Fitness consequences of the selfish supergene *Segregation Distorter*

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Abstract

Segregation distorters are selfish genetic elements that subvert Mendelian inheritance, often 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 distorter alleles defy these predictions, suggesting that we have yet to discover all salient evolutionary forces acting on distorter alleles. 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* remains relatively rare despite its strong distortion. First, we show that the three variants differ in the severity and dominance of the fitness costs they impose on carrier individuals. Second, we found instances in which *SD* parents produce less fit offspring, suggesting that *SD* alleles have non-genetic, transgenerational costs. Third, we found an *SD* variant that altered the offspring sex ratio, perhaps due to off-target effects of *SD* on the Y chromosome. Finally, we use an evolutionary model to investigate the effects of transgenerational costs and sex ratio effects on the evolutionary dynamics of *SD*, and show that these previously undocumented costs potentially explain the puzzingly paucity of *SD* alleles in wild *D. melanogaster* populations.

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

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Introduction

Segregation distorters are genetic elements that manipulate meiosis or gametogenesis to increase their representation in the gametes of heterozygotes above the usual 50% (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 the distorter 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, there is currently great interest in developing natural or artificial segregation distorters that could propagate human-beneficial alleles through a wild animal or plant population, for example to introduce malaria resistance alleles into wild mosquito populations (Gantz et al. 2015). In addition to their promise in 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).

A long-standing puzzle, sometimes called the '*t* paradox' after the well-studied mouse segregation distorter *t* (Carroll and Potts 2007), concerns the failure of many segregation distorters to invade populations as well as simple models predict that they should (Bruck 1957; Lewontin 1968; Charlesworth and Hartl 1978; Taylor and Jaenike 2002; Holman et al. 2015). For example, Bruck (1957) showed that a homozygous lethal distorter that is transmitted to a faction k $(0.5 < k \geq 1)$ of the offspring of heterozygous males should reach an equilibrium allele frequency of $\frac{1}{2} - \sqrt{(k(1-k))}/2k$, if one makes no further complicating assumptions. This prediction is 38.5% if $k = 0.95$, which is substantially higher than the allele frequencies observed for real-world homozygous lethal segregation distorters with $k \approx 0.95$, such as the mouse *t* haplotype (5-14%; Ardlie 1998) and the *Segregation Distorter* gene complex of *Drosophila melanogaster* fruitflies (0-8%; Brand et al. 2015). Several later models sought to resolve this discrepancy by adding additional assumptions to the basic population genetic model. 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 strong, partially recessive fitness costs can maintain a distorter allele at low, stable frequencies. Additionally, males carrying segregation distorters often perform worse in sperm competition due to the loss of half their gametes, and there is some evidence that this can create frequency-dependent selection against distorters (Taylor and Jaenike 2002; Holman et al. 2015).

Here, we measure the magnitude and dominance of the fitness costs to larvae and adults carrying either one or two copies of *Segregation Distorter* (*SD*) in *D. melanogaster*. *SD* is a gene complex or 'supergene' (Thompson and Jiggins 2014) composed of several linked loci on chromosome 2 (an autosome) that 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, preventing the creation of recombinant 'suicide chromosomes' in which the insensitive *Rsp* allele linked to *SD* is replaced by a sensitive allele. The threat of suicide chromosomes selects for reduced recombination, and indeed *SD* is often surrounded by a large non-recombining region (*c.* 10% of the genome; Presgraves et al. 2009) containing deleterious mutations (Temin and Marthas 1984; Larracuente and Presgraves 2012; Brand et al. 2015). *SD* is thought to have a single evolutionary origin around 38,000 years ago (Brand et al. 2015), though it has since diversified into multiple variants which differ in their inversions and associated mutation load (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 (1984).

The evolutionary dynamics of segregation distorters depend strongly on the fitness of drive-carrying individuals. Negative frequency-dependent selection is of particular interest because it can maintain balanced polmorphisms in the face of strong distortion (Holman et al. 2015). If the fitness of carriers declines as the distorter allele becomes more common, the costs and benefits of the distorter can balance, preserving both distorting and non-distorting alleles. Recessive fitness costs create such selection, because distorter homozygotes become more common as the distorter invades. However, some distorters appear healthy when homozygous (Temin and Marthas 1984; Price et al. 2012), meaning that recessive costs cannot provide a complete answer to the *t* paradox. Additionally, models (e.g. Bruck 1957; Lewontin 1968) demonstrate that homozygote lethality is insufficient to explain the low allele frequencies of strong distorters like *SD* or *t*.

Here, we focus on the three best-studied *SD* variants: SD-5, SD-72, and *SD-Mad* (all originally from Wisconsin; Sandler et al. 1959). *SD-5* carries a different set of inversions from the other two and is reportedly homozygous lethal (Larracuente and Presgraves 2012), while some SD-72- and SD-Mad-type alleles are reported to be fit as homozygotes (Temin and Marthas 1984). To our knowledge, the relative fitness of *SD* heterozygotes has not been measured, though this parameter is crucial to the evolutionary dynamics of a distorter allele (Lewontin 1968). As well as measuring these costs, we wished to verify older reports (Hiraizumi and Nakazima 1967; Denell et al. 1969) that males carrying *SD* produce offspring with an atypical sex ratio, and to investigate theoretically how such a sex ratio bias would affect the evolution of *SD*. Lastly, we tested for non-genetic fitness effects of *SD*, e.g. mediated by parental effects or genomic imprinting, and use models to investigate the effects on *SD* evolution. We discuss the evolution of *SD* in wild populations in light of our empirical and theoretical findings.

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 was labelled with one recessive and one dominant phenotypic marker. 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 him 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 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 standardised the balancer to *CyO* (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.

Experiment 1

Experimental crosses

We performed four types of experimental crosses for each of the three *SD* chromosomes (Figure 1). In Cross 1, we mated two *SD/bw-GFP* flies, yielding offspring carrying 0, 1 or 2 *SD* chromosomes. In Cross 2, we mated *SD/bw-GFP* females to *bw* males, yielding offspring carrying 0 or 1 *SD* chromosomes. Cross 3 was the reciprocal: a *bw* mother and *SD/bw-GFP* father. Lastly, to measure the baseline fitness of the *bw-GFP* chromosome, we mated two *bw-GFP* flies (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 sex ratio of individuals that reached adulthood in the juvenile fitness assay.

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. 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 mortality in the embryonic stage. 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 such as harassment, and competition for food. We then recorded the number of surviving focal females, and moved them 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 survival rate 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* chromomes would be largely non-recombining; presgraves2009]. 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 lets us rule out GFP detection errors). 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 non-recombining balancer chromosome), 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 the measured the survival rate of a mixed pool of larvae carrying either 0 or 1 *SD* alleles. Most larvae in this pool carried 1 *SD* allele, rather than 0, because of segregation distortion. Specifically, the proportion of *SD* progeny in the 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 among individuals from the same vial. Female fitness was modelled using the negative binomial distribution, since the data were overdispersed counts. We verified model fit using posterior predictive checks (Gelman and Hill 2006), as shown in the Online Supplementary Material.

For hypothesis testing, we calculated the posterior differences between group means for pairs of means (contrasts) that are informative for this study (e.g. the difference in fitness between individuals with 0 or 1 *SD* allele, or individuals that received *SD* from their father versus their mother). Differences for which most of the posterior lies far from zero are highlighted in Tables 1 and 2. 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 more familiar p-value.

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 a survival rate. 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 in order to estimate the relative survival rates of *SD* and non-SD individuals. This unmeasured variable depends on the gametes produced by the *SD/CyO* parent. Because *SD* is well-documented to only cause 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 'guess' 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 results, thanks to the law of large numbers. We also re-ran the model under the assumption that there is some segregation distortion in fathers (i.e. $k > 0.5$, contradicting the evidence in Ganetzky 1977), and found that all the key results did not change (see Online Supplementary Material).

Population genetic model

Our experiments suggested that some *SD* variants have parent-of-origin-specific effects on fitness and/or cause SD-carrying 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 other genotypes potentially have relative fitness between 0 and 1. We kept track of 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 the possibility that *SD* has a parent-of-origin-specific effect on fitness. We assumed that male heterozygotes transmit the *SD* allele to a fraction $(1 + K)/2$ of their offspring (where $0 < K < 1$), and produce a fraction $(1+s)/2$ female offspring ($0 < s < 1$), while all other genotypes were assumed to show normal Mendelian inheritance and a 50:50 offspring sex ratio. For example, a mating between a wild-type female and a male *SD* heterozygote produces $(1 + K)(1 - s)/4$ heterozygote sons, $(1+K)(1+s)/4$ heterozygote daughters, $(1-K)(1-s)/4$ wild-type sons, and $(1-K)(1+s)/4$ wild-type daughters. Note that for convenience and consistency with other models, the model uses capital K (range: 0-1, where 0 indicates no distortion and 1 complete distortion), rather than the lowercase k discussed earlier (where 0.5 indicates no distortion and 1 complete distortion).

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 genotype frequency using numerical simulations, 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, or until the *SD* allele went extinct (defined as reaching 0.001% frequency) or fixed (>99%).

Availability of data and code

All our raw data plus the R code used for our analyses is provided as supplementary material, and can also be viewed online at https://lukeholman.github.io/fitnessCostSD/.

Results

Experiment 1

Posterior estimates of mean fitness for each group are plotted in Figure 1. Table 1 lists notable pairwise differences between groups, Tables S1-S4 give sample sizes and simple summary statistics, and Tables S6-S9 give results for all the contrasts we examined.

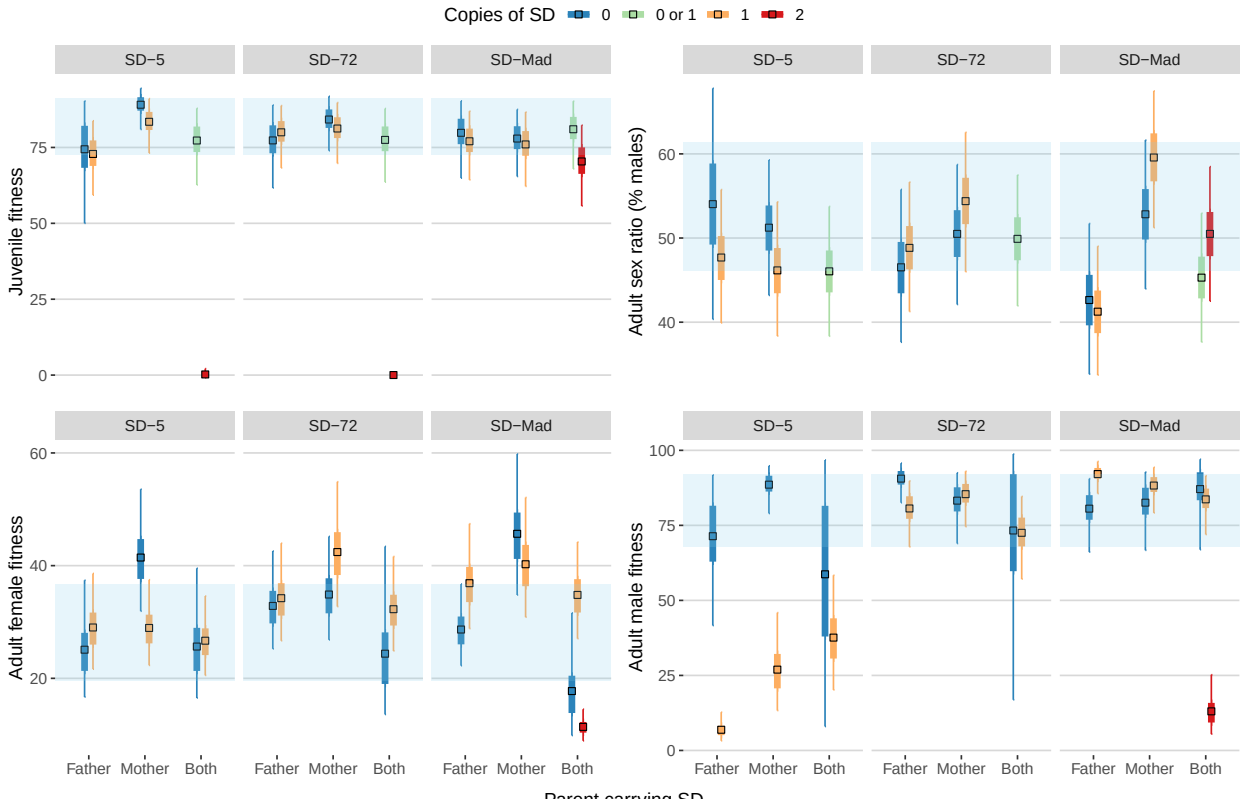

Figure 1: Posterior estimates of the group means for the four different response variables in Experiment 1, for each type of cross (x-axis), *SD* variant (panels), and genotype (colours). The thicker inner bar shows the region containing 50% of the posterior, while the outer bar covers 95% of the posterior. Tables S6-S9 give the accompanying statistcal results. Points labelled as carrying "0 or 1" *SD* allele refer to cases where the genotype of the offspring could not be ascertained due to the brown eye marker not being expressed in larvae; most of these individuals 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 none of these survived to adulthood. The smaller number for *SD-5* indicates that most *SD-5* homozygotes died before hatching, while *SD-72* homozygotes tended to die between hatching and adulthood. By contrast, many larvae homozygous for *SD-Mad* reached adulthood, and there was no detectable fitness effect of *SD-Mad* on juvenile fitness even in homozygotes.

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 no difference in juvenile fitness between individuals with an *SD* mother versus an *SD* father for *SD-72* and *SD-Mad*.

Table 1: List of the all the notable differences between groups in Experiment 1 (posterior probability <0.05). For each contast, we give the parent carrying *SD* (neither, mother, father, or both) and the number of *SD* alleles carried by the offspring. The difference in means is expressed in the original units, i.e. % larvae surviving, % male larvae, number of larvae produced, or % offspring sired, and the parentheses give 95% credible intervals. The difference is positive when the first-listed mean is larger than the second-listed mean, and negative otherwise. The posterior probability (p) has a similar interpretation to a one-tailed p-value.

Trait	SD	Comparison	Difference	Error	p
Larval survival	SD-5	Both parents, 0 or 1 → Both parents, 2	77.1 (62.2 to 87.8)	6.5	0.000
Larval survival	SD-72	Both parents, 0 or 1 → Both parents, 2	77.5 (63.6 to 87.8)	6.1	0.000
Sex ratio	SD-Mad	Neither, 0 → Father, 0	11.1 (-0.8 to 23.0)	6.0	0.034
Sex ratio	SD-Mad	Mother, 1 → Father, 1	18.3 (7.1 to 29.7)	5.7	0.001
Female fitness	SD-5	Neither, 0 → Mother, 0	-14.4 (-28.6 to -0.4)	7.0	0.022
Female fitness	SD-Mad	Neither, 0 → Mother, 0	-18.7 (-34.7 to -3.8)	7.8	0.006
Female fitness	SD-5	Mother, 0 → Father, 0	16.4 (1.0 to 31.0)	7.6	0.019
Female fitness	SD-Mad	Mother, 0 → Father, 0	17.0 (3.2 to 32.6)	7.5	0.009
Female fitness	SD-5	Mother, 0 → Mother, 1	12.5 (-0.2 to 26.1)	6.6	0.027
Female fitness	SD-Mad	Both parents, 1 → Both parents, 2	23.4 (15.0 to 33.1)	4.6	0.000
Male fitness	SD-5	Mother, 1 → Father, 1	20.0 (5.5 to 39.2)	8.8	0.003
Male fitness	SD-5	Mother, 0 → Mother, 1	61.6 (41.0 to 77.7)	9.4	0.000
Male fitness	SD-5	Father, 0 → Father, 1	64.5 (34.6 to 85.6)	13.5	0.000
Male fitness	SD-Mad	Father, 0 → Father, 1	-11.5 (-26.7 to 0.6)	6.9	0.032
Male fitness	SD-Mad	Both parents, 1 → Both parents, 2	70.6 (54.2 to 82.6)	7.2	0.000

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. 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 % male offspring was 54% among the non-*SD* offspring and 48% male among the *SD* offspring; Figure 1).

Adult female fitness

Females homozygous for *SD-Mad* had low fitness. Additionally, females were fitter if their mother, as opposed to their father, carried *SD-5* or *SD-Mad*, irrespective of whether the offspring inherited *SD*.

Adult male fitness

Males homozygous for *SD-Mad* had low fitness. We again observed evidence for non-genetic effects: for *SD-5*, males with a paternally-inherited *SD* chromosome were substantially less fit than males with a maternally-inherited *SD* chromosome. There was also a strong fitness

cost of inheriting an *SD-5* allele from either parent. Interestingly, males with an *SD-Mad* father were fitter if they inherited *SD-Mad* rather than the non-*SD* allele.

Experiment 2

Experiment 2 also 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 S5 and S10). Male larvae with an *SD-5/CyO* mother were significantly less likely to survive than those an *SD-5/CyO* father, irrespective of whether the larva actually inherited *SD-5* (the same was not true for the other *SD* variants, suggesting that *SD-5* not *CyO* mediates this effect). Additionally, female larvae who inherited *SD-5* from the mother survived less well than did females who inherited *CyO*, suggesting that a single copy of *SD-5* reduces survival more than a single copy of *CyO*. The same effect was not observed for males, or for crosses in which *SD-5* came from the father, possibly indicating that *SD-5* has a sex- or parent-of-origin-specific effect on survival (we lack the sample size to be certain). By contrast, male larvae whose father carried *SD-Mad* were fitter if they inherited *SD-Mad* rather than *CyO*, suggesting that *SD-Mad* is less harmful than *CyO*. Lastly, we observed some significant sex differences in survival for all three *SD* chromosomes, with female larvae surviving better than male larvae. We did not find any evidence that the direct genetic effect of *SD* on larval survival is sex-specific: the difference in survival rate for *SD* and *CyO* individuals was similar for males and females (Figure 2).

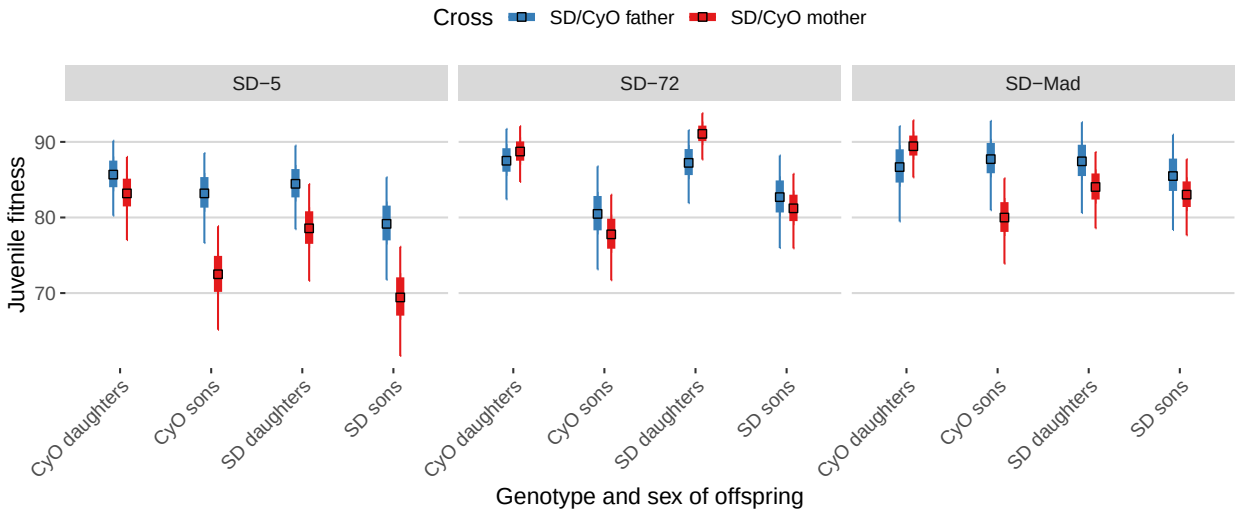

Figure 2: Posterior estimates of % L1 larva-to-adult survival in Experiment 2 for each combination of offspring sex and genotype (x-axis), *SD* variant (panels), and cross (colours). The thicker inner bar shows the region containing 50% of the posterior, while the outer bar covers 95% of the posterior. 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 S1 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 <0.05). 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	Difference	Error	p
SD-5	Daughters, CyO, mother → Daughters, SD, mother	4.6 (-0.6 to 10.0)	2.7	0.041
SD-5	Sons, CyO, mother → Daughters, CyO, mother	-10.7 (-19.4 to -2.0)	4.5	0.008
SD-5	Sons, CyO, mother → Sons, CyO, father	-10.7 (-19.8 to -1.5)	4.6	0.010
SD-5	Sons, SD, mother → Daughters, SD, mother	-9.1 (-18.7 to 0.4)	4.8	0.028
SD-5	Sons, SD, mother → Sons, SD, father	-9.7 (-19.4 to -0.1)	5.0	0.025
SD-72	Sons, CyO, father → Daughters, CyO, father	-7.0 (-15.5 to 1.0)	4.2	0.045
SD-72	Sons, CyO, mother → Daughters, CyO, mother	-11.0 (-17.9 to -4.3)	3.5	0.001
SD-72	Sons, SD, mother → Daughters, SD, mother	-9.9 (-15.8 to -4.2)	3.0	0.000
SD-Mad	Daughters, CyO, mother → Daughters, SD, mother	5.4 (1.2 to 9.9)	2.2	0.007
SD-Mad	Sons, CyO, mother → Daughters, CyO, mother	-9.4 (-16.3 to -2.7)	3.5	0.002
SD-Mad	Sons, CyO, mother → Sons, CyO, father	-7.7 (-15.6 to 0.6)	4.1	0.035

Population genetic model

We first assumed that the *SD* allele had no direct or transgenerational fitness costs (top left, Figure 3), which resulted in the invasion of *SD* 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 a sex ratio bias for SD-carrying males 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* was seldom observed. There was a small zone of polmorphism when drive was very weak and the sex ratio bias was very strong, due to the frequency dependence of the costs associated with the biased sex ratio (i.e. sex ratio-biasing alleles are more costly in populations where they are common, due to the increasing bias in the population sex ratio).

Secondly, when we assumed dominant costs such that all individuals with at least one *SD* allele had a relative fitness of 0.8 (top second left, Figure 3), the *SD* allele could still invade, though it needed a substantially higher transmission bias K. When *SD* did invade, it again proceeded to fixation, except under unrealistically weak drive and extreme sex ratio bias. Notably, invasion was more difficult when *SD* heterozygote males produce a female-biased rather than male-biased sex ratio. Such a female bias is detrimental to *SD* because segregation distortion only happens in males, increasing the critical value of K required for invasion. *SD* invaded slightly more easily when *SD* heterozygote males produced >50% sons, but not so easily as when there was no sex ratio bias (due to the fitness gains of extra transmission bias through sons being opposed by Fisherian sex ratio selection).

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, halting the spread of the *SD* allele once the costs experienced in homozygotes 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 so far, based on our empirical findings). Here, the *SD* allele only invaded when K was high, and it stabilised at medium-high frequencies. 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 additional 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 trans-generational costs further hamper the spread of *SD*, and that paternal costs are worse than maternal costs, because the *SD* allele is inherited from fathers more often than mothers due to its male-limited distortion. 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*).

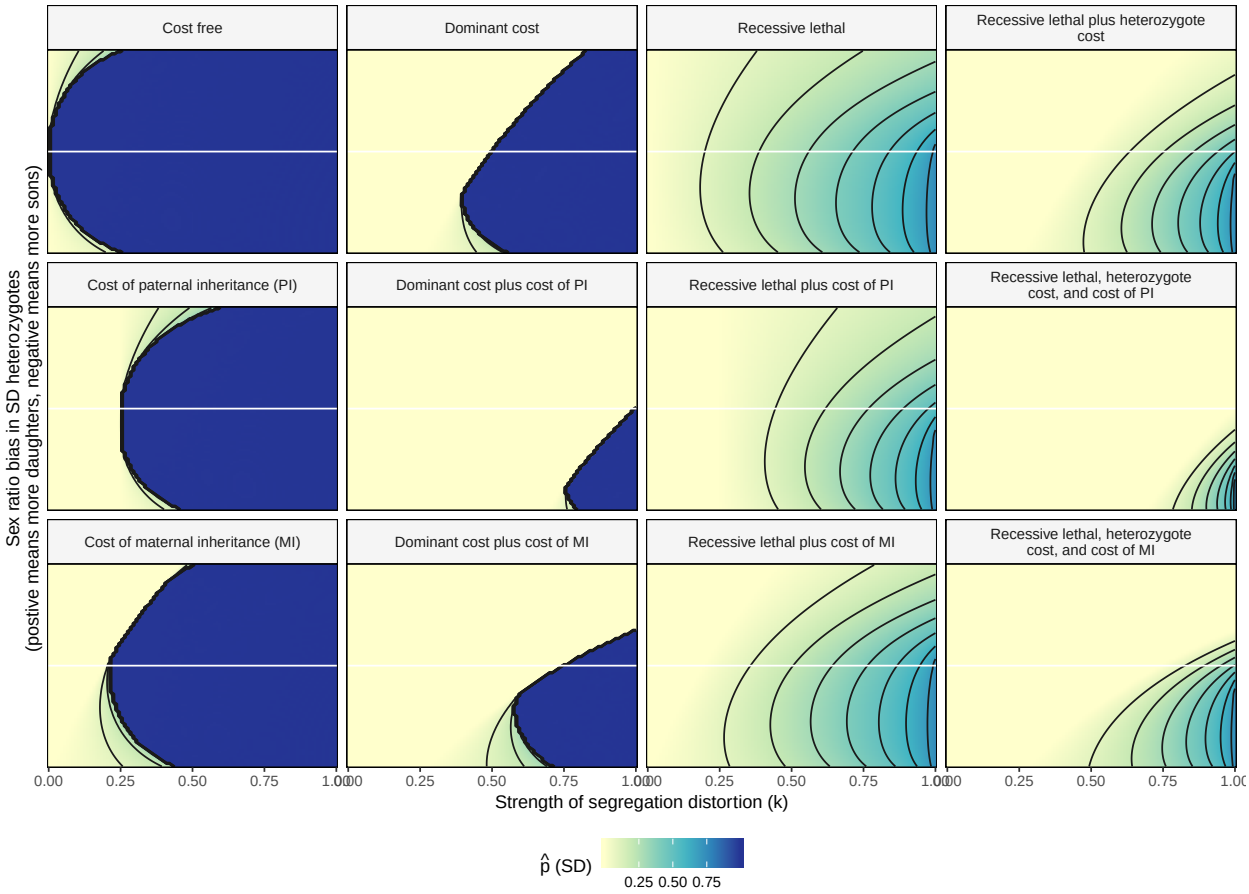

Figure 3: The equilibrium frequency reached by the *SD* allele, $\hat{p}(SD)$, depends on the strength of segregataion distortion ($K = 0$ indicates fair meiosis, $K = 1$ that heterozygotes transmit only the *SD* allele) and the direction and strength of sex ratio bias in the progeny of *SD* heterozygote males. The four columns make different assumptions about the fitness costs to individuals carrying the *SD* allele (see Results), 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).

Discussion

Our results reaffirm that *SD-5* and *SD-72* are homozygous lethal. Most *SD-5* homozygotes died while still in the egg, while *SD-72* homozygotes died after hatching but before adulthood. Although cultures fixed for *SD-Mad* can survive in the lab, we found that male and female *SD-Mad* homozygotes had much lower fitness than the *bw-GFP* competitor flies, suggesting that *SD-Mad* would be effectively homozygous lethal in a natural population. The fitness costs to male adults were dominant for *SD-5* but recessive for *SD-72* and *SD-Mad*. By contrast, the fitness costs to female adults were recessive for all three *SD* variants, illustrating that the dominance of the cost differs between sexes as well as between *SD* variants. Although we did not observe any *SD* variants that were fit as homozygotes in this study, it is possible that such variants exist; an *SD* variant with inversions characteristic of *SD-72* or *SD-Mad*

was observed to reach 98% frequency in a population in Wisconsin (Temin and Marthas 1984).

Interestingly, we found some evidence for costly non-genetic transgenerational effects associated with all three *SD* variants, mediated either by parental effects (sensu Badyaev and Uller 2009) or genomic imprinting (sensu Holman and Kokko 2014). Firstly, female fitness was reduced among the non-SD offspring of *SD-5* or *SD-Mad* heterozygote fathers. One possible mechanism is that chromosomes that escape segregation distortion are epigenetically modified in ways that affect adult fitness; this mechanism is plausible because *SD* seems 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 genotype-dependent paternal effect of *SD-5* (either phenomenon would be consistent with the data). Thirdly, in Experiment 2, we found that male larvae whose mother carried *SD-5* were less likely to reach adulthood than were male larvae whose father carried *SD-5*, irrespective of whether the larva 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 (possibly 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, so we built a simple model incorporating the assumption that *SD* alleles can have parent-of-origin-specific effects on fitness. The model showed that non-genetic transgenerational costs of *SD* can reduce the invasion probability and equilibrium frequency of *SD* (this is an example of evolution via indirect genetic effects, Wolf et al. 1998). Thus, if segregation distorters commonly have transgenerational costs, such costs may help to explain the puzzling rarity of *SD* (Brand et al. 2015) and other autosomal distorters such as the *t*-haplotype (Carroll and Potts 2007) in spite of biased segregation.

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). 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 shortage of sons that we observed. Additionally, we speculate that *SD* might cause a parental effect that affects the relative survival rates of male and female progeny, for example by inducing epigenetic modifications that are more harmful to males than females. 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 help to resolve the *t*-paradox in other systems.

Future studies could compete *SD* alleles with differing costs, and differing cost dominance, in population cages. We predict that *SD* alleles that have 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 relate to their fitness costs in homozygotes and heterozygotes. *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 (Lindholm et al. 2016). For example, we suggest that it is worthwhile to measure 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 thattransgenerational costs can strongly influence the invasion success of a gene drive.

Acknowledgements

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Online Supplementary Material