numbers were encountered in fewer than 5% of the single-pair matings made.

TABLE 1

Strains of Aedes aegypti *used for sex ratio studies*

Name*	Source	Strain history	Remarks
RED	WHO International Reference Centre for Aedes Univ. Notre Dame	Multiple marker strain synthesized at Notre Dame in 1962. All three chromosomes contain genetic markers.	Sex ratio normal. High response to Distorter, homozygous for m^d.
WART	WHO International Reference Centre for Aedes Univ. Notre Dame	Contains the spontaneous mutant wart palp, selected from the NEW TEXAS strain. Maintained since 1960.	Sex ratio normal. Homozygous for Distorter.
MALE-PRODUCING	WHO International Reference Centre for Aedes Univ. Notre Dame	Synthesized from matings between RED and WART strains. F_1 males are male-producing. Strain maintained by outcrossing to sensitive females.	Males predominate. Heterozygous for Distorter; males M^Dm^d.
ROCK	DALE W. JENKINS Army Biological Laboratories, Fort Detrick, Frederick, Maryland	Laboratory reared at Rockefeller Virus Foundation over 20 years. Reared at Fort Detrick in 1958, maintained since 1959.	Sex ratio normal. Little response to Distorter.
KEY WEST	A. O. LEA Entomological Research Center Vero Beach, Florida	Field-collected from Key West, Fla., 1959. Maintained since 1959.	Sex ratio normal. Little response to Distorter. Used as normal strain by CRAIG *et al.* (1960).
OHIO STATE	ROGER MEOLA Ohio State University Columbus, Ohio	Derived from Orlando, Fla., strain maintained at OSU for more than 20 years. Received in 1963.	Sex ratio normal. Contains Distorter.
MASC-O	G. A. H. MCCLELLAND Univ. Notre Dame	Field-collected by R. MAMET in Mauritius, 1962.	Subspecies *mascarensis* of *A. aegypti*. Highly sensitive to Distorter. Sex ratio normal.

* All strains *A. aegypti aegypti*, the type form, unless otherwise indicated.

RESULTS

Crosses within most strains produce a 1:1 sex ratio in subsequent generations, yet crosses between certain strains give F_1 males which produce progeny that are predominantly male. This distortion of sex ratio is characteristic of crosses between RED and WART. Figure 1 shows the distribution of sexes in the progeny of 20 single-pair F_1 matings from an initial pair cross of female RED × male WART. All crosses produced more than 70 offspring. Most pairs had progeny that were less than 10% female. One cross gave 110 males and no females. Of 1,827 progeny from the 20 crosses, only 217, or 12%, were females.

F_1 males from the RED × WART cross regularly produced progeny showing a predominance of males, leading to the observation that the MP phenomenon is reasonably stable. The distortion was not due to postzygotic mortality. There was no significant mortality in eggs, larvae, pupae or adults of male-producing families. Even when these MP families were reared under adverse conditions of temperature, feeding or density, mortality rates were comparable with those in lines with 1:1 ratios. Various lines of evidence have indicated that environmental factors did not influence the relationship between sex ratio of MP and normal families (HICKEY 1965a).

Mode of inheritance: A model is proposed to explain the mode of inheritance of the male-producing factor. First, assume that there is a single factor responsible for sex ratio distortion. This male-producing factor is here designated as Distorter; presence of the factor is indicated by the symbol *D* and the alternative form, *d*, is used to indicate absence of *D*. Second, assume that this factor acts directly at the locus for sex determination, *m*, perhaps reflecting a condition or alternative form of the sex locus. Therefore, the symbol is written as a superscript

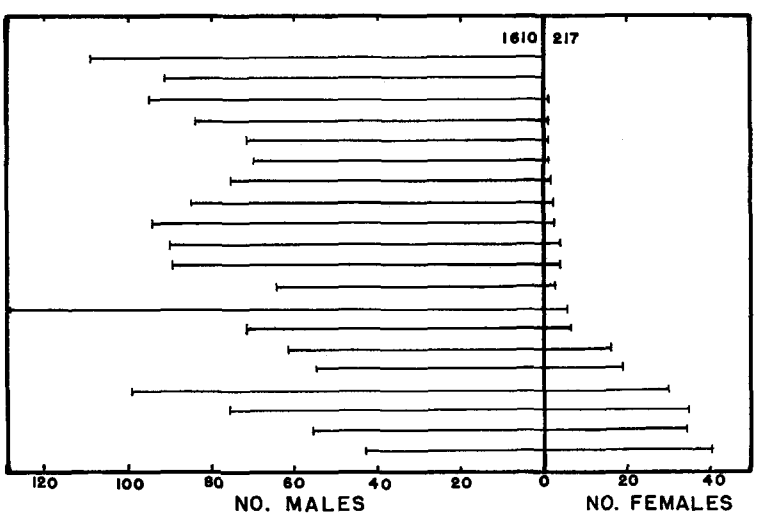

FIGURE 1.—Distribution of sexes in the progeny of 20 single-pair F_1 matings from the original cross of female RED × male WART. Each line represents the number of individuals of each sex from a single-pair mating. Total offspring = 1827; 217 females and 1610 males.

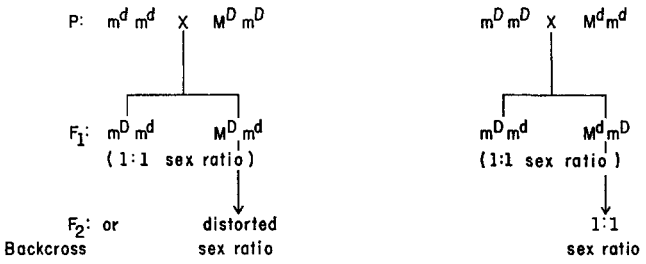

FIGURE 2.—Scheme showing reciprocal crosses between two strains, one homozygous for Distorter and the other lacking Distorter.

of *m*. Figure 2 illustrates results expected in reciprocal crosses between two strains, one *DD* and the other *dd*.

It will be recalled that the male parent is responsible for the sex ratio of the offspring; the genotype of the female plays no direct role. A third and final assumption is required: Assume that distortion occurs only when a male is $M^D m^d$. With this genotype, most of the gametes recovered are male-determining. The reciprocal genotype, $M^d m^D$, gives a 1:1 sex ratio. A more detailed explanation of the model is given in Table 2.

Figures 3 and 4 show the results of reciprocal crosses between two strains (RED and WART) made to test the proposed model. These results support the model if RED is *dd* and WART is *DD*. The F_1 from reciprocal crosses between WART and RED gave 46 and 47% female. However, pair matings using the F_1 males from these two crosses gave very different results. F_1 males from WART × RED gave 51% females when crossed to their sisters for an F_2 and 49% female when backcrossed to RED females (Figure 3). Following the model, these males were $M^d m^D$ and therefore, no distortion would be expected. However, F_1 males from the reciprocal cross (RED × WART, Figure 4) gave 17% female in the F_2 and 16% female in the backcross. This distortion would be expected because these males were $M^D m^d$.

Similar results have been obtained whenever males of the $M^D m^d$ genotype were crossed to any female, regardless of her genotype. These experiments have been

TABLE 2

Genotypes affecting sex ratio in Aedes aegypti

Sex	Genotype*	Gametes	Influence on sex ratio in progeny
Female	$m^d m^d$	m^d	none
	$m^D m^d$	m^D, m^d	none
	$m^D m^D$	m^D	none
Male	$M^d m^d$	1 M^D = 1 m^d	1:1
	$M^D m^d$	M^D (a few m^d)	males predominate
	$M^d m^D$	1 M^d = 1 m^D	1:1
	$M^D m^D$	1 M^D = 1 m^D	1:1

* D=male-producing factor present; d=male-producing factor absent.

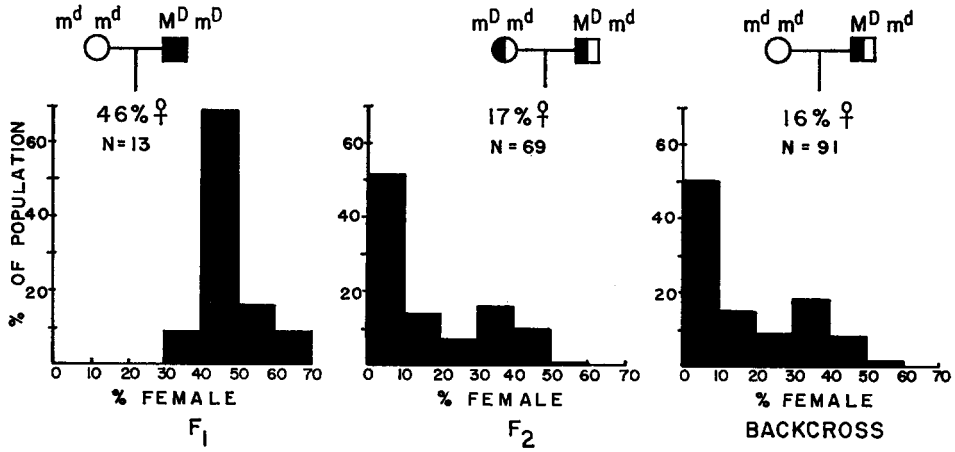

FIGURE 3.—Distribution of sexes in progeny from the cross of female WART × male RED. Data are given for F_1, F_2 and backcross of F_1 male to RED. N = number of single-pair crosses made. Abcissa represents the percent of crosses producing a given percent female (ordinate). Mean percent female for all crosses is also presented.

repeated with numerous other strains and the pattern of inheritance is similar. These findings support the hypothesis that Distorter functions only in the male and is operational only in the heterozygous condition. The Distorter model is in accordance with the data and conclusions of CRAIG, HICKEY and VANDEHEY (1960). Therefore, their term male-producing (MP) will be used here to indicate a male of the genotype M^Dm^d.

As a further test for the proposed model, MP males were tested simultaneously with two kinds of females. Fourteen males were taken from a single family (fifth backcross generation, RED × MP) that had 2% females. A single male (M^Dm^d) was used to inseminate both a WART female (m^Dm^D) and a RED female (m^dm^d). Results of these crosses are given in Table 3. It is interesting to note that every

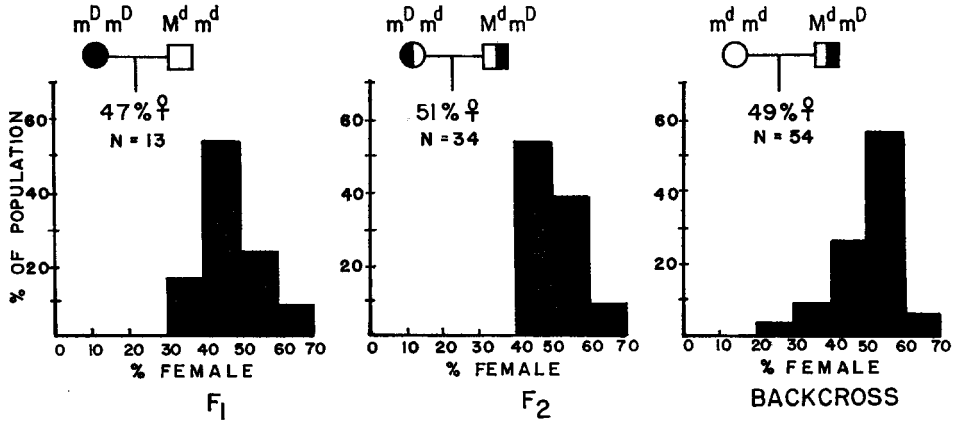

FIGURE 4.—Distribution of sexes in progeny from the cross of female RED × male WART. Legend as in Figure 3.

TABLE 3

Proportion of females in progeny of crosses with individual MALE-PRODUCING males crossed simultaneously to both RED (m^dm^d) *and WART* (m^Dm^D) *females*

Parents		Percent female in F_1													
		Male No.													
Male*	Female	1	2	3	4	5	6	7	8	9	10	11	12	13	14	Mean
M^Dm^d	m^dm^d	0	0	0	2	2	3	4	6	24	25	27	29	31	35	13.4
M^Dm^d	m^Dm^D	0	1	5	5	9	0	0	6	20	20	34	35	37	28	14.3

* All males from a single family which had 2% female.

male produced a similar progeny with each of his two mates. Moreover, different levels of male-producing were evident from different males tested, i.e., 0 and 0% compared to 35 and 28%. When the data for all 14 tested males are pooled, the mean values for the two kinds of female parents are almost identical, 13.4 and 14.3% female. These data further confirm the observation that in any mating in which the male parent is M^Dm^d, the sex ratio of the progeny will be distorted, regardless of the genotype of the female parent.

In order to determine the contribution of the female to sex ratio, the F_1 males obtained from the simultaneous matings in Table 3 were tested further (Figure 5). Both kinds of F_1 males were backcrossed to RED females (m^dm^d) and the progeny were scored for sex ratio (Table 4). When F_1 M^Dm^d males were tested, the sex ratio in progeny was distorted. Tests involving 119 males producing 9956 offspring resulted in a mean percent female of 13.8. Of these 119 males, more than one half (66) produced progenies that were below 10% female. However, when F_1 M^Dm^D were tested, the resultant progeny exhibited a normal distribution of the sexes, 48.7% female. In this cross, 125 males, producing 11,012 offspring,

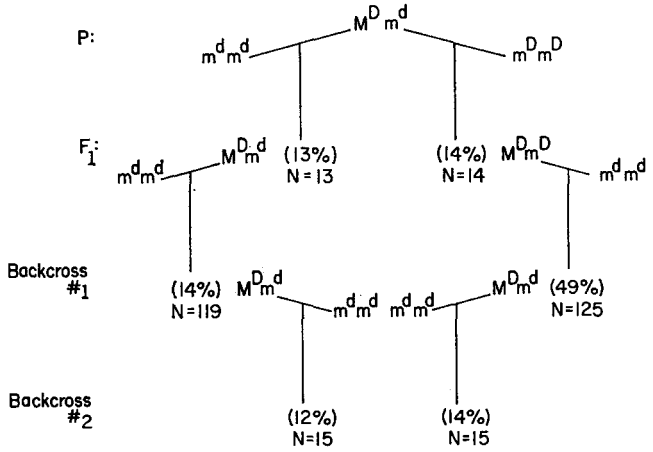

FIGURE 5.—Crossing scheme to demonstrate the influence of the female parent on sex ratio. Figures in parentheses indicate mean percent female obtained. N = number of single-pair crosses scored.

TABLE 4

Results of test crosses with F_1 half brothers resulting from the parental cross of an MP male (M^Dm^d) *to two females,* m^dm^d *and* m^Dm^D

Parental male No.†	F_1 male genotype	No. males tested per family	Distribution of ♂♂ according to % female in progeny*							Mean % ♀ for family	Range
			0	10	20	30	40	50	60		
8	M^Dm^d	12	12	..	..	..	..	..	..	2	0–9
2		14	12	2	..	..	..	..	..	4	0–13
6		12	9	..	2	1	..	..	..	8	0–30
1		8	5	1	..	2	..	..	..	12	0–36
7		10	7	..	1	1	1	..	..	12	1–43
3		14	7	1	3	3	..	..	..	14	0–38
9		13	2	6	4	1	..	..	..	16	0–30
4		15	5	3	4	1	1	1	..	19	0–52
5		15	5	1	3	4	2	..	..	22	0–49
10		6	2	1	..	..	1	2	..	28	3–56
	Total for ten families	119	66	15	17	13	5	3	..	13.8	0–56
8	M^Dm^D	12	..	..	..	1	8	3	..	48	38–59
2		15	..	..	..	1	8	6	..	49	37–54
6		8	..	..	..	1	3	3	1	50	39–66
1		9	..	..	1	..	3	4	1	50	28–66
7		13	..	..	..	1	9	3	..	47	37–59
3		15	..	..	..	..	7	8	..	50	44–57
9		14	..	..	..	3	9	1	1	45	31–60
4		9	..	..	..	1	1	7	..	50	31–55
5		15	..	..	..	..	5	10	..	51	43–56
10		15	..	..	..	..	9	6	..	49	40–54
	Total for ten families	125	..	..	1	8	62	51	3	48.7	28–66

* Each figure represents a category of 10, i.e., 0=0–9% female, 10=10–19% female.
† Table 3.

were tested. More than 90% (113) of these males produced families with the sex ratio approximating normal, i.e., 40% female or higher.

An additional backcross was made in order to demonstrate that Distorter could again be recovered from the apparently normal line (Figure 5). When males from families with normal sex ratios (49% female, backcross #1) were tested, they gave progeny with 14% female. The fact that the male-producing phenomenon can be lost and recovered in sequential generations provides strong support for the model proposed. When males with M^D receive a m^d allele from their mothers, they produce progeny with distorted sex ratio. Males receiving the m^D allele produce normal sex ratios.

Variation in distortion: Different males produce different levels of sex ratio distortion. For a given male, the proportion of the sexes in the progeny is remarkably constant. Table 3 shows that the sex ratio was nearly identical when one male was mated to two different females. However, Table 3 appears to show two distinct categories of males, those that produced 0 to 9% female and those that

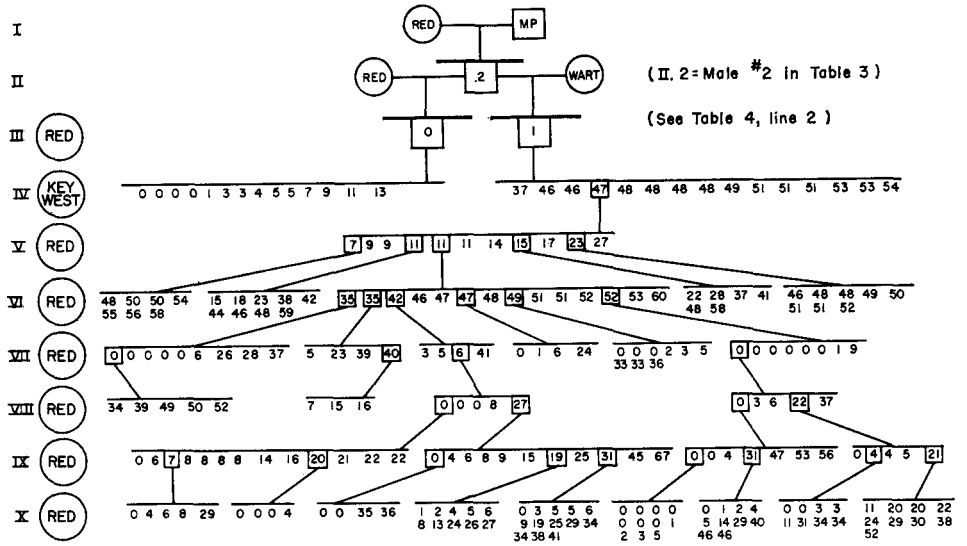

FIGURE 6.—Pedigree of MP, showing variability in sex ratio over ten generations. Numbers indicate percent female in a family from a single-pair cross. Each family of males tested is indicated by a box. These males were crossed to females of strain in circle at left.

gave 20 to 37% female. In another experiment, more completely reported by HICKEY (1965a), single MP males were mated sequentially to five ROCK females at three-day intervals. The sex ratio from all females in the series was essentially the same, indicating no effect of male aging on expression of MP. However, different males again gave different levels. For example, one male gave 1, 6, 1, 3, 0% female and another gave 23, 34, 24, 32, 31% female.

Variability in sequential generations is shown in the pedigree in Figure 6. This line was initiated from the crosses described in Tables 3 and 4. Initial crosses were designed so that MP was expressed, lost, expressed, lost and expressed again in subsequent generations. The normal sex ratios evident in generation VI are due to the fact that the KEY WEST females used in generation IV were primarily $m^D m^D$.

The range of expression of the male-producing phenomenon is evident in generations V and VII-X of Figure 6. Males from families with 0% female may give progeny ranging from 0–5% female or 0–56% female or 34–52% female, albeit the latter is uncommon. There is no consistent pattern of genetic segregation for different levels of distortion. Males from families above 20% female show the same range of variation as do males from families of 0–10% female. Attempts to select lines which consistently give below 10% have been unsuccessful. When males for breeding are taken only from 0% families, a wide range of levels usually reappears in the next generation. This procedure of selection with backcrossing to RED was continued for an additional ten generations beyond those recorded in Figure 6; little reduction in variation was observed.

Somewhat reduced variation was observed when MP males were crossed to

MASC-0 females. The latter represents subspecies *mascarensis* of *A. aegypti*. A single male from a 0% family was crossed to a MASC-0 female; F_1 progeny were 2% female. F_1 single-pair matings gave the following percent female in the F_2: 3, 3, 5, 5, 6, 6, 7, 8, 8, 10, 10, 11, 14, 16, 18. These data give a mean of 8.7% and a variance well below that for most of the family groups in Figure 6.

Chromosomal basis of Distorter: Several lines of evidence show that Distorter does not act on the autosomes. A stock was synthesized with markers on each of the three chromosomes. Mutants used include: sex (*m*) on linkage group 1, wart-palp (*wa*), spot-abdomen (*s*) and yellow larva (*y*) on group 2 and black-tarsi (*blt*) on group 3. MP males heterozygous for *wa, s, y* and *blt* were crossed to females homozygous for all four genes. The progeny were 11% female but showed a 1:1 ratio for *wa, s, y* and *blt*. Thus, MP distorted segregation of chromosome 1 but had no effect on segregation of chromosomes 2 and 3.

Additional crosses were made with red-eye, a gene located about 7 units to the left of sex on linkage group 1. RED females were crossed to MP males heterozygous for *re:*

$$\frac{re\ m^d}{re\ m^d} \times \frac{+\ M^D}{re\ m^d}$$

Almost all of the offspring were males with wild-type eyes. Obviously, the eye color allele was preferentially recovered along with the male-determining allele. In the same cross, markers on linkage groups 2 and 3 showed normal segregation and recovery. One may conclude that Distorter operates only on linkage group 1.

It might be suggested that Distorter is on an autosome, even though it affects recovery of the sex chromosome. If Distorter were indeed autosomal, MP males would have the genotype *M/m;D/d*. When such males were crossed to *d/d* females, only half of the male progeny would be MP. This is not the case. Table 4 shows the results of tests made on the progeny of ten MP males. Of the 119 F_1 males tested, only 8 gave sex ratios above 40%. In one family, all of the 12 F_1 males gave progeny with sex ratios below 10%. Similar results were obtained in each of five sequential generations of backcrossing of MP males to RED females. Therefore, it seems probable that the Distorter locus is on linkage group 1.

The model presented here is based on the assumption that Distorter is located at the sex locus. However, it is also possible that this factor could be at a second locus near that for sex. This problem is best resolved by crossover studies. For example, preliminary results have shown that *D* is not located to the left of the locus for red-eye.

Hypothesis 1: *D* to left of *re*

$$\frac{d\ re\ m}{d\ re\ m} \times \frac{D\ +\ M}{d\ re\ m}$$

Hypothesis 2: *D* elsewhere, possibly at sex locus

$$\frac{re\ m^d}{re\ m^d} \times \frac{+\ M^D}{re\ m^d}$$

In either case, most F_1 progeny would be MP and wild-type for eye color; red-eyed males would occur only from crossovers. If hypothesis 1 is correct, F_1 males that are red-eyed could not be MP (unless double crossovers occurred). With hypothesis 2, F_1 red-eyed males would be MP. Again, MP males heterozygous for

TABLE 5

Linkage data on red-eye and sex, taken from MP and normal males

Male parent*	No. of individuals in progeny	Percent female	Percent crossovers between sex and red-eye
Normal†	1628	46.6	6.7
Normal	1518	48.7	7.2
Normal	3750	53.3	7.8
MP	2633	5.4	2.3
MP	321	7.5	2.4
MP	1391	0.6	1.0
MP	1800	18.3	3.4

* Each line represents a testcross of ♀ *re/re* × ♂ F, *re/+*. Each "normal" represents a different strain tested. MP lines represent tests performed at different times over a two-year period.
† Data of McCLELLAND (1966).

re were backcrossed to RED females. Results obtained support hypothesis 2. Of six red-eyed males from this cross that were tested, five were MP. Therefore, it seems probable that Distorter is located somewhere to the right of *re*. Further experiments with other genes on linkage group 1 are required for exact determination of the position of Distorter.

Preliminary evidence seems to indicate that Distorter interferes with crossing over on linkage group 1. Table 5 gives linkage data between *re* and *m* taken from both normal and MP males. For normal males, about 7% crossovers occurred. Results for MP males showed a reduction to 1–3% crossovers. Apparently, crossing over is suppressed in MP males. Whether this reduction is due to Distorter or to some other factor present in the genome of MP males remains to be demonstrated.

Action of Distorter in the female: The proposed model postulates that Distorter is functional only in the heterozygous condition and only in the male (Table 2). In the heterozygous female, $m^D m^d$, no effect on sex ratio would be evident. If Distorter functions in such a female, its effect could be determined with sex-linked genes. A female heterozygous for *re* and Distorter was derived from the cross of RED × WART. This female was crossed to a RED male:

$$\frac{+\ m^D}{re\ m^d} \times \frac{re\ M^d}{re\ m^d}$$

Among 23 single pairs tested, 1535 progeny were produced:

	Female	Male
wild-type	352	447
red-eye	364	372

If Distorter had affected the segregation of linkage group 1, most of the progeny would have had wild-type eyes. Since this was not the case, one may conclude that Distorter does not operate in the female.

Mechanism of action: Experiments were designed to test the hypothesis that Distorter acts through a prezygotic mechanism which results in reduction in re-

covery of female-determining sperm. This hypothesis might imply that MP males produce fewer functional sperm than do normal males.

First, the insemination capability of MP and normal males was compared. A total of 12 males was tested from each of three strains, MP, RED and ROCK. Each male was exposed to ten virgin females for a five day period. Female spermathecae were removed and examined; the presence of sperm was scored as a successful insemination. The MP males inseminated a mean of 3.9 ± 0.34 females, compared to 4.2 ± 0.34 for RED and 5.2 ± 0.38 for ROCK. A statistically significant difference existed only betweeen ROCK and MP ($P = 0.05$).

Perhaps a female inseminated by an MP male may not contain as many sperm as one inseminated by a normal male. Therefore, an estimate of sperm quantity was also made in the experiment reported above. Three categories were used in scoring: (1) complete absence of sperm, (2) some sperm present, (3) full, i.e., two of the three spermathecae filled. In *A. aegypti* the filling of only two of the three spermathecae is the usual rule, even after repeated matings (ROTH 1948). MP males filled 63% of the positive females, compared to 71% for ROCK and 54% for RED. These data indicate that (1) MP males cannot inseminate quite as many females as can males from some other strains and (2) MP males are as efficient as other males in filling the spermathecae.

It is possible that initial inseminations by MP males are complete, but owing to earlier depletion of sperm supply, older MP males are unable to inseminate as many females. To investigate sperm depletion, individual MP and ROCK males were crossed to ROCK virgin females, with two females provided to each male. After three days, males were transferred to cages containing new virgin females. This procedure was followed for a minimum of five transfers. Several egg clutches were collected from each pair of females. The proportion of hatchable eggs was determined for each oviposition. Figure 7 shows hatchability of eggs from the first oviposition by females from each transfer. There was a trend toward reduction in hatchability as males increased in age. However, the decrease was much greater for MP males (85 to 21%) than for ROCK males (98 to 72%). The points

TABLE 6

Sperm depletion as measured by decline in hatchability of eggs in sequential egg clutches of ROCK females mated to aging males of ROCK and MP

	Mean percent egg hatch from sequential egg clutches of females exposed to aging males							
	ROCK (N=25)				MP (N=35)			
Transfer No.*	1	2	3	4	1	2	3	4
1	98	95	91	91	85	72	64	60
2	92	88	88	79	66	33	29	13
3	94	73	69	..	49	26	15	..
4	90	76	..	..	33	14	..	..
5	88	64	..	..	39	15	..	..
6	69	67	..	..	37	9	..	..
7	72	..	..	..	21	..	..	..

* Each transfer represents exposure of a male to fresh females, a procedure repeated every three days.

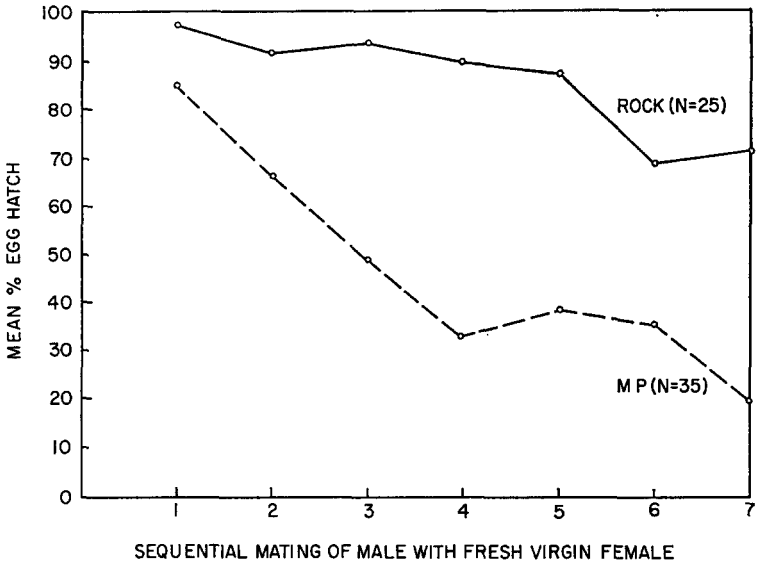

FIGURE 7.—Sperm depletion from aging males of MP and ROCK as measured by hatchability of eggs from sequential matings. Data taken only from the first oviposition in each transfer.

in Figure 7 represent only those females which were still producing some viable eggs after six transfers. If all mated females were included, the decrease for MP males would be much greater. Similar data are provided by hatchability of eggs from successive egg clutches of the same females (Table 6). It is apparent that females mated by MP males were unable to produce as many hatchable eggs as could females mated by ROCK males.

As another index of sperm depletion, the ejaculatory glands of multiply mated ROCK and MP males at different ages were compared (Table 7). No differences were evident in young males, 5 days old; ejaculatory glands of both strains contained abundant sperm. In fact, nearly all ROCK males were positive for sperm during the entire course of the experiment. However, from day 10 through day

TABLE 7

The effect of age on sperm depletion in sequentially mated males of ROCK and MP strains

Age of male in days*	Males examined for sperm in ejaculatory gland			
	ROCK		MP	
	Total	No. with sperm	Total	No. with sperm
5	5	5	5	5
10	5	5	6	1
15	5	4	3	0
20	1	1	3	0
25	4	4	6	0
27	4	4	8	4

* Males provided fresh virgin females every 3 days.

27, most of the MP males examined had no sperm. These data indicate that MP males deplete their sperm supply more rapidly than do ROCK males.

DISCUSSION

Three assumptions were required in the establishment of the model proposed to explain the inheritance of the male-producing phenomenon. It was assumed that (1) a single factor was involved, (2) the factor acted directly at the *m* locus, and (3) distortion occurred only in heterozygous males, M^Dm^d. The model works in practice, giving results that are predictable and reasonably precise, thus generally supporting the validity of these assumptions.

The data on linkage supports assumptions 1 and 2. It was shown that Distorter affected recovery of chromosome 1 but had no effect on the autosomes. It was further shown that Distorter must be located on chromosome 1, somewhere to the right of the sex-linked marker, red-eye. Present data are not sufficient to resolve the problem of the precise location of *D*. For simplicity, we have proceeded on the working hypothesis that *D* is an allele at the *m* locus. If subsequent investigation shows that *D* is at a separate locus, linked to *m*, this finding would not affect operation of the proposed model. Even nomenclatorial problems would be minimal, since the superscript could be written separately. Thus, an MP male would have the genotype *MD/md*. It should be recalled that the designation of sex as a locus is still uncertain and *m* may refer to a small region of a chromosome, perhaps an inversion.

Assumption 3 is supported by the results of reciprocal crosses between RED and WART (Figures 3 and 4), where two different kinds of F_1 males were produced. F_1 males of the genotype M^Dm^d gave progeny with distorted sex ratio, whereas the reciprocal F_1 males, M^dm^D, gave a 1:1 sex ratio. These and other data show that Distorter operates only in heterozygotes and that the *D* must be associated with *M*. Moreover, it was demonstrated that Distorter has no effect on segregation in the female. It would be interesting to test the progeny of a cross where both parents are heterozygous, $m^Dm^d \times M^Dm^d$. Half of the male progeny should be MP and the other half should produce a 1:1 sex ratio.

The female parent influences sex ratio only indirectly. A given male can be MP only if he receives the m^d allele from his mother. TULLIS (1962) attributed high male ratios in *Aedes aegypti* to a maternally-transmitted "sex-ratio" condition. He crossed the OHIO STATE strain, which he considered as normal for sex ratio, with the male-producing TEXAS-D strain of CRAIG *et al.* (1960). When a TEXAS-D female was crossed with OHIO STATE male, the sex ratio in the F_2 and backcross was distorted in favor of males. The reciprocal cross gave normal sex ratios. This led TULLIS to conclude that the "sex-ratio" factor is transmitted only by females, whereas CRAIG *et al.* (1960) claimed that their male-producing factor was passed from MP males to their male offspring.

This apparent disparity can be resolved by the model proposed herein. HICKEY (1965a) found that the so-called normal strain of TULLIS had a high frequency of Distorter. HICKEY crossed ten males of OHIO STATE to RED females. Of 95 F_1 males tested, 78 were MP (below 40% female in progeny). Thus the genotype

of OHIO STATE males was $M^{D}m^{D}$. The females of TEXAS-D were of mixed genotype but primarily $m^{d}m^{d}$. The F_1 male from TULLIS' cross of TEXAS-D × OHIO STATE would be $M^{D}m^{d}$ and distortion of sex ratio would be expected. TULLIS further proposed an hypothesis involving sex-linkage to explain the supposed female transmission. He invoked an XX-XY system of sex-determination, with the "sex-ratio" factor located only on the X chromosome. This explanation is not tenable in view of the demonstration by GILCHRIST and HALDANE (1947) for Culex and MCCLELLAND (1962) for Aedes that sex is apparently determined at a single locus on a pair of homologous chromosomes.

The system proposed by TULLIS cannot explain the data of CRAIG *et al.* (1960). TULLIS attributed this discordance to the use of different normal strains. However, the Distorter system proposed here fits not only the present data but also those of TULLIS (1962) and CRAIG *et al.* (1960). Moreover, this model has been useful in explaining sex ratios encountered in diverse strains and in numerous investigations conducted at our laboratory. WOOD (1961) gave data on single-pair matings with DDT-resistant and susceptible strains. He found progeny varying from 35 to 50% female, with a mean of 43%. He agreed with CRAIG *et al.* (1960) that the male-producing factor is transmitted only through the male parent. However, WOOD may not have been dealing with the Distorter system since, in addition to the high mean value obtained in his sex-ratio strain, there was also differential pupal mortality in favor of the males.

The model propsed here is similar in many respects to several systems of miotic drive described in *Drosophila melanogaster*. The term 'meiotic drive' was used by SANDLER and NOVITSKI (1957) to designate those abnormalities in meiosis in which the two types of gametes from a heterozygote are recovered with unequal frequency. Among the best studied cases of meiotic drive is that involving the Segregation-Distorter (*SD*) locus on chromosome 2 of *D. melanogaster*, discovered by SANDLER, HIRAIZUMI and SANDLER (1959). Males heterozygous for *SD* may pass the *SD*-bearing chromosome to more than 95% of their offspring. *SD* does not appear to have any effect in the female. SANDLER and HIRAIZUMI (1961) found that males showing higher levels of SD deplete their sperm supply and become sterile more rapidly than do those showing a lower level. There was no indication of sterility in younger SD males. In addition, SANDLER and HIRAIZUMI (1960) found that variability in expression of SD in different lines could be reduced by the presence of a stabilizing modifier located elsewhere on chromosome 2. Finally, SANDLER and HIRAIZUMI (1959) showed that SD reduced crossing over in its immediate vicinity.

Another meiotic drive system in *D. melanogaster* distorts sex ratio in favor of females (NOVITSKI and HANKS 1961). Males from RD (Recovery-Disrupter) lines may produce progeny up to 67% female, owing to a reduction in recovery of Y chromosomes (HANKS 1964). No effect is evident in females. Both the autosomes and the X and Y chromosomes affect the level of expression of this sex-ratio distorter. ERICKSON (1965) found that fertility was reduced in RD males.

There are striking similarities between both the SD and RD systems in Drosophila and the Distorter system in *Aedes aegypti*. Among these similarities are:

(1) an abnormal rate of recovery of one of the meiotic products; (2) distortion of ratio limited to a single chromosome pair; (3) functioning of the drive system only in heterozygous males; (4) no effect of the drive system in females; (5) shorter period of fertility in males; (6) variability in recovery rates, possibly due to genetic modifying systems. Certainly it seems reasonable to suggest that meiotic drive is responsible for the high recovery rate of the male-determining sperm in *A. aegypti.*

There are certain differences between the drive systems in Drosophila and in Aedes. SD differs from MP in that the former distorts an autosome. In addition, increase in age of an SD male results in reduction of the segregation distortion phenomenon (SANDLER and HIRAIZUMI 1961), whereas aging had no effect on distortion in MP males (HICKEY 1965a). RD in Drosophila differs from MP in degree of distortion. Maximum distortion for RD was 67% female, whereas MP males commonly give 0% female. The RD effect is much reduced in adults from larvae reared above or below 25°C (ERICKSON and HANKS 1961). However, temperature does not affect expression of MP (HICKEY 1965a). These differences are relatively minor, largely affecting mode of expression. They do not alter the basic similarities between the systems in Drosophila and Aedes.

Although the Distorter model is generally useful, it does not fully account for the variation in levels of MP. Each male produces a constant sex ratio but different males, even from the same family, may give quite different ratios. Different strains show different levels of distortion. The strains described by CRAIG *et al.* (1960) usually produced between 20 and 30% female, whereas the lines used in the present work often showed 0 to 15%. In both studies, we tried to increase the distortion by selection and breeding of males from the family with the lowest proportion of females. Little change was achieved in either study, even after several years of selection. Perhaps these strains contained different Distorter alleles at the *M* locus which result in different levels of sex ratio distortion.

The selection program (Figure 6) had little effect on the range of variation expressed among different males in the same family. This is probably because selection was applied only to the male. The female parent was chosen at random from the RED strain, since this strain was known to be highly responsive to Distorter. We assumed, probably incorrectly, that each RED female was homozygous $m^d m^d$ and hence would give a uniform response. Actually, there may be several forms of m^d, differing in sensitivity to M^D. For example, different males from a single family produced progeny of 0, 0, 0, 0, 0, 6, 26, 28 and 37% female (see Figure 6, first group of families in generation VII). Each of these males had received the same M^D chromosomes from the paternal parent in generation V. However, the maternal parent (RED) might have contributed two kinds of m^d. Let us assume two alleles: $m^{d'}$ is highly sensitive to M^D and $m^{d''}$ is less sensitive. If the female in generation V was $m^{d'}m^{d''}$, males in generation VI would be of two types, $M^D m^{d'}$ and $M^D m^{d''}$. In generation VII, the families with high distortion (0, 0, 0, 0, 0, 6% ♀) may have come from the first type of male and the other families (26, 28, 37% ♀) from the second type. Similar examples can be derived

from other lines in the pedigree in Figure 6. This same system could explain the two levels of distortion reported in Table 3.

If indeed there are several forms of m^d, variation in expression should be reduced by repeated backcrossing of MP males to highly inbred females. Genetic factors at other loci could also be responsible for the variation observed. Perhaps there are genes which act as stabilizers of Distorter. Both of these phenomena have been described for *SD* in *D. melanogaster*, where SANDLER and HIRAIZUMI (1959) found different SD^+ alleles with different levels of sensitivity to *SD*, and (1960) found a stabilizer gene which almost eliminated the variability in expression of *SD*.

The mechanism of action of Distorter is not yet established. It was shown that relatively few female-determining gametes from MP males succeed in fertilizing eggs. It was also shown that MP males deplete their sperm supply earlier than do normal males. These observations lead to the hypothesis that MP males produce a full complement of *M*-bearing sperm but relatively few sperm that are female-determining. Perhaps some abnormal meiotic event incapacitates chromosomes carrying the *m*. As an alternative hypothesis, perhaps *m*-bearing sperm are formed but are nonfunctional.

Either of these hypotheses for the mechanism of Distorter are consonant with meiotic drive systems demonstrated in Drosophila. Earlier workers indicated that *SD* induces a break in the SD^+ chromosome; abnormal spermatogenesis occurs, resulting in the recovery of few SD^+-bearing sperm (SANDLER *et al.*, 1959; CROW *et al.*, 1962). PEACOCK and ERICKSON (1965) have concluded that this cytological model for SD is not valid. They proposed the following: (1) During anaphase I of spermatogenesis, there are functional and nonfunctional poles; only 50% of the sperm (those formed at the functional pole) are capable of fertilizing an egg as suggested earlier by NOVITSKI and SANDLER (1957). (2) In SD males, there is preferential segregation of the *SD* chromosome to the functional pole. (3) Nonfunctional SD^+-bearing sperm are produced and transmitted to the female but are unable to fertilize eggs. The end result is excess recovery of the *SD* chromosome in the progeny.

The RD system in Drosophila appears to act through chromosome breakage during meiosis. In a cytological study of spermatogenesis, ERICKSON (1965) found that fragmentation of the Y chromosome frequently appears during the second meiotic division. He assumed that the fragmented Y had a lethal effect, resulting in reduced recovery of Y-bearing sperm. Sperm bundle counts showed that sperm production was reduced in RD males; the reduction was proportional to the deficiency in Y chromosomes. This mechanism explains the predominance of females characteristic in progeny of RD males.

NOVITSKI, PEACOCK and ENGEL (1965) have suggested that "sex-ratio" in *D. pseudoobscura* involves both preferential segregation *and* aberrant chromosome behavior in meiosis. In this species, progeny of "sex-ratio" males are entirely female. During the first meiotic division, the X and Y chromosomes appear to undergo normal segregation. In the second meiotic division, the Y chromosome

degenerates into a chromatin mass which is not included in a spermatid. Resulting sperm are either X-bearing or contain only autosomes. Only the X-bearing chromosomes can fertilize an egg and hence the progeny are entirely female. NOVITSKI *et al.* suggested that preferential segregation occurs at anaphase I, when the X chromosome moves to the functional pole, while the Y chromosome segregates to the nonfunctional pole and degenerates at the next division.

Perhaps Distorter functions in a manner analogous to RD in *Drosophila*, by causing chromosome breakage during meiosis. The M^D allele may have a deleterious effect on an homologous chromosome bearing m^d. Presuming that this effect is lethal, most sperm would be *M*-bearing. In addition, total sperm productivity would be reduced. The reduced fecundity of MP males has already been noted.

It is equally possible that MP in *A. aegypti* involves polarized segregation to a functional pole, as in SD and "sex-ratio" in Drosophila. Perhaps M^D-bearing chromosomes preferentially segregate to the functional pole. If this is the case, a full complement of gametes could be produced but half would be nonfunctional. The nonfunctional (*m*-bearing) meiotic products could fail at any one of a number of steps. If polarized segregation does occur in MP males, the failure of the nonfunctional products probably takes place prior to the time of exit from the testes. This latter point is supported by the apparent sperm depletion evident in the ejaculatory gland of older MP males. Of course, this reduced fecundity may be due to genetic factors quite unrelated to Distorter.

Further investigations are needed to demonstrate whether any of the Drosophila mechanisms can explain distortion in Aedes. Two areas require immediate attention: cytogenetic analysis of spermatogenesis and investigations to trace the fate of sperm produced. MESCHER and RAI (1966) have recently completed a study of spermatogenesis in normal males of *A. aegypti*. A similar analysis should be performed with MP males. SPIELMAN (1964) and JONES and WHEELER (1965) have initiated studies on the mechanics of mating in *A. aegypti*, including sperm transfer and migration. Further studies on all the steps between spermatogenesis and fertilization should be conducted in order to determine the precise stage where the female-determining chromosomes are lost.

SUMMARY

Although the normal sex ratio in *A. aegypti* approximates 1:1, certain males have very few females in their progeny. High male ratios are not due to post-fertilization mortality. A genetic factor designated Distorter (*D*) causes abnormal segregation of chromosome 1. This system of distortion of segregation ratios is similar to cases of meiotic drive in Drosophila.—Distorter is located on chromosome 1, at or near the sex locus (*m*). Distorter functions only in the heterozygous male and only when located on the male-determining chromosome, M^Dm^d. When functional, the factor distorts segregation of chromosome 1 in favor of the homolog bearing M^D. No distortion occurs in females or in males of the reciprocal genotype, M^dm^D. By appropriate crosses, Distorter can be manipulated so that its effect is expressed, lost and expressed again in sequential generations.—In cur-

rent lines, Distorter gives means of 10–15% female, albeit variation may range from 0–50% female. Each male produces a constant sex ratio but different males, even from the same family, may exhibit quite different levels of distortion. Attempts to reduce variability by selection were unsuccessful.—Distorter males produce fewer functional sperm than do normal males.

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