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An X-linked meiotic drive allele has strong, recessive fitness costs in female *Drosophila pseudoobscura*

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Selfish 'meiotic drive' alleles are transmitted to greater than 50% of offspring, allowing them to rapidly invade populations even if they reduce the fitness of individuals carrying them. Theory predicts that drivers should either fix or go extinct, yet some drivers defy these predictions by persisting at low, stable frequencies for decades. One possible explanation for this discrepancy is that drivers are especially costly when homozygous, although empirical tests of this idea are rare and equivocal. Here, we measure the fitness of female *Drosophila pseudoobscura* carrying zero, one or two copies of the X-linked driver *sex ratio* (*SR*). *SR* had strong negative effects on female offspring production and the probability of reproductive failure, and these effects were largely similar across four genetic backgrounds. *SR* was especially costly when homozygous. We used our fitness measurements to parametrize a population genetic model, and found that the female fitness costs observed here can explain the puzzlingly low allele frequency of *SR* in nature. We also use the model to show how spatial variation in female mating behaviour, fitness costs of *SR* and the reduced siring success of *SR* males can jointly explain the North–South cline in *SR* frequencies across North America.

1. Introduction

Selfish genetic elements (SGEs) are ubiquitous in living organisms and have major impacts on the evolution of sex and genetic systems [1,2]. SGEs increase their transmission by subverting the usual patterns of Mendelian inheritance, ensuring that they are inherited by up to 100% of the progeny of heterozygous individuals, instead of the expected 50% [2,3]. Sex chromosome meiotic drivers cause increased transmission of either the *X* or *Y* chromosome from individuals of the heterogametic sex, by inducing developmental failure in sperm that do not carry the driving chromosome resulting in sex-ratio distortion [4]. This transmission advantage means that drive-bearing chromosomes should spread rapidly to fixation, potentially causing population extinction due to the lack of one sex [5,6]. However, meiotic drivers are often found at stable frequencies in natural populations [7,8].

The factors that maintain stable coexistence between driving and non-driving chromosomes have long been unclear [9]. Any mechanism that imposes negative frequency-dependent selection on the driver will reduce the relative fitness of the drive allele as it spreads through the population. Eventually, selection against the driver may become strong enough to counteract its transmission advantage, leading to an evolutionarily stable polymorphism in which drive and non-drive alleles coexist [10]. One common source of frequency-dependent selection is fitness costs experienced by individuals carrying two copies of the drive allele (e.g. [4,10–13]). If drive homozygotes suffer higher fitness costs than drive heterozygotes, the average fitness of drive-carrying individuals will decline as the driver increases in frequency, due to the increasing frequency of homozygotes.

There is some evidence that drive alleles are indeed more costly to fitness in homozygous form. Many meiotic drivers are found in regions of the genome with little or no recombination [4] and these regions are thought to accumulate deleterious mutations, many of which are likely to be recessive [14]. For example, the 't-haplotype', a large, non-recombining meiotic drive element found in mice, is homozygous-lethal [15], and some *Drosophila* drivers result in reduced homozygote fitness [16,17].

Various aspects of the mating system have also been hypothesized to act as sources of negative frequency-dependent selection. Males carrying meiotic drive produce fewer sperm, and sometimes become sperm-limited more quickly than non-drive males [18]. Many meiotic drivers cause the sex ratio to become female-biased as they invade (due to being X-linked; see below), meaning that male fitness becomes increasingly dependent on being able to fertilize multiple partners. This produces negative frequency-dependent selection on drive, potentially halting its invasion [11]. A subtly different hypothesis involves sperm competition and polyandry. Drive males are often disadvantaged in sperm competition relative to non-drive males, due to producing fewer sperm, and possibly also to other fitness costs of the drive allele [18–22]. As a consequence, the average fitness of drive males declines as the average number of mates per female increases.

Theoretical models have found that polyandry can stabilize allele frequencies and preserve polymorphism for drive, but only if there are high fitness costs to females homozygous for drive [10]. This model was based on the biology of *sex ratio* (abbreviated *SR*), a meiotic driving X chromosome in the fruit fly *Drosophila pseudoobscura*. *SR* kills the Y chromosome-bearing sperm of male carriers during spermatogenesis [4,23], resulting in all female broods. Flies that carry non-driving X chromosomes are referred to as 'standard' (*ST*) flies. All else being equal, *SR* is predicted to outcompete *ST* due to its large transmission advantage, yet in reality, *SR* has persisted at stable, intermediate frequencies in natural populations for many decades [24,25].

SR reduces the number of sperm male carriers produce, causing *SR* carriers to have reduced sperm competitive ability [20]. Thus, the relative fitness of *SR* will be lower in populations in which most females mate multiply [26], and polyandry may be regarded as an adaptation that reduces the number of eggs fertilized by *SR*-carrying sperm, which incidentally reduces the risk of extinction due to a shortage of males [5]. Accordingly, *SR* exhibits a latitudinal cline in frequency across the USA, which correlates negatively with another cline in the frequency of polyandry [25]. Specifically, in northern populations, females have high re-mating frequencies and *SR* frequency is low, whereas in southern populations, the reverse is true [25].

In contrast with males, the relative fitness of females carrying the *SR* distorter is relatively little-studied. The *SR* chromosome carries three inversions that greatly reduce recombination [27], and therefore, *SR* may have accumulated more deleterious mutations than standard *ST* X chromosomes [14]. Additionally, *SR* is found at low frequencies (approx. 1–30%; [7,12,24,25]), and hence has a low effective population size [28]. This reduces the efficacy of selection on competing driving X haplotypes, allowing more mutations to accumulate. *SR* may therefore impose fitness costs on female carriers, particularly those homozygous for *SR*. However, Beckenbach [12] only detected minor differences fitness costs in *SR* females in one of two examined *D. psedoobscura* populations, but concluded that this difference was insufficient to prevent *SR* from fixing. In general, while this hypothesis has previously been examined, no consistent substantial differences in fitness between *SR* and *ST* females were found [29]; however, the study had a low statistical power.

Here, we quantify the fitness cost to females carrying *SR*, by comparing the number of offspring produced of females carrying 0, 1 or 2 copies of *SR*. The fitness of the three female genotypes are a crucial determinant of the evolutionary dynamics of the *SR* allele. In particular, if the costs of *SR* to females are at least partly recessive, such that *SR* homozygotes are less fit than heterozygotes, then *SR* is predicted to be maintained in a balanced polymorphism by frequency-dependent selection (e.g. [10,12,14,27,30,31]). With this in mind, we also analysed a simple population genetic model of *SR* parametrized with our genotypic fitness values, and show that clinal variation in the frequency of polyandry can explain some but perhaps not all of the observed clinal variation in the frequency of *SR*.

2. Material and methods

(a) Origin and maintenance of the isofemale lines

To avoid the risk that our measure of fitness of *SR* is influenced by the fitness of the *ST* X chromosomes it is compared against, or by epistatic interactions with the genetic background, we backcrossed *SR* into four distinct genotypes derived from two populations. Two isolines came from the Northern USA, where *SR* is absent (Lewiston, Montana, 35°05′00″ N, 111°44′10″ W). The other two isolines are from the Southern USA (Show Low, Arizona, 34°15′ N, 110°0′ W), where *SR* naturally occurs at high frequency (approx. 20%), and where we obtained the *SR* chromosome examined in this study. The isofemale lines were established from individual wild-caught female *D. pseudoobscura* caught between May and June 2008 (see [25]). We propagated each isoline by inbreeding sibs for approximately 80 generations prior to beginning the present study. All stocks in this study were maintained in an incubator at 23°C, with a 14 : 10 light : dark photocycle, in 25 × 75 mm plastic *Drosophila* vials on a medium of rolled oats, brown sugar, dried yeast, agar, nipagin, proprionic acid and water [32]. Due to repeated inbreeding, each isofemale line is expected to be homozygous at almost all loci, preserving a 'snapshot' of naturally occurring genetic variation, since homozygosity prevents adaptation to the laboratory environment [33]. Using introgressed isolines, and comparing inbred *ST*/*ST*, *ST*/*SR* and *SR*/*SR* females makes this experiment a very conservative test of the putative costs of *SR*, as in nature *ST*/*ST* females are unlikely to have two near-identical X chromosomes, as they do in the present experiment.

(b) Introgression of *SR* into the four isofemale lines

All the *SR* chromosomes used in this study are derived from a single male caught in Show Low at the same time as the isofemale lines were collected. We introgressed the *SR* X chromosome into each of our four isofemale lines for nine generations. Standard introgression techniques, crossing an XY male from one line to an XX female from another, produces heterozygote females. Unfortunately, heterozygous females in this case would be *SR*/*ST*, and which would risk us losing *SR* from the introgressed line. Hence, we used a two-stage introgression procedure (see electronic supplementary material, figure S1) to prevent *ST* X chromosomes entering the *SR* line. First, we crossed *SR*/*SR* females to an *ST*/*Y* male from the target isoline. This produced heterozygote females

that were discarded, and *SR*/*Y* sons that carried a mix of *SR* stock and target isoline autosomes. These sons were then crossed to *SR*/*SR* females to produce the next generation of partially introgressed *SR*/*SR* females. Over nine generations of introgression, this is expected to result in 93% of autosomal DNA being derived from the isoline. Homozygous *SR*/*SR* females were confirmed by genotyping using PCR (methods and primers reported in [34]). Our introgression technique also has the advantage that, as offspring from heterozygous females were never used, recombination between *SR* and *ST* chromosomes could not occur.

(c) Mating assays and offspring counts

After introgression, we generated experimental females with 0, 1 or 2 *SR* X chromosomes from each of the four isolines, to measure their offspring production. We collected experimental flies within 18 h of eclosion, to ensure they were virgin. All flies were transferred without anaesthesia to ensure normal copulation behaviour [35]. A minimum of 30 females were mated for each of the three female genotypes, for each of the four introgressed lines, giving 12 treatment combinations in total.

We placed each virgin female in a new food vial with an *ST*/*ST* male from the same isoline. All males and females were 3–5 days old at the time of mating, at which age they are fully sexually mature [36], and the males were aged in individual vials, because male–male interactions prior to mating have been shown to affect mating behaviour and success in male *Drosophila* [37]. We observed the pairs of flies for 2 h, and pairs that failed to mate were discarded. After the 2 h mating period, we removed the male from each vial, and transferred all successfully mated females to a fresh vial. We allowed females to oviposit for 12 days in total, moving them onto fresh food every 3 days. This minimizes the potential effect of larval crowing on offspring viability.

To measure female offspring production, we counted all offspring from each vial; offspring production in the first 12 days of life correlates strongly with the lifetime number of offspring produced [38]. We allowed 7 days between the first adult eclosion and offspring count, to ensure that all offspring had eclosed. We counted the number of sons and daughters produced. It is worth noting that since we did not measure fecundity (number of eggs laid by females) or hatching success (fertility), but the number of emerging offspring, we are not able to quantify separate female fitness components (i.e. fecundity, fertility and viability). However, offspring production is the most suitable measure of the combined fitness cost to females carrying *SR* as it captures the genetic contribution to subsequent generations and therefore the frequency of *SR*. To obtain a measure of body size, we removed the focal females' wings and photographed them at 20 × magnification under a Leica L2 microscope, then measured the posterior cross vein to the distal extreme of the fourth longitudinal vein from the resulting digital photograph using ImageJ [39]. All focal females were genotyped. DNA was extracted from each focal female, amplified using PCR and then screened for both *SR* and *ST* chromosomes. This procedure ensured that the *SR* chromosome had been successfully introgressed. All females whose *SR* genotype was not as expected ($n = 23$ out of 463) were excluded from the data analysis.

(d) Statistical analysis

All analyses were conducted using R v. 3.5.1 [40]. Thirteen per cent of females (58/440) failed to produce any offspring, so we elected to analyse the progeny count data with a Bayesian hurdle model. Hurdle models assume the data are generated by a two-step process: in our case, the model assumes that females reproduce with some probability (which is estimated from the data), and if they do reproduce, they produce a variable number of offspring which follows a negative binomial distribution. In the most complex model, we allowed both the hurdle and offspring count components to vary between genotypes (a fixed factor with 3 levels: *ST*/*ST*, *SR*/*ST* and *SR*/*SR*), isolines (fixed factor, 4 levels), female ages (a covariate); the model also included the genotype-by-isoline interaction, as well as experimental block as a random factor. In our main analyses, we did not fit body size as a covariate, because we regard body size as a mediator variable rather than something to be controlled for. That is we hypothesize that genotype affects body size, and body size affects fecundity, and so 'controlling for body size' masks part of the effect of genotype on offspring production. Also, we have no body size data for 102/440 females in the study, and so we would need to discard a quarter of the data to include body size in our models. However, for completeness, we also consider a model that includes body size as a covariate (see Results). We compared competing models using posterior model probability (i.e. the probability that the focal model is the best one in the set, given the data and the prior), computed via bridge sampling. The hurdle model was implemented in the R package *brms* [41], and we used conservative, 'regularizing' priors to help prevent overfitting [42]. Using the posterior model parameters, we calculated the posterior estimates for each genotype and isoline mean for a female of average age, adjusting for block effects. We also calculated pairwise differences between the means for each genotype in order to calculate effect sizes and assess statistical significance (using a 'Bayesian *p*-value', defined as the probability that the true effect size is actually of the opposite sign to the reported effect size). All R code can be viewed at https://lukeholman.github.io/cost_of_SR_Dpseudo/.

(e) Population genetic model

The effect of *SR* on female relative fitness is likely to be important to the evolutionary dynamics of *SR* in natural populations, and so we wrote a population genetic model that incorporated the estimates of relative fitness from our experiment. The model considers an infinite, panmictic population with non-overlapping generations. Meiosis proceeds normally in females and *ST* males, but *SR* males were assumed to pass on the *SR* chromosome to 96% of their offspring (as in [12]). Females mate with either one male or two, with probabilities $(1 - p)$ and p, respectively. We assume that *ST*/*ST* females and *ST* males both have a fitness of 1, while the fitness of the other three genotypes (*SR*/*ST*, *SR*/*SR* and *SR*) are potentially less than 1, where 'fitness' describes a genotype's ability to survive to adulthood and produce offspring relative to the other genotypes. In each generation, we first implement selection by multiplying each genotype frequency by its relative fitness and renormalizing the genotype frequencies to sum to 1. Next, we determine the frequencies of each possible mating type among single-mated females, by taking the product of each possible combination of male and female genotypes multiplied by $(1 - p)$; that is, we assume that mating occurs at random (with respect to *SR* genotype) among the individuals that survive and successfully breed. We similarly found the frequencies of each mating type for twice-mated females by multiplying the genotype frequencies of the female, her first mate, and her second mate, and multiplying by p. With the frequencies of each mating type defined, we can calculate the expected offspring genotype frequencies for the whole population: the offspring genotype frequencies replace the parental ones, bringing us back to the start of the life cycle. For doubly mated females that mated with one *SR* male and one *ST* male, we assumed that the *SR* male potentially sired a percentage C of the offspring where $C \leq 50\%$. In nature, C is approximately 21% (i.e. the average of P1 and P2 in [20,43]), and we used this value when fitting the model to our fitness data. Offspring sired by an *SR* male inherited the *SR* allele with probability k; k is approximately 0.96 in nature [12]. We also compared our data with polyandry and *SR* frequency estimates from Price *et al.* [25], who measured these two variables in seven populations in

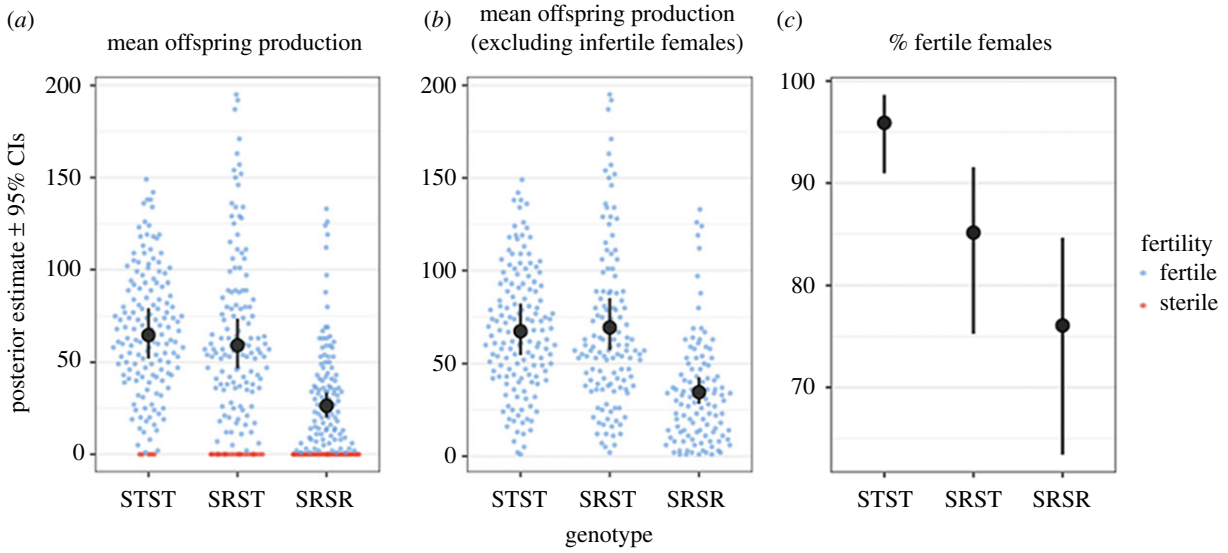

Figure 1. The black points and error bars show the posterior estimates of the genotype means for (*a*) offspring production ($N = 440$), (*b*) offspring production among the set of females that produced at least one offspring ($N = 382$) and (*c*) the percentage of females that produced offspring. The estimates are all derived from a single hurdle model which adjusts for variation due to female age and experimental block, and each estimate is the average across the four isolines (see electronic supplementary material, figure S1 for estimates split by isoline). The points show the raw values of offspring production for individual females, and are coloured purple for females that produced no offspring. The error bars show the 95% credible intervals on each estimate. (Online version in colour.)

a North–South cline across North America. We found the equilibrium frequency of *SR* numerically by iterating the model until *SR* fixed, went extinct or until 10 000 generations had elapsed, since the analytical solution to the model would be unwieldy. The simulation was written in R, and the code used to run it can be viewed at https://lukeholman.github.io/cost_of_SR_Dpseudo/.

3. Result

(a) Effect of *SR* on offspring production

To test whether the genetic background affects fitness and/or the fitness costs of carrying *SR*, we first compared the fit of three models. The full model contained the genotype × isoline interaction and both main effects, the second model lacked the two-way interaction and the third model additionally lacked the main effect of isoline (all three models additionally contained female age as a covariate and block as a random effect, total sample size $N = 440$; electronic supplementary material, table S1). The simplest model had by far the highest posterior model probability (greater than 99%); this means that we found no evidence that females from different isolines vary in fitness more than expected by chance, or that the costs of *SR* vary between the four genetic backgrounds examined. Electronic supplementary material, figure S1 presents the same information as figure 1, split by isoline, illustrating this null result. Electronic supplementary material, tables S2 and S3 summarize the posterior parameter estimates for the top model and the full model, respectively. Electronic supplementary material, figure S1 and table S3 highlight a trend for the Slo B3 isoline to be more sensitive to the costs of carrying *SR* than the others, but since the genotype × isoline effect did not improve model fit, this result is provisional.

Females carrying two driving X chromosomes (genotype: *SR*/*SR*) had substantially lower expected offspring production, and were more likely to fail to produce any offspring, relative to the other genotypes (figure 1 and table 1). Specifically, *SR*/*SR* females produced an estimated 38 fewer progeny than *ST*/*ST* females, meaning that their productivity was only 41% as high as the *ST*/*ST* genotype ($p < 0.0001$; electronic supplementary material, table S1). The fitness of *SR*/*SR* females was also only 45% as high as the fitness of heterozygotes (*SR*/*ST*), illustrating that the fitness costs imposed by *SR* are at least partly recessive ($p < 0.0001$). There was no statistically significant difference in offspring number between the *ST*/*ST* and *SR*/*ST* genotypes ($p = 0.11$).

Much of the reduction in the progeny number of *SR*/*SR* females was due to their significantly greater rate of reproductive failure. Twenty-three per cent of *SR*/*SR* females failed to produce any offspring (33/142), compared to 13.7% of *SR*/*ST* females (20/146) and 3.3% of *ST*/*ST* females (5/152) (figure 1*c*). These three failure rates were all statistically significantly different from one another (electronic supplementary material, table S2), indicating that inheriting a single copy of *SR* is sufficient to increase the rate of reproductive failure, while inheriting two copies increases the failure rate further still. However, *SR*/*SR* females produced significantly fewer offspring than the other genotypes even within the subset of females that did produce offspring ($p < 0.0001$; figure 1*b*). Interestingly, there was a significant difference in the rate of reproductive failure, but not in the number of progeny produced when fertile, between the *SR*/*ST* and *ST*/*ST* genotypes (figure 1; electronic supplementary material, table S2).

Fitting body size as a covariate ($n = 338$ females; electronic supplementary material, table S4) had no qualitative effect on the results: as before, we found that *SR*/*SR* females had lower offspring production and failed to reproduce more often, while *SR*/*ST* females had more frequent reproductive failure, but were equally productive if they did reproduce (electronic supplementary material, table S4). As expected, there was a positive relationship between body size and productivity ($p = 0.025$).

(b) Effect of *SR* on female body size

Body size (as measured by wing vein length) differed between genotypes. Surprisingly, the *ST*/*ST* females were smallest

Table 1. Pairwise comparisons of genotypes for the three measures of female fitness shown in figure 1. The 'difference in means' column shows the posterior estimate of the difference between the genotype means, in the original units (i.e. offspring number, or percentage points). A negative difference indicates that the genotype with more copies of *SR* has lower female fitness, the parentheses show the error and 95% quantiles of the posterior difference in means. The 'relative difference' column expresses each difference in relative terms; e.g. the first row shows that the mean number of offspring produced by *SR*/*ST* females was 92% as much as the number produced by *ST*/*ST* females, with 95% confidence limits of 70–110%. Finally, *p* is the posterior probability that the true difference in means is zero or of the opposite sign to the estimate shown here (similar to a conventional *p*-value).

fitness trait	comparison	difference in means	relative difference	*p*
mean offspring production	STST → SRST	−5.53 (6.23; −18.0 to 6.5)	0.92 (0.09; 0.7–1.1)	0.1842
	STST → SRSR	−38.37 (5.91; −50.5 to −27.6)	0.41 (0.05; 0.3–0.5)	0.0000
	SRST → SRSR	−32.84 (5.67; −44.6 to −22.6)	0.45 (0.05; 0.4–0.6)	0.0000
Mean offspring production (excluding infertile females)	STST → SRST	2.04 (6.12; −9.9 to 14.2)	1.03 (0.09; 0.9–1.2)	0.3693
	STST → SRSR	−32.88 (5.70; −44.5 to −22.3)	0.51 (0.05; 0.4–0.6)	0.0000
	SRST → SRSR	−34.93 (5.81; −47.0 to −24.6)	0.50 (0.05; 0.4–0.6)	0.0000
% fertile females	STST → SRST	0.11 (0.04; 0.0 to 0.2)	4.42 (2.45; 1.6–10.6)	0.0007
	STST → SRSR	0.20 (0.05; 0.1 to 0.3)	7.17 (3.87; 2.8–16.9)	0.0000
	SRST → SRSR	0.09 (0.05; 0.0 to 0.2)	1.69 (0.46; 1.0–2.8)	0.0278

(1.53 ± 0.009 mm, $N = 110$), followed by *SR*/*SR* (1.57 ± 0.008 mm, $N = 113$), and then *SR*/*ST* (1.63 ± 0.005, $N = 115$); all pairwise differences were statistically significant (mixed model containing genotype, isoline and block: $p < 0.0001$). These body size differences were large in magnitude: relative to *ST*/*ST* females, females carrying a single *SR* chromosome had wings that were 1.10 standard deviations longer (s.e. = 0.11), while females carrying two *SR* chromosomes had wings that were 0.46 standard deviations longer (s.e. = 0.11). There were also differences in body size between the isolines ($p < 0.0001$).

(c) Effect of maternal genotype on sex ratio of offspring that reached adulthood

Among the subset of offspring that survived to adulthood, there was a significant excess of daughters for all three female genotypes, and this excess was especially strong when the mother carried at least one copy of *SR* (figure 2). To test for effects of isoline and genotype, we compared the fit of three models: genotype only, genotype and isoline, and genotype, isoline and their two-way interaction. The model containing genotype and isoline without their interaction was the best-fitting of the three (posterior probability greater than 99%), indicating that although the isolines differed, the effect of *ST* on the sex ratio did not differ significantly between isolines (see electronic supplementary material, figure S3). *ST*/*ST* females produced fewer daughters than either of the *SR* genotypes (posterior difference in % daughters compared to *SR*/*SR*: 7.7%, 95% CIs: 5.9–9.5%, $p < 0.0001$; versus *SR*/*ST*: 6.11%, 95% CIs: 4.7–7.5%, $p < 0.0001$), and there was also weak evidence that for a more female-biased sex ratio for *SR*/*SR* females compared to *SR*/*ST* (1.6%, 95% CIs: −0.25 to 3.5, $p = 0.046$).

(d) Population genetic model

The model reaffirmed earlier findings (e.g. [10,44]) that recessive fitness costs of *SR* to females can maintain a balanced polymorphism of *SR* and *ST* chromosomes (figure 3). The reason for this result is that recessive fitness costs impose negative frequency-dependent selection on *SR*. When *SR* is rare, it is rarely found in homozygotes, and thus, *SR* carriers rarely experience the full fitness cost, but when *SR* is common, so too are *SR* homozygotes. Furthermore, we found that *SR* is predicted to reach a lower equilibrium frequency in populations in which most females mate multiply, particularly when *SR* males perform poorly in sperm competition (figure 3).

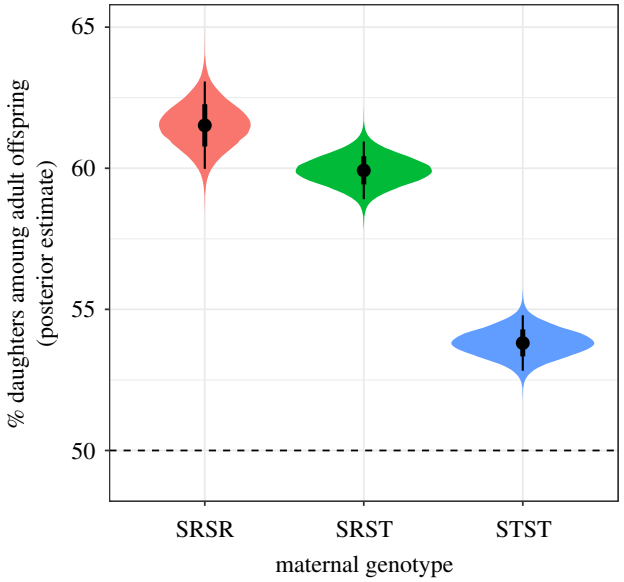

Figure 2. Distribution of proportion of daughters in offspring for each genotype. (Online version in colour.)

Next, we parametrized the model with female relative fitness values that equal the relative offspring production estimated here (i.e. *ST*/*ST* = 1, *SR*/*ST* = 0.92, *SR*/*SR* = 0.41; table 1). We also incorporated estimates of the frequency of polyandry (p) in seven North American populations of *D. pseudoobscura*, and the sperm competitiveness of *SR* males under laboratory conditions, and calculated the expected equilibrium allele frequency of the *SR* allele for three values of the only remaining unmeasured parameter in the model (i.e. the fitness of *SR* males; figure 3). The allele frequencies predicted by the model were a fairly close match to the real-world observed allele frequencies, suggesting that the model captures most

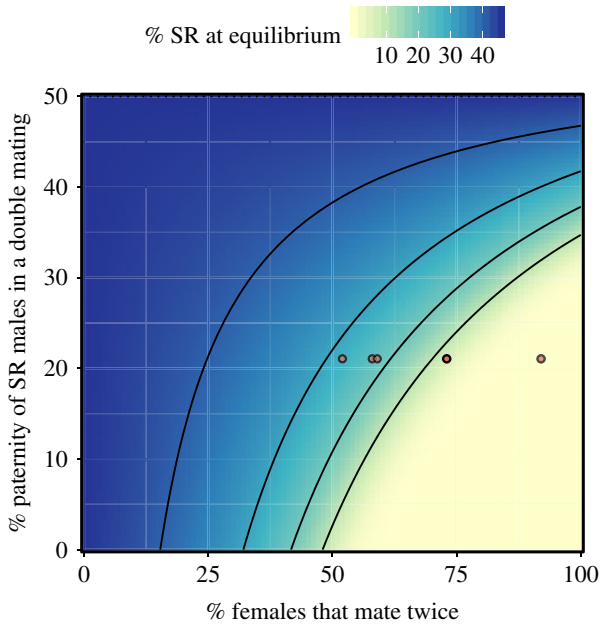

Figure 3. Predicted equilibrium frequency of the *SR* allele, calculated from the population genetic model. The model shows that *SR* is predicted to reach a lower equilibrium frequency when a high proportion of females mate multiply (*x*-axis), and when *SR* males are inferior sperm competitors to *ST* males (*y*-axis). These two predictors interact, because sperm competition becomes more selectively important as polyandry becomes more common. The seven ~~red~~ points illustrate the range of female mating frequencies observed across seven North American populations, and their position on the *y*-axis is based on [25]. The figure further assumes that *SR* males pass on the *SR* chromosome to 96% of their offspring [12], and that *ST* and *SR* males have equal survival and mating success. (Online version in colour.)

of the salient biological variables, and that our offspring production estimates are a reasonable approximation of the genotypic fitnesses in the wild. The model also implies that the relative survival and mating success of *SR* males is in the range 90–100% as for *ST* males, since the *SR* allele was predicted to be unrealistically rare when we assumed that *SR* males have a relative fitness lower than this.

Assuming that the three possible female genotypes have fitness equal to the relative progeny production values observed in our experiment (table 1), in combination with estimates of meiotic drive strength and *SR* male sperm competitiveness from earlier research (see Methods), we find that *SR* is expected to reach an equilibrium frequency of 0% to almost 30%, for a range of natural polyandry frequencies (red points in figure 3). Figure 3 assumes that *SR* and *ST* males are equally likely to survive and mate; relaxing this assumption by adding male-specific costs of *SR* reduces the expected frequencies of *SR* considerably (figure 4). The population frequencies of *SR* that best matched the real-world data when the fitness of *SR* males was assumed to be 90–100% as much as an *ST* male, though the match to the data was not especially strong, suggesting that this simple model is missing one or more predictors of *SR* evolutionary dynamics.

4. Discussion

Here, we show that female *D. pseudoobscura* homozygous for *SR* produce fewer than half as many offspring as heterozygous *SR*/*ST* or standard *ST*/*ST* females. This reduction in fitness was similarly large across all four isoline backgrounds. We also found that the number of driving X chromosomes a female carried predicted whether she would fail to produce any offspring following a single mating. This finding is unlikely to be affected by sperm limitation. While we did not quantify the possible impact of differential sperm allocation by *(ST)* males to females with respect to the number of *SR* chromosomes they carry, female fertility is not limited by the number of sperm received even when mating to an *SR* male that transfer half as many sperm as an *ST* males [20]. Twenty-three per cent of *SR*/*SR* and 14% of *SR*/*ST* females failed to produce offspring following an apparently normal copulation, compared to a 3% failure rate in *ST*/*ST* females. Additionally, we found that females carrying one or two copies of *SR* were substantially larger than the *ST*/*ST* females, with *SR*/*ST* females being the largest genotype. However, this difference in body size did not predict differences in the number of offspring produced between genotypes, indicating that this difference is due to carrying *SR*.

We also found there was a significant difference in the sex ratio of emerging adults between females, with *SR*/*SR* and *SR*/*ST* females producing significantly more female-biased offspring that survived to adulthood compared to *ST*/*ST* females. This suggests that male larvae carrying an *SR* chromosome were suffering increased mortality. However, differential mortality of *SR* male offspring is unlikely to be the main driver of the reduced offspring production by *SR*/*SR* females, as the change in sex ratio was too small to explain the 45% fecundity difference between *SR*/*SR* and *SR*/*ST* females. Moreover, *SR*/*ST* females showed the same female-biased sex ratio as *SR*/*SR* females (60% versus 61%), but produced similar numbers of offspring as *ST*/*ST* females. The absence of a substantial difference in the sex ratio of surviving offspring of *SR*/*ST* and *SR*/*SR* females suggests that viability differences of *SR*/*Y* sons cannot solely explain the reduced offspring production observed in *SR*/*SR* females. As we only measured total offspring production and not egg production, hatching success and viability of individual females, we cannot infer the main cause of the reduced productivity of *SR*/*SR* females.

The results from previous studies of fitness costs to *D. pseudoobscura* females carrying *SR* are inconsistent. Wallace [27] evaluated the lifetime fecundity of groups of five females, and found that heterozygote females laid more eggs than homozygous females, and that *SR*/*SR* and *ST*/*ST* females laid similar numbers of eggs at 25°C, but that *SR*/*SR* females were disadvantaged at 16.5°C. However, Wallace pooled the fecundity of several females making his estimate less reliable. Wallace [27] also looked at hatching success of eggs finding no difference between females, but showed there was strong viability selection against *SR* homozygous females. Curtsinger & Feldman [14] set up cages of *SR*/*ST* and *ST*/*ST*, or *SR*/*SR* and *SR*/*ST* at randomized genotype proportions. They assayed the resulting eggs, then estimated the frequency of parental genotypes, to calculate eggs laid by each genotype. Similar to Wallace's results, they also argue that *SR*/*ST* females were more fecund than both *SR*/*SR* and *ST*/*ST* females, with *SR*/*SR* females being most disadvantaged. However, these experiments were at high density, and present only total offspring numbers summed across all vials, not means and deviations, making them hard to interpret. Nonetheless, Curtsinger & Feldman [14] also found that *SR*/*SR* females had lower viability than heterozygote *SR*/*ST* females. By contrast, Beckenbach [12] found no difference in egg production

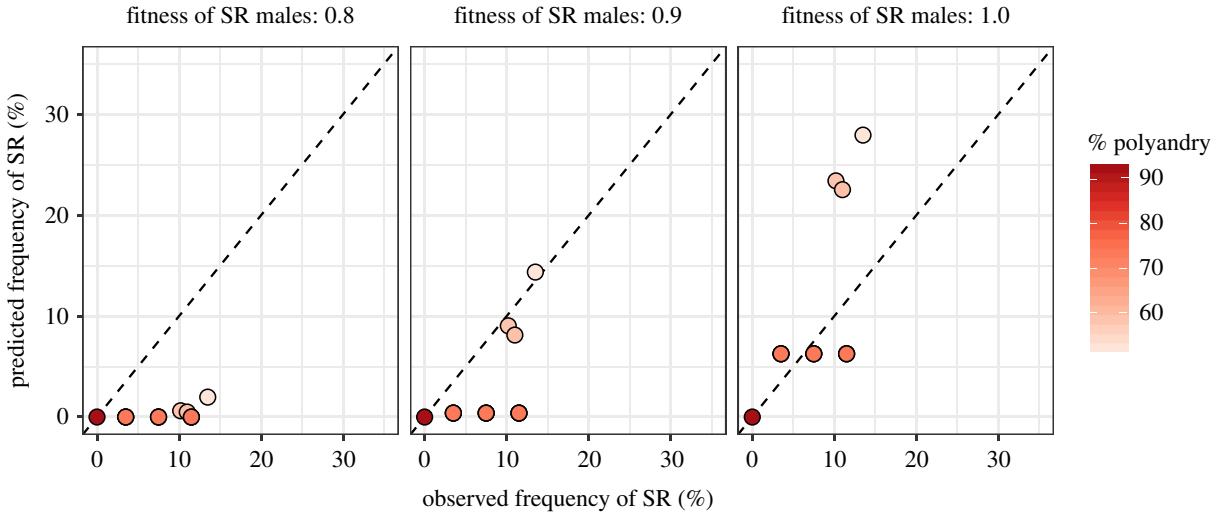

Figure 4. Comparison of the *SR* frequencies predicted by the model with the frequencies observed in the wild across seven North American populations. Each point represents one of the populations plotted in figure 3, and the colour of the point indicates the frequency of female multiple mating in that population. More polyandrous populations contain a lower frequency of *SR* chromosomes, both in nature and in the model predictions, and the predictions are most accurate when we assume that *SR* males have similar or equal survival and mating success (i.e. abbreviated in the figure as 'fitness') to *ST* males (middle and right panel). The dashed line shows $y = x$, such that plots in which the points are closer to the line indicate a better match between the predicted and observed allele frequencies. (Online version in colour.)

between females of the three genotypes in one population, and only a minor reduction in egg production in *SR*/*SR* females in a second population, but concluded that this difference was insufficient to prevent *SR* from fixing. While we did not quantify potential differences in fecundity, fertility or offspring viability of females, and hence do not know which variable is responsible for the observed large reduction in offspring production of *SR*/*SR* females in the current study, this finding mirrors the reduced viability reported for homozygous *SR* females in all the previous studies [14,27,31]. One potential criticism of all of these studies is that they used laboratory-adapted populations, whose genetic make-up might differ from that of wild flies in a manner that alters the fitness effects of *SR*. The larval density also differs in these studies, with a cost to heterozygous females being greatest at higher densities [27,31], whereas in the current study, we aimed to minimize the effect of larval crowding by rearing the offspring of individ-ual females under surplus food. It is also not clear how many *ST* X chromosomes were used in these studies. Hence, the differences in their results might be due to chance sampling of particularly high or low fecundity *ST* X chromosomes. In the current study, we quantify the impact of expressing one or two copies of *SR* across four different genetic backgrounds and therefore take into consideration potential variation in fitness of the *ST* X chromosomes it is compared against.

Our earlier modelling work [10] found that polyandry alone was insufficient to maintain stable polymorphisms of driving and non-driving X chromosomes, as previously hypothesized [18,45]. However, Holman *et al.*'s model [10] reaf-firmed that high fitness costs to drive homozygotes can prevent the driver from fixing, and showed that such costs affect the equilibrium frequency of *SR*, in combination with polyandry and the relative success of *SR* males facing sperm competition. Using a similar, simpler model parametrized with the relative fitness values implied by the present study, we found that real-world frequencies of SR closely match those predicted by our model. For example, in the Southern-most population of *D. pseudoobscura* sampled by Price *et al.* [25], around half of females mate multiply and *SR* has a frequency of 25%, while in the northern population, 90% of females were polyandrous and the *SR* frequency was approximately 0%. Using our data on female fitness, *SR* male sperm competitiveness and the relevant polyandry frequencies, we were able to reproduce the naturally observed *SR* frequencies. At present, we believe that the best-supported explanation for the North–South cline in *SR* frequency runs as follows. Firstly, *SR* is prevented from fixing (in spite of its ability to distort segregation in males) by strong fitness costs to *SR*/*SR* females, which reduce the relative fitness of the *SR* allele whenever it becomes too common. Secondly, variation in the environment along the North–South cline causes females to display different levels of polyandry, reducing the relative fitness of the *SR* allele (and thus, its equilibrium frequency) in areas where females are more likely to mate multiply.

Interestingly, costs associated with being homozygous for meiotic drive have been observed in other species. The mouse *t*-haplotype has high costs in homozygotes, including complete lethality or sterility depending on the variant [15,46], and also reduces the sperm competitive ability of male heterozygote car-riers [21,22]. In the stalk-eyed fly *Teleopsis dalmanni*, sex-ratio drive is also associated with a reduction in egg-to-adult viabi-lity of 21–24% linked to the *SR* X chromosome in both sexes [47]. Driving X chromosomes in *Drosophila recens* also impose costs in homozygotes [16], and it seems likely fitness costs will also be found in other systems once they are studied in more detail. One possible reason for this pattern is that drivers with no costs in homozygotes are more likely to go to fixation, and thus never be detected. Another reason is that many drivers reside in a non-recombining region of the genome [4]. Thus, drivers may tend to accumulate multiple deleterious mutations, resulting in homozygous costs [4]. However, despite the costs to homozygotes observed in these systems, predic-tions of drive frequencies in natural populations have had very little success ([9]; but see [48]). Our combination of costs to homozygote SR female together with a natural cline in polyandry in *D. pseudoobscura* is a substantial improvement on only considering homozygosity costs.

Our finding that females carrying *SR* were larger than *ST*/*ST* females is surprising, given that *SR* reduces fitness and presumably carries a number of deleterious mutations. The difference between *ST*/*ST* and *SR*/*ST* females might simply reflect hybrid vigour, since the *ST*/*ST* females tested here were largely homozygous across the X, while the *SR*/*ST* females were not. However, there was also a large difference between the two homozygous genotypes, implying that the driving X might genuinely carry alleles that encode larger body size than a typical X chromosome. Meiotic drive alleles have been theoretically predicted to evolve linkage with sexually antagonistic alleles that benefit the sex in which drive occurs, i.e. males in the case of *SR* [49,50]. Males are much smaller than females in *D. pseudoobscura*, implying that large body size alleles would be female-beneficial, male-detrimental and so our results appear opposite to what one might predict, or indicate that body size may not be subject to sexually antagonistic selection in this species.

In conclusion, we verified the theoretical prediction of substantial costs to *SR*/*SR D. pseudoobscura* females in terms of reduced offspring production. Hence, negative frequency-dependent selection resulting from costs to *SR* homozygotes is likely to be a key reason why *SR* does not go to fixation in natural populations. We find that a combination of these costs with a natural cline in polyandry could produce the observed cline in *SR* frequency across North America, but only if there are additional costs to *SR*, as *SR* is present at lower frequency in the wild than the model predicts. We still do not fully understand what generates the *SR* frequency cline: we do not understand what drives the observed cline in polyandry, nor what the additional costs are to *SR*. Given that many other natural drive systems are found at stable frequencies, or in stable clines, we predict that a combination of fitness costs to homozygotes and variation in polyandry will be key to the dynamics of drive in nature.

Data accessibility. Data available from the Dryad Digital Repository: https://datadryad.org/stash/share/KgmM18ZUuu6_Qse6-NJRZxurA29Y5AuQqD6p1iZPet8

Authors' contributions. W.L., T.P. and N.W. designed the experiment, W.L. and N.W. carried out the experiment and L.H. performed the statistical analysis and modelling. All authors analysed and wrote the MS.

Competing interests. We declare we have no competing interests.

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