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Fitness effects of X chromosome drive in the stalk-eyed fly, *Cyrtodiopsis dalmanni*

G. S. WILKINSON,* P. M. JOHNS,* E. S. KELLEHER,† M. L. MUSCEDERE‡ & A. LORSONG*

*Department of Biology, University of Maryland, College Park, MD, USA
†Department of Ecology and Evolutionary Biology, University of Arizona, Tucson, AZ, USA
‡Department of Biology, Boston University, Boston, MA, USA

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Abstract

Sex-ratio (SR) males produce predominantly female progeny because most Y chromosome sperm are rendered nonfunctional. The resulting transmission advantage of X^{SR} chromosomes should eventually cause population extinction unless segregation distortion is masked by suppressors or balanced by selection. By screening male stalk-eyed flies, *Cyrtodiopsis dalmanni*, for brood sex ratio we found unique SR alleles at three X-linked microsatellite loci and used them to determine if SR persists as a balanced polymorphism. We found that X^{SR}/X^{ST} females produced more offspring than other genotypes and that SR males had lower sperm precedence and exhibited lower fertility when mating eight females in 24 h. Adult survival was independent of SR genotype but positively correlated with eye span. We infer that the SR polymorphism is likely maintained by a combination of weak overdominance for female fecundity and frequency dependent selection acting on male fertility. Our discovery of two SR haplotypes in the same population in a 10-year period further suggests that this SR polymorphism may be evolving rapidly.

Introduction

Sex chromosome meiotic drive occurs naturally in diverse taxa including some plants, insects and vertebrates (Hurst & Pomiankowski, 1991; Taylor *et al.*, 1999; Jaenike, 2001). In all known cases, sperm carrying drive differentially survive, causing as much as a twofold transmission advantage for regions of the chromosome associated with the drive factor. This results in an increasingly skewed sex ratio and eventual population extinction unless opposed by selection or suppressed by modifiers (Hamilton, 1967). Y chromosome drive can cause rapid extinction because males transmit a Y chromosome directly to their sons. Not surprisingly, Y chromosome drive polymorphisms are rare with mosquitoes providing the best studied examples (Wood & Newton, 1991; Mori *et al.*, 2004). X chromosome drive, often referred to as *sex-ratio* (SR), takes longer to cause extinction because heterogametic males only transmit X chromosomes to daughters and segregation distortion only occurs in males. SR polymorphisms, in which both drive and nondrive (ST) X chromosomes persist in populations, appear to be relatively common among Diptera, with examples described in at least 15 *Drosophila* species (Jaenike, 2001; Yang *et al.*, 2004) and in several species from other families (e.g. Mori *et al.*, 1979; Gariou-Papalexiou *et al.*, 2002), including diopsid stalk-eyed flies (Presgraves *et al.*, 1997; Wilkinson *et al.*, 2003).

The mechanisms maintaining SR in natural populations remain poorly understood (de Carvalho & Vaz, 1999; Jaenike, 2001) despite 70 years of study on *Drosophila pseudoobscura* (e.g. Sturtevant & Dobzhansky, 1936; Wallace, 1948; Dobzhansky, 1958; Beckenbach, 1996). Population genetic analysis indicates that frequency independent selection can stabilize an SR polymorphism if female carriers have reduced fitness or exhibit overdominance (Edwards, 1961; Curtsinger & Feldman, 1980). In contrast, constant selection on males leads either to SR fixation or loss (Curtsinger & Feldman, 1980). Given that SR is caused by defects in sperm development (Policansky & Ellison, 1970; Hauschteck-Jungen & Maurer, 1976; Montchamp-Moreau & Joly, 1997; Cazemajor *et al.*, 2000; Wilkinson & Sanchez, 2001), for selection to act on female carriers the SR

Correspondence: Gerald S. Wilkinson, Department of Biology, University of Maryland, College Park, MD 20742, USA.
Tel.: +1 301 405 6942; fax: +1 301 314 9358;
e-mail: wilkinso@umd.edu

factor must either have pleiotropic effects or be in linkage disequilibrium with genes that influence female fitness. Linkage disequilibrium can result from association of SR with one or more inversions that restrict recombination. Inversions occur in many, but not all (e.g. James & Jaenike, 1990; Mercot *et al.*, 1995) cases of SR. For example, in *D. pseudoobscura*, three nonoverlapping inversions restrict recombination over a 20 cM portion of the X chromosome (Beckenbach, 1996). Studies using lab- and field-reared flies indicate that viability selection operates against SR in both sexes (Curtsinger & Feldman, 1980; Beckenbach, 1996).

Frequency dependent selection on males can also stabilize an SR polymorphism through drive-dependent effects on male fertility. As SR increases in a population, the proportion of females goes up and males must mate more often. If the fertility of SR males declines as male mating rate increases, then a balanced polymorphism is possible as long as SR male fertility is sufficiently reduced to offset SR segregation distortion (Jaenike, 1996). Whereas this scenario seems reasonable given that SR effectively eliminates half of a male's sperm, previous studies (Wu, 1983c; Atlan *et al.*, 2004) indicate that SR male fertility may not be reduced enough for this mechanism to operate alone (de Carvalho & Vaz, 1999).

Alternatively, an SR polymorphism can persist if male mating rate differentially influences SR and ST males when there is sperm competition (Taylor & Jaenike, 2002, 2003). This mechanism is similar to the male fertility effect except in this case the competitive ability of SR sperm must decline as male mating rate increases. In addition, the frequency of sperm competition must vary with the sex ratio. Depending on the relative magnitudes of these effects, frequency-dependent sperm competition can either lead to a balanced polymorphism or fixation of the SR factor and extinction of the population (Taylor & Jaenike, 2002). SR sperm tend not to be as successful as ST sperm at fertilizing eggs in double-mated females (Wu, 1983b; Taylor *et al.*, 1999; Wilkinson & Fry, 2001), but how sperm competitive success changes with male mating rate has yet to be measured for any species.

The magnitude of selection required to stabilize an SR polymorphism can be influenced by modifiers that suppress drive because they influence segregation distortion (Jaenike, 1996; de Carvalho *et al.*, 1997; de Carvalho & Vaz, 1999; Jaenike, 1999a,b; Vaz & Carvalho, 2004). Both Y-linked and autosomal suppressors are expected to evolve to counteract the effects of X drive (Leigh, 1977; Crow, 1991). Both types of suppressors have been found in several SR-carrying species (e.g. Stalker, 1961; Voelker, 1972; Mercot *et al.*, 1995; Jaenike, 1999a; Yang *et al.*, 2004), with *D. pseudoobscura* being a notable exception (Wu, 1983a). If suppressors are not costly, they can go to fixation and mask the presence of SR, as has been observed in *Drosophila simulans* (Mercot *et al.*, 1995; Atlan *et al.*, 1997, 2003) and possibly *Drosophila albomicans* (Yang *et al.*, 2004). When suppressors have negative fitness consequences, then both drive and suppressor can be maintained in balanced polymorphisms (Clark, 1987; de Carvalho & Vaz, 1999) or undergo cycling (Hall, 2004).

Stalk-eyed flies have evolved X chromosome drive independently of *Drosophila* (Presgraves *et al.*, 1997; Jaenike, 2001) and provide an interesting case for comparison given their life history and extraordinary sexual dimorphism in eye span (Wilkinson & Dodson, 1997). In Southeast Asia several populations of two species, *Cyrtodiopsis dalmanni* and *C. whitei*, contain SR males at frequencies between 8% and 34% (Wilkinson *et al.*, 2003). SR males typically produce broods with 95% or more females. In contrast to most *Drosophila*, these *Cyrtodiopsis* species are highly promiscuous, with males and females mating multiple times every day (Wilkinson *et al.*, 2003), living for many months as adults (Wilkinson & Reillo, 1994) and reaching reproductive maturity slowly (Wilkinson & Johns, 2005). The mechanism for X chromosome drive resembles *Drosophila* in that SR affects sperm development (Wilkinson & Sanchez, 2001) and influences sperm competition in *C. whitei* (Wilkinson & Fry, 2001). The effect on sperm competition is not due to a reduced number of sperm transferred to a female but rather to SR sperm being incapacitated by seminal fluid from an ST male (Fry & Wilkinson, 2004). Moreover, genetic mapping studies on *C. dalmanni* have revealed that SR is associated with eye span and recombination rarely occurs between drive and nondrive X chromosomes (Johns *et al.*, 2005). These results are consistent with one or more inversions that contain both the drive factor and a major quantitative trait locus for eyestalk length. Furthermore, SR has persisted for over 10 years in laboratory populations of both dimorphic *Cyrtodiopsis* species and in lines of *C. dalmanni* under selection for increased and decreased relative eye span (Wilkinson *et al.*, 1998b, 2005). Given the intensity of sex ratio distortion observed in *Cyrtodiopsis*, strong selection must occur to maintain the SR polymorphism in field and lab populations.

Here we report on a series of experiments designed to estimate the relative fitness of drive (X^{SR}) and nondrive (X^{ST}) chromosomes in *C. dalmanni*. To identify different drive chromosomes, we first tested for associations between X-linked microsatellite genotypes and female-biased brood sex ratios among males from a large lab population. We then bred flies to generate all possible drive genotypes and used them to estimate female fecundity, progeny production, male fertility and adult survival. Because the fertility of SR males is expected to vary with mating frequency, we estimated male fertility when mating rate is both low and high as well as when there is sperm competition. We used microsatellite genotypes to identify the SR chromosome in all experiments. We then discuss whether the fitness effects we measured are sufficient to stabilize an SR polymorphism by constant or frequency-dependent selection.

Materials and methods

Fly stocks and SR assignment

The flies used in this study descended from *C. dalmanni* collected near Ulu Gombak, Malaysia (3°19′ N, 101°43′ E, 350 m elevation) in 1999 (Wilkinson *et al.*, 2003). This population is kept in a Plexiglas cage (120 × 30 × 30 cm) at 25 ± 1 °C and greater than 75% relative humidity on a 12-h L : D cycle. Twice per week sterile pureed yellow sweet corn treated with a solution of 10% methylparaben to inhibit mould growth is provided on trays as food and in 100-mL cups for oviposition to maintain the population at several hundred individuals.

In a previous study (Johns *et al.*, 2005) linkage analysis of a selected line intercross derived from Gombak *C. dalmanni* collected in 1989 indicated that SR is associated with a single haplotype defined by three X-linked microsatellite loci. Therefore, to determine if the same alleles are associated with SR we collected progeny for 2 weeks from 81 males each mated to three virgin females and then genotyped 62 of them at diagnostic X-linked loci (Table 1). Brood sex ratios were based on 90 ± 8 offspring per male. We designated males producing significantly female-biased brood sex ratios that contained fewer than 10% sons as SR and all other males as ST.

To genotype flies for drive we extracted genomic DNA by grinding and incubating a fly's thorax at 65 °C for at least 2 h in 400 μL of 6.25% (w/v) Chelex 100 solution with 2 U proteinase K (Fisher Scientific, Pittsburgh, PA, USA), followed by incubation at 95 °C for 20 min and centrifuging at 371 *g* on a microcentrifuge for 3 min. Three X-linked microsatellite loci (Wright *et al.*, 2004; Johns *et al.*, 2005) were amplified by polymerase chain reaction (PCR) in 10 μL volume reactions containing 1 μL template DNA, 0.125 U *Taq* polymerase (Invitrogen, Carlsbad, CA, USA), 1× PCR buffer (Invitrogen), 0.2 mM of each dNTP, 2.5 mM $MgCl_2$ and 0.5 μM of primers, one of which was labelled with a fluorescent dinucleotide. Amplification was initiated at 95 °C for 2 min, followed by 30 cycles of 95 °C for 30 s, the annealing temperature for 30 s, and 72 °C for 30 s. Annealing temperatures differed by primer pair: 50 °C for ms-125, 56 °C for ms-244 and 59 °C for ms-395. The program terminated with 3 min at 72 °C. Labelled PCR products were separated on a 3100 Genetic Analyzer (Applied Biosystems, Foster City, CA, USA) using Pop-4 polymer and evaluated using GENESCAN 3.1.2 software (Applied Biosystems). We sized alleles using GENOTYPER 2.5 software (Applied Biosystems).

Fecundity

Female fecundity was measured by counting egg loads in unmated females across a range of ages. To create all three possible drive genotypes we first mated Gombak population females either to SR or ST males and then backcrossed the resulting female offspring to males with the same drive type as their father (see Fig. 1). We then dissected an average of 28 ± 4 female progeny each week for 10 weeks beginning at 2 weeks of age. For this and

Table 1 X haplotypes* and brood sex ratios for Gombak male *Cyrtodiopsis dalmanni*.

Microsatellite loci (bp)			Brood sex ratio	Number of	Inferred drive
ms-125	ms-244	ms-395	(prop. male ± SE)	males	chromosome
150	**240**	**238**	0.01 ± 0.01	15	X^{SR2}
152	222	195	0.45 ± 0.03	5	X^{ST}
152	220	202	0.46	1	X^{ST}
158	230	202	0.46 ± 0.02	2	X^{ST}
158	230	205	0.47 ± 0.03	3	X^{ST}
160	220	205	0.50	1	X^{ST}
158	222	195	0.50 ± 0.04	3	X^{ST}
158	230	195	0.51 ± 0.02	5	X^{ST}
152	230	195	0.54 ± 0.04	13	X^{ST}
160	220	195	0.54	1	X^{ST}
158	216	195	0.56 ± 0.03	6	X^{ST}
152	230	205	0.59 ± 0.13	2	X^{ST}
152	216	195	0.64 ± 0.11	5	X^{ST}
–	–	–	0.51 ± 0.02	19	X^{ST}
148	**236**	**238**	0.05 ± 0.01	26	X^{SR1}
152	226	202	0.45 ± 0.01	24	X^{ST}
152	226	216	0.44 ± 0.02	12	X^{ST}

*Values in bold indicate polymerase chain reaction product sizes (Wright *et al.*, 2004) uniquely associated with *SR* males. Genotypes and brood sex ratios identified in a previous study (Johns *et al.*, 2005) using flies collected from Gombak in 1989 are included below the dotted line for comparison.

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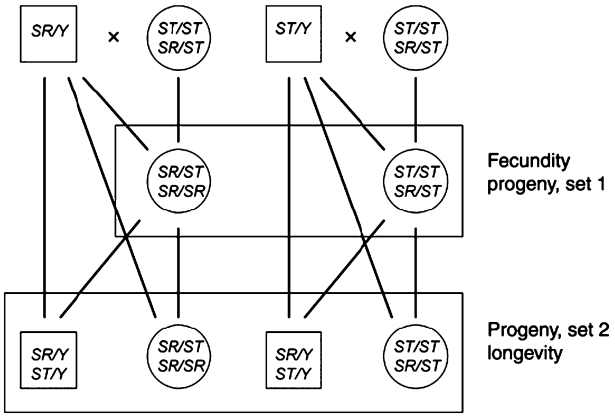

Fig. 1 Breeding design used to create males and females of each drive genotype for use in fitness component experiments, as indicated. Males are denoted by boxes and females by circles. Drive and nondrive X chromosome (SR and ST) males were identified by brood sex ratios (cf. Table 1) and then mated to population stock females. Female types were determined using diagnostic X-linked microsatellite loci.

subsequent experiments flies were measured for eye span between the outer edges of the eye bulbs, body length from head to wing tip and thorax width at the widest point of the body using NIH IMAGE V. 1.61 (National Institutes of Health, Bethesda, MD, USA) and then frozen.

To count eggs, we thawed females and then separated the ovaries from viscera after dissecting abdomens in several drops of phosphate-buffered saline (PBS). Ovaries were teased apart and eggs were scored as 'mature' or 'immature.' Mature eggs were greater than 0.7 mm in length, had longitudinal ridges, and appeared opaque and ovular, which made them indistinguishable from oviposited eggs. Immature eggs varied between 0.3 and 0.7 mm in length and were often partially opaque. Transparent eggs less than 0.3 mm in length were not counted. Each fly was genotyped for drive after dissection.

The number of mature eggs carried by each female was analysed using a general linear model, which included age, thorax width and number of immature eggs as covariates and female genotype and type of cross, i.e. whether mated to an SR or ST male, as factors. Because we did not find any eggs in 14-day-old flies, but flies in all subsequent age classes contained both size classes of eggs, we only analysed egg loads from flies 3 weeks of age or older.

Progeny production

We pair-mated virgin females of each SR genotype with Gombak population males to score progeny production. We obtained females from two sets of breeding sources (Fig. 1). The first set contained 43 females of which 30 were offspring of SR males and 13 were offspring of ST males. The second set consisted of 45 female offspring chosen from parents in the first set to provide nearly equal numbers of each X chromosome type. When female genotype was uncertain, genotypes were obtained for the diagnostic X-linked microsatellite loci.

For both sets we housed male and female flies together in 2.5 L jars and collected food cups every 3 or 4 days for 6 weeks. All eclosing flies were counted and sexed and cages were checked daily for dead flies. If a female died, we included the number of days each female could have oviposited in a general linear model on progeny number to account for variation in laying interval. We also included brood sex ratio and experimental set in the model to control for female age. In the first set females were, on average, 78 ± 3 days old when first mated, whereas in the second set all females were mated at 45 days of age. Cages were excluded if males were infertile or if female genotype could not be determined.

Sperm competition and male fertility

To determine the degree to which SR males could fertilize eggs in the presence or absence of sperm from ST males we conducted a sperm competition experiment. Because previous work on the closely related species, *C. whitei*, revealed that SR sperm do not survive as well as ST sperm when in the presence of seminal fluid from ST males (Wilkinson & Fry, 2001; Fry & Wilkinson, 2004), in this experiment we compared sperm offence of SR to ST, i.e. we mated a female first to an ST male and then 24 h later to either an SR or ST male. This situation mimics what would happen when the X^{SR} chromosome is at low frequency in a population and population density is high enough that females routinely remate. We also mated each male a single time to either one or two females to measure each male's fertility in the absence of sperm competition and in a situation that mimics low mating rates. We mated 60 pairs of males (30 pairs of ST males and 30 pairs of SR and ST males) to a total of 290 virgin females. Matings between pairs of flies were observed in 1-L cages and if the mating lasted at least 60 s, females were isolated in 2.5-L plastic jars with food cups for 2 weeks. All pupae produced during this period were counted and the sex of eclosed progeny was recorded. For females mated to two males all offspring up to a maximum of 40 were frozen and genotyped to assign paternity.

To determine sperm precedence in double-mated females, we genotyped one X-linked microsatellite (ms-125) to determine paternity of female offspring and two autosomal microsatellites (ms-480 and ms-95) to determine the paternity of male offspring (Wright *et al.*, 2004). We also genotyped all three parents at all three loci. Paternity of female offspring was unambiguous when mated by SR and ST males because the SR male always carried the 150-bp allele whereas the ST male

carried either the 152 or 158 bp allele (cf. Haplotype association in Results below).

Because SR males produce half as many viable sperm as ST males (Johns *et al.*, 2005), SR males should experience more sperm depletion than ST males when mating rates are high. To quantify this effect we placed a reproductively mature male into a 2.5-L cage with eight virgin females 3–4 weeks of age for 24 h. We set up 10 replicates each containing six to eight cages. In each replicate SR and ST males were matched for age. All males were housed with females 1 week prior to testing and then oviposition cups with food were collected for 2 weeks. If no progeny eclosed, males were genotyped at two X-linked markers to determine their SR genotype. We then fit a general linear model to the number of progeny produced with replicate and SR genotype as factors, both including and excluding infertile males. The results were nearly identical, so only the analysis excluding infertile males is presented.

Adult survival

Survival of adult flies was determined by following the fates of age-matched cohorts containing all drive genotypes in replicate cages for each sex. Flies for testing were bred as described above (Fig. 1), i.e. females were backcrossed to either SR or ST males for one generation, and their progeny isolated by sex in 2.5-L plastic jars every 3 days after eclosion. Single sex replicates (four female, six male) were then initiated in $48 \times 27 \times 20$ cm cages using approximately equal numbers of flies from each source. Female cages initially contained 72 ± 16 flies that eclosed within 3 days while male cages contained 47 ± 5 flies that eclosed within 10 or fewer days. Food was provided twice a week. Each cage was checked once a day for dead flies, which were removed, measured and frozen for subsequent genotyping. Flies were allowed to die naturally except for the last 10 flies, which were sacrificed at 250 days of age.

Differences in survival between genotypes, sexes and replicates were examined using Log Rank tests on cumulative survival. To control for differences between replicates and body sizes, proportional hazards regression analysis was performed. Because body length correlates with eye span within each sex and eye span differs between the sexes, analyses were conducted with each covariate separately, using deviations from the mean within each sex, to avoid collinearity. The best fitting model, as assessed by likelihood ratio tests, was then used to determine if genotype influenced survival by including genotype as a nested effect within replicate.

All statistical analyses were conducted using JMP v. 5.0.1.2 (SAS Institute, Cary, NC, USA). Interaction terms were examined when possible and removed from the model when they were not significant.

Results

Haplotype association

Brood sex ratio varied significantly among X haplotypes (ANOVA: $F_{12,49} = 19.9$, $P < 0.0001$). Fifteen males exhibited extremely female-biased brood sex ratios and shared a single X haplotype (denoted X^{SR2}) that contained unique alleles at all loci whereas unbiased brood sex ratios were found for 12 other X haplotypes (Table 1). Alleles at two of the diagnostic SR loci differed between the X^{SR2} haplotype and an SR haplotype identified from the Gombak population in a previous study (X^{SR1}, Table 1). Because we never found the X^{SR1} haplotype in the stock population, in subsequent analyses we assume that any male possessing at least one SR allele carries the X^{SR2} chromosome.

Fecundity

A general linear model revealed that age and cross-type significantly predicted the number of mature eggs carried by unmated females (Table 2). Egg counts increased linearly with age ($F_{1,298} = 33.0$, $P < 0.0001$, $r^2 = 0.10$; quadratic term did not improve fit). Female progeny of SR males carried fewer mature eggs (12.1 ± 1.32) than females fathered by ST males (15.2 ± 0.96) after controlling for age and body size. Although egg counts increased with body size in a univariate regression ($F_{1,298} = 13.2$, $P < 0.001$, $r^2 = 0.04$), after controlling for age and cross-type, body size was not significant (Table 2). Female drive genotype also did not influence egg counts significantly. Examination of least squares means revealed that X^{SR}/X^{ST} females tend to have more eggs than either X^{SR}/X^{SR} females or X^{ST}/X^{ST} females (Fig. 2).

Progeny production

A general linear model on the number of offspring produced by all possible SR mating combinations revealed a significant effect of egg-laying interval and maternal drive genotype, but no effect of paternal drive

Table 2 General linear model for number of mature eggs in unmated females.

Source	d.f.	*F*-ratio	*P*
Model	6	7.22	<0.0001
Ovarioles*	1	0.02	0.89
Age	1	22.8	<0.0001
Cross-type†	1	4.45	0.036
Sex-ratio genotype	2	0.81	0.45
Body size	1	3.18	0.076
Error	291		

*Ovarioles refer to immature egg counts.
†Cross-type refers to whether females were created by backcrossing to SR or ST males.

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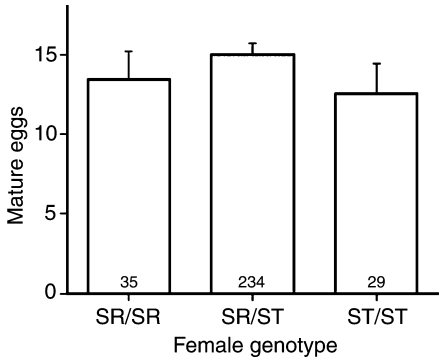

Fig. 2 Mean value (±SE) number of mature eggs carried in a female, after correcting for age, body length, cross-type and number of immature eggs.

Table 3 General linear model for offspring produced by females pair-mated to males.

Source	d.f.	*F*-ratio	*P*
Model	6	6.77	0.0003
Maternal SR genotype	2	3.61	0.0328
Paternal SR genotype	1	2.82	0.0982
Brood sex ratio	1	3.90	0.0526
Laying interval	1	21.17	<0.0001
Set	1	0.45	0.5033
Error	62		

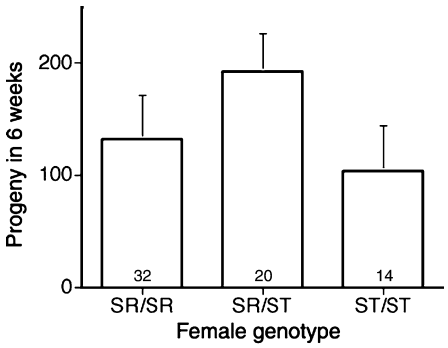

Fig. 3 Mean value (±SE) number of male and female offspring produced in 6 weeks, after correcting for experimental set, paternal genotype and egg laying interval. Female genotype was inferred from three X-linked microsatellite loci. Numbers in each bar indicate sample sizes.

genotype, experimental set or brood sex ratio (Table 3). Thus, when males were able to mate females without competition or constraint, no difference in fertility was detected between SR and ST males. However, *post hoc* Student's *t*-tests ($P < 0.05$) indicated that X^{SR}/X^{ST} females produced more offspring than X^{SR}/X^{SR} females but did not differ from X^{ST}/X^{ST} females (Fig. 3).

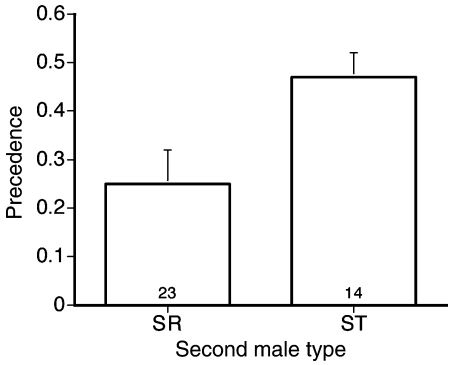

Fig. 4 Mean value (±SE) sperm precedence in double-mated females expressed as the proportion of offspring sired by type of male mating second. All females were mated first by an ST male. Paternity was determined using three microsatellite markers (see Materials and methods). Sample sizes indicate the number of mating groups for which all offspring could be unambiguously assigned to one of the males.

Male fertility with sperm competition

We obtained offspring from 25 females mated successively to ST and SR males and 15 females mated successively to two ST males. The number of offspring produced by each of these 40 females was 31.9 ± 4.0 and did not differ between the two mating conditions ($t = 0.28$, $P = 0.78$). However, brood sex ratios depended on the type of males mating with a female. Females mated to an SR male after mating an ST male had a lower proportion of male progeny (0.35 ± 0.05) than females mated to two ST males (0.53 ± 0.03; $t = 2.71$, $P = 0.010$). Given that the proportion of male progeny in single-mated SR broods was 0.02 ± 0.01 whereas the sex ratio of single-mated ST males was 0.46 ± 0.01, the intermediate sex ratios of females double-mated to ST and SR males indicates that paternity was either shared by both males in each brood or split among broods with either ST or SR males fathering all young.

We used genotypes at three microsatellite loci from 1130 offspring of multiply mated females to assign paternity and estimate sperm precedence. We found that the second-mate precedence of SR sperm was significantly lower ($t = 2.11$, $P = 0.042$) than the second-mate precedence of ST sperm (Fig. 4). Mixed paternity broods were equally common (average 49%) in both mating situations. However, in the 14 single paternity broods from females mated to both ST and SR males, the ST male was the sole father in 11 cases (79%). Thus, the lower sex ratio produced by females mated to both ST and SR males was due to a combination of mixed and split paternities.

Male fertility with low mating rate

We assessed the fertility of the males used for the sperm competition experiment by mating each male a single

time to two separate females. Under this type of mating regime, male fertility is highly variable. Thirty per cent of all males failed to produce offspring with either female and 27% succeeded in producing offspring with both females. The proportion of matings that produced offspring was significantly higher (60%) for females mated second than for females mated first (41%; $\chi^2_1 = 8.17$, $P < 0.01$).

ANOVA revealed no difference between SR and ST males in the average number of pupae produced per female ($F_{1,115} = 1.28$, $P = 0.26$). Moreover, the ability of each type of male to produce any offspring did not differ between SR and ST males for the first ($\chi^2_1 = 0.35$, $P = 0.55$) or second ($\chi^2_1 = 1.54$, $P = 0.21$) female. Across both females ST males produced offspring in 31% of matings in comparison to 19% for SR males ($\chi^2_1 = 1.43$, $P = 0.23$).

Male fertility with high mating rate

Although more SR males (eight of 39) failed to produce offspring when allowed to mate with eight females in 24 h than ST males (three of 37), this apparent difference in infertility was not statistically significant ($\chi^2_1 = 2.36$, $P = 0.12$). However, there was a significant difference in the number of progeny produced by fertile SR and ST males ($F_{1,53} = 5.84$, $P = 0.019$), as well as among experimental replicates ($F_{10,53} = 5.48$, $P < 0.0001$). After controlling for replicate, ST males produced more offspring than SR males (Fig. 5).

Adult survival

Examination of cumulative survival plots (Fig. 6) reveals that survival was independent of sex (Log-Rank test, $\chi^2_1 = 0.01$, $P = 0.94$) and drive genotype within each sex (Log-Rank tests, females: $\chi^2_2 = 0.99$, $P = 0.61$; males: $\chi^2_1 = 0.04$, $P = 0.85$) but not independent across replicates of either sex (Log-Rank tests, females: $\chi^2_3 = 11.41$, $P = 0.010$; males: $\chi^2_5 = 42.2$, $P < 0.0001$). Proportional hazards regression analysis indicated that after controlling for differences between replicates, flies with longer eye span survived longer (likelihood ratio $\chi^2_1 = 13.06$, $P < 0.001$) independent of body length (likelihood ratio $\chi^2_1 = 0.0004$, $P = 0.98$). When the data were analysed separately by sex, eye span and replicate

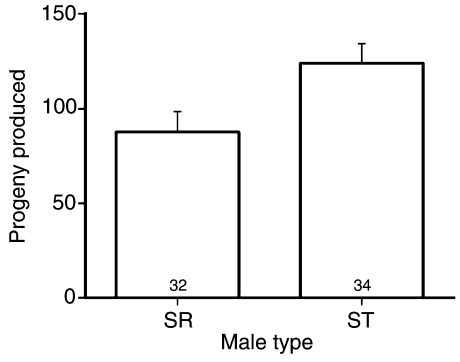

Fig. 5 Mean value (±SE) number of pupae produced in 2 weeks by eight females after being allowed to mate for 24 h. Numbers in each bar indicate the number of males.

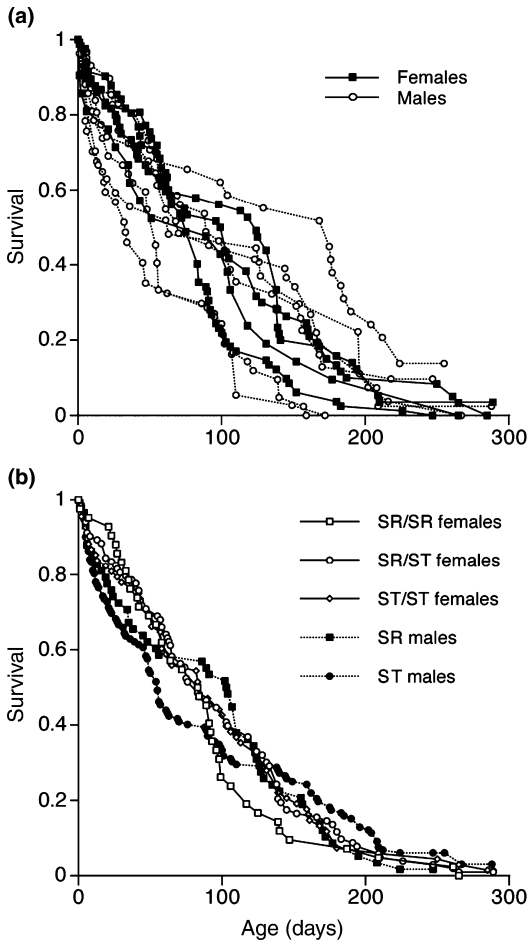

Fig. 6 (a) Cumulative survival for four replicate cages of females and six replicate cages of males. (b) Cumulative survival for males and females by genotype.

Table 4 Proportional hazards regression model results for survival by sex.

Sex	Source	d.f.	Likelihood ratio χ^2	*P*
Females	Replicate	3	11.69	0.009
	Eye span	1	8.11	0.004
	Genotype (replicate)*	8	15.18	0.056
Males	Replicate	5	28.24	<0.0001
	Eye span	1	4.72	0.0299
	Genotype (replicate)*	6	5.96	0.4281

*Genotype is nested within replicate (see Materials and methods).

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remained significant for each sex, and drive genotype approached significance in females but not males (Table 4).

Discussion

As outlined in the Introduction, unsuppressed X chromosome drive can be maintained in a stable polymorphism if there is either constant selection against female carriers or frequency-dependent selection acting via male fertility (Jaenike, 1996) or sperm competition (Taylor & Jaenike, 2002). Our results suggest that both of these mechanisms likely contribute to stabilizing the SR polymorphism in *C. dalmanni*. Whereas adult survival did not vary by *sex-ratio* genotype in either sex, we did find evidence for weak overdominance for female fecundity. Females with X^{SR}/X^{ST} chromosomes produced more progeny than X^{SR}/X^{SR} females. In addition, we found evidence that male fertility was influenced by drive when mating rates were high or when there was sperm competition, either of which could generate frequency-dependent selection.

For frequency-dependent selection to maintain an SR polymorphism, SR male fertility has to decline faster than ST male fertility as SR frequency increases and the population sex ratio becomes more female biased. This scenario could occur if sperm or accessory gland material is more limiting in SR than ST males. We found that sperm precedence of SR males is approximately half that of ST males in *C. dalmanni* on the second day of mating when females are allowed to mate only once with each male. When we housed single males with eight females we found that SR males also fathered fewer offspring. These results are consistent with SR males having fewer sperm available for insemination than ST males. SR sperm may also be less competitive than ST sperm. In the closely related species, *C. whitei*, females mated to SR males store the same number of sperm as females mated to ST males but SR sperm do not survive as well as ST sperm in the presence of seminal fluid from ST males (Fry & Wilkinson, 2004). Thus, SR males may not produce a sufficient quantity or quality of seminal material. This possibility is consistent with results that implicate male accessory gland size as being more important than testis size in determining male mating frequency in *C. dalmanni* (Baker *et al.*, 2003).

Selection could, therefore, act against SR by sperm competition at low frequencies and by sperm depletion at high frequencies when each male must mate with many females due to a female-biased sex ratio. In the wild, population sex ratios of *C. dalmanni* are typically biased, 2 : 1 or more in favour of females. Males fight for access to nocturnal aggregations where, if they are successful, they will mate with multiple females in rapid succession the following morning (Wilkinson & Reillo, 1994). Females remate frequently within and between days (Wilkinson *et al.*, 1998a). Thus, the selection against SR sperm we detected in the lab seems likely to be operating in the wild.

We also have indirect evidence that sperm competition influences SR persistence. After 30 generations of selection for increased and decreased eye span (Wolfenbarger & Wilkinson, 2001) we relaxed selection by choosing 25, rather than 10, males from each line to mate with 25 females each generation. Sometime after this change, the X^{SR1} haplotype was lost from all the lines (Wilkinson *et al.*, 2005). We made the change to minimize inbreeding, but we undoubtedly also increased sperm competition by changing the sex ratio. Consequently, X^{SR1} sperm were likely less able to fertilize offspring. If this interpretation is correct, SR would be most able to invade a population when the density is low and multiple mating is infrequent. As SR increases in frequency, the population should increase in density and become more female biased (Hamilton, 1967). If mating rate is influenced by population density, then seasonal variation in population density could cause oscillations in the intensity of sperm competition and concomitantly, SR frequency (cf. Atlan *et al.*, 2004). Thus, complete understanding of SR dynamics will require measuring SR male mating success as a function of population sex ratio and density. To interpret such experiments, it would be helpful to quantify mating rates and sperm competitive abilities for SR and ST males in the wild.

One possibility that we did not test directly in this paper is that egg-to-adult viability is lower for flies with SR than with ST chromosomes. Two lines of evidence suggest that larval survival is largely independent of SR. First, the number of progeny produced by different female genotypes exhibited an overdominant pattern that was similar to the number of eggs carried by unmated females (cf. Figs 2 and 3). If, for example, X^{SR}/X^{SR} larvae had lower survival than X^{ST}/X^{ST} larvae, then we would have expected X^{SR}/X^{SR} females to produce fewer progeny than X^{ST}/X^{ST} females, but they did not. Second, for a recent study (Johns *et al.*, 2005) we genotyped male and female offspring from three families created by pair-mating an ST male with an X^{SR}/X^{ST} female. In the absence of differential larval viability this cross should produce equal proportions of SR and ST males as well as X^{SR}/X^{ST} and X^{ST}/X^{ST} females. After examining 768 genotypes, only one comparison deviated from expectations. In one family there were more X^{SR}/X^{ST} than X^{ST}/X^{ST} female progeny ($\chi^2 = 5.85$, $P < 0.05$). All other comparisons, including SR and ST males from that same family, did not differ from 1 : 1. Thus, we have no evidence that selection acting against SR during development will reduce the equilibrium SR frequency.

Another possibility is that sexual selection may operate against SR. In *C. dalmanni*, females prefer to mate with males that have long eye span relative to their body length (Wilkinson *et al.*, 1998a) and brood sex ratios have been shown to change as a consequence of artificial selection for increased or decreased relative eye span

(Wilkinson *et al.*, 1998b). This change occurred because a major quantitative trait locus that affects eye span is linked to SR in *C. dalmanni* (Johns *et al.*, 2005). Thus, sexual selection should act to decrease the fertility of SR males relative to ST males below our estimates, at least when SR is at low frequencies, to the extent that females are able to identify SR males by eye span (Lande & Wilkinson, 1999). In this study we also found that eye span, independent of body length, correlates positively with adult survival, which may provide an additional reason for females to select mates based on eye span. Nevertheless, eye span is not a perfect predictor of *sex ratio*, so it is unlikely that mate selection for eye span could eliminate SR from a population. Furthermore, novel X^{SR} chromosomes that are not associated with eye span would be expected to invade such a population as they would not be susceptible to elimination by sexual selection (Lande & Wilkinson, 1999).

Finally, our evidence suggests that novel drive chromosomes may evolve rapidly in this system. As indicated in Table 1, we have found two SR chromosomes in flies that were collected from the same location 10 years apart. Although, we cannot be certain that the X^{SR2} haplotype replaced the X^{SR1} haplotype in the field, we can conclude that multiple X^{SR} chromosomes have recently existed in a single local population. Furthermore, all males that carried the X^{SR2} haplotype produced extremely biased sex ratios (cf. Table 1). In other words, we have not yet detected evidence of any modifiers of the X^{SR2} haplotype. In contrast, we do have evidence for at least one autosomal modifier of X^{SR1} (Johns *et al.*, 2005). We also observed weakly distorted sex ratios, i.e. the brood sex ratio was approximately equal to 0.25, in some males, but these cases of partial SR were never uniquely associated with a single X haplotype. We suspect that these cases of partial sex ratio distortion are likely caused by modifiers that interact with older X^{SR} chromosomes. By the methods used in this study we would score these as X^{ST} chromosomes. Future studies are needed to determine if such cryptic X^{SR} chromosomes can be detected by varying genetic background as has been done for *D. simulans* (Mercot *et al.*, 1995).

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