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Stalk-eyed flies carrying a driving X chromosome compensate by increasing fight intensity

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Exaggerated ornaments provide opportunities to understand how selection can operate at different levels to shape the evolution of a trait. While these features aid their bearer in attracting mates or fending off competitors, they can also be costly and influenced by the environment and genetic variation. The eyestalks of the stalk-eyed fly, *Teleopsis dalmanni*, are of interest because eyestalk length is the target of both intra- and intersexual selection and is also reduced by loci on a highly-divergent sex ratio X chromosome (X^{SR}), a meiotic driver accounting for up to 30% of wild X chromosomes. Male stalk-eyed flies fight to control access to females and over food using a combination of low-intensity displays and high-intensity physical fights. We staged, filmed, and scored contests between pairs of eyespan-matched males to evaluate whether X chromosome type impacts the behavior and outcome of aggressive interactions. While our results broadly match expectations from previous studies, we found that X^{SR} males used more high-intensity behaviors than males carrying a non-driving, standard X chromosome (X^{ST}), particularly when their eyestalks were of similar size or smaller than their opponents. Additionally, we found that when X^{SR} males use high-intensity behaviors, they win more bouts than when they use low-intensity behaviors. Taken together, these results suggest that X^{SR} impacts male aggressive behavior to compensate for the shorter eyestalks of X^{SR} males and may help to explain how this selfish chromosome is maintained.

1 Introduction

Theoretical models explaining the evolution of sexually dimorphic traits by either inter- or intrasexual selection are legion (Maynard Smith, 1991; Andersson, 1994; Kokko et al., 2006), but often ignore the consequences of sex-linked inheritance of ornaments or weapons (but see Hastings, 1994; Reeve and Pfennig, 2003; Kirkpatrick and Hall, 2004). Sex linkage can influence how sexual selection operates, especially if there is a mechanism

for the sex ratio to vary, such as meiotic drive. Meiotic drivers are alleles that are overrepresented in an individual's gametes, causing deviations from Mendelian expectations. When a driver is on a sex chromosome and acts in the heterogametic sex, it distorts the sex ratio of the offspring and can either cause extinction or be maintained by balancing selection (reviewed in Jaenike, 2001; Lindholm et al., 2016). Models of sexual selection that include sex-linked inheritance and X chromosome meiotic drive suggest that female choice can evolve in response to the presence of drive (Lande and Wilkinson, 1999; Reinhold et al., 1999)—either females prefer not to mate with males carrying a drive element or prefer males carrying drive suppressors (Lyth et al., 2023).

Stalk-eyed flies (*Teleopsis dalmanni*) are a model for understanding how X-chromosome meiotic drive can influence both inter- and intrasexual selection. Male carriers of a drive X chromosome have a *sex ratio* phenotype, i.e., they produce broods that are 90%–100% female (Presgraves et al., 1997), but have reduced eyestalk length (Johns et al., 2005; Cotton et al., 2014) and are less competitive in both pre-and post-mating interactions (Wilkinson et al., 2006; Paczolt et al., 2023, but see Meade et al., 2020; Bates et al., 2023). Females are highly promiscuous (Wilkinson et al., 1998a, 2003) but prefer males with longer eyestalks (Wilkinson et al., 1998a; Hingle et al., 2001). Males also aggressively compete for control of aggregations of females (Wilkinson and Reillo, 1994), and fights are typically won by males with longer eyestalks (Panhuis and Wilkinson, 1999; Egge et al., 2011). Stalk-eyed fly fights are consistent with sequential assessment (Enquist and Leimar, 1983), a mutual assessment model in which individuals assess their opponents and themselves through a series of escalating behaviors until one individual retreats (Egge et al., 2011).

Recent genomic analyses (Paczolt et al., 2017; Reinhardt et al., 2023) have revealed that the *T. dalmanni sex ratio* X (X^{SR}) diverged from the standard X (XST) hundreds of thousands of years ago. Due to a series of inversions, recombination was greatly reduced, which has led to extensive genetic differentiation between XST and X^{SR} chromosomes, similar to many other drive systems (Lindholm et al., 2016). The X chromosome also represents 20% of the genome (Baker and Wilkinson, 2010; Reinhardt et al., 2023), providing ample material for the evolution of drive-associated differences in eyespan, sperm length (Johns and Wilkinson, 2007), and female sperm storage sizes (Wilkinson, 2005). Furthermore, the weak selection of male X-linked traits (Kirkpatrick and Hall, 2004) and reduced population size and recombination may have led to the accumulation of deleterious alleles on X^{SR} relative to XST (Muller, 1964).

While previous work on sexual selection in *T. dalmanni* has focused on how X^{SR} affects female mate choice (Wilkinson et al., 1998b; Lande and Wilkinson, 1999; Johns et al., 2005) or sperm competition (Wilkinson et al., 2006; Meade et al., 2020; Bates et al., 2023), the influence of X^{SR} on behaviors involved in male–male competition prior to mating has not yet been examined. In this study, we report the results of two types of trials investigating if *T. dalmanni* males that carry X^{SR} exhibit differences in aggressive behavior during trials against an opponent of similar size. One set of trials involved pairing outbred flies, which allowed direct

comparisons of matches involving either or both X^{SR} and XST individuals with comparable outbred genetic backgrounds. The other set of trials paired an outbred fly with a standardized, inbred opponent, thus allowing the comparison of individual X^{SR} and XST focal males between trials. We hypothesized that X^{SR} male aggressive behavior in size-matched contests may be more intense if they compensate for the disadvantage of their reduced ornament, or conversely, may be less intense if deleterious alleles on X^{SR} negatively impact behaviors.

2 Methods

2.1 Aggression trials

Following previous studies of fighting in stalk-eyed flies (Panhuis and Wilkinson, 1999; Egge et al., 2011; Bubak et al., 2014), we quantified male aggressive behavior in two sets of trials conducted either at SUNY Geneseo (outbred pair trials) or at the University of Maryland, College Park (standardized opponent trials). We conducted trials with outbred and inbred flies as opponents to confirm that the results were robust to the opponents' genetic background. At both sites, outbred male *T. dalmanni* were reared from stock populations descended from a collection made at Ulu Gombak, Malaysia, in 2012 (Paczolt et al., 2017) and maintained in population cages with approximately 100 individuals. The temperature was kept at 25°C on a 12-h light:dark cycle in a walk-in chamber, and humidity was held at 70%–90% by automated humidifiers or by twice-weekly manual watering. Flies were fed twice weekly with chopped and autoclaved whole-cob sweet corn treated with 0.1% methylparaben.

Sexually mature males at least 4 weeks post-eclosion were anesthetized using carbon dioxide; their eyespan and body length were measured from video-captured images to the nearest 0.01 mm using ImageJ; and a dot of paint was applied to the ventral thorax before each male was placed in a cage to recover. Because more aggression is displayed between males with similar eyespan (Panhuis and Wilkinson, 1999; Egge et al., 2011), males were paired with partners who differed in eyespan by no more than 5%. Each fly was used in only one trial.

All trials were conducted in transparent plastic arenas (10 cm × 3.5 cm × 6 cm) lined with moist blotting paper and initially divided into three sections by opaque plastic barriers. Each male was placed into an outer compartment of the arena and fasted for 20–24 h. At the beginning of the trial, a piece of agar gel made with corn juice was placed into the middle compartment as a food source and the barriers were removed and the barriers were removed (Supplementary Video 1). Trials were video recorded for 10 min. Scoring of each video was done blind to genotype. The same person scored all videos for each type of trial (outbred pairs—MP; standardized opponents—KP).

The occurrence of aggressive behaviors performed by each fly in each trial was scored from video recordings following a published ethogram (Egge et al., 2011), except for rear-up behavior, which was omitted because it always occurred with another behavior. Within a trial, we defined fighting "bouts" as beginning when either fly

performed an aggressive behavior and ending when the flies were more than one body length apart or not facing each other for 3 s without exhibiting an aggressive behavior. A male was scored as losing a bout if he moved away from his opponent at the end of a bout either slowly (away) or quickly (retreat) and his opponent did not. A mutual move away or retreat was considered a tied bout. The majority of the trials involved multiple bouts.

Aggressive behaviors were categorized as either high-intensity (HI) behaviors that involved physical contact (tussle, attack/lunge, and jump attack) or low-intensity (LI) behaviors that involved males mutually displaying their eyestalks in close proximity (approach, flex and extend, and line-up eyestalks). Escalations were tallied for each individual as a transition from an LI to an HI behavior with no other behaviors or the start or end of a bout separating them. Similarly, de-escalations were defined as a transition from an HI to an LI behavior.

Males were collected immediately after each trial for DNA extraction and genotyping for X chromosome type (X^{SR} or X^{ST}) using polymerase chain reaction (PCR) to amplify one of two markers (Supplementary Table S1) each of which predicts a drive phenotype in this population with a 95% accuracy (Paczolt et al., 2017; Paczolt et al., 2023).

2.2 Outbred pair trials

In a set of 36 trials, we used eyespan to match outbred males reared from a population in which X^{SR} is segregating or from a family that shared an X^{SR} grandfather from the same population. Mature males were chosen from mixed-sex cages of similar age containing 20–50 flies. We anesthetized 10–20 males, marked each with a colored dot on the thorax using one of five colors of paint pen, and then placed them into temporary holding cages. Each male was then paired with an opponent that had the least difference in eyespan and a different color. Videos were recorded on personal mobile phones, and behaviors were scored using JWatcher, v 0.9 (Blumstein and Daniel, 2007). DNA was extracted using Qiagen DNeasy or Puregene, Qiagen, Germany. PCR was performed using the comp162710 marker, and genotypes were distinguished by 1% agarose gel electrophoresis or, for some samples, by automated fragment analysis and the consensus of the comp162710 and ms-395 markers. Because flies were taken from a population polymorphic for a drive, three match types occurred: X^{ST}–X^{ST} (N = 19), X^{SR}–X^{ST} (N = 13), and X^{SR}–X^{SR} (N = 4). Because X^{SR}–X^{SR} pairings were rare, we contrasted trials that included only X^{ST} males with those that included at least one X^{SR} male.

2.3 Standardized opponent trials

In a separate set of 31 trials, focal outbred males competed against standardized inbred opponents. Focal males were lab-reared from the same source used above, while inbred males came from the *T. dalmanni* "2A" inbred line (Paczolt et al., 2017; Reinhardt et al., 2023), which includes only flies with X^{ST} chromosomes. The 2A inbred strain was derived from flies initially collected in 1989 and

then used as a control line in a selection experiment (Wilkinson, 1993) prior to being inbred for 20 generations of brother–sister mating. Males were group-housed as virgins until at least 4 weeks post-eclosion. Outbred focal males and inbred opponents were then measured and housed individually in cages (13.5 cm × 12 cm × 13 cm) with food, to control for possible loser effects. Within each pairing, size matches were allowed to vary up to 5% of the focal male's eyespan, and only the inbred flies were painted on the thorax. Males were kept for a median of 7 days (range: 5–8 days) in individual cages before being placed in an arena with each member of a pair waiting at the same time. Trials were recorded with a digital video camera, and behaviors were scored using Boris v. 8.25 (Friard and Gamba, 2016). DNA was extracted using a squish protocol (Gloor et al., 1993) followed by PCR and fragment analysis of amplicons from both the comp162710 and ms-395 markers (Applied BioSystems 3730xl). There were 11 focal males that carried the X^{SR} chromosome. Differences in the methods used for the two trial types are summarized in Supplementary Table S2.

2.4 Data analysis

Statistical analyses were performed in JMP 17.0.0. Differences between trial type and matched pairs were assessed using Student's t-tests for continuous variables, the Wilcoxon test for count data, and the Wilcoxon signed-rank test for paired analyses of counts. We used a linear model to evaluate whether the duration of time in which aggressive behavior was observed was influenced by the absolute value of the percent difference in eyespan, the trial type, or their interaction.

To analyze the outbred pair trials, we used generalized linear models (GLMs) assuming a Poisson distribution (log-link function) to determine whether the number of HI behaviors in a trial was influenced by the presence of an X^{SR} male, the absolute value of the percent difference in eyespan, or their interaction. The same analysis was repeated for the number of LI behaviors and escalations. In the subset of X^{SR}–X^{ST} trials, we used non-parametric paired tests to test the hypothesis that the X^{SR} individuals performed different numbers of HI, LI, or escalations than their X^{ST} opponents.

For the standardized opponent trials, we similarly used GLMs (Poisson distribution, log-link function) to evaluate whether the number of HI or LI behaviors or escalations performed by the focal male in a trial was affected by his X type, the percent difference in eyespan between him and his opponent, or the interaction of these effects. To determine if aggressive behaviors or escalations occurred more often when the difference in eyespan was small, we included a quadratic term for the percent eyespan difference in the model.

In addition, we evaluated the importance of factors influencing the number of bouts won by the focal male using a binomial GLM. Factors in this model included the percent difference in eyespan, the focal male's X type, the natural log ratio of the focal male's HI to LI behaviors (focal HI:LI), and all two-way interactions between these effects. To interpret significant interactions, we repeated the analysis separately for X^{SR} and X^{ST} males.

3 Results

3.1 Trial-type comparisons

Despite following similar methods, fly pairings were not identical in the two types of trials (Supplementary Table S2). Pairs in the outbred trials were more closely matched for absolute percent difference in eyespan than in the standardized opponent trials (Supplementary Table S3, t = −3.65, P = 0.0006). Body length did not differ between X^{SR} and X^{ST} males in either trial type (Supplementary Table S3), but in the outbred trials, the eyespan of X^{SR} males was smaller than that of X^{ST} males, whereas, in the standardized opponent trials, the eyespan of X^{SR} and X^{ST} males did not differ (Supplementary Table S3). For both trial types, no aggressive behaviors were observed in three trials, so those trials were omitted from further analysis.

As expected from prior work, relative ornament size was an important determinant of fight duration across both types of trials (Figure 1A, L-R χ^2 = 15.24, DF = 3, P = 0.0016). Total time spent fighting was greater when pairs of males were more closely matched, as measured by the absolute percent difference in eyespan (L-R χ^2 = 7.33, P = 0.0068). Duration did not differ between trial types (L-R χ^2 = 1.76, P = 0.18), and there was no detectable interaction between eyespan differences and trial types (L-R χ^2 = 0.27, P = 0.6). Moreover, within trials of both types, escalations were more common than de-escalations (Figure 1B, Wilcoxon signed-rank test, $S_{outbred}$ = −275.5, $P_{outbred}$ < 0.0001; $S_{standardized}$ = −189.00, $P_{standardized}$ < 0.0001), and no trial had more de-escalations than escalations.

All behaviors were observed in both types of trials, although not all behaviors were observed in all trials (Supplementary Tables S4, S5). The most common LI behavior was line-up, and the most common HI behavior was attack/lunge. Trial types did not differ in the total number of HI behaviors (Supplementary Figure S1A,

Wilcoxon test, W = 371, P = 0.19), LI behaviors (Supplementary Figure S1B, W = 355, P = 0.12), bouts (Supplementary Figure S1C, W = 357.5, P = 0.13), or escalations (Figure 1B, W = 393, P = 0.32), although de-escalations were more common in the standardized opponent trials (Figure 1B, W = 298, P = 0.01).

3.2 Outbred pair trials

A GLM predicting HI behaviors revealed significant effects of the absolute percent difference in eyespan, the presence of an X^{SR} male, and their interaction (Table 1). Specifically, trials including X^{SR} males had more HI behavior overall, but especially when the difference in eyespan between competitors was small (Figure 2A). An identical GLM predicting LI behavior detected a significant effect only of the difference in eyespan, with closer matches having more LI behavior (Figure 2B, Table 1). The number of escalations from LI to HI behaviors exhibited similar patterns to those seen for HI behavior (Figure 2C, Table 1). Within 11 X^{SR}–X^{ST} trials, we did not detect a difference in behaviors of paired X^{SR} and X^{ST} males (Wilcoxon signed-rank test, Supplementary Table S6).

3.3 Standardized opponent trials

In trials where outbred males competed against a standardized opponent from an inbred line, focal males exhibited more HI behaviors (Wilcoxon signed rank test, S = −137.0, P = 0.0007, Supplementary Figure S2) and more escalations (Wilcoxon signed rank test, S = −122.0, P = 0.0017) than their opponent, but there was no difference in the number of LI behaviors between focal males and their opponents (Wilcoxon signed rank test, S = 52.5, P = 0.24, Supplementary Figure S2).

GLMs that included the percent difference in eyespan as both a linear and quadratic effect (Table 1) revealed that focal male HI
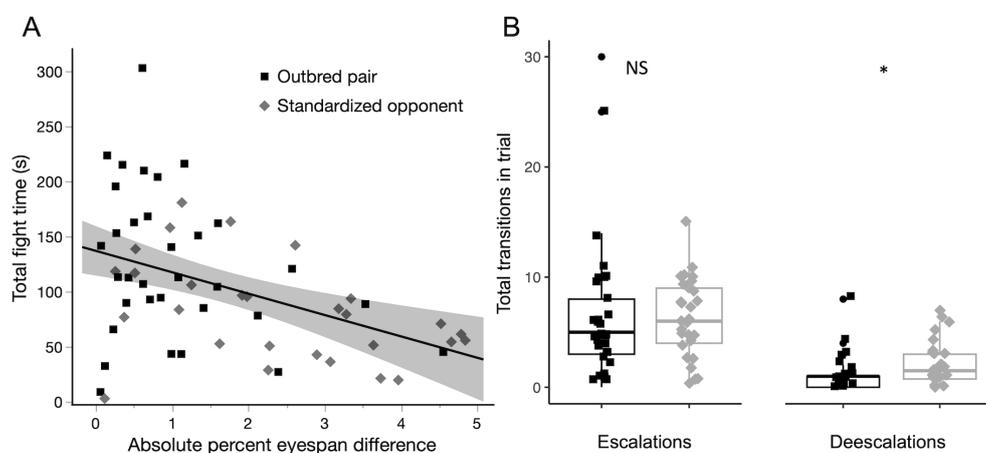

FIGURE 1

Analysis of both trial types show that, consistent with previous experiments in stalk-eyed flies, **(A)** flies fight significantly longer when they have a similarly-sized ornament and **(B)** within a bout of fighting, behaviors tend to escalate from low- to high-intensity behaviors significantly more often than they de-escalate from high- to low-intensity behaviors. There was no significant (NS) difference in the number of escalations between the trial types, but there were fewer de-escalations in the outbred pair trials than in the standardized opponent trails (* indicates P < 0.05).

TABLE 1 Results of Poisson GLMs on the number of behaviors exhibited overall (outbred pair) or by the focal male (standardized opponent trials).
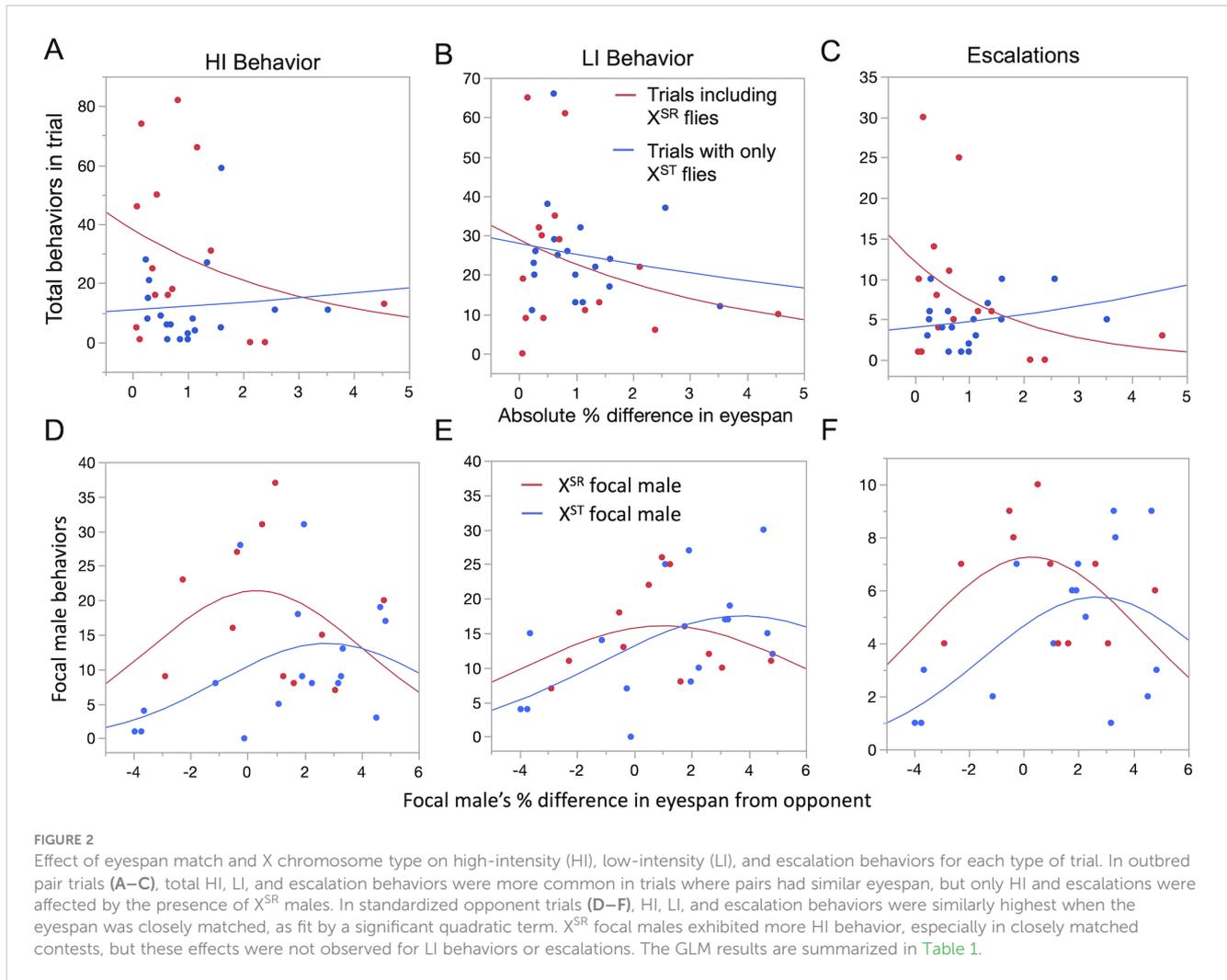
Behavior	Effects	Df	Estimate	L-R χ2	P
Outbred pair trials					
High-intensity	Model	3		156.00	**< 0.0001**
	X^{SR} male present	1	0.4071	96.67	**< 0.0001**
	Absolute % difference in eyespan	1	−0.0970	4.53	**0.0332**
	Interaction	1	−0.2001	17.44	**< 0.0001**
Low-intensity	Model	3		24.10	**< 0.0001**
	X^{SR} male present	1	−0.0560	2.37	0.1235
	Absolute % difference in eyespan	1	−0.1719	18.34	**< 0.0001**
	Interaction	1	−0.0690	2.69	0.1009
Escalations	Model	3		37.68	**< 0.0001**
	X^{SR} male present	1	0.2038	7.10	**0.0077**
	Absolute % difference in eyespan	1	−0.1632	3.95	**0.0468**
	Interaction	1	−0.3292	15.08	**0.0001**
Standardized opponent trials					
High-intensity	Model	4		64.61	**< 0.0001**
	X chromosome type	1	−0.2621	22.74	**< 0.0001**
	% eyespan difference	1	0.0349	1.88	0.1700
	% eyespan difference * X type	1	0.0875	12.32	**0.0004**
	(% eyespan diff)^2	1	−0.0355	16.02	**< 0.0001**
Low-intensity	Model	4		28.10	**< 0.0001**
	X chromosome type	1	0.0314	0.34	0.5591
	% eyespan difference	1	0.0533	4.89	**0.0271**
	% eyespan difference * X type	1	−0.0543	5.24	**0.0221**
	(% eyespan difference)^2	1	−0.0196	5.96	**0.0146**
Escalations	Model	4		14.88	**0.0050**
	X chromosome type	1	0.1421	2.47	0.1157
	% eyespan difference	1	0.0223	0.30	0.5868
	% eyespan difference * X type	1	−0.0710	3.22	0.0726
	(% eyespan difference)^2	1	−0.0297	4.57	**0.0326**

Values in bold denote statistical significance.

behaviors were more common when the focal male had an X^{SR} chromosome (Figure 2D). A significant quadratic term indicates that HI behavior was also higher when the focal male had an eyespan similar to his opponent. A significant interaction between X type and percent difference in eyespan was also detected; males with the X^{ST} chromosome showed more HI behaviors when they had longer eyestalks than their opponent, whereas X^{SR} males did not (Figure 2D). Focal male LI behaviors did not depend on X type but were less common when the focal male had a smaller eyespan than his opponent, as indicated by the significant terms for the effect of eyespan and the additional significant quadratic term (Table 1, Figure 2E). The significant interaction between X chromosome type

and percent eyespan difference was due to X^{ST} males exhibiting more LI behaviors than X^{SR} males when they were larger than their opponents. Focal males were also more likely to escalate when the eyespan difference was small (Table 1, Figure 2F).

A binomial GLM identified several predictors of focal male fight outcomes (Table 2). Focal males won more bouts if they used relatively more HI behaviors. Although a direct effect of X chromosome type was not detected, there were significant interactions between X type and percent difference in eyespan and between X type and focal HI:LI. We therefore fit separate GLMs for focal males of each type of X chromosome. The results of those models (Table 2) reveal that X^{ST} males won more bouts when

FIGURE 2
Effect of eyespan match and X chromosome type on high-intensity (HI), low-intensity (LI), and escalation behaviors for each type of trial. In outbred pair trials **(A–C)**, total HI, LI, and escalation behaviors were more common in trials where pairs had similar eyespan, but only HI and escalations were affected by the presence of X^{SR} males. In standardized opponent trials **(D–F)**, HI, LI, and escalation behaviors were similarly highest when the eyespan was closely matched, as fit by a significant quadratic term. X^{SR} focal males exhibited more HI behavior, especially in closely matched contests, but these effects were not observed for LI behaviors or escalations. The GLM results are summarized in Table 1.

they were larger than their opponents. The ratio of HI to LI behavior did not directly affect the outcome for X^{ST} males, but we did detect a weak effect of the interaction between the percent difference in eyespan and the ratio of HI to LI behaviors. In contrast, X^{SR} males won more bouts when their eyespan was larger than their opponents' and when they used more HI behaviors than LI behaviors (Table 2; Supplementary Figure S3).

4 Discussion

Head appendages, such as eye stalks, antlers, or bristles, have evolved in many different families of flies (Wilkinson and Dodson, 1997). These structures are typically exaggerated in males and are used in head-to-head confrontations when competing over access to mates or food. In the stalk-eyed fly, *T. dalmanni*, males with longer eyestalks win contests more often (Panhuis and Wilkinson, 1999; Egge et al., 2011), even when they are matched for body size. However, eyestalk length is influenced by the presence of a *sex-ratio* drive X chromosome (X^{SR}), which reduces eyespan relative to body length (Wolfenbarger and Wilkinson, 2001; Johns et al., 2005; Cotton et al., 2014), and as a result, is expected to reduce the fighting success of X^{SR} males. However, across two independent

experiments, we found that X^{SR} males exhibit more high-intensity behaviors than X^{ST} males when they have similar eyestalks as their opponents. Furthermore, we found that engaging in a higher ratio of high- to low-intensity aggressive behavior results in well-matched X^{SR} males winning more bouts in contests against standardized opponents, a behavioral shift that may increase the mating success of X^{SR} males despite their reduced ornament.

There are several plausible interpretations of these findings. One possibility is that the X^{SR} chromosome carries alleles that increase high-intensity aggressive behavior. Previous studies have revealed that HI behaviors are impacted by serotonin (5-hydroxytryptamine or 5-HT), as flies with high 5-HT levels after ectopic administration exhibited more high-intensity behaviors and won more fights (Bubak et al., 2014), and RNAi of 5-HT receptors impacts aggressive behavior in males (Bubak et al., 2019). If a gene for a 5-HT receptor was on the X chromosome, X^{SR}-associated differences in behavior might have evolved—however, all four 5-HT receptor genes (*5-HT1B*, *5-HT2A*, *5-HT2B*, and *5-HT7*) in the *T. dalmanni* genome are autosomal (Reinhardt et al., 2023). RNA sequencing of the brains of males carrying each X-chromosome type could help identify unknown X^{SR}-associated gene expressions that might influence aggressive behavior.

TABLE 2 Results of binomial GLMs on focal male wins per trial in standardized opponent trials.

Model	Effects	DF	Estimate	L-R χ2	P
Overall	Model	6		36.27	**< 0.0001**
	X chromosome type	1	0.0726	0.16	0.6874
	ln(Focal HI:LI)	1	0.7330	6.65	**0.0099**
	Percent difference eyespan	1	0.4244	26.88	**< 0.0001**
	Focal HI:LI * Percent eyespan	1	−0.2290	3.52	0.0607
	Percent eyespan * X type	1	−0.2214	4.50	**0.0339**
	Focal HI:LI * X type	1	−0.6240	4.71	**0.0300**
X^{ST}	Model	3		18.67	**0.0003**
	ln(Focal HI:LI)	1	0.0822	0.12	0.7313
	Percent difference eyespan	1	0.2460	6.36	**0.0117**
	Focal HI:LI * Percent eyespan	1	−0.2642	4.19	**0.0406**
X^{SR}	Model	3		18.19	**0.0004**
	ln(Focal HI:LI)	1	1.4118	7.14	**0.0075**
	Percent difference eyespan	1	0.5376	12.98	**0.0003**
	Focal HI:LI * Percent eyespan	1	0.1253	0.09	0.7691

Values in bold denote statistical significance.

The difference could also be due to how X^{SR} and X^{ST} males differently assess their own resource-holding potential (RHP) (Parker, 1974) and that of their opponents. RHP is expected to be related to ornament size due to the strong allometry between eyespan and body length (Wilkinson, 1993; Wilkinson and Dodson, 1997). It has been suggested that stalk-eyed flies use their own body size for self-assessment (Brandt and Swallow, 2009), and subsequent work (Egge et al., 2011) suggested that aggressive behavior is most consistent with mutual assessment due to the pattern of behavioral escalation with very little de-escalation, as seen here (Figure 1). Eyespan may be a better target than body length for assessment of the opponent as the allometry exceeds 1.0, so subtle differences in male eyespan can be observed more easily than subtle differences in body size (see Panhuis and Wilkinson, 1999). Eyespan may also be more visible than body size during the line-up behavior, which is often seen early in a bout prior to escalation. Because X^{SR} males have reduced allometry of eyespan to body size, when matched for eyespan, X^{SR} males should have marginally larger body sizes than their opponents, although we did not observe such a difference in these trials (Supplementary Table S3). Larger body size would both increase a male's self-assessed RHP and allow them to better tolerate the period of fasting prior to fighting. Eyespan-matched X^{ST} opponents, meanwhile, would assess X^{SR} males as having similar RHP and thus be willing to escalate to more HI behavior, explaining why both males are more aggressive in trials, including an X^{SR} male (Figure 2). Female stalk-eyed flies also engage in aggressive interactions over food (Bubak et al., 2019), and the X^{SR} chromosome also affects female eyespan, but the magnitude of the effect is approximately 25% that of the male effect (Johns et al., 2005). A test of the effect of X^{SR} on female aggressive behavior would therefore be a useful additional test of these allometry-based hypotheses.

Previous studies have found that HI behavior correlates with the propensity of a fly to win an aggressive contest, but we found complex results (Table 2) in terms of whether the increased HI behavior associated with X^{SR} impacted how likely a fly was to win a bout. Overall, we found no direct effect of X type on the number of bouts won (Supplementary Table S6, Table 2). However, in standardized opponent trials, X^{SR} focal males that performed a higher proportion of high-intensity behaviors to low-intensity behaviors won more bouts. Meanwhile, among X^{ST} males, there was only a weak interaction effect between HI:LI behavior and percent difference in eyespan (Supplementary Figure S3).

These results have implications for the ecology and evolution of aggressive behavior in the stalk-eyed fly, as well as the prevalence of X^{SR} in natural populations. Males typically compete for territories on root hairs underneath riverbanks and sometimes perch on the same hair as other males, whereas females approach and land near males with long eyestalks. If X^{SR} males inaccurately self-assess, they may engage with X^{ST} males with larger eyestalks, providing proximity to females attracted to his ornament and providing more opportunities for mating. This may be balanced by the survival risks of engaging in more high-intensity behavior. One prediction of this finding would be that populations with a higher frequency of the X^{SR} chromosome should exhibit more intensely aggressive behaviors than those with a lower frequency of X^{SR}. These results may also help to explain how the X^{SR} chromosome is maintained stably in the laboratory and the field (Wilkinson et al., 2003). Experimental evolutionary studies have revealed that in the absence of sexual selection, the X^{SR} chromosome rapidly increases in frequency. In contrast, when females are allowed multiple mating opportunities, the X^{SR} chromosome is maintained at frequencies similar to those found in the wild (Paczolt et al., 2023), indicating

that some X^{SR} males gain reproductive success, which may be aided by their willingness to engage in intense fighting behaviors.

Data availability statement

The original contributions presented in the study are publicly available. This data can be found here: https://doi.org/10.5061/dryad.79cnp5j51.

Ethics statement

The manuscript presents research on animals for which ethical approval is not required.

Author contributions

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationship that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fetho.2024.1461681/full#supplementary-material

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