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/or 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. Dominance also affects the predicted role of sexual antagonism in maintaining polymorphism. Dominant fitness effects that maintain polygenic sex determiantion, for example, are predicted to have sexually antagonistic multi-chromosomal genotypes. However, the individual proto-sex chromosomes are not expected to have sexually antagonstic effects, demonstrating that sexual antagonism can be an emergent property of the multi-chromosome genotype. Overdominant fitness effects, in contrast, are expected to maintain polygenic sex determination with proto-Y chromosomes that have sexually antagonstic effects even though the multi-chromosome genotypes do not have signatures of sexual antagonism, My results therefore demonstrate that 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 its maintenance.

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 and sometimes even polymorphic within populations (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 within natural populations (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 many 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 population (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 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 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

91 (Hamm *et al.* 2015). The *M. domestica male determiner* (*Mdmd*) can be found on at least four of
92 the six house fly chromosomes in natural populations (Sharma *et al.* 2017). *Mdmd* causes the
93 house fly ortholog of *transformer* (*Md-tra*) to be spliced into a non-functional isoform, which
94 leads to male-specific splicing and expression of downstream target genes, thereby producing
95 fertile males (Hediger *et al.* 2010). In the absence of *Mdmd*, *Md-tra* is spliced into a
96 female-determining transcript that regulates the splicing of downstream targets, which promote
97 the development of female morphological and behavioral traits (Hediger *et al.* 2004; Meier *et al.*
98 2013).

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

112 There is evidence that natural selection maintains PSD in house fly populations. First,
113 the proto-Y chromosomes have remained at stable frequencies within house fly populations
114 over decades (Kozielska *et al.* 2008; Meisel *et al.* 2016). Second, Y^M and III^M are distributed

115 along north-south clines on multiple continents (Denholm *et al.* 1986; Tomita and Wada 1989;
116 Hamm *et al.* 2005; Kozielska *et al.* 2008), and temperature is the best predictor of their
117 frequencies (Feldmeyer *et al.* 2008). This suggests that heterogenous (temperature-dependent)
118 selection pressures maintain the Y^M and III^M chromosomes across populations. The stable
119 frequencies within populations, yet divergent frequencies across populations, suggest that there
120 are specific fitness effects associated with the house fly proto-Y chromosomes within each
121 population. Third, males carrying multiple proto-Y chromosomes (e.g., both Y^M and III^M, or two
122 copies of III^M) can be found in some populations (e.g., Hamm and Scott 2009). The frequency of
123 *Md-tra*^{D} is positively correlated with the frequency of these multi-Y males across populations,
124 suggesting that selection for balanced sex ratios may further maintain PSD (Meisel *et al.* 2016).
125 The rich body of observational data in house fly makes it a well-suited system around
126 which to develop population genetic models to determine how PSD can be maintained in natural
127 populations. For example, Kozielska et al. (2006) showed that, even in the complex house fly
128 PSD system, sex ratios are not expected to deviate substantially from 1:1 male:female. In
129 addition, despite no experimental evidence for sexually antagonistic effects of any house fly
130 proto-sex chromosomes, another population genetic model demonstrated that the frequencies
131 of the proto-sex chromosomes in natural populations are consistent with sexual antagonism
132 (Meisel *et al.* 2016). However, that model assumed that the proto-Y chromosomes (Y^M and III^M)
133 have additive fitness effects, which may not be true if, for example, beneficial mutations are
134 recessive or dominant (Orr 2010). In addition, Y chromosomes are expected to carry recessive
135 deleterious alleles (Charlesworth and Charlesworth 2000), which can further violate the
136 assumption of additive fitness effects. To address the shortcomings of previous models, I used
137 population genetic simulations to investigate whether sex chromosomes with non-additive
138 fitness effects can maintain the complex house fly PSD system. I specifically tested if these

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

142 **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

143 **Materials and Methods**

144 ***General overview***

145 I used a simulation approach to identify fitness effects of two proto-Y chromosomes (Y^M
146 and III^M) and one proto-W chromosome (IV^F) that maintain PSD under four different dominance
147 scenarios. To do so, I first randomly assigned fitness values to each of the single chromosome
148 genotypes in males and females separately (Figure 1A), assuming either additive, dominant,
149 recessive, or overdominant (i.e., heterozygote advantage) fitness effects of the proto-Y
150 chromosomes (Y^M and III^M). I then calculated the fitness of each of the 18 multi-chromosome
151 genotypes based on the single chromosome fitness effects (Table 1). I next determined the
152 frequency of each genotype and proto-sex chromosome after 1,000 generations of random
153 mating with the assigned fitness values (Figure 1A). I did this using simulations for one million
154 different fitness values and four possible starting genotype frequencies for each dominance
155 model. I retained fitness effects in which all three proto-sex chromosomes remained
156 polymorphic after 1,000 generations, and from those I selected the 1,000 fitness effects that
157 produced proto-sex frequencies closest to those observed in natural populations (Figure 1A). In
158 doing so, I am essentially applying an approximate Bayesian computation approach to fit
159 selection pressures to natural populations (Beaumont *et al.* 2002). I also evaluated how well
160 those fitness effects maintain PSD in a finite population (Figure 1B).

161 I used simulations to test if fitness effects maintain PSD because the model parameters
162 were too complex to analytically solve for equilibrium conditions. In my simulations, I tested if
163 PSD persists for 1,000 generations, which may not reflect an equilibrium state. In the Results, I

show how this approach can be problematic if proto-Y chromosomes are on a path to fixation or loss but have not yet reached fixation at 1,000 generations. I do this by simulating 1,000,000 generations for a subset of the fitness effects.

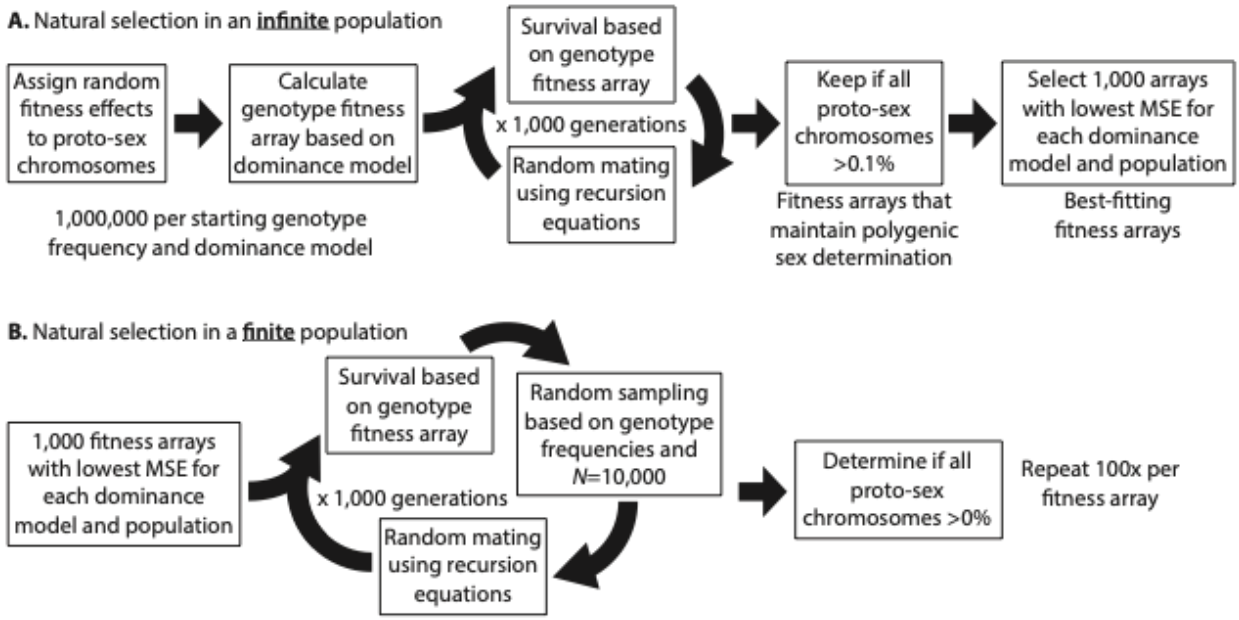


Figure 1. Steps in forward population genetic simulations with **(A)** infinite or **(B)** finite populations. The best-fitting arrays are those with the lowest mean squared error (MSE) comparing simulated proto-sex chromosome frequencies to those observed in each natural population.

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.

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	$1+s_{ij}$

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.

208 I performed a normalization to ensure that the the maximum multi-chromosome
209 genotype fitness in each sex is equal to 1. This is necessary because not all multi-chromosome
210 genotypes are possible for both males and females, which means that the maximum product of
211 all single chromosome genotypes can be <1 even though the maximum single chromosome
212 genotype fitness values are set to 1. To ensure that the maximum female multi-chromosome
213 genotype is 1, I divided each female multi-chromosome genotype fitness by the maximum
214 female multi-chromosome genotype fitness. I did the same for males.
215 All four dominance scenarios (additive, dominant, recessive, or overdominant) attribute a
216 single sex-specific fitness effect to each proto-Y and proto-W chromosome, whether it is Y^M, III^M,
217 or IV^F. I am therefore assuming that all copies of each proto-sex chromosome in the population
218 carry an identical suite of beneficial and deleterious alleles (i.e., all copies of III^M are identical, all
219 copies of Y^M are identical, and all copies of IV^F are identical). In addition, all models assume
220 complete linkage between any allele(s) under selection and the *Mdmd* locus on the Y^M and III^M
221 chromosomes. Similarly, I assume complete linkage between the female-determining *Md-tra*^{D}
222 locus and any alleles on the IV^F chromosome. Complete linkage may be achieved if, for
223 example, chromosomal inversions suppress recombination between the proto-Y and proto-X (or
224 proto-Z and proto-W) chromosomes in heterozygotes (Bergero and Charlesworth 2009; Wright
225 *et al.* 2016). Free recombination in sex chromosome homozygotes would not affect genetic
226 variation on a sex chromosome because all copies of each chromosome are assumed to carry
227 the same alleles.

228 ***Simulations with infinite population size***

229 I used forward simulations to determine how the fitness effects of the proto-sex
230 chromosomes affect their frequencies in populations. These simulations were performed with

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

242 I considered four possible initial genotype frequencies for each simulation. The first three
243 initial frequencies are based on the observed frequencies of Y^M, III^M, and *Md-tra*^{D} from three
244 North American populations (Meisel *et al.* 2016). These populations were sampled in Chino, CA
245 (Meisel *et al.* 2016), Wake County, NC (Hamm and Scott 2008), and Chemung County, NY
246 (Scott *et al.* 2013). Initial genotype frequencies were calculated based on the observed
247 frequencies of Y^M, III^M, and *Md-tra*^{D}, assuming random mating (Meisel *et al.* 2016). These three
248 populations were chosen because Y^M, III^M, and *Md-tra*^{D} have all remained at a frequency >1%
249 across multiple years of sampling (Hamm *et al.* 2005; Hamm and Scott 2008; Meisel *et al.*
250 2016). I used frequencies from actual populations as the initial frequencies because I am
251 specifically interested in how selection can maintain PSD at the frequencies observed in natural
252 populations. The fourth starting values consist of all 18 genotypes at the same initial frequency
253 (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

302 sum to 1. This array of genotype frequencies (after selection and multinomial sampling) was
303 next used as input into the same recursion equations described above to calculate genotype
304 frequencies after random mating (Supplemental Table S1). The process (selection, multinomial
305 sampling, and random mating) was repeated for 1,000 generations. I simulated 100 replicate
306 finite populations for each fitness array. I then used the frequency with which fixation and loss
307 occur in those 100 replicates as an estimate of the probability of fixation and loss of proto-sex
308 chromosomes within 1,000 generations.

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

319 **Results**

320 I used forward population genetic simulations to assess how natural selection can
321 maintain PSD in a species with two proto-Y chromosomes (Y^M and III^M) and one proto-W
322 chromosome (IV^F), modeled after the house fly (Hamm *et al.* 2015). Simulations were performed
323 assuming either additive, dominant, recessive, or overdominant fitness effects of the Y^M and III^M
324 proto-Y chromosomes. Simulations were started with genotype frequencies based on proto-sex

chromosome counts in each of three natural populations (CA, NC, or NY) or equal frequencies of all 18 genotypes. The general trends are the same regardless of the starting genotype frequencies, and I only present the results of simulations starting with equal frequencies in the main text (other results are presented in the Supplemental Material).

Dominance of fitness effects and the maintenance of polygenic sex determination

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

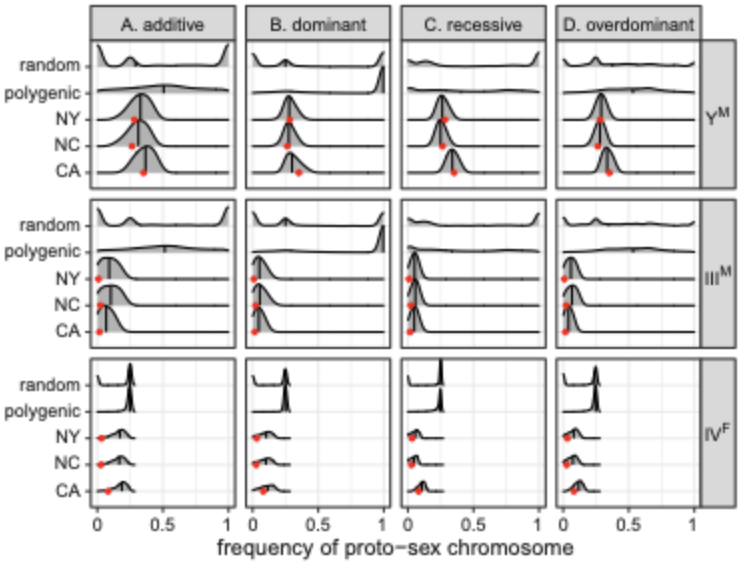
Most dominant fitness effects that appear to maintain PSD are paths to the eventual fixation of Y^M and III^M. More than 25% of the dominant fitness effects that I considered maintain PSD for 1,000 generations (Supplemental Figures S1A, S2A), but most of those produce very high frequencies of the Y^M and III^M chromosomes (Figure 2B; Supplemental Figure S3). A proto-Y chromosome with a dominant fitness effect segregating at very high frequency is

349 consistent with a beneficial effect that masks the recessive variant (in this case the homologous
350 proto-X), allowing the proto-X chromosome to persist at a low frequency for a long time (Hartl
351 and Clark 2007). To test this prediction, I ran simulations for 1,000,000 generations with the
352 best-fitting selection pressures, and I considered a proto-sex chromosome to reach "fixation" or
353 "loss" if it achieves a frequency >99.9% or <0.1%, respectively (i.e., the same threshold for
354 maintaining PSD after 1,000 generations). Consistent with the prediction, >50% of dominant
355 fitness effects that maintain PSD for 1,000 generations eventually cause the Y^M or III^M proto-Y
356 chromosomes to reach fixation (frequency >99.9%) within 1,000,000 generations, regardless of
357 the starting genotype frequencies (Supplemental Figures S4–S7). In contrast, ≤1% of additive,
358 recessive, or overdominant fitness effects of proto-Y chromosomes that maintain PSD for 1,000
359 generations eventually result in fixation of a proto-Y chromosome after 1,000,000 generations.
360 Despite the eventual fixation of proto-Y chromosomes in many parameterizations of dominant
361 fitness effects, there are a subset of dominant fitness effects that maintain Y^M and III^M at lower
362 frequencies, resembling those observed in natural populations (Figure 2B). For these dominant
363 fitness effects that produce proto-sex chromosome frequencies similar natural populations,
364 fixation of proto-Y chromosomes never occurs after 1,000,000 generations (Supplemental
365 Figures S4–S7). Therefore, dominant fitness effects can maintain PSD at equilibrium, although
366 most dominant fitness effects result in fixation or loss of a proto-Y chromosome.

367 Selecting only the 1,000 fitness effects that produce proto-sex chromosome frequencies
368 most similar to natural populations further affects the relationships between dominance and
369 proto-sex chromosome frequencies. For example, proto-sex chromosome frequencies are more
370 similar to those in natural populations when fitness effects of the proto-Y chromosomes are
371 dominant, recessive, or overdominant, as opposed to additive fitness effects (Figure 2;
372 Supplemental Figures S1, S2, S3). The poorer fit of additive models arises because III^M and IV^F

373 segregate at lower frequencies in the CA, NC, and NY populations than they do in the

374 best-fitting simulations with additive fitness effects (Figure 2; Supplemental Figure S3).



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

382 The low frequency of the III^M chromosome in the CA, NC, and NY populations has

383 consequences for the ability of natural selection to maintain the polymorphism. As above, I

384 define loss of the III^M chromosome (or fixation of the III chromosome) as a frequency below

385 0.1% (or above 99.9% for III). Additive fitness effects that maintain the III^M chromosome at

386 frequencies similar to those in CA, NC, or NY for 1,000 generations often (9–17% of the time)

387 result in the loss of III^M within 1,000,000 generations (Supplemental Figures S8, S9). In contrast,

388 <7% of dominant, recessive, or overdominant fitness effects that maintain III^M at frequencies

389 observed in natural populations for 1,000 generations lead to the eventual loss of III^M within

390 1,000,000 generations. III^M is especially likely to be lost within 1,000,000 generations when it is
391 segregating at a lower frequency after 1,000 generations, regardless of the dominance of fitness
392 effects (Supplemental Figures S8, S9). The Y^M chromosome, in comparison, segregates at a
393 higher frequency that III^M all three populations (Figure 2B). Y^M is lost in ≤2% of 1,000,000
394 generation simulations when I apply fitness effects that maintain proto-sex chromosomes at
395 frequencies similar to those observed in natural populations, regardless of the dominance of
396 fitness effects (Supplemental Figure S10). I therefore conclude that low frequency proto-sex
397 chromosomes, such as III^M, are difficult to maintain as polymorphisms at equilibrium under the
398 model I have applied here.

399 The majority of fitness effects that maintain PSD cause IV^F to segregate at a frequency
400 of 25%, regardless of the dominance of the proto-Y chromosomes (Figure 2; Supplemental
401 Figure S3). A frequency of 25% is the expectation for a W chromosome in a randomly mating
402 population with monogenic ZW sex determination. Therefore, in a system with 2 proto-Y
403 chromosomes and one proto-W, PSD is expected to be maintained by the (near) fixation of the
404 ZW female genotype. In contrast, IV^F makes up 2–9% of all fourth chromosomes in the CA, NC,
405 and NY populations (Hamm and Scott 2008; Scott *et al.* 2013; Meisel *et al.* 2016). A subset of
406 fitness arrays in my simulations maintain IV^F at <25% for all dominance models, including those
407 that produce proto-sex chromosome frequencies most similar to those observed in natural
408 populations (Figure 2; Supplemental Figure S3). It is thus possible for PSD to be maintained
409 with a single proto-W chromosome at a frequency <25%. However, even with those best fitting
410 selection parameters, the frequency of IV^F tends to be greater than observed in natural
411 populations (Figure 2).

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

413 I next used simulations to examine how well natural selection maintains PSD when
414 population sizes are finite (N=10^4 individuals). I calculated the proportion of simulations (out of
415 100) in which a given proto-sex chromosome reached fixation for each fitness array to estimate
416 of the probability of fixation (P_{fix}). The P_{fix} value for a given proto-Y or proto-W chromosome
417 (Y^M, III^M, or IV^F) is equal to the probability of loss (P_{loss}) of the homologous proto-X or proto-Z
418 (X, III, or IV), and *vice versa*. I used Fisher's exact test to determine if the number of fixations of
419 a proto-sex chromosome is greater or less in a finite population of the same size and without
420 selection (i.e., genetic drift only).

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

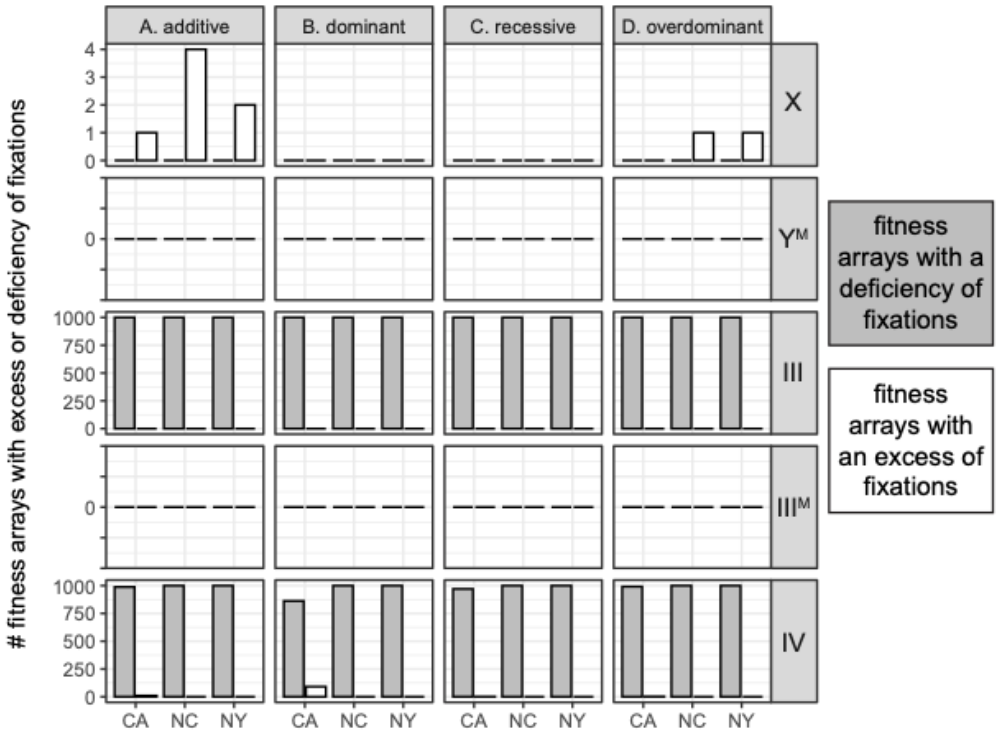


Figure 3. Bar plots show the number of fitness arrays that result in a deficiency (gray) or excess (white) of fixations of each proto-sex chromosome (X, Y^M, III, III^M, or IV) relative to simulations in which there are no fitness differences across proto-sex chromosomes (i.e., drift only). The fitness 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 pressures that should maintain the polymorphism (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 the best-fitting arrays (<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

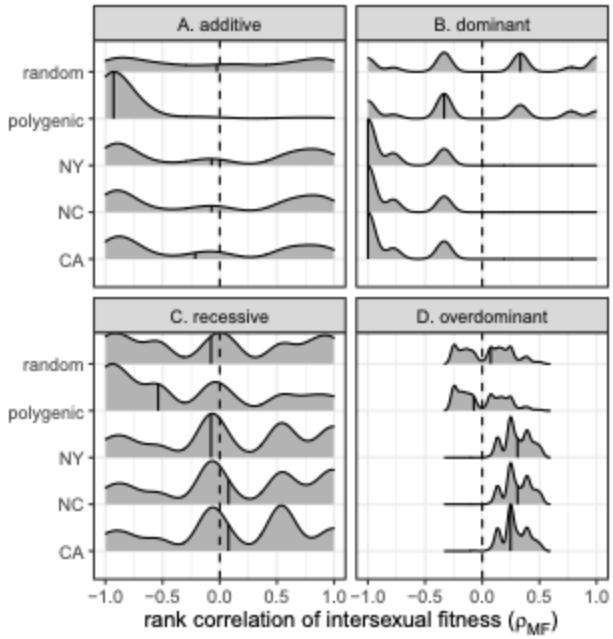
445 chromosomes with selection is observed for all four dominance models and regardless of how
446 the best-fitting arrays are identified (Supplemental Figure S13). Therefore, when fixation or loss
447 is unlikely via drift (because the proto-Y chromosome is at high frequency), selection pressures
448 that maintain PSD do not decrease P_{fix} or P_{loss} of a proto-sex chromosome in a finite
449 population.

450 ***Sexual antagonism and the maintenance of polygenic sex determination***

451 I next inspected if there is a negative correlation between male and female fitness when
452 PSD is maintained. A negative intersexual fitness correlation is a hallmark of sexually
453 antagonistic genetic variation (Rice and Chippindale 2001; Chippindale *et al.* 2001;
454 Bonduriansky and Chenoweth 2009). Each of the eight male house fly proto-sex chromosome
455 genotypes has a corresponding female genotype that differs only because females carry a copy
456 of the IV^F proto-W chromosome and males do not (Table 1). There are two additional female
457 genotypes (X/X; III/III; IV/IV and X/X; III/III; IV^F/IV) that do not have a corresponding male
458 genotype because they do not have a proto-Y chromosome. To test for sexually antagonistic
459 multi-chromosome genotypes, I calculated Spearman's rank order correlation of fitness between
460 males and females (ρ_{MF}) for the eight pairs of male and female genotypes in each fitness
461 array.

462 Additive fitness effects that maintain PSD (i.e., all proto-sex chromosomes at frequency
463 >0.1%) tend to have $\rho_{MF} < 0$ (Figure 4A; Supplemental Figure S14). However, when
464 proto-sex chromosome frequencies resemble those in natural populations and fitness effects
465 are additive, ρ_{MF} can be either positive or negative. There is also no consistent signal of
466 $\rho_{MF} < 0$ for random fitness effects, regardless of dominance (Figure 4; Supplemental Figure
467 S14), demonstrating that $\rho_{MF} < 0$ is not an intrinsic property of the model. In comparison,

468 $\rho_{MF} < 0$ when I previously applied a model with additive fitness effects both within and across
469 chromosomes (Meisel *et al.* 2016). In the model presented here, multi-chromosome genotype
470 fitness is calculated as the product of single chromosome genotype fitness values, rather than
471 by summing across chromosomes. Therefore, there is some evidence that additive fitness
472 effects that maintain PSD result in $\rho_{MF} < 0$, but it depends on specifics of how the model is
473 parameterized.



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

480 When fitness effects are dominant, $\rho_{MF} < 0$ when proto-sex chromosome frequencies
481 resemble those in natural populations. In contrast, ρ_{MF} can be positive or negative for
482 dominant fitness effects that maintain PSD regardless of the proto-sex chromosome frequency

483 (Figure 4B; Supplemental Figure S14B). However, as noted above, many of the cases where
484 dominant fitness effects appear to maintain PSD are instead on a path to fixation that is not yet
485 reached at 1,000 generations. Indeed, $\rho_{MF} < 0$ when I only sample the dominant fitness
486 effects that maintain PSD for 1,000,000 generations, and not for the dominant fitness effects
487 that eventually reach fixation within 1,000,000 generations (Supplemental Figure S15).
488 Therefore, assuming 1,000,000 generations adequately approximates the equilibrium state,
489 $\rho_{MF} < 0$ when dominant fitness effects maintain PSD.

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

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

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

1987). In contrast to this hypothesis, I do not observe a consistent signal of male-beneficial and female-detrimental proto-Y chromosomes (Figure 5; Supplemental Figure S16).

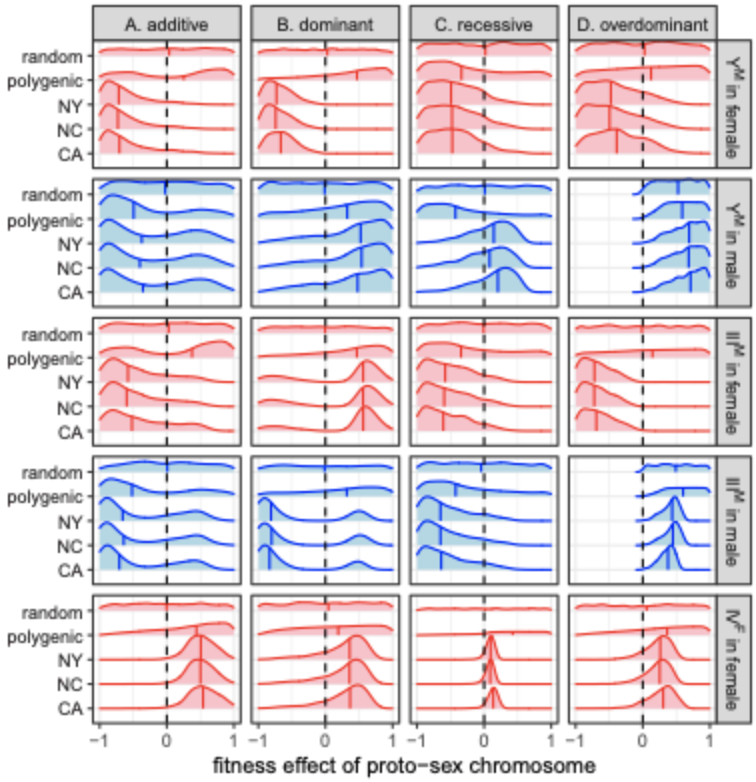


Figure 5. Smoothed histograms show the distributions of fitness effects of each proto-sex chromosome in each sex for 1,000 random fitness arrays (random), fitness arrays that maintain PSD (polygenic), or the 1,000 best-fitting fitness arrays for each population (CA, NC, or NY). Simulations were started with equal frequencies of all genotypes. 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.

Additive fitness effects that maintain PSD often have female-beneficial and male-detrimental effects of the proto-Y chromosomes (Figure 5A; Supplemental Figure S16A). Female-beneficial and male-detrimental Y chromosomes are opposite of the expected effects under most models (Rice 1987, 1996). However, some previous population genetic modeling

517 has suggested the possibility of “feminization” of the Y chromosome (Cavoto *et al.* 2018). Within
518 individual additive parameterizations that maintain PSD, the proto-Y chromosomes do not
519 consistently confer a greater fitness benefit to either males or females (Supplemental Figures
520 S17, S18).

521 There is some evidence that Y^M is male-beneficial and female-detrimental when
522 dominant fitness effects maintain PSD (Figure 5B; Supplemental Figure S16B). Additionally,
523 both Y^M and III^M often confer a greater fitness benefit to males than females when dominant
524 fitness effects maintain PSD (Supplemental Figures S17, S18, S19). However, III^M does not
525 tend to have male-beneficial and female-detrimental effects in the models with dominant fitness
526 effects (Figure 5B; Supplemental Figure S16B). Therefore, when dominant fitness effects
527 maintain PSD the multi-chromosome genotypes tend to have sexually antagonistic fitness
528 effects (Figure 4; Supplemental Figures S14, S15), but the individual chromosomes are not
529 necessarily sexually antagonistic themselves.

530 Overdominant fitness effects that maintain proto-sex chromosomes at frequencies
531 similar to those observed in natural populations have the strongest evidence of male-beneficial
532 and female-detrimental proto-Y chromosomes (Figure 5D; Supplemental Figures S16D, S17D,
533 S18D). The overdominant model is constrained to require male-beneficial effects of the proto-Y
534 chromosomes in heterozygotes, but female fitness effects can be beneficial or deleterious.
535 When proto-sex chromosomes are at frequencies similar to those in the CA, NC, or NY
536 populations, the proto-Y chromosomes are almost always female-deterious. These sexually
537 antagonistic effects of the Y^M and III^M proto-Y chromosomes in the overdominant models does
538 not cause negative intersexual correlations of multi-chromosomal genotype fitness values
539 (Figure 4; Supplemental Figure S14) because of the concave relationship between male
540 genotype and fitness (i.e., heterozygous males are defined as the most fit).

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

542 The IV^F proto-W chromosome is nearly always beneficial to females in fitness arrays that
543 maintain PSD (Figure 5). I observe the female-beneficial effect of IV^F in all cases when PSD is
544 maintained, regardless of the dominance of fitness effects of the proto-Y chromosomes,
545 whether or not proto-sex chromosomes frequencies are similar to those in natural populations,
546 and independently of the genotype frequencies used to start the simulations (Figure 5;
547 Supplemental Figure S16). There also tends to be a positive correlation between the fitness
548 effect and frequency of IV^F when proto-Y chromosomes have additive, dominant, recessive, or
549 overdominant effects, regardless of the starting genotype frequencies used in the simulations
550 (Supplemental Figures S20, S21). A positive correlation indicates that the more
551 female-beneficial the IV^F chromosome, the higher its frequency. The only exception to this rule
552 is when dominant fitness effects maintain PSD at frequencies not similar to those observed in
553 natural populations; however, as described above, these cases are on a path to fixation and
554 therefore not at equilibrium

555 **Discussion**

556 I used forward simulations of a complex PSD system—with two proto-X/Y pairs and one
557 proto-Z/W, modeled after the house fly—to investigate how the dominance of alleles under
558 selection affects the maintenance of PSD. I found that the ability of natural selection to maintain
559 PSD does indeed depend on the dominance of fitness effects. Specifically, PSD is more likely to
560 be maintained if proto-Y chromosomes have overdominant fitness effects, and least likely if the
561 fitness effects are additive (Supplemental Figures S1, S2). Dominance also affects how similar
562 proto-sex chromosome frequencies are to those in natural populations (Figure 2; Supplemental
563 Figure S3). Moreover, the extent of sexually antagonism depends on the dominance of fitness

effects: dominant effects of proto-Y chromosomes result in the strongest signatures of sexually antagonistic multi-chromosomal genotypes (Figure 4; Supplemental Figures S14, S15), whereas overdominant fitness effects produce the strongest signatures of sexually antagonistic proto-Y chromosomes (Figure 5; Supplemental Figure S16). Despite these differences across dominance models, selection pressures that maintain PSD are all effective at preventing the fixation or loss of proto-sex chromosomes via genetic drift, regardless of the dominance of fitness effects (Figure 3; Supplemental Figures S11–S13).

Sexually antagonistic genotypes can maintain polygenic sex determination without sexually antagonistic proto-Y chromosomes

Sexual antagonism has previously been identified as a key component of the maintenance of PSD within populations (Bull 1983; Rice 1986; van Doorn and Kirkpatrick 2007; Meisel *et al.* 2016). A negative inter-sexual correlation of fitness across genotypes is a hallmark of sexually antagonistic selection (Rice and Chippindale 2001; 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, the sexually antagonistic multi-chromosomal genotypes that maintain PSD are emergent properties of cumulative fitness effects across chromosomes. This is evidence for a decoupling between single chromosome sexually antagonistic effects and sexual antagonism across multi-chromosome genotypes. It

587 also suggests that testing for sexually antagonistic effects of individual proto-sex chromosomes
588 may not be a sufficient assay of the role sexual antagonism plays in the maintenance of
589 complex, multi-chromosomal PSD.

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

591 Overdominant fitness effects of proto-Y chromosomes in males are more likely to
592 maintain PSD than any other dominance scenario that I tested. Nearly half of the 4,000,000
593 overdominant fitness effects that I tested maintain all three proto-sex chromosomes at a
594 frequency >0.1% for at least 1,000 generations (Supplemental Figures S1A, S2A). In addition,
595 nearly all of those overdominant fitness effects that maintain PSD for 1,000 generations
596 continue to do so for at least 1,000,000 generations (Supplemental Figures S4–S7), suggesting
597 they are indeed stable polymorphisms at equilibrium. The frequencies with which overdominant
598 fitness effects can maintain multiple polymorphic proto-sex chromosomes covers the full range
599 of possible values, from close to 0 to nearly 1 (Figure 2D; Supplemental Figure S3D).
600 The extent of overdominant genetic variation—and balancing selection more
601 generally—has been a topic of considerable debate in population genetics (Crow 1987; Delph
602 and Kelly 2014; Fijarczyk and Babik 2015), with a few classic examples of heterozygote
603 advantage (e.g., Dobzhansky 1947; Allison 1956). Contemporary evidence for overdominant
604 fitness effects in natural populations is mixed, including some work that suggests
605 overdominance is rare (Andrés *et al.* 2009; Sellis *et al.* 2011, 2016; Hedrick 2012; Bitarello *et al.*
606 2018). Notably, in platyfish, which has a single chromosome PSD system, heterozygous males
607 (XY) and females (WY or WX) have higher fitness than homozygotes (Orzack *et al.* 1980).
608 Regardless of the prevalence of overdominance in natural populations, it is one of the most
609 straightforward mechanisms by which polymorphisms can be maintained (Hartl and Clark 2007).
610 In my overdominant model, I assume that the proto-Y chromosomes carry male-beneficial

611 additive or dominant alleles (Rice 1984, 1992), along with recessive deleterious alleles that
612 have opposing effects equal in magnitude to the beneficial alleles (Charlesworth and
613 Charlesworth 2000). Testing if these assumptions are biologically realistic would require
614 measuring the magnitude and dominance of beneficial and deleterious alleles on proto-Y
615 chromosomes.

616 When overdominant fitness effects maintain proto-sex chromosomes at frequencies
617 most similar to those observed in natural populations, I find that they do so with
618 female-deterious effects of the proto-Y chromosomes (Figure 5D; Supplemental Figure S16D).
619 This is the strongest evidence I observe for sexually antagonistic fitness effects of individual
620 proto-Y chromosomes (as opposed to multi-chromosomal genotypes) in any dominance
621 scenario. It is also consistent with previous work demonstrating that overdominance in one sex
622 can maintain genetic variants at individual loci even if there is directional selection against one
623 allele in the other sex (Kidwell *et al.* 1977). In contrast to the additive and dominant fitness
624 effects, there is no evidence for $\rho_{MF}<0$ when overdominant fitness effects maintain PSD
625 (Figure 4; Supplemental Figure S14). This provides additional evidence for a decoupling
626 between single chromosome sexually antagonistic effects and sexual antagonism across
627 multi-chromosome genotypes.

628 Sexually antagonistic proto-Y chromosomes require there to be segregating sexually
629 antagonistic genetic variation. Male-beneficial and female-deleterious alleles are likely to
630 accumulate on Y chromosomes because of their male-limited inheritance (Rice 1984). It is not
631 clear, however, if that prediction applies when the Y chromosome can be transmitted through
632 females in a complex PSD system such as in house fly. A more appropriate point of comparison
633 for PSD systems may be autosomal and X-linked sexually antagonistic genetic variation, of
634 which there is evidence for a substantial amount (Rice 1992; Chippindale *et al.* 2001; Calsbeek

635 and Sinervo 2004; Fedorka and Mousseau 2004; Foerster *et al.* 2007; Brommer *et al.* 2007;
636 Innocenti and Morrow 2010).

637 My results are also consistent with theory that suggests sexually antagonistic selection is
638 important for the early evolution of sex chromosomes, as well as empirical studies that identified
639 sexually antagonistic variants on young sex chromosomes (Lindholm and Breden 2002; van
640 Doorn and Kirkpatrick 2007, 2010; Roberts *et al.* 2009). It remains to be determined, however,
641 whether young sex chromosomes are enriched for sexually antagonistic variants, if those
642 variants are maintained by balancing selection, and if the sexually antagonistic alleles contribute
643 to the maintenance of PSD. Investigating these problems could follow the approaches
644 previously developed to test if balancing selection maintains sexually antagonistic alleles
645 throughout the genome (e.g., Dutoit *et al.* 2018; Ruzicka *et al.* 2019).

646 ***The dominance of sexually antagonistic alleles***

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

657 In most of the models I consider here, the dominance of fitness effects of the proto-Y
658 chromosomes is the same in both sexes. However, the dominance of fitness effects may be

659 sex-specific (Barson *et al.* 2015; Grieshop and Arnqvist 2018). Such dominance reversals could
660 be important for the maintenance of sexually antagonistic alleles (Spencer and Priest 2016;
661 Connallon and Chenoweth 2019), including at sex-linked loci (Fry 2010). Therefore, it is
662 possible that sex-specific dominance could create a broader range of fitness values that
663 maintain PSD via sexually antagonistic selection. Future work could consider this possibility by
664 including sex-specific dominance as an additional randomly chosen variable within my modeling
665 framework.

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

667 My model makes assumptions about recombination on the sex chromosomes that may
668 affect my conclusions about the maintenance of PSD. Specifically, I assume that there is no
669 recombination between the male- or female-determining locus on each proto-Y or proto-W
670 chromosome and the allele(s) under selection. X-Y and Z-W recombination may be suppressed
671 by chromosomal inversions that create tight genetic linkage between the sex-determining locus
672 and sexually antagonistic alleles (Bergero and Charlesworth 2009; Wright *et al.* 2016). While
673 there is no direct evidence for inversions or other recombination suppressors on the house fly
674 sex chromosomes, male meiosis in flies is thought to occur without crossing over (Gethmann
675 1988). A lack of crossing over in males would prevent X-Y recombination in males without the
676 need for sex-chromosome-specific modifiers. However, there is evidence for male
677 recombination in house fly (Feldmeyer *et al.* 2010), which opens up the possibility for X-Y
678 recombination. Furthermore, in the absence of Y-linked suppressors, X-Y recombination could
679 also occur in the multiple female genotypes that carry a proto-Y chromosome (Table 1).
680 There are at least three other consequences of recombination on the sex chromosomes
681 that could affect how sex-specific selection maintains PSD. First, my model assumes that all
682 copies of each proto-Y and proto-W chromosome have identical fitness effects. This assumption

683 is consistent with population genetics theory that predicts Y-linked variants cannot segregate as
684 protected polymorphisms when there is no X-Y recombination (Clark 1987). In contrast to this
685 prediction, there are numerous examples of Y-linked polymorphisms with phenotypic effects in
686 *Drosophila*, where the X and Y do not recombine (Clark 1990; Zhang *et al.* 2000; Lemos *et al.*
687 2008, 2010; Griffin *et al.* 2015; Brown *et al.* 2020). Relevant to the house fly, these examples
688 include Y-linked variation across heterogeneous environments over the species' geographic
689 range (Rohmer *et al.* 2004). Future work could incorporate variation in fitness effects of each
690 proto-Y and proto-W chromosome into my model to assess how segregating polymorphisms on
691 the proto-sex chromosomes affect the maintenance of PSD.

692 A second consequence of sex chromosome recombination could affect how
693 overdominant fitness effects maintain PSD. I assume that overdominance arises from proto-Y
694 chromosomes that carry recessive deleterious alleles. This assumption is based on the
695 prediction that suppressed X-Y recombination leads to the "degeneration" of the Y chromosome
696 via accumulation of (recessive) deleterious alleles because of Muller's ratchet and hitchhiking
697 (Charlesworth and Charlesworth 2000; Bachtrog 2013). In contrast, X-Y recombination in males
698 and/or females could help purge recessive deleterious mutations from the Y^M and III^M
699 chromosomes (Muller 1964; Felsenstein 1974). Moreover, there are multiple genotypes that are
700 homozygous for a proto-Y chromosome (Table 1), which would expose recessive deleterious
701 mutations to selection. Selection in these proto-Y chromosome homozygotes would further
702 remove recessive deleterious alleles from populations (Charlesworth *et al.* 1990; Barrett and
703 Charlesworth 1991). Therefore, while overdominant fitness effects are likely to maintain PSD,
704 proto-Y chromosomes may not possess the necessary alleles for such selection pressures to
705 act upon. However, overdominance can also occur by other mechanisms besides degeneration

706 of the Y chromosome and independent of accumulation of recessive deleterious Y-linked alleles
707 (Sellis *et al.* 2011; Connallon and Clark 2014).

708 Third, X-Y recombination would introduce X-linked alleles onto the proto-Y
709 chromosomes. Male-limited inheritance of a Y chromosome is expected to lead to the
710 accumulation of male-beneficial alleles in Y-linked genes, even if they have female-deleterious
711 effects (Rice 1987, 1996). Conversely, female-biased inheritance of the X chromosome is
712 expected to result in stronger selection on X-linked alleles in females if those alleles are partially
713 dominant or segregating at moderate frequencies (Rice 1984; Charlesworth *et al.* 1987; Orr and
714 Betancourt 2001), potentially feminizing the X chromosome. If there is X-Y recombination, those
715 feminized X-linked alleles would be continuously introduced onto the Y chromosome, possibly
716 feminizing the Y as well (Cavoto *et al.* 2018). This could provide a biological mechanism that
717 allows for female-beneficial and male-detrimental effects of the proto-Y chromosomes that I
718 predict when additive fitness effects maintain PSD (Figure 5A; Supplemental Figure S16A).

719 ***Proto-sex chromosomes are reminiscent of pseudoautosomal regions, with some***
720 ***notable differences***

721 Proto-sex chromosomes have some superficial similarities to pseudoautosomal regions
722 (PARs) of heteromorphic sex chromosomes. The PAR is a region within which there is X-Y (or
723 Z-W) recombination, even if recombination is suppressed between the remainder of the X and Y
724 (Otto *et al.* 2011). Notably, PARs are capable of maintaining genetic variation, including sexually
725 antagonistic alleles, under broader conditions than autosomes or even non-recombining regions
726 of sex chromosomes (Jordan and Charlesworth 2012). There is evidence for sexually
727 antagonistic alleles in PARs from plants (Delph *et al.* 2010; Spigler *et al.* 2011) and fish (Tripathi
728 *et al.* 2009; Kitano *et al.* 2009), but it is not yet clear whether PARs are generally enriched for
729 sexually antagonistic alleles. Furthermore, the unique evolutionary dynamics of PARs is

730 generally limited to loci close to the boundary with the non-recombining region of the sex
731 chromosomes (Charlesworth *et al.* 2014).

732 Despite the superficial similarities, there are important differences that make PARs
733 imperfect analogs to proto-sex chromosomes. First, when there is an epistatic dominant female
734 determining proto-W chromosome (as in house fly), the proto-Y chromosome itself can be
735 transmitted through females. In contrast, the Y PAR cannot ever be carried by females, but
736 ancestrally Y-linked PAR alleles can recombine onto the X PAR and thus be carried by females.
737 Moreover, males can be homozygous for the proto-Y chromosome, which is superficially similar
738 to a male carrying an X PAR that has an ancestrally Y-linked allele. However, as above, this
739 ancestral Y allele on the X PAR must recombine onto the X PAR, whereas, when there is PSD,
740 homozygosity for the proto-Y occurs without X-Y recombination. Additional theoretical analyses
741 are required to directly compare the invasion dynamics, fixation probabilities, and regions of
742 stable polymorphisms across PARs and proto-sex chromosomes.

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

744 My analysis suggests that it is difficult for selection to maintain proto-sex chromosomes
745 at low frequencies within populations. The III^M proto-Y is the lowest frequency proto-sex
746 chromosome I consider here, segregating at ≤2% in all three populations (Hamm *et al.* 2005;
747 Meisel *et al.* 2016). Selection pressures that maintain III^M at a frequency similar to those
748 observed in the populations for 1,000 generations allow for the loss of III^M within 1,000,000
749 generations (Supplemental Figures S8, S9). Additive fitness effects are especially likely to allow
750 for the loss of III^M. In addition, when PSD is maintained with proto-sex chromosomes at
751 frequencies similar to natural populations, III^M tends to be at a higher frequency in my
752 simulations than in any of the actual populations (Figure 2; Supplemental Figure S3). III^M is also
753 frequently lost when population size is finite, even when selection pressures exist that should

754 maintain the polymorphism (Supplemental Figures S11, S12). IV^F is also found at a low
755 frequency (2–9% of all fourth chromosomes in the natural populations); it is similarly found at a
756 higher frequency in the simulations than in the actual populations (Figure 2; Supplemental
757 Figure S3), and it is often lost in finite populations with selection (Supplemental Figures S11,
758 S12).

759 My results suggest that factors in addition to natural selection within populations may be
760 required for the maintenance of low frequency proto-sex chromosomes. Variation in proto-sex
761 chromosome frequencies across house fly populations are suggestive of what those factors
762 may be. For example, spatially variable, temporally fluctuating, and other heterogeneous
763 selection pressures can contribute to the maintenance of genetic variation across populations
764 (Levene 1953; Hedrick 2006), including maintaining PSD (Bateman and Anholt 2017). The
765 house fly Y^M and III^M proto-Y chromosomes are distributed along north-south clines on multiple
766 continents (Denholm *et al.* 1986; Tomita and Wada 1989; Hamm *et al.* 2005; Kozielska *et al.*
767 2008), suggesting spatially heterogeneous selection pressures may be important for maintaining
768 PSD across house fly populations. Migration between these populations would continuously
769 introduce rare proto-Y and proto-W chromosomes, potentially overwhelming selection pressures
770 that would otherwise allow for the loss of those proto-sex chromosomes within populations
771 (Lenormand 2002; Tigano and Friesen 2016). Future work should directly model the effect of
772 migration across populations with different selection pressures on the maintenance of PSD
773 within populations.

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

775 I anticipate that many of the general patterns I observe here would be similar in other
776 complex PSD systems with at least three different proto-sex chromosomes. For example, in a
777 system with more than two proto-Y chromosomes, I expect to observe similar effects of

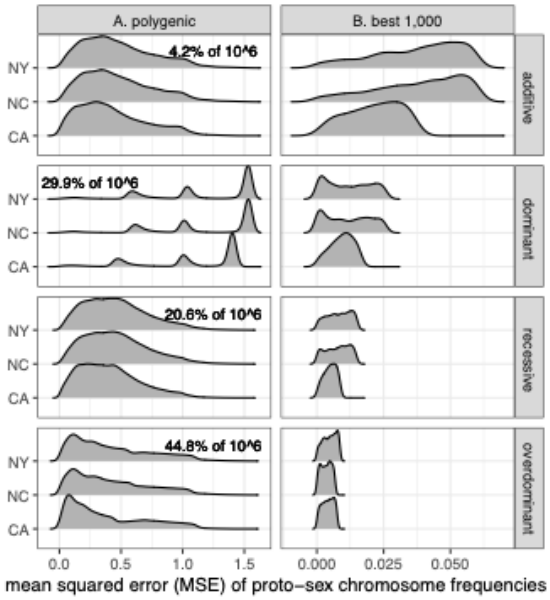
778 dominance on the maintenance of PSD and sexual antagonism as in the system I modeled with
779 two proto-Y chromosomes. In addition, if there are multiple proto-W chromosomes and a single
780 (epistatic) proto-Y (i.e., the opposite of the house fly system), I would expect similar patterns as
781 I observe in the house fly system, but with the sexes reversed. These expectations could be
782 tested in future work. It is not clear, however, what would be expected if there were both multiple
783 proto-W and multiple proto-Y chromosomes in a population. It is worth noting that polygenic
784 systems with many sex-determining loci are expected to be evolutionarily unstable (Rice 1986).
785 Zebrafish may represent a promising model organism for combining empirical and theoretical
786 approaches to make additional progress in understanding the maintenance of many sex
787 determining loci (Liew *et al.* 2012; Wilson *et al.* 2014).

788 ***Conclusions***

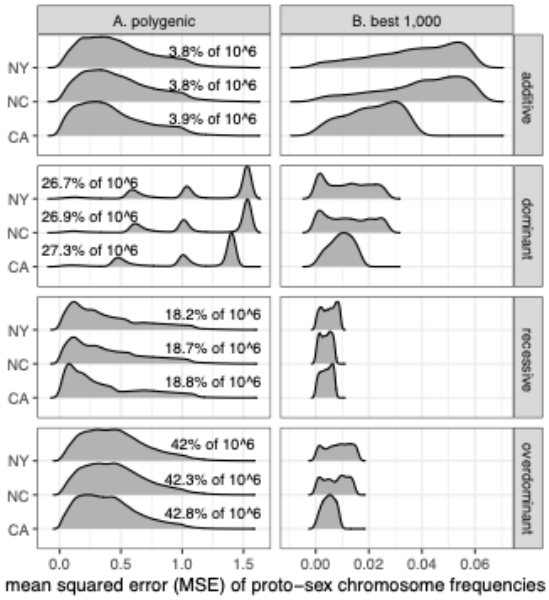
789 My results demonstrate that the maintenance of PSD depends on the dominance of
790 fitness effects, which are further predictive of the sexually antagonistic effects of proto-sex
791 chromosomes and genotypes. Notably, overdominant fitness effects are most likely to maintain
792 PSD, but that conclusion requires at least one of two important assumptions: genetic variation
793 with overdominant fitness effects must exist in natural populations, or proto-Y chromosomes
794 must carry recessive deleterious alleles (in addition to additive or dominant beneficial alleles).
795 My results also suggest that sexual antagonistic multi-chromosomal genotypes should be most
796 prominent when dominant fitness effects (and to a lesser extent additive fitness effects) maintain
797 PSD. This sexual antagonism is an emergent property of multi-chromosomal genotypes, and
798 not intrinsic to the fitness effects of all individual proto-Y chromosomes. In contrast, when
799 overdominant fitness effects maintain PSD, the proto-Y chromosomes tend to be
800 male-beneficial and female-detrimental, but the multi-chromosomal genotypes do not have
801 opposing fitness effects in males and females. Lastly, future work is needed to evaluate how

802 X-Y (and Z-W) recombination, heterogeneous selection pressures, and migration across demes
803 affect the ability of sex-specific selection pressures to maintain complex PSD, including in
804 systems with other combinations of proto-sex chromosomes.

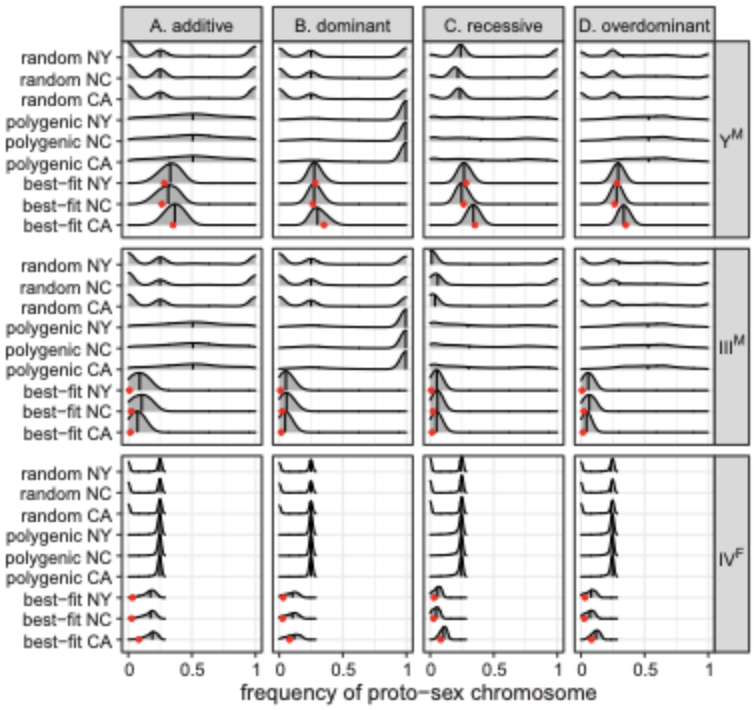
Supplemental Material



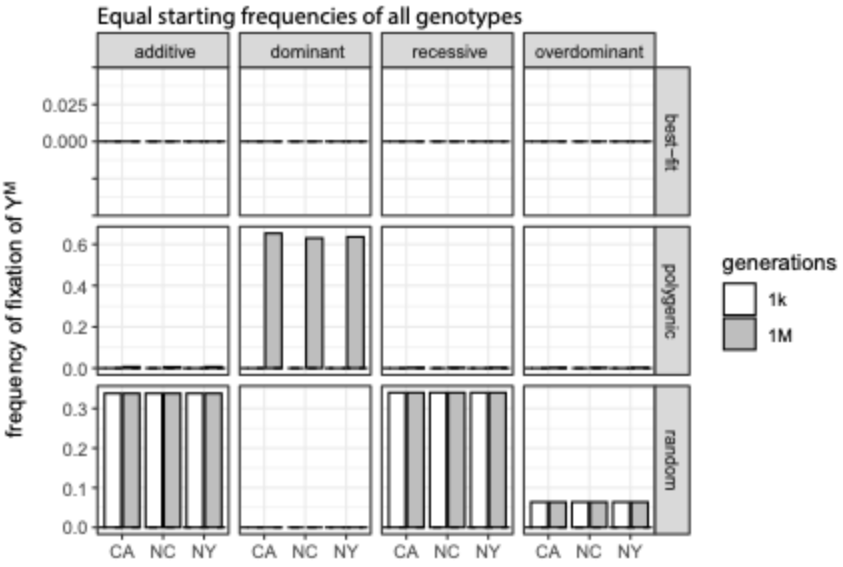
Supplemental Figure S1. Smoothed histograms show the distributions of mean squared error (MSE) values for fitness arrays that maintain PSD (i.e., all proto-sex chromosomes at a frequency >0.1%). MSE is calculated based on the frequencies of Y^M, III^M, and IV^F found in either CA, NC, or NY. Simulations 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.



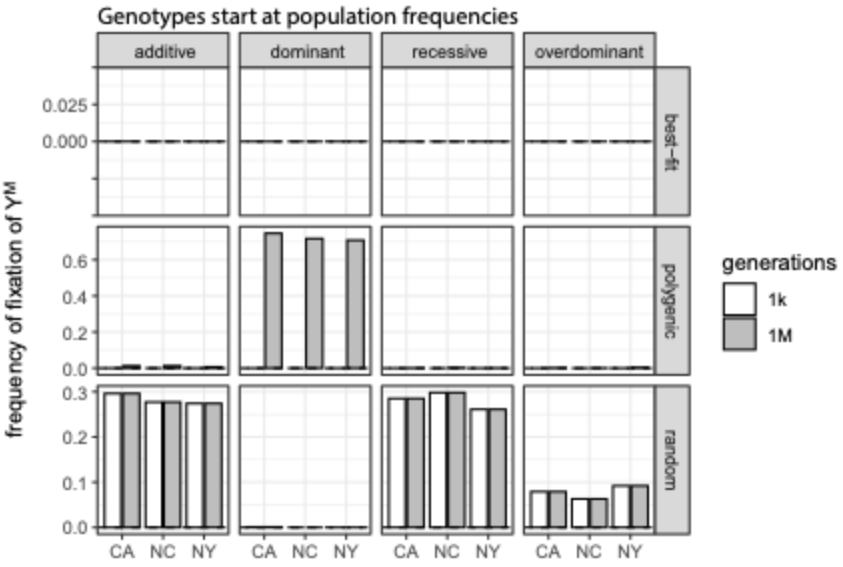
Supplemental Figure S2. Smoothed histograms show the distributions of mean squared error (MSE) values for fitness arrays that maintain PSD (i.e., all proto-sex chromosomes at a frequency >0.1%). MSE is calculated based on the frequencies of Y^M, III^M, and IV^F found in either CA, NC, or NY. Simulations 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.



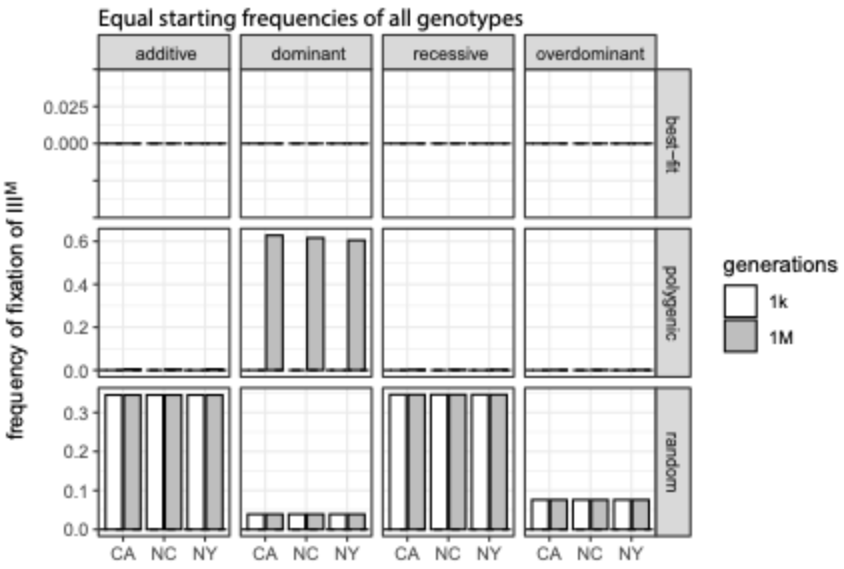
Supplemental Figure S3. Smoothed histograms show the frequency of each proto-sex chromosome (Y^M, III^M, and IV^F) after 1,000 generations in simulations using either 1,000 random fitness arrays (random), fitness arrays that maintain PSD (polygenic), or the 1,000 best-fitting fitness arrays for each 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.



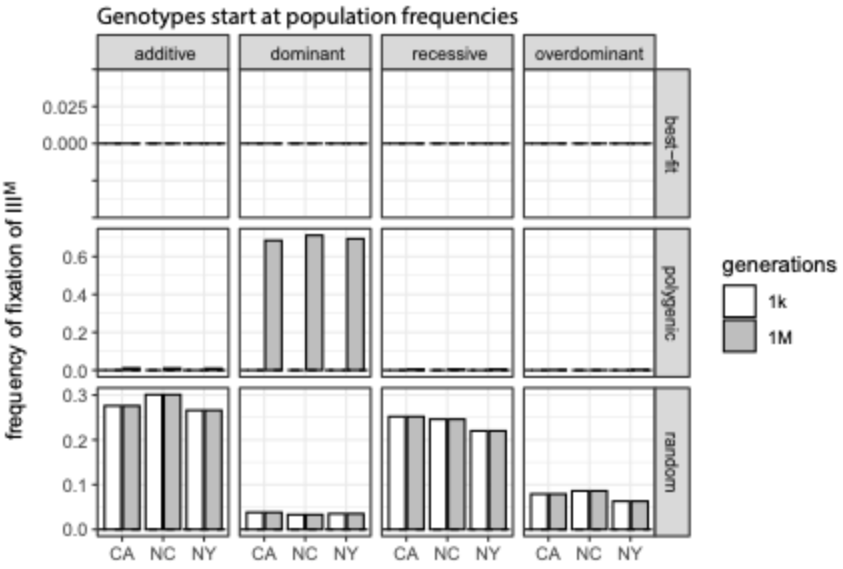
Supplemental Figure S4. The frequency of fixation of the Y^M proto-Y chromosome after 1,000 generations (white) and 1,000,000 generations (gray) is shown for the 1,000 best-fitting genotype fitness arrays (best-fit), 1,000 fitness arrays that maintain PSD (polygenic), and 1,000 randomly chosen fitness 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.



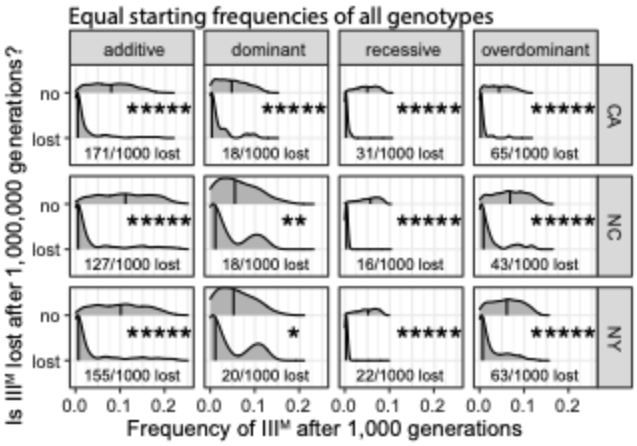
836 **Supplemental Figure S5.** The frequency of fixation of the Y^M proto-Y chromosome after 1,000
837 generations (white) and 1,000,000 generations (gray) is shown for the 1,000 best-fitting genotype fitness
838 arrays (best-fit), 1,000 fitness arrays that maintain PSD (polygenic), and 1,000 randomly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



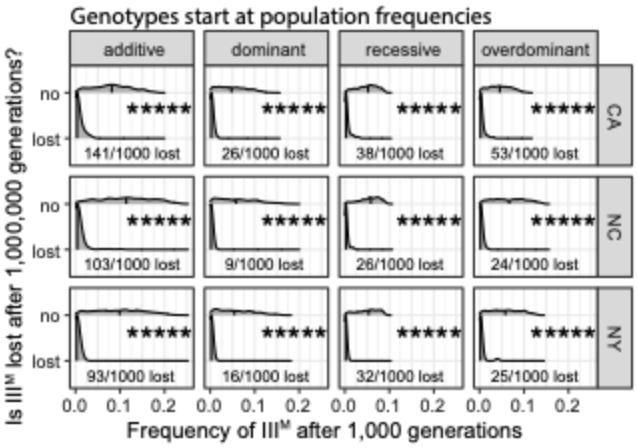
843 **Supplemental Figure S6.** The frequency of fixation of the III^M proto-Y chromosome after 1,000
844 generations (white) and 1,000,000 generations (gray) is shown for the 1,000 best-fitting genotype fitness
845 arrays (best-fit), 1,000 fitness arrays that maintain PSD (polygenic), and 1,000 randomly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



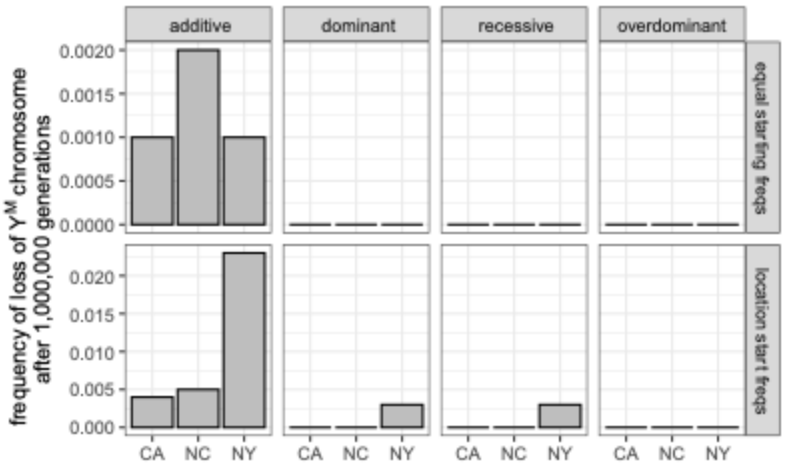
Supplemental Figure S7. The frequency of fixation of the III^M proto-Y chromosome after 1,000 generations (white) and 1,000,000 generations (gray) is shown for the 1,000 best-fitting genotype fitness arrays (best-fit), 1,000 fitness arrays that maintain PSD (polygenic), and 1,000 randomly chosen fitness 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.



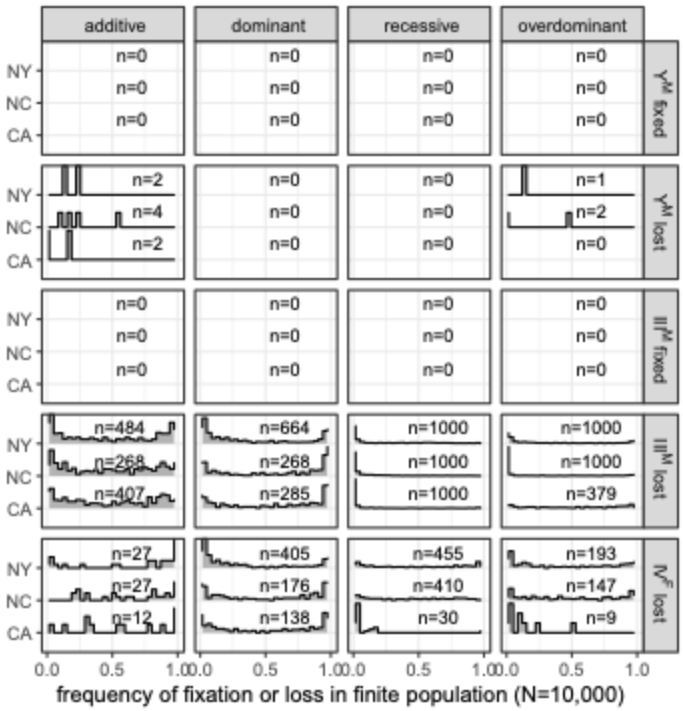
Supplemental Figure S8. Histograms of the frequency of the III^M proto-Y chromosome after 1,000 generations are shown for the 1,000 fitness arrays that produce proto-sex chromosomes most similar to those observed in each natural population (CA, NC, or NY). Fitness arrays are divided into those in which 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.



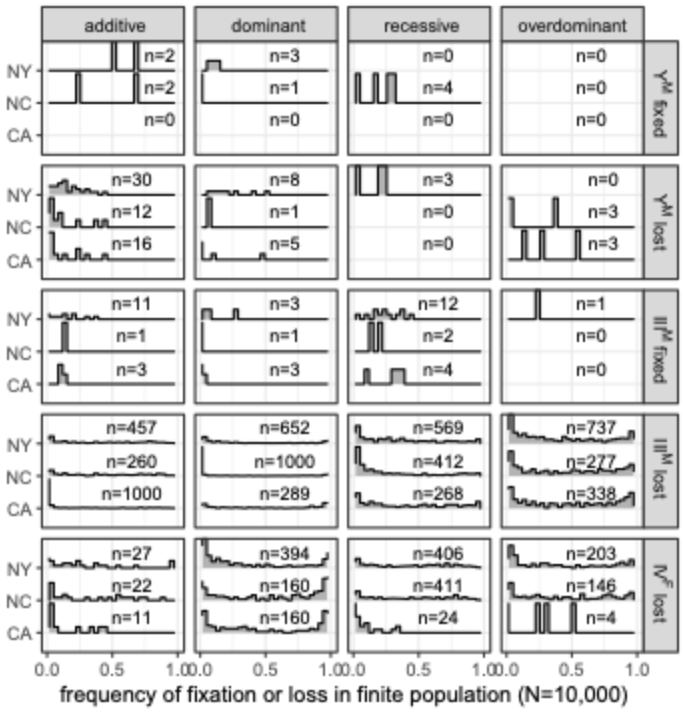
Supplemental Figure S9. Histograms of the frequency of the III^M proto-Y chromosome after 1,000 generations are shown for the 1,000 fitness arrays that produce proto-sex chromosomes most similar to those observed in each natural population (CA, NC, or NY). Fitness arrays are divided into those in which 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.



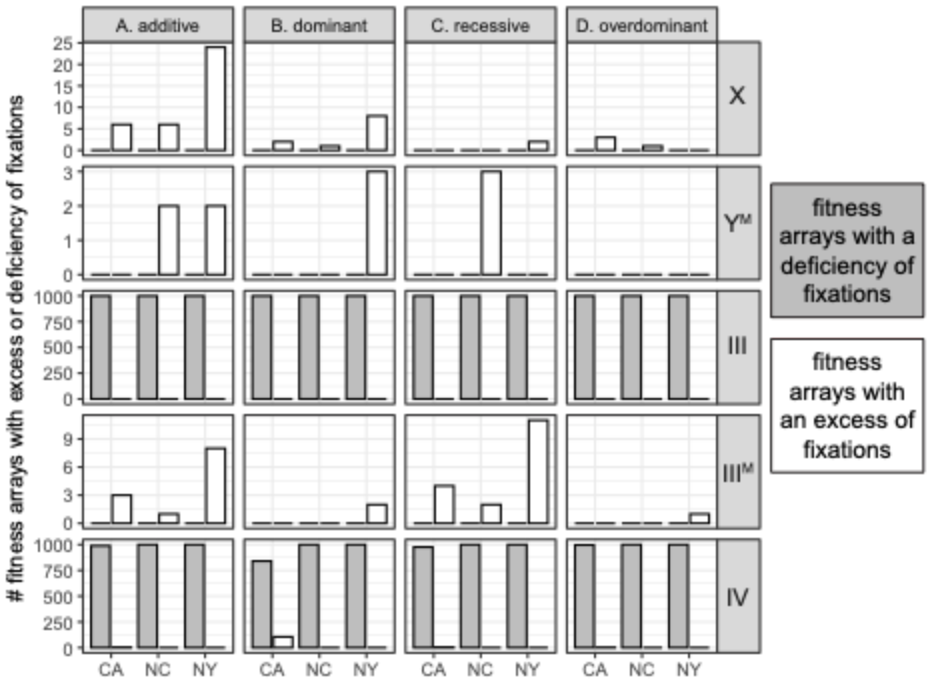
Supplemental Figure S10. Bar graphs show the frequency with which the Y^M chromosome is lost after 1,000,000 generation using fitness effects that maintain proto-sex chromosome frequencies similar to those observed in each natural population (CA, NC, or NY). The best-fitting arrays were identified with 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.



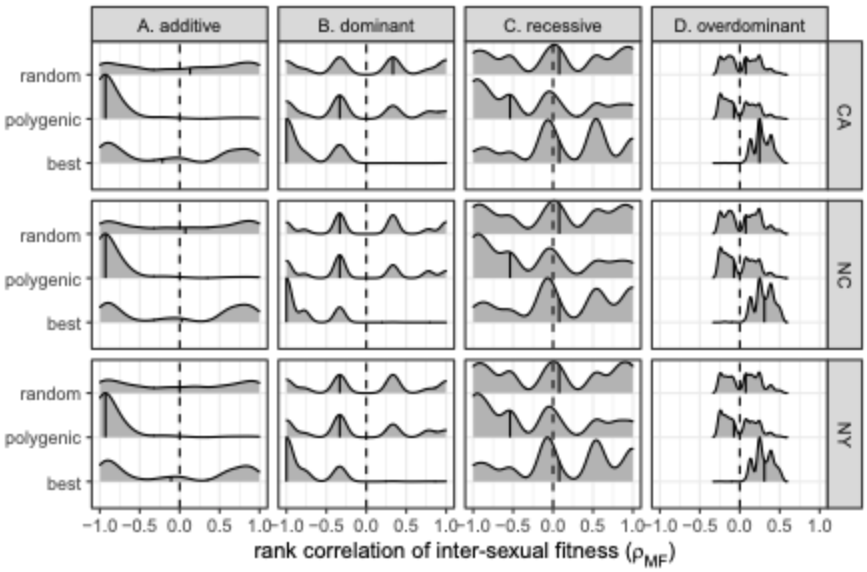
878 **Supplemental Figure S11.** Histograms show the distributions of frequency of fixation or loss each
879 proto-Y or proto-W chromosome in each population when N=10^4. Numbers in each panel (n) indicate the
880 number of fitness arrays with at least one fixation in 100 simulations. The x-axis is the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



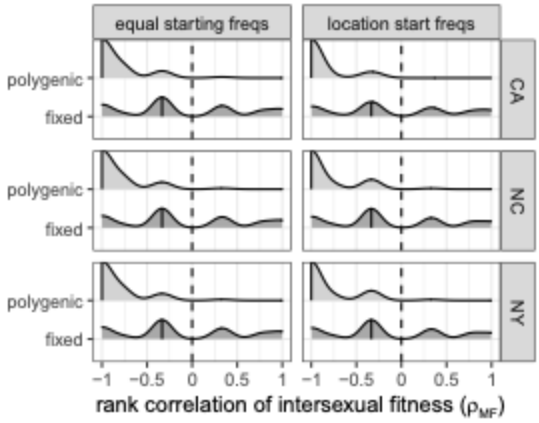
Supplemental Figure S12. Histograms show the distributions of frequency of fixation or loss each proto-Y or proto-W chromosome in each population when N=10^4. Numbers in each panel (n) indicate the number of fitness arrays with at least one fixation in 100 simulations. The x-axis is the frequency of 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.



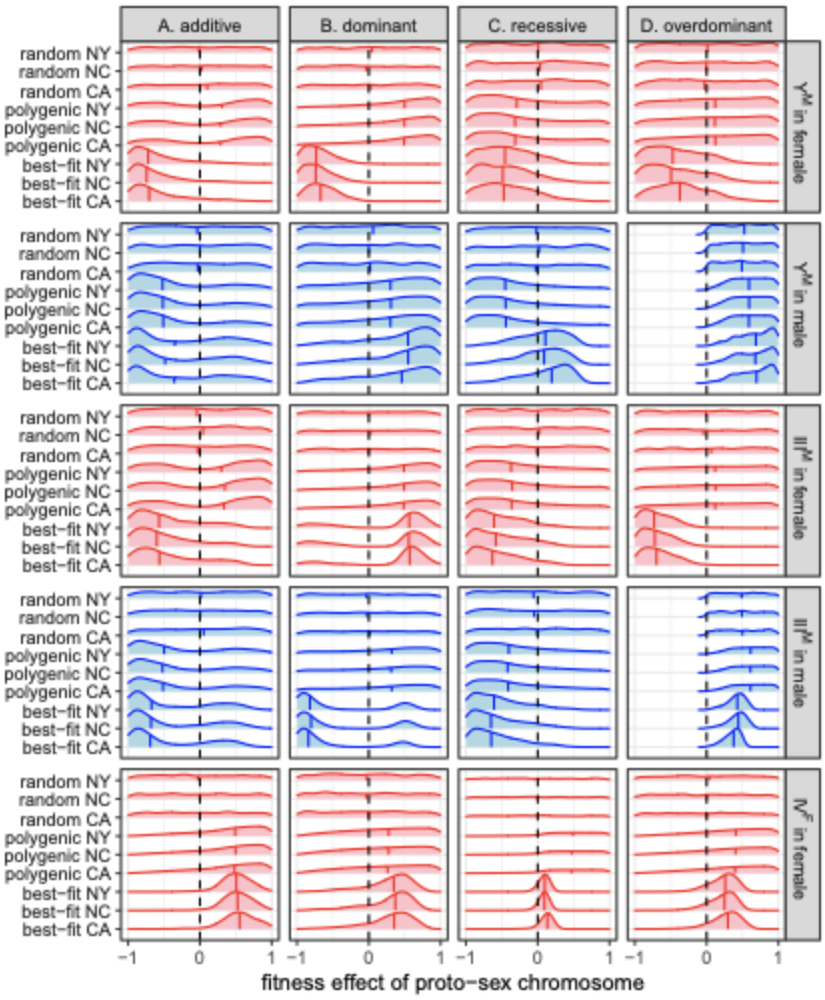
Supplemental Figure S13. Bar plots show the number of fitness arrays that result in a deficiency (gray) or excess (white) of fixations of each proto-sex chromosome (X, Y^M, III, III^M, or IV) relative to simulations in which there are no fitness differences across proto-sex chromosomes (i.e., drift only). 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.



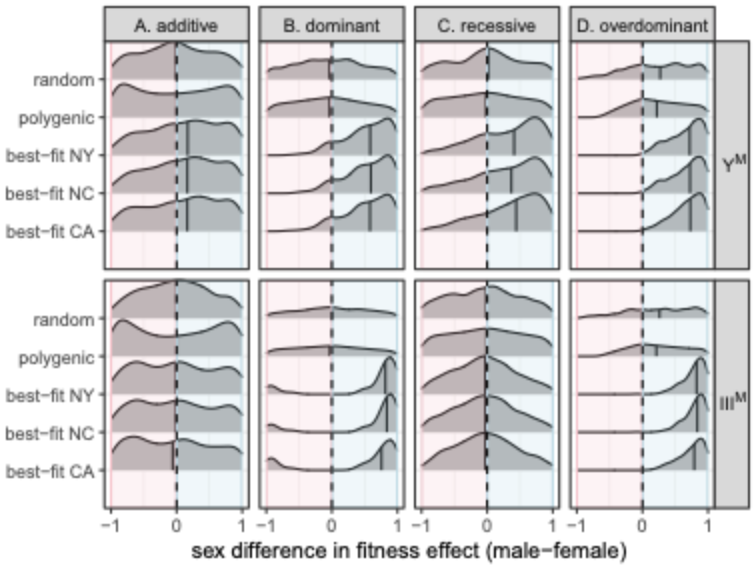
Supplemental Figure S14. Smoothed histograms show intersexual fitness correlations (ρ_{MF}) for the 1,000 best-fitting genotypic fitness arrays for the CA, NC, and NY populations (best), all fitness arrays that maintain PSD (polygenic), and 1,000 random fitness arrays. Simulations were started with genotype 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.



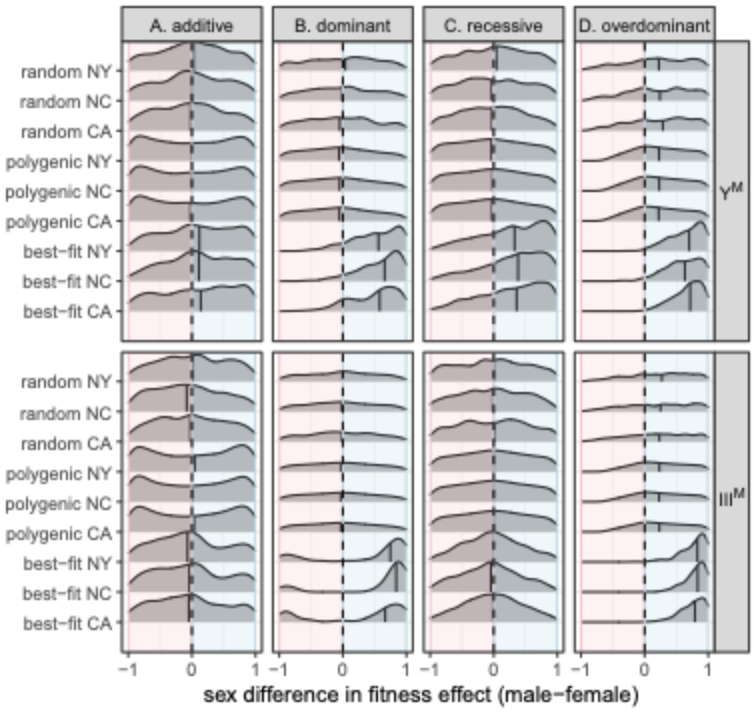
Supplemental Figure S15. Smoothed histograms show intersexual fitness correlations (ρ_{MF}) for the 1,000 best-fitting genotypic fitness arrays based on dominant fitness effects of the proto-Y chromosomes for the CA, NC, and NY populations after 1,000,000 generations. Fitness arrays that maintain PSD 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.



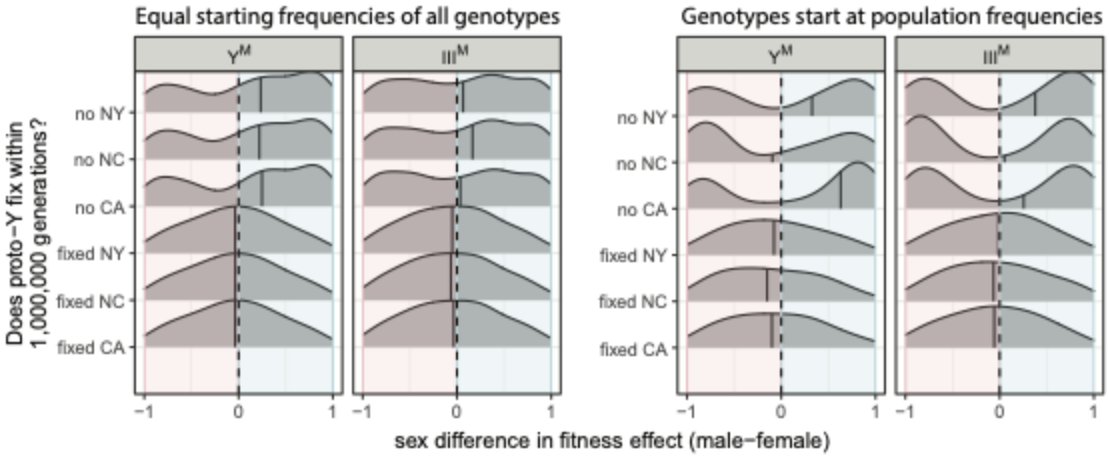
Supplemental Figure S16. Smoothed histograms show the distributions of fitness effects of each proto-sex chromosome in each sex for 1,000 random fitness arrays (random), fitness arrays that maintain PSD (polygenic), or the 1,000 best-fitting fitness arrays for each population (CA, NC, or NY). Simulations 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.



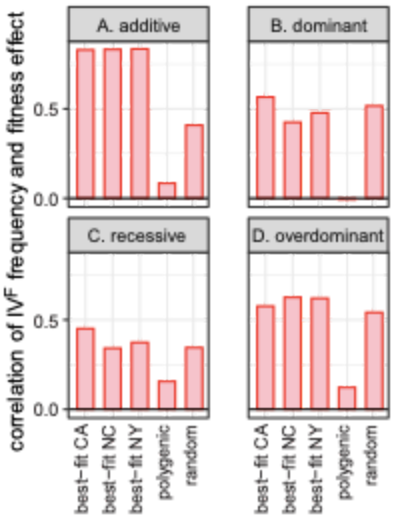
Supplemental Figure S17. Smoothed histograms show the distributions of the difference in the fitness effect of each proto-Y chromosome (Y^M or III^M) between males and females within individual parameterizations of the model. If the fitness effect is greater in males, the values are positive. If the 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.



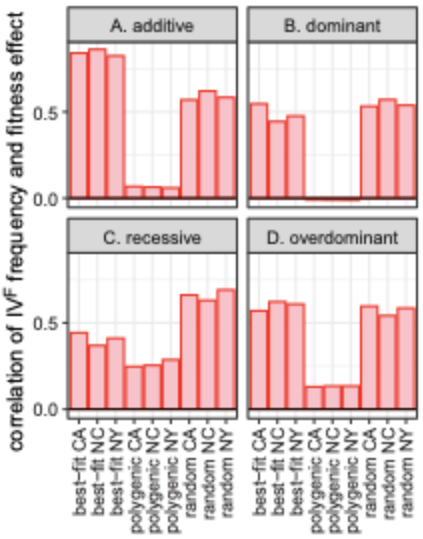
Supplemental Figure S18. Smoothed histograms show the distributions of the difference in the fitness effect of each proto-Y chromosome (Y^M or III^M) between males and females within individual parameterizations of the model. If the fitness effect is greater in males, the values are positive. If the 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 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	$f_6m_4/32 + f_6m_5/16 + f_6m_7/16 + f_6m_8/8$ $+f_7m_4/16 + f_7m_5/8 + f_7m_7/8 + f_7m_8/4$ $+f_9m_4/16 + f_9m_5/8 + f_9m_7/8 + f_9m_8/4$ $+f_{10}m_4/8 + f_{10}m_5/4 + f_{10}m_7/4 + f_{10}m_8/2$

References cited

Abbott, J. K., A. K. Nordén, and B. Hansson, 2017 Sex chromosome evolution: historical insights and future perspectives. Proc. R. Soc. B. 284: 20162806.

Allison, A. C., 1956 The sickle-cell and haemoglobin C genes in some African populations. Ann. Hum. Genet. 21: 67–89.

Andrés, A. M., M. J. Hubisz, A. Indap, D. G. Torgerson, J. D. Degenhardt *et al.*, 2009 Targets of balancing selection in the human genome. Mol. Biol. Evol. 26: 2755–2764.

Bachtrog, D., 2013 Y-chromosome evolution: emerging insights into processes of Y-chromosome degeneration. Nat. Rev. Genet. 14: 113–124.

Bachtrog, D., J. E. Mank, C. L. Peichel, M. Kirkpatrick, S. P. Otto *et al.*, 2014 Sex determination: why so many ways of doing it? PLoS Biol. 12: e1001899.

Barrett, S. C., and D. Charlesworth, 1991 Effects of a change in the level of inbreeding on the genetic load. Nature 352: 522–524.

Barson, N. J., T. Aykanat, K. Hindar, M. Baranski, G. H. Bolstad *et al.*, 2015 Sex-dependent dominance at a single locus maintains variation in age at maturity in salmon. Nature 528: 405–408.

Bateman, A. W., and B. R. Anholt, 2017 Maintenance of polygenic sex determination in a fluctuating environment: an individual-based model. J. Evol. Biol. 30: 915–925.

Beaumont, M. A., W. Zhang, and D. J. Balding, 2002 Approximate Bayesian computation in population genetics. Genetics 162: 2025–2035.

Bergero, R., and D. Charlesworth, 2009 The evolution of restricted recombination in sex chromosomes. Trends Ecol. Evol. 24: 94–102.

Beukeboom, L. W., and N. Perrin, 2014 *The Evolution of Sex Determination*. Oxford University

Press.

Bitarello, B. D., C. de Filippo, J. C. Teixeira, J. M. Schmidt, P. Kleinert *et al.*, 2018 Signatures of long-term balancing selection in human genomes. Genome Biol. Evol. 10: 939–955.

Bonduriansky, R., and S. F. Chenoweth, 2009 Intralocus sexual conflict. Trends Ecol. Evol. 24: 280–288.

Brommer, J. E., M. Kirkpatrick, A. Qvarnström, and L. Gustafsson, 2007 The intersexual genetic correlation for lifetime fitness in the wild and its implications for sexual selection. PLoS One 2: e744.

Brown, E. J., A. H. Nguyen, and D. Bachtrog, 2020 The Y chromosome may contribute to sex-specific aging in *Drosophila*. Nat Ecol Evol 4: 853–862.

Bull, J. J., 1983 *Evolution of Sex Determining Mechanisms*. Benjamin/Cummings.

Calsbeek, R., and B. Sinervo, 2004 Within-clutch variation in offspring sex determined by differences in sire body size: cryptic mate choice in the wild. J. Evol. Biol. 17: 464–470.

Cavoto, E., S. Neuenschwander, J. Goudet, and N. Perrin, 2018 Sex-antagonistic genes, XY recombination and feminized Y chromosomes. J. Evol. Biol. 31: 416–427.

Charlesworth, B., and D. Charlesworth, 2000 The degeneration of Y chromosomes. Philos. Trans. R. Soc. Lond. B Biol. Sci. 355: 1563–1572.

Charlesworth, B., J. A. Coyne, and N. H. Barton, 1987 The relative rates of evolution of sex chromosomes and autosomes. Am. Nat. 130: 113–146.

Charlesworth, B., C. Y. Jordan, and D. Charlesworth, 2014 The evolutionary dynamics of sexually antagonistic mutations in pseudoautosomal regions of sex chromosomes. Evolution 68: 1339–1350.

Charlesworth, D., M. T. Morgan, and B. Charlesworth, 1990 Inbreeding depression, genetic load, and the evolution of outcrossing rates in a multilocus system with no linkage.

Evolution 44: 1469–1489.

Chippindale, A. K., J. R. Gibson, and W. R. Rice, 2001 Negative genetic correlation for adult fitness between sexes reveals ontogenetic conflict in *Drosophila*. Proc. Natl. Acad. Sci. U. S. A. 98: 1671–1675.

Clark, A. G., 1990 Two tests of Y chromosomal variation in male fertility of *Drosophila melanogaster*. Genetics 125: 527–534.

Clark, A. G., 1987 Variation in Y chromosome segregation in natural populations of *Drosophila melanogaster*. Genetics 115: 143–151.

Connallon, T., and S. F. Chenoweth, 2019 Dominance reversals and the maintenance of genetic variation for fitness. PLoS Biol. 17: e3000118.

Connallon, T., and A. G. Clark, 2014 Balancing selection in species with separate sexes: insights from Fisher's geometric model. Genetics 197: 991–1006.

Connallon, T., N. D. Singh, and A. G. Clark, 2012 Impact of genetic architecture on the relative rates of X versus autosomal adaptive substitution. Mol. Biol. Evol. 29: 1933–1942.

Crow, J. F., 1987 Muller, Dobzhansky, and overdominance. J. Hist. Biol. 20: 351–380.

Delph, L. F., A. M. Arntz, C. Scotti-Saintagne, and I. Scotti, 2010 The genomic architecture of sexual dimorphism in the dioecious plant *Silene latifolia*. Evolution 64: 2873–2886.

Delph, L. F., and J. K. Kelly, 2014 On the importance of balancing selection in plants. New Phytol. 201: 45–56.

Denholm, I., M. G. Franco, P. G. Rubini, and M. Vecchi, 1986 Geographical variation in house-fly (*Musca domestica* L.) sex determinants within the British Isles. Genet. Res. 47: 19–27.

Dobzhansky, T., 1947 Genetics of natural populations. XIV. A response of certain gene arrangements in the third chromosome of *Drosophila pseudoobscura* to natural selection.

Genetics 32: 142–160.

van Doorn, G. S., and M. Kirkpatrick, 2010 Transitions between male and female heterogamety caused by sex-antagonistic selection. Genetics 186: 629–645.

van Doorn, G. S., and M. Kirkpatrick, 2007 Turnover of sex chromosomes induced by sexual conflict. Nature 449: 909–912.

Dutoit, L., C. F. Mugal, P. Bolívar, M. Wang, K. Nadachowska-Brzyska *et al.*, 2018 Sex-biased gene expression, sexual antagonism and levels of genetic diversity in the collared flycatcher (*Ficedula albicollis*) genome. Mol. Ecol. 27: 3572–3581.

Fedorka, K. M., and T. A. Mousseau, 2004 Female mating bias results in conflicting sex-specific offspring fitness. Nature 429: 65–67.

Feldmeyer, B., M. Kozielska, B. Kuijper, F. J. Weissing, L. W. Beukeboom *et al.*, 2008 Climatic variation and the geographical distribution of sex-determining mechanisms in the housefly. Evol. Ecol. Res. 10: 797–809.

Feldmeyer, B., I. Pen, and L. W. Beukeboom, 2010 A microsatellite marker linkage map of the housefly, *Musca domestica*: evidence for male recombination. Insect Mol. Biol. 19: 575–581.

Felsenstein, J., 1974 The evolutionary advantage of recombination. Genetics 78: 737–756.

Fijarczyk, A., and W. Babik, 2015 Detecting balancing selection in genomes: limits and prospects. Mol. Ecol. 24: 3529–3545.

Fisher, R. A., 1931 The evolution of dominance. Biol. Rev. Camb. Philos. Soc. 6: 345–368.

Foerster, K., T. Coulson, B. C. Sheldon, J. M. Pemberton, T. H. Clutton-Brock *et al.*, 2007 Sexually antagonistic genetic variation for fitness in red deer. Nature 447: 1107–1110.

Fry, J. D., 2010 The genomic location of sexually antagonistic variation: some cautionary comments. Evolution 64: 1510–1516.

1061 Gethmann, R. C., 1988 Crossing over in males of higher Diptera (Brachycera). J. Hered. 79:
1062 344–350.
1063 Grieshop, K., and G. Arnqvist, 2018 Sex-specific dominance reversal of genetic variation for
1064 fitness. PLoS Biol. 16: e2006810.
1065 Griffin, R. M., D. Le Gall, H. Schielzeth, and U. Friberg, 2015 Within-population Y-linked genetic
1066 variation for lifespan in *Drosophila melanogaster*. J. Evol. Biol. 28: 1940–1947.
1067 Hamm, R. L., 2008 Exploring the population genetics of the house fly sex determining genes, M
1068 and F [Ph.D.]: Cornell University.
1069 Hamm, R. L., R. P. Meisel, and J. G. Scott, 2015 The evolving puzzle of autosomal versus
1070 Y-linked male determination in *Musca domestica*. G3 5: 371–384.
1071 Hamm, R. L., and J. G. Scott, 2009 A high frequency of male determining factors in male *Musca*
1072 *domestica* (Diptera: Muscidae) from Ipswich, Australia. J. Med. Entomol. 46: 169–172.
1073 Hamm, R. L., and J. G. Scott, 2008 Changes in the frequency of Y^M versus III^M in the housefly,
1074 *Musca domestica* L., under field and laboratory conditions. Genet. Res. 90: 493–498.
1075 Hamm, R. L., T. Shono, and J. G. Scott, 2005 A cline in frequency of autosomal males is not
1076 associated with insecticide resistance in house fly (Diptera: Muscidae). J. Econ. Entomol.
1077 98: 171–176.
1078 Hartl, D. L., and A. G. Clark, 2007 *Principles of Population Genetics*. Sinauer.
1079 Hediger, M., G. Burghardt, C. Siegenthaler, N. Buser, D. Hilfiker-Kleiner *et al.*, 2004 Sex
1080 determination in *Drosophila melanogaster* and *Musca domestica* converges at the level of
1081 the terminal regulator *doublesex*. Dev. Genes Evol. 214: 29–42.
1082 Hediger, M., C. Henggeler, N. Meier, R. Perez, G. Saccone *et al.*, 2010 Molecular
1083 characterization of the key switch *F* provides a basis for understanding the rapid divergence
1084 of the sex-determining pathway in the housefly. Genetics 184: 155–170.

1085 Hedrick, P. W., 2006 Genetic polymorphism in heterogeneous environments: the age of
1086 genomics. Annu. Rev. Ecol. Evol. Syst. 37: 67–93.
1087 Hedrick, P. W., 2012 What is the evidence for heterozygote advantage selection? Trends Ecol.
1088 Evol. 27: 698–704.
1089 Innocenti, P., and E. H. Morrow, 2010 The sexually antagonistic genes of *Drosophila*
1090 *melanogaster*. PLoS Biol. 8: e1000335.
1091 Jordan, C. Y., and D. Charlesworth, 2012 The potential for sexually antagonistic polymorphism
1092 in different genome regions. Evolution 66: 505–516.
1093 Kallman, K. D., 1973 The sex-determining mechanism of the platyfish, *Xiphophorus maculatus*,
1094 pp. 19–28 in *Genetics and Mutagenesis of Fish*, Springer Berlin Heidelberg.
1095 Kidwell, J. F., M. T. Clegg, F. M. Stewart, and T. Prout, 1977 Regions of stable equilibria for
1096 models of differential selection in the two sexes under random mating. Genetics 85:
1097 171–183.
1098 Kitano, J., J. A. Ross, S. Mori, M. Kume, F. C. Jones *et al.*, 2009 A role for a neo-sex
1099 chromosome in stickleback speciation. Nature 461: 1079–1083.
1100 Kozielska, M., B. Feldmeyer, I. Pen, F. J. Weissing, and L. W. Beukeboom, 2008 Are autosomal
1101 sex-determining factors of the housefly (*Musca domestica*) spreading north? Genet. Res.
1102 90: 157–165.
1103 Kozielska, M., I. Pen, L. W. Beukeboom, and F. J. Weissing, 2006 Sex ratio selection and
1104 multi-factorial sex determination in the housefly: a dynamic model. J. Evol. Biol. 19:
1105 879–888.
1106 Lemos, B., L. O. Araripe, and D. L. Hartl, 2008 Polymorphic Y chromosomes harbor cryptic
1107 variation with manifold functional consequences. Science 319: 91–93.
1108 Lemos, B., A. T. Branco, and D. L. Hartl, 2010 Epigenetic effects of polymorphic Y

1109 chromosomes modulate chromatin components, immune response, and sexual conflict.
1110 Proc. Natl. Acad. Sci. U. S. A. 107: 15826–15831.
1111 Lenormand, T., 2002 Gene flow and the limits to natural selection. Trends Ecol. Evol. 17:
1112 183–189.
1113 Levene, H., 1953 Genetic equilibrium when more than one ecological niche is available. Am.
1114 Nat. 87: 331–333.
1115 Liew, W. C., R. Bartfai, Z. Lim, R. Sreenivasan, K. R. Siegfried *et al.*, 2012 Polygenic sex
1116 determination system in zebrafish. PLoS One 7: e34397.
1117 Lindholm, A., and F. Breden, 2002 Sex chromosomes and sexual selection in poeciliid fishes.
1118 Am. Nat. 160 Suppl 6: S214–24.
1119 McDonald, I. c., P. Evenson, C. A. Nickel, and O. A. Johnson, 1978 House fly genetics: isolation
1120 of a female determining factor on chromosome 4. Ann. Entomol. Soc. Am. 71: 692–694.
1121 Meier, N., S. C. Käppeli, M. Hediger Niessen, J.-C. Billeter, S. F. Goodwin *et al.*, 2013 Genetic
1122 control of courtship behavior in the housefly: evidence for a conserved bifurcation of the
1123 sex-determining pathway. PLoS One 8: e62476.
1124 Meisel, R. P., T. Davey, J. H. Son, A. C. Gerry, T. Shono *et al.*, 2016 Is multifactorial sex
1125 determination in the house fly, *Musca domestica* (L.), stable over time? J. Hered. 107:
1126 615–625.
1127 Meisel, R. P., C. A. Gonzales, and H. Luu, 2017 The house fly Y Chromosome is young and
1128 minimally differentiated from its ancient X Chromosome partner. Genome Res. 27:
1129 1417–1426.
1130 Moore, E. C., and R. B. Roberts, 2013 Polygenic sex determination. Curr. Biol. 23: R510–2.
1131 Muller, H. J., 1964 The relation of recombination to mutational advance. Mutat. Res. 106: 2–9.
1132 Orr, H. A., 2010 The population genetics of beneficial mutations. Philos. Trans. R. Soc. Lond. B

Biol. Sci. 365: 1195–1201.

Orr, H. A., and A. J. Betancourt, 2001 Haldane's sieve and adaptation from the standing genetic variation. Genetics 157: 875–884.

Orzack, S. H., J. J. Sohn, K. D. Kallman, S. A. Levin, and R. Johnston, 1980 Maintenance of the three sex chromosome polymorphism in the platyfish *Xiphophorus maculatus*. Evolution 34: 663–672.

Otto, S. P., J. R. Pannell, C. L. Peichel, T.-L. Ashman, D. Charlesworth *et al.*, 2011 About PAR: the distinct evolutionary dynamics of the pseudoautosomal region. Trends Genet. 27: 358–367.

Rice, W. R., 1996 Evolution of the Y sex chromosome in animals. Bioscience 46: 331–343.

Rice, W. R., 1986 On the instability of polygenic sex determination: the effect of sex-specific selection. Evolution 40: 633–639.

Rice, W. R., 1984 Sex chromosomes and the evolution of sexual dimorphism. Evolution 38: 735–742.

Rice, W. R., 1992 Sexually antagonistic genes: experimental evidence. Science 256: 1436–1439.

Rice, W. R., 1987 The accumulation of sexually antagonistic genes as a selective agent promoting the evolution of reduced recombination between primitive sex chromosomes. Evolution 41: 911–914.

Rice, W. R., and A. K. Chippindale, 2001 Intersexual ontogenetic conflict. J. Evol. Biol. 14: 685–693.

Roberts, R. B., J. R. Ser, and T. D. Kocher, 2009 Sexual conflict resolved by invasion of a novel sex determiner in Lake Malawi cichlid fishes. Science 326: 998–1001.

Rohmer, C., J. R. David, B. Moreteau, and D. Joly, 2004 Heat induced male sterility in

1157 *Drosophila melanogaster*: adaptive genetic variations among geographic populations and
1158 role of the Y chromosome. J. Exp. Biol. 207: 2735–2743.
1159 Ruzicka, F., M. S. Hill, T. M. Pennell, I. Flis, F. C. Ingleby *et al.*, 2019 Genome-wide sexually
1160 antagonistic variants reveal long-standing constraints on sexual dimorphism in fruit flies.
1161 PLoS Biol. 17: e3000244.
1162 Scott, J. G., C. A. Leichter, F. D. Rinkevich, S. A. Harris, C. Su *et al.*, 2013 Insecticide resistance
1163 in house flies from the United States: resistance levels and frequency of pyrethroid
1164 resistance alleles. Pestic. Biochem. Physiol. 107: 377–384.
1165 Scott, J. G., W. C. Warren, L. W. Beukeboom, D. Bopp, A. G. Clark *et al.*, 2014 Genome of the
1166 house fly, *Musca domestica* L., a global vector of diseases with adaptations to a septic
1167 environment. Genome Biol. 15: 466.
1168 Sellis, D., B. J. Callahan, D. A. Petrov, and P. W. Messer, 2011 Heterozygote advantage as a
1169 natural consequence of adaptation in diploids. Proc. Natl. Acad. Sci. U. S. A. 108:
1170 20666–20671.
1171 Sellis, D., D. J. Kvitek, B. Dunn, G. Sherlock, and D. A. Petrov, 2016 Heterozygote advantage is
1172 a common outcome of adaptation in *Saccharomyces cerevisiae*. Genetics 203: 1401–1413.
1173 Sharma, A., S. D. Heinze, Y. Wu, T. Kohlbrenner, I. Morilla *et al.*, 2017 Male sex in houseflies is
1174 determined by *Mdmd*, a paralog of the generic splice factor gene *CWC22*. Science 356:
1175 642–645.
1176 Spencer, H. G., and N. K. Priest, 2016 The Evolution of Sex-Specific Dominance in Response to
1177 Sexually Antagonistic Selection. Am. Nat. 187: 658–666.
1178 Spigler, R. B., K. S. Lewers, and T.-L. Ashman, 2011 Genetic architecture of sexual dimorphism
1179 in a subdioecious plant with a proto-sex chromosome. Evolution 65: 1114–1126.
1180 Tigano, A., and V. L. Friesen, 2016 Genomics of local adaptation with gene flow. Mol. Ecol. 25:

1181 2144–2164.

1182 Tomita, T., and Y. Wada, 1989 Multifactorial sex determination in natural populations of the
1183 housefly (*Musca domestica*) in Japan. Jpn. J. Genet. 64: 373–382.

1184 Tripathi, N., M. Hoffmann, E.-M. Willing, C. Lanz, D. Weigel *et al.*, 2009 Genetic linkage map of
1185 the guppy, Poecilia reticulata, and quantitative trait loci analysis of male size and colour
1186 variation. Proc. Biol. Sci. 276: 2195–2208.

1187 Wilkins, A. S., 1995 Moving up the hierarchy: a hypothesis on the evolution of a genetic sex
1188 determination pathway. Bioessays 17: 71–77.

1189 Wilson, C. A., S. K. High, B. M. McCluskey, A. Amores, Y.-L. Yan *et al.*, 2014 Wild sex in
1190 zebrafish: loss of the natural sex determinant in domesticated strains. Genetics 198:
1191 1291–1308.

1192 Wright, A. E., R. Dean, F. Zimmer, and J. E. Mank, 2016 How to make a sex chromosome. Nat.
1193 Commun. 7: 12087.

1194 Zhang, P., B. Timakov, R. L. Stankiewicz, and I. Y. Turgut, 2000 A trans-activator on the
1195 *Drosophila* Y chromosome regulates gene expression in the male germ line. Genetica 109:
1196 141–150.