Sex Chromosome Meiotic Drive in Stalk-Eyed Flies

Daven C. Presgraves,*[,1] Emily Severance[†] and Gerald S. Wilkinson*

**Department of Zoology, University of Maryland, College Park, Maryland 20742 and [†]Department of Biology, University of South Florida, Tampa Bay, Florida 33620*

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ABSTRACT

Meiotically driven sex chromosomes can quickly spread to fixation and cause population extinction unless balanced by selection or suppressed by genetic modifiers. We report results of genetic analyses that demonstrate that extreme female-biased sex ratios in two sister species of stalk-eyed flies, *Cyrtodiopsis dalmanni* and *C. whitei*, are due to a meiotic drive element on the *X* chromosome (X^d). Relatively high frequencies of X^d in *C. dalmanni* and *C. whitei* (13–17% and 29%, respectively) cause female-biased sex ratios in natural populations of both species. Sex ratio distortion is associated with spermatid degeneration in male carriers of X^d. Variation in sex ratios is caused by *Y*-linked and autosomal factors that decrease the intensity of meiotic drive. *Y*-linked polymorphism for resistance to drive exists in *C. dalmanni* in which a resistant *Y* chromosome reduces the intensity and reverses the direction of meiotic drive. When paired with X^d, modifying *Y* chromosomes (Y^m) cause the transmission of predominantly *Y*-bearing sperm, and on average, production of 63% male progeny. The absence of sex ratio distortion in closely related monomorphic outgroup species suggests that this meiotic drive system may predate the origin of *C. whitei* and *C. dalmanni*. We discuss factors likely to be involved in the persistence of these sex-linked polymorphisms and consider the impact of X^d on the operational sex ratio and the intensity of sexual selection in these extremely sexually dimorphic flies.

MEIOTIC drive occurs when a gene violates the Mendelian law of equal segregation by interfering with the transmission of its homologue thus enhancing its own representation in the gamete pool (SANDLER and NOVITSKY 1957). When a drive element is located on one of the sex chromosomes in the heterogametic sex, it causes distortion of progeny sex ratios. Sex chromosome drive is of special interest because the drive element alters individual and population fitness. First, the reproductive values of male carriers of driving *X*-chromosomes, and those females inseminated by them, are reduced due to the overproduction of the abundant sex (FISHER 1958). Second, the frequency of a sex-linked drive element affects the population sex ratio, which in turn impacts the effective size and intrinsic growth rate of the population, as well as the intensity of sexual selection. Ultimately, a driving sex chromosome, unconstrained by selection or modifier alleles that suppress the intensity of drive, will quickly go to fixation, resulting in the absence of individuals of one sex and extinction of the population (HAMILTON 1967).

Meiotic drive requires the interaction of at least two loci: a drive locus with *driving* and *non-driving* alleles, and a target locus with *resistant* and *sensitive* alleles. Conditions of limited recombination allow *driving* alleles to become coupled with *resistant* target alleles and are thus conducive to the evolution of drive elements (HAIG and GRAFEN 1991). Drive and target loci are expected to be tightly linked because a recombination event that couples a *driving* allele with a *sensitive* target allele creates a suicidal chromosome that drives against itself. Not surprisingly then, linked complexes of genes that interact to cause meiotic drive often localize to chromosomal inversions (STURTEVANT and DOBZHANSKY 1936; WU and BECKENBACH 1983; but see JAMES and JAENIKE 1990) or centromeric regions (PIMPINELLI and DIMITRI 1989; CABOT *et al.* 1993) where interchromosomal recombination is rare. Due to the general absence of recombination and lack of homology between the two sex chromosomes, sex chromosome drive is expected to be more common than autosomal drive (HURST and POMIANKOWSKI 1991; WU and HAMMER 1991). In fact, driving sex chromosomes have been reported in several species of Drosophila (for reviews see JAMES and JAENIKE 1990; WU and HAMMER 1991) as well as for several other Dipteran species including *Aedes aegypti* (HICKEY and CRAIG 1966; WOOD 1976), *Culex pipiens* (SWEENEY and BARR 1978), *Glossina morsitans* (RAWLINGS and MAUDLIN 1984), and *Musca domestica* (FOOT 1972).

These drive polymorphisms may persist when drive elements are associated with reduced fitness (EDWARDS 1961) or interact with genetic modifiers of the intensity of drive (WU 1983), but the conditions for stability are extraordinarily stringent (CLARK 1987). Several fitness studies have detected density-dependent viability and

Corresponding author: Gerald S. Wilkinson, Department of Zoology, University of Maryland, College Park, MD 20742.
E-mail: wilkinson@zool.umd.edu

[1]*Present address:* Department of Biology, University of Rochester, Rochester, NY 14627.

fecundity selection against sex ratio (*SR*) in male and female *D. pseudoobscura* (WALLACE 1948; CURTSINGER and FELDMAN 1980; BECKENBACH 1983; BECKENBACH 1996). POLICANSKY and ELLISON (1970) first demonstrated that *X*-linked drive elements cause spermiogenic failure of the targeted *Y*-bearing spermatids in the testes of *SR* males in *D. pseudoobscura,* and similar patterns have been shown for *SR* in *D. subobscura* (HAUSCHTECK-JUNGEN and MAURER 1976), the mosquito *A. aegypti* (HASTINGS and WOOD 1978), as well as for the autosomal drive element, segregation distorter (*SD*) in *D. melanogaster* (PEACOCK and MIKLOS 1973). This sort of spermiogenic failure can cause reduced male fertility and limit the spread of drive elements (BECKENBACH 1978; JAENIKE 1996). For example, in *D. pseudoobscura, SR* males suffer a sperm displacement disadvantage relative to wild-type males (WU 1983b).

Theoretical work indicates that in populations with *X*-linked drive, the targeted *Y* chromosomes are under the strongest pressure to evolve drive-resistance, but autosomal modifiers that suppress drive are also expected to increase in frequency due to their tendency to be found in the rarer sex (WU 1983c). Both *D. paramelanica* (STALKER 1961) and *D. mediopunctata* (CARVALHO and KLACZKO 1993; CARVALHO and KLACZKO 1994; CARVALHO *et al.* 1997) have major *Y*-linked and minor autosomal modification. In *D. affinis,* when the driving *X* chromosome was placed in an *XO* genetic background (*XO* males are fertile in *D. affinis*), the sex ratio was reversed resulting in *MSR* or male sex ratio (VOELKER 1972). Hybridizations between isolated populations of *D. simulans* revealed the presence of hidden driving *X* chromosomes whose expression was otherwise masked by modifiers (MERCOT *et al.* 1995). Sex ratio distortion occurred in hybrid males when the driving *X* was placed on a foreign genetic background that lacked modifiers that suppress drive.

In this article we present evidence for a sex-linked drive element (X^d) in two sister species of sexually dimorphic Malaysian stalk-eyed flies, *Cyrtodiopsis dalmanni* and *C. whitei.* We use field captured flies to determine that the drive element is relatively common in natural populations of both species. Examination of developing sperm bundles in male testes reveals that carriers of X^d experience a high degree of spermiogenic failure, as has been reported for other drive systems. The absence of sex ratio distortion from the congener, *C. quinqueguttata* (G. S. WILKINSON, D. C. PRESGRAVES and L. CRYMES, unpublished results), suggests that the drive element may have evolved before the divergence of *C. dalmanni* and *C. whitei.* Remarkably, this ancient polymorphism appears to be maintained, at least in part, by the presence of autosomal modifiers that reduce drive intensity and a *Y*-linked counter-drive factor (Y^m) that suppresses and reverses the direction of *X*-chromosome drive.

MATERIALS AND METHODS

Inheritance of X^d: Large stock population cages are maintained for each species in 40 × 40 × 120 cm cages in temperature-controlled (25°) rooms with 12 hr light-dark cycles. For more detailed description of general lab methods, see LORCH *et al.* (1993). Controlled matings were conducted in inverted ventilated Nalgene mouse cages (13 × 13 × 23 cm). Females were subsequently isolated in 500-ml containers lined with moist cotton containing plastic cups of processed corn where they were allowed to feed and oviposit for ~10 days or until they died. *C. whitei* and *C. dalmanni* both have generation times of ~5 weeks.

Sex ratio distortion was first detected while conducting quantitative genetic breeding studies using *C. dalmanni* and *C. whitei* (WILKINSON 1993; G. S. WILKINSON, unpublished data). Some individual males mated to four to 12 females each consistently produced female-biased progeny sex ratios. To test for *X* linkage we crossed known sex ratio biasing males to three or more stock population females to generate expected heterozygous daughters. We retained a sample of F_1 males and tested their fertility. Presumed heterozygous F_1 females were crossed to non-biasing males, and to determine if the predicted 1:1 expectation of sex ratio biasing and non-biasing F_2 males was produced, we then crossed F_2 males to three or more females and scored the sex ratios of their progenies.

Females used in crosses (Figures 2 and 4) were not known to be homozygous for the non-distorting *X* chromosome. However, contamination of any cross by a driving *X* would not be expected to alter the results reported here. Furthermore, in the three crosses where presumed homozygous non-distorter females were used (see Figures 2 and 4), we found no evidence for the inadvertent introduction of a driving *X.*

Sex ratios were tested for goodness of fit to 1:1 using two-tailed chi-square tests corrected for continuity (SOKAL and ROHLF 1981). The continuity correction makes the chi-square a conservative test for small samples sizes, *i.e.,* $N < 200$ (SOKAL and ROHLF 1981).

Frequency of X^d in natural populations: *C. dalmanni* and *C. whitei* were collected along streams in 1989 and 1996 near the village of Ulu Gombak (~36 km north of Kuala Lumpur, 3° 19′ latitude, 101° 43′ longitude) in peninsular Malaysia. The frequency of X^d was estimated for *C. dalmanni* and *C. whitei* from these field-collected flies. First, we determined the fraction of field-collected *C. whitei* sires producing sex ratios that deviated significantly from 1:1. Second, 15 female *C. dalmanni* inseminated in the field were returned to the lab and allowed to oviposit. Each of their sons were mated to three virgin females and the sex ratios of the F_2 progeny determined. All of the F_1 sons were expected to inherit one of their field-collected mother's *X* chromosomes, therefore females producing both X^dY and *XY* sons must be heterozygous X^dX, while those producing only X^dY sons must be homozygous X^dX^d.

Genetic modification of X^d: Two series of crosses designed to place X^d in different genetic backgrounds were conducted to detect the presence of genetic modification of the driver in *C. dalmanni* (Figure 2A). In the first set of crosses (parental generation through F_2), we mated a single known X^dY male (male 1) to five females. Four heterozygous daughters from each of three (of the original five) females were then all mated to a single *XY* male (male 2). The sex ratio produced by three F_2 sons from each of the 12 crosses was then determined. These F_2 males all shared the *Y* chromosome of male 2 and half possessed the driving *X* of male 1.

F_3 daughters were collected from F_2 males that produced modified sex ratios, *i.e.,* sex ratios differing significantly from 1:1, and mated to *XY* males (Figure 2A). Five of the resulting F_4 males were then mated to four females each and their

TABLE 1

Average sex ratios for multiple females mated to sex-distorting male *C. dalmanni* and *C. whitei*

Species	No. of female mates	Total progeny	Sex ratio (percent males)	χ^{2a}
C. dalmanni	6	262	0.004 ± 0.004	5.6
	18	1207	0.109 ± 0.010	18.1
	7	210	0.156 ± 0.017	2.5
C. whitei	9	268	0.000 ± 0.000	—
	12	392	0.000 ± 0.000	—
	10	380	0.004 ± 0.003	4.3
	13	421	0.011 ± 0.008	21.2
	9	153	0.256 ± 0.058	5.7

Values are ± SE.

[a] Heterogeneity in sex ratio among females. In no case is $P < 0.05$.

progeny sex ratios scored. These crosses were done to characterize the inheritance of modified sex ratio distortion and determine if sex ratio variation is caused by different X^d alleles or by autosomal and *Y*-chromosome loci that modify X^d expression.

Chromosome preparations: Mitotic chromosome preparations were made from the testes of *C. dalmanni* males to determine karyotype and if cytological differences are associated with sex ratio variation. Chromosome preparation methods follow MATSUDA *et al.* (1983). Testes were dissected from males in Ringer's solution and placed in fresh 1% sodium citrate solution on a depression slide for 5 min at room temperature. The testes were then transferred to a precleaned slide where they were macerated with needles, fixed, and stained for 19 min using freshly prepared 3% Giemsa solution diluted with phosphate buffer (pH 6.8).

Spermiogenesis preparations: To determine the mechanism of sex ratio distortion, we made preparations of sperm bundles from the testes of *C. dalmanni* males with known sex ratio phenotypes. Testes were dissected in phosphate buffer solution (PBS pH 6.8) and transferred to a glass microscope slide with one drop of PBS. The contents of the testes were released in the PBS droplet and stained with Hoechst 33258 following the methods described in SAKALUK and O'DAY (1985). Because Hoechst 33258 is a DNA-specific stain, we were able to determine sperm morphology throughout the various stages of spermatogenesis using a Zeiss microscope equipped with epifluorescence illumination. We scored the number of sperm bundles present and quantified the occurrence of degenerate sperm bundles in males differing in sex ratio phenotype.

RESULTS

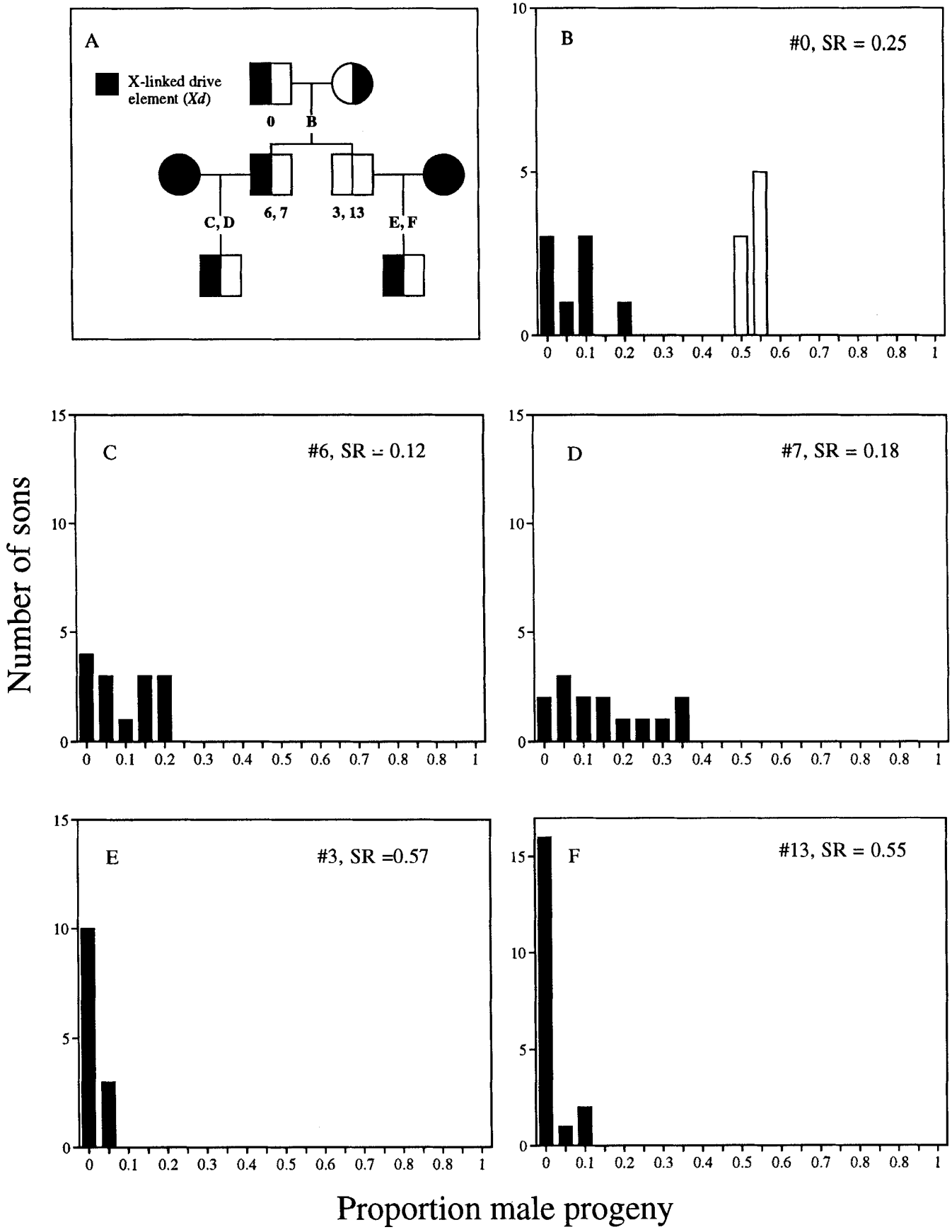
Inheritance of X^d: Three lines of evidence indicate that distorted sex ratios are caused by a driving *X* chromosome. First, several crosses involving both *C. dalmanni* and *C. whitei* demonstrated that the sex ratio produced from a given mating is determined by the genotype of the male, not the female. For example, three sex ratio distorting male *C. dalmanni* mated to between six and 18 females produced consistent sex ratios among all females (Table 1). Similarly, among five *C. whitei* sex-distorting males that were mated to nine or more females, no significant heterogeneity in sex ratio was found among females for any cross (Table 1).

Second, crosses using progeny from a *C. dalmanni* X^dY male indicate that the male sex ratio phenotype alternates generations, as expected for a sex-linked trait. This male was mated to six females and produced a total of four sons and 131 daughters. One of his male offspring was subsequently mated to several sisters, each of which produced progeny with equal sex ratios. These crosses, as well as others for *C. dalmanni* and *C. whitei* not presented, also demonstrated that male progeny from X^dY males are typically fertile. Three of eight F_2 sons mated to virgin females from the lab population produced significantly female-biased sex ratios [proportion male progeny (total progeny): 0.03 (73), 0.07 (107), and 0.10 (133)]. In other words, about half of the grandsons of a sex-distorting male produced similarly sex-biased progeny. Figure 4C illustrates a separate series of crosses that produced 1:1 segregation pattern of sons with normal and female-biased sex ratios.

Third, the sex ratio genotype of females, not males, influences the sex ratio produced by sons. Figure 1 illustrates that crosses between both non-biasing (XY) and female-biasing (X^dY) males to X^dX^d females produced sons that sired female-biased progeny sex ratios. The apparent similarity between father and son sex ratios for the two female-biased X^dY males may be coincidental because the sex ratios of the sons from the male with an 0.12 (3136) sex ratio ranged from 0.00 (327) to 0.21 (275) (Figure 1C). Similarly, the sex ratios of the sons from the 0.18 (3214) sex ratio male ranged from 0.00 (95) to 0.38 (139) (Figure 1E). Thus, these results strongly suggest that the genotype of the mother determines the sex ratio phenotype of her sons, as predicted if sex ratio distortion is caused primarily by an *X*-linked genetic factor.

X^d frequencies in natural populations: Biased sex ratios occurred among 29% of 17 *C. whitei* males captured at the Gombak field site and mated to virgin females in the lab. Four males produced female-biased progenies [0.00 (10), 0.09 (23), 0.26 (103), 0.36 (53)] and one produced male-biased progeny [0.69 (35)]. In addition to these 17 *C. whitei* males, one field-caught male was sterile. Distorted sex ratios were also obtained from some or all of the male progeny screened from four of 15 *C. dalmanni* females that were inseminated in the field. Three females produced sons with both normal and female-biased sex ratios [0.035 (57), 0.31 (132), 0.39 (117)], while one female produced two sons, both of which had female-biased sex ratios [0.00 (176)]. These results indicate that four or five, depending on whether the female with two female-biasing sons was X^dX or X^dX^d, of 30 (13–17%) *C. dalmanni* *X* chromosomes produced altered sex ratios.

Biased progeny sex ratios among field-caught flies were not caused by differential survival by sex. The aver-


A
X-linked drive element (Xd)
0
B
C, D
6, 7
3, 13
E, F
B
#0, SR = 0.25
C
#6, SR = 0.12
D
#7, SR = 0.18
E
#3, SR =0.57
F
#13, SR = 0.55
Number of sons
Proportion male progeny

age number of progeny produced per female did not differ between sex-biased and non-sex-biased sires for either *C. dalmanni* ($t = -1.733$, d.f. = 51, $P = 0.089$, sex ratio biased: 80.3 ± 20.0, unbiased: 55.7 ± 4.4) or *C. whitei* ($t = 1.619$, d.f. = 15, $P = 0.126$, sex ratio biased: 47.3 ± 20.7, unbiased: 95.2 ± 15.0).

Genetic modification of sex ratio: Direct evidence for genetic modification of X^d was obtained by comparing the sex ratios of F_2 sons from an initial cross involving a single female-biasing *C. dalmanni* X^dY male (Figure 2A). Male 1 (X^dY) produced no males out of 70 F_1 progeny obtained from matings with five stock population females. Four F_1 daughters (X^dX) from each of three females (12 total females) were then mated to male 2 (XY) (Figure 2A). The sex ratios of three sons from each of the 12 F_1 females were obtained by mating each to three females. We expected a 1:1 ratio of X^dY (female-biased) and XY (unbiased) from these 36 F_2 males. Because all of the F_2 sons carry male 2's Y chromosome, significant variation in sex ratio among X^dY sons from differing maternal lineages would indicate autosomal modification of the sex ratio. In contrast, two sex ratio phenotypes among the sons suggest either no modification, if the progeny are female-biased, or Y-chromosome modification, if the sex-ratio bias differs from male 1.

As expected, significant differences in sex ratio occurred among 30 F_2 males ($\chi^2 = 85.3$, $P < 0.0001$) with 14 producing sex ratios that did not deviate from 1:1. However, the remaining 16 F_2 males produced significantly male-biased, rather than female-biased, sex ratios. Six F_2 males failed to produce any progeny. The distribution of sex ratios was bimodal with relatively little sex ratio variation among non-biasing and male-biasing sons (Figure 2B). No significant difference in sex ratio could be detected among sons from the three different females ($\chi^2 = 5.6$, $P = 0.061$). Approximately equal numbers of two sex ratio phenotypes are most consistent with Y-chromosome modification of sex ratio distortion.

Chromosome preparations from the testes of adult male *C. dalmanni* were made to determine if male-biased sex ratios are due to a modifying Y chromosome or the absence of a Y as found in *D. affinis* (VOELKER 1972). These preparations show two pairs of medium telocentric chromosomes, three pairs of large submetacentric chromosomes, and a pair of heteromorphic sex chromosomes. Thus, male-biasing males have $2n = 12$ chromosomes, including the X and Y chromosomes (Figure 3).

Expression of the Y-linked modifier (Y^m) depends upon the presence of X^d, because in male 2 and in 14 of the F_2 males Y^m did not produce aberrant sex ratios (Figure 2B). Furthermore, two of five F_4 males produced significantly female-biased sex ratios, with one male exhibiting complete sex ratio distortion and the second showing only partial distortion (Figure 2C). Of the other three F_4 males, one was sterile and the progeny sex ratios of two did not differ from 1:1. No male-biased progenies were observed. These results are consistent with X-linked inheritance of the drive element and replacement of Y^m with a non-modifying, drive-susceptible Y inherited from male 3 (Figure 2A).

A second set of crosses involving three *C. dalmanni* population stock males each mated to several carrier females provided further evidence for Y-linked modification (Figure 4A). One of the three males produced a male-biased sex ratio (0.58, $n = 212$, $\chi^2 = 4.5$, $P < 0.05$), and the other two produced unbiased sex ratios. We predicted that half of the sons from the male-biasing male (X^dY^m) should produce unbiased sex ratios, while half should produce male-biased sex ratios. We found that nine of the 36 sons produced significantly male-biased sex ratios (Figure 4B). This apparent deviation from the expected 1:1 ratio of male-biasing and non-biasing sons is due to small progeny sample sizes. Because the magnitude of the deviation from a 1:1 sex ratio for male-biasing genotypes is small, large sample sizes are required to detect biased sex ratios. Therefore, as an alternative approach, we ranked the 36 test males by sex ratio and found that the mean proportion of sons produced by the top 18 males was 0.59 ± 0.01 while that of the lower 18 males was 0.52 ± 0.01. Note that the sex ratio of the top 18 males does not differ from the sex ratio of their father (see above and Figure 4B), as would be expected for a Y-chromosome effect.

The recovery of male-bias in the sons of a male-biasing sire (X^dY^m) crossed with four carrier females (X^dX) (Figure 4B) further indicates that the male-biased phenotype requires both X^d and Y^m and rules out the possibility of a cytoplasmic modifier. Again, there was no evidence of heterogeneity among the sex ratios produced across the four females ($\chi^2 = 1.741$, d.f. = 3, $P = 0.628$). In contrast, the sons of the XY males produced either unbiased or female-biased sex ratios. For example, a single XY male by

FIGURE 1.—(A) Pedigree of crosses in *C. dalmanni* used to determine X-linked inheritance of meiotic drive element (X^d). Squares, males; circles, females. Half filled and completely filled symbols indicate individuals heterozygous and homozygous for X^d, respectively. Histograms show the distribution of sex ratios (proportion males) produced by (B) F_1 and (C–F) F_2 males resulting from the corresponding crosses found in the pedigree. In this and subsequent figures, SR indicates the sex ratio of the parental male. Filled and open bars indicate $P < 0.05$ and $P > 0.05$, respectively, for chi-square tests of deviation from 1:1 sex ratio. Occurrence of dark and open bars for the same sex ratio is due to differences in progeny sample sizes. Due to sample size differences among males, our ability to detect deviations from 1:1 among sires with the same sex ratio differed. Variation in sex ratio among sons from single male-female crosses indicates the presence of autosomal modifiers of X^d expression.

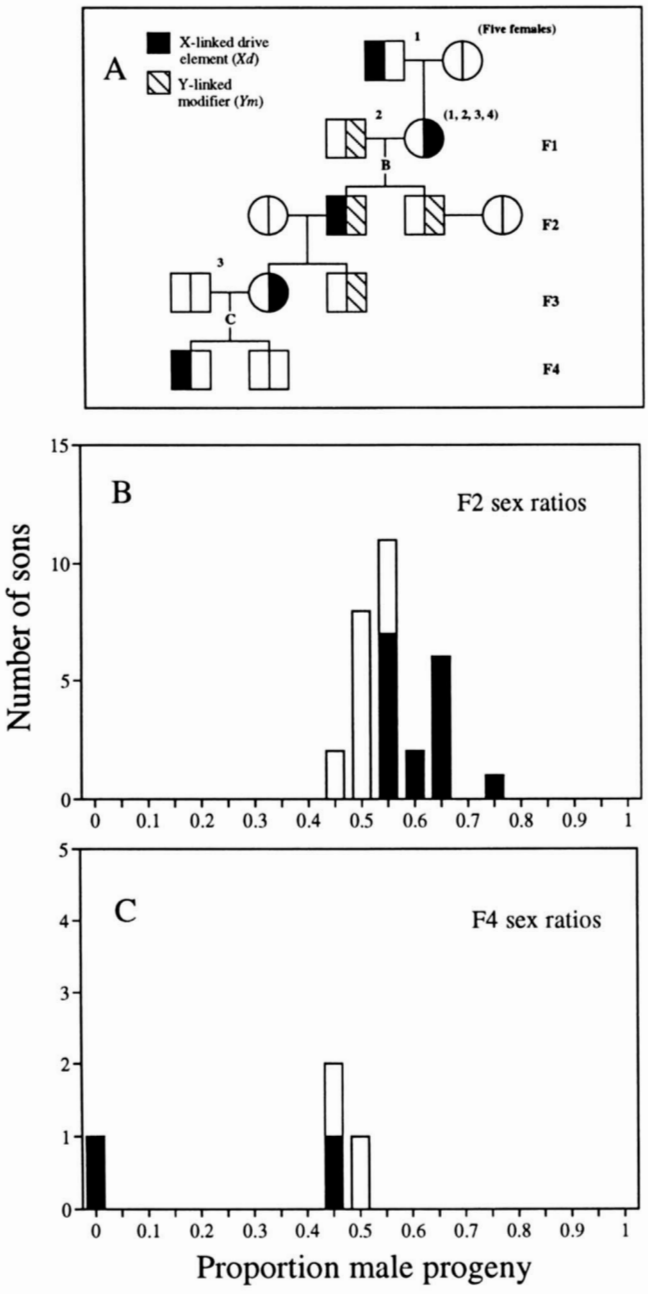


FIGURE 2.—(A) Pedigree of crosses in *C. dalmanni* demonstrating modification of X^d expression by a *Y*-linked modifier (Y^m). Symbols as in Figure 1 with the exception that hatched rectangles in male symbols indicate the modifying *Y* chromosome (Y^m). Histograms show the distribution of sex ratios produced by (B) F_2 and (C) F_4 males resulting from the corresponding crosses found in the pedigree. (B) Bimodal distribution of sex ratios is a result of the effect of Y^m when paired with *X* (unbiased sex ratios) and X^d (male-biased sex ratios). (C) Recovery of female bias in F_4 males demonstrates that the same X^d chromosome causing male-biased sex ratios in F_2 males causes female-biased sex ratios when paired with a susceptible *Y* chromosome.

X^dX female cross produced nine unbiased, 12 strongly female-biased, and two intermediate female-biased sex ratio sons (Figure 4C). Significant sex ratio phenotypes >0.10 and <0.50 in sons produced from a single male-female cross indicate that autosomal loci affect X^d expression.

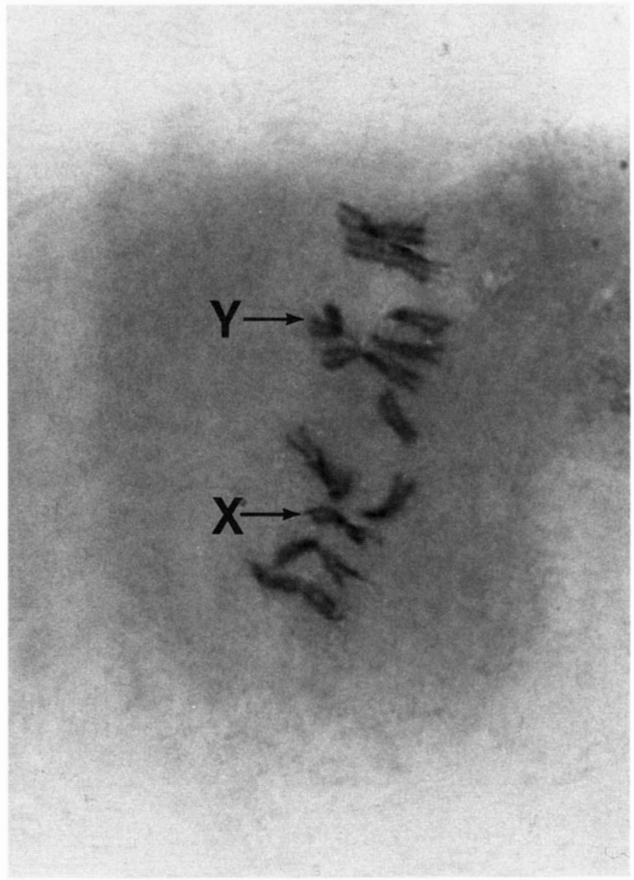


FIGURE 3.—Karyotype of a *C. dalmanni* F_1 male (XY^m) produced by a male-biasing (X^dY^m) sire. Five pairs of autosomes are present with heteromorphic *X* and *Y* chromosomes indicated ($2n = 12$).

Spermiogenesis in male carriers of X^d: We found that spermiogenesis in *C. dalmanni* is generally similar to that in Drosophila. Maturation of 128 spermatids per bundle occurs within two cyst cells, where spherical postmeiotic nuclei are transformed into the elongate heads of the spermatids. After elongation, individualization begins at the head region and extends down the length of the sperm bundle until individual spermatids are released from the receding cyst cells (Figure 5A). During individualization, malformed or degenerate sperm heads may be observed in two general forms: (1) sperm heads in degenerate bundles appear to deteriorate before the spermatids are released from the bundle (Figure 5, B and C) and (2) sperm heads in degenerate bundles appear overextended forming rows of chromatin "dots" (Figure 5C; see also HAUSCHTECK-JUNGEN and MAURER 1976). We scored the number of sperm bundles that showed normal development and those that were degenerate for five *XY*, three X^dY, and two X^dY^m males (Figure 6).

We discovered that *C. dalmanni* males are sperm heteromorphic, that is, two discrete size classes of sperm

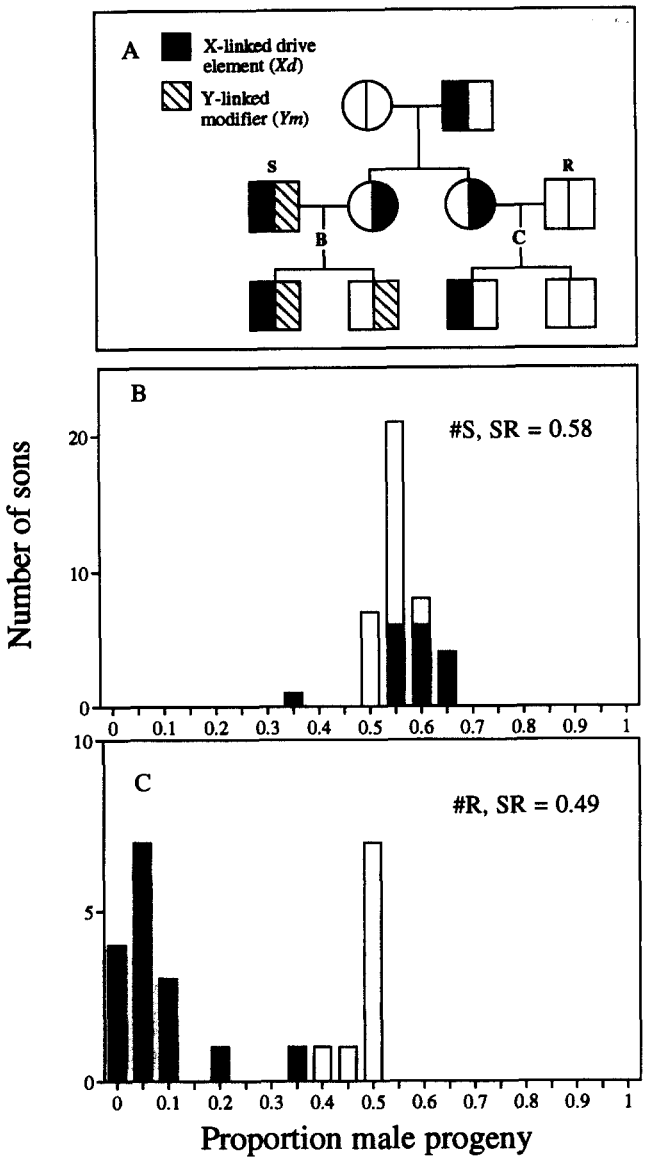


FIGURE 4.—(A) Pedigree of crosses in *C. dalmanni* demonstrating modification of the expression of a single X^d chromosome by Y^m and autosomal factors. Symbols as in Figures 1 and 2. (B) Histogram showing sex ratios produced by sons resulting from a cross between the male-biasing sire S (X^dY^m) and a heterozygous female (X^dX) demonstrating that both X^d and Y^m are necessary to cause male-biased sex ratios and that Y^m has no effect on sex ratio when paired with a standard *X*. (C) Variation in sex ratios produced by sons resulting from a cross between the non-biasing sire R (*XY*) and a heterozygous female demonstrate autosomal modification of X^d.

occur within the testes of individual males, with individual bundles of sperm giving rise to only one morph of sperm. To determine if the number of degenerate sperm differ by genotype we used repeated measures analysis of variance since counts of short and long sperm bundles and counts of normal and degenerate sperm bundles within subject males are nonindependent. We found that there are significantly more long sperm (247.1 ± 48.8) than short sperm (25.8 ± 4.5) for all males (Table 2), with long bundles comprising 89.8% of all sperm bundles produced (Figure 6). Genotype has no effect on the total number of sperm bundles in the testes of individual males (Table 2), and the absence of a sperm bundle morph by genotype interaction further indicates that males differing in sex ratio genotype produce similar numbers of short and long sperm (Table 2). However, the highly significant sperm degeneracy by genotype interaction (Table 2) indicates that X^d affects sperm degeneration (Figure 6). The significant three-way interaction (Table 2) occurs because there were more degenerate long sperm bundles in X^dY males (Figure 6A), but sex ratio genotype had no effect on the number of short sperm bundles that were degenerate (Figure 6B). A much greater proportion of short (67%; Figure 6B) than long (22%; Figure 6A) sperm bundles were degenerate across all genotypes.

Together, these results suggest that short sperm do not influence sex ratio. Therefore, to determine if sperm degeneration explains sex ratio phenotype, we regressed the absolute deviation of sex ratio on the proportion of degenerate long sperm bundles (arcsine transformed). The resulting highly significant regression (Figure 7; $F_{1,7} = 68.4$, $r^2 = 0.91$, $P < 0.0001$) suggests that X^d causes degeneration of long sperm, while sex ratio modification by Y^m occurs by reducing the sperm degeneration effect and restoring normal sperm development.

DISCUSSION

Sex ratio distortion in stalk-eyed flies is caused by a driving *X*-linked factor that disrupts the transmission of *Y*-bearing spermatids. The meiotic drive system described here is unique in several respects. First, in contrast to most Drosophila systems, the rare sons produced by strongly female-biasing sires are fertile, suggesting either that they are not the products of nondisjunctive sperm (*i.e.*, lacking a *Y*) or that *XO* males are fertile in *C. dalmanni* and *C. whitei*. Second, there is extraordinary variation in sex ratio phenotypes among males. In *C. dalmanni*, this is due to the dual action of *Y*-linked and autosomal modifiers that alter the expression of the driver. In this respect, the modification system of *C. dalmanni* is superficially similar to that described for *D. paramelanica* (STALKER 1961) and *D. mediopunctata* (CARVALHO and KLACZKO 1993; CARVALHO and KLACZKO 1994; CARVALHO *et al.* 1997). Third, the modifying *Y* chromosome (Y^m) of *C. dalmanni* has no obvious phenotypic effects on the sex ratio unless it is coupled with a driving *X*, in which case it is not only resistant to meiotic drive, but reverses the direction of drive, resulting in the production of male-biased sex ratios. Unlike the male-biasing sires in *D. affinis* (VOELKER 1972), *C. dalmanni* sires producing male-biased progenies are not *XO* (Figure 3). Finally, genetic analysis of the drive element and its modifiers reveals a polymorphism for the *X*-linked driver, the resistant *Y* chromosome, and modifying autosomal loci.

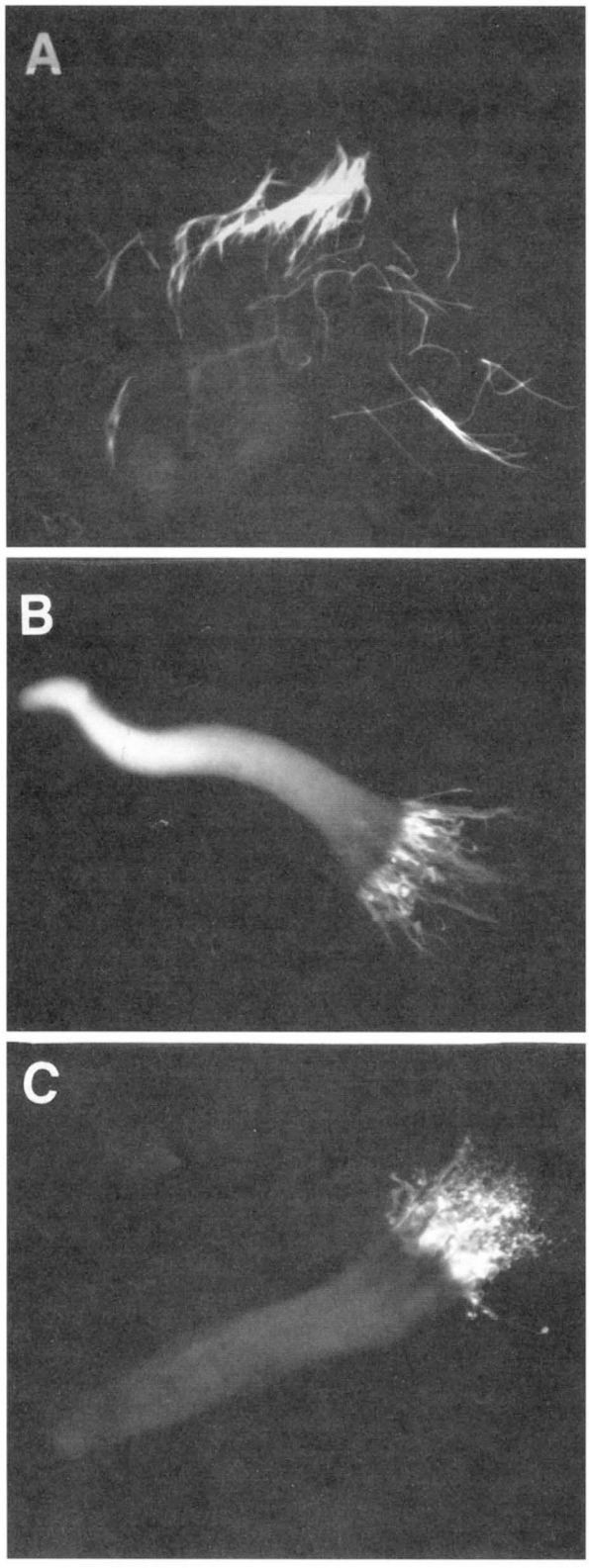


FIGURE 5.—*C. dalmanni* sperm bundles showing the effects of *X* and X^d on spermiogenesis. (A) Sperm bundle from *XY* male during late development and individualization of normal spermatids. (B) Sperm bundle from X^dY male showing degeneration of approximately half of spermatids. (C) Sperm bundle from X^dY male showing complete degeneration of all spermatids.

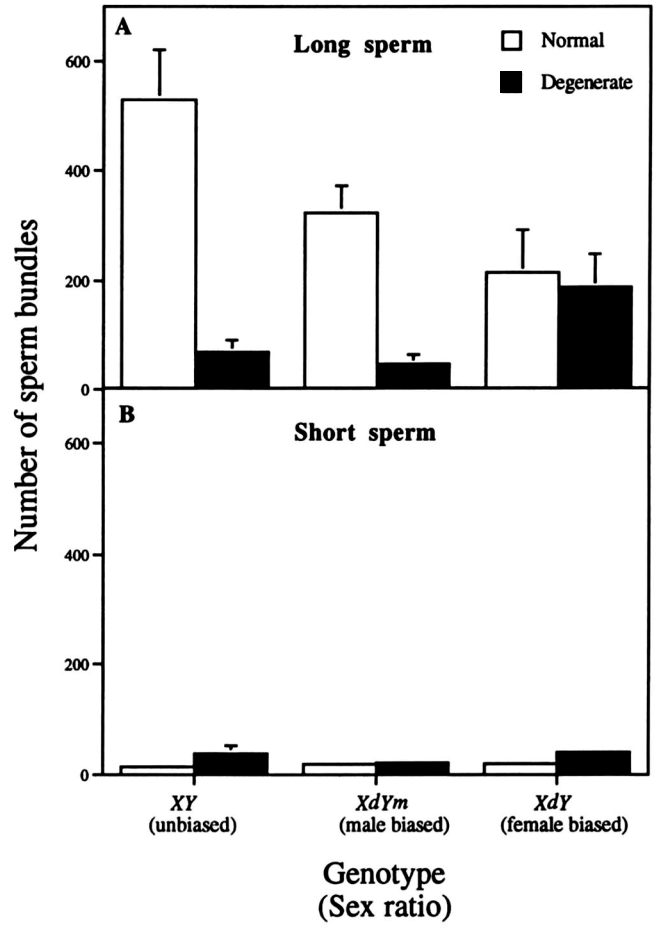


FIGURE 6.—Effect of sex ratio genotype on number (±SE) of normal and degenerate mature sperm bundles in testes for (A) long and (B) short sperm.

Mechanism of meiotic drive and the effects of X^d on male reproductive success: We found significant differences in the number of sperm bundles containing malformed or degenerate spermatids between sex ratio genotypes. We presume that the vast majority of spermatids lost in X^dY males are *Y*-bearing, but this interpretation is complicated by the fact that in 66% of degenerate bundles in X^dY males all spermatids were affected (Figure 5C). Since each bundle of sperm contains the meiotic products of a single diploid spermatogonium (assuming similarity with Drosophila spermatogenesis; for review see LINDSLEY and TOKUYASU 1980), both *X*- and *Y*-bearing sperm must be affected in bundles exhibiting complete degeneration. Similar patterns were observed for spermiogenesis in the *SR* system of the *D. subobscura* (HAUSCHTECK-JUNGEN and MAURER 1976).

General models of meiotic drive typically require a *trans*-acting drive element that codes directly or indirectly for a product that binds to the *cis*-acting target locus and somehow inhibits chromatin condensation and proper spermatid packaging (for review see TEMIN *et al.* 1991). The counter-drive effect of Y^m suggests either an active conditional response mechanism, or a

TABLE 2

Repeated measures ANOVA of the effects of sex ratio genotype and sperm morph on the number of normal and degenerate sperm bundles in *C. dalmanni*

Source	d.f.	Mean squares	F (MS_s/MS_e)
a. Sex ratio genotype	2	13,895	0.98 (a/b) (NS)
b. Subject	7	14,233	
c. Sperm morph	1	360,924	31.49 (c/e)***
d. Sperm morph × genotype	2	13,493	1.18 (d/e) (NS)
e. Sperm morph × subject	7	11,461	
f. Degenerate	1	124,932	29.99 (f/h)***
g. Degenerate × genotype	2	43,659	10.48 (g/h)**
h. Degenerate × subject	7	4,166	
i. Degenerate × sperm morph	1	159,519	30.29 (i/k)***
j. Degenerate × genotype × sperm morph	2	33,882	8.52 (j/k)*
k. Degenerate × genotype × subject	7	5,267	

NS, not significant. * $P < 0.025$. ** $P < 0.01$. *** $P < 0.001$.

passive-resistant Y coupled with a self-detrimental driving X. For example, Y^m may be activated by a product of X^d, and once activated causes counter-drive. However, a more likely alternative is that for X^dY males X^d may cause complete elimination of the Y and, as a side effect, simultaneously suffer some self-inflicted X^d chromosome loss. In this scenario all drive-susceptible Y chromosomes and some fraction of X^d chromosomes are eliminated, resulting in the production of extremely female-biased progenies. This second scenario is consistent with the observed degeneration of entire sperm bundles in X^dY males. However, when X^d is coupled with a strictly resistant Y^m chromosome, it may still suffer self-inflicted degeneration resulting in a net transmission advantage for Y^m. This model is consistent with the intermediate proportion of degenerate long sperm observed in male-biasing X^dY^m males (Figure 6).

High rates of promiscuity and remating in both sexes of *C. dalmanni* and *C. whitei* are likely to magnify the effects of reduced sperm production in male carriers of X^d by increasing the rate of sperm depletion caused by rapid remating and reducing sperm competitive ability since sperm number is likely to be an important determinant of fertilization success (PARKER 1982). Both species form nocturnal roosting aggregations on root threads that hang from the eroded banks of small streams, and at dusk dominant males vigorously defend roosting sites with between one to 24 females per site for *C. whitei* and one to 15 females per site for *C. dalmanni*. As many as 90% of all copulations take place in these aggregations during a brief 1-hr period at dawn, and individual male *C. whitei* have been observed to mate sequentially up to 24 times in that 1-hr period (LORCH *et al.* 1993). Females can store sperm for up to 30 days (LORCH *et al.* 1993; unpublished data), which creates the opportunity for sperm competition. Interestingly, fertility selection against male carriers of the driver is expected to increase with the frequency of the driver in the population (JAENIKE 1996). This negative frequency-dependent selection against X^d occurs because the average rate of male mating, and thus the likelihood of sperm depletion, increases as the population becomes increasingly female-biased.

Specific predictions regarding the effects of X^d on male fertility and sperm competitive ability are complicated by the discovery of sperm heteromorphism in *C. dalmanni* and *C. whitei*. One study of spermatogenesis in the *SR* system of *D. subobscura* reported that *SR* males do not suffer reduced fertility despite loss of *Y*-bearing long spermatids because short sperm morph production is increased (BIRCHER *et al.* 1995). However, this conclusion requires that short sperm are capable of fertilization and is at odds with the findings of SNOOK *et al.* (1994), who have shown that only long sperm morphs successfully penetrate eggs in *D. pseudoobscura*. In *C. dalmanni*, short sperm bundles exhibited relatively high levels of degeneracy for all sex ratio genotypes in contrast to the pattern of degeneracy observed for long sperm bundles (Figure 6, A and B). Our data, therefore, strongly suggest that short sperm are not involved in fertilization in *C. dalmanni*. Remarkably, the finding that X^d affects long, but not short, sperm morph production in *C. dalmanni* is paralleled in the *D. subobscura* system: approximately half (55%) as many long sperm bundles reach individualization in *SR* males as non-*SR* males, but individualization of short sperm bundles is unaffected (see Table 1 in BIRCHER *et al.* 1995). Thus, the presence of sperm heteromorphism and the relative sensitivity of each sperm morph to meiotic drive appear to have evolved independently in *C. dalmanni* and *D. subobscura*.

Factors maintaining sex-linked meiotic drive polymorphisms: The evolution of a complex modification system, and the presence of X^d in *C. whitei* and *C. dalmanni*, but not in closely related species (G. S. WILKINSON, D. C. PRESGRAVES and L. CRYMES, unpublished results), both suggest an ancient origin and long-term persistence of the *X*-linked polymorphism. Such poly-

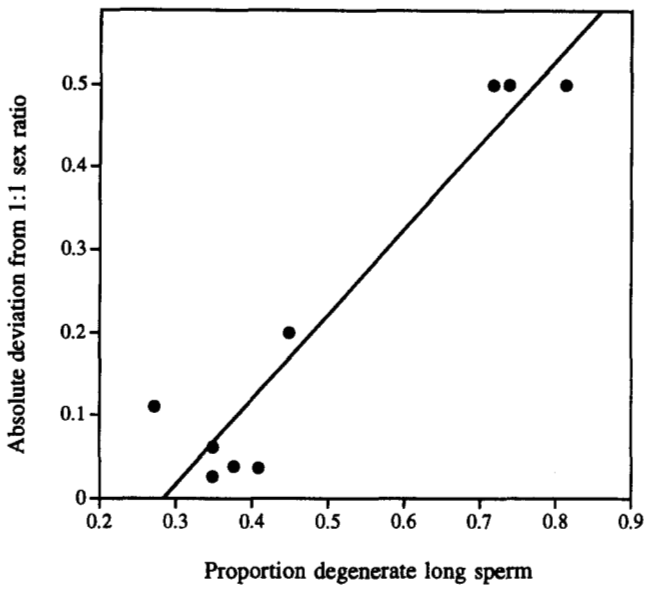


FIGURE 7.—Absolute deviation from a 1:1 sex ratio regressed on the proportion of degenerate long sperm (arcsine-transformed) ($y = 1.025x - 0.290$). The absolute deviation from 1:1 is calculated as the absolute value of the expected proportion male (0.5) minus the observed proportion male progeny.

morphism at the drive locus and autosomal modifier loci may not be unusual, but *Y*-chromosome polymorphism for drive modification within a single population is unexpected (WU 1983c; CLARK 1987). In *D. paramelanica*, *Y* chromosomes that differed in their resistance to driving *X*'s had different geographic origins possibly reflecting adaptation to local drive elements (STALKER 1961). All alleles under consideration in this study originated from a single geographic region suggesting (1) that the population is not at equilibrium and the observed allele frequencies are transient, and/or (2) that the *Y* polymorphism is maintained by a balance between drive resistance and viability or fertility selection. Theoretical studies indicate that the conditions for the stable or unstable maintenance of *Y*-linked polymorphisms are extremely restrictive even with meiotic drive and selection acting simultaneously (CLARK 1987). However, in addition to *C. dalmanni*, polymorphism for *Y*-linked modification of drive has also been reported in *D. mediopunctata* (CARVALHO and KLACZKO 1994; CARVALHO *et al.* 1997), indicating that *Y*-linked polymorphism may be more common than expected.

The forces involved in the origin and maintenance of such *Y*-linked polymorphisms may be revealed by consideration of a more well understood meiotic drive system. The target locus, *Responder*, in the autosomal *SD* system of *D. melanogaster* consists of highly repetitive satellite DNA in which susceptibility to drive is positively related to copy number (for review see TEMIN *et al.* 1991). The copy number mutation rate of repetitive DNA is often extremely high (CABOT *et al.* 1993), and it is not unlikely that target loci on the characteristically heterochromatic *Y* are similarly composed of such highly repetitive sequences. Taking these high mutation rates into consideration, it is possible that *Y*-linked repeat copy number polymorphism may exist as a balance between mutation, selection and meiotic drive. Previous theoretical work on *Y*-linked polymorphism, which considered selection and meiotic drive simultaneously, did not consider such high recurrent mutational input (CLARK 1987).

The selective factors maintaining polymorphism for *Y*-linked modification of drive in *C. dalmanni* are probably complex. The *Y*-linked modifier may have deleterious pleiotropic effects (see WU *et al.* 1989), or it may be permanently linked with other deleterious mutations since there is generally no opportunity for recombination on the *Y*. Furthermore, the fitness of the *Y*-linked modifier relative to susceptible *Y* chromosomes can be broken down into two frequency-dependent components. First, since Y^m has no on the sex ratio effects unless it co-occurs with X^d, its transmission advantage via drive-resistance and counter-drive declines with decreasing frequency of X^d. Second, the counter-drive effect of Y^m produces a male-biased sex ratio that is advantageous for individuals in the female-biased populations of stalk-eyed flies, but as X^d decreases in frequency the population sex ratio becomes less female-biased and the advantage of producing male-biased progeny diminishes.

Finally, while our discussion has focused primarily upon the interplay of *X* and *Y* chromosomes, this simple two chromosome system does not sufficiently account for all of the variation in observed sex ratios. Our results provide strong evidence for autosomal modifiers in both *C. dalmanni* and *C. whitei*, but much future work will be required to map these elements and characterize their effects on sex ratio.

Coevolution of meiotic drive and exaggerated eyespan: Of particular interest in the case of sexually dimorphic stalk-eyed flies is the impact of the *X*-linked drive element on the operational sex ratio (OSR) and the intensity of sexual selection. Classical theory predicts that intensity of sexual selection on males increases as the OSR becomes male-biased (EMLEN and ORING 1976). However, contrary to this prediction, female water striders, *Gerris odontogaster*, modify their mating behavior in relation to the OSR in such a way that reduces, rather than increases, the intensity of sexual selection as the OSR becomes increasingly male-biased (ARNQVIST 1992a,b). These studies suggest that the relationship between sex ratio and sexual selection intensity may not always be straightforward.

In the Cyrtodiopsis clade, sex ratio distortion occurs in *C. dalmanni* and *C. whitei* but not in the outgroup species *C. quinqueguttata* (G. S. WILKINSON, D. C. PRESGRAVES and L. CRYMES, unpublished results) and *Teleopsis quadriguttata* (G. S. WILKINSON, unpublished data), which are both sexually monomorphic for eye-

span. Thus, X^d may have evolved before the divergence of *C. dalmanni* and *C. whitei*. The resulting increased production of females is likely to increase the intensity of sexual selection in stalk-eyed flies if males of large eyespan are able to monopolize disproportionately greater numbers of females per aggregation, thus leading to an increased positive covariance between eyespan and reproductive fitness. In agreement with this model, the frequency of X^d, the degree of female-bias in natural populations (BURKHARDT and DE LA MOTTE 1983), the mean aggregation size (WILKINSON and REILLO 1994), and the degree of sexual dimorphism are all greater in *C. whitei* than *C. dalmanni* (BURKHARDT and DE LA MOTTE 1985). Since these two species are sympatric, these trends cannot be explained by environmental differences in microhabitat quality or predation. Preliminary screening of two African species of stalk-eyed flies, *Diasemopsis sylvatica* and *D. dubia*, further supports the association between the presence of sex ratio distortion and sexual dimorphism. These patterns suggest that the morphological evolution of these extremely elaborated secondary sexual ornaments may, in part, be influenced by the presence of a selfish genetic element.

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