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Sexual selection can partly explain low frequencies of *Segregation Distorter* alleles

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The *Segregation Distorter* (*SD*) allele found in *Drosophila melanogaster* distorts Mendelian inheritance in heterozygous males by causing developmental failure of non-*SD* spermatids, such that greater than 90% of the surviving sperm carry *SD*. This within-individual advantage should cause *SD* to fix, and yet *SD* is typically rare in wild populations. Here, we explore whether this paradox can be resolved by sexual selection, by testing if males carrying three different variants of *SD* suffer reduced pre- or post-copulatory reproductive success. We find that males carrying the *SD* allele are just as successful at securing matings as control males, but that one *SD* variant (*SD-5*) reduces sperm competitive ability and increases the likelihood of female remating. We then used these results to inform a theoretical model; we found that sexual selection could limit *SD* to natural frequencies when sperm competitive ability and female remating rate equalled the values observed for *SD-5*. However, sexual selection was unable to explain natural frequencies of the *SD* allele when the model was parameterized with the values found for two other *SD* variants, indicating that sexual selection alone is unlikely to explain the rarity of *SD*.

1. Introduction

In sexually reproducing organisms, meiosis ensures that autosomal alleles are divided evenly between the haploid gametes. However, this equitable transmission can be subverted by 'selfish genetic elements' which encode phenotypes that are selected to increase their own propagation, at the expense of other alleles in the genome [1]. These selfish alleles have manifold ecological and evolutionary consequences [2], and given their potential to spread even when they lower the fitness of individuals carrying them, efforts are under way to develop synthetic selfish alleles that mimic their effects, with the aim to modify or suppress populations [3]. This highlights a need to understand the evolutionary dynamics of naturally occurring selfish alleles.

One well-studied selfish allele is *Segregation Distorter* (*SD*), a male gamete killer found in *Drosophila melanogaster* [4]. *SD* is a large multigenic locus making up approximately 40% of the second chromosome, a large autosome which itself comprises over a third of the genome. It contains a distorter locus, multiple loci that enhance distortion and a target site that is insensitive to distortion [5]. In heterozygous *SD*/+ males (that carry one *SD* allele and one homologous non-distorting allele), *SD* causes spermatids that carry the non-distorting, sensitive allele to die before completing development [5]. The result is that greater than 90% of the male's functional sperm carry *SD*, rather than the 50% expected for a typical heterozygous locus [6].

This large advantage in within-individual sperm competition should cause the *SD* allele to reach fixation [7]. Contrary to this prediction, *SD* was only found on 0–8% of second chromosomes in a sample of wild *D. melanogaster* populations [6]. A possible explanation for this is that some variants of the *SD* allele accumulate harmful, recessive mutations causing lethality, sterility or greatly reduced fitness in *SD*/*SD* homozygotes [8,9]. These recessive mutations impose negative frequency-dependent selection on *SD*: as *SD* becomes more common, the within-individual benefits of distortion are increasingly offset by the costs

to *SD* alleles in homozygotes, creating a balanced polymorphism of *SD* and non-distorting alleles. However, population genetic models that consider recessive lethality (e.g. [7,10]) still overestimate the equilibrium frequency of *SD*. For example, Bruck [7] found that the equilibrium frequency for a homozygous lethal segregation distorter is $\frac{1}{2} - \sqrt{\frac{k(1-k)}{2k}}$, where k is the proportion of a heterozygous male's functional sperm that carry the distorting allele. When $k = 0.9$, the predicted equilibrium frequency is 33%, suggesting there are unconsidered fitness consequences associated with *SD* alleles.

Here, we test whether sexual selection acting on males might partly explain why *SD* is rare in natural populations. The population genetic effects of sexual selection have been well-explored in other species harbouring segregation distorters (reviewed in [2,11]). Moreover, a recent study of *SD* showed that *SD*/+ males were sometimes weak competitors in sexual selection, but did not determine whether *SD*/+ males have reduced success in pre- or post-copulatory competition (or both [9]). Theoretically, pre-copulatory sexual selection might help to explain the rarity of *SD* if females tend to avoid mating with *SD*/+ males if, for example, females have been selected to avoid males that produce non-viable or *SD*-carrying offspring [12]. *SD*/+ males may also have reduced overall condition relative to +/+ males, because the large *SD* gene complex experiences little to no recombination, and is thus predicted to accumulate deleterious mutations [13]. If either or both of these hold and because male mating success often relies on condition-dependent traits [14], we predict females to mate preferentially with non-*SD* males.

Post-copulatory sexual selection may also explain the discrepancy between predicted and observed *SD* frequencies. Segregation distorters increase their relative within-individual frequency by destroying or incapacitating sperm carrying non-distorting homologous alleles. This means that *SD*/+ males should produce half as many sperm as +/+ males [5], assuming no compensatory increase in sperm production by the male (see [15]). The deleterious mutations carried by *SD*, or off-target effects of the sperm incapacitation mechanism, might reduce the number of sperm still further and/or reduce their average competitive ability [16]. Sperm number and quality are key determinants of post-copulatory mating success [17,18], such that *SD* alleles might have reduced fitness in populations where females mate multiply (as hypothesized for other distorters; e.g. [19,20]). In support of this hypothesis, segregation distorters reduce sperm competitive ability in other fly species and mice [21–25]. Building upon earlier models [7,10], evolutionary simulations accounting for sperm competition costs paired with homozygous viability costs have produced distorter frequency estimates that match observations from wild populations [26,27]. However, the effect of *SD* on sperm competitive ability has never been measured.

Here we examined pre- and post-copulatory success for *SD*/+ males and also measured whether females preferentially re-mate after mating with *SD*/+ males. *D. melanogaster* has strong last-male sperm precedence [28], and so effects of male genotype on female remating latency could strongly affect the fitness of the *SD* allele. In *Drosophila*, females tend to remate faster when their sperm storage organs are comparatively empty (e.g. because stored sperm steadily release chemicals such as sex peptide that suppress remating [29]). One might therefore expect *SD*/+ males, which probably transfer fewer sperm (as found for a segregation distorter in *D. simulans*; [30]), to create a shorter post-mating refractory period in their mates. Female remating is also strongly affected by seminal fluid proteins from the male ejaculate [31], and it is also possible that the deleterious mutations linked to *SD* affect seminal fluid quantity or quality.

Finally, we present a population genetic model incorporating these effects in conjunction with segregation distortion and homozygote lethality, which we parameterized with our empirical results. We use the model to explore the effects that pre-copulatory mating success, sperm competitive ability and female remating propensity have on the allele frequency of *SD*, and to test whether the fitness costs we identified are sufficient to explain the observed rarity of *SD* in nature [6].

2. Methods

(a) Fly stocks

We maintained all stocks at 25°C under a 16 : 8 h photoperiod in *Drosophila* vials (95 × 25 mm) on food medium (recipe in electronic supplementary material, table S1; approximately 8 cm^3 in each vial), supplemented with dry yeast. We used four genotypes in this study: three of these were heterozygous for three different variants of *SD*, all of which were originally collected in Madison, Wisconsin [4]. The *SD* variants are named *SD-5* (Bloomington stock number: 64322), *SD-72* (64323) and *SD-Mad* (64324). Each variant is characterized by the inversions it carries and/or its viability effects [5]; *SD-5* and *SD-72* are homozygous lethal, while *SD-Mad* is not (though its homozygotes have low fitness [9]). To minimize extraneous genetic differences between the three *SD* genotypes, we first standardized the genotype of both of the sex chromosomes, the non-*SD* copy of chromosome 2, and both copies of chromosome 3 using a crossing scheme involving balancers (electronic supplementary material, figure S1). This scheme produced experimental lines (hereafter *SD*/+ lines) that carried one copy of an *SD*-variant chromosome and one copy of the w^{1118} chromosome 2 and were otherwise genetically uniform, with the possible exception of the tiny fourth chromosome. We confirmed that each of the *SD*/+ lines exhibited segregation distortion in a pilot experiment (see electronic supplementary material, figure S2). The fourth genotype (hereafter +/+) was a non-*SD* control, which we generated in an identical fashion, except that the flies carried a copy of chromosome 2 from the isogenic w^{1118} line (and were therefore homozygous for both major autosomes), instead of an *SD*-bearing chromosome. The *SD-5* line was not included in Experiment 1 because it went extinct when access to the laboratory was restricted due to COVID-19 (Experiment 1 was the last to be completed).

We also used three other fly stocks to compete or mate with the *SD*/+ and +/+ lines. In our experiments, we used males from two outbred strains to provide a standardized source of competition against the *SD*/+ and +/+ males. For Experiment 1, we sourced males from a LH_m population that is homozygous for the *bw* mutation and therefore expresses a brown eye phenotype (hereafter *Lbw*). For Experiment 2, we used males from another LH_m population, that is homozygous for the transgenic construct *Ubi-GFP* (hereafter LH_m^{Ubi}). The *Ubi-GFP* construct is attached to chromosome three and causes ubiquitous expression of green fluorescence in *D. melanogaster* when viewed under fluorescent light. Females that mated with experimental and competitor males were sourced from a large, outbred population of the LH_m line that does not harbour the *Ubi-GFP* construct.

For our experiments, we reared the four experimental genotypes at a density of 100 larvae per vial. Each genotype was sired by parents 2–4 days old that had also developed under density-controlled conditions. We collected virgin males from the *SD*/+, +/+ and competitor male LH_m^{Ubi} and *Lbw* populations,

and virgin females from the LH_m population. All virgins were collected within 8 h of eclosion and housed in same-sex environments until they were themselves 2–4 days old, to ensure sexual maturity at the onset of the experiments. To minimize differences in male mating investment caused by the social environment during the days preceding the experiment, we standardized the number of adult experimental virgin males (and *Lbw* males, for Experiment 1) to approximately 10 per vial. In Experiment 2, we housed adult LH_m^{Ubi} competitor males at 80 per vial, due to the larger number of males required.

(b) Experiment 1: testing whether *SD*/+ males exhibit reduced mating success

To assess whether *SD*/+ males suffer reduced mating success when competing with other males, we employed a two-choice test design. We aspirated two males into a vial containing food medium; first a brown-eyed *Lbw* male, followed by a white-eyed male carrying one of the experimental genotypes (either *SD-72*, *SD-Mad* or the control). We then introduced a single virgin LH_m female and noted the time. Once the female mated with one of the males, we recorded the genotype of the successful male and the time at which mating started. After the mating pair separated, we immediately ended the trial, recorded the time mating finished and discarded the three flies. We recorded the mating outcomes from 124 triads and conducted the experiment blind to male genotype, to prevent observer bias affecting the results [32].

We note that eye colour may affect mating success, and as such we expect greater than 50% of females to mate with the brown-eyed competitor male over the white-eyed focal male [33]. However, the purpose of this experiment is to compare the relative mating successes of the four types of experimental males, and this comparison is not confounded by differences in eye colour.

(c) Experiment 2: testing sperm competitive success and female remating propensity

The aims of this experiment were to (i) measure sperm competitive success of *SD*/+ males and (ii) test whether female remating propensity is affected by male genotype. We ran the experiment across three blocks made up of flies from three consecutive generations and again conducted the experiment blind to male genotype.

To mimic natural conditions and accentuate any effects of *SD* on sperm production, we mated all *SD*/+ and control males once, shortly before starting the experiment. To do this, we paired individual virgin *SD*/+ or control males with a virgin LH_m female, allowed the pair to interact for 2 h and recorded that mating occurred. Males that did not mate were discarded, and the mated males were used in the experiment 2–3 h after mating.

To measure P1 (the proportion of offspring sired by *SD*/+ males when mating first), as well as female remating propensity, we first paired a single *SD*/+ or control male with a virgin LH_m female and allowed them 3 h to mate. We confirmed mating and discarded the male once they disengaged from copula. After 4 days, we allowed females a single opportunity to remate—we aspirated a single 6- to 8-day-old LH_m^{UBI} male and the previously mated female into a new food vial, and observed the pair for a maximum of 3 h. For both mating interaction periods, we recorded whether mating occurred, the time taken for mating to begin (hereafter 'mating latency'), and the copulation duration; 94/196 females remated, and we collected no further mating data on females that did not remate. Throughout the experiment, we observed 11 females mating after 3 h had passed, before we could discard them from their vial. We recorded these females as failing to re-mate, but we did include them in the subsequent sperm competitive ability measurements in order to maximize sample size. Upon completion of the female's second mating, we discarded males and transferred females into a vial containing grape juice agar and a small amount of yeast paste, and left them to oviposit for 72 h.

We recorded the number of offspring sired by the *SD*/+ (or +/+) male and the LH_m^{UBI} competitor to estimate P1. We determined paternity by first counting the number of offspring produced by each female using a light microscope, then counting the number of these offspring expressing GFP fluorescence (using a Leica M165 FC fluorescence microscope): the offspring of *SD* males did not express GFP, while offspring of LH_m^{UBI} competitor males exhibited strong fluorescence. We measured P2 (the proportion of offspring produced by the *SD*/+ male when the *SD*/+ male mated second) for *SD*/+ males in identical fashion, except that the order of matings was reversed, with LH_m^{UBI} males mated to females first and *SD*/+ or control males mated to females second. This time, 119/246 females remated within the 3 h observation period (and were scored as having remated), and 16 females were observed remating after this time (and were scored as not having remated, but were included in subsequent sperm competition progeny counts).

(d) Statistical analysis

We analysed the results using Bayesian generalized linear mixed models implemented in the *brms* package for R [34]. For all models, we specified a prior distribution of $N(\mu = 0, \sigma = 3)$ for fixed effect estimates and $N(\mu = 0, \sigma = 5)$ for intercept estimates. We ran four chains per model, each with 8000 iterations (2000 discarded as a warm-up), and confirmed model convergence and fit with R statistics and posterior predictive checks. To make inferences about our models, we calculated posterior differences between the means of the *SD*-variant treatment groups and the control treatment group. We interpret differences between the *SD* lines and the control line for which the 95% uncertainty intervals exclude zero as noteworthy.

For Experiment 1, we modelled whether or not each male mated using a binomial model. We fitted *SD* variant as a fixed effect and rearing vial as a random effect (to model and control for similarities between individuals that developed in the same vial). We also modelled the mating latency and copulation duration for the subset of trials in which the *SD*/+ or control male mated, in two separate models, both using the Weibull distribution and with the same fixed and random effects as the mating success model.

For Experiment 2, we modelled P1 and P2 separately using binomial models, with the proportion of offspring sired as the response variable. We fitted the P1 model using the progeny count data for females that mated with an *SD*/+ or +/+ male first, and the P2 model using data from females that mated with these males second. We fitted *SD* variant as a fixed effect, as well as Block (which models the variance produced by the replication of the experiment across three generations). We also included rearing vial and individual ID as random effects. Second, we used another binomial model to estimate the likelihood of female remating after mating with each type of male. Third, we modelled remating latency to further explore the effects of male genotype on female remating. These data were modelled using a Weibull distribution with right censoring, where females that did not re-mate within 3 h were censored. Both models of remating contained the same fixed effects as the sperm competition models and rearing vial as a random effect. Finally, we modelled copulation duration using two separate models, where the duration of the first and second matings was used as response variables. We specified a Weibull distribution for each and used the same fixed and random effects as the remating models.

The raw data and R code used to run all analyses are presented at https://tomkeaney.github.io/SD_sexual_selection/.

(e) Population genetic model

The effect that *SD* has on a male's sperm competitive ability and its capacity to limit female remating is likely to affect the frequency of *SD* in natural populations. We therefore built a one-locus, two-allele population genetic model—parameterized with our estimates of segregation distortion, mating success, sperm competitive ability and female remating probability—to assess how these variables affect the evolutionary trajectory of the *SD* allele.

The model considers an infinite, panmictic population composed of two sexes with non-overlapping generations. The population contains distorting *SD* alleles and non-distorting wild-type alleles. Beginning with the fertilized zygotes, all genotypes survive to breeding age with equal probability, except for *SD* homozygotes, which we assume to be inviable (electronic supplementary material, table S3 shows that our model returns the same equilibrium frequencies as earlier analytical models, e.g. [7], if we only include segregation distortion and homozygote lethality). This assumption simplifies the model considerably and reflects reality for at least two of the *SD* variants (the third has low but non-zero fitness in homozygotes [9]). Removing this assumption would result in elevated allele frequencies for *SD*, while modelling a viability cost to *SD*/+ individuals would lower the frequency of *SD* (see [9,26]).

After removing non-viable genotypes, the population matures to adulthood and breeds. We implement pre-copulatory sexual selection on males via a parameter S_{precop}. When $S_{precop} = 1$, the two male genotypes are selected as mates randomly (i.e. with probabilities equal to their frequencies in the population). Values of S_{precop} below 1 indicate that *SD*/+ males are poor pre-copulatory competitors, while values above indicate they are superior competitors. S_{precop} includes the short-range sexual selection we measured in Experiment 2, as well as longer-range processes like mate searching. We explored the evolution of *SD* for parameter spaces where $0.8 \leq S_{precop} \leq 1.2$.

With S_{precop} defined and the genotype frequencies among the surviving adults known, we next calculated the frequencies of each possible mating type. We make the simplifying assumption that females mate with a maximum of two males, which is likely to be reasonable given that *D. melanogaster* has a long post-mating refractory period and thrice-mated females produce very few offspring sired by the first-mated male [35]. The proportion of females that mate twice is $p_{+/+}$ among females whose first mate was +/+, or $p_{SD/+}$ for females whose first mate was *SD*/+. We focus on parameter spaces where $p_{SD/+} \geq p_{+/+}$ (i.e. where females are equally or more likely to remate after mating with *SD*/+ males). The mating types therefore consist either of a male–female pair, or triads containing a female, her first mate and her second mate. We began by multiplying the population frequency of *SD*/+ males by S_{precop} then renormalizing all of the genotype frequencies to again sum to 1 (this step lowers or raises the frequencies of mating types involving *SD*/+ males). Then, for singly mated females, the frequency of each mating type was calculated as $F_i M_j (1-p_i)$, where F_i and M_j are the female and male parental genotype frequencies, and p_j is the probability of female remating following the first mating with a male of genotype *j*. Similarly, we found the expected frequencies of each possible mating type for females that mated with two males via the formula $F_i M_j N_k p_j$, where N_k represents the genotype frequency of the second male to mate.

We next model (order-specific) sperm competition, which is only necessary for females that mated with one *SD*/+ and one +/+ male. We set the normal *P1* value for the population, $P1_{normal}$, to 0.1 (i.e males mating first sire 10% of the offspring produced by a twice-mated female), which is broadly consistent with our empirical estimates and those from other studies of *D. melanogaster* (e.g. [28,36]). We also explored the parameter space where $P1_{normal} = 0.5$, which represents a scenario where first-mating males sire half the offspring produced by twice mating females. We assume that first-mating *SD*/+ males suffer a cost to their sperm competitive ability when the female mates second with a +/+ male, such that the *SD*/+ male sires a proportion $P1_{normal} - (P1_{normal} \times P1_{cost})$ of the offspring. When they occupy the second mating role and a +/+ male mates first, *SD*/+ males suffer a cost to P2 and sire a proportion $1 - (P1_{normal} + (1 - P1_{normal}) \times P2_{cost})$ of the offspring. We investigated the full range of possible values for $P1_{cost}$ and $P2_{cost}$, i.e. 0–1, where 0 indicates that *SD*/+ males are equally effective in sperm competition, and 1 indicates a complete loss of paternity for the *SD*/+ male when females mate twice.

After determining the mating-type frequencies and the outcome of sperm competition, zygotes are produced and the adults are removed, starting the next generation. We assume standard Mendelian inheritance except for zygotes fertilized by *SD*/+ males, where 86.8%, 90.9% or 94.4% of zygotes inherit their father's *SD* allele (these values correspond to the k_c estimates found in our pilot experiment; see electronic supplementary material, methods and table S2), instead of the typical 50%.

We calculated the genotype frequencies of each generation immediately after removing the inviable *SD*/*SD* genotype. We found the equilibrium allele frequencies numerically, by setting the initial frequency of *SD* to 0.01 and iterating for multiple generations until *SD* approached extinction (freq > 0.0001), fixation (freq < 0.99) or until 1000 generations had elapsed. We wrote the model in R; the code and a detailed explanation of it can be found at https://tomkeaney.github.io/SD_sexual_selection/.

3. Results

(a) Experiment 1: no evidence for an effect of *SD* on male mating success

There was no difference between the proportion of successfully mating males carrying either of the *SD* variants and the +/+ male control (figure 1*a*,*b*). Moreover, we found weak evidence that males carrying either *SD-Mad* or *SD-72* had *shorter* mating latencies than the control males (*SD-Mad* odds difference from +/+ males = −0.65, 95% CIs: −1.36 to 0.09, *SD-72* odds difference from +/+ males = −0.49, 95% CIs: −1.22 to 0.24; electronic supplementary material, figure S3), the opposite of predicted if *SD* reduces male attractiveness to females. There was no difference in mating duration between males carrying *SD-72*, *SD-Mad* or the control allele (electronic supplementary material, figure S4).

(b) Experiment 2: *SD* reduces sperm competitive success and female remating propensity

We found strong mating order effects on fertilization success: males of all genotypes (both experimental and competitor males) that mated second sired 6556 of the 7158 offspring (92%) produced by the 227 females. *SD*/+ males exhibited reduced P1 values compared to experimental control males (figure 1*c*,*d*). +/+ control males sired 8.2% (95% CIs: 1–44.4%) of offspring when their mates subsequently mated with an LH_m^{UBI} male. The negative effect of *SD* on fitness was greatest in males carrying a copy of *SD-5* (log-odds mean difference from +/+ males = −2.47, 95% CIs: −4.46 to −0.57) who only sired 0.8% (CIs: 0.1–5.8%) of offspring when mating first. Males heterozygous for *SD-72* and *SD-Mad* appeared to suffer an intermediate reduction in P1, siring 2.2% (CIs: 0.2–17%) and 1.8% of offspring (CIs: 0.2–16.3%). Their P1 estimates did not differ significantly from

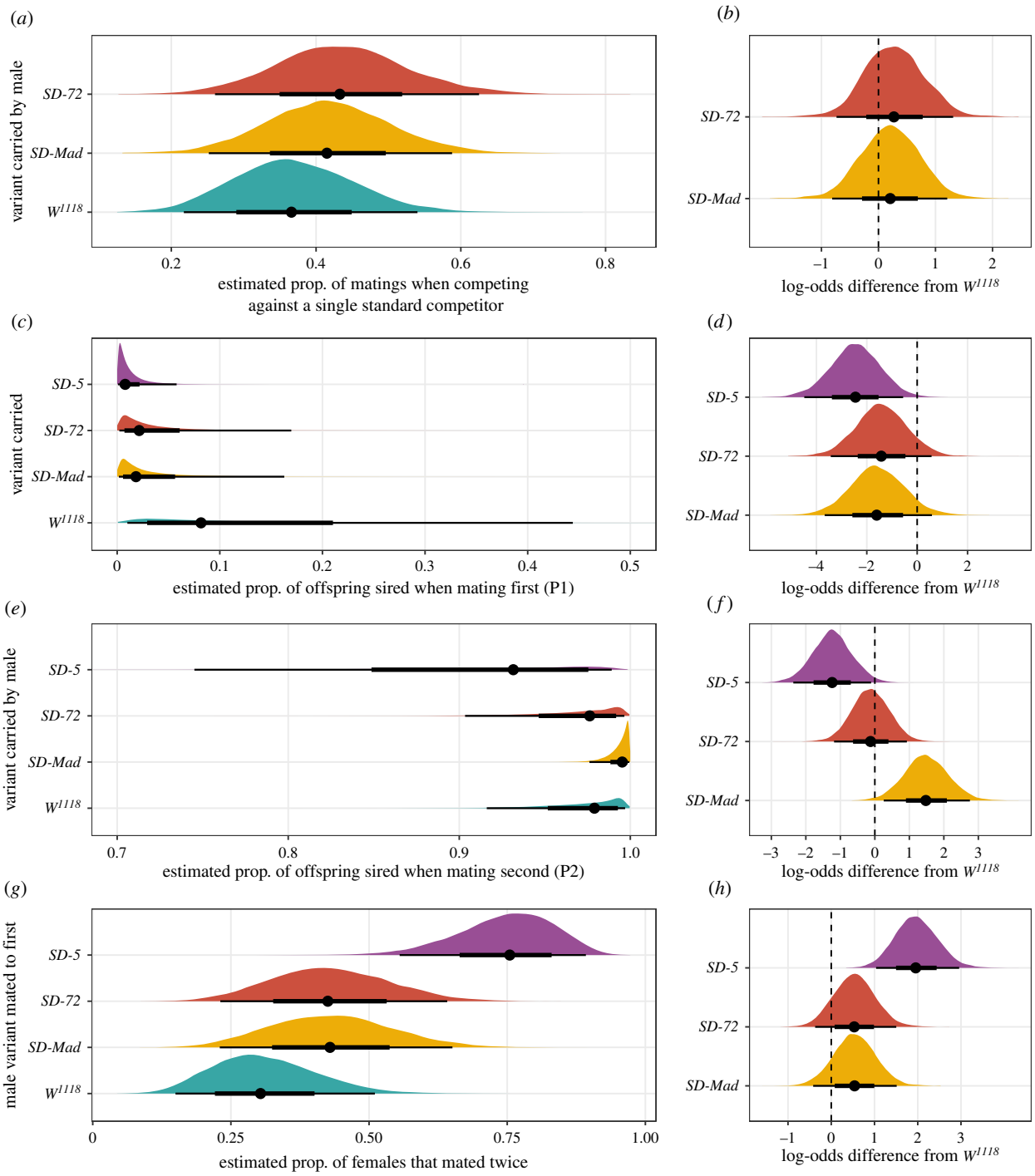

Figure 1. The effect of *SD* on male mating success, fertilization success and female remating propensity. Black points indicate the estimated mean, with associated 66 and 95% uncertainty intervals, while coloured areas show the posterior distribution; (*a*,*c*,*e*,*g*) show results on the response scale, while (*b*,*d*,*f*,*h*) show log-odds differences between the *SD* variants and the control allele; 95% uncertainty intervals that do not overlap zero indicate a significant effect.

+/+ males (*SD-72* log-odds mean difference: −1.42, CIs: −3.45 to 0.59; *SD-Mad*: −1.57, CIs: −3.67 to 0.55; figure 1*d*), though we note that detecting a significant difference between two small proportions requires a very large sample size.

The proportion of offspring sired by a *SD*/+ male when mating second (P2) depended on the variant of *SD* he carried (figure 1*e*,*f*). Males heterozygous for *SD-5* sired 93.2% (CIs: 74.5–98.9%) of the offspring produced by a female that had previously mated with an LH_m^{UBI} male. This was significantly lower P2 than we recorded for +/+ males (CIs: 97.9%, 91.6–99.7%; log-odds mean difference: −1.25, CIs: −2.38 to −0.12). However, males heterozygous for the *SD-Mad* allele sired 99.5% (CIs: 97.6–99.9%) of offspring when mating second, which was significantly higher than the P2 estimated for +/+ males (log-odds mean difference: 1.5; CIs: 0.29 to 2.76). There was no difference between the percentage of offspring sired by males carrying the *SD-72* and the w^{1118} allele when mating second (log-odds mean difference: −0.13; CIs: −1.2 to 0.92; figure 1*f*).

A total of 94 of 196 (48%) females mated a second time, 4 days after initially mating with a *SD*/+ male. The genotype of the female's first mate significantly affected the probability of remating (figure 1*g*,*h*). Specifically, 75.5% (CIs: 55.5–89.2%) of females that originally mated with a *SD-5*/+ male mated again, while only 30.4% (CIs: 15%–51.1%) of females that had originally mated with +/+ males mated again

(odds mean difference: 1.97, CIs: 1.03 to 2.98). There was no difference in the proportion of females remating that had originally mated with males carrying a copy of the *SD-72* (42.5% remating, CIs: 23.1–64.1%), *SD-Mad* (42.9% remating, CIs: 23–65.1%) or control alleles (figure 1*h*). Additionally, females that originally mated with *SD-5*/+ males remated more quickly than females that had mated with +/+ males when presented with an opportunity to remate. The estimated mean remating latency of these females was 58 min (CIs: 37–95 min), about half the estimated mean for those females that originally remated with +/+ males (115 min, CIs: 65–213 min). We found no difference between the remating latencies of females that originally mated with males possessing a copy of the *SD-72*, *SD-Mad* or control allele (electronic supplementary material, figure S5).

There was no variation in mating duration between *SD*/+ and +/+ males when in the first-mating role (electronic supplementary material, figure S6). However, males carrying the *SD-72* allele mated for significantly longer than did +/+ males, when occupying the second mating role (odds mean difference: 0.29, CIs: 0.01 to 0.57; electronic supplementary material, figure S7). We found no difference between the mating durations of males carrying the *SD-5*, *SD-Mad* or control allele when in the second mating role.

(c) Population genetic model

We found many parameter spaces in which *SD* and wild-type alleles coexisted in a balanced polymorphism (figure 2). As in earlier models (e.g. [7,10]), *SD* was unable to drive to fixation because we assumed that it is lethal in homozygous form, which creates negative frequency-dependent selection. At low frequencies, *SD* alleles rarely pay the cost of homozygous lethality, so they increase in frequency due to their within-individual distortion advantage. However, as *SD* becomes more common, *SD*/*SD* zygotes are formed more commonly, which removes *SD* from the population. This opposes the effects of segregation distortion, creating a balanced polymorphism.

Furthermore, we found that both pre- and post-copulatory sexual selection affect the equilibrium frequency of *SD*. Varying the mating success of *SD*/+ males (controlled by the parameter S_{precop}) within the parameter space that equates with our empirical data simply shifts the equilibrium frequency of *SD* (figure 2; the mating success of *SD*/+ males increases as panels move left to right). Put simply, detrimental effects of *SD* from pre-copulatory sexual selection reduce its equilibrium frequency, while benefits increase it. In combination with our empirical findings, the model suggests that pre-copulatory sexual selection against *SD* is not strong enough to explain the rarity of *SD* in natural populations.

Figure 2 shows that post-copulatory sexual selection can stop the *SD* allele from invading when it is also homozygous lethal. When there is strong second male sperm precedence ($P1_{\text{normal}} = 0.1$), as in *Drosophila*, a proportional reduction in P2 for *SD* males matters more to the equilibrium allele frequency of *SD* than a correspondingly large proportional reduction in P1, as shown by figure 2's relatively horizontal isobars (as compared to electronic supplementary material, figure S8). When there is no second male sperm precedence ($P1_{\text{normal}} = 0.5$), costs to P1 and P2 are of equal importance for the equilibrium allele frequency of *SD* (electronic supplementary material, figure S8; note the diagonal isobars). However, when the mates of *SD*/+ males remate more often than the population mean ($p_{SD/+} > p_{+/+}$), *SD*/+ males become increasingly likely to occupy the first-mating role. This has two general effects on the evolutionary outcome. First, with strong second male sperm precedence, the first-mating male sires few offspring, and so *SD* becomes rarer when females mated to *SD*/+ males are more likely to remate; this is true even if we assume that *SD* does not affect a male's success in sperm competition. If there is no second male sperm precedence, the effect of remating likelihood becomes less pronounced (figure 2; electronic supplementary material, figure S8). Secondly, as $p_{SD/+}$ increases, the effect of $P1_{\text{cost}}$ on *SD* frequencies becomes increasingly influential, because *SD*/+ males occupy the first-mating role more often (figure 2; compare the three rows).

To estimate how sexual selection might affect the frequencies of the three *SD* variants we studied, we plotted the points in the sperm competition parameter space where *SD-5*, *SD-72* and *SD-Mad* occupy, based on our estimates from Experiment 2. Figure 2*h* best represents the parameter space relevant to *SD-5*, as $p_{SD/+} = 0.75$ (meaning that females are approximately 2.5 times more likely to remate relative to females that mated with a standard male), and $S_{\text{precop}} = 1$, matching our empirical estimates. Here, the equilibrium frequency for *SD-5* falls below 5%, which is within the range of frequencies that *SD* is found to occur in real-world populations. However, the predicted allele frequencies for *SD-72* and *SD-Mad* fell between 25 and 35% when we observed the parameter space informed by our estimates of $p_{SD/+}$ and mating success for these two genotypes (figure 2*e*,*f*); this frequency is higher than observed in natural populations. This probably reflects the simplifications made by of our model, especially our assumption that *SD*/+ males are equally fit as +/+ males in all other contexts besides pre-copulatory sexual selection and sperm competition, which is probably not correct (see [9]).

4. Discussion

We evaluated whether sexual selection might explain the observed low allele frequencies of the *SD* selfish allele, using experiments and a model. In Experiment 1, we found no evidence that a single copy of *SD* reduces male mating success, suggesting that *SD* is not held at low frequencies by pre-copulatory sexual selection. However, Experiment 2 revealed that males carrying *SD-5* are poor sperm competitors, and that their mates are subsequently more likely to mate again. Using a population genetic model, we found that if these effects on remating and sperm competition are sufficiently large, they can fully explain the rarity in natural populations. However, males carrying the *SD-72* or *SD-Mad* allele do not suffer sexually selected costs of the same sufficient magnitude, and so these costs seem unlikely to fully explain the rarity of *SD* in nature. Overall, our results provide limited empirical support for the hypothesis that post-copulatory sexual selection constrains the spread of *SD*.

We found no support for the hypothesis that male pre-copulatory competitive ability is adversely affected by the distorting genes of *SD* or deleterious mutations found in the *SD* locus. Furthermore, given that mating success is determined both by male–male competition and female choice, our data suggest that females are unable to identify and/or discriminate against *SD*-carrying males, as might be expected given the fitness costs of selecting *SD*-carrying mates [12].

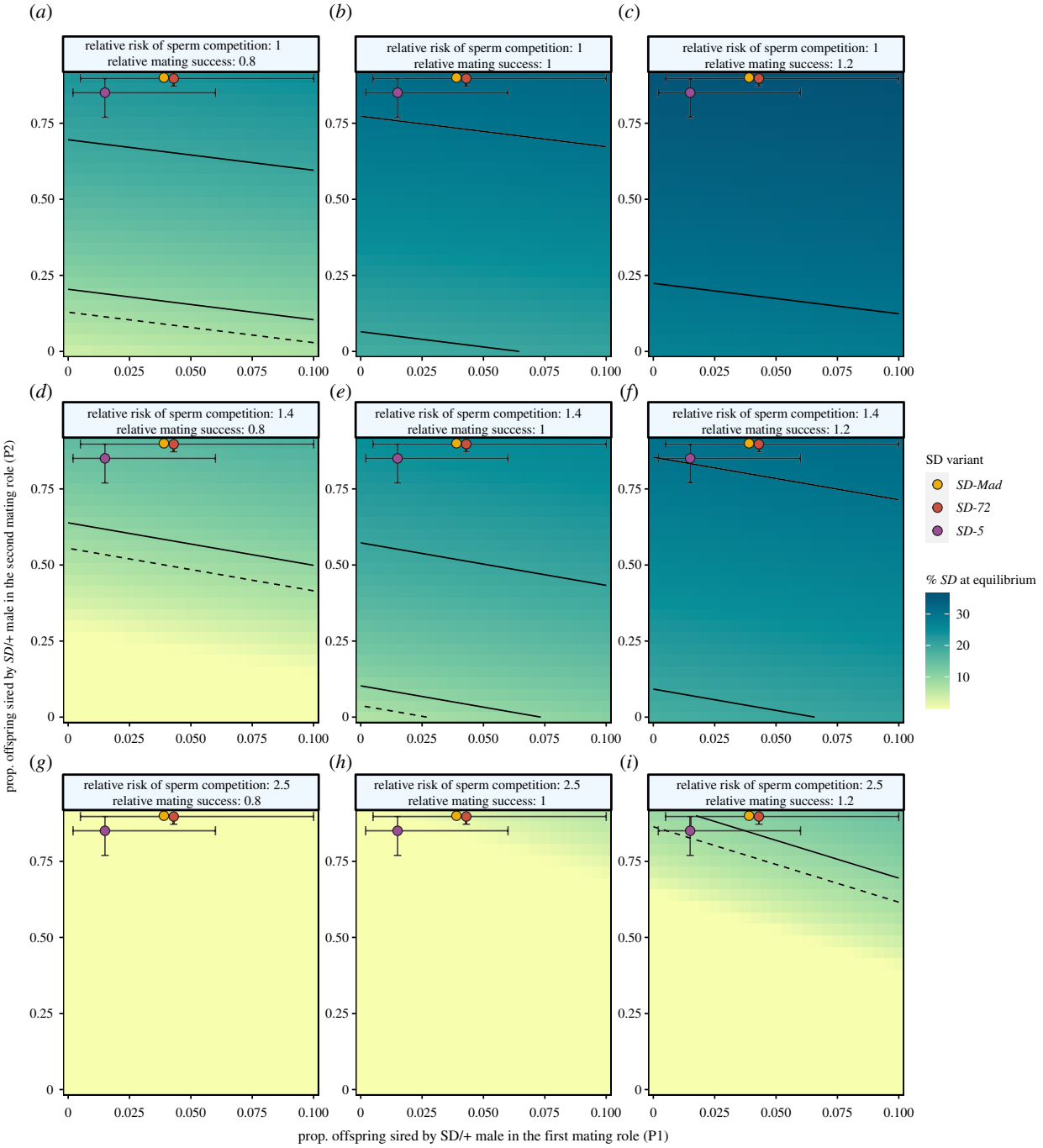

Figure 2. Predicted equilibrium frequency of the *SD* allele, calculated from the population genetic model. The plot depicts the interaction between the P1 and P2 costs suffered by *SD*/+ males in their effects on the equilibrium frequency of *SD* (shown by the colour scale and 10% contour lines). The dashed line shows an equilibrium frequency of 8%, the upper estimate for *SD* in natural populations. *SD*/+ male mating success (S_{precop}) increases across the columns and the risk of sperm competition caused by a female remating after first mating to an *SD*/+ male, $p_{SD/+}$, increases down the rows (values correspond to the risk of sperm competition we estimated in Experiment 2). The three points (with associated 95% credible intervals) in each panel show where males carrying each *SD* variant fall in the figure's parameter space. In the parameter space presented, $k = 0.944$, $P1_{normal} = 0.1$ and *SD* homozygotes are non-viable.

However, as with all other laboratory studies that have measured the effects of segregation distorters on mating success, our experimental design removes the need for males to locate females within a larger landscape. If the mutations hitchhiking within the *SD* complex affect condition, this may reduce the mate-searching ability of males, in which case we may underestimate pre-copulatory sexual selection against *SD* alleles. Nevertheless, our findings align with explicit investigations of male mating success conducted on the other well-known segregation distorters: *SR* elements in other *Drosophila* species [37,38] and the *t* haplotype in mice [39], with one notable exception. Female *Teleopsis dalmanni* stalk-eyed flies have been found to avoid mating with *SR* males [40,41]. In these systems, *SR* is genetically linked to a locus that affects eye-stalk width, a trait that is under sexual selection due to female choice [42]. Here, it appears there are mutations hitchhiking within the *SR* complex that reduce eye-stalk width, causing *SR* males to be disfavoured by females [43]. It is unclear whether this female preference has been strengthened by the indirect fitness benefits of mating with non-*SR* males, or if the female preference has evolved entirely through conventional 'good genes' or 'sexy sons' processes, and *SR* males are coincidentally affected because they carry deleterious mutations.

In Experiment 2, we found some evidence that *SD*/+ males suffer reduced sperm competitive ability. Males carrying *SD-5* sired significantly fewer offspring than +/+ males when competing against the sperm of a rival male, both in the P1 and P2 role. When paired with homozygote lethality and an increased risk of sperm competition (resulting from elevated rates of female remating), our model suggests that the observed sperm competition costs for *SD-5* can explain the low *SD* frequencies found in wild populations. The poor sperm competitive ability of *SD-5* males is consistent with previous work on other segregation distorters [21–25]. Together, these studies suggest that a reduction in sperm number caused by the targeted gamete killing of a segregation distorter has direct individual-level costs to male fitness in polyandrous mating systems [2,19]. Interestingly, while we observe mild reductions in P1 for the *SD-72* and *SD-Mad* male carriers, we observe no costs to P2, and even a small increase in P2 for *SD-Mad*/+ males. Unlike for *SD-5*, our model suggests that the P1 and P2 values observed for these variants are not sufficient to explain the low frequency at which they are found in natural populations. There are several potential explanations for the competitive P1 and P2 values observed for males carrying *SD-72* and *SD-Mad*. First, it is unknown how many sperm are inseminated by *SD*/+ males, and how much variation there is between variants. Males might compensate for the sperm lost to distortion by investing more in spermatogenesis, as demonstrated for stalk-eyed fly populations harbouring *SR* [15]. Under this scenario, *SD* would incur a direct material cost to the male, but not to his sperm competitive ability. It is also possible that while *SD*/+ males suffer a reduced absolute sperm number, they 'strategically allocate' their sperm towards early matings [44]. If true, we might not observe large deficits in sperm competition, as the maximum number of matings for a male in our experiments was two. In Experiment 2, we found that males carrying the *SD-72* allele, but not the *SD-5* or *SD-Mad* alleles, mated for significantly longer in the second mating role than did males carrying non-distorting alleles. This may suggest variation between males carrying different *SD* alleles in ejaculate investment; however, while mating duration is positively correlated with the transfer of accessory seminal proteins in *D. melanogaster* [45], there is no clear relationship between mating duration and sperm transfer [46]. Finally, it is also possible that our control males, which were homozygous at most loci for the w^{1118} genotype, have much lower sperm competitive ability than wild-type males, which would lead to underestimation of the costs of *SD*.

In our model, we show that P1 becomes increasingly important for the evolutionary trajectory of *SD* when *SD*/+ males disproportionately occupy the first-mating role. We also show that this is a particularly plausible scenario, because we observed cryptic female choice (as defined in [47]) against *SD*/+ males: the mates of *SD*/+ males were more likely to remate than females first mated to control males when given the opportunity. Interestingly, even in the absence of sperm competition costs, the ability of males to reduce the risk of subsequent sperm competition remains an important determinant of the *SD* equilibrium frequency. This is probably because by inhibiting a female from remating, a male can avoid losing the majority of any subsequently produced offspring to the second male (approximately 90% in *D. melanogaster* [28,36]). Accordingly, our model confirms that female remating behaviour may be a more important determinant of *SD* frequencies than sperm competitive ability.

In sum, we show for the first time that post-copulatory sexual selection, combined with homozygote lethality, is sufficient to explain the rarity of a particularly costly variant of *SD* in wild populations. However, sexual selection alone seems unable to explain the rarity of the two other *SD* variants studied here, implying that other evolutionary or ecological factors are involved. For example, there may be alleles that confer resistance to segregation distortion [5]. Other sources of selection against *SD* are likely to be important too, such as costs of *SD* to survival, longevity or mate-searching in heterozygotes. Higher-order levels of selection may also play a role, for example, if *SD* reduces the size of a population relative to populations that do not harbour the selfish allele [48]. Future empirical studies could manipulate the strength of sexual selection acting on laboratory populations and test whether this affects the invasion success of the *SD* allele, for example, by manipulating female remating frequency (as in [49]) and/or the opportunity for pre-copulatory sexual selection. There is also scope to further our understanding of how segregation distorters affect population dynamics [2], which incidentally might inform the development of synthetic selfish genetic elements for population control [3].

Data accessibility. All the raw data, supplementary material and R scripts can be found at https://tomkeaney.github.io/SD_sexual_selection/. Raw data are also available from the Dryad Digital Repository: https://doi.org/10.5061/dryad.pzgmsbcmt [50].

Authors' contributions. T.A.K.: conceptualization, data curation, formal analysis, investigation, methodology, visualization, writing-original draft, writing-review and editing; T.M.J.: conceptualization, methodology, resources, supervision, writing-review and editing; L.H.: conceptualization, formal analysis, methodology, resources, supervision, writing-review and editing.

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