The maintenance of polygenic sex determination depends on the dominance of fitness effects which are predictive of the role of sexual antagonism

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Abstract

In species with polygenic sex determination, multiple male- and female-determining loci on different proto-sex chromosomes segregate as polymorphisms within populations. The extent to which these polymorphisms are at stable equilibria is not yet resolved. Previous work demonstrated that polygenic sex determination is most likely to be maintained as a stable polymorphism when the proto-sex chromosomes have opposite (sexually antagonistic) fitness effects in males and females. However, these models usually consider polygenic sex determination systems with only two proto-sex chromosomes, or they do not broadly consider the dominance of the alleles under selection. To address these shortcomings, I used forward population genetic simulations to identify selection pressures that can maintain polygenic sex determination under different dominance scenarios in a system with more than two proto-sex chromosomes (modeled after the house fly). I found that overdominant fitness effects of male-determining proto-Y chromosomes are more likely to maintain polygenic sex determination than dominant, recessive, or additive fitness effects. The overdominant fitness effects that maintain polygenic sex determination tend to have proto-Y chromosomes with sexually antagonstic effects (male-beneficial and female-detrimental). In contrast, dominant fitness effects that maintain polygenic sex determination tend to have sexually antagonistic multi-chromosomal genotypes, but the individual proto-sex chromosomes do not have sexually antagonstic effects. These results demonstrate that sexual antagonism can be an emergent property of the multi-chromosome genotype without individual sexually antagonistic chromosomes. My results further illustrate how the dominance of fitness effects has consequences for both the likelihood that polygenic sex determination will be maintained as well as the role sexually antagonistic selection is expected to play in maintaining the polymorphism.

Introduction

Sex determination is the developmental process by which a genetic signal or environmental cue initiates sexually dimorphic gene regulatory pathways to produce phenotypically different males and females (Bachtrog *et al.* 2014). The master regulators of sex determination evolve fast, often differing between closely related species (Bull 1983; Wilkins 1995; Beukeboom and Perrin 2014). Master regulators can be found on sex chromosomes, and the evolutionary transitions of sex determiners can drive turnover of the sex chromosomes (Abbott *et al.* 2017). These evolutionary transitions include a period of polygenic sex determination (PSD), during which multiple master sex determining loci on different chromosomes segregate as polymorphisms within species (Moore and Roberts 2013). Understanding the population dynamics of PSD is informative of the factors responsible for the evolutionary divergence of sex determination pathways and sex chromosomes.

PSD has been observed in multiple animal and plant species (Moore and Roberts 2013; Beukeboom and Perrin 2014; Bachtrog *et al.* 2014). Most population genetic models predict that PSD will be an unstable intermediate between monogenic equilibria (Rice 1986; van Doorn and Kirkpatrick 2007). Models that do allow for stable PSD often predict that opposing selection pressures in males and females (i.e., sexually antagonistic selection) may be important for maintaining the polymorphism (Bull 1983; Rice 1986; van Doorn and Kirkpatrick 2007; Meisel *et al.* 2016). It is also possible that variable selection pressures across heterogeneous environments could maintain PSD (Bateman and Anholt 2017).

Most previous theoretical treatments of the selection pressures that maintain PSD considered specific cases with a small number of sex chromosomes segregating within a population. For example, in the platyfish, *Xiphophorus maculatus*, a single chromosome can be an X, Y, or Z (Kallman 1973). Orzack et al. (1980) showed that this polymorphism is maintained

because heterogametic females (WY or WX) and males (XY) have higher fitness than their homogametic counterparts (XX females or YY males). In addition, van Doorn and Kirkpatrick (2007, 2010) found that sexually antagonistic selection could maintain a polymorphism in which one chromosome segregates as an XY pair and another chromosome is either an XY or ZW pair. However, these models do not capture the full diversity of PSD, which can include more than two XY or ZW pairs segregating within a single species (Moore and Roberts 2013).

Previous models also did not broadly consider how the maintenance of PSD depends on the dominance of fitness effects. The dominance of sexually antagonistic alleles affects their ability to segregate as stable polymorphisms (Kidwell *et al.* 1977; Connallon and Chenoweth 2019), suggesting that dominance may be important for the maintenance of PSD under sexually antagonistic selection. Dominance also differentially affects the evolutionary fate of X-linked and autosomal alleles that experience sex-specific selection pressures (Rice 1984; Charlesworth *et al.* 1987; Fry 2010; Connallon *et al.* 2012). In a species with PSD, there are proto-sex chromosomes that behave neither like autosomes nor like conventional heteromorphic sex chromosomes; proto-sex chromosomes are diploid in both sexes (like autosomes) but have sex-biased modes of inheritance (like conventional sex chromosomes). This is superficially similar to freely recombining pseudoautosomal regions on sex chromosomes (Otto *et al.* 2011), except that in PSD a proto-Y (or proto-W) chromosome can be directly carried by females (males) without X-Y (or Z-W) recombination. Therefore, the expected effects of dominance and sexual antagonism on the evolution of PSD cannot be determined from existing models of autosomal or sex-linked alleles.

The house fly, *Musca domestica*, is a well-suited organism for investigating the maintenance of complex PSD because it has a highly polymorphic sex determination system (Hamm *et al.* 2015). The *M. domestica male determiner* (*Mdmd*) can be found on at least four of

90 the six house fly chromosomes in natural populations (Sharma *et al.* 2017). *Mdmd* causes the
91 house fly ortholog of *transformer* (*Md-tra*) to be spliced into a non-functional isoform, which
92 leads to male-specific splicing and expression of downstream genes in the sex determination
93 pathway, thereby producing fertile males (Hediger *et al.* 2010). In the absence of *Mdmd*, *Md-tra*
94 is spliced into a female-determining transcript that regulates the splicing of downstream targets,
95 which promote the development of female morphological and behavioral traits (Hediger *et al.*
96 2004; Meier *et al.* 2013).

97 There are two common male-determining proto-Y chromosomes and one
98 female-determining proto-W chromosome in house fly populations. *Mdmd* is most commonly
99 found on the Y (Y^M) and third (III^M) chromosomes in natural populations (Hamm *et al.* 2015).
100 Both Y^M and III^M are young proto-Y chromosomes that arose recently and are nearly identical in
101 gene content to their homologous proto-X chromosomes, known as X and III (Meisel *et al.* 2017;
102 Son and Meisel 2021). In addition, there is a dominant allele of *Md-tra* that is found in many
103 house fly populations (*Md-tra*^{D}), which is resistant to *Mdmd* and causes female development
104 regardless of whether there are copies of *Mdmd* in the genotype (McDonald *et al.* 1978; Hediger
105 *et al.* 2010). *Md-tra* is found on the fourth chromosome, and thus fourth chromosomes carrying
106 *Md-tra*^{D} (which I will refer to as IV^F) are W chromosomes. All sequences of *Md-tra*^{D} from around
107 the world are identical (Scott *et al.* 2014), suggesting that *Md-tra*^{D} arose recently and IV^F is a
108 young proto-W chromosome. In populations where Y^M, III^M, and *Md-tra*^{D} all segregate, there are
109 18 possible sex chromosome genotypes (Table 1).

110 **Table 1**. House fly sex chromosome genotypes.

genotype

	X or Y^M	III or III^M	IV or IV^F	sex
f_1	X/X	III/III	IV/IV	female
f_2	X/X	III/III	IV/IV^F	female
f_3	X/X	III/III^M	IV/IV^F	female
f_4	X/X	III^M/III^M	IV/IV^F	female
f_5	X/Y^M	III/III	IV/IV^F	female
f_6	X/Y^M	III/III^M	IV/IV^F	female
f_7	X/Y^M	III^M/III^M	IV/IV^F	female
f_8	Y^M/Y^M	III/III	IV/IV^F	female
f_9	Y^M/Y^M	III/III^M	IV/IV^F	female
f_{10}	Y^M/Y^M	III^M/III^M	IV/IV^F	female
m_1	X/X	III/III^M	IV/IV	male
m_2	X/X	III^M/III^M	IV/IV	male
m_3	X/Y^M	III/III	IV/IV	male
m_4	X/Y^M	III/III^M	IV/IV	male
m_5	X/Y^M	III^M/III^M	IV/IV	male
m_6	Y^M/Y^M	III/III	IV/IV	male
m_7	Y^M/Y^M	III/III^M	IV/IV	male
m_8	Y^M/Y^M	III^M/III^M	IV/IV	male

There is evidence that natural selection maintains PSD in house fly populations. First, the proto-Y chromosomes have remained at stable frequencies within house fly populations over decades (Kozielska *et al.* 2008; Meisel *et al.* 2016). Second, Y^M and III^M are distributed along north-south clines on multiple continents (Denholm *et al.* 1986; Tomita and Wada 1989;

115 Hamm *et al.* 2005; Kozielska *et al.* 2008), and temperature is the best predictor of their
116 frequencies (Feldmeyer *et al.* 2008). This suggests that heterogenous (temperature-dependent)
117 selection pressures maintain the Y^M and III^M chromosomes across populations. The stable
118 frequencies within populations, yet divergent frequencies across populations, suggest that there
119 are specific fitness effects associated with the house fly proto-Y chromosomes within each
120 population. Third, males carrying multiple proto-Y chromosomes (e.g., both Y^M and III^M, or two
121 copies of III^M) can be found in some populations (e.g., Hamm and Scott 2009). The frequency of
122 *Md-tra*^{D} is positively correlated with the frequency of these multi-Y males across populations,
123 suggesting that selection for balanced sex ratios may further maintain PSD (Meisel *et al.* 2016).
124 The rich body of observational data in house fly makes it a well-suited system around
125 which to develop population genetic models to determine how PSD can be maintained in natural
126 populations. For example, Kozielska et al. (2006) showed that, even in the complex house fly
127 PSD system, sex ratios are not expected to deviate substantially from 1:1 male:female. In
128 addition, despite no experimental evidence for sexually antagonistic effects of any house fly
129 proto-sex chromosomes, another population genetic model demonstrated that the frequencies
130 of the proto-sex chromosomes in natural populations are consistent with sexually antagonistic
131 selection maintaining the polymorphism (Meisel *et al.* 2016). However, that model assumed that
132 the proto-Y chromosomes (Y^M and III^M) have additive fitness effects, which may not be true if,
133 for example, beneficial mutations are recessive or dominant (Orr 2010). In addition, Y
134 chromosomes are expected to carry recessive deleterious alleles (Charlesworth and
135 Charlesworth 2000), which can further violate the assumption of additive fitness effects. To
136 address the shortcomings of previous models, I used population genetic simulations to
137 investigate whether sex chromosomes with non-additive fitness effects can maintain the
138 complex house fly PSD system. I specifically tested if these selection pressures can produce

proto-sex chromosome frequencies similar to those observed in natural populations of house fly, and I determined the conditions under which sexually antagonistic effects of those proto-sex chromosomes are expected.

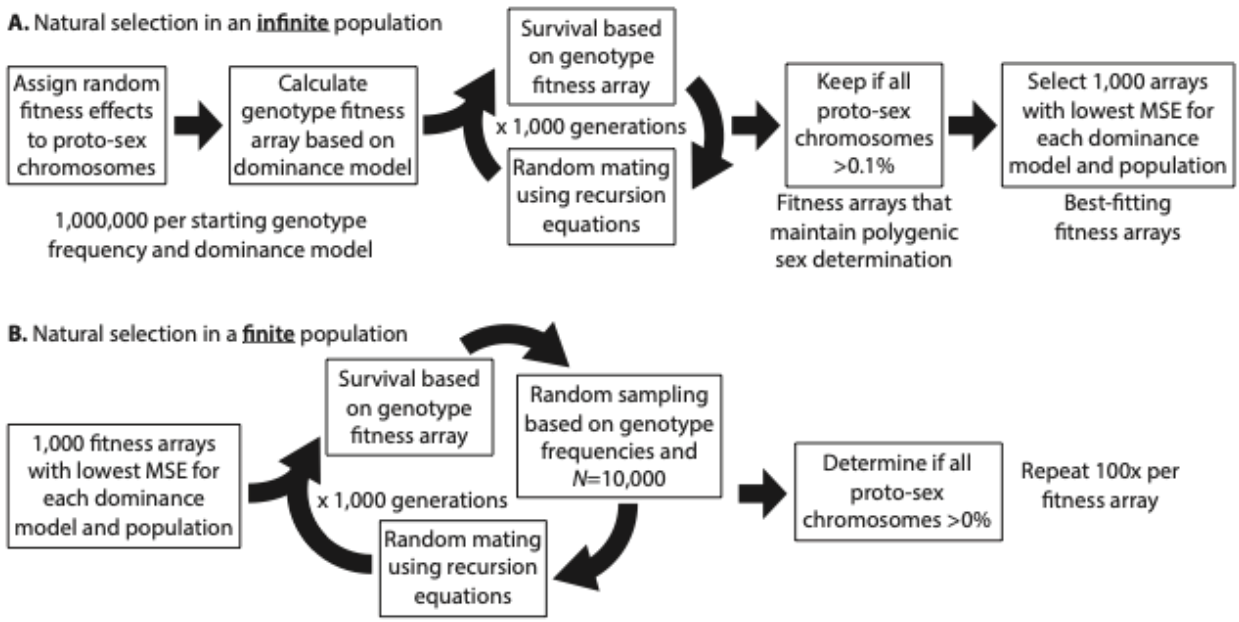


Figure 1. Steps in forward population genetic simulations with **(A)** infinite or **(B)** finite populations. The best-fitting arrays are those with the lowest mean squared error (MSE) comparing simulated proto-sex chromosome frequencies to those observed in each natural population.

Materials and Methods

I used a simulation approach (Figure 1A) to identify fitness effects of two proto-Y chromosomes (Y^M and III^M) and one proto-W chromosome (IV^F) that maintain PSD under four different dominance scenarios. I used simulations to test if fitness effects maintain PSD because the model parameters were too complex to analytically solve for equilibrium conditions. To do so, I first assigned sex-specific fitness effects, s_{ij}, for each j proto-Y or proto-W chromosome in each sex i by drawing from a random uniform distribution between -1 and 1 (negative values indicate beneficial effects, and positive indicate deleterious). I then used those fitness effects to

153 calculate single chromosome genotype fitness values assuming either additive, dominant,
154 recessive, or overdominant (i.e., heterozygote advantage) fitness effects of the proto-Y
155 chromosomes (Table 2). I calculated the fitness of each of the 18 multi-chromosome genotypes
156 (Table 1) by multiplying all single chromosome fitness values.

157 To test if the fitness effects result in stable PSD, I determined the frequency of each
158 genotype and proto-sex chromosome after 1,000 generations of random mating with the
159 assigned fitness values (Figure 1A). Simulations were started with either equal frequencies of all
160 18 genotypes or the genotype frequencies observed in one of three natural populations (CA,
161 NC, or NY) sampled from North America (Hamm and Scott 2008; Scott *et al.* 2013; Meisel *et al.*
162 2016). I performed simulations for one million different fitness values for each set of starting
163 genotype frequencies and each dominance model using previously developed recursion
164 equations (Hamm 2008; Meisel *et al.* 2016). I retained fitness effects in which all three proto-sex
165 chromosomes remained polymorphic at a frequency >0.1% after 1,000 generations. From
166 those, I selected the 1,000 fitness effects with the smallest mean squared error (MSE) between
167 simulated proto-sex frequencies and those observed in each natural population (CA, NC, or NY)
168 for each dominance model, population, and starting genotype frequency (Figure 1A). In doing
169 so, I am essentially applying an approximate Bayesian computation approach to fit selection
170 pressures to natural populations (Beaumont *et al.* 2002). I observed the same general trends
171 regardless of the starting genotype frequencies, and I only present the results of simulations
172 starting with equal frequencies in the main text (other results are presented in the Supplemental
173 Material). I additionally evaluated how well those fitness effects maintain PSD in a finite
174 population of 10,000 individuals (Figure 1B). I also selected 1,000 random fitness effects for
175 each set of starting genotype frequencies and each dominance model to determine null
176 expectations under my model. Additional details are provided in the Supplemental Methods.

Table 2. Single proto-Y/proto-X chromosome genotype fitness values with different dominance scenarios.

	XX	XY	YY
additive			
$s_{ij}>0$	$1-s_{ij}$	$1-0.5s_{ij}$	1
$s_{ij}<0$	1	$1+0.5s_{ij}$	$1+s_{ij}$
dominant			
$s_{ij}>0$	$1-s_{ij}$	1	1
$s_{ij}<0$	1	$1+s_{ij}$	$1+s_{ij}$
recessive			
$s_{ij}>0$	$1-s_{ij}$	$1-s_{ij}$	1
$s_{ij}<0$	1	1	$1+s_{ij}$
overdominant (male only)			
$s_{ij}>0$	$1-s_{ij}$	1	$1-s_{ij}$
$s_{ij}<0$	$1+s_{ij}$	1	$1+s_{ij}$

Calculations are shown for determining single chromosome genotype fitness values from the selective effect s_{ij} of proto-Y chromosome j in sex i. Three genotypes are shown (XX, XY, and YY). The Y chromosome could either be Y^M or III^M, and the X chromosome could either be X or III.

Results

Dominance of fitness effects and the maintenance of polygenic sex determination

I find that the probability that PSD is maintained (with a frequency of each proto-sex chromosome >0.1% after 1,000 generations) is affected by the dominance of the fitness effects

185 of the Y^M and III^M proto-Y chromosomes (Supplemental Figures S1A, S2A). When fitness effects
186 of proto-Y chromosomes are additive, <5% of fitness values maintain PSD. In contrast, when
187 fitness effects of the proto-Y chromosomes are recessive, ~20% of fitness values maintain PSD.
188 Overdominant fitness effects are the most likely to maintain PSD, with >40% of fitness values
189 maintaining proto-sex chromosomes at a frequency >0.1% after 1,000 generations. When
190 additive, recessive, or overdominant (but not dominant) fitness effects maintain PSD, there is a
191 broad range of frequencies at which the proto-Y chromosomes (Y^M and III^M) can segregate as
192 polymorphisms (Figure 2; Supplemental Figure S3). Randomly chosen fitness effects, in
193 comparison, tend to produce one of three proto-Y chromosome frequency classes: 0 (loss of a
194 proto-Y, because a different XY system has taken over), 1 (fixation of the proto-Y chromosomes,
195 corresponding to a monogenic ZW system), and 0.25 (the expectation for a monogenic XY
196 system).

197 Most dominant fitness effects that appear to maintain PSD are paths to the eventual
198 fixation of the proto-Y chromosomes. More than 25% of dominant fitness maintain PSD for
199 1,000 generations (Supplemental Figures S1A, S2A), but most of those produce very high
200 frequencies of the Y^M and III^M chromosomes (Figure 2B; Supplemental Figure S3). A proto-Y
201 chromosome with a dominant fitness effect segregating at very high frequency is consistent with
202 a beneficial effect that masks the recessive variant (in this case the homologous proto-X),
203 allowing the proto-X chromosome to persist at a low frequency for a long time (Hartl and Clark
204 2007). To test this prediction, I selected fitness effects that maintain PSD for 1,000 generations,
205 and I ran simulations with those fitness effects for 1,000,000 generations. I considered a proto-Y
206 chromosome to reach “fixation” after 1,000,000 generations if it achieves a frequency >99.9%
207 (i.e., the same threshold for maintaining PSD after 1,000 generations). Consistent with the
208 prediction, >50% of dominant fitness effects that maintain PSD for 1,000 generations eventually

209 cause the Y^M or III^M proto-Y chromosomes to reach fixation within 1,000,000 generations,
210 regardless of the starting genotype frequencies (Supplemental Figures S4–S7). In contrast,
211 ≤1% of additive, recessive, or overdominant fitness effects of proto-Y chromosomes that
212 maintain PSD for 1,000 generations eventually result in fixation of a proto-Y chromosome after
213 1,000,000 generations. Despite the eventual fixation of proto-Y chromosomes with many
214 dominant fitness effects, there are a subset of dominant fitness effects that maintain Y^M and III^M
215 at lower frequencies, resembling those observed in natural populations (Figure 2B). For these
216 dominant fitness effects that produce proto-sex chromosome frequencies similar natural
217 populations, fixation of proto-Y chromosomes never occurs after 1,000,000 generations
218 (Supplemental Figures S4–S7). Therefore, dominant fitness effects can maintain PSD at
219 equilibrium, although most dominant fitness effects result in fixation or loss of the proto-Y
220 chromosomes. Below, I describe how dominant fitness effects that can or cannot maintain PSD
221 differ in their signatures of sexual antagonism.

222 ***Evaluating if selection pressures can produce proto-sex chromosome frequencies***
223 ***observed in natural populations***

224 Selecting only the 1,000 fitness effects that produce proto-sex chromosome frequencies
225 most similar to natural populations further affects the relationships between dominance and
226 proto-sex chromosome frequencies. For example, dominant, recessive, and overdominant
227 fitness effects can produce proto-sex chromosome frequencies that are more similar to those in
228 natural populations when compared to additive fitness effects (Figure 2; Supplemental Figures
229 S1, S2, S3). The poorer fit of additive models arises because they struggle to produce
230 frequencies of III^M and IV^F as low as those observed in the CA, NC, and NY populations (Figure
231 2; Supplemental Figure S3).

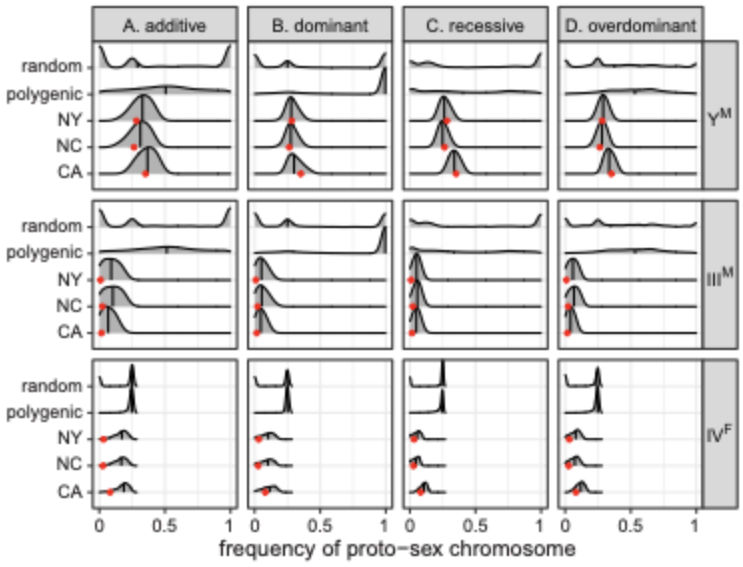


Figure 2. Smoothed histograms show the frequency of each proto-sex chromosome (Y^M, III^M, and IV^F) after 1,000 generations in simulations using either 1,000 random fitness arrays (random), fitness arrays that maintain PSD (polygenic), or the 1,000 best-fitting fitness arrays for each population (CA, NC, or NY). Simulations were started with equal frequencies of all genotypes. The vertical line within each histogram shows the median. Red dots show the observed proto-sex chromosome frequencies in each natural population. Fitness arrays were calculated assuming either **(A)** additive, **(B)** dominant, **(C)** recessive, or **(D)** overdominant fitness effects of the proto-Y chromosomes.

The low frequency of the III^M chromosome in the CA, NC, and NY populations has consequences for the ability of natural selection to maintain the polymorphism. As above, I define loss of the III^M chromosome (or fixation of the III chromosome) as a frequency below 0.1% (or above 99.9% for III). Additive fitness effects that maintain the III^M chromosome at frequencies similar to those in CA, NC, or NY for 1,000 generations often (9–17% of the time) result in the loss of III^M within 1,000,000 generations (Supplemental Figures S8, S9). In contrast, <7% of dominant, recessive, or overdominant fitness effects that maintain III^M at frequencies observed in natural populations for 1,000 generations lead to the eventual loss of III^M within 1,000,000 generations. III^M is especially likely to be lost within 1,000,000 generations when it is segregating at a lower frequency after 1,000 generations, regardless of the dominance of fitness effects (Supplemental Figures S8, S9). The Y^M chromosome, in comparison, segregates at a

250 higher frequency that III^M all three populations (Figure 2B). Y^M is lost in ≤2% of simulations after
251 1,000,000 generations when I apply fitness effects that maintain proto-sex chromosomes at
252 frequencies similar to those observed in natural populations, regardless of the dominance of
253 fitness effects (Supplemental Figure S10). I therefore conclude that low frequency proto-Y
254 chromosomes, such as III^M, are difficult to maintain as polymorphisms under the model I have
255 applied here. Moderate frequency proto-Y chromosomes, such as Y^M, on the other hand, can be
256 maintained under a variety of dominance scenarios.

257 The majority of fitness effects that maintain PSD cause IV^F to segregate at a frequency
258 of 25%, regardless of the dominance of the proto-Y chromosomes (Figure 2; Supplemental
259 Figure S3). A frequency of 25% is the expectation for a W chromosome in a randomly mating
260 population with monogenic ZW sex determination. Therefore, in a system with 2 proto-Y
261 chromosomes and one proto-W, PSD is expected to be maintained by the (near) fixation of the
262 ZW female genotype. In contrast, IV^F makes up 2–9% of all fourth chromosomes in the CA, NC,
263 and NY populations (Hamm and Scott 2008; Scott *et al.* 2013; Meisel *et al.* 2016). A subset of
264 fitness arrays in my simulations maintain IV^F at <25% for all dominance models, including those
265 that produce proto-sex chromosome frequencies most similar to those observed in natural
266 populations (Figure 2; Supplemental Figure S3). It is thus possible for PSD to be maintained
267 with a single proto-W chromosome at a frequency <25%. However, the frequency of IV^F is still
268 greater in simulated populations than in natural populations (Figure 2).

269 ***Selection pressures can maintain polygenic sex determination in finite populations***

270 I next used simulations to examine how well natural selection maintains PSD when
271 population sizes are finite (N=10^4 individuals). I calculated the proportion of simulations (out of
272 100) in which a given proto-sex chromosome reached fixation in a finite population for each

fitness array to estimate of the probability of fixation (P_{fix}). The P_{fix} value for a given proto-Y or proto-W chromosome (Y^M, III^M, or IV^F) is equal to the probability of loss (P_{loss}) of the homologous proto-X or proto-Z (X, III, or IV), and *vice versa*. I also simulated finite populations of the same size but without selection (i.e., genetic drift only). I used Fisher's exact test to determine if the P_{fix} or P_{loss} of a proto-sex chromosome is significantly different between populations with and without selection.

When a proto-sex chromosome is at low frequency, selection pressures that maintain PSD decrease P_{fix} and P_{loss} in finite populations, relative to populations without selection. For example, III^M is rare in all three populations, and it is the most likely proto-sex chromosome to be lost when population size is finite (Supplemental Figures S11, S12). In turn, selection pressures that maintain PSD, decrease P_{fix} of chromosome III relative to drift alone (Figure 3; Supplemental Figure S13), which is equivalent to decreasing P_{loss} of III^M. The IV^F proto-W chromosome is also at a low frequency in all three populations, and the maintenance of the IV^F polymorphism (i.e., the standard IV chromosome does not fix) is substantially greater when there is selection (Figure 3; Supplemental Figure S13). Therefore, when loss via drift is likely because a proto-sex chromosome is at low frequency (such as III^M and IV^F), selection pressures that maintain PSD decrease the probability of loss.

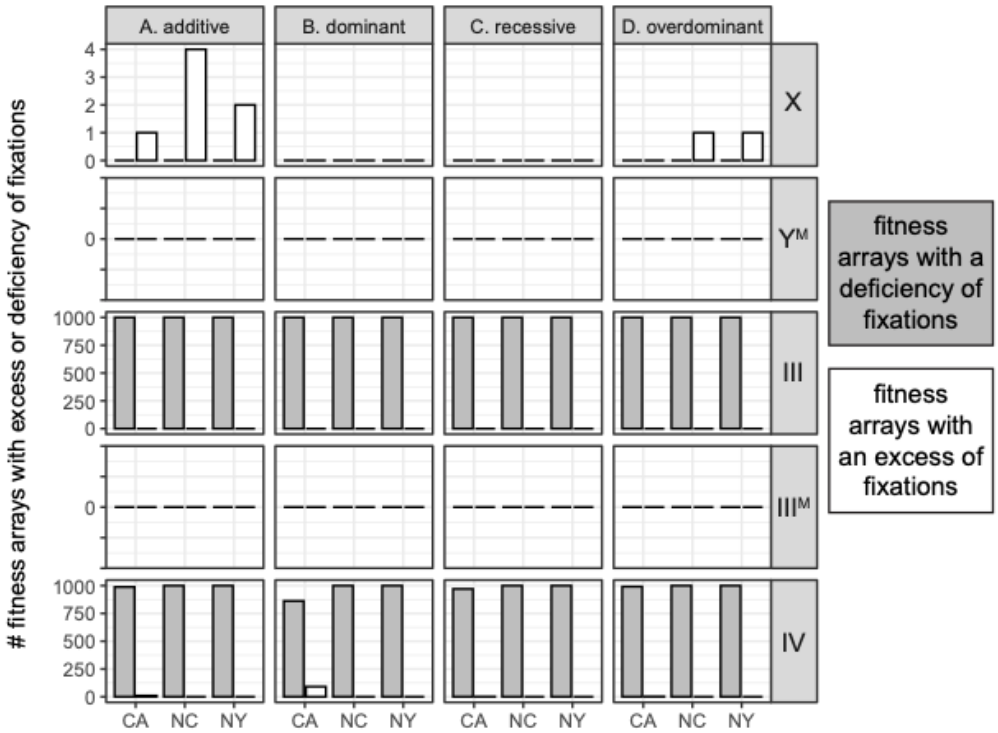


Figure 3. Bar plots show the number of fitness arrays that result in a deficiency (gray) or excess (white) of fixations of each proto-sex chromosome (X, Y^M, III, III^M, or IV) in a finite population, relative to simulations in which there are no fitness differences across proto-sex chromosomes (i.e., drift only). The fitness arrays are the 1,000 best-fitting fitness values for each population (CA, NC, or NY) when simulations were started with equal frequencies of all genotypes. Fitness effects of the proto-Y chromosomes are either **(A)** additive, **(B)** dominant, **(C)** recessive, or **(D)** overdominant.

A different effect is observed for sex chromosomes segregating at higher frequencies. The X and Y^M chromosomes rarely fix in finite populations with selection (Supplemental Figures S11, S12). Y^M is at a higher frequency than III^M and IV^F across all three populations (Meisel *et al.* 2016), and the high frequency of Y^M is likely responsible for its low P_{loss} within 1,000 generations. However, for a small fraction of fitness effects that maintain proto-sex chromosomes at frequencies similar to those in natural populations (<10%), P_{fix} for both the X and Y^M chromosomes is greater with selection and drift than under drift alone (Figure 3). The

slightly elevated P_{fix} of the X and Y^M chromosomes with selection is observed for all four dominance models (Supplemental Figure S13). Therefore, when fixation or loss is unlikely via drift (because the proto-Y chromosome is at high frequency), selection pressures that maintain PSD do not decrease P_{fix} or P_{loss} of a proto-sex chromosome in a finite population.

Sexual antagonism and the maintenance of polygenic sex determination

Sexually antagonistic selection is predicted to maintain PSD (Bull 1983; Rice 1986; van Doorn and Kirkpatrick 2007; Meisel *et al.* 2016). A negative intersexual fitness correlation is a hallmark of sexually antagonistic genetic variation (Rice and Chippindale 2001; Chippindale *et al.* 2001; Bonduriansky and Chenoweth 2009). I therefore inspected if there is a negative correlation between male and female fitness when PSD is maintained. Each of the eight male house fly proto-sex chromosome genotypes has a corresponding female genotype that differs only because females carry a copy of the IV^F proto-W chromosome and males do not (Table 1). There are two additional female genotypes (X/X; III/III; IV/IV and X/X; III/III; IV^F/IV) that do not have a corresponding male genotype because they do not have a proto-Y chromosome. To test for sexually antagonistic multi-chromosome genotypes, I calculated Spearman's rank order correlation of fitness between males and females (ρ_{MF}) for the eight pairs of male and female genotypes in each fitness array.

Additive fitness effects that maintain PSD (i.e., all proto-sex chromosomes at frequency >0.1%) tend to have $\rho_{MF} < 0$ (Figure 4A; Supplemental Figure S14). However, when proto-sex chromosome frequencies resemble those in natural populations and fitness effects are additive, ρ_{MF} can be either positive or negative. There is also no consistent signal of $\rho_{MF} < 0$ for random fitness effects, regardless of dominance (Figure 4; Supplemental Figure S14), demonstrating that $\rho_{MF} < 0$ is not an intrinsic property of the model. In comparison,

$\rho_{MF} < 0$ when I previously applied a model with additive fitness effects both within and across chromosomes (Meisel *et al.* 2016). In the model presented here, multi-chromosome genotype fitness is calculated as the product of single chromosome genotype fitness values, rather than by summing across chromosomes. Therefore, there is some evidence that additive fitness effects that maintain PSD result in $\rho_{MF} < 0$, but it depends on specifics of how the model is parameterized. I address the causes and effects of $\rho_{MF} < 0$ in more detail in the following section.

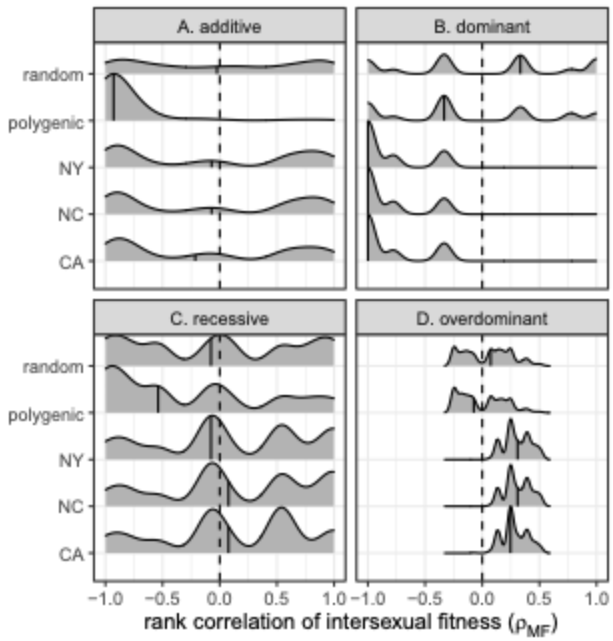


Figure 4. Smoothed histograms show distributions of intersexual fitness correlations (ρ_{MF}) for genotype fitness arrays used in simulations started with equal frequencies of all genotypes. Correlations are shown for the 1,000 best-fitting genotypic fitness arrays for the CA, NC, and NY populations. Correlations are also shown for all fitness arrays that maintain PSD (polygenic), as well as 1,000 random fitness arrays. Dashed vertical lines show a correlation of 0, and solid vertical lines within histograms show the median.

Intersexual fitness correlations tend to be negative ($\rho_{MF} < 0$) when fitness effects are dominant and proto-sex chromosome frequencies resemble those in natural populations

(Figure 4B; Supplemental Figure S14B). In contrast, ρ_{MF} can be positive or negative for dominant fitness effects that maintain PSD regardless of the proto-sex chromosome frequency. However, as noted above, many of the cases where dominant fitness effects appear to maintain PSD are instead on a path to fixation that has not yet been reached at 1,000 generations. When I only sample the dominant fitness effects that maintain PSD for 1,000,000 generations, I find that $\rho_{MF} < 0$ (Supplemental Figure S15). Therefore, assuming 1,000,000 generations adequately approximates the equilibrium state, $\rho_{MF} < 0$ when dominant fitness effects maintain PSD.

When fitness effects are overdominant or recessive, ρ_{MF} does not provide evidence for sexually antagonistic selection. First, there is no clear pattern of $\rho_{MF} < 0$ when fitness effects are recessive (Figure 4C; Supplemental Figure S14C). Second, overdominant fitness effects that produce the best-fitting genotype fitness arrays nearly all have $\rho_{MF} > 0$ (Figure 4D; Supplemental Figure S14D), but this result is difficult to interpret because overdominant effects produce a concave relationship between the number of copies of a proto-Y chromosome and male fitness (i.e., heterozygous males are defined as the most fit). Therefore, a simple rank order correlation (i.e., ρ_{MF}) does not adequately capture the relationship between male and female fitness when the proto-Y chromosomes have overdominant effects in males.

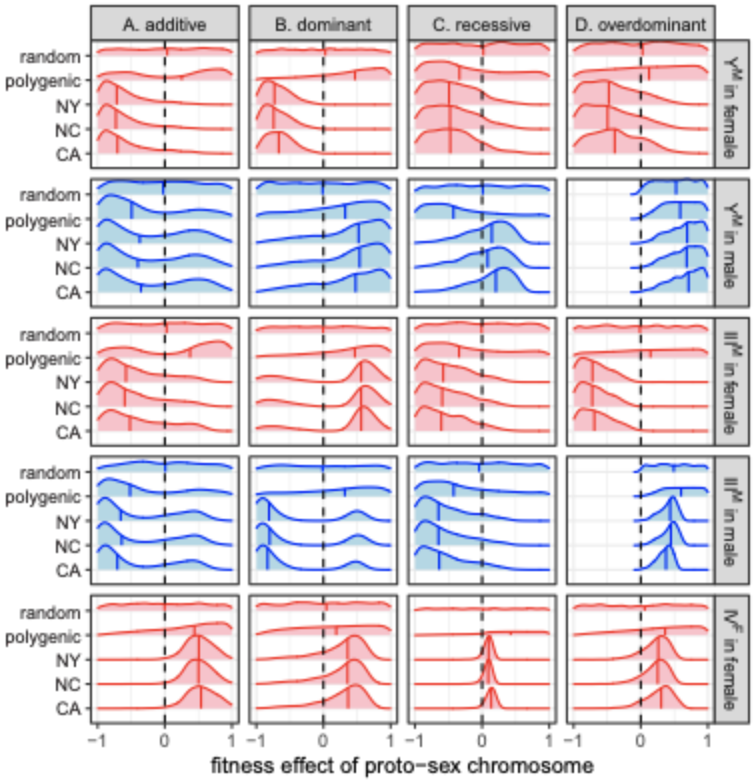
Negative intersexual fitness correlations are not caused by sexually antagonistic proto-Y chromosomes

The negative ρ_{MF} that I observe for additive and dominant fitness effects that maintain PSD (Figure 4; Supplemental Figure S14) are suggestive of sexually antagonistic selection. A potential cause of $\rho_{MF} < 0$ is sexually antagonistic effects of the proto-Y chromosomes if, for example, Y^M and III^M carry male-beneficial and female-detrimental alleles (Fisher 1931; Rice

364 1987). In contrast to this hypothesis, I do not observe a consistent signal of male-beneficial and

365 female-detrimental proto-Y chromosomes when fitness effects are additive or dominant

366 (Figure 5; Supplemental Figure S16).



367 **Figure 5**. Smoothed histograms show the distributions of fitness effects of each proto-sex chromosome in
368 each sex for 1,000 random fitness arrays (random), fitness arrays that maintain PSD (polygenic), or the
369 1,000 best-fitting fitness arrays for each population (CA, NC, or NY). Simulations were started with equal
370 frequencies of all genotypes. The vertical line within each histogram shows the median, and dashed lines
371 in each panel are at fitness value of 0. Fitness arrays were calculated assuming either (**A**) additive, (**B**)
372 dominant, (**C**) recessive, or (**D**) overdominant fitness effects of the proto-Y chromosomes.

373 There is some evidence that Y^M is male-beneficial and female-detrimental when

374 dominant fitness effects maintain PSD (Figure 5B; Supplemental Figure S16B). Additionally,

375 both Y^M and III^M often confer a greater fitness benefit to males than females when dominant

376 fitness effects maintain PSD (Supplemental Figures S17, S18, S19). However, III^M does not
377 tend to have male-beneficial and female-detrimental effects in the models with dominant fitness
378 effects (Figure 5B; Supplemental Figure S16B). Therefore, when dominant fitness effects
379 maintain PSD, the multi-chromosome genotypes tend to have sexually antagonistic fitness
380 effects (Figure 4; Supplemental Figures S14, S15), but the individual chromosomes are not
381 necessarily sexually antagonistic themselves.

382 In contrast to most expectations, additive fitness effects that maintain PSD often have
383 female-beneficial and male-detrimental effects of the proto-Y chromosomes (Figure 5A;
384 Supplemental Figure S16A). In addition, the proto-Y chromosomes do not consistently confer a
385 greater fitness benefit to males than females when PSD is maintained by additive fitness effects
386 (Supplemental Figures S17, S18). Female-beneficial and male-detrimental Y chromosomes are
387 opposite of the expected effects under most models of sex chromosome evolution (Rice 1987,
388 1996). However, some previous population genetic modeling has suggested the possibility of
389 "feminization" of the Y chromosome (Cavoto *et al.* 2018).

390 Overdominant fitness effects that maintain proto-sex chromosomes at frequencies
391 similar to those observed in natural populations have the strongest evidence of male-beneficial
392 and female-detrimental proto-Y chromosomes (Figure 5D; Supplemental Figures S16D, S17D,
393 S18D). The overdominant model is constrained to require male-beneficial effects of the proto-Y
394 chromosomes in heterozygotes, but female fitness effects can be beneficial or deleterious (see
395 Supplemental Methods). Despite the full range of possible female fitness effects in the
396 overdominant model, when proto-sex chromosomes are at frequencies similar to those in the
397 CA, NC, or NY populations, the proto-Y chromosomes are almost always female-deterious
398 (Figure 5D; Supplemental Figures S16D). These sexually antagonistic (female-deleterious and
399 male-beneficial) effects of the Y^M and III^M proto-Y chromosomes in the overdominant model

400 does not cause negative intersexual correlations of multi-chromosomal genotype fitness values
401 (Figure 4; Supplemental Figure S14) because heterozygous males are defined as the most fit.

402 ***Female-beneficial effects of the proto-W chromosome***

403 The IV^F proto-W chromosome is nearly always beneficial to females when PSD is
404 maintained. This is the case regardless of the dominance of fitness effects of the proto-Y
405 chromosomes, whether or not proto-sex chromosomes frequencies are similar to those in
406 natural populations, and independently of the genotype frequencies used to start the simulations
407 (Figure 5; Supplemental Figure S16). There also tends to be a positive correlation between the
408 fitness effect and frequency of IV^F when PSD is maintained (Supplemental Figures S20, S21). A
409 positive correlation indicates that the more female-beneficial the IV^F chromosome, the higher its
410 frequency. The only exception to this rule is when dominant fitness effects maintain PSD for
411 1,000 generations; however, as described above, these cases are on a path to fixation and
412 therefore not at equilibrium

413 **Discussion**

414 ***Dominance of fitness effects, sexually antagonistic sex chromosomes, and sexually***
415 ***antagonistic genotypes***

416 I used multiple approaches to test if there is evidence of sexually antagonistic selection
417 when PSD is maintained in my simulations. Sexual antagonism has previously been identified
418 as a key component of the maintenance of PSD within populations (Bull 1983; Rice 1986; van
419 Doorn and Kirkpatrick 2007; Meisel *et al.* 2016). A negative inter-sexual correlation of fitness
420 across genotypes is a hallmark of sexually antagonistic selection (Rice and Chippindale 2001;
421 Chippindale *et al.* 2001; Bonduriansky and Chenoweth 2009). I find strong evidence for

$\rho_{MF}<0$ when dominant fitness effects maintain PSD (Figure 4B; Supplemental Figure S15). There is also some evidence that additive fitness effects that maintain PSD tend to have negative ρ_{MF}, although it is dependent on the frequency at which the proto-sex chromosomes are maintained (Figure 4A; Supplemental Figure S14A). However, neither dominant nor additive fitness effects that maintain PSD have male-beneficial and female-detrimental effects of all individual proto-Y chromosomes (Figure 5; Supplemental Figure S16). Therefore, sexually antagonistic multi-chromosomal genotypes that maintain PSD can be emergent properties of cumulative fitness effects across chromosomes, without each chromosome having the same sexually antagonistic effects. This is evidence for a decoupling between single chromosome sexually antagonistic effects and sexual antagonism across multi-chromosome genotypes. It also suggests that testing for sexually antagonistic effects of individual proto-sex chromosomes may not be a sufficient assay of the role sexual antagonism plays in the maintenance of complex, multi-chromosomal PSD.

It has generally been observed that the dominance of fitness effects is an important parameter affecting the ability sexually antagonistic selection to maintain genetic variation (Connallon and Chenoweth 2019). For example, a sexually antagonistic allele is more likely to be maintained as a polymorphism if the sex-specific deleterious effects are recessive (Kidwell *et al.* 1977). In contrast, when fitness effects are dominant, stronger selection pressures are required to maintain the polymorphism (Kidwell *et al.* 1977). My work presented here contributes to the special case where the alleles under selection are on proto-sex chromosomes (as opposed to autosomes or heteromorphic sex chromosomes). Consistent with Kidwell *et al.* (1977), I find that dominant fitness effects that maintain PSD do indeed have strong selection in favor of or against proto-Y chromosomes in one sex (Figure 5; Supplemental Figure S16).

445 In most of the models I consider here, the dominance of fitness effects of the proto-Y
446 chromosomes is the same in both sexes. However, the dominance of fitness effects may be
447 sex-specific (Barson *et al.* 2015; Grieshop and Arnqvist 2018). Such dominance reversals could
448 be important for the maintenance of sexually antagonistic alleles (Spencer and Priest 2016;
449 Connallon and Chenoweth 2019), including at sex-linked loci (Fry 2010). Therefore, it is
450 possible that sex-specific dominance could create a broader range of fitness values than I
451 observe to maintain PSD via sexually antagonistic selection. Consistent with this prediction, I
452 observed PSD maintained most frequently when fitness effects of proto-Y chromosomes are
453 overdominant in males and additive in females (see below for more discussion of overdominant
454 fitness effects). Future work should further examine how sex-specific dominance affects the
455 maintenance of PSD.

456 Sexually antagonistic proto-Y chromosomes require there to be segregating sexually
457 antagonistic genetic variation. Male-beneficial and female-deleterious alleles are likely to
458 accumulate on Y chromosomes because of their male-limited inheritance (Rice 1984). It is not
459 clear, however, if that prediction applies when the Y chromosome can be transmitted through
460 females in a complex PSD system. A more appropriate point of comparison for PSD systems
461 may be autosomal and X-linked sexually antagonistic genetic variation, of which there is
462 evidence for a substantial amount (Rice 1992; Chippindale *et al.* 2001; Calsbeek and Sinervo
463 2004; Fedorka and Mousseau 2004; Foerster *et al.* 2007; Brommer *et al.* 2007; Innocenti and
464 Morrow 2010). The fact that such variation exists in a broad range of taxa suggests that sexually
465 antagonistic proto-Y chromosomes may be possible in a PSD system.

466 My results are consistent with theory and data that suggest sexually antagonistic
467 selection is important for the early evolution of sex chromosomes. For example, van Doorn and
468 Kirkpatrick (2007, 2010) showed that selection on linked sexually antagonistic alleles can favor

469 a new sex determining locus because sex-limited inheritance can resolve the sexual conflict.
470 Empirical studies have also identified sexually antagonistic variants on young Y and W
471 chromosomes (Lindholm and Breden 2002; Roberts *et al.* 2009). In addition, once a new sex
472 chromosome is established, fixation of additional alleles with sex-specific beneficial effects can
473 be favored on the sex-specific Y or W chromosome (Abbott *et al.* 2017). It remains to be
474 determined, however, whether young sex chromosomes are enriched for sexually antagonistic
475 variants, if those variants are maintained by balancing selection, and if the sexually antagonistic
476 alleles contribute to the maintenance of PSD. Investigating these problems could follow the
477 approaches previously developed to test if balancing selection maintains sexually antagonistic
478 alleles throughout the genome (e.g., Dutoit *et al.* 2018; Ruzicka *et al.* 2019).

479 ***Overdominance and the maintenance of polygenic sex determination***

480 Overdominant fitness effects of proto-Y chromosomes in males are more likely to
481 maintain PSD than any other dominance scenario that I tested. Nearly half of the 4,000,000
482 overdominant fitness effects that I tested maintain all three proto-sex chromosomes at a
483 frequency >0.1% for at least 1,000 generations (Supplemental Figures S1A, S2A). In addition,
484 nearly all of those overdominant fitness effects that maintain PSD for 1,000 generations
485 continue to do so for at least 1,000,000 generations (Supplemental Figures S4–S7), suggesting
486 they are indeed stable polymorphisms at equilibrium. The frequencies with which overdominant
487 fitness effects can maintain multiple polymorphic proto-sex chromosomes covers the full range
488 of possible values, from close to 0 to nearly 1 (Figure 2D; Supplemental Figure S3D).
489 The extent of overdominant genetic variation—and balancing selection more
490 generally—has been a topic of considerable debate in population genetics (Crow 1987; Delph
491 and Kelly 2014; Fijarczyk and Babik 2015), with a few classic examples of heterozygote
492 advantage (e.g., Dobzhansky 1947; Allison 1956). Contemporary evidence for overdominant

493 fitness effects in natural populations is mixed, including some work that suggests
494 overdominance is rare (Andrés *et al.* 2009; Sellis *et al.* 2011, 2016; Hedrick 2012; Bitarello *et al.*
495 2018). Notably, in platyfish, which has a single chromosome PSD system, heterozygous males
496 (XY) and females (WY or WX) have higher fitness than homozygotes (Orzack *et al.* 1980),
497 providing evidence for overdominant fitness effects of sex chromosomes. Future work should
498 evaluate the prevalence of proto-sex chromosomes with overdominant fitness effects in order to
499 determine if the appropriate genetic variation exists for overdominance to maintain PSD more
500 generally.

501 Regardless of the prevalence of overdominance in natural populations, it is one of the
502 most straightforward mechanisms by which any polymorphism can be maintained (Hartl and
503 Clark 2007). In my overdominant model, I assume that the proto-Y chromosomes carry
504 male-beneficial additive or dominant alleles (Rice 1984, 1992), along with recessive deleterious
505 alleles that have opposing effects equal in magnitude to the beneficial alleles (Charlesworth and
506 Charlesworth 2000). Testing if these assumptions are biologically realistic would require
507 measuring the magnitude and dominance of beneficial and deleterious alleles on proto-Y
508 chromosomes. I also assume that the two homozygous genotypes (i.e., XX and YY) have equal
509 fitness (Table 2), which may not be biologically realistic. Future work should consider how
510 different fitness values of the two homozygotes affects the ability of overdominance to maintain
511 polygenic sex determination.

512 When overdominant fitness effects in males maintain proto-sex chromosomes at
513 frequencies most similar to those observed in natural populations, I find that they do so with
514 female-deterious (additive) effects of the proto-Y chromosomes (Figure 5D; Supplemental
515 Figure S16D). This is the strongest evidence I observe for sexually antagonistic fitness effects
516 of individual proto-Y chromosomes (as opposed to multi-chromosomal genotypes) in any

517 dominance scenario. It is also consistent with previous work demonstrating that overdominance
518 in one sex can maintain genetic variants at individual loci even if there is directional selection
519 against one allele in the other sex (Kidwell *et al.* 1977). In contrast to the additive and dominant
520 fitness effects, there is no evidence for $\rho_{MF}<0$ when overdominant fitness effects maintain
521 PSD (Figure 4; Supplemental Figure S14). This provides additional evidence for a decoupling
522 between single chromosome sexually antagonistic effects and sexual antagonism across
523 multi-chromosome genotypes.

524 ***Recombination on the proto-sex chromosomes and Y-linked alleles***

525 My model makes assumptions about recombination on the sex chromosomes that may
526 affect my conclusions about the maintenance of PSD. Specifically, I assume that there is no
527 recombination between the male- or female-determining locus on each proto-Y or proto-W
528 chromosome and the allele(s) under selection. Suppressed recombination is predicted to evolve
529 in order to ensure that a sex-determining locus and sexually antagonistic alleles are inherited
530 together on a young Y or W chromosome (Rice 1987) Suppressed recombination can also
531 facilitate the invasion of a new sex determiner by increasing the effects of indirect selection on
532 linked sexually antagonistic alleles, and it can further stabilize an ancestral sex chromosome
533 through similar indirect effects (van Doorn and Kirkpatrick 2007, 2010). X-Y and Z-W
534 recombination may be suppressed by chromosomal inversions that create tight genetic linkage
535 between the sex-determining locus and sexually antagonistic alleles (Bergero and Charlesworth
536 2009; Wright *et al.* 2016).

537 While there is no direct evidence for inversions or other recombination suppressors on
538 the house fly sex chromosomes, male meiosis in flies is thought to occur without crossing over
539 (Gethmann 1988). A lack of crossing over in males would prevent X-Y recombination in males

540 without the need for sex-chromosome-specific modifiers. However, there is evidence for male
541 recombination in house fly (Feldmeyer *et al.* 2010), which opens up the possibility for X-Y
542 recombination. Furthermore, in the absence of Y-linked suppressors, X-Y recombination could
543 also occur in the multiple female genotypes that carry a proto-Y chromosome (Table 1).

544 There are at least three consequences of X-Y or Z-W recombination that could affect
545 how sex-specific selection maintains PSD in my model. First, my model assumes that all copies
546 of each proto-Y and proto-W chromosome have identical fitness effects. This assumption is
547 consistent with population genetics theory that predicts Y-linked variants cannot segregate as
548 protected polymorphisms when there is no X-Y recombination (Clark 1987). In contrast to this
549 prediction, however, there are numerous examples of Y-linked polymorphisms with phenotypic
550 effects in *Drosophila*, where the X and Y do not recombine (Clark 1990; Zhang *et al.* 2000;
551 Lemos *et al.* 2008, 2010; Griffin *et al.* 2015; Brown *et al.* 2020). Relevant to the house fly, these
552 examples include Y-linked variation with temperature-dependent effects that are distributed
553 across the species' geographic range (Rohmer *et al.* 2004). Future work could incorporate
554 context-dependent variation in fitness effects of each proto-Y and proto-W chromosome into my
555 model to assess how segregating polymorphisms on the proto-sex chromosomes affect the
556 maintenance of PSD.

557 A second consequence of X-Y recombination could affect how overdominant fitness
558 effects maintain PSD in my model. I assume that overdominance arises from proto-Y
559 chromosomes that carry recessive deleterious alleles. This assumption is based on the
560 prediction that suppressed X-Y recombination leads to the "degeneration" of the Y chromosome
561 via accumulation of (recessive) deleterious alleles because of Muller's ratchet and hitchhiking
562 (Charlesworth and Charlesworth 2000; Bachtrog 2013). In contrast, X-Y recombination in males
563 and/or females could help purge recessive deleterious mutations from the Y^M and III^M

564 chromosomes (Muller 1964; Felsenstein 1974). Moreover, there are multiple house fly
565 genotypes that are homozygous for a proto-Y chromosome (Table 1), which would expose
566 recessive deleterious mutations to selection. Selection in these proto-Y chromosome
567 homozygotes would further remove recessive deleterious alleles from populations
568 (Charlesworth *et al.* 1990; Barrett and Charlesworth 1991). Therefore, while overdominant
569 fitness effects are likely to maintain PSD, proto-Y chromosomes may not possess the necessary
570 alleles for such selection pressures to act upon. However, overdominance can also occur by
571 other mechanisms besides degeneration of the Y chromosome and independent of
572 accumulation of recessive deleterious Y-linked alleles (Sellis *et al.* 2011; Connallon and Clark
573 2014). These other causes of overdominance may allow for heterozygote fitness advantages to
574 maintain PSD without degeneration of the proto-Y chromosomes.
575 Third, X-Y recombination would introduce X-linked alleles onto the proto-Y
576 chromosomes. Male-limited inheritance of a Y chromosome is expected to lead to the
577 accumulation of male-beneficial alleles in Y-linked genes, even if they have female-deleterious
578 effects (Rice 1987, 1996). Conversely, female-biased inheritance of the X chromosome is
579 expected to result in stronger selection on X-linked alleles in females if those alleles are partially
580 dominant or segregating at moderate frequencies (Rice 1984; Charlesworth *et al.* 1987; Orr and
581 Betancourt 2001), potentially feminizing the X chromosome. If there is X-Y recombination, those
582 feminized X-linked alleles would be continuously introduced onto the Y chromosome, possibly
583 feminizing the Y as well (Cavoto *et al.* 2018). This could provide a biological mechanism that
584 allows for female-beneficial and male-detrimental effects of the proto-Y chromosomes that I
585 predict when additive fitness effects maintain PSD (Figure 5A; Supplemental Figure S16A).

586 ***Low frequency proto-sex chromosomes are unlikely to be maintained by selection***

587 My simulations suggest that it is difficult for selection to maintain proto-sex
588 chromosomes at low frequencies within populations. For example, the III^M proto-Y represents
589 <3% of all third chromosomes in each of the three natural populations I considered (Hamm *et al.*
590 2005; Meisel *et al.* 2016). In my simulations, many of the selection pressures that maintain III^M
591 at a frequency similar to those observed in each of the populations for 1,000 generations
592 eventually allow for the loss of III^M within 1,000,000 generations (Supplemental Figures S8, S9).
593 Additive fitness effects are especially likely to allow for the loss of III^M. In addition, when PSD is
594 maintained with proto-sex chromosomes at frequencies similar to natural populations, III^M tends
595 to be at a higher frequency in my simulations than in any of the actual populations (Figure 2;
596 Supplemental Figure S3). III^M is also frequently lost when population size is finite, even when
597 selection pressures exist that should maintain the polymorphism (Supplemental Figures S11,
598 S12). IV^F is also found at a low frequency (2–9% of all fourth chromosomes in the natural
599 populations); it is similarly found at a higher frequency in the simulations than in the actual
600 populations (Figure 2; Supplemental Figure S3), and it is often lost in finite populations with
601 selection (Supplemental Figures S11, S12).

602 The behavior of III^M and IV^F in my simulations suggests that natural selection within
603 populations may not be sufficient to maintain low frequency proto-sex chromosomes, and other
604 factors may be required. Variation in proto-sex chromosome frequencies across house fly
605 populations are suggestive of what those factors may be. In particular, spatially variable,
606 temporally fluctuating, and other heterogeneous selection pressures can contribute to the
607 maintenance of genetic variation across populations (Levene 1953; Hedrick 2006), including
608 maintaining PSD (Bateman and Anholt 2017). The house fly Y^M and III^M proto-Y chromosomes
609 are distributed along north-south clines on multiple continents (Denholm *et al.* 1986; Tomita and

610 Wada 1989; Hamm *et al.* 2005; Kozielska *et al.* 2008), suggesting spatially heterogeneous
611 selection pressures may be important for maintaining PSD across house fly populations.
612 Migration between these populations could continuously introduce rare proto-Y and proto-W
613 chromosomes, potentially overwhelming selection pressures that would otherwise allow for the
614 loss of those proto-sex chromosomes within populations (Lenormand 2002; Tigano and Friesen
615 2016). Future work should directly model the effect of migration across populations with different
616 selection pressures on the maintenance of PSD within populations.

617 ***Proto-sex chromosomes are reminiscent of pseudoautosomal regions, with some***
618 ***notable differences***

619 Proto-sex chromosomes have some superficial similarities to pseudoautosomal regions
620 (PARs) of heteromorphic sex chromosomes, within which there is X-Y (or Z-W) recombinatioin
621 (Otto *et al.* 2011). Recombination in the PAR moves Y-linked alleles onto the X where they can
622 be exposed to selection in females, reminiscent of how proto-Y chromosomes can be carried by
623 females in a PSD system. Notably, PARs are capable of maintaining genetic variation, including
624 sexually antagonistic alleles, under broader conditions than autosomes or even
625 non-recombining regions of sex chromosomes (Jordan and Charlesworth 2012). These unique
626 evolutionary dynamics of PARs are limited to loci close to the boundary with the
627 non-recombining region of the sex chromosomes (Charlesworth *et al.* 2014). There is evidence
628 for sexually antagonistic alleles in PARs from plants (Delph *et al.* 2010; Spigler *et al.* 2011) and
629 fish (Tripathi *et al.* 2009; Kitano *et al.* 2009), but it is not yet clear whether PARs are enriched for
630 sexually antagonistic alleles. Nonetheless, the possibility that PARs and proto-Y chromosomes
631 are both hotspots of sexually antagonistic variation suggests they may experience similar
632 selection pressures.

633 Despite the superficial similarities, there are important differences that make PARs
634 imperfect analogs to proto-sex chromosomes. For example, when there is an epistatic dominant
635 female determining proto-W chromosome (as in house fly), the proto-Y chromosome itself can
636 be transmitted through females. In contrast, the Y PAR cannot ever be carried by females.
637 Instead, Y-linked PAR alleles must first recombine onto the X PAR in order to be carried by
638 females. Moreover, males can be homozygous for the proto-Y chromosome, which is
639 superficially similar to a male carrying an X PAR that has an ancestrally Y-linked allele.
640 However, as above, this ancestral Y allele on the X PAR must recombine onto the X PAR,
641 whereas, when there is PSD, homozygosity for the proto-Y occurs without X-Y recombination.
642 Additional theoretical analyses are required to directly compare the invasion dynamics, fixation
643 probabilities, and regions of stable polymorphisms across PARs and proto-sex chromosomes.

644 ***Generalizing to even more complex polygenic sex determination systems***

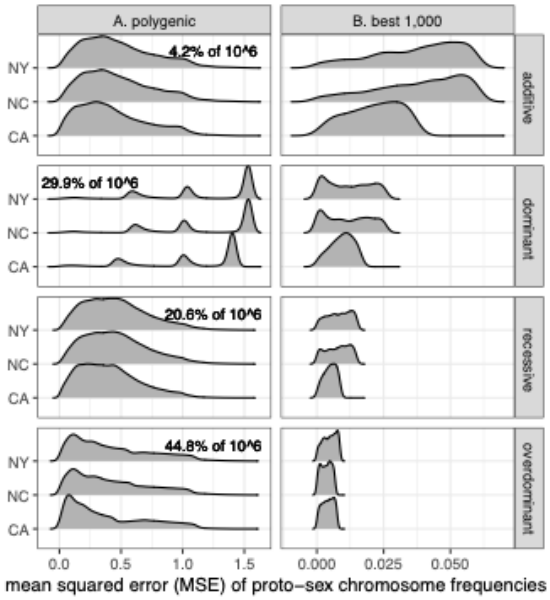
645 I anticipate that many of the general patterns I observe here would be similar in other
646 complex PSD systems with at least three different proto-sex chromosomes. For example, in a
647 system with more than two proto-Y chromosomes, I expect to observe similar effects of
648 dominance on the maintenance of PSD and sexual antagonism as in the system I modeled with
649 two proto-Y chromosomes. In addition, if there are multiple proto-W chromosomes and a single
650 (epistatic) proto-Y (i.e., the opposite of the house fly system), I would expect similar patterns as
651 I observe in the house fly system, but with the sexes reversed. These expectations could be
652 tested in future work. It is not clear, however, what would be expected if there were both multiple
653 proto-W and multiple proto-Y chromosomes in a population. It is worth noting that polygenic
654 systems with many sex-determining loci are expected to be evolutionarily unstable (Rice 1986).
655 Zebrafish may represent a promising model organism for combining empirical and theoretical

656 approaches to make additional progress in understanding the maintenance of many sex
657 determining loci (Liew *et al.* 2012; Wilson *et al.* 2014).

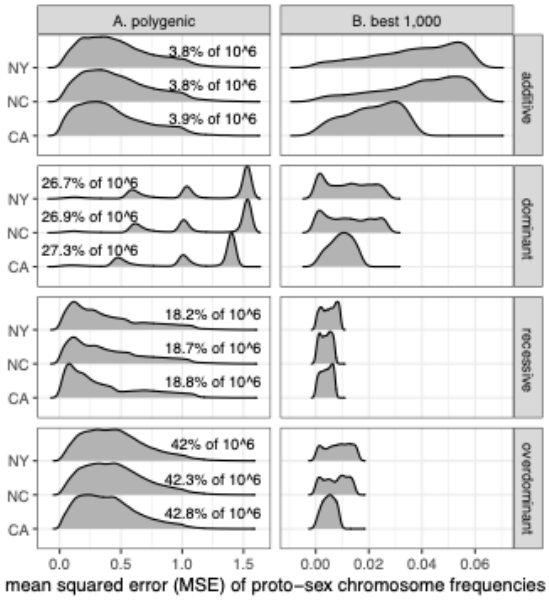
658 ***Conclusions***

659 My results demonstrate that the maintenance of PSD depends on the dominance of
660 fitness effects, which are further predictive of the sexually antagonistic effects of proto-sex
661 chromosomes and genotypes. Notably, overdominant fitness effects are more likely to maintain
662 PSD than fitness effects with other types of dominance. However, that conclusion requires at
663 least one of two important assumptions: genetic variation with overdominant fitness effects must
664 exist in natural populations, or proto-Y chromosomes must carry recessive deleterious alleles (in
665 addition to additive or dominant beneficial alleles). My results also suggest that sexually
666 antagonistic multi-chromosomal genotypes should be most prominent when dominant fitness
667 effects (and to a lesser extent additive fitness effects) maintain PSD. This sexual antagonism is
668 an emergent property of multi-chromosomal genotypes, and not intrinsic to the fitness effects of
669 all individual proto-Y chromosomes. In contrast, when overdominant fitness effects in males
670 maintain PSD, the proto-Y chromosomes tend to be male-beneficial and female-detrimental, but
671 the multi-chromosomal genotypes do not have opposing fitness effects in males and females.
672 Lastly, future work is needed to evaluate how X-Y (and Z-W) recombination, heterogeneous
673 selection pressures, and migration across demes affect the ability of sex-specific selection
674 pressures to maintain complex PSD, including in systems with other combinations of proto-sex
675 chromosomes.

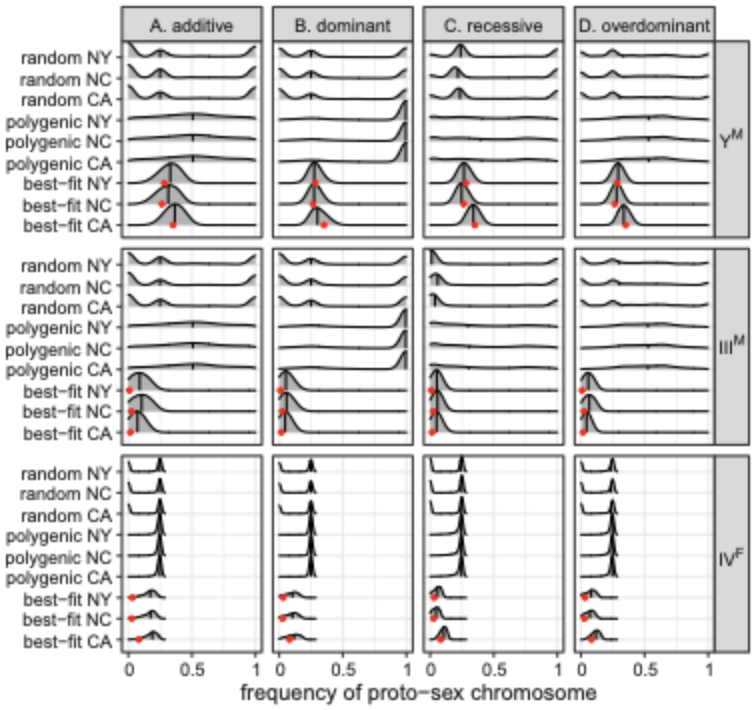
Supplemental Figures



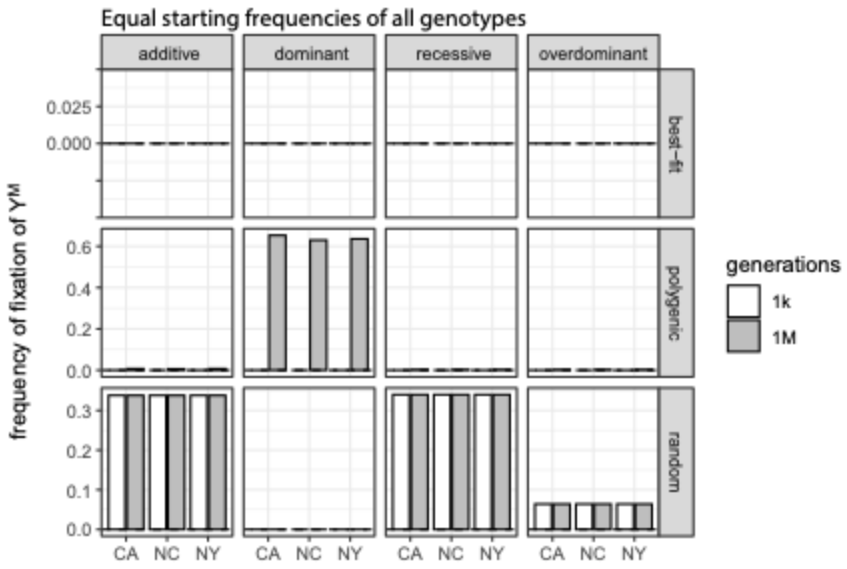
Supplemental Figure S1. Smoothed histograms show the distributions of mean squared error (MSE) values for fitness arrays that maintain PSD (i.e., all proto-sex chromosomes at a frequency >0.1%). MSE is calculated based on the frequencies of Y^M, III^M, and IV^F found in either CA, NC, or NY. Simulations were started with equal frequencies of all genotypes. Fitness arrays are based on additive, dominant, recessive, or overdominant fitness effects of Y^M and III^M. **(A)** Distributions of MSE are shown for all fitness arrays that maintain PSD, with the percent of those arrays that maintain PSD shown in each panel. **(B)** Distributions of MSE are shown for the 1,000 best-fitting arrays with the lowest MSE.



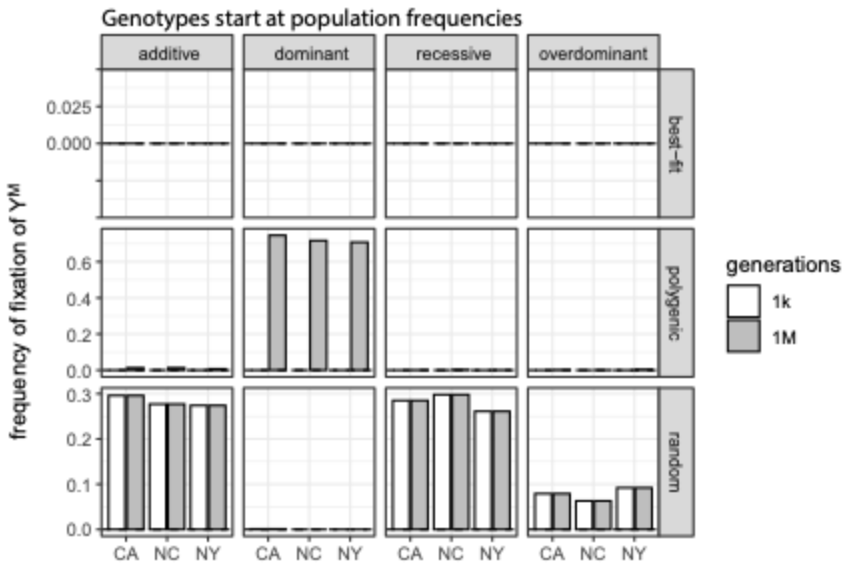
Supplemental Figure S2. Smoothed histograms show the distributions of mean squared error (MSE) values for fitness arrays that maintain PSD (i.e., all proto-sex chromosomes at a frequency >0.1%). MSE is calculated based on the frequencies of Y^M, III^M, and IV^F found in either CA, NC, or NY. Simulations were started with genotype frequencies observed in natural populations. Fitness arrays are based on additive, dominant, recessive, or overdominant fitness effects of Y^M and III^M. **(A)** Distributions of MSE are shown for all fitness arrays that maintain PSD, with the percent of those arrays that maintain PSD shown in each panel. **(B)** Distributions of MSE are shown for the 1,000 best-fitting arrays with the lowest MSE.



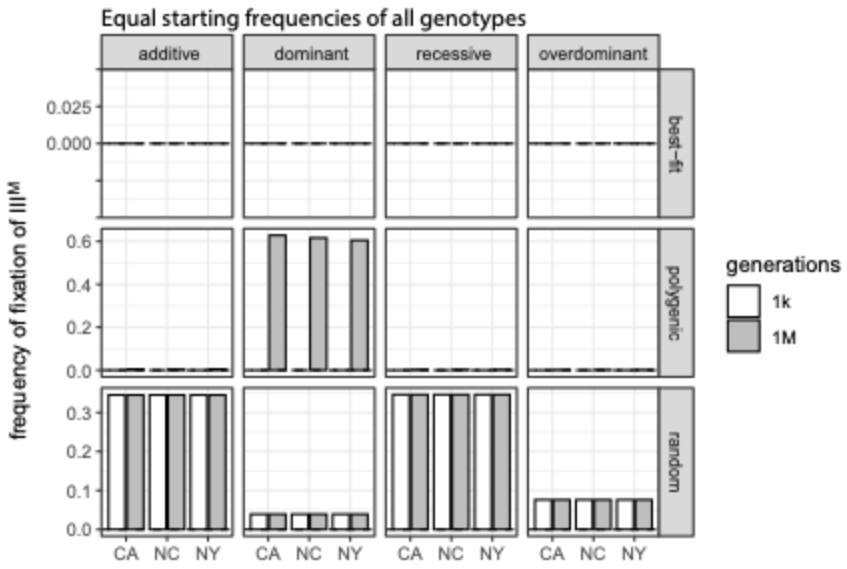
Supplemental Figure S3. Smoothed histograms show the frequency of each proto-sex chromosome (Y^M, III^M, and IV^F) after 1,000 generations in simulations using either 1,000 random fitness arrays (random), fitness arrays that maintain PSD (polygenic), or the 1,000 best-fitting fitness arrays for each population (CA, NC, or NY). Simulations were started with genotype frequencies observed in each population. The vertical line within each histogram shows the median. Red dots show the observed proto-sex chromosome frequencies in each natural population. Fitness arrays were calculated assuming either (**A)** additive, **(B)** dominant, **(C)** recessive, or **(D)** overdominant fitness effects of the proto-Y chromosomes.



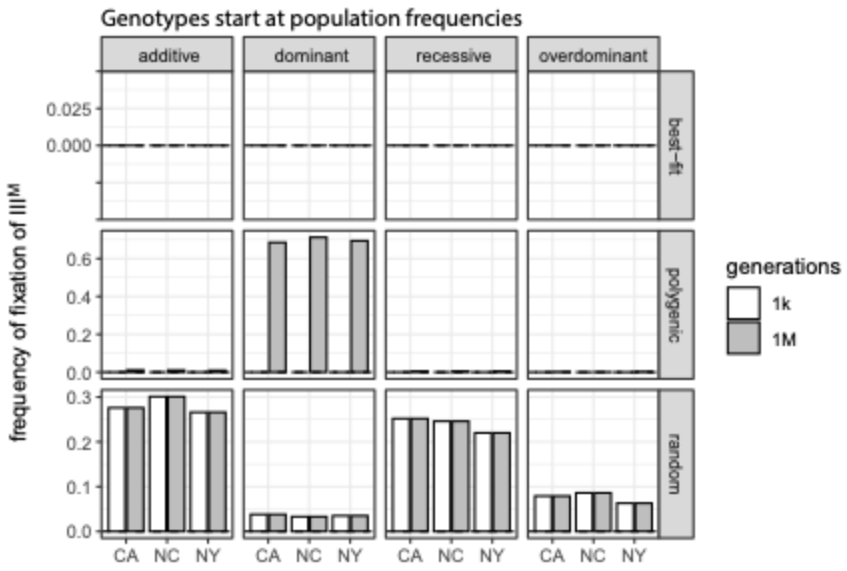
Supplemental Figure S4. The frequency of fixation of the Y^M proto-Y chromosome after 1,000 generations (white) and 1,000,000 generations (gray) is shown for the 1,000 best-fitting genotype fitness arrays (best-fit), 1,000 fitness arrays that maintain PSD (polygenic), and 1,000 randomly chosen fitness arrays (random) in each population (CA, NC, or NY). Best-fitting fitness values and fitness values that maintain PSD were identified using simulation in which all genotypes started at equal frequencies. Fitness effects of each proto-Y chromosome were either additive, dominant, recessive, or overdominant.



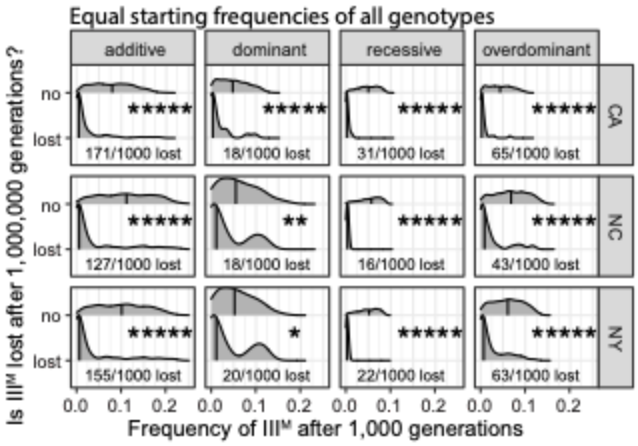
Supplemental Figure S5. The frequency of fixation of the Y^M proto-Y chromosome after 1,000 generations (white) and 1,000,000 generations (gray) is shown for the 1,000 best-fitting genotype fitness arrays (best-fit), 1,000 fitness arrays that maintain PSD (polygenic), and 1,000 randomly chosen fitness arrays (random) in each population (CA, NC, or NY). Best-fitting fitness values and fitness values that maintain PSD were identified using simulation in which all genotypes started at the frequencies observed in each population. Fitness effects of each proto-Y chromosome were either additive, dominant, recessive, or overdominant.



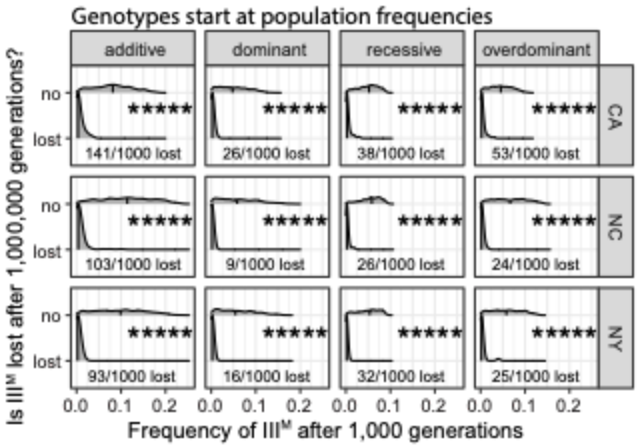
Supplemental Figure S6. The frequency of fixation of the III^M proto-Y chromosome after 1,000 generations (white) and 1,000,000 generations (gray) is shown for the 1,000 best-fitting genotype fitness arrays (best-fit), 1,000 fitness arrays that maintain PSD (polygenic), and 1,000 randomly chosen fitness arrays (random) in each population (CA, NC, or NY). Best-fitting fitness values and fitness values that maintain PSD were identified using simulation in which all genotypes started at equal frequencies. Fitness effects of each proto-Y chromosome were either additive, dominant, recessive, or overdominant.



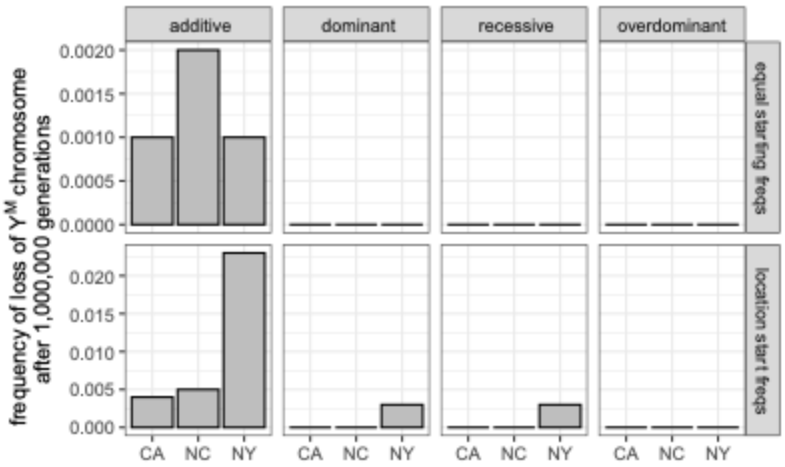
Supplemental Figure S7. The frequency of fixation of the III^M proto-Y chromosome after 1,000 generations (white) and 1,000,000 generations (gray) is shown for the 1,000 best-fitting genotype fitness arrays (best-fit), 1,000 fitness arrays that maintain PSD (polygenic), and 1,000 randomly chosen fitness arrays (random) in each population (CA, NC, or NY). Best-fitting fitness values and fitness values that maintain PSD were identified using simulation in which all genotypes started at the frequencies observed in each population. Fitness effects of each proto-Y chromosome were either additive, dominant, recessive, or overdominant.



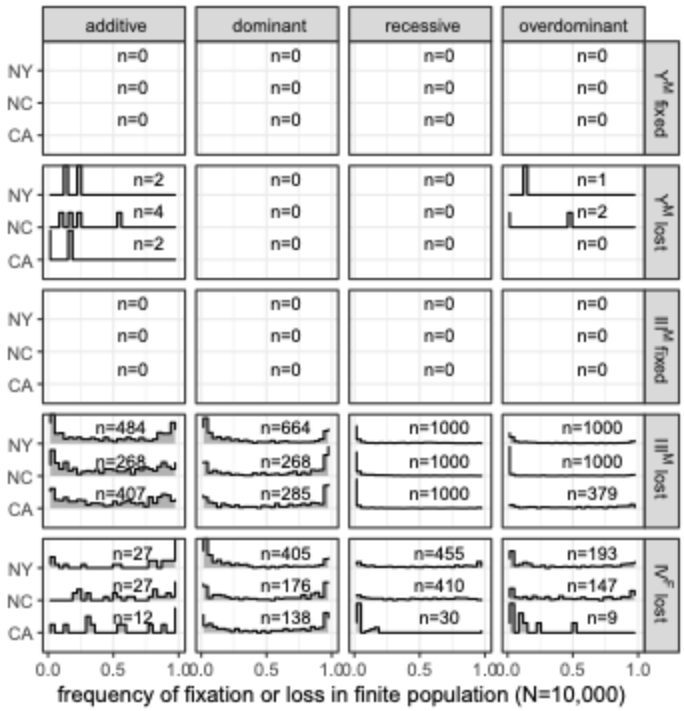
Supplemental Figure S8. Histograms of the frequency of the III^M proto-Y chromosome after 1,000 generations are shown for the 1,000 fitness arrays that produce proto-sex chromosomes most similar to those observed in each natural population (CA, NC, or NY). Fitness arrays are divided into those in which the III^M chromosome eventually reaches a frequency <0.1% after 1,000,000 generations (lost) and those in which the III^M chromosome is maintained at a frequency >0.1% (no). Fractions in each panel indicate how often the III^M chromosome was lost after 1,000,000 generations in the 1,000 best-fitting arrays. Simulations were started with equal frequencies of all genotypes, and fitness effects of proto-Y chromosomes were either additive, dominant, recessive, or overdominant.



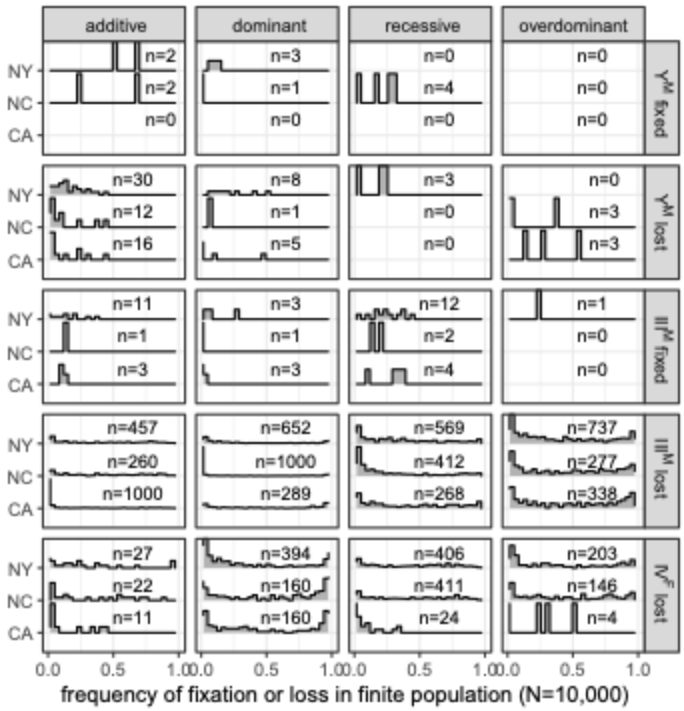
Supplemental Figure S9. Histograms of the frequency of the III^M proto-Y chromosome after 1,000 generations are shown for the 1,000 fitness arrays that produce proto-sex chromosomes most similar to those observed in each natural population (CA, NC, or NY). Fitness arrays are divided into those in which the III^M chromosome eventually reaches a frequency <0.1% after 1,000,000 generations (lost) and those in which the III^M chromosome is maintained at a frequency >0.1% (no). Fractions in each panel indicate how often the III^M chromosome was lost after 1,000,000 generations in the 1,000 best-fitting arrays. Simulations were started with genotypes frequencies observed in each population, and fitness effects of proto-Y chromosomes were either additive, dominant, recessive, or overdominant.



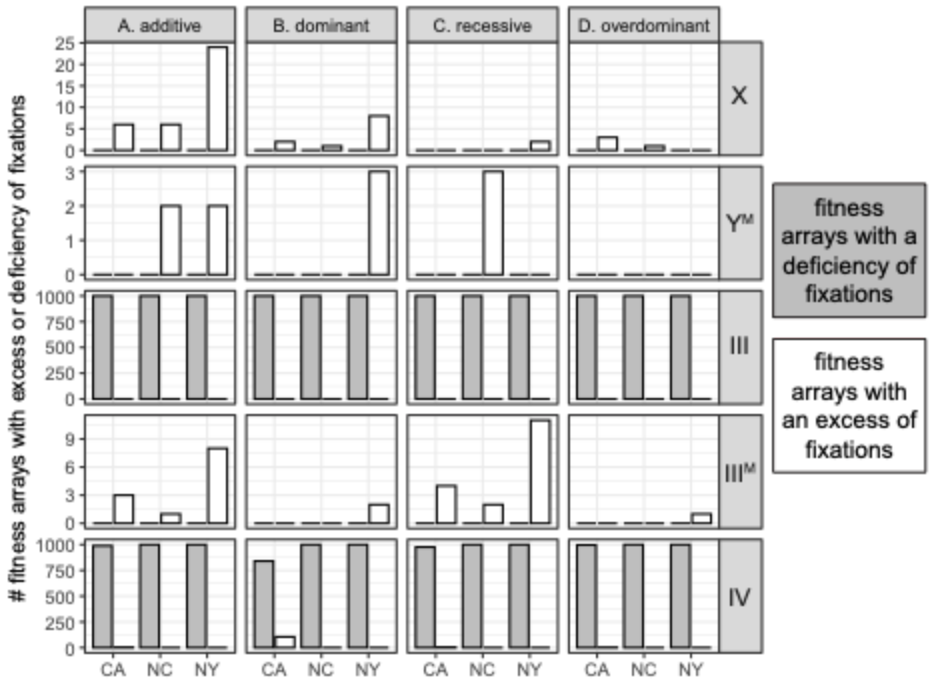
Supplemental Figure S10. Bar graphs show the frequency with which the Y^M chromosome is lost after 1,000,000 generation using fitness effects that maintain proto-sex chromosome frequencies similar to those observed in each natural population (CA, NC, or NY). The best-fitting arrays were identified with simulations starting with equal frequencies of all genotypes (equal starting freqs) or frequencies observed in each population (location start freqs). Genotype fitness values were calculating assuming either additive, dominant, recessive, or overdominant fitness effects.



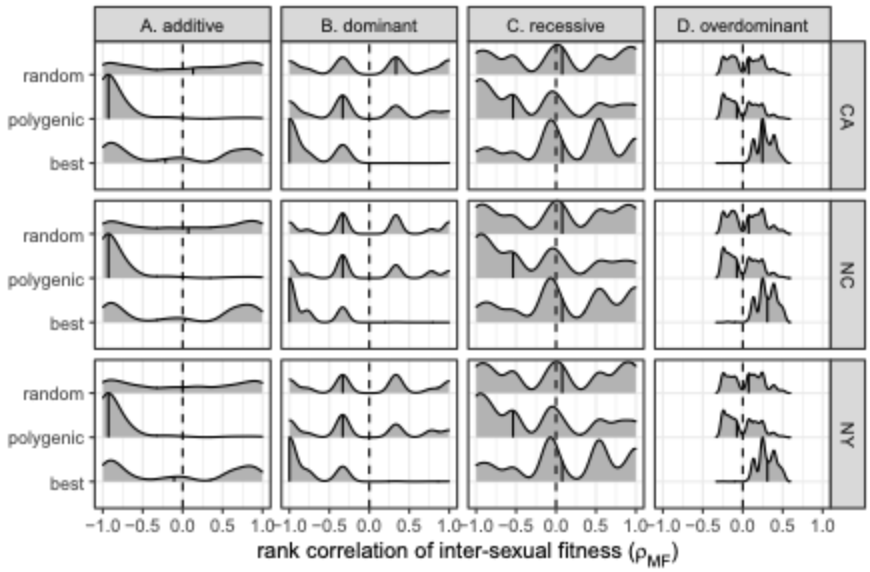
Supplemental Figure S11. Histograms show the distributions of frequency of fixation or loss each proto-Y or proto-W chromosome in each population when N=10^4. Numbers in each panel (n) indicate the number of fitness arrays with at least one fixation in 100 simulations. The x-axis is the frequency of fixations in 100 simulations for those arrays with at least one fixation. Simulations were performed with the 1,000 best-fitting selection pressures for each population (CA, NC, or NY) that were identified from simulations started with equal frequencies of all genotypes, using either **(A)** additive, **(B)** dominant, **(C)** recessive, or **(D)** overdominant fitness effects of the proto-Y chromosomes.



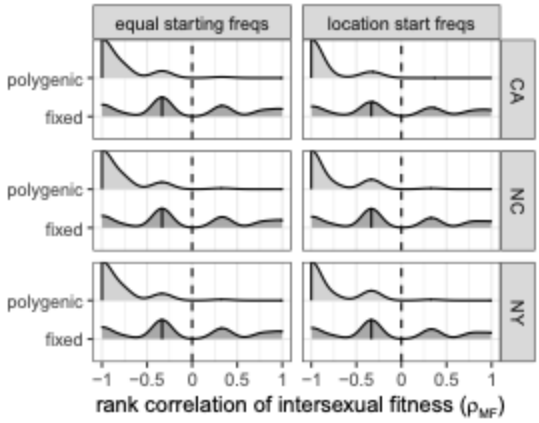
Supplemental Figure S12. Histograms show the distributions of frequency of fixation or loss each proto-Y or proto-W chromosome in each population when N=10^4. Numbers in each panel (n) indicate the number of fitness arrays with at least one fixation in 100 simulations. The x-axis is the frequency of fixations in 100 simulations for those arrays with at least one fixation. Simulations were performed with the 1,000 best-fitting selection pressures for each population (CA, NC, or NY) that were identified from simulations started with the observed frequencies of each genotype in each population, using either **(A)** additive, **(B)** dominant, **(C)** recessive, or **(D)** overdominant fitness effects of the proto-Y chromosomes.



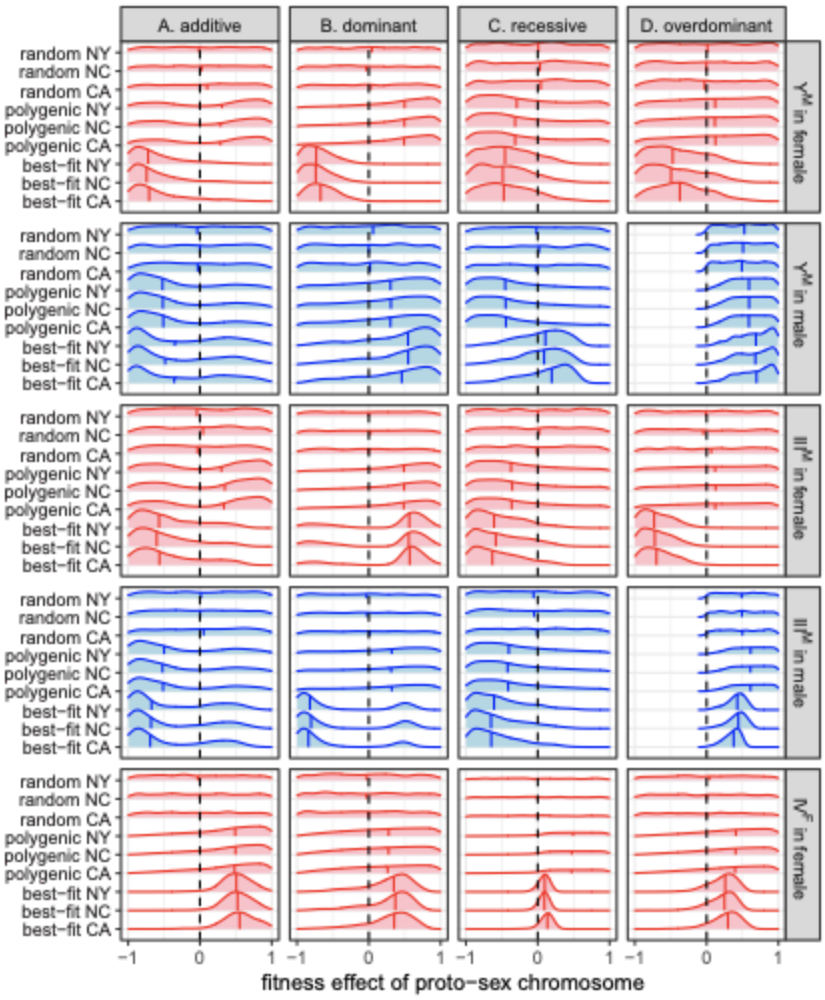
Supplemental Figure S13. Bar plots show the number of fitness arrays that result in a deficiency (gray) or excess (white) of fixations of each proto-sex chromosome (X, Y^M, III, III^M, or IV) relative to simulations in which there are no fitness differences across proto-sex chromosomes (i.e., drift only). The fitness arrays are the 1,000 best-fitting fitness values for each population (CA, NC, or NY) when simulations were started with genotype frequencies observed in natural populations. Fitness effects of the proto-Y chromosomes are either **(A)** additive, **(B)** dominant, **(C)** recessive, or **(D)** overdominant.



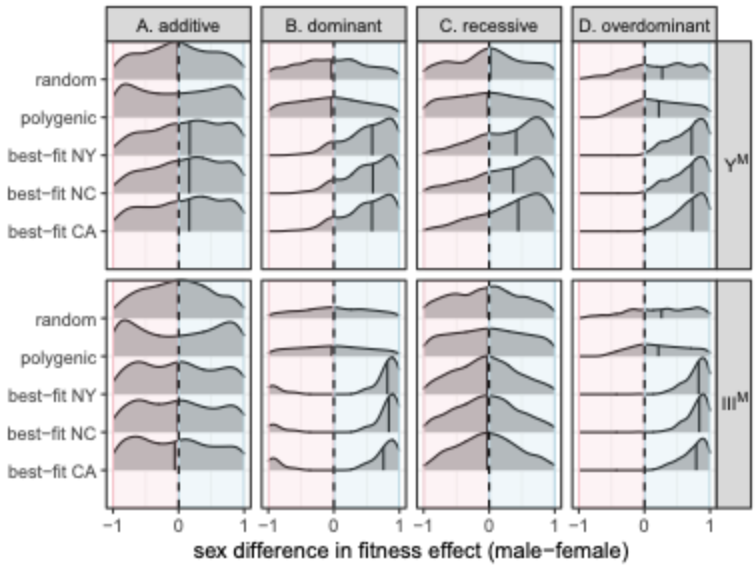
Supplemental Figure S14. Smoothed histograms show intersexual fitness correlations (ρ_{MF}) for the 1,000 best-fitting genotypic fitness arrays for the CA, NC, and NY populations (best), all fitness arrays that maintain PSD (polygenic), and 1,000 random fitness arrays. Simulations were started with genotype frequencies observed in natural populations. Dashed vertical lines show $\rho_{MF} = 0$, and solid vertical lines within histograms show the median. Fitness arrays were calculated assuming either (**A)** additive, **(B)** dominant, **(C)** recessive, or **(D)** overdominant fitness effects of the proto-Y chromosomes.



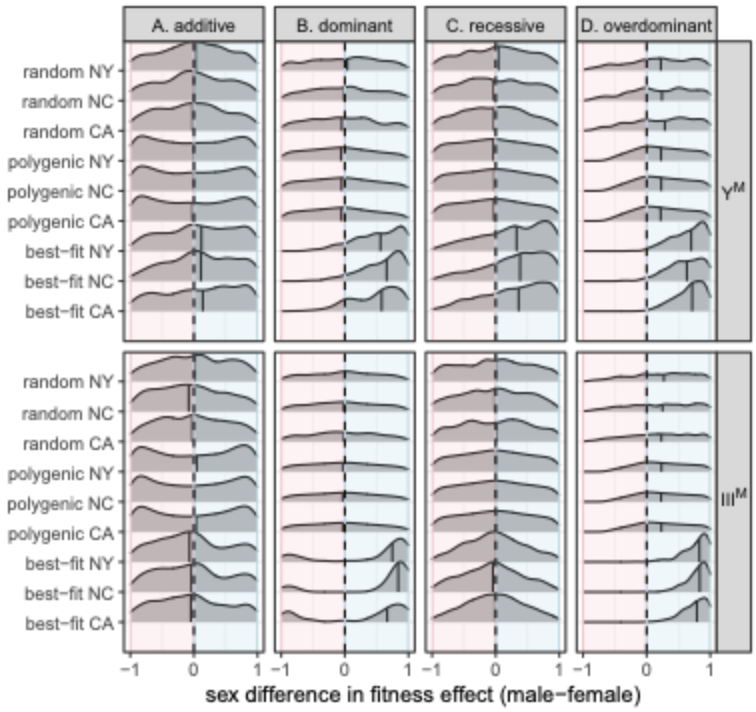
Supplemental Figure S15. Smoothed histograms show intersexual fitness correlations (ρ_{MF}) for the 1,000 best-fitting genotypic fitness arrays based on dominant fitness effects of the proto-Y chromosomes for the CA, NC, and NY populations after 1,000,000 generations. Fitness arrays that maintain PSD for 1,000 generations are divided into those the maintain PSD for 1,00,000 generations (polygenic) and those that allow at least one proto-sex chromosome to reach fixation (fixed). Simulations were started with either equal frequencies of all genotypes (left) or genotype frequencies observed in natural populations (right). Dashed vertical lines show $\rho_{MF} = 0$, and solid vertical lines within histograms show the median.



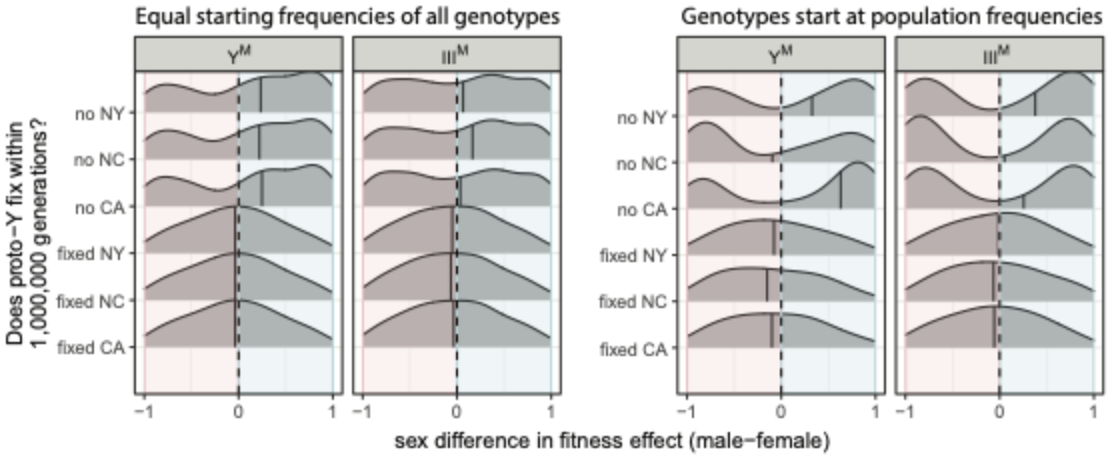
Supplemental Figure S16. Smoothed histograms show the distributions of fitness effects of each proto-sex chromosome in each sex for 1,000 random fitness arrays (random), fitness arrays that maintain PSD (polygenic), or the 1,000 best-fitting fitness arrays for each population (CA, NC, or NY). Simulations were started with genotype frequencies observed in natural populations. The vertical line within each histogram shows the median, and dashed lines in each panel are at fitness value of 0. Fitness arrays were calculated assuming either (**A)** additive, **(B)** dominant, **(C)** recessive, or **(D)** overdominant fitness effects of the proto-Y chromosomes.



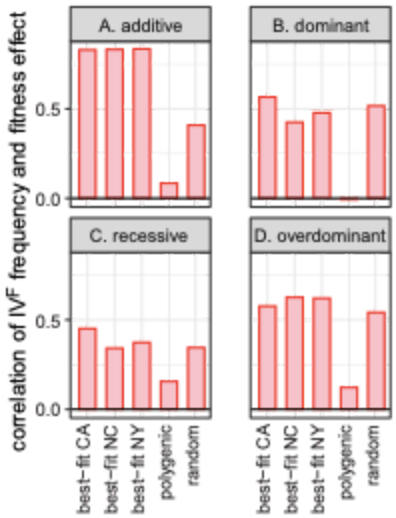
Supplemental Figure S17. Smoothed histograms show the distributions of the difference in the fitness effect of each proto-Y chromosome (Y^M or III^M) between males and females within individual parameterizations of the model. If the fitness effect is greater in males, the values are positive. If the fitness effect is greater in females, the values are negative. Fitness effects of each proto-Y chromosome come from the 1,000 random fitness arrays (random), fitness arrays that maintain PSD (polygenic), or the 1,000 best-fitting fitness arrays for each population (CA, NC, or NY). Simulations were started with equal frequencies of all genotypes. The vertical line within each histogram shows the median, and dashed lines in each panel are a difference of 0 (i.e., equal fitness effects in males and females). Genotype fitness arrays were calculated assuming either (**A)** additive, **(B)** dominant, **(C)** recessive, or **(D)** overdominant fitness effects of the proto-Y chromosomes.



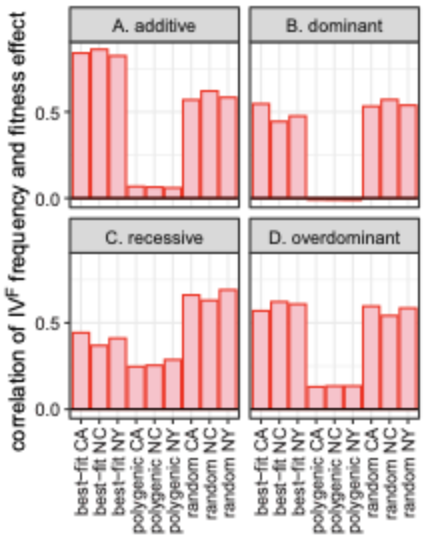
Supplemental Figure S18. Smoothed histograms show the distributions of the difference in the fitness effect of each proto-Y chromosome (Y^M or III^M) between males and females within individual parameterizations of the model. If the fitness effect is greater in males, the values are positive. If the fitness effect is greater in females, the values are negative. Fitness effects of each proto-Y chromosome come from the 1,000 random fitness arrays (random), fitness arrays that maintain PSD (polygenic), or the 1,000 best-fitting fitness arrays for each population (CA, NC, or NY). Simulations were started with genotype frequencies observed in natural populations. The vertical line within each histogram shows the median, and dashed lines in each panel are a difference of 0 (i.e., equal fitness effects in males and females). Genotype fitness arrays were calculated assuming either (**A)** additive, **(B)** dominant, **(C)** recessive, or **(D)** overdominant fitness effects of the proto-Y chromosomes.



Supplemental Figure S19. Smoothed histograms show the distributions of the difference in the fitness effect of each proto-Y chromosome (Y^M or III^M) between males and females within individual parameterizations of the model. If the fitness effect is greater in males, the values are positive. If the fitness effect is greater in females, the values are negative. Simulations were performed using 1,000 fitness arrays based on dominant fitness effects of the proto-Y chromosomes that maintain PSD for 1,000 generations. Fitness arrays that maintain PSD for 1,000 generations are divided into those in which the proto-Y chromosome reaches fixation within 1,00,000 generations (fixed) and those in which it does not reach fixation (no) in each population (CA, NC, or NY). Simulations were started with either equal frequencies of all genotypes (left) or genotype frequencies observed in natural populations (right). The vertical line within each histogram shows the median, and dashed lines in each panel are a difference of 0 (i.e., equal fitness effects in males and females).



Supplemental Figure S20. Bar plots show Spearman's rank order correlation between the frequency the IV^F proto-W chromosome across simulated populations and its fitness effect in the populations. Simulated populations include those that best-fit proto-sex chromosome frequencies in natural populations (CA, NC, or NY), those that maintain PSD (polygenic), and 1,000 random populations (random). Simulations were started with equal frequencies of all genotypes. Genotype fitness in populations was calculated assuming **(A)** additive, **(B)** dominant, **(C)** recessive, or **(D)** overdominant effects of the proto-Y chromosomes.



Supplemental Figure S21. Bar plots show Spearman's rank order correlation between the frequency the IV^F proto-W chromosome across simulated populations and its fitness effect in the populations. Simulated populations include those that best-fit proto-sex chromosome frequencies in natural populations (CA, NC, or NY), those that maintain PSD (polygenic), and 1,000 random populations (random). Simulations were started with the observed frequencies of each genotype in each population. Genotype fitness in populations was calculated assuming **(A)** additive, **(B)** dominant, **(C)** recessive, or **(D)** overdominant effects of the proto-Y chromosomes.

Supplemental Table

Supplemental Table S1. Recursion equations to simulate random mating.

genotype	frequency	frequency after random mating
X/X; III/III; IV/IV	f_1	$f_1m_1/2 + f_1m_3/2 + f_1m_4/4$ $+ f_2m_1/4 + f_2m_3/4 + f_2m_4/8$ $+ f_3m_1/4 + f_3m_3/8 + f_3m_4/16$ $+ f_5m_1/8 + f_5m_3/8 + f_5m_4/16$ $+ f_6m_1/16 + f_6m_3/16 + f_6m_4/32$
X/X; III/III; IV/IV^F	f_2	$f_2m_1/4 + f_2m_3/4 + f_2m_4/8$ $+ f_3m_1/8 + f_3m_3/8 + f_3m_4/16$ $+ f_5m_1/8 + f_5m_3/8 + f_5m_4/16$ $+ f_6m_1/16 + f_6m_3/16 + f_6m_4/32$
X/X; III/III^M; IV/IV^F	f_3	$f_2m_1/4 + f_2m_2/2 + f_2m_4/8 + f_2m_5/4$ $+ f_3m_1/4 + f_3m_2/4 + f_3m_3/8$ $+ f_3m_4/8 + f_3m_5/8$ $+ f_4m_1/4 + f_4m_3/4 + f_4m_4/8$ $+ f_5m_1/8 + f_5m_2/4 + f_5m_4/16 + f_5m_5/8$ $+ f_6m_1/8 + f_6m_2/8 + f_6m_3/16$ $+ f_6m_4/16 + f_6m_5/16$ $+ f_7m_1/8 + f_7m_3/8 + f_7m_4/16$
X/X; III^M/III^M; IV/IV^F	f_4	$f_3m_1/8 + f_3m_2/4 + f_3m_4/16 + f_3m_5/8$ $+ f_4m_1/4 + f_4m_2/2 + f_4m_4/8 + f_4m_5/4$ $+ f_6m_1/16 + f_6m_2/8 + f_6m_4/32 + f_6m_5/16$ $+ f_7m_1/8 + f_7m_2/4 + f_7m_4/16 + f_7m_5/8$
X/Y^M; III/III; IV/IV^F	f_5	$f_2m_3/4 + f_2m_4/8 + f_2m_6/2 + f_2m_7/4$ $+ f_3m_3/8 + f_3m_4/16 + f_3m_6/4 + f_3m_7/8$ $+ f_5m_1/8 + f_5m_3/4 + f_5m_4/8$ $+ f_5m_6/4 + f_5m_7/8$ $+ f_6m_1/16 + f_6m_3/8 + f_6m_4/16$ $+ f_6m_6/8 + f_6m_7/16$ $+ f_8m_1/4 + f_8m_3/4 + f_8m_4/8$ $+ f_9m_1/8 + f_9m_3/8 + f_9m_4/16$

X/Y^{M}; III/III^{M}; IV/IV^{F}	f_6	$f_2m_4/8 + f_2m_5/4 + f_2m_7/4 + f_2m_8/2$ $+ f_3m_3/8 + f_3m_4/8 + f_3m_5/8$ $+ f_3m_6/4 + f_3m_7/4 + f_3m_8/4$ $+ f_4m_3/4 + f_4m_4/8 + f_4m_6/2 + f_4m_7/4$ $+ f_5m_1/8 + f_5m_2/4 + f_5m_4/8$ $+ f_5m_5/4 + f_5m_7/8 + f_5m_8/4$ $+ f_6m_1/8 + f_6m_2/8 + f_6m_3/8 + f_6m_4/8$ $+ f_6m_5/8 + f_6m_6/8 + f_6m_7/8 + f_6m_8/8$ $+ f_7m_1/8 + f_7m_3/4 + f_7m_4/8$ $+ f_7m_6/4 + f_7m_7/8$ $+ f_8m_1/4 + f_8m_2/2 + f_8m_4/8 + f_8m_5/4$ $+ f_9m_1/4 + f_9m_2/4 + f_9m_3/8$ $+ f_9m_4/8 + f_9m_5/8$ $+ f_{10}m_1/4 + f_{10}m_3/4 + f_{10}m_4/8$
X/Y^{M}; III^{M}/III^{M}; IV/IV^{F}	f_7	$f_3m_4/16 + f_3m_5/8 + f_3m_7/8 + f_3m_8/4$ $+ f_4m_4/8 + f_4m_5/4 + f_4m_7/4 + f_4m_8/2$ $+ f_6m_1/16 + f_6m_2/8 + f_6m_4/16$ $+ f_6m_5/8 + f_6m_7/16 + f_6m_8/8$ $+ f_7m_1/8 + f_7m_2/4 + f_7m_4/8$ $+ f_7m_5/4 + f_7m_7/8 + f_7m_8/4$ $+ f_9m_1/8 + f_9m_2/4 + f_9m_4/16 + f_9m_5/8$ $+ f_{10}m_1/4 + f_{10}m_2/2 + f_{10}m_4/8 + f_{10}m_5/4$
Y^{M}/Y^{M}; III/III; IV/IV^{F}	f_8	$f_5m_3/8 + f_5m_4/16 + f_5m_6/4 + f_5m_7/8$ $+ f_6m_3/16 + f_6m_4/32 + f_6m_6/8 + f_6m_7/16$ $+ f_8m_3/4 + f_8m_4/8 + f_8m_6/2 + f_8m_7/4$ $+ f_9m_3/8 + f_9m_4/16 + f_9m_6/4 + f_9m_7/8$
Y^{M}/Y^{M}; III/III^{M}; IV/IV^{F}	f_9	$f_5m_4/16 + f_5m_5/8 + f_5m_7/8 + f_5m_8/4$ $+ f_6m_3/16 + f_6m_4/16 + f_6m_5/16$ $+ f_6m_6/8 + f_6m_7/8 + f_6m_8/8$ $+ f_7m_3/8 + f_7m_4/16 + f_7m_6/4 + f_7m_7/8$ $+ f_8m_4/8 + f_8m_5/4 + f_8m_7/4 + f_8m_8/2$ $+ f_9m_3/8 + f_9m_4/8 + f_9m_5/8$ $+ f_9m_6/4 + f_9m_7/4 + f_9m_8/4$ $+ f_{10}m_3/4 + f_{10}m_4/8 + f_{10}m_6/2 + f_{10}m_7/4$

Y^M/Y^M; III^M/III^M; IV/IV^F	f_{10}	$f_6m_4/32 + f_6m_5/16 + f_6m_7/16 + f_6m_8/8$ $+ f_7m_4/16 + f_7m_5/8 + f_7m_7/8 + f_7m_8/4$ $+ f_9m_4/16 + f_9m_5/8 + f_9m_7/8 + f_9m_8/4$ $+ f_{10}m_4/8 + f_{10}m_5/4 + f_{10}m_7/4 + f_{10}m_8/2$
X/X; III/III^M; IV/IV	m_1	$f_1m_1/2 + f_1m_2/1 + f_1m_4/4 + f_1m_5/2$ $+ f_2m_1/4 + f_2m_2/2 + f_2m_4/8 + f_2m_5/4$ $+ f_3m_1/8 + f_3m_2/4 + f_3m_3/8$ $+ f_3m_4/8 + f_3m_5/8$ $+ f_4m_1/4 + f_4m_3/4 + f_4m_4/8$ $+ f_5m_1/8 + f_5m_2/4 + f_5m_4/16 + f_5m_5/8$ $+ f_6m_1/8 + f_6m_2/8 + f_6m_3/16$ $+ f_6m_4/16 + f_6m_5/16$ $+ f_7m_1/8 + f_7m_3/8 + f_7m_4/16$
X/X; III^M/III^M; IV/IV	m_2	$f_3m_1/8 + f_3m_2/4 + f_3m_4/16 + f_3m_5/8$ $+ f_4m_1/4 + f_4m_2/2 + f_4m_4/8 + f_4m_5/4$ $+ f_6m_1/16 + f_6m_2/8 + f_6m_4/32 + f_6m_5/16$ $+ f_7m_1/8 + f_7m_2/4 + f_7m_4/16 + f_7m_5/8$
X/Y^M; III/III; IV/IV	m_3	$f_1m_3/2 + f_1m_4/4 + f_1m_6/1 + f_1m_7/2$ $+ f_2m_3/4 + f_2m_4/8 + f_2m_6/2 + f_2m_7/4$ $+ f_3m_3/8 + f_3m_4/16 + f_3m_6/4 + f_3m_7/8$ $+ f_5m_1/8 + f_5m_3/4 + f_5m_4/8$ $+ f_5m_6/4 + f_5m_7/8$ $+ f_6m_1/16 + f_6m_3/8 + f_6m_4/16$ $+ f_6m_6/8 + f_6m_7/16$ $+ f_8m_1/4 + f_8m_3/4 + f_8m_4/8$ $+ f_9m_1/8 + f_9m_3/8 + f_9m_4/16$ $+ f_5m_6/4 + f_5m_7/8$ $+ f_6m_1/16 + f_6m_3/8 + f_6m_4/16$ $+ f_6m_6/8 + f_6m_7/16$ $+ f_8m_1/4 + f_8m_3/4 + f_8m_4/8$ $+ f_9m_1/8 + f_9m_3/8 + f_9m_4/16$

X/Y^{M}; III/III^{M}; IV/IV	m_4	$f_1m_4/4 + f_1m_5/2 + f_1m_7/2 + f_1m_8/1$ $+f_2m_4/8 + f_2m_5/4 + f_2m_7/4 + f_2m_8/2$ $+f_3m_3/8 + f_3m_4/8 + f_3m_5/8$ $+f_3m_6/4 + f_3m_7/4 + f_3m_8/4$ $+f_4m_3/4 + f_4m_4/8 + f_4m_6/2 + f_4m_7/4$ $+f_5m_1/8 + f_5m_2/4 + f_5m_4/8$ $+f_5m_5/4 + f_5m_7/8 + f_5m_8/4$ $+f_6m_1/8 + f_6m_2/8 + f_6m_3/8 + f_6m_4/8$ $+f_6m_5/8 + f_6m_6/8 + f_6m_7/8 + f_6m_8/8$ $+f_7m_1/8 + f_7m_3/4 + f_7m_4/8$ $+f_7m_6/4 + f_7m_7/8$ $+f_8m_1/4 + f_8m_2/2 + f_8m_4/8 + f_8m_5/4$ $+f_9m_1/4 + f_9m_2/4 + f_9m_3/8$ $+f_9m_4/8 + f_9m_5/8$ $+f_{10}m_1/4 + f_{10}m_3/4 + f_{10}m_4/8$
X/Y^{M}; III^{M}/III^{M}; IV/IV	m_5	$f_3m_4/16 + f_3m_5/8 + f_3m_7/8 + f_3m_8/4$ $+f_4m_4/8 + f_4m_5/4 + f_4m_7/4 + f_4m_8/2$ $+f_6m_1/16 + f_6m_2/8 + f_6m_4/16$ $+f_6m_5/8 + f_6m_7/16 + f_6m_8/8$ $+f_7m_1/8 + f_7m_2/4 + f_7m_4/8$ $+f_7m_5/4 + f_7m_7/8 + f_7m_8/4$ $+f_9m_1/8 + f_9m_2/4 + f_9m_4/16 + f_9m_5/8$ $+f_{10}m_1/4 + f_{10}m_2/2 + f_{10}m_4/8 + f_{10}m_5/4$
Y^{M}/Y^{M}; III/III; IV/IV	m_6	$f_5m_3/8 + f_5m_4/16 + f_5m_6/4 + f_5m_7/8$ $+ f_6m_3/16 + f_6m_4/32 + f_6m_6/8 + f_6m_7/16$ $+ f_8m_3/4 + f_8m_4/8 + f_8m_6/2 + f_8m_7/4$ $+ f_9m_3/8 + f_9m_4/16 + f_9m_6/4 + f_9m_7/8$

Y^M/Y^M; III/III^M; IV/IV	m_7	$f_5m_4/16 + f_5m_5/8 + f_5m_7/8 + f_5m_8/4$ $+f_6m_3/16 + f_6m_4/16 + f_6m_5/16$ $+f_6m_6/8 + f_6m_7/8 + f_6m_8/8$ $+f_7m_3/8 + f_7m_4/16 + f_7m_6/4 + f_7m_7/8$ $+f_8m_4/8 + f_8m_5/4 + f_8m_7/4 + f_8m_8/2$ $+f_9m_3/8 + f_9m_4/8 + f_9m_5/8$ $+f_9m_6/4 + f_9m_7/4 + f_9m_8/4$ $+f_{10}m_3/4 + f_{10}m_4/8 + f_{10}m_6/2 + f_{10}m_7/4$
Y^M/Y^M; III^M/III^M; IV/IV	m_8	$f_6m_4/32 + f_6m_5/16 + f_6m_7/16 + f_6m_8/8$ $+f_7m_4/16 + f_7m_5/8 + f_7m_7/8 + f_7m_8/4$ $+f_9m_4/16 + f_9m_5/8 + f_9m_7/8 + f_9m_8/4$ $+f_{10}m_4/8 + f_{10}m_5/4 + f_{10}m_7/4 + f_{10}m_8/2$

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Supplemental Methods

Fitness effects and genotypic fitness

To generate the fitness of the 18 possible multi-chromosome genotypes (Table 1), I first assigned fitness effects to each single chromosome genotype (e.g., for the X and Y^M chromosomes there are three possible genotypes: X/X, X/Y^M, and Y^M/Y^M). The single chromosome genotype is analogous to a single locus genotype in other population genetic models (e.g., Kidwell *et al.* 1977). I generated the single chromosome genotype fitness values by assigning a sex-specific fitness effect, s_{ij}, to each j proto-Y or proto-W chromosome (Y^M, III^M, or IV^F) in each sex i. The fitness effect of IV^F was only assigned for females because the female-determining proto-W chromosome (carrying *Md-tra*^{D}) cannot be found in male genotypes (Table 1). Thus, there are five fitness effects: Y^M in males ($i{=}\mathrm{male},\ j{=}\mathrm{Y^M}$), Y^M in females ($i{=}\mathrm{female}, j{=}\mathrm{Y^M}$), III^M in males ($i{=}\mathrm{male}, j{=}\mathrm{III^M}$), III^M in females ($i{=}\mathrm{female}, j{=}\mathrm{III^M}$), and IV^F in females ($i{=}\mathrm{female}, j{=}\mathrm{IV^F}$). Each s_{ij} was drawn from a uniform distribution between -1 and 1. Positive fitness effects ($s_{ij}{>}0$) mean that the proto-Y or proto-W chromosome increases fitness (i.e., it is beneficial), and negative values ($s_{ij}{<}0$) mean that it decreases fitness (i.e., it is deleterious).

The fitness effects of the proto-Y and proto-W chromosome were then used to calculate the fitness of each single chromosome genotype, using one of four dominance scenarios. The calculations depend on whether the proto-Y chromosome has beneficial ($s_{ij}{>}0$) or deleterious ($s_{ij}{<}0$) effects, which ensures that the maximum genotypic fitness for a given chromosome and sex is equal to 1 (Table 2). First, in the additive model, the sex-specific single chromosome fitness values are defined such that the heterozygous genotype (X/Y^M or III/III^M) is intermediate between the two homozygous genotypes. Second, when the proto-Y chromosomes have

dominant fitness effects, the single chromosome fitness of the heterozygote is equal to the proto-Y homozygote. Third, when the effects of the proto-Y chromosomes are recessive, the single chromosome fitness of the heterozygote is equal to the proto-X homozygote and different from the proto-Y homozygote (Table 2). Fourth, the Y^M and III^M proto-Y chromosomes have overdominant effects in males, but are additive in females. In the overdominant model, the fitness effect of recessive deleterious proto-Y alleles is equal in magnitude to the fitness benefit of dominant advantageous proto-Y alleles. This simplifying assumption may not be biologically realistic, and future work could examine the implications of this assumption. In all four models, there is a single fitness effect associated with carrying a copy of the IV^F proto-W chromosome in females; only females can carry IV^F and it is impossible to be homozygous for IV^F (Table 1). In all four dominance models, the fitness of each of the multi-chromosomal genotypes (Table 1) is calculated as the product of the three relevant sex-specific single chromosome genotype fitness values.

I performed a normalization to ensure that the the maximum multi-chromosome genotype fitness in each sex is equal to 1. This is necessary because not all multi-chromosome genotypes are possible for both males and females, which means that the maximum product of all single chromosome genotypes can be <1 even though the maximum single chromosome genotype fitness values are set to 1. To ensure that the maximum female multi-chromosome genotype is 1, I divided each female multi-chromosome genotype fitness by the maximum female multi-chromosome genotype fitness. I did the same for males.

All four dominance scenarios (additive, dominant, recessive, or overdominant) attribute a single sex-specific fitness effect to each proto-Y and proto-W chromosome, whether it is Y^M, III^M, or IV^F. I am therefore assuming that all copies of each proto-sex chromosome in the population carry an identical suite of beneficial and deleterious alleles (i.e., all copies of III^M are identical, all copies of Y^M are identical, and all copies of IV^F are identical). In addition, all models assume complete linkage between any allele(s) under selection and the *Mdmd* locus on the Y^M and III^M

chromosomes. Similarly, I assume complete linkage between the female-determining *Md-tra*^{D} locus and any alleles on the IV^{F} chromosome. Complete linkage may be achieved if, for example, chromosomal inversions suppress recombination between the proto-Y and proto-X (or proto-Z and proto-W) chromosomes in heterozygotes (Bergero and Charlesworth 2009; Wright *et al.* 2016). Free recombination in sex chromosome homozygotes would not affect genetic variation on a sex chromosome because all copies of each chromosome are assumed to carry the same alleles.

Simulations with infinite population size

I used forward simulations to determine how the fitness effects of the proto-sex chromosomes affect their frequencies in populations. These simulations were performed with non-overlapping generations and random mating, assuming a population of infinite size (simulations with finite population sizes are described later). Each generation of a simulation consists of two discrete steps (Figure 1A). First, the frequency of each of the 18 genotypes is multiplied by its corresponding fitness value, which models differential survival across genotypes. Second, recursion equations developed by Hamm (2008) and previously implemented by Meisel *et al.* (2016) are used to model the production of progeny by random mating (Supplemental Table S1). After 1,000 generations of selection and random mating, the frequency of each genotype and proto-sex chromosome was calculated. This process was performed for 1,000,000 fitness arrays for each dominance scenario (additive, dominant, recessive, and overdominant) and four possible initial genotype frequencies (16,000,000 total simulations).

I considered four possible initial genotype frequencies for each simulation. The first three initial frequencies are based on the observed frequencies of Y^{M}, III^{M}, and *Md-tra*^{D} from three North American populations (Meisel *et al.* 2016). These populations were sampled in Chino, CA (Meisel *et al.* 2016), Wake County, NC (Hamm and Scott 2008), and Chemung County, NY

(Scott *et al.* 2013). Initial genotype frequencies were calculated based on the observed frequencies of Y^M, III^M, and *Md-tra*^{D}, assuming random mating (Meisel *et al.* 2016). These three populations were chosen because Y^M, III^M, and *Md-tra*^{D} have all remained at a frequency >1% across multiple years of sampling (Hamm *et al.* 2005; Hamm and Scott 2008; Meisel *et al.* 2016). I used frequencies from actual populations as the initial frequencies because I am specifically interested in how selection can maintain PSD at the frequencies observed in natural populations. The fourth starting values consist of all 18 genotypes at the same initial frequency (i.e., 1/18 for each genotype). This allows me to evaluate how selection pressures can drive the proto-sex chromosomes to the frequencies observed in natural populations if they start at arbitrary values that deviate from the observed frequencies.

To determine whether the fitness effects maintain PSD, I first selected fitness arrays that produced frequencies of each proto-sex chromosome (Y^M, III^M, IV^F, and their homologous chromosomes, X, III, and IV) that are all >0.1% after 1,000 generations. This criterion ensures that all three chromosomes are polymorphic with both alleles at a frequency that is measurable (i.e., 1/1,000) given the sampling schemes used in previous studies of natural house fly populations (Hamm *et al.* 2005; Hamm and Scott 2008; Meisel *et al.* 2016). I refer to genotype fitness arrays with all proto-sex chromosomes present at a frequency >0.1% as maintaining PSD, although not necessarily at the frequencies observed in natural populations.

From the genotype fitness arrays that maintain PSD (i.e., all proto-sex chromosomes >0.1%), I selected those that produce proto-sex chromosome frequencies most similar to the frequencies observed in natural populations. To do so, I calculated the mean squared error (MSE) between the frequencies of Y^M, III^M, and IV^F after 1,000 generations in a simulation (S) and the observed frequencies (O) in a natural population (CA, NC, or NY):

$$\mathrm{MSE} = \frac{1}{3}[(O_{Y^M} - S_{Y^M})^2 + (O_{III^M} - S_{III^M})^2 + (O_{tra^D} - S_{tra^D})^2].$$

When using simulations that started with proto-sex chromosome frequencies observed in a population (e.g., CA), I only used MSE to compare with observed frequencies from that same population. When simulations started with equal frequencies of all 18 genotypes, I compared simulated chromosomes frequencies with the observed frequencies in each of the three populations (CA, NC, and NY).

I retained the 1,000 fitness arrays that produce proto-sex chromsome frequencies with the lowest MSE for a given dominance model, population, and starting genotype frequency. I refer to each of these 1,000 fitness arrays as the best-fitting arrays for each population. Simulations for the 1,000 best-fitting arrays were then run for 1,000,000 generations and compared to the 1,000 generation simulations to assess the stability of chromosomal frequencies. I also selected 1,000 random fitness arrays and 1,000 fitness arrays that maintain PSD to perform 1,000,000 generation simulations.

Simulations with finite population sizes

I assessed how well the 1,000 best-fitting fitness arrays for each population, starting frequency, and dominance model maintain PSD when population sizes are finite. Finite population sizes introduce stochasticity to the change in allele frequencies across generations (i.e., genetic drift), in contrast to the deterministic effects of constant fitness values in a population of infinite size (Hartl and Clark 2007). It is not possible to calculate the probability of fixation for alleles in a finite population when there is complex sex-linked inheritance, as in the house fly PSD system (Meisel *et al.* 2016). To overcome this limitation, I estimated the probability of fixation using simulations that modeled finite populations by including multinomial sampling each generation.

I simulated finite populations by adding one additional step to the simulations described above in order to model a population size (*N*) of 10,000 individuals (Figure 1B). This population size was chosen because it is small enough to capture the effects of drift within the time scale of

my simulations. Each simulation was started with the frequencies of the proto-sex chromosomes observed in the population where the fitness array was identified as best-fitting. In each generation, following multiplication by the fitness array (i.e., natural selection), I used multinomial sampling to calculate the new frequencies of all 18 genotypes. Multinomial sampling for all genotype was performed with 10,000 trials (i.e., $N=10^4$) and a probability of success equal to the frequency of each genotype after selection. The resulting array of 18 genotype frequencies was then divided by the sum of all values to ensure that the genotype frequencies sum to 1. This array of genotype frequencies (after selection and multinomial sampling) was next used as input into the same recursion equations described above to calculate genotype frequencies after random mating (Supplemental Table S1). The process (selection, multinomial sampling, and random mating) was repeated for 1,000 generations. I simulated 100 replicate finite populations for each fitness array. I then used the frequency with which fixation and loss occur in those 100 replicates as an estimate of the probability of fixation and loss of proto-sex chromosomes within 1,000 generations.

I determined a null expectation for fixation or loss of proto-sex chromosomes in finite populations by using simulations without selection (i.e., genetic drift and no fitness differences across genotypes). To those ends, I performed simulations with 10,000 individuals for 1,000 generations, as was done in the simulations with selection and drift. I performed 1,000 replicate “drift-only” simulations with starting values at the observed frequencies of the proto-sex chromosomes in each population (CA, NC, and NY). These drift-only simulations included the same steps as the simulations with both selection and drift, except that there is no differential survival (i.e., natural selection) step in the drift-only simulation. After 1,000 generations, I calculated the frequency of each proto-Y and proto-W chromosome (Y^M, III^M, and IV^F), as well as the frequency with which each chromosome was lost, across the 1,000 simulations.