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Inversions are bigger on the X chromosome

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Abstract

In many insects, X-linked inversions fix at a higher rate and are much less polymorphic than autosomal inversions. Here, we report that in *Drosophila*, X-linked inversions also capture 67% more genes. We estimated the number of genes captured through an approximate Bayesian computational analysis of gene orders in nine species of *Drosophila*. X-linked inversions fixed with a significantly larger gene content. Further, X-linked inversions of intermediate size enjoy highest fixation rate, while the fixation rate of autosomal inversions decreases with size. A less detailed analysis in *Anopheles* suggests a similar pattern holds in mosquitoes. We develop a population genetic model that assumes the fitness effects of inversions scale with the number of genes captured. We show that the same conditions that lead to a higher fixation rate also produce a larger size for inversions on the X.

KEYWORDS
evolution, population genetics, sex chromosomes

1 | INTRODUCTION

The evolution of inversions on sex chromosomes is of interest for two reasons. First, chromosomal inversions play a key role in the evolution of sex chromosomes of many groups of animals and plants (Bachtrog, 2013; Bachtrog et al., 2011; Charlesworth, 2013; Charlesworth & Charlesworth, 2005). The nonrecombining sex determination region can expand by fixation of inversions that suppress recombination between the X and Y (or Z and W) chromosomes. In mammals and other taxa, the fixation of multiple inversions has generated "evolutionary strata" that show different levels of divergence between the sex chromosomes (Handley, Ceplitis, & Ellegren, 2004; Lahn & Page, 1999; Liu et al., 2004). Second, sex chromosomes have unique genetic properties (Bachtrog et al., 2011). X-linked genes are hemizygous in males, they have fewer copies in the population than do autosomal genes, and they spend more of their evolutionary lives in females. These characteristics are expected to impact the properties of chromosome rearrangements that fix on sex chromosomes (Charlesworth, Coyne, & Barton, 1987; Pennell et al., 2015). Understanding differences between inversions on sex chromosomes and autosomes may give general insights into how inversions evolve throughout the genome.

Previous studies of inversions in Orthoptera and Diptera have found a "faster X" effect—higher fixation rates on the X chromosome than on autosomes (Bhutkar et al., 2008; Charlesworth et al., 1987; von Grotthuss, Ashburner, & Ranz, 2010; White, 1973). The X chromosome in marsupials also shows more rearrangements by inversions than do autosomes (Charlesworth et al., 1987; Deakin et al., 2012). Likewise in birds, the Z chromosome is subject to much more extensive intrachromosomal rearrangements than are autosomes (Griffin, Robertson, Tempest, & Skinner, 2007; Nanda, Schlegelmilch, Haaf, Schartl, & Schmid, 2008). A second intriguing observation from Orthoptera and Diptera is that there is a striking deficiency of inversion polymorphism on the X compared to autosomes (Charlesworth et al., 1987; Kitzmiller, 1977; Neafsey et al., 2015; Pombi et al., 2008).

Charlesworth et al. (1987) developed a series of models to explain the faster X effect. In their analysis of inversions, they assumed that heterozygotes suffer a disadvantage (e.g. because of meiotic problems). They found that the fixation rates can be higher on the X than the autosomes if inversions that are homozygous or hemizygous have a fitness advantage that is sufficiently large. They also showed that inversions (or any other kind of mutation) have higher fixation rates on the X when they are beneficial and partly or

completely recessive. The conditions that maintain polymorphic inversions have also been studied theoretically (Avery, 1984; Curtsinger, 1980; Pamilo, 1979). Consistent with the deficit of polymorphisms seen on the X, those models show that there is a reduced parameter space for polymorphism on the X relative to the autosomes. To date, other distinctive properties of inversions on the X have not been studied.

In this article, we address the sizes of inversions. This focus is motivated by studies in *Drosophila* and *Anopheles* that show several patterns. Polymorphic inversions that are common and geographically widespread tend to be larger than rare inversions with localized distributions (Wallace, 1954; Olvera et al. 1979; Brehm & Krimbas, 1991; Cáceres, Barbadilla, & Ruiz, 1997; Pombi et al., 2008). A comparison between *Drosophila melanogaster* and *D. yakuba* suggested that the fixation rates of inversions vary with their size (York, Durrett, & Nielsen, 2007). Inversions can also impact patterns of gene expression in the genome (Cassone et al. 2011; Fuller, Haynes, Richards, & Schaeffer, 2016), and the number of differentially expressed genes might scale with inversion size. There are also theoretical reasons to suspect that the sizes of inversions affect how likely they are to become established. If inversions fix because they link together locally adapted alleles, the probability that a new inversion spreads increases with the number of locally adapted loci that it captures (Kirkpatrick & Barton, 2006). In their models for the fixation of inversions, Nei (1967) and Kimura and Ohta (1970) hypothesized that the size of an inversion affects its fitness through the number of deleterious mutations that it is likely to capture.

We use gene order across the genomes of nine *Drosophila* species (von Grotthuss et al., 2010) to study the evolution of inversions. Our approach uses a novel scheme based on approximate Bayesian computation (ABC) to estimate their sizes. Our analysis focuses on fixed inversions that differ between species, and we do not attempt to explain patterns of polymorphism. We measure size in terms of the number of genes that an inversion captures. We find that inversions fixed on X are larger on average. The distribution of sizes on the X is also distinctive. On autosomes, the smallest inversions fix most frequently, while on the X it is inversions of intermediate size that are most frequent. Less detailed analyses of two species of *Anopheles* mosquitoes suggest that inversions are also larger on the X in those taxa.

We develop a population genetic model to explain this "bigger-on-the-X" pattern. The key assumption is that the fitness effects of inversions are proportional to the number of genes they carry. We find the conditions regarding fitness effects and dominance that result in larger inversions becoming fixed on the X. We show that our model also explains other salient features of inversions observed in natural populations.

2 | MATERIALS AND METHODS

Our analyses are based on the gene order for nine species of flies (*Drosophila ananassae*, *D. erecta*, *D. grimshawi*, *D. mojavensis*, *D. pseudoobscura*, *D. virilis*, *D. willistoni*, *D. yakuba and D. melanogaster*) as determined by von Grotthuss et al. (2010). The gene orders, in turn, are based on the reference genomes for those species (*Drosophila* 12 Genomes Consortium 2007). We used the phylogeny of these species estimated by Powell and DeSalle (1995) and shown in Figure 1. This phylogeny is consistent with that estimated from whole genomes (*Drosophila* 12 Genomes Consortium 2007).

We do not account for polymorphism caused by inversions that are currently segregating. We do not, however, expect that to affect the results noticeably. Inversions in *D. melanogaster*, whose ages are the best characterized of any species in the genus, are typically only about 10^5 years old (Corbett-Detig & Hartl 2012). The branches on the phylogeny are 10–100 times longer, and so (assuming that inversions in *melanogaster* are representative), polymorphisms will little impact on estimates of differences between species. For semantic simplicity, we refer to the inversions found in the reference genomes as "fixed," but in reality, some of them are certain to be polymorphic.

One approach to estimate the sizes of inversions fixed in different species would be to reconstruct their breakpoints using parsimony, then count the number of genes between the breakpoints. However, we found using simulations that this strategy greatly underestimates the sizes of inversions. This bias results because the breakpoints of older inversions are covered by younger ones (Bourque & Pevzner, 2002), making the older inversions seem smaller.

We therefore devised the following strategy based on approximate Bayesian computation, or ABC (Beaumont, Zhang, & Balding, 2002). In Step 1, we used parsimony to estimate the distribution of inversion sizes with the software package GRIMM (Tesler, 2002) under default parameter settings, considering the sign of the genes. In Step 2, we simulate the fixation of inversions on the phylogeny of the nine species. The number of inversions fixed along each branch is drawn from a Poisson distribution with a mean given by the

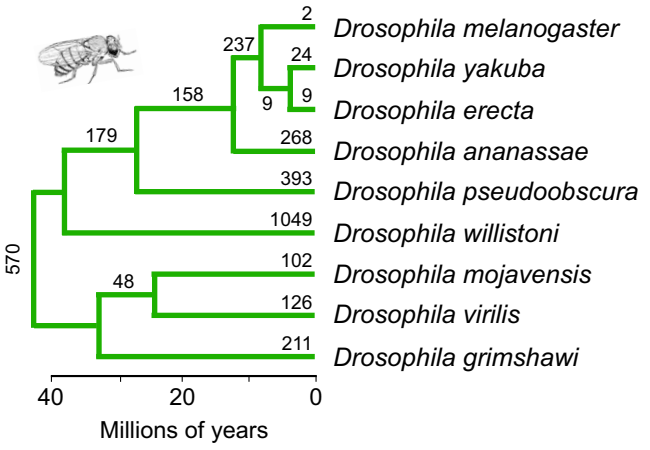

FIGURE 1 The phylogeny of the Drosophila species analysed in this study (Powell & DeSalle, 1995; Drosophila 12 Genomes Consortium 2007; Crosby et al. 2007). The estimated numbers of inversions fixed along each branch are from von Grotthuss et al. (2010)

product of the branch length and the fixation rate. This rate is sampled from a uniform prior distribution, with limits of 0 and 1.2 times the number of inversions estimated for that branch in Step 1. (These limits were chosen for optimal convergence by preliminary analyses.) For each inversion, a breakpoint is randomly chosen between two adjacent genes. The second breakpoint is randomly chosen such that the number of genes between the two breakpoints follows a gamma distribution with given size (μ) and shape (γ) parameters. Note that a new inversion can overlap with or be nested within an inversion that fixed previously. In Step 3, we again use the parsimony method of Step 1 to estimate the distribution of inversion sizes in the simulated data set. In Step 4, we compare the distribution estimated from the simulated data (Step 3) to the distribution estimated from the real data (Step 1). We measure the fit of the simulated data to the real data using the difference in the numbers of inversions that fix and the difference in their mean sizes. We repeat steps 2–4, adjusting the parameters of the gamma distribution (μ and γ) until the simulated data converge on the real data in Step 4.

We repeated this entire procedure 10^8 times. In each run, the parameters for the gamma distribution were sampled from log-uniform distributions, with μ sampled in the range (1, 1,000) and γ sampled in the range (0.1, 10). The posterior distribution was obtained by a rejection algorithm in which the 10^4 simulations with the smallest Euclidean distances to the real data were retained. The posterior distributions of μ and γ were estimated from those simulations.

This procedure was carried out separately for each chromosome arm (Muller element). This allows us to compare the X chromosome with the autosomes and to compare the different autosomal arms. We excluded the small dot chromosome (Muller element *F*) for two reasons: it has only 5% of the genes carried by the other chromosomes, which strongly skews the sizes of inversions downwards, and the quality of this chromosome's assemblies is lower than that for the other chromosomes (Leung et al. 2015).

To test the taxonomic generality of the results, we also studied two species of *Anopheles* mosquitoes. The quality of the genome assemblies for the mosquitoes is inferior to those in the flies, so these results should be treated with caution. We compared 3,958 orthologous genes in *Anopheles gambiae* and *Anopheles stephensi*, the two mosquitoes with the best reference genomes (Neafsey et al., 2015).

3 | RESULTS

3.1 | The sizes of inversions in *Drosophila*

We find that inversions in *Drosophila* that have fixed on the X are on average 67% larger than those on autosomes (Figure 2). The maximum a posteriori (MAP) estimate for the average size of inversions that fix on X is 496 genes (95% credible interval = [382, 575]), while on autosomes it is 297 genes (CI = [198, 378]). This difference is significant at the 0.5% level. The mean size of inversions that fix on the X is also significantly larger than the means of the four autosomal arms when each of the latter are treated separately (Table 1). These trends are also seen when inversion size is measured as a fraction of the genes on its chromosome arm that were captured. On the X, on average inversions capture 30% of the genes, while on autosomes they capture only 12% of the genes ($p < 10^{-15}$, Wilcoxon test).

The shapes of the distributions of inversion sizes also differ between the X and autosomes (Figure 3). The mode of the distribution on the X is 422 genes, which is significantly greater than 0. In contrast, the mode for autosomes is 0. (In reality, inversions of size 0 do not exist. This result is a minor artefact of the gamma distribution that we fit to the data. We interpret this result to mean that inversions with very few genes are most likely to fix.) In sum, the most frequent inversions to fix on the X are intermediate in size, while on autosomes, it is the smallest inversions that have the highest fixation rate.

One of the autosomal arms provides an interesting natural experiment to test the effect of sex linkage on inversion size. Muller Element D is fused to the X chromosome in *D. willistoni* and *D. pseudoobscura*. We found that the mean size of inversions on Element D when fused is 263 genes (CI = [182, 379]), while when it is not fused the mean is 233 genes (CI = [170, 284]). Although the trend is consistent with what we found in the comparison of the X and autosomes, the difference is not statistically significant.

3.2 | Inversions in mosquitoes

The results for inversions in mosquitoes are consistent with those from the flies. In *Anopheles*, the average size of inversions fixed on the X is much larger than those on the autosomes: 26 versus 1 marker gene ($p < 0.01$, one way Wilcoxon test). The result remains significant when the inversion sizes are scaled relative to chromosome size. The pattern is all the more striking when one considers that the autosomes in *A. gambiae* have 3 to 4 times more genes than the X (Neafsey et al., 2015), and so inversions on autosomes have the potential to span many more genes.

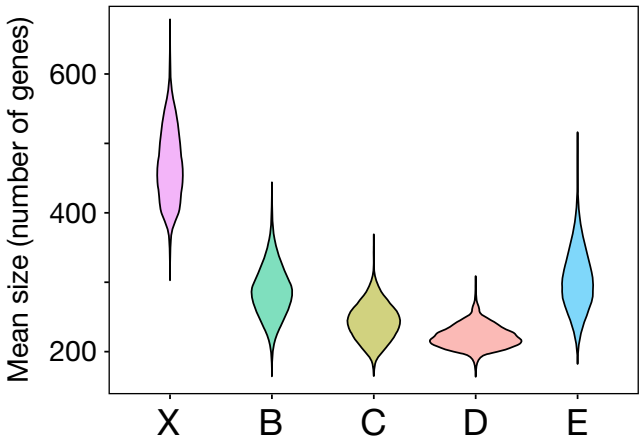

FIGURE 2 Posterior distributions of the mean sizes of inversion on the X chromosome and the arms of the autosomes (Muller elements B, C, D and E)

TABLE 1 The maximum a posteriori (MAP) estimates and 95% credible intervals for the mean sizes of inversions on the five Muller elements (major chromosomal arms) in *Drosophila*

Muller element	MAP estimate	95% CI
X (A)	496	382, 575
B	283	226, 371
C	258	199, 299
D	240	195, 293
E	336	224, 412

Muller element A is the X chromosome, while the others comprise the autosomes.

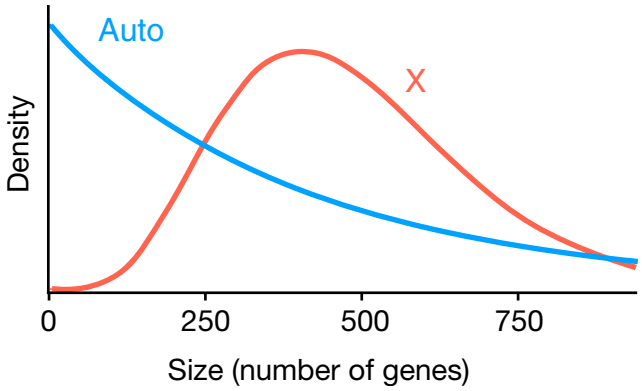

FIGURE 3 The distributions of inversion sizes on the X and the autosomes estimated by the ABC analysis. The mode of inversion size on the X is 422, significantly larger than 0. The mode on autosomes is not significantly larger than 0, however, and so the larger the size of inversion, the less likely it will be fixed

We emphasize that the result is much less robust than those from *Drosophila* because of the quality of the genome assemblies. Nevertheless, they suggest that the bigger-on-the-X pattern may be general.

3.3 | A population genetic model

Next, we used a population genetic model to develop a hypothesis to explain why inversions fixed on the X might be larger. The key assumptions are that an inversion's fitness effects are proportional to its size. Our analysis is a minor extension of models developed by Charlesworth et al. (1987).

On autosomes, let the relative fitnesses of standard (that is, uninverted) homozygotes, heterozygotes and inverted homozygotes be

$$\begin{matrix} W_{SS} & W_{SI} & W_{II} \\ 1 & 1+s_1 y & 1+s_2 y \end{matrix}$$

where y is the size of the inversion. On X chromosomes, we assume full dosage compensation and no sex differences in fitness. Consequently, the relative fitness of males that carry the inverted X chromosome is $(1 + s_2\, y)$ relative to those with the standard chromosome. The selection parameters s_1 and s_2 can be positive or negative, allowing for cases in which inversions are either deleterious or beneficial, and for arbitrary patterns of dominance.

Following Charlesworth et al. (1987), we calculated the fixation rates of inversions on autosomes and the X using Kimura's (1962) diffusion approximation. Assuming weak selection, the fixation rate for inversions of size y on autosomes is

$$K_A = \frac{\mu(y)}{\int_0^1 \exp\{-2Nxy[2(1-x)s_1 + xs_2]\}dx}, \quad (1)$$

where $\mu(y)$ is the rate that inversions of that size originate by mutation and N is the population size. The fixation rate on the X chromosome is

$$K_X = \frac{\mu(y)}{\int_0^1 \exp\{-Nxy[2(1-x)s_1 + (1+x)s_2]\}dx}. \quad (2)$$

We assume that the mutation rates for inversions on the X and autosomes are the same.

We denote the relative fixation rate for inversions on the X compared to those on autosomes as $R = K_X/K_A$. Inspection of equations (1) and (2) shows that R is >1, meaning that inversions have a higher fixation rate on the X, whenever

$$2s_1 < s_2. \quad (3)$$

An analogous result was derived previously by Charlesworth et al. (1987).

This condition can be satisfied when inversion heterozygotes are deleterious and when they are advantageous. When heterozygotes are beneficial, the condition requires that the fitness effects are partly recessive, such that homozygotes are more than twice as fit as heterozygotes. When heterozygotes are deleterious, the condition is met when the inversion is partly dominant ($2s_1 < s_2 < 0$), and when it is underdominant ($s_1 < 0$, $s_2 > 0$).

All else equal, if R increases with the size of inversions, then the mean size of inversions that fix will be larger on the X than on the autosomes. To show that this condition is met, we linearize R in terms of $s_1\, Ny$ and $s_2 Ny$, which gives

$$R = \frac{K_X}{K_A} \approx 1 + \frac{1}{6}(s_2 - 2s_1)Ny. \quad (4)$$

Thus R increases with y, and inversions that fix on the X will be larger on average than those on autosomes, whenever condition (3) is met.

An example of the distributions of inversion sizes predicted by this model is shown in Figure 4. Here, we assumed that new inversions generated by mutation have an exponential distribution with a mean of 200 genes. Inversions are beneficial, with $N_e s_2 = 0.1$, and slightly underdominant, with $N_e s_1 = -0.04$. Under those assumptions, inversions fix more frequently on the X, and their mean size is larger (60 genes on autosomes vs. 309 genes on the X).

4 | DISCUSSION

The evolutionary genetics of inversions has a rich history dating back to the laboratory studies of Sturtevant and the work on natural populations by Dobzhansky (Hoffmann & Rieseberg, 2008; Kirkpatrick, 2010).

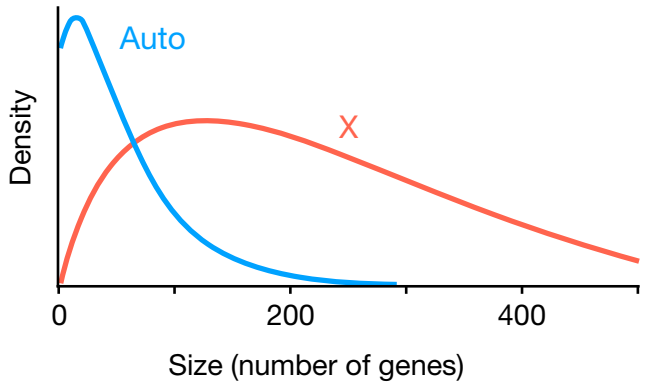

FIGURE 4 Distributions of sizes for inversions that fix on autosomes and the X chromosome predicted by the model. In this example, inversions are assumed to be beneficial ($N_e s_2 = 0.1$) and slightly under dominant ($N_e s_1 = -0.04$), and the sizes of new mutant inversions are exponential with a mean of 200 genes. With those parameters, the model predicts the mean size of inversions that fix will be 60 genes on autosomes and 309 genes on the X

Much of the research has focused on the inversion polymorphisms that are abundant in some species, such as *D. pseudoobscura* (Dobzhansky 1981) and *Anopheles* mosquitoes (Coluzzi, Sabatini, della Torre, Di Deco, & Petrarca, 2002). Another important research theme has been the important role that inversions play in blocking recombination between the X and Y (or Z and W) sex chromosomes (Bachtrog, 2013; van Doorn & Kirkpatrick, 2007).

This article focuses on somewhat less studied aspects: the differences between inversions that have fixed on the X chromosome and the autosomes, and how those differences can inform us about the fitness effects of inversions. Our key finding is that inversions in *Drosophila* that have fixed on the X are larger than those on autosomes. Males are achiasmatic in these flies, and so this contrast cannot involve blocking recombination between the X and the Y. Instead, it must trace back to differences in how selection or mutation acts on those chromosomes. The same pattern is seen in comparisons between two species of *Anopheles* mosquitoes, which have comparable recombination rates in females and males (Zheng, Benedict, Cornel, Collins, & Kafatos, 1996).

These results are consistent with a population genetic model that assumes the fitness effects of an inversion are proportional to its size. When that is true, the bigger-on-the-X pattern is expected under the same fairly general conditions that cause inversions to fix more frequently on the X, an empirical pattern that has been documented previously (Bhutkar et al., 2008; Charlesworth et al., 1987; Neafsey et al., 2015). These conditions are satisfied in several situations: When inversions are beneficial as heterozygotes and more than twice as beneficial as homozygotes, when inversions are underdominant, and when inversions are deleterious (both heterozygotes and homozygotes) and partly or wholly dominant. Thus, the bigger-on-the-X pattern does not require that the inversion be deleterious when heterozygous, as assumed in some other models (Charlesworth et al., 1987; Lande, 1979).

The model suggests what fitness effects can lead to the pattern, but provides no biological insight about what might produce those effects. Several mechanisms can be hypothesized. In one scenario that our model predicts will lead to the bigger-on-the-X effect, inversions are underdominant, and the deleterious fitness effects in heterozygotes increase with the size of the inversion. In many organisms, inversions are underdominant because single cross-overs within the inverted region lead to aneuploid gametes (White, 1973). Because the probability of a cross-over increases with the size of an inversion, this would cause fitness loss in heterozygotes to increase with the size of the inversion, as required by the model. This scenario may not apply to *Drosophila*, however, which have mechanisms that largely suppress the deleterious effects of inversion heterozygotes (White, 1973). Alternatively, inversions could be underdominant simply because of their genetic content, rather than their effects on recombination.

In a second scenario compatible with the predictions of our model, inversions increase fitness and are partly recessive. One situation in which this can occur is when inversions spread because of their effects in suppressing recombination between loci carrying locally adapted alleles (Charlesworth, Barton, & Charlesworth, 2017; Kirkpatrick & Barton, 2006). The selective benefit of suppressing recombination scales with the initial recombination rate and with the number of loci involved. All else equal, larger inversions will span more of the linkage map, so they will have larger fitness advantage from suppressing recombination. Further, inversions on X chromosomes will have greater effects on decreasing recombination than those on autosomes: the X spends two-thirds of its evolutionary life in females, where it can recombine, while autosomes spend only half of their lives in females. Thus if locally adapted loci are partly recessive, we might expect inversions on the X to fix more frequently.

In sum, several biological mechanisms could create the conditions causing inversions that fix on the X to be larger and more frequent than those on autosomes, as predicted by our model. The shapes of the size distributions estimated for the X and autosomes by the ABC analysis also differ: the mode is at the smallest size for inversions on autosomes, but at an intermediate size for those on the X (Figure 3). These shapes are determined by the distribution of sizes of new inversions generated by mutation as well as the fixation probabilities for mutations of different sizes. Figure 4 shows an example of the distributions predicted by the model assuming that the distribution of sizes of inversions arising by mutation is exponential; that is, the smallest inversions are most frequent. Further, in this example inversions are slightly underdominant, and so there is stronger selection against them as heterozygotes when they first appear. A result of these two factors is that the frequency of inversions that fix on autosomes declines with inversion size. In contrast, inversions that fix most frequently on the X are intermediate in size. That is because they have a selective advantage in males (as hemizygotes) even when rare, and that advantage grows with the size of the inversion.

Our model is highly simplified in several regards. Perhaps the most extreme is that we assume all inversions of the same size have the same fitness effects. This means that there is no allowance for the possibility that some inversions are overdominant, for example,

or that their fitnesses can vary in space and time. At best, our model hopes to capture some average features of inversions. Inversions polymorphisms maintained (for example) by overdominance or local adaptation are a fascinating but likely very small subset of all inversions generated by mutation and fixed by selection and drift. Our model does not seek to understand how that set of inversions evolves.

The bigger-on-the-X effect may contribute to patterns involving sterility and other reproductive incompatibilities between populations and species. Inversions can contribute to incompatibilities. When they do, it is plausible that larger inversions will be more likely to carry alleles responsible for incompatibilities. The bigger-on-the-X effect will then cause the X chromosome to contribute to incompatibilities more often than autosomes. This pattern, called the "large X effect," is seen in some taxa (reviewed in Charlesworth et al. (1987), Coyne and Orr (1989), and Presgraves (2008)). Consistent with that trend, segments of X chromosomes introgress between species less often than do segments of autosomes in *Drosophila* flies (Kulathinal, Stevison, & Noor, 2009) and *Anopheles* mosquitoes (Fontaine et al., 2015). Likewise, the X chromosome in mice (*Mus*) (Macholan et al., 2007) and the Z chromosome in flycatchers (*Ficedula*) (Saetre et al. 2003) show less introgress than do autosomes.

eTwo other hypotheses might also explain the bigger-on-the-X pattern seen in flies. First, the genetic content is often quite different on the X chromosome. The large X effect mentioned earlier might result from these differences. Genes with male-biased expression are significantly underrepresented on the X chromosome in flies (Parisi et al., 2003), and sexually antagonistic loci may be enriched on the X in *D. melanogaster* (Innocenti & Morrow, 2010). The expression levels of genes on the X diverge faster than those on the autosome in flies (Meisel, Malone, & Clark, 2012). It is plausible that one or more of these genetic differences between the X and the autosomes drives the pattern.

Second, new inversions generated by mutation might tend to be larger on the X than the autosomes. Transposons and repetitive sequences have been implicated in the mutational origin of inversions in several organisms (Cáceres, Ranz, Barbadilla, Long, & Ruiz, 1999; Coulibaly et al., 2007; Goidts, Szamalek, Hameister, & Kehrer-Sawatzki, 2004). Perhaps differences between the X and autosomes in the distributions of those (and possibly other) genomic elements biases the mutational spectrum towards larger inversions on the X.

Regardless of why bigger inversions establish on the X, our results suggest inversions been fixed may affect more genes on the X chromosome and may have larger evolutionary impacts than those on autosomes.

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AUTHOR CONTRIBUTIONS

C.C. and M.K. contributed to all phases of this project.

DATA ACCESSIBILITY

We used publicly available data for our analyses. Orthologous marker genes in *Drosophila* were taken from von Grotthuss et al. (2010) (https://doi.org/10.1101/gr.103713.109) and FlyBase (flybase.org). Orthologous marker genes in *Anopheles* were taken from Neafsey et al. (2015) (https://doi.org/10.1126/science.1258522) and VectorBase (https://www.vectorbase.org/).

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