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Association of polyandry and *sex-ratio* drive prevalence in natural populations of *Drosophila neotestacea*

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Selfish genetic elements bias their own transmission to the next generation, even at the expense of the fitness of their carrier. *Sex-ratio* (SR) meiotic drive occurs when an X-chromosome causes Y-bearing sperm to die during male spermatogenesis, so that it is passed on to all of the male's offspring, which are all daughters. How SR is maintained as a stable polymorphism in the absence of genetic suppressors of drive is unknown. Here, we investigate the potential for the female remating rate to affect SR dynamics in natural populations, using the fly *Drosophila neotestacea*. In controlled laboratory conditions, females from populations where SR is rare mate more often than females from populations where SR is common. Furthermore, only when males mate multiply does the average fertility of SR males relative to wild-type males decrease to a level that can prevent SR from spreading. Our results suggest that differences in the female mating rate among populations may contribute to SR dynamics in the wild, and thus also affect the outcome of this intragenomic conflict. In line with this, we also present evidence of a localized population crash due to SR that may have resulted from habitat fragmentation along with a reduced mating rate.

1. Introduction

Intragenomic conflict occurs when selection acts in opposing directions on elements within a genome. This type of conflict can be a potent source of evolutionary change and innovation, with consequences for the evolution of sex, recombination, and mating systems (reviewed in [1,2]). An important cause of intragenomic conflict is selfish genetic elements (SGEs), which promote their own transmission into the next generation, and as a result can spread through a population even if they are harmful to their carriers (reviewed in [3]). In this study, we focus on a classic SGE, X-chromosome meiotic drive, which occurs when a driving X-chromosome prevents the maturation of Y-bearing sperm during male spermatogenesis [4]. A male that carries an unsuppressed driving X-chromosome will thus transmit it to all of his offspring, rather than the usual 50%, and all of his offspring will be female. This *sex-ratio* (SR) drive is an especially interesting SGE because a secondary effect of this biased transmission is that it can cause the population-level sex ratio to become female biased in a way that may not be adaptive for the host population. In theory, SR can change the effective population size and growth rate [5] and the direction or strength of sexual selection in a population [6], and if left unchecked, it is expected to drive the host population to extinction owing to a lack of males [7].

SR males are expected to produce half the number of sperm as wild-type males, but if an SR male transfers more sperm to the female than are necessary to fertilize all of her eggs, this may not result in a reduction in the male's fertility. As long as SR males produce at least half the number of offspring as wild-type males, an SR chromosome is expected to increase in frequency in a population as long as there are no other fitness effects [7]. However, in natural populations, SR is often maintained at low frequencies that are stable in both space and time (reviewed in [4]). It is straightforward to account for a stable SR polymorphism when there are suppressors of drive and/or when SR has severe pleiotropic effects on the fitness of female carriers [8–11]. However, we understand much less about how SR can be

maintained as a polymorphism when there are no suppressors of drive present. Because in most species, females cannot discriminate against mating with SR males (but see [12]), in these situations post-mating mechanisms of selection may be key for SR dynamics. For instance, because SR males produce fewer sperm than wild-type males, they may be at a disadvantage in conditions of sperm competition or when males mate frequently [13,14]. This has been demonstrated empirically in several SR systems [15–17].

Multiple mating may allow for a balanced polymorphism of SR through frequency-dependent selection [13,14]. High male mating rates may inhibit the spread of SR: as the rate of male mating increases, SR males may transfer fewer sperm to the female compared to their wild-type counterparts, and they would be expected to have reduced relative fertility. In the absence of any other fitness effects, a balanced polymorphism could occur at the SR frequency where SR males sire half as many offspring as wild-type males [13]. In addition, sperm competition between SR and wild-type males, which is expected to occur when SR is at low-to-moderate frequency in a population, can be sufficient to stabilize an SR polymorphism if the success of SR sperm decreases with an increased male mating rate [14]. Because of these effects, it has been proposed that variation in female multiple mating, or polyandry, may affect the invasion and maintenance of SR and other SGEs (reviewed in [18]). If a female mates multiple times, she is more likely to mate with a non-SR male than if she only mates once, and thus is more likely to produce sons [19]. In addition, increased female mating rates may also intensify sperm competition between SR and wild-type males, further reducing SR male fertility [14,20,21]. Empirical evidence for a potential role of polyandry in SR dynamics has come from experimental evolution in *Drosophila pseudoobscura*, where enforced female multiple mating slowed the rate of extinction due to SR relative to population cages where females were only allowed to mate once [22,23].

Female mating rate varies within and among species and has a genetic component [24,25]. Many factors may affect the evolution of this trait in the wild; for instance, the mating rate may respond to selection from environmental factors, demographic variables, the risk of inbreeding and even the presence of SR drive [6,26–28]. If polyandry is important for SR dynamics, then differences in the multiple mating rate may explain why some species are especially prone to invasion by SR. Furthermore, if the strength and direction of selection pressures on the mating rate also vary among populations within a species, differences in the local level of polyandry may contribute to the among-population variation in SR prevalence that is seen in several systems (reviewed in [4]). A first step towards understanding the role of polyandry for SR dynamics in natural populations is to ask whether natural populations that differ in SR prevalence also vary in the level of polyandry.

Here, we investigate multiple mating in natural populations of the fly *Drosophila neotestacea*. This fly is a non-cosmopolitan, mushroom-feeding species that inhabits temperate and boreal forests across North America. It exhibits a stable cline in SR frequency, ranging from 0 to 30% across populations [29,30]. There is no evidence for any active genetic suppressors or pleiotropic effects of drive in females [29,31,32]. Because levels of gene flow across the species range are moderate to high, natural selection probably maintains the geographical distribution of SR drive

Table 1. Summary of populations used in this study. (*Sex-ratio* (SR) frequency is based on SR-linked microsatellites, with 95% confidence intervals (CIs) calculated using a binomial sampling distribution. The population sex ratio is calculated as the proportion of females out of the total wild flies collected, with 95% CIs calculated using a binomial sampling distribution. Significance from 50:50 was determined using a χ^2 -test.) * $p < 0.05$, ** $p < 0.001$.

population location	SR frequency ($\pm 95\%$ CI)	population sex ratio ($\pm 95\%$ CI)
Edmonton, AB	0.06 (0.03–0.10)	0.51 (0.46–0.56)
Seattle, WA	0.47 (0.40–0.54)	0.91 (0.86–0.95)**
Coeur d'Alene, ID	0.15 (0.08–0.25)	0.45 (0.35–0.54)
Missoula, MT	0.20 (0.11–0.34)	0.72 (0.56–0.85)*
Portland, OR	0.50 (0.41–0.59)	0.77 (0.69–0.83)**

prevalence [29]. The selective force(s) that maintains this cline in SR frequency remains unknown.

In this study, we test for the potential of polyandry to contribute to the maintenance of the cline in SR in *D. neotestacea*. First, we ask whether the fertility of SR males is decreased relative to wild-type males, indicating whether fertility selection against SR males can occur in *D. neotestacea*. Second, we use flies derived from five different natural populations to test for the presence of genetic variation in female polyandry. Finally, we ask whether the variation in polyandry we observe in the laboratory is correlated with the local prevalence of SR. We hypothesize that populations with a higher rate of polyandry would have stronger fertility selection against SR males, and thus a lower prevalence of SR.

2. Material and methods

(a) Fly collections, fly maintenance and *sex-ratio* prevalence

We collected wild adult *D. neotestacea* in 2010 and/or 2011 near Edmonton, Alberta (AB), Coeur d'Alene, Idaho (ID), Missoula, Montana (MT), Portland, Oregon (OR) and Seattle, Washington (WA) (table 1 and the electronic supplementary material, table S1). We created isofemale lines from wild-caught females, and maintained lines for at least seven generations before using them in mating experiments. We maintained cultures on instant *Drosophila* medium (Carolina Biological Supply) with a piece of commercial mushroom (*Agaricus bisporus*) on a 14 L:10 D cycle at 20°C. Virgins were collected within 24 h of emergence using light CO₂, housed 10–15 flies per vial and were 5–10 days old when initially used in an experiment. Air aspiration was used during all mating assays, which commenced within 1 h of the incubator lights turning on.

To estimate the SR frequency in each population, we genotyped wild-caught flies at two X-linked microsatellite markers that are in linkage with SR drive [29]. Based on previous data, Dn8377 and Dn8385 (accession nos. EF199832 and EF199836, respectively) together have 94% accuracy in assigning an X-chromosome as SR or standard (ST) based on different size fragments. Methods for microsatellite genotyping and fragment analysis were as described previously [31]. Estimates of SR prevalence from OR and WA were also obtained by mating wild-caught males or F₁ sons of wild-caught females to

laboratory females; those that produced greater than or equal to 10 offspring, of which were greater than or equal to 90% females, were considered to carry SR. We tested for an association of SR prevalence and population sex ratio using a linear regression, weighted by the number of flies collected. Unless otherwise noted, statistical analyses were performed in JMP v. 10 (SAS Institute, Cary, NC, USA).

(b) Effect of sex-ratio on male fertility

To assess SR male fertility relative to ST, wild-type males, we used laboratory stocks from Rochester NY established in the early 1990s by J. Jaenike, which maintains the SR and ST X-chromosomes on the same genetic background (for crossing scheme see [29]). We paired a single 7-day-old virgin SR or ST male with a single virgin wild-type (ST/ST) female for 1 h, and then transferred each male that mated to a vial that contained 10 additional virgin wild-type (ST/ST) females. After 24 h, we discarded the male from each vial and allowed the females to oviposit individually in food vials, transferring each female to fresh food after 5 days and discarding them after 10 days. We counted the offspring produced from each first and subsequent mating from 24 SR and 28 ST males, and used Wilcoxon rank sum tests to compare the number of offspring produced by SR and ST males.

(c) Variation in female polyandry

To assess variation in female remating behaviour across different locations, we used five isofemale lines from WA; six isofemale lines each from AB, ID and MT, and nine isofemale lines from OR, which several generations earlier had been combined together to make three stocks each comprised three isofemale lines. The lines we used did not carry SR, which we verified by scoring offspring sex ratios of a sample of males. On day 1 of the assay, we combined one virgin female with 10 virgin males from the same population. The line identity of the female was recorded, and the males used were chosen randomly from the lines within each population. We checked each vial every 5 min and recorded the number of times each female mated. In *D. neotestacea*, copulations last about 15 min. After 12 h, we aspirated out each female that successfully mated and allowed each to recover alone overnight. Females that did not mate on the first day were discarded and not included in any analyses. The following morning, we placed each of the mated females with 10 new males from the same population, and recorded whether each female remated within 2 h.

First, we investigated differences among populations in the total number of female copulations on day 1 only and across both mating days. We used a Wilcoxon rank sums test and the Steel–Dwass method for multiple comparisons [33,34], as we were unable to transform the data to normality. Second, we tested for differences among populations in the proportion of females that remated on the second day of the assay, using a contingency analysis with a likelihood ratio test, and an analysis of means for proportions. Finally, we asked whether there was an association between the level of female polyandry and the population prevalence of SR. We performed Spearman rank correlations between the frequency of SR and the different measures of polyandry, including the number of copulations and proportion of females that remated on the second day. We determined significance using statistical tables for small sample sizes.

(d) Genetic differentiation among populations

To test whether the variation we observe in polyandry may be due to the effects of drift rather than local selection, we surveyed the level of population variation and differentiation at five autosomal microsatellite loci. We genotyped wild-caught individuals from

each of the five populations at Neo6003, Neo6429, Neo7013, Neo8380 and Neo8394 from Dyer [35] using methods described previously [29]. We tested for the presence of null alleles, linkage disequilibrium between pairs of loci, and departures from Hardy–Weinberg equilibrium in GENEPOP v. 4.0.10 [36]. We calculated allele richness, observed and expected heterozygosity, and measures of population differentiation (F_{ST} and R_{ST}) using ARLEQUIN v. 3.5 [37], with significance determined from 1000 permutations. To infer the number of genetic clusters (K), we used STRUCTURE v. 2.3 [38]. We used a model that assumed no admixture and correlated allele frequencies, used the collecting location as a prior, and ran the program five times at each $K = 1$ through to $K = 5$, with a burnin of 150 000 steps and a run length of 200 000 steps. We determined the most probable value of K using the highest log-likelihood of the posterior probability of the data across values of K [38] and also via the ΔK method of Evanno *et al.* [39].

3. Results

(a) Sex-ratio prevalence and population-level sex ratio

Using SR-linked microsatellite loci, we estimated the SR frequency to range between 4 and 50% across the five populations (table 1 and electronic supplementary material, table S1). The prevalence of SR at the three locations that were sampled previously, AB, ID and MT (0.06, 0.15 and 0.20, respectively), were similar to the frequencies found in the 2001–2002 collections that were based on offspring sex ratio of wild-caught males (0, 0.12 and 0.18, respectively [29]). The OR and WA populations were not sampled previously, and both estimates of SR prevalence (0.50 and 0.47, respectively) are higher than ever observed in *D. neotestacea* [29,30]. These estimates are consistent with the SR frequencies we obtained using the offspring sex ratio from wild-caught flies, suggesting that they are not artefacts (OR:SR = 0.56, 95% CI 0.30–0.80, $n = 16$; WA:SR = 0.47, 95% CI 0.33–0.60, $n = 58$; electronic supplementary material, figure S1).

Of the five populations, three had a significantly female-biased population-level sex ratio (table 1 and electronic supplementary material, table S1). While caution should be used interpreting these estimates, as females and males may be attracted at different rates to baits, we find in this sample that higher SR prevalence is associated with a more female-biased population-level sex ratio ($r^2 = 0.83$, $F_{1,3} = 14.90$, $p = 0.03$; electronic supplementary material, figure S2). We note that the observed population sex ratio of the WA population is significantly higher than expected based on its SR frequency: with a SR frequency of 0.47 (95% CI 0.40–0.54), the expected population SR is 73.5% female (95% CI 70–77%), the bounds of which are lower than the 95% CIs of the observed population sex ratio (91% female, 95% CI 86–95%). The observed population-level sex ratio of the other four populations is within the expected range given the sample size and observed SR frequency.

(b) Effect of sex ratio on male fertility

Overall, SR males produce significantly fewer offspring than ST males. From the initial mating, SR males produced 64% as many offspring as ST males (Wilcoxon rank sum test $\chi^2_1 = 4.7$, $p = 0.030$; figure 1). This fertility effect is magnified upon repeated matings of the male: summed over the 10 subsequent females, SR males produced about 30% as many offspring as ST males (Wilcoxon rank sum test $\chi^2_1 = 13.1$,

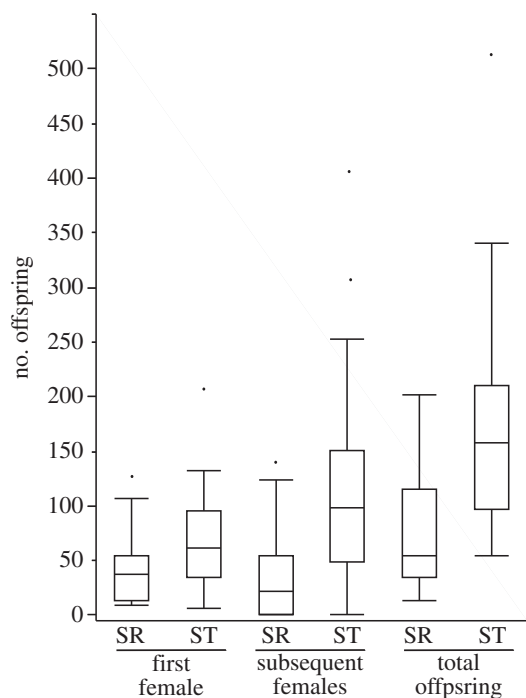


Figure 1. Fertility of sex-ratio (SR) ($n = 24$) and standard (ST) ($n = 28$) males. Shown is a box plot of the total number of offspring produced from the first and subsequent matings and summed over all the females each male had the opportunity to mate with. The box indicates the quartiles, with the median depicted as the horizontal line within each box.

$p = 0.0003$; figure 1). This is owing to a lower number of females that produced offspring (SR: 1.3 ± 0.3 , ST: 2.6 ± 0.2 (mean \pm s.e.); Wilcoxon rank sum test $\chi^2_1 = 11$, $p = 0.0009$). Because the number of offspring produced by each of these females is not significantly reduced for SR males, this suggests that SR males run out of sperm faster than ST males (SR: 28.0 ± 5.8 , ST: 41.5 ± 3.7 ; Wilcoxon rank sum test $\chi^2_1 = 2.1$, $p = 0.15$). Summed over all 11 potential mates, SR males produced 44% as many offspring as ST males (Wilcoxon rank sum test $\chi^2_1 = 16.2$, $p < 0.0001$; figure 1).

Using these fertility estimates, we estimate that after 2.8 matings in a 25 h period using the mean number of offspring and after 2.2 matings using the median number of offspring the relative fertility of SR males is expected to drop below 50% of ST males. Thus, if the only aspect of host biology that is affected by SR is male fertility, we expect that SR could invade a population if males mated only once per day, but would not invade a population where males mate more than two to three times per day. These estimates assume SR and ST males are equally likely to obtain copulations and also do not consider the consequences of sperm competition.

(c) Variation in female remating behaviour

First, we find evidence for variation among populations in polyandry. We find no significant effects within populations on mating rate due to a line or day effect, so therefore we combine data across lines and days for all analyses (Wilcoxon rank sum tests all $p > 0.05$ using a Bonferroni correction). There is significant variation among populations in the number of copulations on the first mating day ($\chi^2_4 = 21.10$, $p = 0.0003$; electronic supplementary material, figure S3a). Using the Steel–Dwass method of multiple comparisons, there are

significant differences between two geographically overlapping groups (AB, ID) and (WA, ID, MT, OR). There is also significant variation in the total number of copulations a female engaged in over the 2 days of the mating assay ($\chi^2_4 = 30.07$, $p < 0.0001$; electronic supplementary material, figure S3b), with significant differences between populations falling into three overlapping groups (AB, ID), (WA, ID, MT) and (WA, MT, OR). Results of an analysis of variance were consistent, though the data violated the assumption of normality (results not shown). There is also significant variation among populations in the fraction of females that remated on the second day ($\chi^2 = 11.2$, $p = 0.0249$; electronic supplementary material, figure S4), with fewer females from OR remating relative to the other populations ($p < 0.05$).

Second, the prevalence of SR correlates with estimates of female polyandry. Females from populations with a lower SR prevalence tended to mate more often than those from higher prevalence populations, when considering either the number of matings on the first assay day only ($\rho_3 = -0.9$, $p < 0.05$; electronic supplementary material, figure S5) or combined across both days of the assay ($\rho_3 = -1.0$, $p < 0.01$; figure 2a). We also find that females from populations with a lower SR prevalence were more likely to remate on the second day ($\rho_3 = -1.0$, $p < 0.01$; figure 2b). Summed together, these results indicate that higher levels of polyandry are associated with lower levels of SR prevalence in natural populations of *D. neotestacea*.

(d) Genetic differentiation among populations

Consistent with previous studies in *D. neotestacea* [29,31], we find substantial levels of genetic diversity within populations and high gene flow among the populations we surveyed in this study. No pairs of loci showed evidence for a signature of linkage disequilibrium (all $p > 0.05$), and none showed consistent evidence for an excess of homozygotes or inbreeding within populations (all $p > 0.05$; electronic supplementary material, table S4). Across populations there is no correlation of either the average observed heterozygosity or the average number of alleles per locus with geographical distance, the prevalence of SR or the population-level sex ratio (all $p > 0.3$).

None of the pairwise estimates of genetic differentiation among populations were significantly greater than zero (F_{ST} and R_{ST} all $p > 0.05$; electronic supplementary material, table S3). Using the program STRUCTURE, the most probable number of genetic clusters (K) was $K = 1$ using the highest averaged likelihood across runs (average $\ln L = -2704.32$), and $K = 3$ using the ΔK method of Evanno *et al.* [39] (average $\ln L = -2715.32$; electronic supplementary material, table S4). For $K = 3$, there is no assignment probability of a population to a cluster of more than 0.6 (see the electronic supplementary material, table S5), and this weak clustering is also evident in the assignment of individuals by population into the genetic clusters (see the electronic supplementary material, figure S6). Concordantly, if the locations are not used as prior information, none of the populations had clear support for any cluster assignment. Thus, consistent with the results of a previous study in *D. neotestacea* [29], which included some of the same populations but used samples collected 10 years earlier, there appears to be moderate to high levels of gene flow among populations, indicating differences in polyandry are probably owing to selection rather than drift.

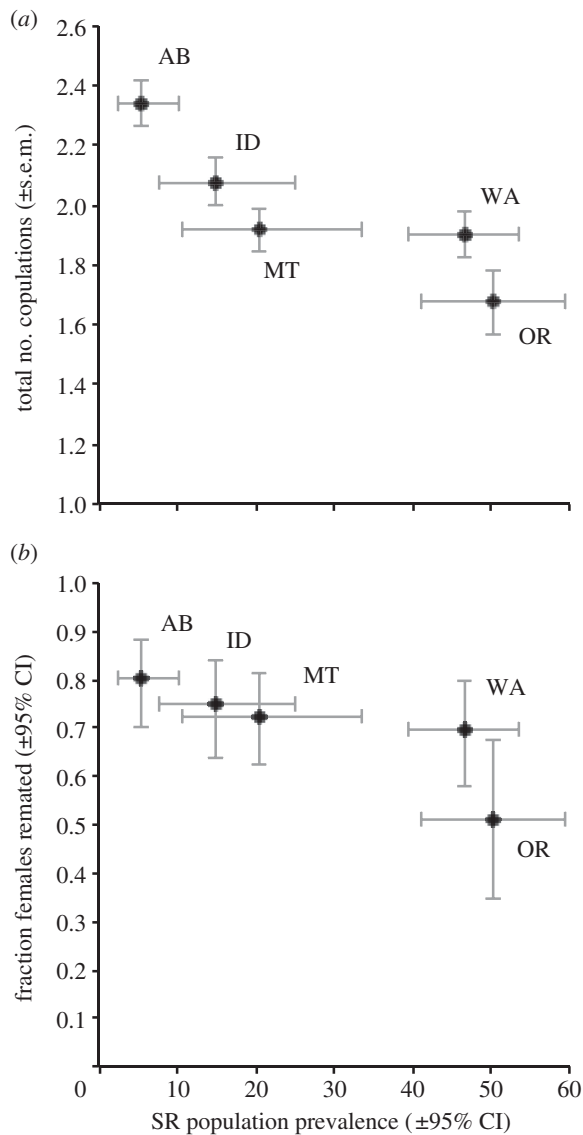


Figure 2. Association of *sex-ratio* (SR) prevalence in natural populations with the rate of female polyandry. Shown is the relationship between SR prevalence and (a) the mean number of female copulations over the 2 days of the mating assay, and (b) the fraction of females that remated on the second day. Population abbreviations are adjacent to the respective data point. Standard errors (s.e.m.) are indicated for copulation number and the 95% CIs, calculated with a binomial distribution, are indicated for SR prevalence and fraction remated.

4. Discussion

The consequences of SGEs for host genome evolution and reproductive biology are significant [3]. For an SGE such as X-chromosome drive, males produce fewer sperm overall as a result of the mechanism to bias transmission, which can make them vulnerable to reduced fertility and sperm competition under conditions of multiple mating. Thus, polyandry has been proposed as a mechanism to counteract the spread of SR (reviewed in [4,18]). *Drosophila neotestacea* is an ideal system to test this in the wild, because populations exhibit stable differences in SR prevalence, and there are no confounding factors such as suppressors of drive or known pleiotropic effects on female fitness [29,31,32]. Importantly, *D. neotestacea* females mate multiply—in this study, females mated up to four times in one day in the laboratory, and others have noted that *D. neotestacea* females also remate readily in the wild (J. Jaenike 2013, personal communication). This sets the

stage for fertility selection against SR males, and for sperm competition between SR and ST males to occur.

(a) Can multiple mating prevent the invasion of *sex ratio*?

When there are no pleiotropic fitness effects on survival or female fecundity, SR is expected to increase in frequency in a population when SR males produce at least half as many offspring as wild-type males [7]. In *D. neotestacea*, we estimate that the relative fertility of SR males drops below 50% of ST males after two to three matings in a 25 h period, which means that if males mate at least this much SR may not be able to invade a population. These relative fertility estimates are similar to other mushroom-feeding *Drosophila* species that harbour SR [13]. However, to determine whether multiple mating is important to SR dynamics in the wild, we must consider not only the relative fertility of SR males but also the amount that females actually mate. To take an extreme example, if it takes 10 matings in a day for the relative fertility of SR males to fall below 50% of ST males, but females only ever mate once a day, then it is unlikely fertility selection due to multiple mating plays a substantial role in SR dynamics.

In our assays, populations varied in how many times females mated per day, with average values between 1.6 and 2.4 copulations in a 26 h period (figure 2). The upper range, for example as is seen in the AB population, overlaps with number of times males must mate for fertility selection to prevent the spread of SR. At the lower end of our observed polyandry values, for example in OR and WA, this is not likely to be the case. Sperm competition in *D. neotestacea* has not been investigated, but may further intensify fertility selection against SR. Severely reduced fertility of SR males in conditions of sperm competition have been shown to occur in several other SR systems, for example *Drosophila simulans*, *D. pseudoobscura* and stalk-eyed flies [15,40,41]. In addition, even in populations where females mate less often, the spread of SR may be inhibited once the population sex ratio is female biased enough such all males mate several times even if females do not [13].

(b) Can polyandry maintain a cline in *sex-ratio* prevalence?

Our data support the scenario hypothesized by others and demonstrated in laboratory cage populations, where low levels of polyandry allows SR to increase in frequency, and higher polyandry may reduce SR frequency or prevent its invasion due to fertility selection and sperm competition against SR males [13,22,23]. *Drosophila pseudoobscura* also exhibits a similar cline in SR frequency; in this system, there are also differences in polyandry among natural populations of *D. pseudoobscura*, such that higher polyandry populations also have lower SR prevalence (T. Price 2013, personal communication). Additionally, a similar situation has also been inferred to explain lower than expected frequencies of the *t*-haplotype segregation distortion system in the mouse [42]. By contrast, this pattern does not seem to be the case in stalk-eyed flies, but owing to segregating suppressors the SR dynamics may be much more complicated in this system [43]. In theory, in the absence of suppressors or pleiotropic effects on female fitness, multiple mating and sperm competition alone can maintain a stable equilibrium of SR in a population, though the conditions are very restrictive

[14]. This also assumes that populations are isolated entities, with SR dynamics operating independently within each population to maintain each at a stable equilibrium. However, populations of *D. neotestacea* show high levels of gene flow, which should homogenize the prevalence of SR among populations. Because we see substantial differences in SR prevalence across populations that appear to be stable over time, we suggest that the cline in SR frequency is more likely maintained by migration–selection balance via local differences in female mating rate. In populations such as AB, female mating rate is high, which may result in strong selection against SR alleles and the observed low frequency of SR. However, where remating is lower, such as in OR, there may be less of a fertility disadvantage for SR males, which may allow SR to spread to a higher prevalence. The constant migration of SR and/or ST alleles into a population may prevent it from ever fixing or eliminating SR. Thus, fertility selection against SR alleles within populations appears to be balancing the high rates of gene flow among populations.

(c) Why might polyandry vary among populations?

We found that polyandry differs among natural populations under laboratory conditions and in the absence of SR, which suggests that there is a genetic basis to this variation. There are several reasons why selection may favour different levels of multiple mating among populations. First, polyandrous behaviour may evolve as a mechanism for inbreeding avoidance, for example, by increasing the diversity of the offspring when there is a cost to inbreeding [44]. While this could occur in some species, we do not think this is the case in *D. neotestacea*. In our study, the mixed isofemale lines from OR may be less inbred than the isofemale lines from the other populations and we do find the lowest level of multiple mating in this population; however, if we exclude the OR population we still find substantial variation in polyandry among the remaining populations. Furthermore, *D. neotestacea* harbours substantial genetic diversity (this study, [29,31]) and does not show evidence for recent inbreeding, as no population had an excess of homozygotes or showed evidence of reduced genetic diversity as SR prevalence increased. Finally, in nature we do not expect a high risk for sibling-mating for this species, as a mushroom host is decomposed by the time that flies emerge from it, and thus each generation of flies must find a new mushroom to mate and oviposit on.

Second, the presence of SR in a population may select for more promiscuous female mating behaviour. For instance, in *D. pseudoobscura* population cages, the rate of polyandry increased in the presence of SR relative to controls, suggesting that SR selected for increased polyandry [26]. Furthermore, a study in stalk-eyed flies found that a species with SR drive had a higher rate of multiple mating than a closely related species without drive, although there was no association of SR prevalence and mating rate among populations within species [43]. If the presence of SR selects for increased polyandry, we would expect that populations with higher polyandry would also have higher SR prevalence. However, this is the opposite pattern to what we observe in *D. neotestacea*, as well as to what is found in *D. pseudoobscura* (this study; T. Price 2013, personal communication). Furthermore, if there is a cost to female polyandry, as is seen in many species [45–47], we predict that populations would fluctuate in SR frequency through time. This is because once higher mating rates

eliminated SR there would be no selective force to maintain the increased polyandry, and thus it would return to lower levels that would allow the re-invasion of SR. However, in many systems, including *D. neotestacea* and *D. pseudoobscura*, the frequencies of SR in natural populations have been remarkably stable through time. Thus, we suggest that selection due to SR probably does not explain the variation in female multiple mating in *D. neotestacea*. It would be useful to quantify empirically the costs associated with female multiple mating in *D. neotestacea* and other species with SR drive as well as to model theoretically how fast the SR dynamics would be expected to change under this scenario. This could also shed light on the more general question of the conditions that permit polyandry to be favoured specifically to combat SGEs [18].

Finally, and we argue most likely, the selective agent may be an environmental or demographic variable that differs among populations but which may not be directly related to the presence of SR. Environmental factors may vary among populations and cause the female mating rate to evolve in the wild; for example, it is conceivable that the local population density, resource availability, day length and temperature could each affect the strength of selection for multiple mating [48–50]. The SR cline in *D. neotestacea* correlates with local temperature, and there are many environmental factors related to temperature that could affect selection on mating rate. If other species that are sympatric with *D. neotestacea* but that do not harbour SR show a similar geographical pattern in polyandry this may indicate that selection is a result of the local environment and is not species specific. In addition, these variables, as well as the presence of SR, may affect aspects of the local mating system such as the operational sex ratio and the intensity of sexual selection and sexual conflict, which may alter local selection on the female mating rate (reviewed in [27,51]). At this point, we can only hypothesize about why the mating rate varies among populations, and it is clear that much work remains to determine what causes differential selection on the female mating rate in this and other species. Comparative studies will also be useful to determine whether certain ecological or demographic variables or aspects of the mating system are common among species that harbour SR versus those that do not.

(d) A potential extinction event due to *sex-ratio*

Prior to this study, SR chromosomes in *D. neotestacea* had been found at a maximum of about 30% across the geographical range in North America and have been stable for at least 20 years [29]. This is comparable to SR clines in *D. pseudoobscura* and *D. subobscura*, in which SR frequencies also range between 0 and 30% [52–54]. Two of the sites we sampled in 2010, OR and WA, harboured an extremely high prevalence of SR, between 40 and 50%, which is higher than that has ever been observed in any species where SR drive is unsuppressed (reviewed in [4]). These SR frequencies may not be typical for this region, as 10 years prior in other locations in Oregon and in British Columbia the SR prevalence was approximately 25–30% [29]. Both of these populations also had highly female-biased population sex ratios and the lowest rates of polyandry in our assays (figure 2 and table 1). Interestingly, it appears that the WA population crashed the year after we collected there. In 2010, we collected 380 *Drosophila*, including 175 *D. neotestacea*, of which 91% were female (see the electronic supplementary material, table S1). We returned to the same

site in 2011 at the same time of year and for the same length of time, and out of 249 *Drosophila*, only three were *D. neotestacea*, all of which were female. Of these three flies only one was inseminated, and all carried SR, one of which was an SR/SR homozygote. This is a strikingly smaller proportion than the year before (2010, 46.1%; 2011, 1.2%; Fisher's exact test, $p < 0.0001$), and may indicate the occurrence of a localized SR-induced extinction. To our knowledge, this is the first time an SR drive-induced population crash may have been witnessed in a natural population.

Both the WA and OR sites are large parks surrounded by an expanse of urban development, whereas the other populations we have sampled in this and other studies were from more continuously forested areas. Based on our population genetic analyses, the WA and OR populations do not appear to be highly differentiated from the other populations in this study, nor do they harbour a lower level of genetic diversity. However, if gene flow has been suppressed only recently, these signatures may not be evident yet. We suggest that a reduced rate of immigration along with a low remating rate may have been sufficient to permit SR to drive the WA population to extinction. In support of this, we note that WA and MT had similar rates of polyandry, but there was a large difference in SR prevalence between these populations (figure 2 and table 1). While this is a limited sample, it highlights the potential for habitat fragmentation to affect the population dynamics of SGEs. It also suggests that SR drive and other SGEs may persist for longer periods of time in species with high levels of gene flow among populations [55].

5. Conclusion

There is still much debate as to the proximate and ultimate causes of multiple mating [27,56]. Recently, multiple mating has been proposed as a force to protect a host against SGEs [18], and here we show that higher polyandry is associated

with lower SR prevalence in natural populations. We do not find evidence, however, that multiple mating has evolved in response to the presence of the SGE. Instead, we suggest it is more likely that the mating rate is affected by local ecological or demographic factors, which vary among populations. However, the selective forces that underlie the observed differences in polyandry remain unknown, and await further study. Nevertheless, the rates of multiple mating we observe are right in the range of plausible values that can prevent or permit SR from spreading in a population, and further theoretical investigations will determine whether selection from polyandry alone can explain the differences we observe in SR prevalence in nature. Furthermore, models can be extended to ask if, and how, multiple mating affects SR drive dynamics when populations are polymorphic for suppressors of drive, as is seen in many SR systems. It will also be of interest to use paternity studies of wild-caught females to infer whether the patterns of polyandry we find in wild-derived flies occur in the wild, which has found to be the case in other *Drosophila* [57]. In summary, we suggest that the interaction between host ecology, mating system, and SGEs has the potential to affect both the population dynamics on an ecological scale as well as the long-term evolution of SGEs. This may have consequences not only for the prevalence of SGEs within a species, but also for the distribution of SGEs across a broader diversity of taxa.

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Data accessibility. Data available from the Dryad Digital Repository: <http://dx.doi.org/10.5061/dryad.g8d5h>.

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