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# LOCAL SELECTION UNDERLIES THE GEOGRAPHIC DISTRIBUTION OF SEX-RATIO DRIVE IN *DROSOPHILA NEOTESTACEA*

**Kelly A. Dyer**[1,2]

[1]*Department of Genetics, University of Georgia, Athens, Georgia 30602*

[2]*E-mail: kdyer@uga.edu*

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**"Selfish" genetic elements promote their own transmission to the next generation, often at a cost to the host individual. A *sex-ratio* (SR) driving X chromosome prevents the maturation of Y-bearing sperm, and as a result is transmitted to 100% of the offspring, all of which are female. Because the spread of a SR chromosome can result in a female-biased population sex ratio, the ecological and evolutionary consequences of harboring this selfish element can be severe. In this study, we show that the prevalence of SR drive in *Drosophila neotestacea* varies between 0% and 30% among populations, and is common in the south whereas rare in the north. The prevalence of SR is not associated with the presence of suppressors of drive, geographic distance, or genetic distance based on autosomal microsatellite loci. Instead, our results indicate that ecological selection on SR drive varies among populations, as the prevalence of SR is highly correlated with climatic factors, with the severity of winter the best determinant of SR frequency. Thus, ecological and demographic factors may have significant consequences for the short and long term evolutionary dynamics of selfish elements and the manner with which they coevolve with the rest of the genome.**

**KEY WORDS:** **Genetic conflict, meiotic drive, X chromosome drive.**

Some genes violate the fundamental genetic law of equal segregation to increase their own transmission to the next generation (Werren et al. 1988). When a heterogametic male carries an X chromosome that harbors such a driving gene, it causes the Y-bearing sperm fail to develop and thus the male sires only daughters (reviewed in Jaenike 2001). Theory predicts that if this selfish *sex-ratio* (SR) element goes unchecked by selection, it will spread rapidly through a population (Hamilton 1967). The consequence of harboring a sex ratio distorting element can be severe for the host population, and may include changes in the strength and direction of sexual selection (Emlen and Orin 1977), the population growth rate, and the effective population size (Charlesworth and Hartl 1978). If the population becomes severely female biased, a sex ratio distorting element may even cause extinction of the host population due to a lack of males (Hamilton 1967; Hatcher et al. 1999).

In addition to these population-level effects, the presence of a sex ratio distorter can create intense conflict among elements of the host genome over the offspring sex ratio (reviewed in Burt and Trivers 2006). Because males have a higher mean fitness when a population is nonadaptively female biased, selection favors autosomal and Y-linked suppressors of X chromosome drive that restore the production of sons (Hamilton 1967). The driving X, in turn, may evolve modifying alleles that restore drive, and the following intragenomic dynamics may resemble a coevolutionary "arms race," with subsequent periods of drive polymorphism and apparent loss as the drive system accumulates modifiers and suppressors (Hurst et al. 1996; Jaenike 1999). Eventually, the drive system may become genetically complicated, with the accumulation of multiple X-linked driving genes and autosomal and/or Y suppressors of drive. Recent work in autosomal and sex ratio drive systems, as well in systems where the sex ratio distorting element

occurs in the cytoplasm, has shown that the rate of this genomic conflict can be rapid (e.g., Charlat et al. 2007; Presgraves et al. 2009; Bastide et al. 2011).

In many populations that harbor SR drive, the frequency of SR appears to be maintained at a stable polymorphism. Three mechanisms have been proposed to explain what might counteract the strong transmission bias of a SR chromosome (reviewed in Carvalho and Vaz 1999; Jaenike 2001). First, balancing selection due to linkage of SR with deleterious mutations can occur: if multiple genes are necessary for the expression of drive, and inversions that maintain linkage among these genes capture linked recessive alleles, these linked mutations may decrease fecundity and/or viability in both male and homozygous female carriers of SR (Edwards 1961; Curtsinger and Feldman 1980). Second, if a suppressor is present and both a SR gene (or linked factors) and the suppressor bears a cost to fitness, an equilibrium may occur such that a population is polymorphic for both SR drive and the suppressor of drive (Clark 1987; Carvalho et al. 1997; Jaenike 1999; Hall 2004). Third, there may be deleterious effects of SR itself on the fitness of the carriers. Most notably, due to the death of the Y-bearing sperm, SR males produce half as many sperm as wild-type Standard (ST) males, and in conditions of frequent mating SR males may transfer fewer sperm and thus sire fewer offspring than Standard males (Jaenike 1996; Jaenike 2001). Although a combination of mechanisms may contribute to maintaining stability, a polymorphism for SR drive can be maintained with only sperm competition operating, which may be especially important when a driving gene first arises or in drive systems where there are no segregating suppressors of drive (Taylor and Jaenike 2002, 2003). Related to this, selection for increased female remating rate has also been suggested as a mechanism to combat the spread of SR drive, because females that mate randomly with more males increase their chances of producing sons (Haig and Bergstrom 1995; Zeh and Zeh 1996, 1997; Price and Wedell 2008).

Although individual populations may have a relatively stable frequency of SR, differences among populations in the frequency of SR drivers and/or suppressors suggest that the interaction between selection and gene flow can also affect the evolutionary trajectory of a drive system. For example, if there is severely restricted gene flow among populations, the evolutionary dynamics of drive may occur at the level of the host population, with each population evolving an independent solution to a common problem, or with each representing a different snapshot in the time course of the coevolutionary dynamics between SR and the rest of the genome. On the other hand, selection on the drive system may be so strong that the coevolutionary dynamics occur at the host metapopulation or species level. For example, this could occur if there are differences in local selection among populations due to ecological or demographic factors that vary among populations, even if gene flow otherwise homogenizes the genetic background.

When a SR distorter first appears in a population, its initial fate is likely to be affected by ecological and demographic forces that facilitate or inhibit its spread. This is because selection on the genome to resist a SR chromosome will only occur once the distorter has succeeded in invading the population (Hamilton 1967). In this study, I investigate the geographic structure of the SR drive system of *Drosophila neotestacea*, a species with a drive system that is thought to be recently evolved relative to other species with drive (James and Jaenike 1990; Jaenike 2001). James and Jaenike (1990) characterized SR drive in populations of *D. neotestacea* from the eastern United States, and found that ~20–30% of males express SR and that the population-level SR was ~65% female. They did not find any chromosomal inversions associated with SR, and they also found no suppressors of drive in these populations (James and Jaenike 1990; James 1992). In addition, James (1992) identified no pleiotropic effects of drive on males or females, except for a decrease in male fertility that arises with repeated copulations.

*Drosophila neotestacea* has among the broadest distributions of the noncosmopolitan *Drosophila* species in North America, and is the only species of mushroom-feeding *Drosophila* found in both the eastern and western portions of the continent. Thus, the portion of the range where James and Jaenike (1990) focused their study represents only a small part of the total geographic range where this species occurs. In this study, I first expand the work of James and Jaenike (1990) to survey SR in natural populations that span the geographic range of *D. neotestacea*. I show that in the southern part of the range SR is relatively common, whereas in northern populations it is nearly absent. I then analyze three factors that may underlie this geographic distribution: suppressors specific to these northern populations, limited gene flow among populations, or an ecological condition which may prevent the establishment of SR in the north. In sum, my results indicate that local selection against carriers of SR drive determines the ability of a driving X to invade a population, with local temperature (or something related to it) the most predictive factor for the frequency of SR. Thus, even though they enjoy a large transmission advantage, variation in local ecological factors can affect the dynamics of selfish elements and the manner with which they coevolve with the rest of the genome.

## Materials and Methods

### SAMPLES AND FREQUENCY OF SR

*Drosophila neotestacea* is in the testacea group in the subgenus Drosophila (Grimaldi et al. 1992), and the geographic range of *D. neotestacea* includes the temperate and boreal forests across North America. In the southeast, the range extends along the Appalachian Mountains south to the Smokies, and in the west it is found from Alaska south through Oregon. Flies were collected from the 16 populations listed in Table 1 during the months of June

**Table 1.** Populations of *D. neotestacea* used in this study, including summary statistics of population sex ratio and frequency of *sex-ratio* as well as sample sizes for these and for microsatellite genotyping. Populations are listed by latitude from north to south.

| Abr. | Site | Date | Latitude | Longitude | Wild-caught flies | Population sex ratio (±95% CI)[1,2] | Assayed for *sex-ratio*[3] | *Sex-ratio* frequency (±95% CI)[1] | Assayed at microsats |
|---|---|---|---|---|---|---|---|---|---|
| AB1 | Winston Churchill, AB | 2002 | 54.82 | −111.98 | 668 | 0.50 (0.46–0.54) | 111 | 0.01 (0– 0.05) | 30 |
| MB | The Pas, MB | 2002 | 53.96 | −101.10 | 104 | 0.74 (0.65–0.82)*** | 59 | 0 (0–0.06) | 30 |
| AB2 | Edmonton, AB | 2002 | 53.51 | −113.54 | 110 | 0.59 (0.49, 0.68) | 27 | 0 (0–0.13) | 30 |
| AB3 | Jasper, AB | 2002 | 52.84 | −118.07 | 385 | 0.60 (0.55–0.65)*** | 77 | 0.05 (0.01–0.13) | 31 |
| BC | Vancouver, BC | 2001 | 49.33 | −122.97 | 215 | 0.58 (0.51–0.65)* | 126 | 0.19 (0.13–0.27) | 34 |
| ON | Dogfly Lake, ON | 2001 | 49.10 | −93.12 | 32 | 0.56 (0.38–0.74) | 0 | na | 20 |
| MT1 | Columbia Falls, MT | 2002 | 48.46 | −113.98 | 157 | 0.61 (0.52–0.68)** | 48 | 0.19 (0.09–0.33) | 30 |
| ND | Minot, ND | 2002 | 48.24 | −101.36 | 80 | 0.40 (0.29–0.52) | 29 | 0.07 (0.01–0.23) | 30 |
| ID | Coeur d'Alene ID | 2001 | 47.61 | −116.67 | 297 | 0.55 (0.49–0.61) | 184 | 0.12 (0.07–0.18) | 36 |
| MN | Bemidji, MN | 2002 | 47.42 | −94.70 | 139 | 0.56 (0.48–0.65) | 52 | 0.25 (0.14–0.39) | 30 |
| MT2 | St. Regis, MT | 2001 | 47.30 | −115.10 | 325 | 0.46 (0.41–0.52) | 201 | 0.18 (0.13–0.24) | 41 |
| PEI | Charlottetown, PEI | 2002 | 46.25 | −63.17 | 26 | 0.62 (0.38–0.81) | 8 | 0 (0–0.37) | 23 |
| OR | MacKenzie Bridge, OR | 2001 | 44.18 | −122.16 | 311 | 0.51 (0.46–0.57) | 172 | 0.26 (0.20–0.33) | 41 |
| NY | Rochester, NY | 2001 | 43.10 | −77.65 | 140 | 0.57 (0.49–0.65) | 133 | 0.23 (0.16–0.31) | 30 |
| TN1 | Gatlinburg, TN | 2001 | 35.68 | −83.50 | 125 | 0.59 (0.50–0.68)* | 68 | 0.25 (0.15–0.37) | 16 |
| TN2 | Clingmans Dome, TN | 2001 | 35.60 | −83.44 | 190 | 0.59 (0.52–0.66)* | 95 | 0.12 (0.06–0.20) | 16 |
| All | Total | | | | 3267 | 0.55 (0.53–0.57)*** | 1390 | 0.16 (0.14– 0.18) | 468 |

[1] Confidence intervals (CI) calculated using a binomial sampling distribution.

[2] Significance from 50:50 using a $\chi^2$ test, with *, **, and *** to indicate $P < 0.05$, $P < 0.01$, and $P < 0.001$, respectively.

[3] Includes only males that sired ≥20 offspring.

and July by sweep-netting over baits of decaying mushrooms or by collecting flies off naturally occurring mushrooms. These populations span most of the known range of *D. neotestacea*; the only part of the range not represented here is northern British Columbia and Alaska. Flies were kept alive from all but the ON population until they were returned to the laboratory. In the laboratory, I established 20 isofemale lines from each of five populations (NY, TN2, OR, BC, and AB2). From each of these isofemale lines, I also created an iso-Y chromosome line, with each started with one wild-type ST male and three virgin females. This resulted in a total of 100 lines, each with a single and potentially different Y chromosome and genetic background. All cultures and crosses were maintained at 21°C on Instant Drosophila Medium (Carolina Biological Supply, Burlington, NC) supplemented with commercial mushroom (*Agaricus bisporus*).

To test for SR expression, I crossed individual wild-caught males or single sons of wild-caught females to 2–3 virgin females each, and scored the offspring sex ratio. For this and all experiments that test the expression of SR, I used virgin ST/ST females that were homozygous for an autosomal recessive bright red eye mutation (*red*) and maintained in a large outbred laboratory population with a genetic background from Rochester, New York. Note that SR is expressed during male spermatogenesis, and thus its phenotype is expected to depend only on the genotype of the male, not the female he mates with. A male was characterized as SR if he sired at least 20 offspring, of which at least 90% were female.

## LOCAL COEVOLUTION OF THE SR SYSTEM AND TEST FOR SUPPRESSORS OF DRIVE

To test for coevolution between SR and the rest of the genome, I paired SR and Y chromosomes from sympatric and allopatric populations, and asked if an SR chromosome drives allopatric Y chromosomes as well as Y chromosomes from their own population. I focused on five geographically distinct populations (NY, TN2, OR, BC, and AB2). From each first four populations, I extracted a naturally occurring SR chromosome into pure laboratory culture (Fig. S1), taking care to maintain the genetic background of each SR chromosome with the geographic location from which it originated. Eventually, SR is straightforward to maintain in the laboratory, and a large number of SR males and SR/SR females can be produced.

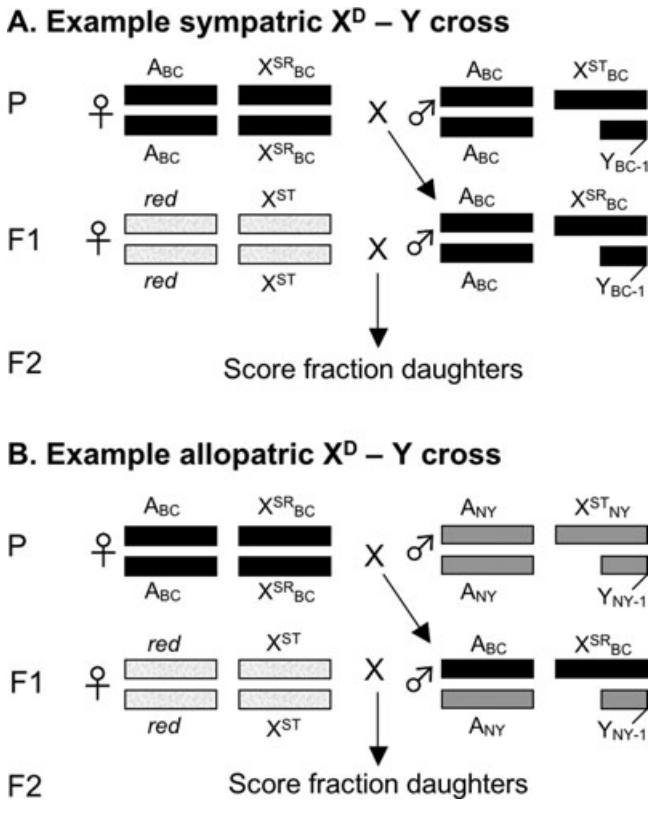


**Figure 1. Crossing scheme to test geographic interactions between *sex-ratio* and Y chromosomes. Shown is an example of (A) sympatric and (B) allopatric crosses. The *sex-ratio* chromosome is indicated as SR, the wild-type Standard is ST, and one autosome is shown as an example, indicated by an A. The populations used in this example, BC and NY, are indicated with the subscript letters.**

To place each SR chromosome into sympatric and allopatric genetic backgrounds, I crossed ST males from each of 17–20 iso-Y lines from the NY, TN2, OR, and BC populations to SR/SR females from of these same populations. One example of each sympatric and allopatric cross is shown in Figure 1. The Y chromosome from each iso-Y chromosome line was tested against one sympatric and three allopatric SR chromosomes, resulting in a total of four SR X chromosomes $\times$ 74 Y chromosomes $=$ 296 crosses. The sons of the 74 sympatric crosses will carry an SR X chromosome, Y chromosome, and 100% of their autosomes from the population they originate, and the sons of the 222 allopatric crosses will carry an SR X chromosome from the maternal population, a Y chromosome from the paternal population, and 50% of their autosomes from each parental population. I also crossed males from the iso-Y lines from the AB2 population to SR/SR females from the NY population.

The presence of a resistant Y chromosome or dominant suppressor of drive will be evident by the production of fertile sons; note that this experimental design will not detect autosomal recessive suppressors. From each cross, I tested at least three F1 sons for SR expression. Only males that sired at least 20 offspring were included in the analyses. The fraction of females among the offspring was square root arcsine transformed, and analyzed using an analysis of covariance as a function of Y chromosome population, individual Y chromosome within population, SR X chromosome population, the interaction of SR and the Y chromosome population, and the interaction of SR population and Y chromosome within Y population. All statistics were completed in JMP version 8.0 (SAS Institute, Cary, NC).

## POPULATION DIFFERENTIATION AT NEUTRAL LOCI

I surveyed variation and population differentiation at autosomal microsatellite loci in all 16 populations in Table 1. I extracted DNA from 16–41 individuals from each population using Qiagen's Puregene kit (Valencia, CA), including roughly equal number of males and females and without regard to SR status. I genotyped each individual at seven autosomal microsatellite loci, which included Neo5270, Neo6003, Neo6428, Neo6429, Neo7013, Neo8380, and Neo8394 from Dyer (2007). Markers were amplified in multiplex with one primer of each locus labeled on the 5′ end with a fluorescent tag, and run concurrent with size standards. See Dyer (2007) for polymerase chain reaction primers and reaction conditions. Genemarker (SoftGenetics, State College, PA) was used for fragment size analyses. Genotypes are deposited in Dryad (doi:10.5061/dryad.sp4hq3rt).

I tested for the presence of null alleles, linkage disequilibrium between pairs of loci, and departures from Hardy–Weinberg equilibrium (HWE) as implemented in Genepop version 4.0.10 (Raymond and Rousset 1995; Rousset 2008). I calculated allele richness, observed and expected heterozygosity, and measures of global and pairwise population differentiation ($F_{ST}$ and $R_{ST}$) for individual loci and across all loci using Arlequin version 3.5 (Excoffier and Lischer 2010). Significance for $F_{ST}$ and $R_{ST}$ was determined with 1000 permutations. To infer the number of genetic clusters ($K$) in the data, I used the program Structure version 2.3 (Pritchard et al. 2000). Based on preliminary analyses, I used a model that assumed no admixture and correlated allele frequencies, used the collecting location as a prior, and I ran the program five times at each value of $K$ (from one to 16), with a burnin of 150,000 steps and a run length of 150,000 steps. I determined the most likely value of $K$ using both the highest log-likehood of the posterior probability of the data [Pr(X|$K$)] across values of $K$ (Pritchard et al. 2000), and also via $\Delta K$, which analyzes the second-order rate of change in ln[Pr(X|$K$)] with respect to $K$ (Evanno et al. 2005).

## GEOGRAPHIC ANALYSIS OF SR

I investigated the relationship between genetic differentiation and geographic proximity among sites using Mantel and partial Mantel tests (Mantel 1967). SR was treated as a single locus,

and estimates of pairwise $F_{ST}$ between populations were obtained using the observed proportions weighted by sample size, as implemented in Arlequin version 3.5 (Excoffier and Lischer 2010). The geographic distance between pairs of populations was determined using the latitude/longitude population coordinates, and pairwise global $R_{ST}$ from the microsatellites as described above. $F_{ST}$ and $R_{ST}$ were adjusted to Slatkin's linearization of $F_{ST}/(1 - F_{ST})$ (likewise for $R_{ST}$), and significance calculated based on 10,000 matrix randomizations (Slatkin 1995). I completed Mantel tests using all sampled populations as well as excluding the TN populations to test for effects specific to the main part of the geographic range.

### ASSOCIATION OF ECOLOGICAL VARIABLES WITH SR

To investigate ecological correlates with SR, I obtained climate records from the weather station closest to each collecting site. Data from sites in the United States were from the National Climatic Data Center (http://www.ncdc.noaa.gov), and data for sites in Canada were from the National Climate Archive (http://climate.weatheroffice.gc.ca). I gathered 30-year means from the period from 1971 to 2000, including annual and monthly mean, minimum, and maximum temperatures, monthly and total annual precipitation, and the monthly and annual cooling and heating degree days (CDD and HDD, base 18°C). Based on preliminary analyses, precipitation had little affect on SR frequency, so I included only measures of temperature in the subsequent analyses. I used the mean temperature ($T_{mean}$) from each month to determine the first and second principle components of temperature using eigenvalue decomposition, and I used the first principle component ($T_{PC1}$) as an index of temperature. I then used linear regression models to analyze the correlation of SR frequency and temperature. Correlations with SR frequency were weighted by the sample size of X chromosomes surveyed for drive.

As a complement to the PCA, I used logistic regression models to investigate which temperature-related variables best explained the observed SR frequency in each population (as in McAllister et al. 2008). Whereas the linear models used in the PCA analysis above consider the population frequency of SR, a logistic model treats each observation independently. I used a logistic regression with only the intercept as the base model, and asked which environmental variables improved the fit of the model to the data, using the difference in log likelihoods. These analyses were completed using JMP version 8.0 (SAS Institute).

## Results

### FREQUENCY OF SR

The number of flies collected from each population and the proportion female are listed in Table 1. There is significant heterogeneity in the population-level sex ratio among populations ($\chi^2 = 24.35$, $P < 0.0001$), and six of the 15 populations had a significant female bias (Table 1). However, because males and females may be attracted at different rates to baits, these numbers may not be a true representation of the actual population-level sex ratio, and caution should be used in their interpretation. From these populations, I surveyed a total of 1390 X chromosomes for SR expression, with a mean of 93 and a range between 8 and 201 chromosomes per population (Table 1). An additional 407 males were surveyed but did not produce at least 20 offspring, and thus are not included. Across all populations, the offspring sex ratio shows a clear bimodal distribution, with one group of males siring roughly a 50:50 offspring sex ratio (Standard) and the other siring close to 100% daughters (SR) (Fig. 2). There was a clear distinction between these groups, as no male produced between 82% and 90% daughters. The overall frequency of SR was 15.5% (95% CI 13.7–17.6%). Among all males, the mean fraction of daughters was 0.614 ± 0.005, among the 1174 standard males, the mean fraction of daughters was 0.545 ± 0.002, and among the 216 SR males, the mean fraction of daughters was 0.993 ± 0.001.

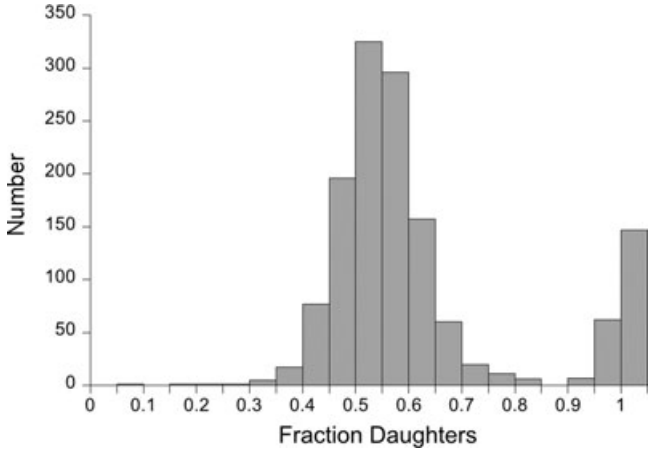


**Figure 2. Distribution of offspring sex ratio of X chromosomes from wild-caught *D. neotestacea*. This figure includes all 1390 X chromosomes from this study that produced at least 20 offspring.**

There is significant heterogeneity among populations in the frequency of SR ($\chi^2 = 97.55$, $P < 0.0001$; Fig. 3, Fig. S2, and Table 1). There is no correlation between the population-level sex ratio and the frequency of SR ($r^2 = 0.028$; $F_{1,13} = 0.37$, $P = 0.55$). As can be seen in Figure 3, the most northern populations appear to harbor the lowest frequency of SR chromosomes. There is a significant positive correlation between latitude and frequency of SR, with prevalence decreasing as latitude increases ($r^2 = 0.357$; $F_{1,13} = 7.22$, $P = 0.019$; Fig. 4A). When the southernmost TN populations are excluded so that only the main part of the geographic range is considered, this correlation becomes extremely strong ($r^2 = 0.779$; $F_{1,11} = 38.74$, $P < 0.0001$; Fig. 4A). There

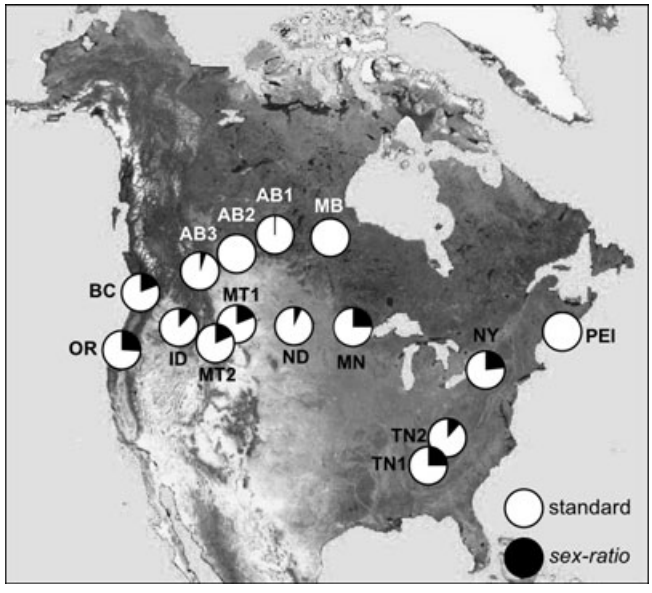


**Figure 3.** Distribution of *sex-ratio* drive in sampled populations. Filled portions of each circle indicate the proportion of sampled X chromosomes that carry *sex-ratio*, with the abbreviation of each population shown.

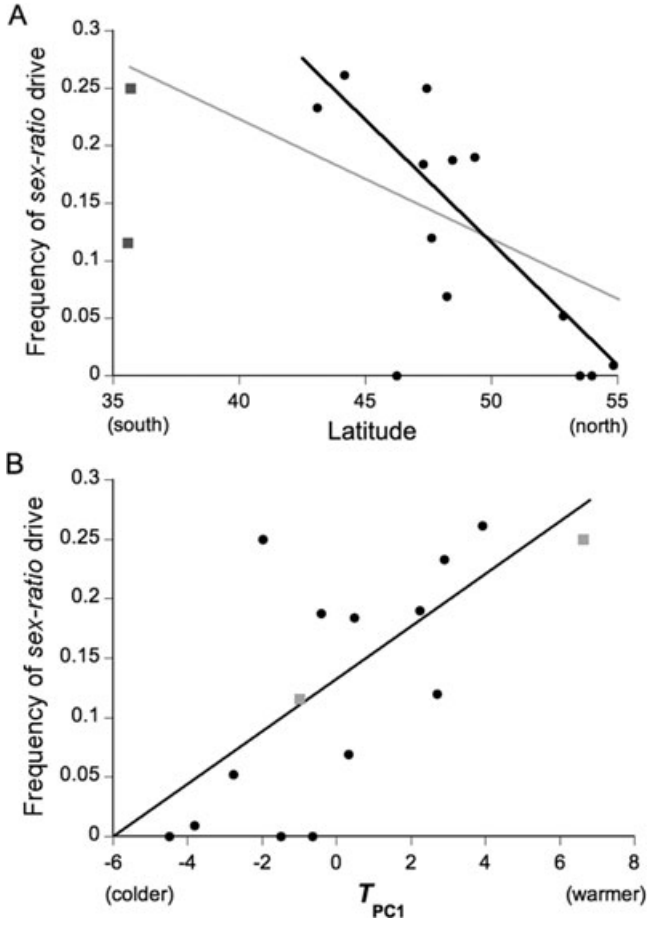


**Figure 4.** Correlation of *sex-ratio* frequency with (A) latitude and (B) temperature index, $T_{PC1}$. In graph A, the gray line is the regression for all populations ($r^2 = 0.357$; $F_{1,13} = 7.22$, $P = 0.019$), and the black line is excluding the two TN populations ($r^2 = 0.779$; $F_{1,11} = 38.74$, $P < 0.0001$; see text for details). In graph B, the line is for all populations ($r^2 = 0.45$; $F_{1,13} = 11.5$, $P = 0.0046$). In both graphs, the TN populations are shown by gray squares.

is no correlation of SR frequency with longitude ($r^2 = 0.007$; $F_{1,13} = 0.09$, $P = 0.76$), indicating that the frequency of SR does not vary in an east–west pattern.

## LOCAL COEVOLUTION OF THE SR SYSTEM AND TEST FOR SUPPRESSORS OF DRIVE

Combinations of sympatric SR X chromosomes and Y chromosomes produced an average fraction of daughters of 0.9887 ± 0.0016, and allopatric combinations of SR and Y chromosomes produced an average offspring SR of 0.9891 ± 0.0008. The fraction of daughters produced by allopatric and sympatric crosses is not significantly different ($F_{1,294} = 0.021$, $P = 0.88$; Fig. 5). Nevertheless, there is significant variation in offspring sex ratio among all SR–Y combinations ($F_{295,1083} = 1.649, P < 0.0001$). As Table S1 shows, the only significant contribution to this variation is the interaction of SR X chromosomes and individual Y chromosomes ($F = 1.481$, $P < 0.0001$), which indicates that the variation among Y chromosomes is concentrated within rather than distributed among populations. To test whether any of the rare sons were fertile, I placed a random selection of 129 F2 males individually with three virgin females each, and found that none produced offspring, indicating these males are likely XO and a consequence of a lack of a sex chromosome in either the sperm or the egg. Thus, given the overall high rate of daughters and the lack of fertile sons, this variation among Y chromosomes is likely due to variation in the rate of aneuploidy. Finally, from the crosses designed to test for suppressors in the northern populations, I found that all 20 Y chromosomes from the AB2 population were completely susceptible to drive (mean fraction daughters = 0.990 ± 0.002). This indicates that the low frequency of SR is not due to local fixation of dominant or Y-linked resistance factors. In sum, this experiment indicates a lack of coevolution between SR and the genetic background, as well as the absence of segregating dominant or Y-linked suppressors of drive.

## POPULATION DIFFERENTIATION AT NEUTRAL LOCI

To investigate the role of gene flow in shaping the SR drive system, I surveyed the level of population differentiation at loci that are not associated with drive. In total, I genotyped 468 individuals (mean = 29 individuals/population) at each of seven autosomal loci (Table 1). The number of alleles per locus per population ranged between 5 and 22, observed heterozygosity between 0.11 and 1.0, and expected heterozygosity between 0.46 and 0.95

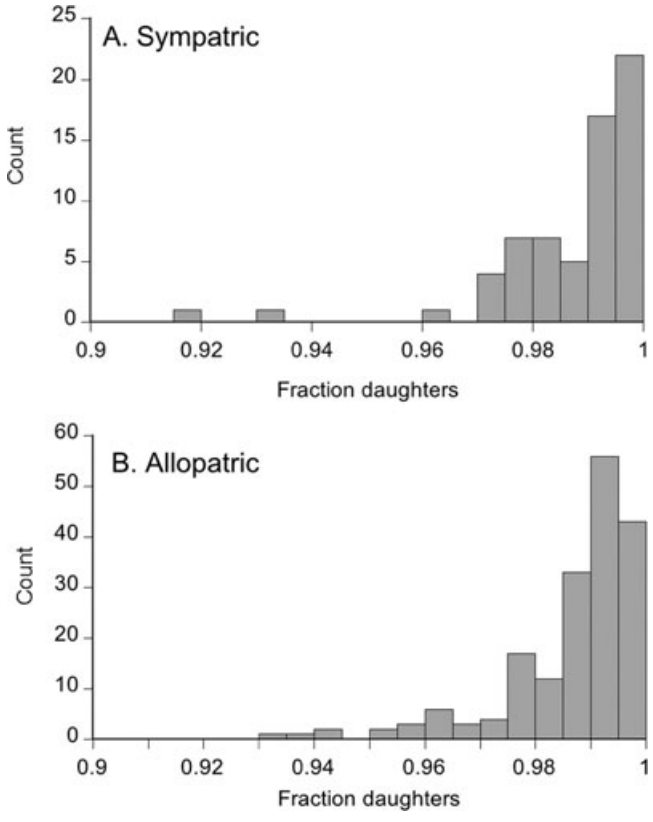


**Figure 5. Offspring sex ratio of (A) sympatric and (B) allopatric crosses. The mean offspring sex ratio of at least three males is shown for each SR–Y combination.**

(Table S2). Across all populations, two loci showed evidence of linkage disequilibrium (Neo5270 and Neo6428), although this is likely due to effects of population structure as only two of the 16 populations (BC and MT1) showed this signature. All other pairs of loci showed no signature of linkage disequilibrium ($P > 0.4$). In the tests of HWE, no locus in any population showed any evidence for an excess of heterozygotes. However, two loci–Neo5270 and Neo6428–showed strong evidence of heterozygote deficiency ($P < 0.001$) within at least 13 of the 16 populations (Table S2). The most common cause of heterozygote deficiency in microsatellite loci is null alleles, and indeed these are the only two loci with evidence of null alleles (mean rate of null alleles: Neo5270 = 0.31 and Neo6428 = 0.19). Thus, I excluded these two loci (Neo5270 and Neo6428) from the analyses of population structure.

The global estimates across 16 populations at the five microsatellites are $F_{ST} = 0.013$ ($P < 0.00001$) and $R_{ST} = 0.008$ ($P = 0.065$). Pairwise estimates of $F_{ST}$ and $R_{ST}$ averaged across the five microsatellites ranged from 0 to 0.044 and 0 to 0.079, respectively (Table S3). Of the 120 pairwise estimates of differentiation among populations, for $F_{ST}$, 39 pairs of populations were significantly greater from zero at $P < 0.001$, and for $R_{ST}$, one pair was significantly greater from zero at $P < 0.001$ (Table S3). TN2 appeared to be the most differentiated from other populations, with 10 of 15 pairwise $F_{ST}$ values significant at $P < 0.001$. In analyses of individual loci, for $F_{ST}$, two loci (Neo6429 and Neo7013) showed significant differentiation across all populations, and for $R_{ST}$, one locus (Neo8380) showed significant differentiation across all populations. Across every locus-specific and global analyses of differentiation, >98% of the variation was contained within populations rather than among populations.

Using the program Structure, the most probable number of genetic clusters ($K$) was three (averaged lnL = −10,595), with the averaged lnL values and the $\Delta K$ for each value of $K$ shown in Table S4. Assignment of individuals to genetic clusters is shown in Figure 6. There is one main genetic cluster that contains much of the range of the species, including most of the eastern and central North America populations, and two smaller clusters, one with populations in Idaho and Montana on the Eastern side of the Coastal Mountain Range, and one that contains two of the most distant populations–PEI and OR–although support for the assignment of OR is the weakest of all population assignments (Table S5). This genetic clustering is subtle, as the values of $F_{ST}$ values calculated by the program are small (<0.1). I also note that if the locations are not used as prior information, the clustering pattern is much less clear, and the only cluster with clear support (i.e., population assignment >0.5 to a cluster) is the one with the ID and MT populations (results not shown). I asked whether this pattern of population clustering was supported using an Analysis of Molecular Variance, as implemented in Arlequin, by placing each population into the group that it had the highest proportion of membership in the Structure analysis. I found that 0.19% of the variation was distributed among the three groups ($F_{CT} = 0.0019$; $P = 0.28$ based on 1000 permutations), and that >99% of the genetic variation was contained within populations. Thus, both the estimates of population differentiation and the Structure analyses show a moderate-to-high level of gene flow among populations, with most of the variation contained within populations.

## GEOGRAPHIC ANALYSIS OF SR

To test formally for a correlation between genetic and geographic distance, I used Mantel and partial Mantel tests between SR frequency, genetic distance based on five autosomal loci, and geographic distance. For the dataset that includes the 15 populations where SR was sampled, there was no association between genetic and geographic distance ($r = -0.11$, $P = 0.75$), of SR frequency and geographic distance ($r = -0.05$, $P = 0.68$), or SR frequency and genetic distance ($r = 0.17$, $P = 0.10$). Partial Mantel tests explained 3.3% of the differentiation in SR frequency, with genetic and geographic distance accounting for 3% and 0.3% of the variation, respectively. When I repeated this analyses excluding the two TN populations, which are geographic outliers, I found no significant positive association between genetic and

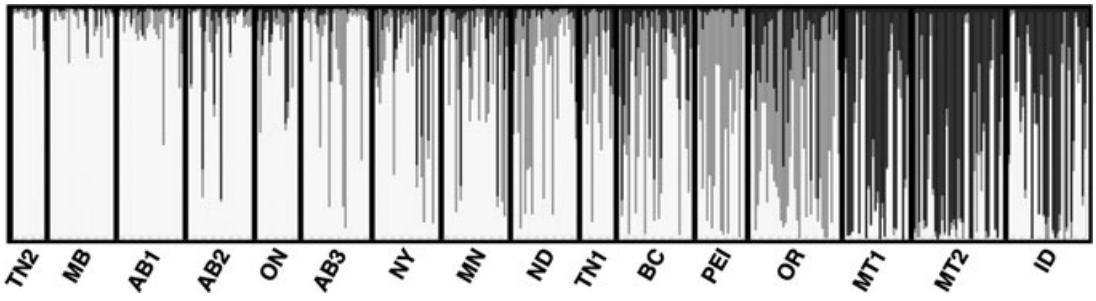


**Figure 6.** Results of Structure analysis with $K = 3$. Individuals are grouped by population, with the proportional composition of each individual from each cluster in white, gray, or dark gray. Populations are sorted by genetic cluster, using the abbreviations in Table 1.

geographic distance ($r = -0.21$, $P = 0.93$) or SR frequency and geographic distance ($r = -0.09$, $P = 0.70$). There was a positive association between SR frequency and genetic distance that was weakly significant ($r = 0.29$, $P = 0.028$), although this does not consider a correction for multiple tests. With this smaller dataset, genetic and geographic distance accounting for 4.7% and 0.6% of the variation in SR frequency, respectively. Although these results are based on a limited number of neutral molecular markers, they suggest that neutral demographic processes, in particular geographic distance and genetic relatedness, account for little, if any, of the differences in SR frequency among populations of *D. neotestacea*.

### ASSOCIATION OF ECOLOGICAL VARIABLES WITH SR

To investigate whether ecological conditions might affect the spread or maintenance of SR, I asked whether the frequency of SR correlates with various climatic factors. I used the average monthly temperatures in a PCA, and found that the first principle component of mean temperature ($T_{PC1}$) explained 76% of the variation in temperature among sites, with positive load bearings (0.22–0.32) for all temperatures. Thus, the large positive values for $T_{PC1}$ indicate relatively warmer locations. The second principle component ($T_{PC2}$) explained 20% of the variation, and described the difference between the summer months (May–September) (0.11–0.46) and the other seasons (−0.32 to −0.03). The $T_{PC1}$ is highly correlated with the PC1 of a principle component analysis that uses the maximum and minimum monthly temperatures ($r^2 = 0.98$, $F_{1,14} = 1363$, $P < 0.0001$), and with annual cooling and heating degree days (CDD: $r^2 = 0.57$; $F_{1,14} = 19$, $P = 0.0006$; HDD: $r^2 = 0.93$; $F_{1,13} = 207$, $P < 0.0001$). Thus, this measure captures much of the variation in temperature among sites.

The temperature index ($T_{PC1}$) strongly correlates with latitude ($r^2 = 0.45$; $F_{1,14} = 11.5$, $P = 0.0046$), whereas the second and higher temperature principle components do not ($T_{PC2}$: $r^2 = 0.007$; $F_{1,14} = 0.1$, $P = 0.76$). As shown in Figure 4, there is a positive correlation between the frequency of SR and $T_{PC1}$ ($r^2 = 0.59$; $F_{1,13} = 19$, $P = 0.0008$), indicating that warmer locations harbor a higher frequency of SR drive chromosomes. It is important to note that the TN populations are no longer outliers, as they are in the correlation of SR frequency with latitude. There is no correlation with $T_{PC2}$ and SR frequency ($r^2 = 0.003$; $F_{1,13} = 0.04$, $P = 0.84$), thus $T_{PC2}$ is not considered further here. I found no correlation of the residuals of the correlation between SR and $T_{PC1}$ with latitude ($r^2 = 0.02$; $F_{1,13} = 0.35$, $P = 0.56$), including when the TN populations are excluded ($r^2 = 0.1$; $F_{1,11} = 1.29$, $P = 0.28$). Importantly, this result indicates that no clinal variation remained after the effect of temperature was removed.

Finally, I used a logistic regression of SR frequency with environmental variables, and asked which variables resulted in the best improvement to the model. The lnL of the null model with only the intercept was 600.4, and the lnL of the model with latitude predicting SR was −587.0 ($2\Delta\text{lnL} = 26.8$). Overall, precipitation and summer temperatures did little to improve the model relative to only using latitude, whereas nonsummer temperatures consistently improved the fit of the model. Considering mean seasonal temperatures was particularly informative: summer (June–August) does not improve the base model (lnL = −586.5, $2\Delta\text{lnL} = 27.9$), whereas the other seasons do (fall [September–November]: lnL = −576.3, $2\Delta\text{lnL} = 48.2$; winter [December–February]: lnL = −578.6, $2\Delta\text{lnL} = 43.6$; spring [March–May]: lnL = −577.32, $2\Delta\text{lnL} = 46.6$). Similar results were found using $T_{PC1}$ (lnL = −576.12, $2\Delta\text{lnL} = 48.6$). These results are consistent with the PCA results above that suggest that the overall annual temperature, in particular factors related to the duration and severity of the winter and not simply the temperature during the summer months when the flies are active, contributes most to differences in SR frequency among populations.

## Discussion

Here, I show that populations of *D. neotestacea* vary in their prevalence of SR drive: although in the most northern populations SR is very rare, in southern populations it is quite common, with a prevalence close to 30%. The prevalence of SR appears to be relatively stable in *D. neotestacea*, at least over short periods of time. First, in the eastern United States, the frequency of SR

is consistent from James and Jaenike (1990) to this survey, more than 10 years later. Second, I sampled from populations in Central Canada in 2010, and found SR to still be very rare (data not shown). Finally, in 2008, I resampled the populations in the Smoky Mountains, and the frequencies are roughly similar to the samples in this study (in 2008: TN1 = 17% SR and TN2 = 10% SR).

Using a series of genetic crosses, I found that the variation in SR prevalence is not due to dominant or Y-linked suppressors of drive, as any SR X chromosome could drive any Y chromosome it was tested against. Thus, the observed variation in SR frequency is not likely due to the isolated presence of suppressors that are limited to the northern populations. Although I cannot rule out the presence of recessive SR suppressors, dominant and Y-linked suppressors will be much more likely to be seen by selection and thus rise to appreciable frequency (Haldane 1924). Based on these results, I can also conclude that there has been no local coevolution between SR and Y chromosomes, and that in *D. neotestacea*, the geographic scale of intragenomic conflict occurs at the level of the species and not at the individual population or metapopulation.

The analysis of population differentiation at autosomal microsatellite markers suggests that *D. neotestacea* has generally high levels of gene flow among populations, which indicates there is ample opportunity for the SR chromosome to move among populations. Because the transmission advantage of SR is so strong, it would probably take an extremely high amount of neutral population structure to impede its spread. I find that while the geographic pattern of genetic clustering is weak (Fig. 6), it is in line with previous work in mushroom-feeding species of *Drosophila*. Specifically, eastern North American species exhibit relatively high levels of historical gene flow, and western species show much more differentiation among populations, with patterns concordant with the presence of mountain ranges (Shoemaker and Jaenike 1997; Jaenike et al. 2006). This phylogeographic pattern is also similar to a suite of other organisms, and is likely due to glacial formations and refugia during the last ice age (Carstens et al. 2005).

Using climate data from each site, I found a strong association of SR prevalence with local temperature, with warmer populations harboring a higher prevalence of SR. Based on both PCA and logistic regression analyses, the local frequency of SR drive is best predicted by the duration and severity of the winter season rather than the local summer conditions. Once temperature was accounted for, there was no clinal variation in SR, which indicates that local temperature, or some factor related to it, may prevent the establishment of SR in some populations but not in others. Two populations that are key to this interpretation are the low and high elevation populations in the Smoky Mountains in Tennessee (TN1 and TN2). These populations are not only outliers in the latitudinal cline (Fig. 4A), but also differ in their frequency of drive: at low elevation, 17 of 68 (25%) X chromosomes carried SR, in contrast to 11 of 95 (12%) X chromosomes at high elevation (Fisher's Exact Test [FET], $P = 0.035$). However, when temperature is taken into account, both of these populations fall into the temperature gradient and are no longer outliers (Fig. 4B). This is strong evidence that selection, and not simply geographic distance, plays an important role in determining the frequency of SR among populations.

Several different mechanisms could create local conditions that permit SR to invade in some populations but not others. First, SR may be genetically linked to or epistatic with a factor that affects the fitness of its carriers. For example, this might occur if SR is linked to cold temperature tolerance, propensity to diapause, or some other physiological trait that is distributed on a temperature gradient. Tolerance of local environmental conditions, in particular cold temperature and winter severity, is associated with species range boundaries (e.g., Addo-Bediako et al. 2000; Kimura 2004), and within a species' range local adaptation can occur across environments (reviewed in Hoffmann 2010). Because the geographic range of *D. neotestacea* is very broad, it would be unsurprising for there not to be local adaptation in environmental tolerance among populations. However, if selection is acting on a linked factor rather than on drive itself, it must be stronger than the opposing transmission bias due to drive to cause such large differences among populations in SR prevalence. In addition, there must be very tight linkage between the selected trait and driving genes for selection on a linked trait to affect SR fitness (Lande and Wilkinson 1999). Strong selection on a linked trait may not be unfeasible given that in other *Drosophila* species parallel clines of inversions quickly establish in new species ranges, and inversion clines move rapidly based on changing climatic patterns (Huey et al. 2000; Balanyà et al. 2006). Furthermore, many species have well-characterized clines in temperature tolerance and related traits (e.g., Hoffmann et al. 2002; Schmidt et al. 2005; Schmidt and Paaby 2008).

Second, local environmental conditions may create population-specific demographies that affect the ability of SR to invade. For example, local conditions may cause population sizes to differ, which may then affect factors such as mating rate and thus relative fitness of SR males. Ecological variables can affect mating behavior, for example, females tend to mate more under high-density conditions (reviewed in Schlötterer et al. 2005), and more available resources can promote egg production (Marks et al. 1988). In this case, if there are more flies present in a population, females may mate more often, which may decrease the relative fitness of SR males due to an increase in sperm competition (Jaenike 1996; Taylor and Jaenike 2003). For this to underlie variation in SR frequency across populations, we would expect for populations in cooler climates to have higher population sizes and thus higher rates of mating. Species in the testacea group

are generally quite cold tolerant (Kimura 2004), and from our collections, we observe that cooler locations have fewer species in general, and both factors may lead to less between-species competition for resources and higher numbers of *D. neotestacea* in colder climates. Likewise, James and Jaenike (1990) found that in New York, the prevalence of SR decreased somewhat over the growing season, which may be a consequence of an increase in the population size as the season progressed. Although my collections were not standardized in a manner to determine the census population size, as a proxy for local population size we can use the local genetic diversity at the microsatellite loci. Perhaps not surprising given the amount of gene flow, I do not find higher genetic diversity in populations with a lower prevalence of SR or which occur in colder climates ($H_O$ vs. SR frequency: $r^2 = 0.014$; $F_{1,13} = 0.15$, $P = 0.18$; $H_O$ vs. $T_{PC1}$: $r^2 = 0.06$; $F_{1,14} = 0.84$, $P = 0.37$). Thus, at least the long-term effective population sizes do not appear to be different among populations that differ in SR prevalence.

Finally, differences in the SR prevalence may be a reflection of local adaptation in the mating system. In this case, populations may not vary in population size, but may still differ in mating rates and thus SR fitness, perhaps as a consequence of local selection on the mating rate itself. Theoretical studies find that females that mate randomly with more males increase their chance of producing sons (Haig and Bergstrom 1995). Thus, if SR or some other factor has selected for an increased female mating rate in some populations, we would expect that in these populations, SR males would suffer an increased cost to fitness and thus the selfish element would be less likely to invade. In experimental evolution studies in *D. pseudoobscura*, in just 10 generations, the rate of female remating increased in response to the presence of a SR X chromosome (Price et al. 2008). Furthermore, these authors also found that enforcing monogamy in experimental populations increased the probability that a SR chromosome would drive the host population extinct, suggesting that increased sperm competition among males due to polyandry may prevent the spread of SR (Price et al. 2010). However, these studies are limited to laboratory populations, and further studies are necessary to determine whether this also occurs in natural populations. If this type of mechanism has occurred in *D. neotestacea*, we would expect that populations with a lower frequency of SR also have a higher rate of mating. However, it could be difficult to disentangle whether this was caused by SR or to some other factor, especially because when SR is absent it cannot directly impose selection on the host.

The geographic nature of SR has been described for several other species (reviewed in Jaenike 2001), and the closest parallel to *D. neotestacea* is the SR system in *D. pseudoobscura*. *Drosophila pseudoobscura* populations also differ in prevalence of SR drive, with populations showing a stable north–south pattern that is not associated with gene flow or suppressors, of which none have been found (reviewed in Powell 1996; Sturtevant and Dobzhansky 1936; Kovacevic and Schaeffer 2000). However, in contrast to *D. neotestacea*, inversions associated with SR have been shown to have pleiotropic effects on both male and female fitness, which suggests that both balancing selection and other factors may govern the SR dynamics in this species (Wallace 1948; Beckenbach 1983). Although the sample size is small, it is interesting to note that *D. neotestacea* and *D. pseudoobscura* are the only two species known that have a relatively high frequency of drive and yet no segregating suppressors. In all other characterized SR drive systems, SR has drastic negative effects on female fitness that keeps the frequency very low (e.g., *D. recens*, Dyer et al. 2007), populations are polymorphic for both suppressors and drivers (e.g., *D. mediopunctata*, Carvalho et al. 1997; *D. quinaria*; Jaenike 1999), or suppressors are fixed locally so that SR is only expressed on a naive genetic background (e.g., *D. simulans*, Altan et al. 1997). The lack of suppressors in *D. neotestacea* and *D. pseudoobscura* presents a bit of a conundrum, as we would expect selection for suppressors to be quite strong because the frequency of SR is so high. Interestingly, these two species are also the only species known to also show a strong geographic cline in SR prevalence, with high levels of gene flow among populations that differ in SR frequency. One possibility that warrants further theoretical investigation is that migration from populations without drive may decrease the strength of selection for suppressors and thus contribute to the local persistence of SR in populations where it has invaded.

In conclusion, in this article, I investigated the geographic distribution of SR drive in the fly *D. neotestacea*, with a goal of understanding how selection and gene flow interact to shape the evolution of this SR drive system. I found that the variation in SR prevalence is not accounted for by genetic or neutral demographic factors, but instead appears to be due to local ecological factors, with temperature the strongest predictor of SR frequency. Variation in a trait in the face of gene flow, as we find here for SR, is a clear sign of local selection (Endler 1977), and these data show that ecological factors can affect the evolutionary outcome of selfish genetic systems, even when they otherwise enjoy a large selective advantage. Future studies are necessary to explore which factors affect the fitness of SR carriers, and how these factors differ among populations to prevent the establishment of SR in some populations but not others. The stability of SR frequency through time and across populations also suggests that the SR system of *D. neotestacea* may not be as evolutionarily young as previously thought, although further investigation is necessary to determine the age of this drive system. Finally, it will also be interesting to explore whether gene flow from other populations can affect the local dynamics of drive, and how this might affect the long-term dynamics of drive by altering the strength of selection on the rest of the genome to resist drive.

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## LITERATURE CITED

Addo-Bediako, A., S. L. Chown, and K. J. Gaston. 2000. Thermal tolerance, climatic variability and latitude. Proc. R. Soc. Lond. B 267:739–745.

Altan, A., H. Mercot, C. Landre, and C. Montchamp-Moreau. 1997. The *sex-ratio* trait in *Drosophila simulans*: geographical distribution of distortion and resistance. Evolution 51:1886–1895.

Balanyà, J., J. M. Oller, R. B. Huey, G. W. Gilchrist, and L. Serra. 2006. Global genetic change tracks global climate warming in *Drosophila subobscura*. Science 313:1773–1775.

Bastide, H., M. Cazemajor, D. Ogereau, N. Derome, F. Hospital, and C. Montchamp-Moreau. 2011. Rapid rise and fall of selfish *sex-ratio* X chromosomes in *Drosophila simulans*: spatio-temporal analysis of phenotypic and molecular data. Mol. Biol. Evol. 28:2461–2470.

Beckenbach, A. T. 1983. Fitness analysis of the sex-ratio polymorphism in experimental populations of *Drosophila pseudoobscura*. Am. Nat. 121:630–648.

Burt, A., and R. Trivers. 2006. Genes in conflict: the biology of selfish genetic elements. Harvard Univ. Press, Cambridge.

Carstens, B. C., S. J. Brunsfeld, J. R. Demboski, J. D. Good, and J. Sullivan. 2005. Investigating the evolutionary history of the Pacific Northwest mesic forest ecosystem: hypothesis testing within a comparative phylogeographic framework. Evolution 59:1639–1652.

Carvalho, A. B., and S. C. Vaz. 1999. Are *Drosophila* SR drive chromosomes always balanced? Heredity 83:221–228.

Carvalho, A. B., S. C. Vaz, and L. B. Klaczko. 1999. Polymorphism for Y-linked suppressors in two natural populations of *Drosophila mediopunctata*. Genetics 146:891–902.

Charlat, S., E. A. Hornett, J. H. Fullard, N. Davies, G. K. Roderick, N. Wedell, and G. D. D. Hurst. 2007. Extraordinary flux in sex-ratio. Science 317:214.

Charlesworth, B., and D. L. Hartl. 1978. Population dynamics of the segregation distorter polymorphism of *Drosophila melanogaster*. Genetics 89:171–192.

Clark, A. G. 1987. Natural selection and Y-linked polymorphism. Genetics 115:569–577.

Curtsinger, J. W., and M. W. Feldman. 1980. Experimental and theoretical analyses of the "sex-ratio" polymorphism in *Drosophila pseudoobscura*. Genetics 94: 445–466.

Dyer, K. A. 2007. Identification and characterization of 21 polymorphic microsatellite loci from the mycophagous fly *Drosophila neotestacea*. Mol. Ecol. Notes 7:1120–1122.

Dyer, K. A., B. Charlesworth, and J. Jaenike. 2007. Chromosome-wide linkage disequilibrium as a consequence of meiotic drive. Proc. Natl. Acad. Sci. USA 104:1587–1592.

Edwards, A. W. F. 1961. The population genetics of "sex-ratio" in *Drosophila pseudoobscura*. Heredity 16:291–304.

Emlen, S. T., and L. W. Oring. 1977. Ecology, sexual selection, and the evolution of mating systems. Science 197:215–223.

Endler, J. 1977. Geographic variation, speciation and clines. Princeton Univ. Press, Princeton, NJ.

Evanno, G., S. Regnaut, and J. Goudet. 2005. Detecting the number of clusters of individuals using the software Structure: a simulation study. Mol. Ecol. 14:2611–2620.

Excoffier, L., and H. E. L. Lischer. 2010. Arlequin suite ver 3.5: a new series of programs to perform population genetics analyses under Linux and Windows. Mol. Ecol. Res. 10:564–567.

Grimaldi, D., A. C. James, and J. Jaenike. 1992. Systematics and modes of reproductive isolation in the Hoarctic *Drosophila testacea* species group (Diptera: Drosophilidae). Ann. Entomol. Soc. Am. 85:671–685.

Haig, D., and C. T. Bergstrom. 1995. Multiple mating, sperm competition, and meiotic drive. J. Evol. Biol. 8:265–282.

Haldane, J. B. S. 1924. A mathematical theory of natural and artificial selection, Part I. Trans. Camb. Philos. Soc. 23:19–41.

Hall, D. W. 2004. Meiotic drive and sex chromosome cycling. Evolution 58:925–931.

Hamilton, W. D. 1967. Extraordinary sex ratios. Science 156:477–488.

Hatcher, M. J., D. E. Taneyhill, A. M. Dunn, and C. Tofts. 1999. Population dynamics under parasitic sex ratio distortion. Theor. Popul. Biol. 56: 11–18.

Hoffmann, A. A. 2010. Physiological climatic limits in *Drosophila*: patterns and predictions. J. Exp. Biol. 213:870–880.

Hoffmann, A. A., A. Anderson, and R. Hallas. 2002. Opposing clines for high and low temperature resistance in *Drosophila melanogaster*. Ecol. Lett. 5:614–618.

Huey, R. B., G. W. Gilchrist, M. L. Carlson, D. Berrigan, and L. Serra. 2000. Rapid evolution of a geographic cline in size in an introduced fly. Science 287:308–309.

Hurst, L. D., A. Atlan, and B. O. Bengtsson. 1996. Genetic conflicts. Q. Rev. Biol. 71:317–364.

Jaenike, J. 1996. Sex-ratio meiotic drive in the *Drosophila quinaria* group. Am. Nat. 148:237–254.

———. 1999. Suppression of sex-ratio meiotic drive and the maintenance of Y chromosome polymorphism in *Drosophila*. Evolution 53:164–174.

———. 2001. Sex chromosome meiotic drive. Ann. Rev. Ecol. Syst. 32:25–49.

Jaenike, J., K. A. Dyer, C. Cornish, and M. Minhas. 2006. Asymmetrical reinforcement and *Wolbachia* infection in *Drosophila*. PloS Biol. 4:1852–1862.

James, A. C. 1992. "Sex-ratio" meiotic drive in *Drosophila neotestacea*. Ph.D. Dissertation. University of Rochester, Rochester, NY.

James, A. C., and J. Jaenike. 1990. "Sex-ratio" meiotic drive in *Drosophila testacea*. Genetics 125:651–656.

Kimura, M. T. 2004. Cold and heat tolerance of Drosophilid flies with reference to their latitudinal distributions. Oecologia 140:442–449.

Kovacevic, M., and S. W. Schaeffer. 2000. Molecular population genetics of *X*-linked genes in *Drosophila pseudoobscura*. Genetics 156:155–172.

Lande, R., and G. S. Wilkinson. 1999. Models of *sex-ratio* meiotic drive and sexual selection in stalk-eyed flies. Genet. Res. 74:245–253.

Mantel, N. 1967. The detection of disease clustering and a generalized regression approach. Cancer Res. 27:209–220.

Marks, R. W., R. D. Seager, F. J. Ayala, and L. G. Barr. 1988. Local ecology and multiple matings in a natural population of *Drosophila melanogaster*. Am. Nat. 131:918–923.

McAllister, B. F., S. L. Sheeley, P. A. Mena, A. L. Evans, and C. Schlötterer. 2008. Clinal distribution of a chromosomal rearrangement: a precursor to chromosomal speciation? Evolution 62:1852–1865.

Powell, J. R. 1997. Progress and prospects in evolutionary biology: the drosophila model. Oxford Univ. Press, New York.

Presgraves, D. C., P. R. Gérard, A. Cherukuri, T. W. Lyttle. 2009. Large-scale selective sweep among *Segregation Distorter* chromosomes in African populations of *Drosophila melanogaster*. PLoS Genet. 5:e1000463.

Price, T. A. R., and N. Wedell. 2008. Selfish genetic elements and sexual selection: their impact on male fertility. Genetica 132:295–307.

Price, T. A. R., A. J. Bretman, T. D. Avent, R. R. Snook, G. D. D. Hurst, and N. Wedell. 2008. Sex ratio distorter reduces sperm competitive ability in an insect. Evolution 62:1644–1652.

Price, T. A. R., G. D. Hurst, and N. Wedell. 2010. Polyandry prevents extinction. Curr. Biol. 20:471–475.

Pritchard, J. K., M. Stephens, and P. Donnelly. 2000. Inference of population structure using multilocus genotype data. Genetics 155: 945–959.

Raymond, M., and F. Rousset. 1995. Genepop, population genetics software for exact tests and ecumenicism. J. Hered. 86:248–249.

Rousset, F. 2008. Genepop'007: a complete reimplementation of the Genepop software for Windows and Linux. Mol. Ecol. Res. 8:103–106.

Schlötterer, C., M. Reiss, A. Schneider, and M. Imhof. 2005. Similar mating and sperm displacement patterns from highly divergent *D. simulans* populations from Africa and Europe. Mol. Ecol. 14:1511–1515.

Schmidt, P. S., L. Matzkin, M. Ippolito, and W. F. Eanes. 2005. Geographic variation in diapause incidence, life history traits, and climatic adaptation in *Drosophila melanogaster*. Evolution 59:1721–1732.

Schmidt, P. S., and A. B. Paaby. 2008. Reproductive diapause and life history clines in North American populations of *Drosophila melanogaster*. Evolution 62:1204–1215.

Shoemaker, D. D. W., and J. Jaenike. 1997. Habitat continuity and the genetic structure of populations. Evolution 51:1326–1332.

Slatkin, M. 1995. A measure of population subdivision based on microsatellite allele frequencies. Genetics 139:457–462.

Sturtevant, A. H., and T. Dobzhansky. 1936. Geographical distribution and cytology of "sex-ratio" in *Drosophila pseudoobscura* and related species. Genetics 21:473–490.

Taylor, J. E., and J. Jaenike. 2002. Sperm competition and the dynamics of X chromosome drive: stability and extinction. Genetics 160: 1721–1731.

———. 2003. Sperm competition and the dynamics of X chromosome drive in finite and structured populations. Ann. Zool. Fenn. 40:195–206.

Wallace, B. 1948. Studies on "sex-ratio" in *Drosophila pseudoobscura*. I. Selection and "sex-ratio." Evolution 2:189–217.

Werren, J. H., U. Nur, and C. I. Wu. 1988. Selfish genetic elements. Trends Ecol. Evol. 3:297–302.

Zeh, J. A., and D. W. Zeh. 1996. The evolution of polyandry I: intragenomic confict and genetic incompatibility. Proc. R. Soc. Lond. B 263:1711–1717.

———. 1997. The evolution of polyandry II: post-copulatory defences against genetic incompatibility. Proc. R. Soc. Lond. B 264:69–75.

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## *Supporting Information*

The following supporting information is available for this article:

**Table S1.** Analysis of variance of fraction of daughters in sympatric and allopatric introgressions (data were arcsin squareroot transformed; see text for details).

**Table S2.** Variability measures for each of the seven microsatellites within each population and across all populations.

**Table S3.** Pairwise population differentiation at five autosomal microsatellite loci.

**Table S4.** Proportion of membership of each population into each of the three clusters, as defined by the Structure analyses.

**Table S5.** Results of the Structure analyses to infer the number of genetic clusters ($K$).

**Figure S1.** Crossing scheme to extract a *sex-ratio* chromosome into laboratory culture.

**Figure S2.** Distribution of offspring sex ratio in each of the sampled 15 populations.

Supporting Information may be found in the online version of this article.

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