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SEX-LINKED EXPRESSION OF A SEXUALLY SELECTED TRAIT IN THE STALK-EYED FLY, *CYRTODIOPSIS DALMANNI*

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Abstract.—Recent theoretical and empirical work has suggested that the X chromosome may play a special role in the evolution of sexually dimorphic traits. We tested this idea by quantifying sex chromosome influence on male relative eyespan, a dramatically sexually selected trait in the stalk-eyed fly, *Cyrtodiopsis dalmanni*. After 31 generations of artificial sexual selection on eyespan:body length ratio, we reciprocally crossed high- with low-line flies and found no evidence for maternal effects; the relative eyespan of F_1 females from high- and low-line dams did not differ. However, F_1 male progeny from high-line dams had longer relative eyespan than male progeny from low-line dams, indicating X-linkage. Comparison of progeny from a backcross involving reciprocal F_1 males and control line females confirmed X-linked inheritance and indicated no effect of the Y chromosome on relative eyespan. We estimated that the X chromosome accounts for 25% (SE = 6%) of the change in selected lines, using the average difference between reciprocal F_1 males divided by the difference between parental males, or 34%, using estimates of the number of effective factors obtained from reciprocal crosses between a high and low line. These estimates exceed the relative size of the X in the diploid genome of a male, 11.9% (SE = 0.3%), as measured from mitotic chromosome lengths. However, they match expectations if X-linked genes in males exhibit dosage compensation by twofold hyperactivation, as has been observed in other flies. Therefore, sex-linked expression of relative eyespan is likely to be commensurate with the size of the X chromosome in this dramatically dimorphic species.

Key words.—Artificial selection, Diopsidae, dosage compensation, sexual dimorphism, X-linkage.

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Theory and evidence (Bull 1983; Charlesworth 1991) indicate that sex chromosomes are derived from a homologous pair of chromosomes, one of which acquired a sex-determination factor. Selection to reduce recombination between sex-determination and sex-specific sterility factors creates conditions that result in loss of function and eventual degeneration of genes on the Y chromosome (Rice, 1994, 1996; Charlesworth 1996). Loss of function in a Y-linked gene selects for dosage compensation of the X-linked homologue (Charlesworth 1996, 1998; Jegalian and Page 1998). Sex chromosome evolution has considerable consequences for the evolution of sexually dimorphic traits because the rate of evolution of sexual dimorphism depends on the degree to which genes on the sex chromosomes influence the trait (Rice 1984; Charlesworth et al. 1987; Reinhold 1999; Rhen 2000), as well as the intensity of sexual selection and the magnitude of the genetic correlation between the sexes (Lande 1980).

When sexual selection favors trait expression in males, but not females, recessive alleles at X-linked loci that affect sexual dimorphism evolve more rapidly (Rice 1984; Charlesworth et al. 1987) and are more likely to persist relative to alleles at autosomal loci under conditions of fluctuating selection (Reinhold 1999). These factors should cause sex chromosomes to have a disproportionate effect on sexually dimorphic traits. In accordance with this prediction, sex chromosomes have been found to influence sexually dimorphic traits in some species (Ewing 1969; Bentley and Hoy 1972; Grula and Taylor 1980; Carson and Lande 1984; Roelofs et al. 1987; Houde 1992; Shaw 1996; Lardon et al. 1999; Saifa and Chandra 1999; Ritchie 2000) but not in others (Henry 1985; Tomaru and Oguma 1994; Butlin 1996; Pugh and Ritchie 1996). A recent review (Reinhold 1998) indicates that traits under sexual selection are influenced more by the X chromosome than are traits under natural selection. However, this conclusion should remain provisional because it rests largely on studies of *Drosophila* and Orthoptera, and few of these studies have tested both types of traits in the same species. Because relative X chromosome size can differ between species, comparison of X-linked effects on traits under natural or sexual selection across species potentially confounds relative chromosome size with form of selection.

Thus, to conclude that the X chromosome has a special role in the evolution of sexually selected traits requires demonstrating that genes on the X disproportionately influence the expression of a trait relative to an appropriate null hypothesis. One null hypothesis is whether the effect of the X is proportional to its relative size in the genome, as would be expected if trait expression is influenced by multiple genes of equal and additive effects. For example, in the tettigoniid bushcricket (*Ephippiger ephippiger*), the inheritance of syllable number for male song is disproportionately affected by the X chromosome because the X accounts for 25% of the variation between song races and this species has 15 equally sized chromosome pairs (Ritchie 2000).

Eyespan exhibits sexual dimorphism and has been shown to be under sexual selection in the stalk-eyed fly, *Cyrtodiopsis dalmanni*, and related species (Burkhardt and delaMotte 1985; Wilkinson and Dodson 1997). Males with larger eyespan displace other males (Panhuis and Wilkinson 1999) and gain preferential mating access to groups of roosting females. In addition, females preferentially choose to mate with males with large eyespan (Wilkinson et al. 1998a). Under bidirectional artificial selection for high and low relative eyespan (i.e., the ratio of eyespan to body length) male bias in progeny sex ratios increased in concert with increases in relative eyespan (Wilkinson et al. 1998b). Female-biased progeny sex

ratios occur in this species due to X chromosome meiotic drive (Presgraves et al. 1997). Recent theory (Lande and Wilkinson 1999; Reinhold et al. 1999) shows that X chromosome meiotic drive can accelerate the evolution of female choice and an ornamental trait if genes that increase the expression of the ornament reliably indicate the nondriving X chromosome. These models predict, therefore, X-linked genetic effects on male eyespan.

Accordingly, here we test whether the sex chromosomes influence change in eyespan in *C. dalmanni*. We used reciprocal crosses between replicate lines of flies that had undergone 31 generations of artificial sexual selection for high or low relative eyespan (Wilkinson 1993; Wilkinson *et al*. 1998b). Male F_1 progeny of a reciprocal cross are identical with respect to autosomes, but differ at the sex chromosomes, that is, either $X_{high}Y_{low}$ or $X_{low}Y_{high}$. Thus, the mean difference between these two types of male progeny can be compared to the mean difference between the parental males to quantify the net effect of the sex chromosomes on relative eyespan (e.g., Carson and Lande 1984). We also estimated the minimum number of genes contributing to the difference in eyespan between the selected lines, with and without the effects of the sex chromosomes, using trait distributions from parental, F_1, and F_2 flies (Lande 1981). We used both of these estimates to test whether the observed effect of the X chromosome was larger than expected if the effects of loci are distributed evenly throughout the genome as measured from mitotic chromosome preparations. This analysis also provides insight into the mechanism of dosage compensation. Most X-linked genes in male *Drosophila* undergo twofold hyperactivation to maintain equal expression between males and females (Baker et al. 1994; Meller 2000). Dosage compensation by X-linked hyperactivation in males predicts, therefore, that the X chromosome effect should equal twice its haploid length relative to the diploid genome, whereas absence of dosage compensation in males predicts that the X chromosome effect will equal its haploid length relative to the diploid genome.

Materials and Methods

Reciprocal Cross: Sex Chromosome or Maternal Effect

F_1 male progeny from a reciprocal cross between two selected lines differ in sex chromosome composition depending on the line origin of the sire and dam, whereas the autosomes come equally from both lines. Thus, any difference between reciprocal F_1 male or female progeny provides evidence for either a sex chromosome or a maternal effect. Maternal effects can be distinguished from X-linked effects by comparing F_1 females, because they cannot differ due to X-linked effects but they can differ due to cytoplasmic factors or other maternally inherited differences.

We used single-pair matings to create four pairs of reciprocal crosses involving each of the two high and low lines that had undergone 31 generations of artificial selection to increase or decrease the ratio of eyespan to body length (Wilkinson 1993; Wilkinson *et al*. 1998b). We replicated each of the eight crosses twice. The 16 pairs of flies were housed in 1-L plastic containers with nylon mesh covering the opening and moist cotton lining the bottom. A 100-ml cup with approximately 50 g of pureed corn was provided for food and as an oviposition site. All food used in this experiment was obtained from a single batch of pureed corn. We replaced food cups twice a week. Larvae were reared at 25 ± 1°C in the 100-ml cups (Wilkinson 1993). We transferred pupae to moist cotton in 500-ml cups. After eclosion, we froze adult flies at −20°C until morphological measurement. We measured eyespan as the distance between the outer edges of the eye bulbs and body length as the maximum distance between the head and wing tip. We used NIH Image version 1.59 to obtain measurements from digitized video images with a resolution of 50 pixels/mm (available at http://rsb.info.nih.gov/nih-image). For each pair of flies we measured a mean of 33 (SE = 5) male and 36 (±6) female progeny. Mean larval density was 16 individuals (±3) in the 85 larval cups collected.

F_1 Male x Control Line Female Backcross: X or Y Effect

To distinguish X-linked from Y-linked effects, we mated F_1 reciprocal cross males to control-line females. In progeny from this backcross, half of the autosomes come from the control line and half represent a random mixture of high and low lines due to segregation and recombination in the F_1 generation. However, depending on the source of the F_1 male's sire, the Y chromosome differs in male progeny and a single X chromosome differs in female progeny. The F_1 males came either from a cross involving a high-line male and a low-line female or from a cross involving a low-line male and a high-line female. Control-line females were collected from seven pairs of reciprocal crosses between the two replicate control lines. We used three replicates for each male source and mated each of the nine males to three control line females. Males and females were housed and bred as described above for the reciprocal crosses, except that a single batch of food was used each week. Measurements were obtained on digitized video images using Measurement TV version 3.0 (Garr Updegraff, San Clemente, CA) with an image resolution of 100 pixels/mm. For each pair, we measured a mean of 70 (±11) male and 60 (±11) female progeny. Larval densities averaged 45 individuals (±6) for the 37 food cups collected.

Statistical Analysis

We used relative, rather than absolute, eyespan in all analyses because artificial selection was exerted on relative eyespan. We analyzed the high- × low-line reciprocal cross data with a three-factor nested analysis of covariance in which source of sex chromosome, high-line replicate, and low-line replicate were factors and nesting was by pair. Because larval density is known to influence eyespan (David et al. 1998), we included larval density as a covariate. This analysis effectively controlled for larval density effects because the results did not change when the data were split into high (> 25 individuals/cup) and low (< 25 individuals/cup) larval density subsets and analyzed separately. For the F_1 male × control-line female backcross, we used a single factor nested analysis of covariance with larval density as a covariate. Offspring from the two sources of F_1 males from low-line sires did not differ morphologically, and these data were combined

into a single category with six replicates (three replicates from each source). Analyses were performed with the combined dataset as well as on each replicate. Results did not differ, so only the results from the combined dataset are presented.

Reciprocal crosses between the high and low lines were also conducted at generation 14. Published analyses (Wilkinson 1993) combined data from the replicate lines and did not test for possible differences between the replicate selected lines. Therefore, we reanalyzed these data using a two-factor (source of sex chromosome, selected line replicate) analysis of variance.

All analyses were performed using SAS version 6.12 (SAS Institute, Inc., Cary, NC). We report means ± SE for all estimates with replication.

Magnitude of an X-Chromosome Effect

We used two methods to quantify the effect of the X chromosome on relative eyespan. We calculated the difference in relative eyespan between F_1 reciprocal-cross male progeny as a fraction of the difference in relative eyespan between the parental-line males. We obtained estimates from four pairs of reciprocal line crosses in generation 32 (H1-L1, H1-L2, H2-L1, H2-L2) and two pairs (H1-L1, H2-L2) in generation 14.

We also calculated the X-chromosome effect from estimates of the number of effective factors, a minimum estimate of the number of genes contributing to the variance of a quanititative trait. The number of effective factors was estimated from the variances in parental, F_1, and F_2 individuals (Lande 1981). Reciprocal crosses between two of the selected lines (H2 and L2) were used to generate F_1 and F_2 flies using breeding methods described above for experiment 1. To generate F_2 progeny, we used four F_1 males from reciprocal crosses between two selected lines. We pooled all F_1 males regardless of sire source to calculate F_1 variances and pooled all F_2 males or females to calculate F_2 variances. For males, we estimated the number of autosomal effective factors by standardizing the mean of F_1 males from each sire to zero, thereby eliminating any X-linked effect on the variance estimate. We estimated variances from 50 males and 25 females for each parental line, from 101 males and 125 females for the F_1 generation, and from 604 males and 608 females for the F_2 generation (Fig. 1). Whereas estimation of the absolute number of effective factors is known to provide a biased estimate of the absolute number of genes affecting a trait (Zeng et al. 1990; Zeng 1992), such biases should be independent of chromosome origin. Therefore, estimation of the relative number of factors influencing the X should provide a useful comparison to the relative difference between reciprocal F_1 trait means.

We tested whether the magnitude of the X-chromosome effect changed between generations 14 and 32 for each reciprocal cross (H1 × L1, H2 × L2) with *t*-tests and combined probabilities from these two tests to obtain an estimate of the overall significance associated with this comparison (Sokal and Rohlf 1981).

For generation 32, we tested whether the magnitude of the effect of the X chromosome on relative eyespan was greater than the relative size of the X in the male genome. We estimated X-chromosome size from pictures of mitotic chromosomes prepared by dissecting in PBS cerebral and ventral ganglia from well-fed, third-instar larvae. One percent sodium citrate was applied for 30 min to swell cells and then two to three drops of 1:1 lactic acid:acetic acid were placed on the tissues. Once the ganglia began to dissociate, slides were fixed in 3:1 methanol:acetic acid and stained with 2% Giemsa. We located anaphase chromosomes at 1000× under oil and used NIH Image version 1.59 to measure the relative length of each chromosome from seven larvae. We then used a Mann-Whitney *U*-test to compare whether the divergence between reciprocal crosses differed significantly from the relative size of the X chromosome, expressed as the length of one (haploid) X chromosome divided by the sum of doubled (diploid) autosome lengths plus haploid sex chromosome lengths (i.e., assuming no dosage compensation of X-linked genes in males). We also compared the X-linked effect to relative chromosome length expressed as 2X/2n, which assumes twofold hyperactivation of X-linked genes in males, as has been reported for many X-linked genes in *Drosophila melanogaster* (Baker *et al*. 1994) and for another Dipteran, *Sciara ocellaris* (Cunha et al. 1994).

RESULTS

Divergence between high- and low-selected lines occurred primarily due to change in eyespan. After 31 generations of selection, the average difference between high- and low-line males was 1.444 mm (±0.053) for eyespan and −0.155 mm (±0.028) for body length (Fig. 2). In females eyespan differed, on average, by 0.683 mm (±0.039) and body length by −0.080 mm (±0.028) between high and low lines.

Reciprocal Cross

The source of the sex chromosomes significantly affected the expression of relative eyespan in male progeny from crosses between the lines selected for high and low relative eyespan. After 13 generations of selection, male progeny with high-line X chromosomes had higher relative eyespan than males with low-line X chromosomes ($\bar{x}_{highX} = 1.266 \pm 0.003$, $\bar{x}_{lowX} = 1.242 \pm 0.003$; Table 1). The difference in relative eyespan between males with high- or low-line X chromosomes was due to change in eyespan ($\bar{x}_{highX} = 9.295 \pm 0.033$ mm, $\bar{x}_{lowX} = 9.135 \pm 0.035$ mm, $F_{1,190} = 10.7$, $P = 0.001$), but not body length ($\bar{x}_{highX} = 7.344 \pm 0.019$ mm, $\bar{x}_{lowX} = 7.357 \pm 0.020$ mm, $F_{1,190} = 0.23$, $P = 0.63$). Female progeny did not differ in morphology with respect to the source of the sex chromosomes except in body length after 13 generations of selection. Females with maternal high-line X chromosomes had significantly longer body length than females with maternal low-line X chromosomes ($\bar{x}_{highX} = 7.013 \pm 0.025$ mm, $\bar{x}_{lowX} = 6.916 \pm 0.025$ mm).

After 31 generations of selection, the differences in relative eyespan between F_1 males with high- or low-line X chromosomes were larger than after 13 generations (Table 2). Male progeny with high-line X chromosomes had mean relative eyespan of 1.298 ± 0.003 compared to 1.244 ± 0.002 for male progeny with low-line X chromosomes. These differences were primarily due to differences in eyespan ($\bar{x}_{highX}$

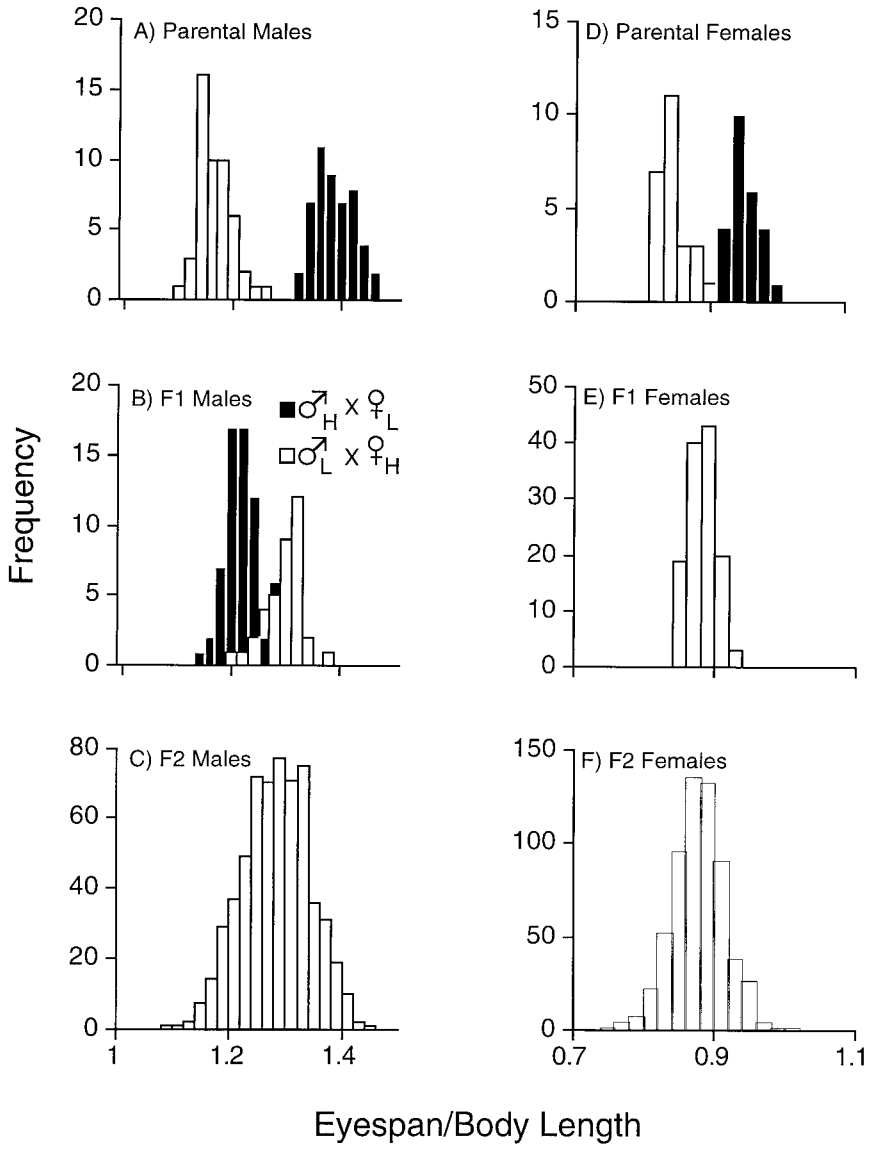

FIG. 1. Distributions of relative eyspan in male (A) and female (D) flies at generation 32 of artificial sexual selection for high (shaded bars) and low (open bars) eyespan to body length ratios. (B, E) F_1 progeny from crossing high- and low-selected lines. (C, F) F_2 progeny created from interbreeding F_1 individuals.

= 9.394 ± 0.027 mm, $\bar{x}_{lowX}$ = 9.098 ± 0.025 mm, $F_{1,11}$ = 8.6, P = 0.01) and to a lesser extent body length ($\bar{x}_{highX}$ = 7.235 ± 0.016, $\bar{x}_{lowX}$ = 7.315 ± 0.015, $F_{1,11}$ = 4.2, P = 0.07). There were no significant effects of the source of the X chromosome on relative eyespan in female progeny (Table 2). Density was not a significant covariate in the analysis of either male or female relative eyespan at generation 32.

Backcross

In the F_1 male × control-line female backcross, the source of the X chromosome significantly influenced relative eyespan of female progeny (Table 3). Female progeny with high-line X chromosomes had significantly higher ratios of eyespan to body length than females with low-line X chromosomes ($\bar{x}_{highX}$ = 0.889 ± 0.002, $\bar{x}_{lowX}$ = 0.866 ± 0.002). As in the reciprocal cross experiment, these differences were due to eyespan ($\bar{x}_{highX}$ = 5.836 ± 0.012, $\bar{x}_{lowX}$ = 5.648 ± 0.015, $F_{1,7}$ = 12.9, P = 0.009) and not body length ($\bar{x}_{highX}$ = 6.570 ± 0.013, $\bar{x}_{lowX}$ = 6.529 ± 0.017, $F_{1,7}$ = 0.5, P = 0.49). The source of the Y chromosome did not, however, have any effect on the relative eyespan of male backcross progeny (Table 3).

Magnitude of the X-Chromosome Effect

Mean difference in relative eyespan between F_1 males from reciprocal crosses in generation 14 was 0.025 ± 0.008 mm and in generation 32 was 0.055 ± 0.025 mm (Fig. 3). The average overall difference in male relative eyespan between the selected lines crossed in generation 14 was 0.174 ± 0.054 mm and in generation 32 was 0.224 ± 0.031 mm (Fig. 3). Therefore, the X chromosome accounted for 14 ± 5.5% at generation 14 and 25 ± 5.6% at generation 32 of the diver-

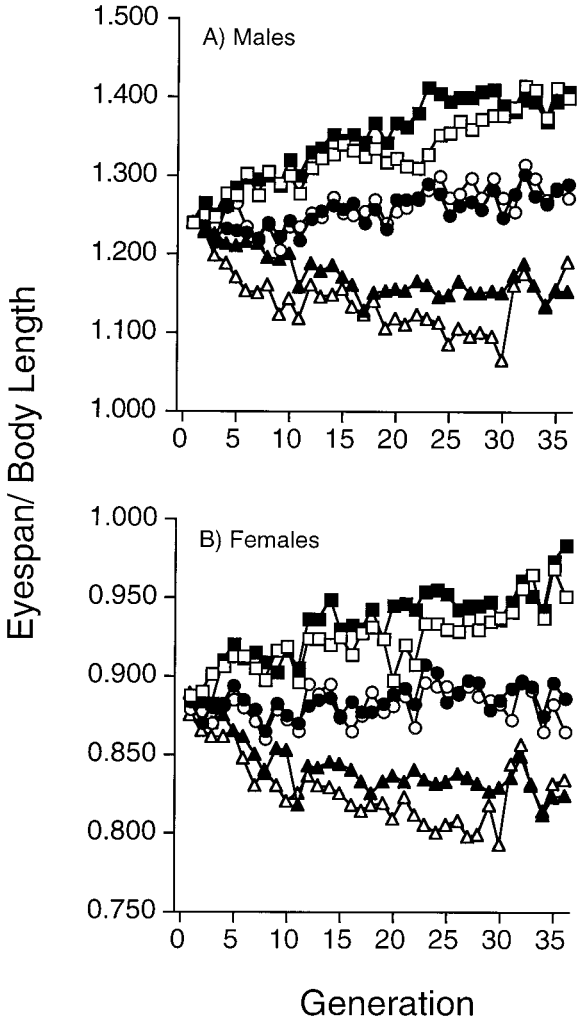

FIG. 2. Response of ratio of eyespan to body length to artificial sexual selection on males by generation in (A) males and (B) females. Squares, circles and triangles indicate response to selection pressure for high, control, and low ratios, respectively. Two replicate lines for each selection regime are shown with open and solid symbols.

gence in relative eyespan between the selected lines. Comparison of identical reciprocal crosses between generation 14 and 32 shows that the effect of the X chromosome differed significantly at these times (H1 × L1 vs. L1 × H1: $t_{61} = 4.56$, $P < 0.0001$; H2 × L2 vs. L2 × H2: $t_{34} = 3.23$, $P = 0.002$; combined probabilities: $\chi^2 = 33.0$, df = 4, $P < 0.0001$).

Preparations of mitotic chromosomes from larval ganglion cells revealed two autosomes and a pair of sex chromosomes (Fig. 4). Linear measurements indicated that the X chromosome is 11.9 ± 0.3% of the diploid *C. dalmanni* male genome. Using its relative size in a diploid genome, the X chromosome has a greater effect on relative eyespan expression than that expected if eyespan genes occur at random throughout the genome ($Z = 2.27$, $P = 0.023$, Mann-Whitney *U*-test). However, if dosage compensation occurs by hyperactivation of X-linked genes in males, the X would represent 20.7 ± 0.6% of the male genome and would not have a disproportionate effect on relative eye stalk expression ($Z = 1.13$, $P = 0.26$, Mann-Whitney *U*-test).

An independent estimate of the effect of the X chromosome on male relative eyespan is consistent with the estimate obtained using divergence between the reciprocal F_1 males compared to that between the selected lines. Using distributions of relative eyespan from reciprocal crosses involving a single pair of lines (H2 and L2), we calculated the minimum number of segregating genetic factors in males to be 4.1 ± 0.8 and in females to be 1.9 ± 0.3. After removing the effects of the X chromosome by using the within-family variance for F_1 males, the number of effective factors for male relative eyespan decreased significantly to 2.7 ± 0.3. Thus, the magnitude of the effect of the X chromosome on male relative eyespan between lines H2 and L2 based on effective factor estimates is 34%.

DISCUSSION

These results show that the X chromosome has a significant effect on the expression of relative eyespan in the stalk-eyed

TABLE 1. *F*-ratios for ANOVAs on relative eyespan in male and female progeny from high- × low-line reciprocal crosses after 13 generations of artificial sexual selection on male relative eyespan. Sire's sex chromosome (from high line or low line) and line replicate were treated as random effects in the ANOVA model ($r^2 = 0.23$ for males and 0.08 for females).

Source	df	Male (n = 193)	Female (n = 100)
Sire's sex chromosome	1	30.48***	0.23
Line replicate	1	27.99***	9.30**

** $P < 0.01$; *** $P < 0.0001$.

TABLE 2. *F*-ratios for nested ANCOVAs on relative eyespan in F_1 male and female progeny from high- × low-line crosses after 31 generations of artificial sexual selection on male relative eyespan. Sire's sex chromosome (from high line or low line), high-line replicate, low-line replicate, and pair replicate were treated as random effects in the ANCOVA model ($r^2 = 0.50$ for males and 0.10 for females).

Source	df	Male (n = 407)	Female (n = 452)
Sire's sex chromosome	1	52.70***	1.27
High-line replicate	1	4.35	4.85
Low-line replicate	1	0.002	0.03
Pair replicate (sex chr, high, low)	10	4.67***	2.92**
Larval density	1	0.0003	0.33

** $P < 0.01$; *** $P < 0.0001$.

TABLE 3. *F*-ratios for nested ANCOVAs on relative eyespan in male and female progeny from backcrosses of F_1 males to control-line females. Sire's sex chromosome (from high line or low line) and pair replicate were treated as random effects ($r^2 = 0.09$ for females and 0.04 for males).

Source	df	Male (n = 770)	Female (n = 659)
Sire's sex chromosome	1	0.03	80.40***
Pair replicate (sex chr)	7	3.12**	0.75
Larval density	1	7.20**	0.59

** $P < 0.01$; *** $P < 0.0001$.

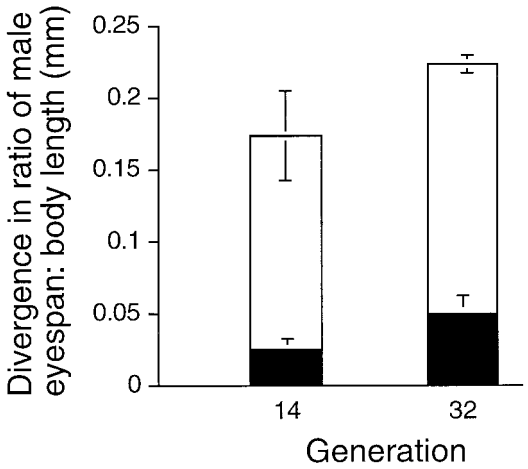

FIG. 3. Mean divergence in relative eyespan among selected lines (open bars) and between reciprocal line crosses (solid inset bars) at generation 14 and 32 of artificial sexual selection on males.

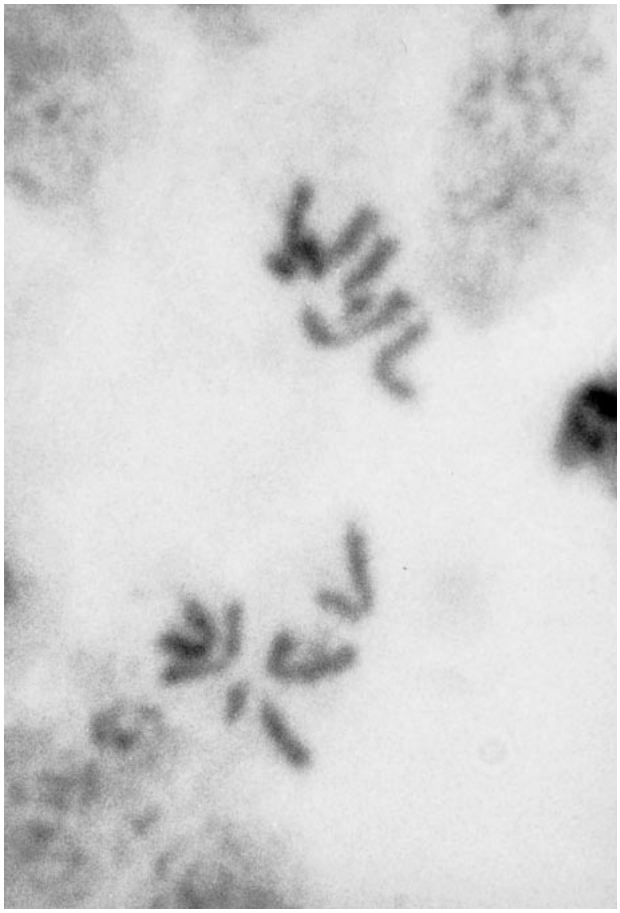

FIG. 4. Representative example of male mitotic anaphase chromosomes prepared from larval ganglia. There are two pairs of homologous chromosomes and a pair of nonhomologous sex chromosomes shown in each half of this dividing cell.

fly, *C. dalmanni*. High-line females mated to low-line males produced male progeny with larger relative and absolute eyespan than male progeny from the reciprocal cross. The absence of any difference in relative eyespan between female progeny from these reciprocal crosses indicates that the difference in eyespan between male progeny must be due to the sex chromosomes, not to a maternal effect. Although these high- $\times$ low-line crosses potentially confound the effects of the X and Y chromosomes, an additional backcross experiment permitted us to separate effects due to each sex chromosome. When F_1 males from the high $\times$ low reciprocal cross were mated to control-line females with uniform genetic background, female progeny with high-line X chromosomes had larger relative and absolute eyespan than female progeny with low-line X chromosomes. In contrast, we detected no difference in relative eyespan between male progeny from this same cross. Thus, in these experiments change in relative eyespan is influenced strongly by genes on the X chromosome, but not by genes on the Y chromosome.

The effect of the X chromosome on relative eyespan increased between 13 and 31 generations of selection. With male heterogamety and selection operating only on males, response to selection should be more rapid for autosomal than X-linked traits, assuming codominant allelic effects (Lande 1980). For autosomal loci, selection is reduced by half because males and females contribute alleles equally to progeny. In contrast, selection is reduced to one-third for X-linked traits because fathers pass X-linked alleles only to daughters. Therefore, response to sexual selection operating only on males for an autosomal trait should occur 1.5 times faster than selection of comparable intensity on an X-linked trait (Lande 1980). As a consequence, genetic variation at autosomal loci should be depleted more rapidly by selection and result in greater proportional X-linked effects after more generations of selection, as we observed. The best estimate of an X chromosome effect should, therefore, be made after response to selection has diminished strongly or ended.

Although a recent review (Reinhold 1998) reported that the X chromosome influences sexually selected traits to a greater extent than traits under natural selection, this conclusion is dependent on studies of *Drosophila* in which the X chromosome ranges in size from 20% to 40% of the genome (Turelli and Begun 1997). For example, Carson and Lande (1984) reported a 29.4% effect of the X chromosome on sex comb cilia number in *D. silvestris*, which has an X chromosome representing approximately 20% of the genome. In contrast, Miller (1958) reported a 41.4% effect of the X chromosome on copulation duration in *D. athabasca*, a species for which the X represents approximately 40% of the genome. Without a comparison between taxa with X chromosomes of similar size, these findings are insufficient to conclude that any X chromosome effect is larger than what would be expected based on a random distribution of genes in the genome. Using male progeny from the reciprocal crosses, we estimated the X chromosome accounts for 25%, on average, of the difference between the selected lines after 31 generations of selection. We also estimated that the X chromosome accounts for 34% of the difference in the number of segregating factors with and without sex-linkage. This latter estimate is close to the 38% obtained by comparing male progeny eyespans from the reciprocal cross involving the same two lines, H2 and L2. Although these are highly significant effects, they are not disproportionately larger than expected based on the rel-

ative size of the X chromosome if eyespan genes exhibit dosage compensation by hyperactivation in males.

Dosage compensation in other Diptera, including five species of *Drosophila* and *Sciara ocellaris*, operates by hyperactivation of genes on the male X chromosome so that expression equals that of females (Baker *et al*. 1994; Cunha *et al*. 1994; Meller 2000). The majority of X-linked loci in these species exhibit this form of dosage compensation, suggesting that X-linked loci affecting relative eyespan in *C. dalmanni* may undergo similar compensation. Nevertheless, in *Drosophila*, not all genes on the male X chromosome are dosage compensated, and the exceptions appear to include genes that either have sex-limited expression in females or have autosomal copies (Baker *et al*. 1994; Meller 2000). Although our results are consistent with X-linked dosage compensation by upregulation in males, not downregulation in females, direct evidence for dosage compensation is needed before ruling out the possibility that some X-linked loci disproportionately affect relative eyespan.

The effective factor estimates suggest that relatively few genes of equal and additive effect caused change in relative eyespan after selection. However, the absolute values of these estimates should be viewed with caution. Effective factor estimates have been severely criticized for having practical maxima at the number of linkage groups and for large sampling variances (Zeng *et al*. 1990), unless several conditions are met, such as large sample sizes, substantial divergence in parental populations, and linkage equilibrium (Zeng 1992). Although we obtained large sample sizes for some of the line crosses and the parental populations exhibit considerable divergence, linkage equilibrium is unlikely because we crossed lines that had undergone 31 generations of selection. A study of quantitative trait loci would be needed to determine the number and effect size of sex-linked genes that influence morphological change in head shape between these selected lines.

The lack of a disproportionate effect of dosage-compensated X-linked loci on relative eyespan in stalk-eyed flies appears to be inconsistent with theory that suggests sexually selected traits evolve more rapidly when genes are on an X chromosome than on an autosome (Rice 1984; Charlesworth *et al*. 1987). Frequencies of recessive alleles favorable to heterogametic males, but deleterious to females, should increase more rapidly at X-linked loci than at autosomal loci (Rice 1984). In addition, fluctuating selection can favor X-linked inheritance of sex-limited traits because X-linked alleles can persist longer than autosomal alleles during periods of countervailing selection pressure, due to their lower selection intensities in heterogametic males (Reinhold 1999).

Several factors may explain the lack of a disproportionate effect of the X chromosome on relative eyespan. In contrast to assumption, relative eyespan shows no evidence of recessive expression (Wilkinson 1993). Furthermore, male eyespan appears to be under directional, not fluctuating, selection as a consequence of female preference (Wilkinson *et al*. 1998a) and male assessment of competitors (Panhuis and Wilkinson 1999). Lastly, when selection pressures differ between the sexes, as studies on flight performance of stalk-eyed flies suggest (Swallow et al. 2000), sex-limited autosomal effects are also expected to evolve (Rhen 2000).

A significant effect of the X chromosome with no effect of the Y chromosome is consistent with theory that has recently demonstrated that X-linked meiotic drive can accelerate the evolution of a female preference and an ornamental trait if choosy females use an X-linked ornament to identify and mate with those males that do not carry a driving X-chromosome (Lande and Wilkinson 1999). An alternative scenario in which eyespan indicates Y-linked suppression of X-chromosome meiotic drive (Wilkinson *et al*. 1998a) does not appear to be supported by theory (Reinhold *et al*. 1999) or by the results of this study. Thus, the changes in sex ratio that were observed between the lines after selection on eyespan (Wilkinson *et al*. 1998b) could have been due either to changes in the frequency of the driving X chromosome or in the frequency of autosomal alleles that influence Y-linked suppression. Epistatic effects on sex ratio have been described before. For example, Y-linked suppression of sex ratio depends on genetic background in both *D. mediopunctata* (Carvalho et al. 1997) and *D. simulans* (Cazemajor et al. 1997). Further study is needed to determine if the genes that influence eyespan exhibit linkage disequilibrium with X chromosomes lacking meiotic drive or with genes that suppress meiotic drive (Pomiankowski and Hurst 1999).

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Literature Cited

Baker, B. S., M. Gorman, and I. Marin. 1994. Dosage compensation in *Drosophila*. Ann. Rev. Genet. 28:491–521.

Bentley, D. R., and R. R. Hoy. 1972. Genetic control of the neuronal network generating cricket (*Teleogryllus gryllus*) song patterns. Anim. Behav. 20:478–492.

Bull, J. J. 1983. Evolution of sex determining mechanisms. Benjamin Cummings, Menlo Park, CA.

Burkhardt, D., and I. delaMotte. 1985. Selective pressures, variability, and sexual dimorphism in stalk-eyed flies (Diopsidae). Naturwissenschaften 72:204–206.

Butlin, R. K. 1996. Co-ordination of the sexual signalling system and the genetic basis of differentiation between populations in the brown planthopper, *Nilaparvata lugens*. Heredity 77: 369–377.

Carson, H. L., and R. Lande. 1984. Inheritance of a secondary sexual character in *Drosophila silvestris*. Proc. Natl. Acad. Sci. USA 81:6904–6907.

Carvalho, A. B., S. C. Vaz, and L. B. Klaczko. 1997. Polymorphism for Y-linked suppressors of sex-ratio in two natural populations of *Drosophila mediopunctata*. Genetics 146:891–902.

Cazemajor, M., C. Landre, and C. Montchamp-Moreau. 1997. The sex-ratio trait in *Drosophila simulans*: genetic analysis of distortion and suppression. Genetics 147:635–642.

Charlesworth, B. 1991. The evolution of sex chromosomes. Science 251:1030–1033.

———. 1996. The evolution of chromosomal sex determination and dosage compensation. Curr. Biol. 6:149–162.
———. 1998. Sex chromosomes: evolving dosage compensation. Curr. Biol. 8:R931–933.
Charlesworth, B., J. A. Coyne, and N. H. Barton. 1987. The relative rates of evolution of sex chromosomes and autosomes. Am. Nat. 130:113–146.
Cunha, P. R. d., B. Granadino, A. L. Perondini, and L. Sanchez. 1994. Dosage compensation in sciarids is achieved by hypertranscription of the single X chromosome in males. Genetics 138:787–790.
David, P., A. Hingle, D. Greig, A. Rutherford, A. Pomiankowski, and K. Fowler. 1998. Male sexual ornament size but not asymmetry reflects condition in stalk-eyed flies. Proc. R. Soc. Lond. B 265:2211–2216.
Ewing, A. W. 1969. The genetic basis of sound production in *Drosophila pseudoobscura* and *D. persimilis*. Anim. Behav. 1: 555–560.
Grula, J. W., and O. R. Taylor. 1980. Some characteristics of hybrids derived from the sulfur butterflies, *Colias eurytheme* and *C. philodice*: phenotypic effects of the X-chromosome. Evolution 34:673–687.
Henry, C. S. 1985. Sibling species, call differences, and speciation in green lacewings (Neuroptera: Chrysopidae: *Chrysoperla*). Evolution 39:965–984.
Houde, A. E. 1992. Sex-linked heritability of a sexually selected character in a natural population of *Poecilia reticulata* (Pisces, Poecilidae). Heredity 69:229–235.
Jegalian, K., and D. C. Page. 1998. A proposed pathway by which genes common to mammalian X and Y chromosomes evolve to become X inactivated. Nature 394:776–780.
Lande, R. 1980. Sexual dimorphism, sexual selection, and adaptation in polygenic characters. Evolution 34:292–305.
———. 1981. The minimum number of genes contributing to quantitative variation between and within populations. Genetics 99: 541–553.
Lande, R., and G. S. Wilkinson. 1999. Models of sex-ratio meiotic drive and sexual selection in stalk-eyed flies. Genet. Res. 74: 245–253.
Lardon, A., S. Georgiev, A. Aghmir, G. Le Merrer, and I. Negrutiu. 1999. Sexual dimorphism in white campion: complex control of carpel number is revealed by Y chromosome deletions. Genetics 151:1173–1185.
Meller, V. H. 2000. Dosage compensation: making 1X equal 2X. Trends Cell Biol 10:54–59.
Miller, D. D. 1958. Sexual isolation and variation in mating behavior within *Drosophila athabasca*. Evolution 12:72–81.
Panhuis, T. M., and G. S. Wilkinson. 1999. Exaggerated male eyespan influences contest outcome in stalk-eyed flies (Diopsidae). Behav. Ecol. Sociobiol. 46:221–227.
Pomiankowski, A., and L. D. Hurst. 1999. Driving sexual preference. Trends Ecol. Evol. 14:425–426.
Presgraves, D. C., E. Severance, and G. S. Wilkinson. 1997. Sex chromosome meiotic drive in stalk-eyed flies. Genetics 147: 1169–1180.
Pugh, A. R. G., and M. G. Ritchie. 1996. Polygenic control of a mating signal in *Drosophila*. Heredity 77:378–382.
Reinhold, K. 1998. Sex linkage among genes controlling sexually selected traits. Behav. Ecol. Sociobiol. 44:1–7.
———. 1999. Evolutionary genetics of sex-limited traits under fluctuating selection. J. Evol. Biol. 12:897–902.
Reinhold, K., L. Engqvist, B. Misof, and J. Kurtz. 1999. Meiotic drive and the evolution of female choice. Proc. R. Soc. Lond. B 226:1341–1346.
Rhen, T. 2000. Sex-limited mutations and the evolution of sexual dimorphism. Evolution 54:37–43.
Rice, W. R. 1984. Sex chromosomes and the evolution of sexual dimorphism. Evolution 38:735–742.
———. 1994. Degeneration of a nonrecombining chromosome. Science 263:230–232.
———. 1996. Evolution of the Y sex chromosome in animals. Bioscience 46:331–343.
Ritchie, M. G. 2000. The inheritance of female preference functions in a mate recognition system. Proc. R. Soc. Lond. B 267:1–6.
Roelofs, W., T. Glover, X.-H. Tang, I. Sreng, P. Robbins, C. Eckenrode, C. Löfstedt, B. S. Hansson, and B. O. Bengtsson. 1987. Sex pheromone production and perception in European corn borer moths is determined by both autosomal and sex-linked genes. Proc. Natl. Acad. Sci. USA 87:7585–7589.
Saifa, G. M., and H. S. Chandra. 1999. An apparent excess of sex- and reproduction-related genes on the human X chromosome. Proc. R. Soc. Lond. B 266:203–209.
Shaw, K. L. 1996. Polygenic inheritance of a behavioral phenotype: interspecific genetics of song in the Hawaiian cricket genus *Laupala*. Evolution 50:256–266.
Sokal, R. R., and F. J. Rohlf. 1981. Biometry. W. H. Freeman, New York.
Swallow, J. G., J. H. Marden, and G. S. Wilkinson. 2000. Aerial performance of stalk-eyed flies that differ in eyespan. J. Comp. Physiol. B 170:481–487.
Tomaru, M., and Y. Oguma. 1994. Genetic basis and evolution of species-specific courtship song in the *Drosophila auraria* complex. Genet. Res. 63:11–17.
Turelli, M., and D. J. Begun. 1997. Haldane's rule and X-chromosome size in *Drosophila*. Genetics 147:1799–1815.
Wilkinson, G. S. 1993. Artificial sexual selection alters allometry in the stalk-eyed fly *Cyrtodiopsis dalmanni* (Diptera: Diopsidae). Genet. Res. 62:213–222.
Wilkinson, G. S., and G. N. Dodson. 1997. Function and evolution of antlers and eye stalks in flies. Pp. 310–328 *in* J. C. Choe and B. J. Crespi, eds. The evolution of mating systems in insects and arachnids. Cambridge Univ. Press, Cambridge, U.K.
Wilkinson, G. S., H. Kahler, and R. H. Baker. 1998a. Evolution of female mating preferences in stalk-eyed flies. Behav. Ecol. 9: 525–533.
Wilkinson, G. S., D. C. Presgraves, and L. Crymes. 1998b. Male eyespan in stalk-eyed flies indicates genetic quality by meiotic drive suppression. Nature 391:276–279.
Zeng, Z.-B. 1992. Correcting a bias of Wright's estimates of the number of genes affecting a quantitative character: a further improved method. Genetics 131:987–1001.
Zeng, Z.-B., D. Houle, and C. C. Cockerham. 1990. How informative is Wright's estimator of the number of genes affecting a quantitative character? Genetics 126:235–247.

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