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Fitness consequences of a non-recombining *sex-ratio* drive chromosome can explain its prevalence in the wild

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Understanding the pleiotropic consequences of gene drive systems on host fitness is essential to predict their spread through a host population. Here, we study *sex-ratio* (SR) X-chromosome drive in the fly *Drosophila recens*, where SR causes the death of Y-bearing sperm in male carriers. SR males only sire daughters, which all carry SR, thus giving the chromosome a transmission advantage. The prevalence of the SR chromosome appears stable, suggesting pleiotropic costs. It was previously shown that females homozygous for SR are sterile, and here, we test for additional fitness costs of SR. We found that females heterozygous for SR have reduced fecundity and that male SR carriers have reduced fertility in conditions of sperm competition. We then use our fitness estimates to parametrize theoretical models of SR drive and show that the decrease in fecundity and sperm competition performance can account for the observed prevalence of SR in natural populations. In addition, we found that the expected equilibrium frequency of the SR chromosome is particularly sensitive to the degree of multiple mating and performance in sperm competition. Together, our data suggest that the mating system of the organism should be carefully considered during the development of gene drive systems.

1. Introduction

Selfish genetic elements promote their own transmission to the next generation even if there is a cost to the fitness of the host organism [1]. The intragenomic conflict they cause is thought to have consequences for the evolution of recombination, sex, speciation, mating systems and genome architecture [2]. Meiotic drivers are a type of selfish element that act by manipulating gametogenesis to favour their own transmission at the expense of the homologous locus or chromosome [3]. These are especially common on sex chromosomes but also occur on autosomes, and are known from a variety of taxa [4]. When on a sex chromosome, a meiotic driver also has a secondary effect of distorting the offspring sex ratio of the carrier. Here, we focus on sex-ratio (SR) drive, which occurs when an X-chromosome prevents the maturation of the Y-bearing sperm in a male carrier. An SR male thus transmits the selfish SR chromosome to all of his offspring, all of which are female. The consequences of SR can be severe, and at the most extreme include extinction of the host population owing to a lack of males [5,6]. However, in many natural populations, SR chromosomes are maintained at a low-to-moderate prevalence [3,4,7].

Selection on the rest of the genome to suppress SR can be very strong, especially if the SR chromosome spreads and the population-level SR becomes female-biased [5]. Factors on the Y-chromosomes and/or autosomes that suppress drive will be favoured, and in return, SR chromosomes may evolve enhancers that restore drive [8–10]. If multiple loci are necessary for drive expression, selection will favour chromosomal inversions that maintain genetic linkage between the driver and enhancer loci. However, when a chromosomal inversion forms, it will also capture a random snapshot of the linked segregating variation in addition to the drive loci. If some of these captured linked mutations are deleterious, they

can reduce the fecundity or viability of male and female carriers of SR. Reduced fitness of SR carriers can be sufficient to prevent the spread of SR through a population and allow it to persist as a balanced polymorphism [11,12].

Reduced fitness of SR carriers can also occur via pleiotropic effects of the SR-causing mutations themselves. For instance, because half of an SR male's sperm fail to develop, male fertility is the most obvious trait for SR to affect. Reduced male fertility, especially upon repeated matings, is a common feature of SR systems (reviewed in [4,13]). How often males mate, how fast SR males are depleted of and replenish sperm, how SR males fare in sperm competition and whether females discriminate against SR males either pre- or post-mating can both directly and indirectly affect SR male fertility [14–17]. A reduction in male fertility and poor performance of SR males in sperm competition can also balance an SR polymorphism, though for the latter case, the conditions are stringent [18–20].

In this study, we examine the fitness consequences of SR drive in the fly *Drosophila recens*. The SR X-chromosome in *D. recens* contains multiple inversions that presumably keep drivers and modifiers of drive in linkage disequilibrium by preventing crossing over between SR and wild-type standard (ST) chromosomes in females [21]. Females homozygous for SR are sterile, and SR chromosomes are fixed for this sterility mutation that was probably captured in the formation of an inversion [21,22]. In addition to limiting the upper frequency of SR to 25% in a population [12], this mutation also means that the SR chromosome does not have the opportunity to recombine in homozygous SR/SR females. As a consequence of not recombining with either ST or other SR chromosomes, the SR chromosome is a single haplotype that extends for more than 130 cM [21]. Because it does not enjoy the benefits of recombination, the SR chromosome is unable to purge deleterious mutations.

In natural populations of *D. recens*, only about 2.8% of males carry an SR chromosome (95% confidence interval (CI) 0.014–0.05) [21,22], which is nearly 10-fold lower than expected if homozygous female sterility was the only deleterious fitness consequence. The prevalence appears to be stable across both space and time. There is no evidence for segregating suppressors of drive in this species, suggesting they are rare or absent [21]. Thus, SR carriers must suffer an additional reduction in fitness beyond female homozygous sterility relative to wild-type flies. Other than female fertility, the only other fitness effect of SR that has been assayed in *D. recens* is male fertility, which is reduced relative to ST males, especially upon repeated mating [22]. In this study, we examine multiple components of fitness of carriers of SR in *D. recens*, including female fecundity, male and female longevity, female discrimination against mating with SR males, and sperm competition. We then parametrize theoretical models of SR to investigate whether the fitness effects we identify are sufficient to account for the observed frequency in the wild.

2. Material and methods

(a) Fly stocks and maintenance

We made use of a geographically diverse sample of ST strains, each of which was founded with many wild-caught individuals. These are from Munising, MI (collected in 2004; denoted as MI); Bemidji, MN (2004, MN); Charlottetown, Prince Edward Island (2003; PEI) and Rochester, NY (NY1 from 2004; NY2 from 2002). Population genetic work in this species suggests that there is minimal population structure among these locations [23]. We created a geographically diverse wild-type stock by crossing together the MI, MN, NY1 and PEI stocks. This 'mixed' stock was maintained for three generations before being used in experiments. We also backcrossed this mixed stock twice into two different stocks that each carried a recessive eye colour mutation, including *brown* (X-linked) and *dark* (autosomal).

We examined two different wild-caught SR X-chromosomes, each of which originated from a single wild-caught male: one was collected in 2004 in Chebeague Island, ME (denoted as SR_{ME}), and the other in 2004 in Rochester, NY (denoted as SR_{NY}). Both stocks were maintained in the laboratory 'balanced' over an ST X-chromosome that carries both *yellow* (*y*) and *brown* (*b*) X-linked mutations. Each SR stock was maintained by crossing *y, b*/SR females to *y, b* males each generation. Over many years of maintaining these stocks, we have never observed a recombination event between *y, b* and SR. Before being used in experiments, each SR chromosome was outcrossed for two generations to the mixed *brown* stock described above and maintained by crossing *b*/SR females with *b* males.

Fly cultures and crosses were maintained on instant *Drosophila* food (Carolina Biological, Burlington, NC, USA) supplemented with commercial mushroom (*Agaricus bisporus*) and reared at 20°C on a 14 : 10 light cycle and 60% relative humidity. Light CO_2 anaesthesia was used to collect virgins, and air aspiration was used during experiments. Mating assays started within 1 h of the incubator lights on and used 7–8-day-old virgin flies. In some experiments, all individuals were wild-type for eye colour regardless of SR carrier status. In these cases, we determined SR status using a single nucleotide polymorphism that is fixed between SR and ST in the X-linked *cp36* gene [21]. We incubated each amplicon with the enzyme *bsrgI* (New England Biolabs, Ipswich, MA, USA), which cuts the SR but not the ST allele, and visualized the fragments on an agarose gel.

(b) Female fecundity

We used a series of genetic crosses to produce morphologically indistinguishable ST/ST and SR/ST females with a similar genetic background (electronic supplementary material, figure S1). These crosses were completed separately for the two SR stocks (SR_{ME} and SR_{NY}). Virgin 7-day-old ST/ST or SR/ST females were placed individually in a vial with three 7-day-old ST virgin males from the wild-type mixed stock, and the flies were transferred to a fresh vial every 6 days. After 18 days, the adult flies were removed, and females that survived the full 18 days were genotyped for SR carrier status and their offspring counted. We used an analysis of variance to test for the effects of SR strain and SR carrier status nested within SR strain on the number of offspring produced. We note that this experiment does not directly measure egg-to-adult survival. Unless otherwise noted, statistics used JMP v. 14.1.0 (SAS Institutes, Cary, NC, USA).

(c) Longevity

We used genetic crosses with the mixed *brown* stock to create ST and SR flies that were genetically similar except for the X-chromosome (electronic supplementary material, figure S2). These crosses were completed separately for the two SR stocks (SR_{ME} and SR_{NY}) and four wild-type stocks (MI, NY1, NY2, PEI). Males used in the experiment carry an ST or SR X-chromosome from the tester stock, and females are heterozygous at the X-chromosome, with one *b* X-chromosome and the other from the tester ST or SR stock (electronic supplementary material, figure S2). Flies were collected as virgins and stored 10 flies $vial^{-1}$ on regular culture food. At one week of age, flies were transferred

to vials containing a blended mushroom-agar food. Survival was assayed every 1–2 days, and the flies were transferred to fresh food every 4 days. Survival analyses used a Cox proportional hazard model with PROC PHREG in SAS v. 9.3 (SAS Institutes), with SR carrier status and line nested within SR carrier status as fixed effect variables, and the vial as a random effect variable. Analyses were completed separately for females and males.

(d) Female mate discrimination

We tested for virgin female discrimination against mating with SR males using both no-choice and choice assays. First, we conducted no-choice trials with SR_{ME} and ST males. Using a series of genetic crosses, we created morphologically indistinguishable males that were either SR_{ME} or ST with an otherwise similar genetic background (electronic supplementary material, figure S3). Mate trials of a female from the mixed wild-type stock with either a single SR_{ME} or ST male were observed for 2 h, with mating latency noted. After the trial, males were genotyped as SR_{ME} or ST as described above. Analyses used Fisher's exact tests (FET) and non-parametric Wilcoxon rank-sum tests.

Second, we used a mass mating cage experiment to test whether females discriminate against mating with male SR carriers under high male–male competition conditions. Each cage included 40 females from the *dark* mixed stock, 40 ST males from the *dark* mixed stock, and 40 tester males that were wild-type for eye colour and carried ST, SR_{ME} or SR_{NY}. Females were placed in the cage first, and after 5 min, all the males were added; copulating pairs were removed by aspiration and each male was scored for eye colour. Each cage was observed until half of the females mated. A total of 35 cage experiments were carried out, which included 16 with SR_{ME} males, 14 with SR_{NY} males and five controls with wild-type ST males from the mixed stock. We used a Cochran–Mantel–Haenszel test within each male type to test for a deviation from 50 : 50 random mating.

(e) Sperm competition assay

We used cage assays to examine the rate of female remating and whether SR males suffer reduced fertility in conditions of potentially high sperm competition. We used small cages (10 cm diameter × 15 cm tall) that contained instant *Drosophila* food and a mushroom cap. In each cage, we placed 20 *dark* females, 20 *dark* ST males, and 20 of either wild-type ST males from the mixed stock or SR_{ME} males. All flies were 7-day-old virgins when placed in the cage. Each cage was left for 1, 2, 3, 4, 6 or 10 days, after which the flies were removed and females were placed individually in culture vials for 10 days, after which they were discarded. The offspring of each female were scored for eye colour to determine paternity. Between 4 and 10 cages were set up for each day and male SR status ($n = 65$ cages total).

Females that produced both *dark* and wild-type offspring were inferred to have mated with both types of males. Females that produced only *dark* or wild-type offspring could have mated with one male, multiple males of the same phenotype, or males of different phenotypes but then only used the sperm of one type of male. Thus, this experiment does not disentangle pre- and post-mating female discrimination against SR males; our goal was to estimate whether females mate multiply, and if in conditions of both mate competition and female choice, whether SR males sire fewer offspring than ST males.

We asked whether the pattern of paternity differed between days and SR status of the tester male type. First, we compared the phenotypes present in the offspring using a nominal logistic regression on the paternity of each female's offspring (all *dark*, all wild-type, both *dark* and wild-type), with days in the cage, SR status nested within days, and the cage replicate nested within SR status and days as effects in the model. Second, we asked whether SR males sire fewer offspring than ST males under conditions of sperm competition. For each day, we asked if the fraction of wild-type offspring differed among females that were with SR or ST males as the tester male. Using females that were with males for 3 or more days, we asked if the proportion of wild-type offspring was different than 0.5 using a *t*-test. If sperm competitive ability is equal between *dark* and the wild-type tester SR or ST males, we expect that each male genotype sires about half the offspring on average.

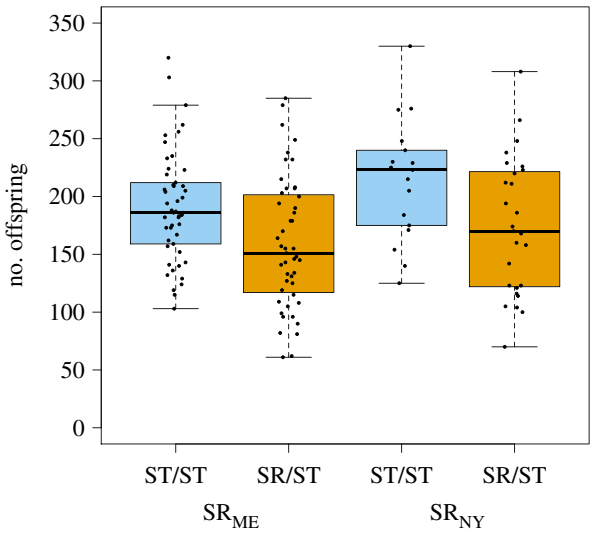

Figure 1. Female fecundity of SR carriers is reduced when compared with wild-type females. Two different SR chromosomes were assayed (SR_{ME} and SR_{NY}) in heterozygous females, and each was compared to wild-type (ST/ST) females with an otherwise similar genetic background. The dark line in the box indicates the median and the bottom and top of the box indicate the first and third quartiles, respectively. (Online version in colour.)

3. Results

(a) *Sex-ratio* reduces female fecundity

We find that SR/ST females suffer reduced fecundity relative to ST/ST females (figure 1). Overall, SR/ST females produce fewer offspring than ST/ST females ($F_{2,2} = 7.0$, $p = 0.0013$), and this is significant for each SR strain (SR_{ME}: $t = -2.9$, $p = 0.0049$; SR_{NY}: $t = -2.4$, $p = 0.018$). However, there was not a significant difference in the effect of SR strain on female fecundity (SR_{ME}: 159 ± 8.3 (mean ± s.e.), SR_{NY}: 174 ± 11.5; $F_{1,1} = 3.7$, $p = 0.056$). Across both SR strains combined, SR/ST females produced an average of 165 ± 6.7 offspring ($n = 74$) whereas ST/ST females produced an average of 197 ± 6.1 offspring ($n = 66$), a 16.3% reduction in fecundity (95% CI 0.074–0.245).

(b) No effect of *sex-ratio* on longevity

We find segregating variation for longevity, but we do not find that SR carriers show reduced survival relative to ST carriers. We assayed 2669 flies for longevity, which included female and male carriers of two different SR chromosomes and four different ST chromosomes (electronic supplementary material, table S1). SR carrier status did not contribute significantly to variation in longevity (figure 2; electronic supplementary material, figure S4; females: Wald $\chi^2_1 = 2.5$, $p = 0.1$; males: Wald $\chi^2_1 = 0.8$, $p = 0.7$), but there was a significant effect of line within chromosome type (females: Wald $\chi^2_4 = 24$, $p = 0.0004$; males: Wald $\chi^2_4 = 3$, $p < 0.0001$). Within chromosome type, there was no significant difference

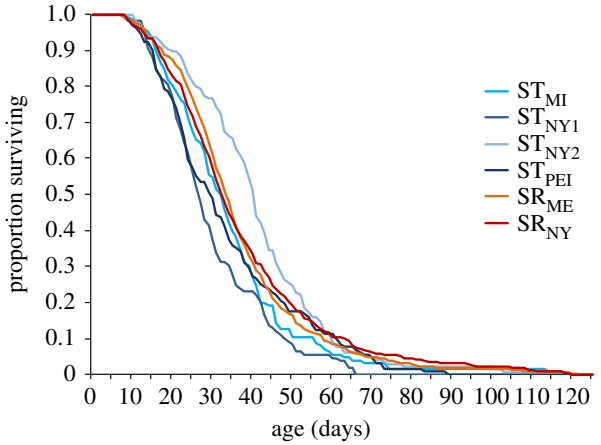

Figure 2. The longevity of female SR carriers (SR/ST) is not decreased relative to wild-type (ST/ST) females. Flies are separated by strain, which included four ST strains and two SR strains. (Online version in colour.)

between the two SR strains (females: Wald $\chi^2_1 = 0.2$, $p = 0.6$; males: Wald $\chi^2_1 = 0.3$, $p = 0.6$), though there was among ST strains, which is owing to a longer lifespan of the NY2 line when compared with the other lines (females: Wald $\chi^2_1 = 6$, $p = 0.01$; males: Wald $\chi^2_1 = 13$, $p = 0.0004$).

(c) Females do not discriminate against mating with *sex-ratio* males

We do not find evidence that virgin females discriminate against mating with SR males. First, in no-choice trials, females mated at similar rates with SR_{ME} and ST males (SR_{ME}: 97/128 (76%) mated, ST: 40/49 (82%) mated in 2 h; FET, $p = 0.55$). Of the pairs that copulated, the mating latency was not significantly different between SR_{ME} and ST males (SR_{ME}: 34.0 ± 2.6 min (mean ± s.e.), ST: 29.9 ± 4.5; Wilcoxon rank-sum test, $Z = 1.69$, $p = 0.09$; electronic supplementary material, figure S5). Second, we do not find evidence that females discriminate against mating with SR males in situations with the extensive male–male competition. In our cage experiments, none of the three tester male genotypes (ST, SR_{ME} and SR_{NY}) showed a consistent difference from 50 : 50 random mating against the *dark* males (ST: $\chi^2_{1\,MH} = 0.18$, $p = 0.7$; SR_{ME}: $\chi^2_{1\,MH} = 0.01$, $p = 0.9$; SR_{NY}: $\chi^2_{1\,MH} = 1.5$, $p = 0.2$; electronic supplementary material, figure S6).

(d) Multiple mating is common and *sex-ratio* suffers in sperm competition

Most *D. recens* females remate within a few days and use the sperm of multiple males to sire their offspring. We found that the proportion of females that produced both wild-type and *dark*-eyed offspring, indicative of multiple mating, increased with the number of days the flies were held together ($\chi^2_5 = 27$, $p < 0.0001$), and that this was owing to a lower number of multiply mated females in the 1-day treatment ($\chi^2 = 20$, $p < 0.0001$; figure 3; electronic supplementary material, figure S7). Whether the wild-type eyed males in the cage were ST or SR did not affect this pattern ($\chi^2_6 = 10$, $p = 0.13$). If a female mates twice and each mating is with a random male, half of the time she would be expected to mate with both *dark* and wild-type males (and half of the time she would mate with two wild-type or two *dark*-eyed

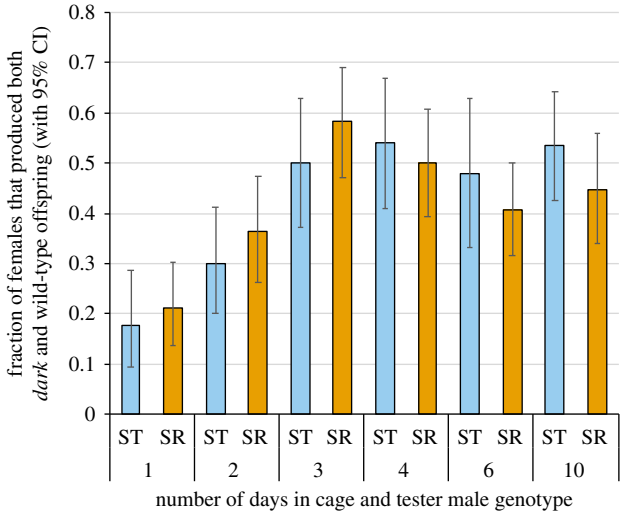

Figure 3. Paternity analyses from the sperm competition cage experiment indicate females mate multiply. Shown is the fraction of females that produced both *dark* and wild-type offspring, indicating they had mated with both types of males, separated by the number of days the flies were held together and the male tester genotype (SR or ST). The error bars indicate the 95% binomial confidence interval. (Online version in colour.)

males). If she uses sperm from both males, then she will produce a mixed brood containing both wild-type and *dark*-eyed offspring. Using the occurrence of mixed broods is a conservative estimate of remating rate, as we cannot detect when females mate with males of the same eye colour genotype or mate twice but do not use the sperm of both males. Combining SR and ST cages within each day, when flies are kept together for 24 h, 20% of females produced mixed broods, indicating they mated with both *dark* and wild-type males. This suggests that about 40% of females mated at least twice within 24 h, because we only detected half of the broods produced from double matings (95% CI 0.32–0.47). The proportion of females that produced mixed broods increased to 33% after 2 days (approx. 66% mated at least twice) and increased to approximately 50% after 3 days, suggesting that all of the females had mated twice (figure 3).

These data also provide information about how females use sperm. If females that mate more than twice use the sperm of those males equally, then most or all of the females should eventually sire mixed broods. This is because when a female mates multiply and uses the sperm of all of her mates equally, the probability that she produces offspring sired by n males of the same type $= (1/2)^{(n-1)}$, where n is the number of matings. Importantly, this number rapidly declines to zero as n becomes large. However, we observe that the frequency of females producing mixed broods levels off around 50% (figure 3). This suggests that the effective number of mates in such females is $n = 2$ and that females only use the sperm of the last two males they mated with.

We find that in conditions of sperm competition, SR males sire fewer offspring than ST males. In cages where flies were kept together for 2, 3, 4 or 6 days, ST males sired a greater proportion of the offspring than SR males (each $p < 0.05$; figure 4). The fraction of offspring by SR versus ST males did not differ significantly in cages where flies were kept together for 1 or 10 days (each $p > 0.05$). In the cages where the flies were kept together for 1 day, the *dark* males sired more offspring regardless of the SR status of the tester wild-type males (figure 4); it is unclear why this is because

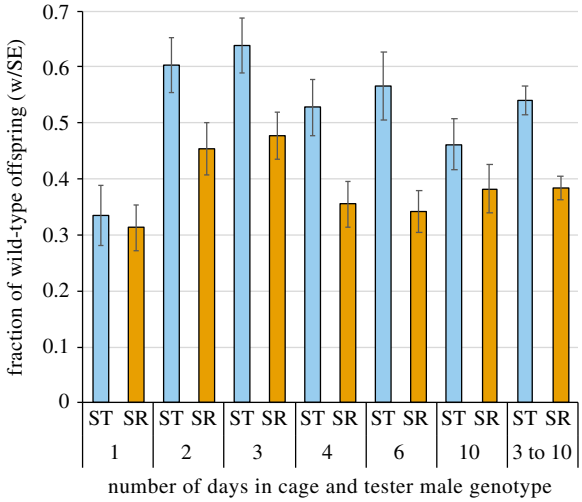

Figure 4. Paternity analyses from the sperm competition cage experiment indicate that SR males suffer in sperm competition relative to ST males. Shown is the average fraction of wild-type offspring produced among females separated by the number of days the flies were held together and the tester male genotype (SR or ST). The error bars indicate the standard error of the mean. (Online version in colour.)

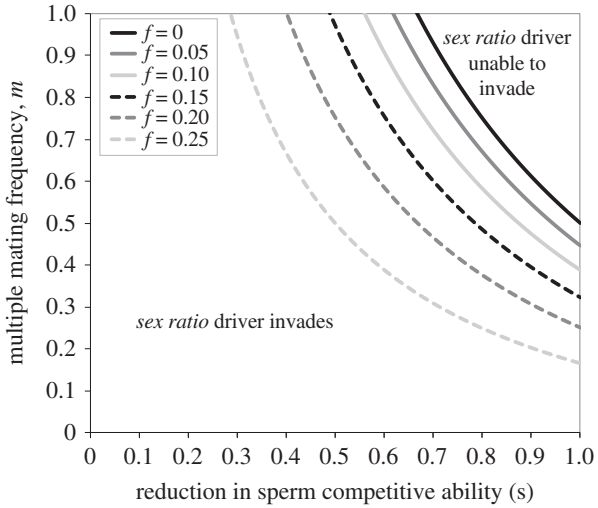

Figure 5. The region of the parameter space allowing invasion of a newly arising *sex-ratio* driving X-chromosome. Larger reductions in sperm competitive ability (larger s), higher incidence of multiple mating (larger m) and higher fecundity cost for heterozygous females (larger f) all reduce the likelihood that a driving X-chromosome will invade.

our mating experiments described above found no preference of *dark* females for *dark* males. This effect goes away after day 1, where all of the cages with ST tester males have approximately 50% wild-type offspring, as expected if there was no preference for male eye colour.

To infer sperm competition ability, we consider only cages that were left for 3 or more days, where most or all females are multiply mated. We find that the proportion of wild-type offspring produced by females in cages where ST males competed with *dark* males does not differ significantly from 0.5 (0.54 ± 0.025; $t_{260} = 1.6$, $p = 0.12$; figure 4), indicating that ST and *dark* males perform equally in sperm competition. By contrast, the proportion of offspring produced by SR males when in competition with *dark* males is significantly lower than 0.50 (0.38 ± 0.02, $t_{376} = -5.6$, $p < 0.0001$; figure 4), indicating that SR males perform poorly in sperm competition. Overall, SR males sire 30% fewer offspring than ST males when in sperm competition with *dark* males.

4. Theory

To determine whether the fitness effects we have measured are sufficient to explain the low frequency of SR observed in the wild, we modelled the evolution of the SR chromosome in *D. recens*. The model adds sperm competition to the classic model of SR evolution [12], with two additional assumptions that capture the specifics of the *D. recens* SR system. These assumptions include complete drive, such that SR-bearing males produce no Y-bearing sperm, and complete sterility of females homozygous for SR (mathematically equivalent to zero viability). The model includes three parameters: f—the reduction in the fecundity of heterozygous females, s—the reduction in sperm competitive ability of SR males and m—the female multiple mating rate ($0 \leq f, s, m \leq 1$). Females mate singly with probability $(1 - m)$ and doubly with probability m. When a female mates twice, she could mate with two identical males, i.e. both ST or both SR, in which case there is no opportunity for sperm competition between SR and ST, or she may mate with one of each type of male, i.e. a mixed mating, in which case sperm competition occurs. SR males contribute $(1 - s)$ as many sperm as ST males, so they fertilize a proportion $(1 - s)/(2 - s)$ of a female's eggs. If there is no reduction in sperm competitive ability $s = 0$, and SR males fertilize half the eggs. If $s > 0$, SR males will sire fewer than half the eggs in mixed mating females. We do not include a decrease in egg-to-adult viability for SR carriers because our data from the crosses in the electronic supplementary material, figures S1–S3, indicates no such cost (discussed below).

With these assumptions, we determined the recursions in the frequencies of the five genotypes within a population. These and additional details are in the electronic supplementary material, appendix. We first used these recursions to determine the conditions for the invasion of the SR chromosome. SR fixation is prevented because of female sterility in SR homozygotes. A stability analysis at the fixation of the ST chromosome indicates that the SR chromosome can only invade if

$$m < \frac{(2 - s)(1 - 3f)}{2(1 - f)s}. \tag{4.1}$$

Several implications are immediately apparent from this condition. First, a reduction in heterozygote female fecundity of one-third will prevent the invasion of the driving chromosome, in agreement with previous work with appropriate parameter value substitutions [12]. If the right-hand side of equation (4.1) is greater than 1, the SR chromosome will always invade the population. Biologically, this means that the driving SR chromosome is favoured when the female fecundity cost (f) is small, the sperm competition cost (s) is small and the frequency of multiple mating (m) is low. In figure 5, we show the region of the parameter space that allows invasion of the SR chromosome.

We also used the recursions to run simulations. Using various parameter combinations, we ran forward simulations until an equilibrium was reached, at which point the frequency of the SR chromosome no longer changed (details in the electronic supplementary material, appendix). Our interest is in

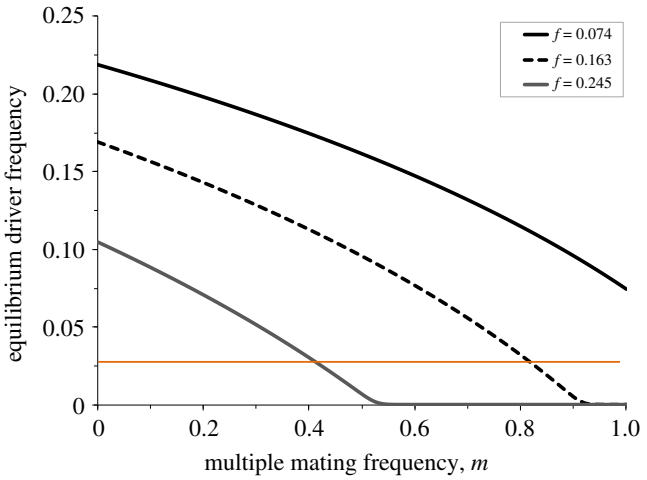

Figure 6. The equilibrium frequency of a *sex-ratio* driving X-chromosome as a function of the rate of multiple mating (m) for the mean and 95% confidence interval limit values of the reduction in the fecundity of heterozygous females (f). Larger reductions in fecundity (larger f) and higher rates of multiple mating (larger m) both reduce the expected equilibrium allele frequency. In all cases, the reduction in sperm competition, s, is set to 0.5, which is the expectation if the competition is purely owing to the number of viable sperm present in an ejaculate. The horizontal orange line at 0.028 indicates the frequency of the driving X-chromosome in nature. (Online version in colour.)

whether our fitness estimates can explain the observed allele frequency. We used the forward simulations to calculate the relationship between equilibrium SR frequency and the multiple mating rate, which is the one parameter that we have not estimated empirically. The result of these simulations is shown in figure 6 for our estimate of female fecundity ($f = 0.163$) and its 95% CIs. In these simuations, we set s equal to 0.5, implying sperm competitive ability is proportional to sperm numbers with SR males producing half as many sperm owing to the death of Y-bearing sperm. As such, SR male will fertilize one-third of the eggs in a mixed mating. In our sperm competition experiment, SR males would thus be expected to sire $(0.25 \times 1) + (0.5 \times 0.33)$ of the total offspring, because 25% of the time a female is expected to mate with two SR males and half the time a female is expected to mate with ST and SR males. This value of 0.417 is not significantly different from what we observe in our sperm competition assay, where 38% of offspring are sired by SR males ($t_{376} = -1.75$, $p = 0.08$). As we demonstrated for virgin males and was suggested by our sperm competition assay (figure 4), we assume that *D. recens* females do not discriminate between ST and SR males when multiply mating. Using our best estimates of $f = 0.163$, and setting $s = 0.5$, consistent with sperm mixing, the observed equilibrium allele frequency of 0.028 is predicted if multiple mating is high ($m = 0.815$).

5. Discussion

Meiotic drivers are often found at low-to-moderate prevalence in the wild even though they enjoy a substantial benefit during transmission from one generation to the next. Natural drive systems such as the SR system in *D. recens*, which is thought to be at about 300 000 years old [21], can provide insight into the long-term consequences of coevolution of the host and driver. Even though the SR chromosome in *D. recens* benefits from being transmitted to 100% of the offspring by the males that carry it, it remains at low prevalence in natural populations that span the geographical range of this species [21]. Here, we investigate various fitness costs of SR to its carriers and ask how these may contribute to the observed equilibrium prevalence.

Drosophila recens females homozygous for SR are sterile [21,22], which limits its maximum frequency to 25%. Here, we show that heterozygous female carriers suffer a 16% reduction in fecundity when compared with their wild-type counterparts (figure 1). Because extensive inversions differentiate the SR and ST chromosomes, we suggest that this reduced fecundity is probably because the resulting products of crossing over are not viable owing to chromosomal abnormalities of the X-chromosome (e.g. [24,25]). In other species with SR, fecundity effects are mixed. For instance, in one population of *Drosophila pseudoobscura*, fecundity of both SR/SR and SR/ST females is as high as wild type, whereas in a different location, SR/SR fecundity is half that of flies that carry ST [26]. By contrast, in the stalk-eyed fly *Teleopsis dalmanni* SR/ST females have higher fecundity than SR/SR and ST/ST females [27].

The second deleterious fitness effect we identify is on male fertility. Unless balanced by other fitness effects, SR will increase in frequency as long as it produces more than half the offspring of ST males. Jaenike [22] assayed male fertility in *D. recens* and found that after a single mating, an SR male sired about 30% fewer offspring as an ST male, and after subsequent matings that led to sperm depletion, SR males sired 70% fewer offspring than ST males. Thus, only when an SR male mates with many females in a relatively short time period is SR fertility lower than 50% of ST males and sufficient to prevent the spread of SR. However, whether this happens will depend on how often the males actually mate and how the sperm fare in sperm competition against ST males. No paternity studies have been completed on wild *D. recens*, but the results of our cage experiment suggest that females remate within 1 day and use the sperm of the last two males they mated with to sire their offspring. Because SR is rare in the wild and females mate multiply, SR will probably always compete with ST in sperm competition (i.e. m close to 1). We note that our cages contained twice as many males as females. Thus, if females mate once per day, then males mate on average once every other day, implying sperm depletion is not likely (see [22]). Thus, our sperm competition experiment probably did not lead to conditions of male sperm depletion in either SR or ST males. One additional aspect that may affect sperm competition is whether females remate faster after mating with SR males compared to ST males. We note that figure 3 suggests a very slight but non-significant increase in the rate of mixed matings when SR males are the tester males compared to ST; this warrants further investigation in future studies.

Our cage experiment simulates the condition of high sperm competition, and it also incorporates the preferences of both virgin and mated females, as well as any post-mating prezygotic and postzygotic differences between SR and ST males. We find that ST males sired about 54% of the offspring when in competition with *dark* males, which is not a significant difference from the expected 50% (figure 4). However, SR males sired only about 38% of the offspring when in competition with *dark* males, which is a reduction of 30% relative to ST. We note that this result is probably not owing to the decreased viability of SR carriers in the offspring. First, in the 1-day cages

where many females mated only once, the proportion of wild-type offspring is not different between females kept with SR versus ST tester males (figure 4). Second, in our crosses of SR/ST females to ST/Y males in other experiments (e.g. the F_1 cross in the electronic supplementary material, figures S1–S3), we have not seen a deviation from the expected Mendelian 1 : 1 offspring ratios. Specifically, among 2866 offspring scored from these crosses, the fraction of SR carriers at eclosion was 0.505, and the CI includes the 0.5 Mendelian expectation (95% CI 0.48–0.52). This is in contrast with some other recent studies, for instance, in stalk-eyed flies, where egg-to-adult viability is lower for SR than ST carriers [28]. Instead, in our sperm competition experiment, we find that the fraction of offspring sired by SR males is not significantly different from the expected 42% expected if SR males produce half the sperm as ST males ($s = 0.5$). Thus, our data are consistent with reduced SR male fertility owing to the lower number of sperm transferred and not the ability of those sperm to compete for fertilizations. In other systems, both the number of sperm and the ability of sperm to compete for fertilizations contribute to reduced fertility of SR males [27,29–32].

We did not find a fitness effect of SR on the other traits that we assayed. The longevity of SR carriers was similar to wild-type flies (figure 2), though we did find segregating variation among wild-type strains, as is common in other species (e.g. [33,34]). A similar result was found in stalk-eyed flies [27]. Second, we find that SR does not compromise the attractiveness of male carriers, at least by virgin females, as females mated with ST and SR males at similar rates and with similar latencies in both no-choice and choice assays (electronic supplementary material, figures S5 and S6). A similar result has been found in the SR system of *D. pseudoobscura* and the *t*-haplotype system of *Mus* [35,36], though in stalk-eyed flies, females can discriminate against SR males owing to genetic linkage with eye stalk length [37]. Overall, we consider our fitness estimates of fitness reductions to be conservative because they are assayed in the laboratory and over a single generation. A single generation may be insufficient to observe small fitness differences that would be seen by selection in the wild. For instance, significant effects of multiple mating have been observed in multi-generation cage experiments [38,39].

Are the fitness consequences we and others have identified sufficient to account for the observed prevalence of SR in natural populations of *D. recens*? The female sterility mutation limits SR to 25% prevalence, but in the wild, SR is found at almost a 10-fold lower frequency than this. Our theoretical inferences suggest that a 16% reduction in female fecundity of SR carriers and a reduction in sperm competition of 50% for SR can account for the 2.8% prevalence that is found in the wild if the multiple mating rate is around 82% (figure 6). If all females mate once, then the reduction in female fecundity (f) determines the maximum equilibrium frequency of SR (figure 5), and if the fecundity effect is minimal then the driver will invade. However, like most insects, *D. recens* mates multiply, in which case both the frequency of multiple mating (m) and sperm competitive ability of SR (s) have a significant effect on SR dynamics (as also seen in [11,12]). For instance, if m is high, even a small perturbation in sperm competitive ability of SR (s) will determine whether the driver invades or is lost from the population (figure 5). In addition, the expected equilibrium frequency is very sensitive to both m and s (electronic supplementary material, figure S8).

Incorporating multiple components of fitness into models of meiotic drive dynamics is a promising route to understand how drivers are maintained at an intermediate frequency in the wild. In addition to our system, this approach has helped to explain the prevalence of centromere drive in *Mimulus* plants [40], Ab10 in maize [41], *t*-haplotype in mouse [42], Segregation Distorter in *Drosophila melanogaster* [43] and SR drive in *D. pseudoobscura* [12]. These studies indicate that estimating all of the fitness consequences of natural gene drivers and the parameters that contribute to their prevalence is critical when developing synthetic gene drive systems (e.g. [3,44,45]). Whether a driver will invade, and if so what its equilibrium frequency will be, determine whether the development of a gene drive system is worth it in terms of achieving its intended effect. If a gene drive system operates by targeting and eliminating half the sperm, then multiple mating is a key parameter that should be considered (e.g. [46]). While it may be possible to create a driver that has few fitness effects other than male fertility, it may be difficult, or probably impossible, to create a gene drive system that also prevents multiple mating. If multiple mating is high and there is a cost to the driver in sperm competition, it may be difficult for a gene driver to invade to high frequency under any conditions.

Data accessibility. Raw data are deposited in the Dryad Digital Repository: https://doi.org/10.5061/dryad.3tx95x6br [47].

Authors' contributions. K.A.D. performed the experiments and D.W.H. performed the theory. Both authors wrote the manuscript.

Competing interests. We declare we have no competing interests.

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