Fitness consequences of the selfish supergene *Segregation Distorter*

Abstract

Segregation distorters are selfish genetic elements that subvert Mendelian inheritance, for example by destroying gametes that do not carry the distorter. Simple theoretical models predict that distorter alleles will either spread to fixation, or stabilise at some high intermediate frequency. However, many distorters have substantially lower allele frequencies than predicted by simple models, suggesting that key sources of selection remain to be discovered. Here, we measured the fitness of *Drosophila melanogaster* adults and juveniles carrying zero, one, or two copies of three different variants of the naturally-occurring supergene *Segregation Distorter* (*SD*), in order to investigate why *SD* alleles remain relatively rare within populations despite being preferentially inherited. First, we show that the three *SD* variants differ in the severity and dominance of the fitness costs they impose on individuals carrying them. Second, *SD*-carrying parents produced less fit offspring in some crosses, independent of offspring genotype, indicating that *SD* alleles can have non-genetic, transgenerational costs in addition to their direct costs. Third, we found that *SD* carriers sometimes produce a biased offspring sex ratio, perhaps due to off-target effects of *SD* on the sex chromosomes. Finally, we used a theoretical model to investigate how sex ratio and transgenerational effects alter the population genetics of distorter alleles; accounting for these additional costs helps to explain why real-world segregation distorter alleles are rarer than predicted.

Keywords: gene drive, meiotic drive, population genetic model, selfish genes, *t* paradox.

Introduction

Segregation distorters are genetic elements that manipulate meiosis or gametogenesis such that they are present in more than the usual 50% of the gametes (Burt and Trivers 2006; Lindholm et al. 2016). Because of this bias in transmission, segregation distorters are predicted to spread rapidly to fixation assuming that individuals carrying the distorter are equally fit as non-carriers (Bruck 1957). Even if a distorter allele reduces the fitness of individuals that carry it, it can still be favoured by selection provided that its individual-level fitness costs are outweighed by the within-individual advantage conferred by segregation distortion (Lindholm et al. 2016). For this reason, it has been proposed that natural or artificially-created segregation distorters be used to spread human-beneficial alleles through wild populations, for example to introduce malaria resistance alleles into mosquitos (Gantz et al. 2015). In addition to their promise for applied science, the study of segregation distorters has led to multiple advances in our understanding of evolution, genetics, and speciation (Rice 2013; Lindholm et al. 2016; Manser et al. 2017; Lin et al. 2018; Verspoor et al. 2018). The best-studied naturally-occurring distorters are the *t* allele in mice (Carroll and Potts 2007) and the *Segregation Distorter* (*SD*) allele of *Drosophila melanogaster* (Larracuente and Presgraves 2012), both of which caused biased tranmission in heterozygous males by preventing the development of sperm that do not carry the distorter.

The '*t* paradox' (Carroll and Potts 2007) is a long-standing evolutionary puzzle. Though it is named after the mouse *t* allele, the paradox applies to many other segregation distorters that have similar properties (reviewed in Lindholm et al. 2016). The paradox is that many distorter alleles are quite rare within populations despite their strong transmission advantage. For example, the *t* allele occurs at frequencies of 5-14% depending on the population (Ardlie 1998), and *SD* occurs at frequencies of 0-8% (Brand et al. 2015), both of which are substantially lower than predicted by simple population genetic models (Bruck 1957; Lewontin 1968; Charlesworth and Hartl 1978; Taylor and Jaenike 2002; Holman et al. 2015). Taking the *t* allele as an example, we know that *t* is transmitted to a fraction k of the offspring of heterozygous males where $k \approx 0.95$, and that individuals homozygous for *t* generally have close to zero fitness (Ardlie 1998). Assuming no other effects on fitness or inheritance, a distorter like *t* is predicted to reach an equilibrium allele frequency of $\frac{1}{2} - \sqrt{(k(1-k))}/2k$ (Bruck 1957), which is 38.5% for $k = 0.95$. The discrepancy between this prediction and real-world allele frequencies indicates that something is missing from the model, and so several subsequent models sought to resolve the puzzle by incorporating additional biological details. For example, Lewontin (1962) argued that population structure can reduce the equilibrium frequency of a distorter allele (see also Bull et al. 2019), and Lewontin (1968) showed that the drive allele will reach a lower equilibrium frequency if it is costly when heterozygous rather than only being costly when homozygous. Additionally, males carrying segregation distorters often perform worse in sperm competition due to the loss of half their sperm, which can affect evolution of the distorter allele under certain conditions (Taylor and Jaenike 2002; Holman et al. 2015; Lindholm et al. 2016).

Here, we attempt to explain the puzzling rarity of *Segregation Distorter* (*SD*) in *D. melanogaster*. Similar to *t* in mice, *SD* is a gene complex or 'supergene' (Thompson

and Jiggins 2014) composed of several linked loci on an autosome (chromosome 2). *SD* causes strong segregation distortion in heterozygous males by disrupting the development of non-*SD*-carrying spermatids (reviewed in Larracuente and Presgraves 2012). The *SD* supergene contains an 'insensitive' allele at the *Responder* locus (*Rsp*), while most chromosomes that lack *SD* carry a 'sensitive' *Rsp* allele that makes them susceptible to distortion. Chromosomal inversions in the *SD* region help to keep the component loci in linkage by suppressing recombination, which prevents the creation of recombinant 'suicide chromosomes' in which the insensitive *Rsp* allele linked to *SD* is replaced by a sensitive *Rsp* allele. The threat of suicide chromosomes appears to have selected for reduced recombination, and the small number of loci that cause segregation distortion are usually embedded in a large non-recombining region comprising *c.* 10% of the genome (Presgraves et al. 2009), which has accumulated deleterious mutations that have hitchhiked along with the distorter (Temin and Marthas 1984; Larracuente and Presgraves 2012; Brand et al. 2015). All *SD* alleles are thought to descend from a single common ancestor from around 38,000 years ago (Brand et al. 2015), though *SD* has since diversified into multiple variants that differ in their inversions and in their load of deleterious mutations (Presgraves et al. 2009; Larracuente and Presgraves 2012; Brand et al. 2015). In some populations, *SD* chromosomes are present at low, stable frequencies that suggest balancing selection (e.g. 0-8% in 14 populations; Brand et al. 2015), although high and unstable allele frequencies have also been reported: one *SD* variant increased in frequency from 17% to 98% over 23 years in Wisconsin (Temin and Marthas 1984).

The evolutionary dynamics of distorters such as *SD* depend strongly on the fitness of drive-carrying individuals (e.g. Lewontin 1968). Negative frequency-dependent selection is of particular interest, because it can maintain a balanced polmorphism of distorting and non-distorting alleles. If selection on the distorter is not negatively frequency-dependent, the distorter will eventually fix or go extinct (Holman et al. 2015). Recessive fitness costs are one likely source of negative frequency-dependent selection, because recessive costs are expressed more often when the distorter allele (and thus distorter homozygotes) is common. However, some distorter alleles have no obvious fitness cost (Temin and Marthas 1984; Price et al. 2012), meaning that recessive costs probably cannot provide a complete answer to the *t* paradox. Additionally, models (e.g. Bruck 1957; Lewontin 1968) demonstrate that homozygote lethality alone is insufficient to explain the low allele frequencies of strong distorters like *SD* or *t*. For these two reasons, we also tested whether *SD* has fitness costs besides being harmful when homozygous.

Here, we focus on the three best-studied variants of *SD*, which are named *SD-5*, *SD-72*, and *SD-Mad* (all originally collected in Wisconsin; Sandler et al. 1959). *SD-5* carries a different set of inversions than the other two, and is thought to be homozygous lethal (Larracuente and Presgraves 2012), while some *SD-72*- and *SD-Mad*-type alleles are reportedly fit as homozygotes (Temin and Marthas 1984). Indeed, the *SD-Mad* allele studied here was previously reported to be fully viable and fertile in both sexes when homozygous (Brittnacher and Ganetzky 1983), making it especially puzzling that this *SD* variant is not more common. To our knowledge, the relative fitness of *SD* heterozygotes has never been measured, and homozygotes have only been scored as viable or non-viable; we thus sought to measure the three genotypes' relative fitnesses, which are crucial to the evolutionary dynamics of *SD*

(Lewontin 1968). We measured the fitness of each *SD* genotype in juveniles, as well as in male and female adults. We also investigated older reports (Hiraizumi and Nakazima 1967; Denell et al. 1969) that the offspring sex ratio of males carrying *SD* deviates from the usual 50:50. If autosomal distorter alleles like *SD* alter the sex ratio in addition to their other effects, there would be presumably be evolutionary consequences (since there is strong, "Fisherian" selection on the sex ratio; Fisher 1930). We therefore wrote a model to predict how sex ratio bias would affect allele frequencies of *SD*. Lastly, we tested whether *SD* has non-genetic, transgenerational fitness effects, e.g. mediated by parental effects or genomic imprinting, and used a model to investigate how *SD* evolves in the presence of such transgenerational effects. Our empirical and theoretical findings have implications for the evoluton of *SD* and other natural and human-engineered distorter alleles, and help to resolve the *t* paradox.

Methods

Fly stocks

All flies were reared at 25°C under natural light (*c.* 14h day length) in 25mm plastic vials containing food medium (yeast-soy-cornmeal-agar-corn syrup). All stocks were obtained from the Bloomington *Drosophila* Stock Centre unless otherwise stated (*SD* stock numbers: 64322, 64324, and 64323).

In order to generate a non-*SD* reference allele which also allowed us to visually distinguish flies carrying 0, 1 or 2 copies of *SD*, we created a stock carrying an isogenic copy of chromosome 2 that carried one recessive and one dominant 'marker' mutation. The recessive marker was a mutant allele of *bw* encoding brown eye colour (obtained from a teaching laboratory in Melbourne; unknown origin), while the dominant marker was the transgene *Ubi-GFP* (stock 5826), which expresses green fluorescent protein (GFP) throughout the body. To recombine these markers, we crossed F1 *bw/Ubi-GFP* females to *bw* males and collected male progeny expressing brown eyes and GFP. From these recombinants, we selected a single male and crossed it to a female carrying wild-type X chromosomes (one from the *bw* stock and one from the *SD-72* stock) as well as the balancer chromosome *SM5*, collected +/+; *bw-GFP/SM5* progeny, and crossed them to create what we hereafter call the *bw-GFP* stock.

In the adult fitness assays, we used opposite-sex *bw* individuals as mates, and *Gla/CyO* individuals (stock 44227) as same-sex competitors. The offspring of *Gla/CyO* flies express a dominant mutant phenotype (either curly wings or atypical eyes), distinguishing them from the offspring of the focal flies.

Lastly, the three SD-bearing Bloomington stocks had different balancer chromosomes (*SD-5* used *CyO*, *SD-72* used *SM5*, and *SD-Mad* was not balanced), so we first re-balanced the three *SD* stocks to use the *CyO* balancer (from the *Gla/CyO* stock) to remove this potential confounding effect. We then crossed *SD/CyO* progeny to the *bw-GFP* stock to create *SD/bw-GFP* individuals.

Reaffirming that *SD* shows biased inheritance

We first ran a pilot to confirm that *SD* is inherited by >50% of the adult progeny of heterozygote males. We mated 45 pairs, each consisting of a *bw*/*bw* female and *SD*/*bw* male, and recorded the sex and eye colour of each of the 4,016 resulting progeny (n = 16 crosses involved *SD-5*, 14 *SD-72*, and 15 *SD-Mad*). We then fit a binomial GLMM (with family as a random effect) to estimate the average % *SD* progeny carrying *SD* among the F1 sons and daughters reaching adulthood for each of the three *SD* variants. Note that this method will underestimate the strength of segregation distortion if *SD* progeny are more likely to die before reaching adulthood: it thus provides a lower bound on the proportion of *SD*-bearing sperm produced by heterozygote males.

Experiment 1

Experimental crosses

We performed four types of experimental crosses for each of the three *SD* alleles (Figure S1). In Cross 1, we mated two *SD/bw-GFP* flies, yielding offspring carrying 0, 1 or 2 *SD* alleles. In Cross 2, we mated *SD/bw-GFP* females to *bw* males, yielding offspring carrying 0 or 1 *SD* alleles. Cross 3 was the reciprocal of Cross 2: a *bw* mother and *SD/bw-GFP* father. Lastly, to measure the baseline fitness of non-*SD* genotypes in the same experimental conditions, we mated *bw* females and *bw-GFP* males (Cross 4).

All of these crosses were performed in parallel on a common cohort of flies under identical conditions in a randomised order, minimising confounding effects. We ran all four crosses (and their associated fitness assays; see below) in each of three experimental blocks, with equal representation of crosses within blocks. We measured three components of fitness: survival rate from first-instar larva (hereafter 'L1 larvae') to adult, adult male competitive fertilisation success, and adult female fecundity following social interaction. For brevity, we term these juvenile, male, and female fitness. We also recorded the adult sex ratio produced by each cross.

Juvenile fitness and sex ratio assays

Mated females from the four experimental crosses were placed separately onto egg collection plates (grape-agar medium with live yeast) for 24h, then removed. We waited 24h, then collected L1 larvae and sorted them by GFP phenotype. The reason for beginning the assay with L1 larvae, not eggs, was that we could correctly classify the GFP phenotype of L1 larvae (100/100 successes in a pilot) but not eggs, and because it is difficult to distinguish unfertilised eggs from fertilised eggs in which the embryo died before hatching. We placed the sorted larvae in fresh vials in groups of up to 100. It was difficult to obtain 100 larvae for every class of progeny because some progeny classes are rare due to segregation distortion

and/or pre-hatching mortality. We subsequently quantified juvenile fitness and the sex ratio by counting, sexing, and phenotyping the adults that eclosed from these vials.

Adult female and male fitness assays

Flies that survived to adulthood in the juvenile fitness assay were sorted by phenotype/genotype into single-sex vials, left to mature for 48-72h, and then used in adult fitness assays.

To measure female fitness, we placed 5 same-genotype females in an 'interaction vial' with 15 *bw* males and 10 *Gla/CyO* females (all flies were 48- to 72-hour-old virgins), and allowed them to interact for 48h to facilitate mating, courtship, behavioural interactions, and competition for food. We then recorded the number of surviving focal females, and moved them as a group to a new yeasted food vial (without the non-focal flies), where they oviposited for 24h. We then removed the females and counted the number of larvae eclosing from their eggs, and used this as our measure of female fitness. Thus, our measure of female fitness measure is the product of female fecundity, the proportion of eggs that are fertilised, and offspring survival in the zygote-to-L1 stage.

To measure male fitness, we placed 5 same-genotype males in an interaction vial with 15 *bw* females and 10 *Gla/CyO* males (again, all flies were 48- to 72-hour-old virgins), where they interacted and mated for 48h. We then moved all surviving individuals (focal and non-focal) to a new food vial where they continued to interact and oviposit for 24h. We then removed all adults and allowed their offspring to develop to adulthood, then counted the number of progeny sired by the focal males and the competitor *Gla/CyO* males. We used the proportion of progeny sired by the focal males as a measure of adult male fitness. This fitness measure encompasses pre- and post-copulatory sexual selection, as well as the survival rate of focal males' offspring relative to those of *Gla/CyO* males.

Limitations of Experiment 1's juvenile fitness assay

Upon phenotyping adult flies emerging from Crosses 1-4, we observed unexpected recombination between the *bw* and *Ubi-GFP* loci for the *SD-72* and *SD-Mad* (but not *SD-5*) chromosomes (we had assumed that *SD* chromosomes would be largely non-recombining in light of previous data; e.g. Presgraves et al. 2009). Specifically, in Cross 2, some GFP-negative larvae developed brown eyes, and some GFP-positive ones developed red eyes, indicating recombination in the *SD/bw-GFP* mother (recombinants were never seen in Cross 3, because there is no recombination in male *Drosophila*; this shows that recombination rather than phenotyping errors explains the results). The proportion of recombinant adults in Cross 2 was 3.6% (95% CIs: 2.4-4.9%) for *SD-5*, 36.1% (33-39%) for *SD-72*, and 32.8% (30-36%) for *SD-Mad*. The *bw* locus is at the terminal end of the right arm of chromosome 2 (2R), and *SD-5* is distinguished from the other two variants by an additional inversion on 2R; we therefore hypothesise that the *Ubi-GFP* transgenic insertion lies somewhere on 2R between the *SD* complex and *bw*, probably close to the *SD-5*-specific inversion (Figure 1 in Larracuente

and Presgraves 2012). As a consequence of this unexpected recombination, we cannot be certain how many larvae of each genotype were present at the start of the juvenile fitness assay for Cross 2, at least for *SD-72* and *SD-Mad* – we simply removed the recombinant individuals from the dataset, and made the simplistic assumption that all of the larvae that did not reach adulthood were non-recombinants. We interpret the relevant part of the Results in light of the resulting bias. This limitation is offset by data from Experiment 2 (which does not rely on these markers, and uses a balancer chromosome to suppress recombination), as well as data from Cross 3 (since there is no recombination in male *Drosophila*).

Additionally, for Cross 1, individuals carrying 0 or 1 *SD* chromosomes were phenotypically indistinguishable until they reached adulthood and developed eyes, and so we simply measured the survival rate of a mixed pool of larvae carrying either 0 or 1 *SD* alleles. The great majority of larvae in this pool will carry 1 *SD* allele, rather than 0, because of segregation distortion. Specifically, the proportion of *SD* progeny in this pool will be $1/(k + 2(1 - k))$, or 95.2% for $k = 0.95$. This limitation is offset by data from Crosses 2 and 3 and Experiment 2.

Experiment 2

Experiment 2 was designed to measure the direct and transgenerational effects of *SD* on sex-specific larval survival, and to address the limitations of Experiment 1. Experiment 2 used the transgenic construct *P{Sxl-Pe-EGFP.G}G78b* (extracted from stock 24105, backcrossed into the w^{1118} genotype for 5 generations, and made homozygous), which allows discrimination of males and females at the egg stage (female-destined embryos express GFP while males do not; Thompson et al. 2004). We conducted six types of crosses using parents bred at standardised density: in each cross, one parent was *SD/CyO* and the other was homozygous for *P{Sxl-Pe-EGFP.G}G78b*; we performed this cross with the three *SD* variants, with either the mother or the father providing *SD* (10-24 replicates per cross). We then collected embryos of both sexes (mean: 48 embryos per sex per cross), placed them in single-sex vials to develop, and then counted and phenotyped the eclosing adults to infer the survival rates of different progeny classes.

Statistical analysis

We analysed Experiment 1 using Bayesian hierarchical models implemented in the R package `brms` (Bürkner 2017). The data on juvenile fitness, male fitness, and adult sex ratio were treated as binomially distributed, and we fit 'vial' as a random effect to account for nonindependence of measurements from the same vial (this random effect was unnecessary for the female fitness data, which had one observation per vial). Female fitness was modelled using the negative binomial distribution, since the data were overdispersed counts. For all fixed effects, we used a moderately informative prior (a normal distribution centered on zero with SD = 5), in order to regularise the parameter estimates and reduce overfitting (McElreath 2018). We verified model fit using posterior predictive checks (Gelman and Hill 2006).

For hypothesis testing, we calculated the posterior differences between pairs of means for contrasts that we deemed informative for this study. For example, we calculated the posterior difference between the mean fitnesses of individuals with 0 or 1 *SD* allele, or individuals that received *SD* from their father versus their mother, and thereby tested for genetic and parental effects respectively. We also calculated the posterior probability that the group with the larger posterior mean actually has a smaller mean than the other group; this provides a metric with a similar interpretation to the p-value (contrasts for which >95% of the posterior lies on one side of zero were considered notable). It is not necessary to correct for multiple testing when calculating these pairwise differences, since the contrasts are all calculated using the posterior from the same model and thus are not independent tests.

The aim of Experiment 2 is to estimate the proportion of *SD* and non-*SD* male and female larvae that survive to adulthood. However, because the genotype of larvae could not be visually determined at the start of Experiment 2, we had to estimate the initial numbers of larvae belonging to each genotype in order to calculate the survival rates of each genotype. For example, if we placed 50 larvae in a vial and 20 non-SD and 20 *SD* individuals reached adulthood, we inferred the genotypes of the 10 dead ones. This unmeasured variable depends on the gametes produced by the *SD/CyO* parent. Because *SD* only causes distortion in males (Larracuente and Presgraves 2012), we assumed that the *SD/CyO* mothers transmitted *SD* to 50% of their progeny. We also assumed 50% transmission in *SD/CyO* fathers (i.e. $k = 0.5$), in light of evidence that *CyO* carries an insensitive allele of *Rsp* that makes it immune to segregation distortion (Ganetzky 1977). We then used a binomial random number generator with $p = 0.5$ to stochastically 'fill in' the genotypes of the dead larvae. Our sample size was sufficiently large that generating a new set of random numbers and re-running the model gave near-identical parameter estimates and identical qualitative conclusions, thanks to the law of large numbers. We also re-ran the model under the assumption that there is some segregation distortion in *SD/CyO* fathers (i.e. $k > 0.5$, contradicting the evidence in Ganetzky 1977), and found that all the key results did not change (Figure S2).

Population genetic model

Our experiments suggested that some *SD* variants have parent-of-origin-specific effects on fitness, and that some *SD* variantsc cause males to produce a biased offspring sex ratio. We therefore constructed a simple one-locus, two-allele population genetic model to examine the effect of these two factors on the evolution of *SD*.

The model considers the spread of an autosomal segregation distorter in an infinitely large, panmictic population with discrete generations. We assume that individuals carrying two wild type alleles have a relative fitness of 1, while genotypes carrying *SD* potentially have relative fitness betwen 0 and 1. We tracked the parental origin of the *SD* allele in heterozygotes, to allow heterozygotes with a maternally-inherited *SD* to have a different fitness than heterozygotes with a paternally-inherited *SD*, and thereby allow for parent-of-origin-specific effects on fitness. We assumed that male heterozygotes transmit *SD* to a fraction k offspring (where $0.5 < k < 1$), and produce a fraction $(1 + s)/2$ female offspring ($-1 < s < 1$), while all

other genotypes were assumed to show normal Mendelian inheritance and a 50:50 offspring sex ratio.

For each parameter space, we determined the evolutionary fate of an *SD* allele in a starting population with 1% *SD* alleles at Hardy-Weinberg genotype frequencies. We calculated the equilibrium allele frequencies numerically, since the analytical solution would be unwieldy. In each generation, we first multiplied the frequency of each genotype by its relative fitness (representing the combined action of natural and sexual selection across all life stages) and then renormalised the genotype frequencies to sum to one. We then determined the frequency of each of the possible mating types as the product of each possible pair of maternal and paternal genotype frequencies. From these, we determined the offspring genotype frequencies, and replaced the parental generation with the offspring. The simulation ran for 10,000 generations to ensure that *SD* had reached equilibrium, though it was terminated early if *SD* went extinct (defined as reaching 0.001% frequency) or fixed (>99%).

Results

SD show biased inheritance

The pilot study found that the percentage of adult progeny carrying *SD* in crosses where the father was an *SD* heterozygote was 88% (95% credible intervals: 85-90%) for *SD-5*, 89% (86-91%) for *SD-72*, and 82% (79-86%) for *SD-Mad* (Figure S3). The percentage was significantly lower for *SD-Mad* ($p = 0.009$), implying that this variant has weaker segregation distortion and/or better egg-to-adult survival. The proportion of *SD* progeny was similar among the sons and daughters of *SD* males, and there was no interation between *SD* variant and offspring sex (all $p > 0.084$).

Experiment 1

Posterior estimates of mean fitness for each group are plotted in Figure 1. Tables S1-S4 give sample sizes and summary statistics, and Tables S5-S8 present estimated differences between means. Table 1 summarises Tables S5-S8 by listing only the differences for which >95% of the posterior lies on one side of zero.

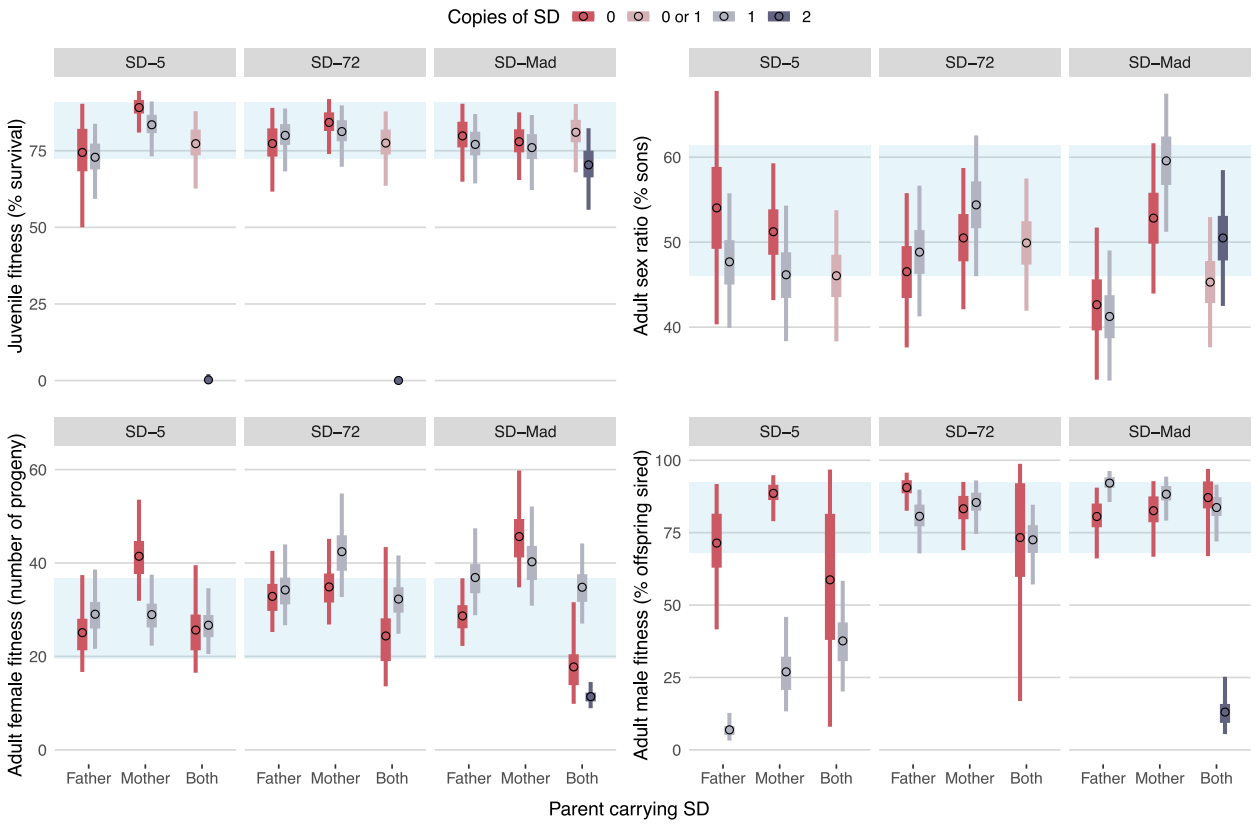

Figure 1: Posterior estimates of the group means for the four different response variables in Experiment 1, for each type of cross (x-axis), *SD* variant (panels), and offspring genotype (colours). Juvenile fitness was measured as % L1 larva-to-adult survival, adult sex ratio refers to the number of males and females among the individuals that reached adulthood, female fitness is the estimated number of progeny produced per female, and male fitness is the siring success relative to competitior males. The thicker inner bar shows the region containing 50% of the posterior, the outer bar covers 95% of the posterior, and the circle marks the median. Tables S6-S9 give the accompanying statistical results. Points labelled as carrying "0 or 1" *SD* allele refer to cases where the genotype of the offspring could not be ascertained; most of these individuals ($> 90\%$) probably carried 1 *SD* allele because of segregation distortion.

Juvenile fitness

When collecting larvae we observed 40 L1 larvae homozygous for *SD-5*, and over 600 carrying two copies of *SD-72*, but not one of these larvae survived to adulthood. Since we collected approximately the same number of eggs for each *SD* variant (over 600 eggs; precise number not recorded), the smaller number of *SD-5* larvae indicates that most *SD-5* homozygotes died as embryos (i.e. before hatching from the egg), while *SD-72* homozygotes primarily died after developing into L1 larvae but before adulthood. The heterozygotes survived equally well as larvae that did not carry *SD*, for all three *SD* variants, showing that the detrimental effects of *SD-5* and *SD-72* on juvenile fitness are recessive. By contrast, many larvae homozygous for *SD-Mad* reached adulthood, and there was no statistically significant effect of *SD-Mad* on larval survival, even in homozygotes (Table S5).

Table 1: List of all the notable differences between groups in Experiment 1 (posterior probability, p, <0.05; see Tables S6-S9 for results that did not meet this arbitrary cutoff). For each contast, we list the parent(s) that carried *SD* (neither, mother, father, or both) and the number of *SD* alleles carried by the offspring. The absolute difference in means is expressed in the original units (i.e. % larvae surviving, % sons, per-female progeny production, or % offspring sired), and the parentheses give its 95% credible intervals. The difference is positive when the second-listed mean is higher than the first one, and negative otherwise (e.g. the first row indicates that the fitness of females carrying 0 copies of *SD-5* is higher when the mother rather than the father carries *SD-5*). The Relative difference column shows the second-listed mean divided by the first-listed one, and thus gives the difference as a ratio. The posterior probability p is the chance that this difference is actually zero or has the opposite sign, given the priors, model, and data. The final column gives a biological interpretation for each difference; note that our experimental design cannot distinguish genomic imprinting from parental effects that differentially affect *SD* and non-*SD* offspring.

SD	Trait	Comparison	Absolute diff.	Relative diff.	p	Implication
SD-5	Female fitness	Mother, 0 → Father, 0	-16.4 (-31.0 to -1.0)	0.62 (0.38 to 0.97)	0.019	Transgenerational effect
SD-5	Female fitness	Neither, 0 → Mother, 0	14.4 (0.4 to 28.6)	1.57 (1.01 to 2.31)	0.022	Transgenerational effect
SD-5	Female fitness	Mother, 0 → Mother, 1	-12.5 (-26.1 to 0.2)	0.71 (0.49 to 1.00)	0.027	Costs of SD to heterozygotes
SD-5	Larval survival	Both, 0 or 1 → Both, 2	-77.1 (-87.8 to -62.2)	0.00 (0.00 to 0.03)	0.000	Extra costs when homozygous
SD-5	Male fitness	Mother, 0 → Mother, 1	-61.6 (-77.7 to -41.0)	0.30 (0.15 to 0.52)	0.000	Costs of SD to heterozygotes
SD-5	Male fitness	Father, 0 → Father, 1	-64.5 (-85.6 to -34.6)	0.10 (0.04 to 0.21)	0.000	Costs of SD to heterozygotes
SD-5	Male fitness	Mother, 1 → Father, 1	-20.0 (-39.2 to -5.5)	0.28 (0.10 to 0.64)	0.003	Transgenerational effect
SD-72	Larval survival	Both, 0 or 1 → Both, 2	-77.5 (-87.8 to -63.6)	0.00 (0.00 to 0.00)	0.000	Extra costs when homozygous
SD-Mad	Female fitness	Both, 1 → Both, 2	-23.4 (-33.1 to -15.0)	0.33 (0.23 to 0.47)	0.000	Extra costs when homozygous
SD-Mad	Female fitness	Neither, 0 → Mother, 0	18.7 (3.8 to 34.7)	1.73 (1.11 to 2.56)	0.006	Transgenerational effect
SD-Mad	Female fitness	Mother, 0 → Father, 0	-17.0 (-32.6 to -3.2)	0.64 (0.43 to 0.91)	0.009	Transgenerational effect
SD-Mad	Male fitness	Both, 1 → Both, 2	-70.6 (-82.6 to -54.2)	0.16 (0.06 to 0.31)	0.000	Extra costs when homozygous
SD-Mad	Male fitness	Father, 0 → Father, 1	11.5 (-0.6 to 26.7)	1.15 (0.99 to 1.40)	0.032	Benefits of SD to heterozygotes

The limitations of this assay (see Methods) mean that Figure 1 might underestimate the survival rate of individuals carrying a maternally-inherited *SD* allele, for *SD-72* and *SD-Mad*. Therefore, we cannot be certain that there is really no difference in juvenile fitness between individuals with an *SD* mother versus an *SD* father for *SD-72* and *SD-Mad*.

Sex ratio among individuals reaching adulthood

For crosses in which the father carried *SD-Mad*, the sex ratio of the emerging adults was significantly more female-biased than for crosses in which the mother carried *SD-Mad*, irrespective of offspring genotype (difference in % sons: 18.3, 95% CIs: 7.1 to 29.7, p = 0.0014; Table S6). The results did not replicate earlier findings that the non-*SD* offspring of *SD* heterozygote fathers show a female-biased sex ratio (Hiraizumi and Nakazima 1967; Denell et al. 1969); indeed, there was a nonsignificant trend in the opposite direction for *SD-5* (the posterior median was 54% sons among the non-*SD* offspring, and 48% sons among the *SD* offspring; Table S6).

Adult female fitness

Although *SD-Mad* homozygotes were viable and fertile, female homozygotes produced far fewer progeny than female heterozygotes from the same cross (homozygote productivity was only 33% that of heterozygotes; Table S7). There was evidence that *SD-Mad* had non-genetic

transgenerational effects on female fitness: the non-*SD* daughters of *SD* fathers were only 64% (95% CIs: 43 to 91%) as productive as non-*SD* daughters whose *mother* carried *SD* ($p = 0.009$). Indeed, the non-*SD* daughters of *SD* mothers were actually fitter than daughters from the control cross in which neither parent carried *SD* ($p = 0.009$). The same results were found for *SD-5*: the non-*SD* daughters of *SD-5* mothers were more fit than those of *SD-5* fathers ($p = 0.019$), or daughters from the control cross ($p = 0.021$). *SD-5* also had a direct genetic effect on female fitness: females carrying *SD-5* had 71% productivity (95% CIs: 0.49 to 1.00) relative to females from the same cross that did not inherit it, although this effect was only observed when *SD-5* was maternally inherited. *SD-72* had no detectable effects on female fitness, other than the aforementioned homozygous lethality in larvae of both sexes.

Adult male fitness

Males homozygous for *SD-Mad* had low fitness. We again observed evidence for non-genetic transgenerational effects: for *SD-5*, males with a paternally-inherited *SD* chromosome were substantially less fit than males with a maternally-inherited *SD* chromosome ($p = 0.0034$). Additionally, male fitness was reduced by more than half (Table S8) when the male inherited a single *SD-5* allele from the mother or the father (both $p < 0.0001$), suggesting that *SD-5* has a dominant direct genetic effect on male fitness. Interestingly, the sons of *SD-Mad* fathers were fitter if they inherited *SD-Mad* rather than the non-*SD* allele; a similar though non-sigificant result ($p = 0.080$) was observed in the female fitness assay.

Experiment 2

Experiment 2 suggested that *SD* chromosomes can have both direct and transgenerational effects on L1 larva-to-adult survival (Figure 2; Table 2; full results in Tables S9-10). Male larvae with an *SD-5/CyO* mother were significantly less likely to survive than those with an *SD-5/CyO* father, irrespective of whether the larva actually inherited *SD-5*. A similar result was observed for *SD-Mad*, though only among offspring that inherited *CyO* rather than *SD*. Also, for crosses in which the mother carried either *SD-5* or *SD-Mad*, survival was lower among daughters that inherited *SD* rather than *CyO* (and since *CyO* itself carries deleterious mutations, this implies that *SD* would also lower fitness relative to the wild type). The same effect was not observed for male larvae, or for crosses in which *SD* was inherited from the father, possibly indicating that *SD* alleles can have sex- or parent-of-origin-specific effects on larval survival. Lastly, we observed some significant sex differences in survival for all three *SD* chromosomes, with female larvae surviving better than male larvae for six different combinations of offspring and parental genotypes. We did not find any evidence that the direct genetic effect of *SD* on larval survival is sex-specific: the (small) differences in survival between *SD* and *CyO* progeny were similar in sons and daughters (Figure 2).

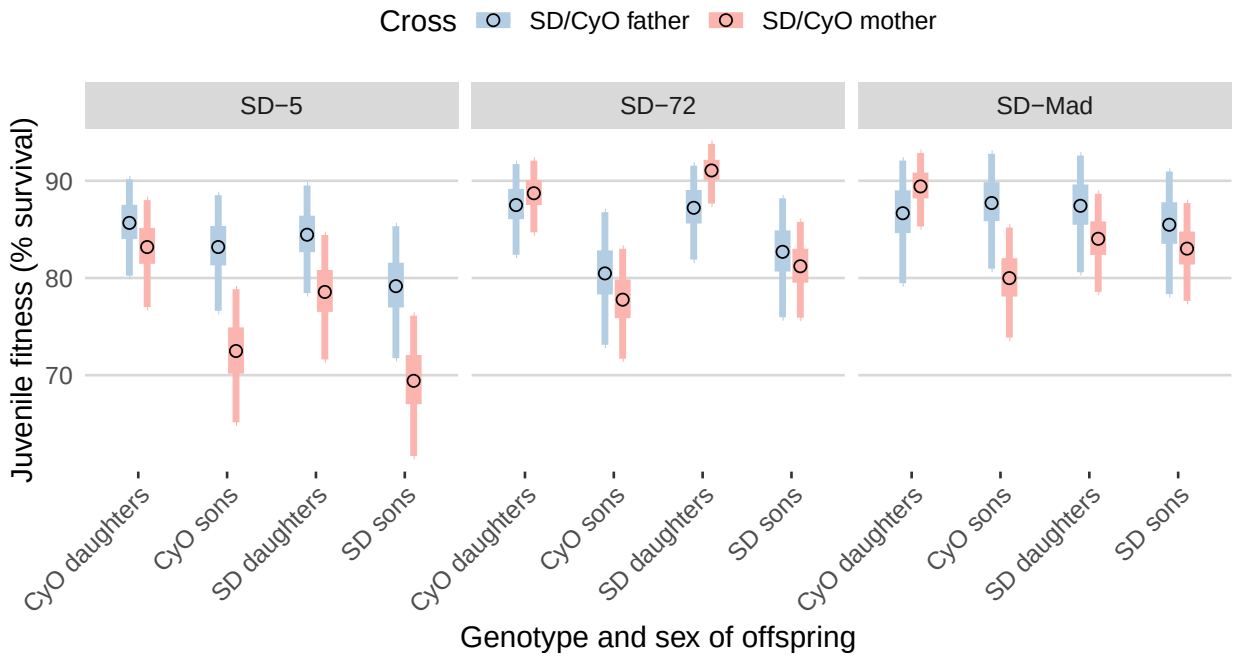

Figure 2: Posterior estimates of % L1 larva-to-adult survival in Experiment 2 for each combination of offspring sex and genotype (x-axis), *SD* variant (panels), and whether the mother or father had the genotype *SD*/*CyO* ('cross'; colours). The thicker inner bar shows the region containing 50% of the posterior, the outer bar covers 95% of the posterior, and the circle marks the median. See Tables 2 and S10 for associated hypothesis tests. The model underlying this plot assumed fair meiosis ($k = 0.5$) in *SD/CyO* males; see Figure S2 for equivalent plots made using different assumed values of k.

Table 2: List of the all the notable differences between groups in Experiment 2 (posterior probability, p, <0.05; see Table S10 for results that did not meet this arbitrary cutoff). For each group, we list the sex of the focal larvae, their genotype (*SD* or *CyO*), and the parent that carried *SD* (mother or father). The difference in means is expressed in % larvae surviving; other details are as in Table 1.

SD	Comparison	Absolute diff.	Relative diff.	p	Implication
SD-5	Sons, CyO, mother → Daughters, CyO, mother	10.7 (2.0 to 19.4)	1.15 (1.03 to 1.29)	0.008	Sons have lower survival
SD-5	Sons, CyO, mother → Sons, CyO, father	10.7 (1.5 to 19.8)	1.15 (1.02 to 1.30)	0.010	Transgenerational effect
SD-5	Sons, SD, mother → Sons, SD, father	9.7 (0.1 to 19.4)	1.14 (1.00 to 1.31)	0.025	Transgenerational effect
SD-5	Sons, SD, mother → Daughters, SD, mother	9.1 (-0.4 to 18.7)	1.13 (0.99 to 1.30)	0.028	Sons have lower survival
SD-5	Daughters, CyO, mother → Daughters, SD, mother	-4.6 (-10.0 to 0.6)	0.94 (0.88 to 1.01)	0.041	SD lowers survival
SD-72	Sons, SD, mother → Daughters, SD, mother	9.9 (4.2 to 15.8)	1.12 (1.05 to 1.21)	0.000	Sons have lower survival
SD-72	Sons, CyO, mother → Daughters, CyO, mother	11.0 (4.3 to 17.9)	1.14 (1.05 to 1.25)	0.001	Sons have lower survival
SD-72	Sons, CyO, father → Daughters, CyO, father	7.0 (-1.0 to 15.5)	1.09 (0.99 to 1.21)	0.045	Sons have lower survival
SD-Mad	Sons, CyO, mother → Daughters, CyO, mother	9.4 (2.7 to 16.3)	1.12 (1.03 to 1.22)	0.002	Sons have lower survival
SD-Mad	Daughters, CyO, mother → Daughters, SD, mother	-5.4 (-9.9 to -1.2)	0.94 (0.89 to 0.99)	0.007	SD lowers survival
SD-Mad	Sons, CyO, mother → Sons, CyO, father	7.7 (-0.6 to 15.6)	1.10 (0.99 to 1.21)	0.035	Transgenerational effect

Population genetic model

We first assumed that the *SD* allele had no direct or transgenerational fitness costs (top left, Figure 3), which allowed *SD* to invade even if segregation distortion (k) was very weak.

However, if the *SD* allele caused males carrying it to produce a highly biased sex ratio (unrealistically high, based on our data), *SD* required a higher k to invade. The reason that this sex ratio bias hinders the spread of *SD* is that autosomal loci usually maximise their fitness by producing a 50:50 sex ratio, due to 'Fisherian' selection on the sex ratio, which disfavours alleles causing unequal production of sons and daughters (Fisher 1930). In cases where the *SD* allele was able to invade, it generally went to fixation: a balanced polymorphism of *SD*- and non-*SD* alleles was seldom observed. There was a small zone of polymorphism when drive was very weak and sex ratio bias was very strong (both of which are unrealistic for any known distorter alleles). This polymorphism results from the frequency-dependent selection on alleles that affect the sex ratio: over-producing one sex is especially costly if that sex is over-represented in the population.

Secondly, when we assumed that all individuals with at least one *SD* allele had a relative fitness of 0.8 (dominant costs, top second left of Figure 3), the *SD* allele could still invade, though it needed a substantially higher transmission bias k to do so. When *SD* could invade, it again proceeded to fixation, except under unrealistically weak drive and extreme sex ratio bias. Notably, invasion was more difficult (i.e. a higher k was required) when we assumed that *SD* heterozygote males produce a female-biased rather than male-biased sex ratio; this is because *SD* can only bias segregation in males. *SD* invaded slightly more easily when *SD* heterozygote males produced >50% sons, but invasion was still harder than when *SD* did not bias the sex ratio (due to Fisherian sex ratio selection against *SD*).

Thirdly, when we assumed that *SD* is recessive-lethal but cost-free in heterozygotes (top second right, Figure 3), the *SD* allele stabilised at high, intermediate frequencies for realistic (i.e. high) values of k (as expected; Bruck 1957). This is because recessive fitness costs create negative frequency-dependent selection on *SD*, halting the spread of the *SD* allele once homozygotes become common enough to cancel out the effect of segregation distortion (Holman et al. 2015). A female-biased sex ratio reduced the equilibrium frequency of *SD* while a male-biased sex ratio had little effect, due to the opposing effects of Fisherian selection and the benefits of producing more sons (i.e. the sex in which distortion occurs).

Fourthly, we modelled a recessive-lethal *SD* that reduces the relative fitness of heterozygotes to 0.8 (top right, Figure 3 – this assumption is probably the most realistic of the four, based on our empirical findings). Here, the *SD* allele only invaded when k was (realistically) high, and *SD* reached a medium-high equilibrium frequency. Interestingly, *SD* alleles that induced a male-biased sex ratio invaded for substantially lower k and reached a higher equilibrium frequency for any given k than those that did not affect the sex ratio. Presumably this occurred because when *SD* is kept rare by its direct fitness costs, the population sex ratio stays close to 50:50, and so Fisherian sex ratio selection against *SD* remains weak (while the benefits of extra transmission bias stay the same).

For all four of these scenarios, we produced similar graphs under the additional assumption that offspring suffer an extra cost when the *SD* allele is inherited from a particular parent. In the middle row of Figure 3, genotypes carrying a paternally-inherited *SD* allele have their fitness reduced by an additional 0.2, while in the bottom row, the same applies to genotypes with a maternally-inherited *SD*. Comparison of the three rows shows that these transgenerational costs further hamper the spread of *SD*, and that paternal costs are worse

than maternal costs. The reason that paternal costs are worse is that they primarily afflict *SD*-carrying offspring (because of segregation distortion in males), while maternal costs harm a mixture of *SD* and non-*SD* offspring, reducing the impact of the transgenerational cost on the relative fitness of *SD*. By combining recessive lethality with some mixture of heterozygote fitness costs, sex ratio bias, or transgenerational costs, we could get *SD* chromosomes to persist at low, stable frequencies as they often do in nature (e.g. the middle right panel of Figure 3 near $k = 0.95$, which approximates the costs and k value for *SD-5*).

The oval in Figure 3 show the region that best approximate our empirical findings: strong fitness costs in homozygotes, moderate costs in heterozygotes, weak sex ratio bias, and extra fitness costs when *SD* is paternally inherited. The allele frequencies in this area are similar to those observed in nature (0-8%; Brand et al. 2015). Although we should be wary of affirming the consequent, and of extrapolating our lab-based fitness estimates to the wild, the model results suggest that the direct and transgenerational fitness costs documented in Experiments 1 and 2 are likely an important reason for the puzzling rarity of *SD* in the wild.

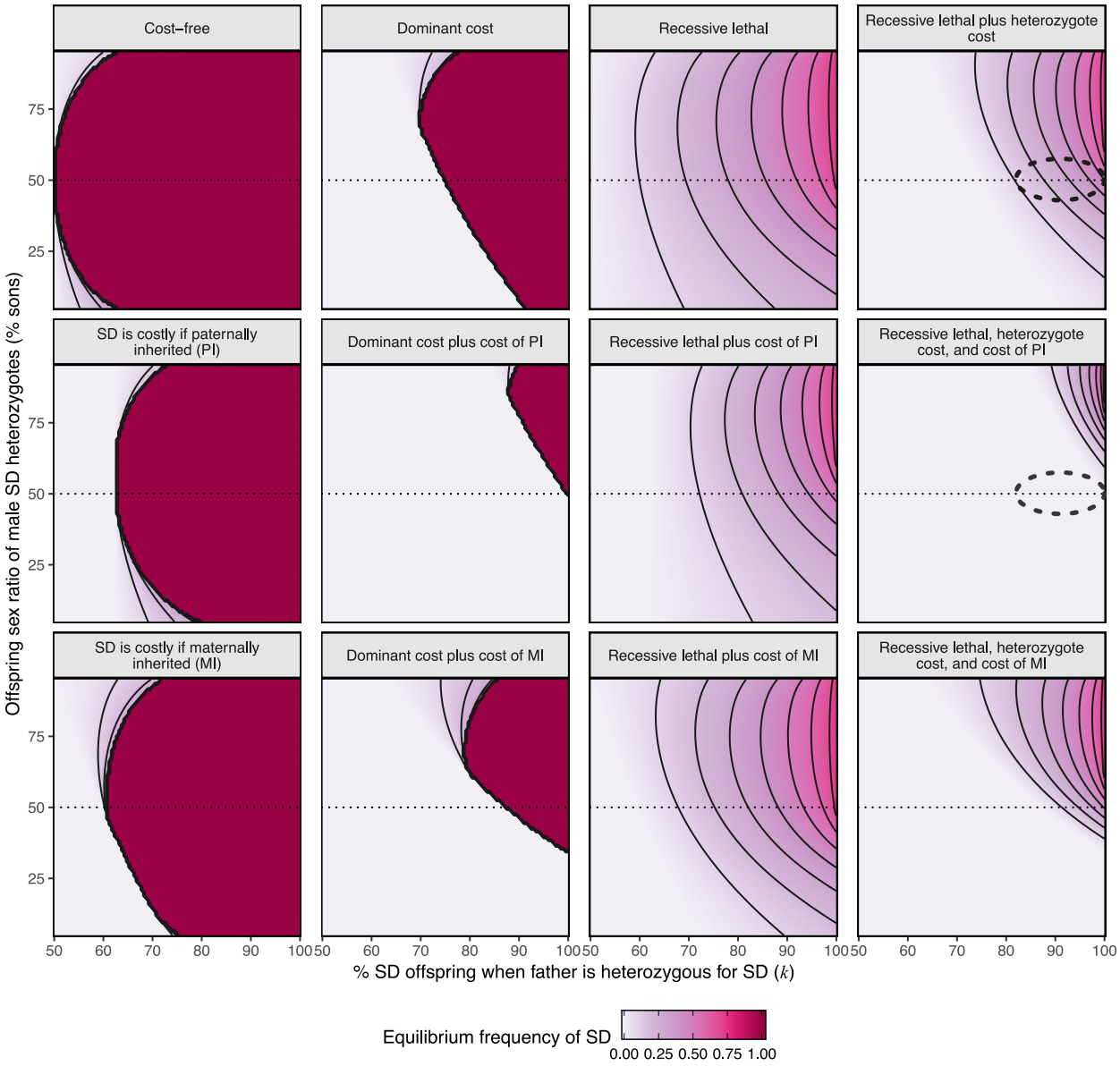

Figure 3: The equilibrium frequency reached by the *SD* allele depends on the strength of segregation distortion (x-axis), as well as the direction and strength of sex ratio bias in the progeny of *SD* heterozygote males (y-axis). The four columns make different assumptions about the fitness of individuals carrying the *SD* allele, while the three rows assume either that *SD* has no parent-of-origin-specific effects on fitness (top row), or that *SD* is especially costly when paternally inherited (middle row) or maternally inherited (bottom row). In the 'Dominant costs' column and the fourth column, individuals with one copy of *SD* had a relative fitness of 0.8, while 'Recessive lethal' means that *SD* homozygotes had zero fitness. The ovals show the parts of the parameter space that most closely approximate the values suggested by our data (i.e. strong drive, weak sex ratio bias, homozygote lethality, moderate costs in heterozygotes, and possible transgenerational effects), indicating that the model's predictions are not far off the reported real-world allele frequencies of *SD*.

Discussion

Our results reaffirmed that *SD-5* and *SD-72* are homozygous lethal. Most *SD-5* homozygotes died in the egg stage, while *SD-72* homozygotes died after hatching but before adulthood. Although populations of *SD-Mad* homozygotes can be cultured in the lab, and most homozygotes survived until adulthood in our experiments, we found that adult *SD-Mad* homozygotes had far lower male and female fitness than the comparison genotype (which was an inbred lab strain carrying two visible mutations). Thus, it seems plausible that *SD-Mad* homozygotes might have close to zero fitness in the wild. The fitness costs to female and male adults were dominant for *SD-5* but recessive for *SD-72* and *SD-Mad*, suggesting that *SD-5* carries additional dominant mutations that the others lack. Although we did not observe any *SD* variants that had high fitness as homozygotes, it is possible that such variants do exist; an *SD* variant with inversions characteristic of *SD-72* or *SD-Mad* was reportedly present in 98% of individuals in a population in Wisconsin (Temin and Marthas 1984).

Interestingly, we found some evidence for costly non-genetic transgenerational effects associated with *SD-5* and *SD-Mad*. These transgenerational effects might represent parental effects (i.e. non-genetic effects of parental phenotype on offspring phenotype; Badyaev and Uller 2009), genomic imprinting (i.e. when the effect of a genotype depends on the parental origins of the alleles; Holman and Kokko 2014), or a combination of both. Firstly, fitness was reduced among the non-*SD* daughters of *SD-5* or *SD-Mad* heterozygote fathers, relative to heterozygote mothers. One possible mechanism is that non-*SD*-carrying chromosomes that escape segregation distortion are epigenetically modified in ways that affect adult fitness; this mechanism is plausible because *SD* is thought to function by altering the chromatin of sensitive chromosomes (Larracuente and Presgraves 2012). Secondly, *SD-5* was especially harmful to adult male fitness when paternally inherited, hinting at either genomic imprinting or a paternal effect of *SD-5* that varies based on offspring genotype. Thirdly, in Experiment 2, we found that male larvae were less likely to reach adulthood when their mother carried *SD-5* than when their father did, irrespective of whether the larva actually inherited *SD-5*. This result again suggests that *SD-5* has a transgenerational effect on offspring fitness, though puzzlingly the harmful effect was associated with mothers rather than fathers this time (likely because Experiments 1 and 2 used a different non-*SD* reference chromosome and genetic background). To our knowledge, all previous theoretical models of segregation distorters implicitly assume that transgenerational effects are absent. We therefore incorporated parent-of-origin-specific effects on fitness into our model, and found that such costs can reduce the invasion probability and equilibrium frequency of *SD*. Thus, if segregation distorters commonly have harmful transgenerational effects in addition to their direct cost to the individual carrying them, transgenerational costs may help to explain the puzzlingly low allele frequencies of *SD* (Brand et al. 2015) and other autosomal distorters such as the *t*-haplotype (Carroll and Potts 2007).

We also observed that fathers heterozygous for *SD-Mad* produced an excess of daughters, while *SD-5* and *SD-72* parents produced a similar sex ratio to controls. Our results thus differ from earlier studies of *SD-5* and *SD-72*, which found an excess of daughters but only among the non-*SD* progeny (Hiraizumi and Nakazima 1967; Denell et al. 1969). In light of

those earlier results, Larracuente and Presgraves (2012) proposed that Y-bearing spermatids might be eliminated in *SD* males as a result of 'collateral damage' arising because of sequence homology between Y-linked loci and *Responder*, which could explain the observed shortage of sons in crosses where the father carries *SD*. As an alternative or complementary hypothesis, we speculate that *SD* might cause a parental effect that differentially affects the survival of sons and daughters, for example by inducing epigenetic modifications that are more harmful in males (this hypothesis was not supported by Experiment 2, but it was not definitively ruled out either). Our modelling results suggest that *SD* alleles invade less easily, and reach a lower equilibrium frequency, when they cause male heterozygotes to produce a female-biased sex ratio. There are two reasons for this result: firstly, autosomal alleles that skew the sex ratio away from 50:50 are usually disfavoured by selection (Fisher 1930), and secondly, *SD* alleles can only distort segregation in sons. The model also showed that producing a male-biased sex ratio was disadvantageous for *SD* alleles, except in populations where *SD* was kept rare by its fitness costs. When *SD* is rare, the population-wide sex ratio remains close to 50:50, reducing the Fisherian cost to *SD* of producing extra sons. Assuming that other autosomal segregation distorters also cause imbalanced sex ratios, this finding may be relevant to resolving the *t*-paradox for other species' distorter alleles.

In a somewhat unexpected result, we found that the adult sons and daughters of *SD-Mad*-bearing fathers were fitter if they inherited *SD-Mad*, relative to those that did not inherit it. We also found that the larvae of *SD-Mad*-bearing fathers were more likely to survive until adulthood if they inherited *SD-Mad* rather than the alternative *CyO* chromosome in Experiment 2. Assuming these results are genuine and not statistical flukes, we can infer either that *SD-Mad* heterozygotes were fitter than both *SD*-free test genotypes, or that *SD-Mad* has transgenerational effects when transmitted by fathers. The *SD* allele is thought to inactivate non-*SD*-bearing spermatids by altering their chromatin (Larracuente and Presgraves 2012), and so it is possible that the few non-*SD* gametes that do survive being inactivated carry epigenetic 'scars'. Assuming that sperm that escape segregation distortion produce lower-fitness progeny, we predict that *SD* alleles will reach slightly higher equilibrium alleles frequencies than they otherwise would, since only non-*SD* alleles would be harmed in this way.

Future studies could compete *SD* alleles with differing costs, and differing cost dominance, in population cages. We predict that *SD* alleles with dominant costs will either fail to spread (if the costs are sufficiently high relative to the strength of segregation distortion, k), or will sweep to fixation, while alleles with recessive costs will potentially reach an evolutionary equilibrium. Similarly, we predict that the stability and allele frequencies of *SD* chromosomes in natural populations will correlate with their fitness costs in homozygotes and heterozygotes. In line with this prediction, *SD-5* is more costly, has more dominant costs, and was rarer than other the other two variants in the original Wisonsin population (Temin and Marthas 1984), and it would be interesting to see if the frequencies of competing *SD* variants can be similarly explained in other populations. Our results also have implications for the design of artificial gene drives, or attempts to use natural gene drives like *t* to deliver human-beneficial 'payloads' (e.g. there are proposals to modify the *t* allele to control invasive populations of mice; Backus and Gross 2016). We suggest considering the fitness of drive-carrying individuals' offspring (not just the fitness of the carriers themselves) when testing a newly-designed gene drive

in the lab, since our model shows that transgenerational costs can strongly influence the invasion success of the gene drive.

Acknowledgements

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Availability of data and code

All raw data and R code is available at https://lukeholman.github.io/fitnessCostSD/.

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Online Supplementary Material

Fitness consequences of the selfish supergene *Segregation Distorter*

The figures and tables in this document, along with the with the R code used to generate them, can also be viewed online:
https://lukeholman.github.io/fitnessCostSD

Supplementary figures

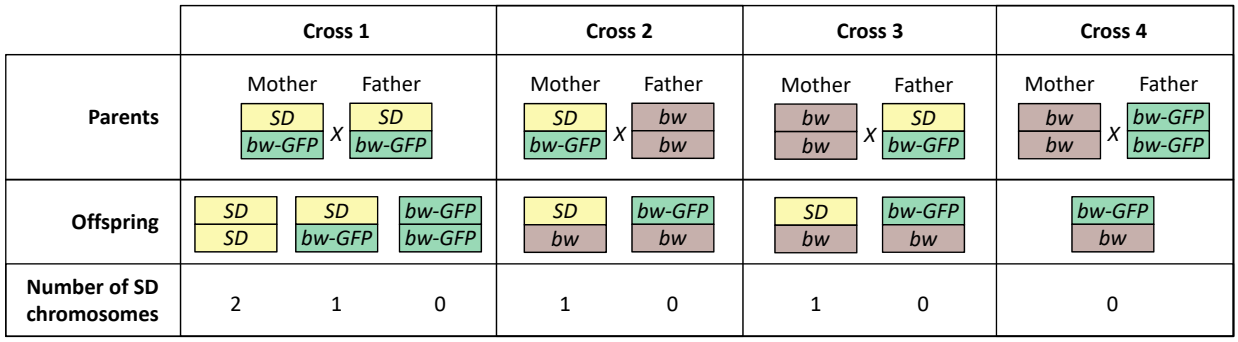

Figure S1: Crossing scheme used in Experiment 1 to generate offspring carrying 0, 1, or 2 SD chromosomes. The rectangles represent copies of chromosome 2, and are coloured and labelled according the mutations they carry.

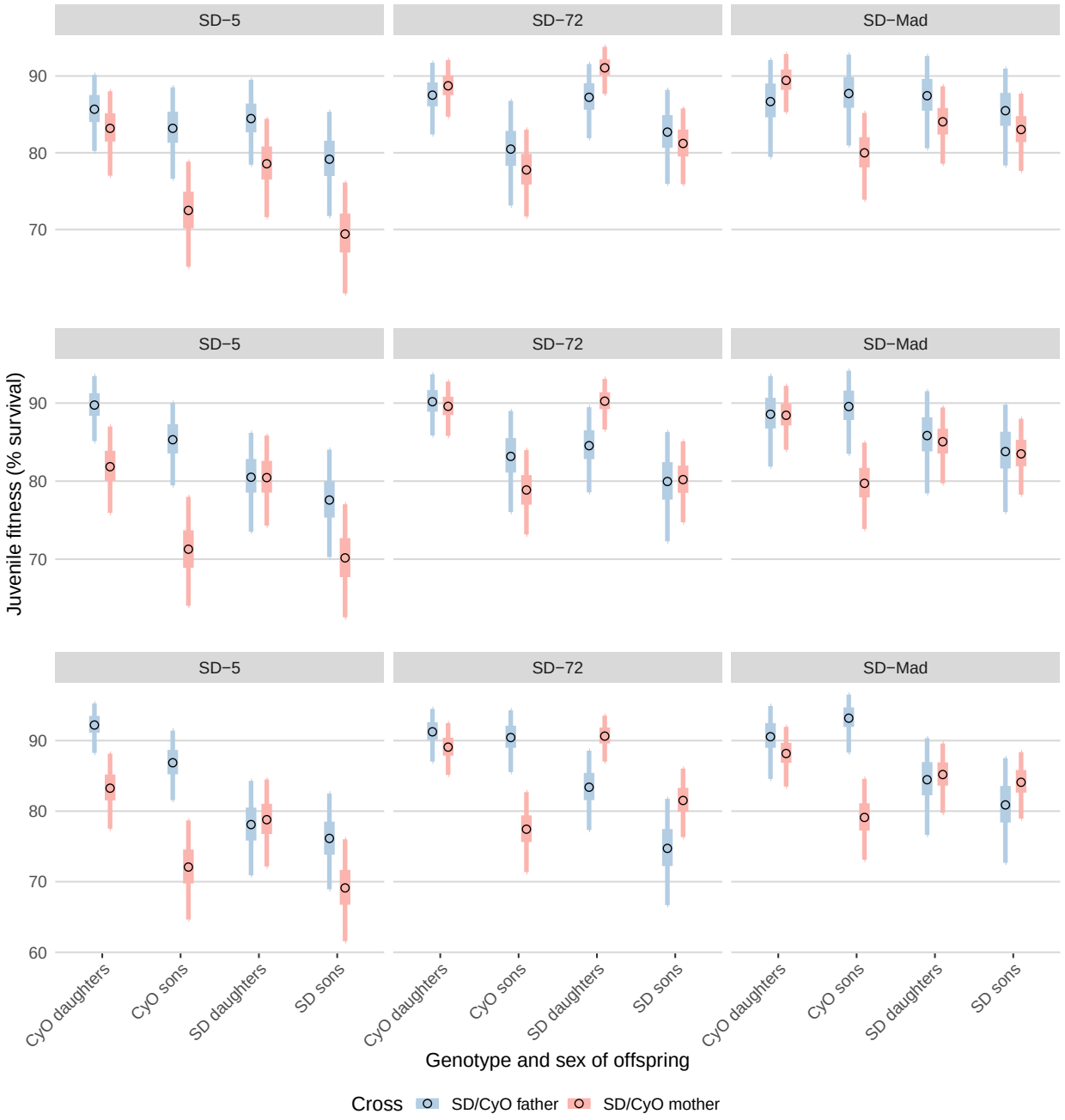

Figure S2: Equivalent plots to Figure 2, under the assumption that meiosis is fair ($k = 0.5$, top row, same as Figure 1), slightly biased ($k = 0.6$, middle row), and more strongly biased ($k = 0.7$, bottom row) in SD/CyO males. Note that the significant results for Figure 2 mostly stay the same or increase in magnitude, suggesting that the results are not strongly influenced by our assumptions about the strength of segregation distortion in SD/CyO males.

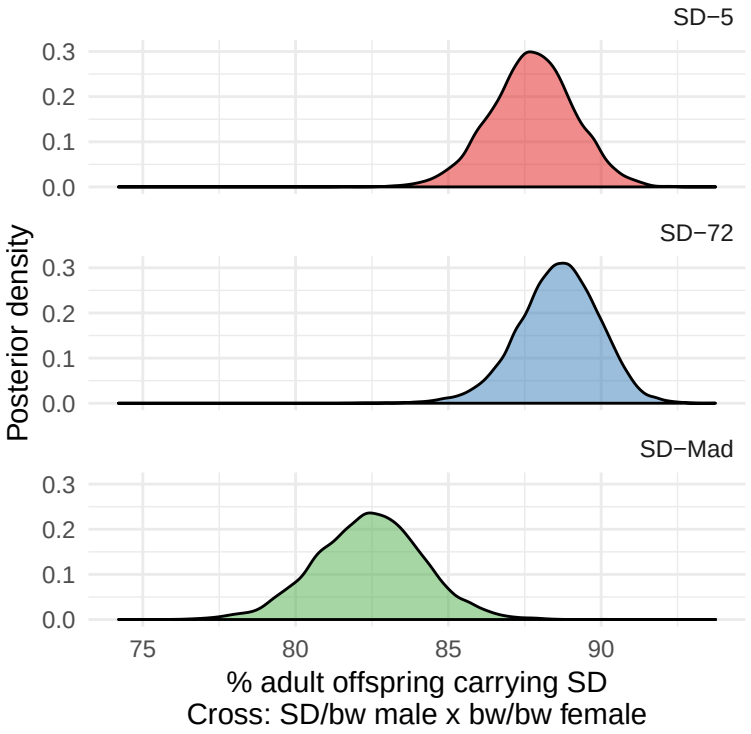

Figure S3: Posterior estimates of the percentage of adult progeny that carried *SD* in the pilot experiment, which crossed SD/bw males and bw/bw females. All estimates lie well above the 50% expected under Mendelian inheritance, and the estimate for *SD-Mad* is significantly lower than the other two. Note that the elevated percentage of *SD* progeny is the net result of segregation distortion and pre-adult mortality, and so the strength of segregation distortion might be stronger than suggested by these estimates if *SD* progeny are less likely to survive to adulthood.

Supplementary tables

Table S1: Number and percentage of L1 larvae surviving to adulthood for each *SD* genotype and cross type.

SD	Copies of SD	Parent with SD	Number larvae counted	n survivors	% surviving
No SD chromosome	0	Neither parent	600	495	82.5
SD-5	0	Father	113	89	78.8
SD-5	0	Mother	459	408	88.9
SD-5	0 or 1	Both parents	700	520	74.3
SD-5	1	Father	563	412	73.2
SD-5	1	Mother	494	415	84.0
SD-5	2	Both parents	40	0	0.0
SD-72	0	Father	287	226	78.7
SD-72	0	Mother	396	333	84.1
SD-72	0 or 1	Both parents	700	542	77.4
SD-72	1	Father	600	477	79.5
SD-72	1	Mother	423	342	80.9
SD-72	2	Both parents	600	0	0.0
SD-Mad	0	Father	296	239	80.7
SD-Mad	0	Mother	371	279	75.2
SD-Mad	0 or 1	Both parents	700	558	79.7
SD-Mad	1	Father	600	462	77.0
SD-Mad	1	Mother	436	320	73.4
SD-Mad	2	Both parents	585	413	70.6

643 **Table S2**: Number and percentage of male and female adults emerging from the juvenile
644 fitness assay vials.

SD	Copies of SD	Parent with SD	n males	n females	n total	% male
No SD chromosome	0	Neither parent	267	228	495	53.9
SD-5	0	Father	48	41	89	53.9
SD-5	0	Mother	206	202	408	50.5
SD-5	0 or 1	Both parents	239	281	520	46.0
SD-5	1	Father	196	216	412	47.6
SD-5	1	Mother	193	222	415	46.5
SD-5	2	Both parents	0	0	0	NaN
SD-72	0	Father	105	121	226	46.5
SD-72	0	Mother	169	164	333	50.8
SD-72	0 or 1	Both parents	272	270	542	50.2
SD-72	1	Father	233	244	477	48.8
SD-72	1	Mother	186	156	342	54.4
SD-72	2	Both parents	0	0	0	NaN
SD-Mad	0	Father	102	137	239	42.7
SD-Mad	0	Mother	145	134	279	52.0
SD-Mad	0 or 1	Both parents	253	305	558	45.3
SD-Mad	1	Father	190	272	462	41.1
SD-Mad	1	Mother	184	136	320	57.5
SD-Mad	2	Both parents	209	204	413	50.6

645 **Table S3**: Average fecundity of adult females for each SD genotype and cross type. The
646 last two columns give the sample size in terms of number of oviposition vials (each of which
647 contained up to 5 focal females), and number of males.

SD	Copies of SD	Parent with SD	Average fecundity	SE	n vials	n females
No SD chromosome	0	Neither parent	26.55	3.874	10	48
SD-5	0	Father	25.06	8.127	6	28
SD-5	0	Mother	41.13	3.700	15	71
SD-5	0	Both parents	24.95	3.767	5	22
SD-5	1	Father	28.88	3.337	12	55
SD-5	1	Mother	29.74	3.118	16	69
SD-5	1	Both parents	26.83	2.583	15	67
SD-72	0	Father	32.68	3.258	14	65
SD-72	0	Mother	35.10	3.023	15	68
SD-72	0	Both parents	22.53	6.671	3	15
SD-72	1	Father	33.97	2.743	16	77
SD-72	1	Mother	41.90	3.792	14	68
SD-72	1	Both parents	31.85	2.885	15	73
SD-Mad	0	Father	28.25	3.769	16	79
SD-Mad	0	Mother	44.71	3.723	13	65
SD-Mad	0	Both parents	16.50	1.762	3	14
SD-Mad	1	Father	36.85	3.968	16	77
SD-Mad	1	Mother	40.58	4.602	14	64
SD-Mad	1	Both parents	34.88	3.478	16	76
SD-Mad	2	Both parents	11.26	1.631	17	83

Table S4: Average relative fitness of adult males for each SD genotype and cross type, expressed as the average proportion of offspring sired. The last two columns give the sample size in terms of number of vials (each of which contained 5 focal males), and number of males.

SD	Copies of SD	Parent with SD	Average relative fitness	SE	n vials	n males
No SD chromosome	0	Neither parent	0.79	0.040	13	65
SD-5	0	Father	0.68	0.147	5	25
SD-5	0	Mother	0.82	0.045	17	85
SD-5	0	Both parents	0.59	NA	1	5
SD-5	1	Father	0.14	0.055	18	90
SD-5	1	Mother	0.32	0.072	12	60
SD-5	1	Both parents	0.39	0.074	13	65
SD-72	0	Father	0.88	0.027	16	80
SD-72	0	Mother	0.77	0.054	13	65
SD-72	0	Both parents	0.79	NA	1	5
SD-72	1	Father	0.76	0.045	18	90
SD-72	1	Mother	0.80	0.039	17	85
SD-72	1	Both parents	0.67	0.051	19	95
SD-Mad	0	Father	0.75	0.055	14	70
SD-Mad	0	Mother	0.75	0.069	11	55
SD-Mad	0	Both parents	0.82	0.078	5	25
SD-Mad	1	Father	0.87	0.028	18	90
SD-Mad	1	Mother	0.81	0.037	17	85
SD-Mad	1	Both parents	0.75	0.053	18	90
SD-Mad	2	Both parents	0.19	0.072	14	70

651 **Table S5**: The results of hypothesis tests computed using the model of larval survival in
652 Experiment 1. Each row gives the posterior estimate of a difference in means, such that
653 the estimate is positive if the first mean is larger than the second, and negative otherwise
654 (expressed in % larval survival). The error column gives the average absolute residual, and
655 other details are as in Tables 1 and 2.

SD	Comparison	Difference	Relative difference	Posterior probability	
SD-5	Neither, 0 → Father, 0	-8.9 (-34.9 to 11.6)	0.90 (0.59 to 1.15)	0.225	
SD-72	Neither, 0 → Father, 0	-6.0 (-24.3 to 10.5)	0.93 (0.72 to 1.14)	0.246	
SD-Mad	Neither, 0 → Father, 0	-3.5 (-21.0 to 12.5)	0.96 (0.76 to 1.17)	0.341	
SD-5	Neither, 0 → Mother, 0	5.8 (-5.3 to 18.1)	1.07 (0.94 to 1.25)	0.156	
SD-72	Neither, 0 → Mother, 0	0.9 (-12.1 to 14.0)	1.01 (0.86 to 1.19)	0.444	
SD-Mad	Neither, 0 → Mother, 0	-5.4 (-20.0 to 9.3)	0.94 (0.77 to 1.12)	0.218	
SD-5	Mother, 0 → Father, 0	-14.7 (-39.3 to 2.8)	0.84 (0.56 to 1.03)	0.057	
SD-72	Mother, 0 → Father, 0	-6.9 (-25.0 to 9.6)	0.92 (0.71 to 1.13)	0.214	
SD-Mad	Mother, 0 → Father, 0	1.9 (-15.9 to 18.1)	1.03 (0.81 to 1.27)	0.399	
SD-5	Mother, 1 → Father, 1	-10.6 (-26.4 to 4.4)	0.88 (0.70 to 1.06)	0.080	
SD-72	Mother, 1 → Father, 1	-1.2 (-15.4 to 13.1)	0.99 (0.82 to 1.18)	0.429	
SD-Mad	Mother, 1 → Father, 1	1.0 (-15.2 to 17.9)	1.02 (0.81 to 1.27)	0.449	
SD-5	Mother, 0 → Mother, 1	-5.6 (-17.4 to 5.2)	0.94 (0.81 to 1.06)	0.156	
SD-72	Mother, 0 → Mother, 1	-3.0 (-16.9 to 10.2)	0.97 (0.81 to 1.13)	0.326	
SD-Mad	Mother, 0 → Mother, 1	-1.9 (-19.4 to 14.4)	0.98 (0.77 to 1.21)	0.413	
SD-5	Father, 0 → Father, 1	-1.6 (-23.4 to 25.5)	1.00 (0.73 to 1.50)	0.415	
SD-72	Father, 0 → Father, 1	2.7 (-15.1 to 21.5)	1.04 (0.82 to 1.34)	0.394	
SD-Mad	Father, 0 → Father, 1	-2.8 (-20.4 to 16.7)	0.97 (0.76 to 1.25)	0.366	
SD-5	Both parents, 0 or 1 → Both parents, 2	-77.1 (-87.8 to -62.2)	0.00 (0.00 to 0.03)	0.000	*
SD-72	Both parents, 0 or 1 → Both parents, 2	-77.5 (-87.8 to -63.6)	0.00 (0.00 to 0.00)	0.000	*
SD-Mad	Both parents, 0 or 1 → Both parents, 2	-10.6 (-27.7 to 6.7)	0.87 (0.67 to 1.09)	0.105	

Table S6: The results of hypothesis tests computed using the model of adult sex ratio in Experiment 1. Each row gives the posterior estimate of a difference in means, such that the estimate is positive if the first mean is larger than the second, and negative otherwise (expressed in % males). The error column gives the average absolute residual, and other details are as in Tables 1 and 2.

SD	Comparison	Difference	Relative difference	Posterior probability	
SD-5	Neither, 0 → Father, 0	0.3 (-15.7 to 15.9)	1.01 (0.73 to 1.32)	0.482	
SD-72	Neither, 0 → Father, 0	-7.2 (-19.1 to 4.9)	0.87 (0.67 to 1.10)	0.114	
SD-Mad	Neither, 0 → Father, 0	-11.1 (-23.0 to 0.8)	0.80 (0.61 to 1.02)	0.034	*
SD-5	Neither, 0 → Mother, 0	-2.5 (-13.7 to 9.0)	0.96 (0.77 to 1.19)	0.323	
SD-72	Neither, 0 → Mother, 0	-3.2 (-14.5 to 7.9)	0.94 (0.75 to 1.16)	0.284	
SD-Mad	Neither, 0 → Mother, 0	-0.9 (-12.4 to 11.0)	0.99 (0.79 to 1.23)	0.437	
SD-5	Mother, 0 → Father, 0	2.8 (-13.4 to 18.4)	1.06 (0.76 to 1.40)	0.362	
SD-72	Mother, 0 → Father, 0	-4.0 (-16.1 to 8.5)	0.93 (0.71 to 1.19)	0.260	
SD-Mad	Mother, 0 → Father, 0	-10.2 (-22.6 to 2.7)	0.81 (0.61 to 1.06)	0.056	
SD-5	Mother, 1 → Father, 1	1.5 (-9.9 to 13.0)	1.04 (0.81 to 1.32)	0.391	
SD-72	Mother, 1 → Father, 1	-5.6 (-17.0 to 5.8)	0.90 (0.72 to 1.12)	0.159	
SD-Mad	Mother, 1 → Father, 1	-18.3 (-29.7 to -7.1)	0.70 (0.54 to 0.87)	0.001	*
SD-5	Mother, 0 → Mother, 1	-5.1 (-16.6 to 6.2)	0.91 (0.70 to 1.14)	0.187	
SD-72	Mother, 0 → Mother, 1	3.9 (-7.9 to 15.7)	1.08 (0.86 to 1.36)	0.251	
SD-Mad	Mother, 0 → Mother, 1	6.7 (-5.0 to 18.6)	1.14 (0.92 to 1.41)	0.128	
SD-5	Father, 0 → Father, 1	-6.3 (-22.4 to 9.9)	0.90 (0.66 to 1.23)	0.208	
SD-72	Father, 0 → Father, 1	2.3 (-9.5 to 13.8)	1.06 (0.82 to 1.35)	0.343	
SD-Mad	Father, 0 → Father, 1	-1.4 (-13.2 to 10.2)	0.98 (0.73 to 1.29)	0.410	
SD-Mad	Both parents, 1 → Both parents, 2	5.2 (-5.9 to 16.1)	1.12 (0.88 to 1.41)	0.164	

Table S7: The results of hypothesis tests computed using the model of female fitness in Experiment 1. Each row gives the posterior estimate of a difference in means, such that the estimate is positive if the first mean is larger than the second, and negative otherwise (expressed as the number of offspring produced). The error column gives the average absolute residual, and other details are as in Tables 1 and 2.

SD	Comparison	Difference	Relative difference	Posterior probability	
SD-5	Neither, 0 → Father, 0	-1.9 (-14.7 to 12.3)	0.95 (0.56 to 1.54)	0.368	
SD-72	Neither, 0 → Father, 0	5.8 (-6.5 to 18.5)	1.25 (0.81 to 1.84)	0.161	
SD-Mad	Neither, 0 → Father, 0	1.7 (-9.9 to 12.7)	1.09 (0.71 to 1.59)	0.371	
SD-5	Neither, 0 → Mother, 0	14.4 (0.4 to 28.6)	1.57 (1.01 to 2.31)	0.022	*
SD-72	Neither, 0 → Mother, 0	7.9 (-4.5 to 20.5)	1.32 (0.87 to 1.93)	0.105	
SD-Mad	Neither, 0 → Mother, 0	18.7 (3.8 to 34.7)	1.73 (1.11 to 2.56)	0.006	*
SD-5	Mother, 0 → Father, 0	-16.4 (-31.0 to -1.0)	0.62 (0.38 to 0.97)	0.019	*
SD-72	Mother, 0 → Father, 0	-2.0 (-14.7 to 10.9)	0.96 (0.65 to 1.36)	0.371	
SD-Mad	Mother, 0 → Father, 0	-17.0 (-32.6 to -3.2)	0.64 (0.43 to 0.91)	0.009	*
SD-5	Mother, 1 → Father, 1	0.1 (-10.8 to 11.5)	1.02 (0.69 to 1.45)	0.500	
SD-72	Mother, 1 → Father, 1	-8.2 (-22.8 to 5.5)	0.82 (0.57 to 1.15)	0.125	
SD-Mad	Mother, 1 → Father, 1	-3.4 (-17.7 to 10.5)	0.93 (0.64 to 1.31)	0.321	
SD-5	Mother, 0 → Mother, 1	-12.5 (-26.1 to 0.2)	0.71 (0.49 to 1.00)	0.027	*
SD-72	Mother, 0 → Mother, 1	7.5 (-6.9 to 22.5)	1.24 (0.83 to 1.75)	0.146	
SD-Mad	Mother, 0 → Mother, 1	-5.4 (-22.3 to 10.9)	0.90 (0.60 to 1.29)	0.255	
SD-5	Father, 0 → Father, 1	3.9 (-10.1 to 16.9)	1.21 (0.71 to 1.91)	0.264	
SD-72	Father, 0 → Father, 1	1.4 (-11.4 to 13.9)	1.06 (0.72 to 1.51)	0.412	
SD-Mad	Father, 0 → Father, 1	8.2 (-3.4 to 20.4)	1.31 (0.90 to 1.83)	0.080	
SD-Mad	Both parents, 1 → Both parents, 2	-23.4 (-33.1 to -15.0)	0.33 (0.23 to 0.47)	0.000	*

Table S8: The results of hypothesis tests computed using the model of male fitness in Experiment 1. Each row gives the posterior estimate of a difference in means, such that the estimate is positive if the first mean is larger than the second, and negative otherwise (expressed in % offspring sired). The error column gives the average absolute residual, and other details are as in Tables 1 and 2.

SD	Comparison	Difference	Relative difference	Posterior probability	
SD-5	Neither, 0 → Father, 0	-10.9 (-42.5 to 13.9)	0.87 (0.50 to 1.19)	0.238	
SD-72	Neither, 0 → Father, 0	8.3 (-4.4 to 23.7)	1.11 (0.95 to 1.35)	0.110	
SD-Mad	Neither, 0 → Father, 0	-1.7 (-19.4 to 15.5)	0.98 (0.78 to 1.22)	0.420	
SD-5	Neither, 0 → Mother, 0	6.2 (-7.6 to 22.2)	1.08 (0.91 to 1.32)	0.195	
SD-72	Neither, 0 → Mother, 0	0.9 (-16.6 to 18.2)	1.02 (0.81 to 1.26)	0.452	
SD-Mad	Neither, 0 → Mother, 0	0.2 (-18.0 to 18.2)	1.01 (0.79 to 1.26)	0.483	
SD-5	Mother, 0 → Father, 0	-17.2 (-47.4 to 5.8)	0.81 (0.47 to 1.07)	0.091	
SD-72	Mother, 0 → Father, 0	7.3 (-4.7 to 22.6)	1.09 (0.95 to 1.32)	0.133	
SD-Mad	Mother, 0 → Father, 0	-2.0 (-19.3 to 16.1)	0.98 (0.78 to 1.23)	0.394	
SD-5	Mother, 1 → Father, 1	-20.0 (-39.2 to -5.5)	0.28 (0.10 to 0.64)	0.003	*
SD-72	Mother, 1 → Father, 1	-4.8 (-19.9 to 9.6)	0.95 (0.78 to 1.13)	0.254	
SD-Mad	Mother, 1 → Father, 1	3.8 (-5.1 to 13.8)	1.05 (0.94 to 1.17)	0.201	
SD-5	Mother, 0 → Mother, 1	-61.6 (-77.7 to -41.0)	0.30 (0.15 to 0.52)	0.000	*
SD-72	Mother, 0 → Mother, 1	2.2 (-12.7 to 18.4)	1.03 (0.86 to 1.26)	0.399	
SD-Mad	Mother, 0 → Mother, 1	5.7 (-8.5 to 23.0)	1.08 (0.91 to 1.34)	0.229	
SD-5	Father, 0 → Father, 1	-64.5 (-85.6 to -34.6)	0.10 (0.04 to 0.21)	0.000	*
SD-72	Father, 0 → Father, 1	-9.9 (-23.9 to 2.2)	0.89 (0.74 to 1.03)	0.055	
SD-Mad	Father, 0 → Father, 1	11.5 (-0.6 to 26.7)	1.15 (0.99 to 1.40)	0.032	*
SD-Mad	Both parents, 1 → Both parents, 2	-70.6 (-82.6 to -54.2)	0.16 (0.06 to 0.31)	0.000	*

671 **Table S9**: Number and percentage of L1 larvae surviving to adulthood in Experiment 2, for
672 each SD genotype, cross type, and offspring sex.

SD	Parent with SD	Offspring sex	% surviving SD larvae	% surviving CyO larvae	n larvae counted	n crosses
SD-5	Father	Female	83.4	83.8	763	16
SD-5	Father	Male	79.0	79.3	727	17
SD-5	Mother	Female	76.4	81.9	871	18
SD-5	Mother	Male	70.8	67.8	972	20
SD-72	Father	Female	85.5	85.9	744	16
SD-72	Father	Male	81.0	78.6	615	15
SD-72	Mother	Female	90.2	87.4	1123	23
SD-72	Mother	Male	78.9	76.8	1186	24
SD-Mad	Father	Female	87.2	83.9	457	10
SD-Mad	Father	Male	87.8	83.1	480	11
SD-Mad	Mother	Female	84.4	85.7	942	20
SD-Mad	Mother	Male	82.3	78.4	1010	21

673 **Table S10**: Complete version of Table 2, showing all the contrasts that were tested in
674 Experiment 2.

SD	Comparison	Difference	Relative difference	Posterior probability	
SD-5	Sons, CyO, mother → Daughters, CyO, mother	10.7 (2.0 to 19.4)	1.15 (1.03 to 1.29)	0.008	*
SD-5	Sons, CyO, mother → Sons, CyO, father	10.7 (1.5 to 19.8)	1.15 (1.02 to 1.30)	0.010	*
SD-5	Sons, SD, mother → Sons, SD, father	9.7 (0.1 to 19.4)	1.14 (1.00 to 1.31)	0.025	*
SD-5	Sons, SD, mother → Daughters, SD, mother	9.1 (-0.4 to 18.7)	1.13 (0.99 to 1.30)	0.028	*
SD-5	Daughters, CyO, mother → Daughters, SD, mother	-4.6 (-10.0 to 0.6)	0.94 (0.88 to 1.01)	0.041	*
SD-5	Daughters, SD, mother → Daughters, SD, father	5.9 (-2.5 to 14.6)	1.08 (0.97 to 1.20)	0.078	
SD-5	Sons, CyO, father → Sons, SD, father	-4.0 (-10.0 to 1.8)	0.95 (0.88 to 1.02)	0.087	
SD-5	Sons, SD, father → Daughters, SD, father	5.3 (-3.7 to 14.5)	1.07 (0.96 to 1.20)	0.118	
SD-5	Sons, CyO, mother → Sons, SD, mother	-3.1 (-9.0 to 2.8)	0.96 (0.88 to 1.04)	0.149	
SD-5	Daughters, CyO, mother → Daughters, CyO, father	2.5 (-4.8 to 9.8)	1.03 (0.94 to 1.12)	0.245	
SD-5	Sons, CyO, father → Daughters, CyO, father	2.5 (-4.9 to 10.4)	1.03 (0.94 to 1.13)	0.260	
SD-5	Daughters, CyO, father → Daughters, SD, father	-1.2 (-6.3 to 3.5)	0.99 (0.93 to 1.04)	0.310	
SD-72	Sons, SD, mother → Daughters, SD, mother	9.9 (4.2 to 15.8)	1.12 (1.05 to 1.21)	0.000	*
SD-72	Sons, CyO, mother → Daughters, CyO, mother	11.0 (4.3 to 17.9)	1.14 (1.05 to 1.25)	0.001	*
SD-72	Sons, CyO, father → Daughters, CyO, father	7.0 (-1.0 to 15.5)	1.09 (0.99 to 1.21)	0.045	*
SD-72	Sons, CyO, mother → Sons, SD, mother	3.4 (-1.2 to 8.1)	1.04 (0.98 to 1.11)	0.074	
SD-72	Daughters, SD, mother → Daughters, SD, father	-3.8 (-9.9 to 1.6)	0.96 (0.89 to 1.02)	0.085	
SD-72	Daughters, CyO, mother → Daughters, SD, mother	2.3 (-1.1 to 5.9)	1.03 (0.99 to 1.07)	0.088	
SD-72	Sons, SD, father → Daughters, SD, father	4.5 (-3.3 to 12.7)	1.06 (0.96 to 1.17)	0.134	
SD-72	Sons, CyO, father → Sons, SD, father	2.2 (-3.8 to 8.8)	1.03 (0.95 to 1.12)	0.243	
SD-72	Sons, CyO, mother → Sons, CyO, father	2.7 (-6.3 to 11.4)	1.04 (0.92 to 1.16)	0.274	
SD-72	Daughters, CyO, mother → Daughters, CyO, father	-1.2 (-7.4 to 4.7)	0.99 (0.92 to 1.05)	0.340	
SD-72	Sons, SD, mother → Sons, SD, father	1.5 (-6.4 to 9.3)	1.02 (0.92 to 1.12)	0.354	
SD-72	Daughters, CyO, father → Daughters, SD, father	-0.3 (-5.0 to 4.4)	1.00 (0.94 to 1.05)	0.449	
SD-Mad	Sons, CyO, mother → Daughters, CyO, mother	9.4 (2.7 to 16.3)	1.12 (1.03 to 1.22)	0.002	*
SD-Mad	Daughters, CyO, mother → Daughters, SD, mother	-5.4 (-9.9 to -1.2)	0.94 (0.89 to 0.99)	0.007	*
SD-Mad	Sons, CyO, mother → Sons, CyO, father	7.7 (-0.6 to 15.6)	1.10 (0.99 to 1.21)	0.034	*
SD-Mad	Sons, CyO, mother → Sons, SD, mother	3.0 (-1.9 to 8.0)	1.04 (0.98 to 1.10)	0.112	
SD-Mad	Daughters, SD, mother → Daughters, SD, father	3.4 (-4.9 to 11.0)	1.04 (0.94 to 1.14)	0.196	
SD-Mad	Sons, CyO, father → Sons, SD, father	-2.2 (-8.7 to 4.1)	0.98 (0.90 to 1.05)	0.232	
SD-Mad	Daughters, CyO, mother → Daughters, CyO, father	-2.8 (-10.7 to 4.3)	0.97 (0.88 to 1.05)	0.236	
SD-Mad	Sons, SD, mother → Sons, SD, father	2.5 (-6.3 to 10.3)	1.03 (0.93 to 1.13)	0.258	
SD-Mad	Sons, SD, father → Daughters, SD, father	1.9 (-6.7 to 10.9)	1.02 (0.92 to 1.14)	0.335	
SD-Mad	Sons, SD, mother → Daughters, SD, mother	1.0 (-6.1 to 8.0)	1.01 (0.93 to 1.10)	0.385	
SD-Mad	Daughters, CyO, father → Daughters, SD, father	0.8 (-5.2 to 6.9)	1.01 (0.94 to 1.09)	0.409	
SD-Mad	Sons, CyO, father → Daughters, CyO, father	-1.1 (-9.8 to 7.8)	0.99 (0.89 to 1.09)	0.413	