bioRxiv preprint doi: https://doi.org/10.1101/638684; this version posted May 24, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

Female meiotic drive preferentially segregates derived metacentric chromosomes in *Drosophila*

Nicholas B. Stewart[1,2,*], Yasir H. Ahmed-Braimah[1,3], Daniel G. Cerne[1,4], Bryant F. McAllister[1]

1. Department of Biology, University of Iowa, Iowa City, IA 52240
2. Department of Biological Sciences, Fort Hays State University, Hays, KS 67601
3. Department of Molecular Biology and Genetics, Cornell University, Ithaca, NY 14853
4. Integrated DNA Technologies Inc., Coralville, IA 52241

[* nbstewart2@fhsu.edu](mailto:nbstewart2@fhsu.edu)

Abstract

A vast diversity of karyotypes exists within and between species, yet the mechanisms that shape this diversity are poorly understood. Here we investigate the role of biased meiotic segregation—i.e., meiotic drive—in karyotype evolution. The closely related species, *Drosophila americana* and *D. novamexicana*, provide an ideal system to investigate mechanisms of karyotypic diversification. Since their recent divergence, *D. americana* has evolved two centromeric fusions: one between the 2nd and 3rd chromosomes, and another between the X and 4th chromosomes. The 2-3 fusion is fixed in *D. americana*, but the X-4 fusion is polymorphic and varies in frequency along a latitudinal cline. Here we evaluate the hypothesis that these derived metacentric chromosomes segregate preferentially to the egg nucleus during female meiosis in *D. americana*. Using two different methods, we show that the fused X-4 chromosome is transmitted at an average frequency of ~57%, exceeding expectations of 50:50 Mendelian segregation. Three paracentric inversions are found in the vicinity of the X-4 fusion and could potentially influence chromosome segregation. Using crosses between lines with differing inversion arrangements, we show that the transmission bias persists regardless of inversion status. Transmission rates are also biased in *D. americana*/*D. novamexicana* hybrid females, favoring both the X-4 and 2-3 fused arrangements over their unfused homologs. Our results show that meiotic drive influences chromosome segregation in *D. americana* favoring derived arrangements in its reorganized karyotype. Moreover, the fused centromeres are the facilitators of biased segregation rather than associated chromosomal inversions.

bioRxiv preprint doi: https://doi.org/10.1101/638684; this version posted May 24, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

1 **Introduction**

2

3 Evolution has produced remarkable diversity in karyotypes among species, but the underlying
4 mechanisms remain unclear. Various mutational events, such as fusion of non-homologous
5 chromosomes, inversions, deletions, duplications or translocation of chromosomal regions are known to
6 alter chromosome number and/or form. Such rearrangements are common but can lead to aneuploidy
7 and/or negative fitness effects that decrease the likelihood of fixation in a population (Bengtsson 1980).
8 One possible mechanism by which these rearrangements can become fixed is centromere-associated
9 meiotic drive (Sandler and Novitski 1957; Pardo-Manuel de Villena and Sapienza 2001a). This
10 hypothesis posits that female meiotic drive shapes karyotype structure through preferential segregation
11 of specific centromere forms. Indeed, in a survey of >1000 mammalian karyotypes, Pardo-Manuel de
12 Villena and Sapienza found that most species either have nearly all metacentric or all acrocentric
13 chromosomes indicating biases that may be shaped by preferential meiotic segregation (Pardo-Manuel
14 de Villena and Sapienza 2001b).

15

16 For meiotic drive to operate during gametogenesis, three requirements must be fulfilled: (1)
17 asymmetric meiosis that produces less than four gametes from the four meiotic products, (2)
18 karyotypically heterozygous homologs that pair during meiosis, and (3) asymmetry in the spindle
19 attachment strength between meiotic poles (Sandler and Novitski 1957; Pardo-Manuel de Villena and
20 Sapienza 2001a). Female meiosis is asymmetric because it produces a single functional product; one
21 of four meiotic products forms the functional egg nucleus and the remaining three form the polar
22 bodies. Thus, nonrandom segregation of a given chromosomal variant to either of these two fates
23 indicates the presence of female meiotic drive.

24

25 Nonrandom segregation during meiosis can be caused by differences in the strength and/or
26 number of meiotic spindles deployed by the opposing centrioles during metaphase I, or by distinct
27 characteristics of centromere sequences and centromere-associated proteins (Henikoff *et al.* 2001;
28 Chmátal *et al.* 2017). This mechanistic model is supported by recent evidence in mice (Chmátal *et al.*
29 2014), where the strength of centromeres is determined by the levels of kinetochore proteins localized
30 at the centromere. These proteins are the centromeric attachment site for spindle microtubules, and

bioRxiv preprint doi: https://doi.org/10.1101/638684; this version posted May 24, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

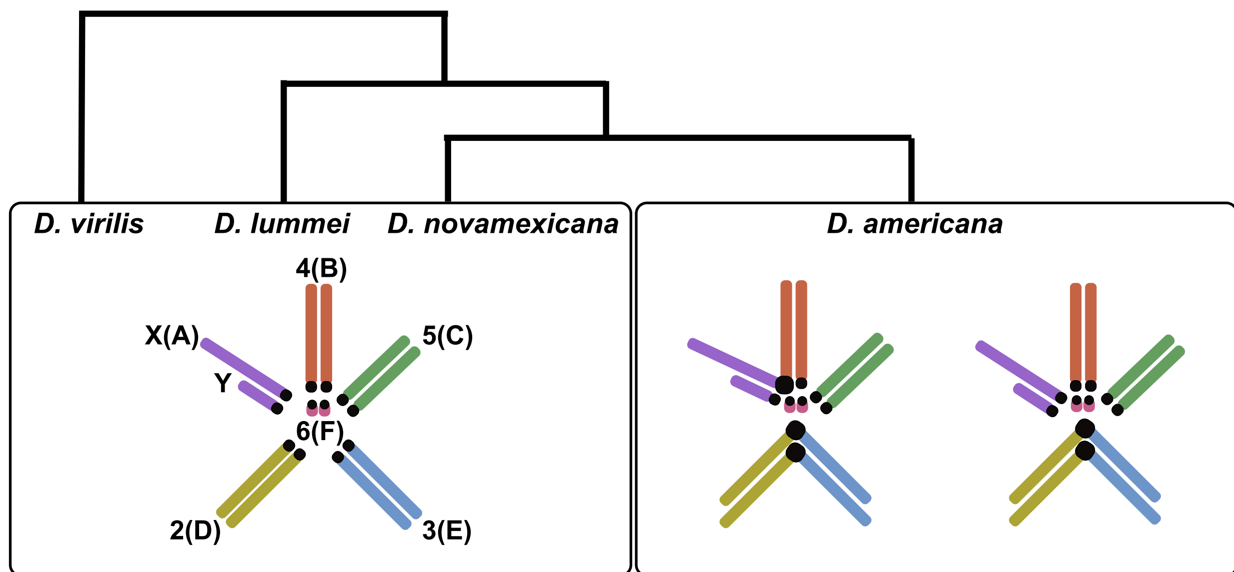

Figure 1. *D. virilis*, *D. lummei*, and *D. novamexicana* have the ancestral karyotype, which consists of five acrocentric, rod-shaped chromosomes and a single "dot" chromosome. *D. americana* has evolved two centromeric fusions between chromosomes 2 and 3 (fixed) and chromosomes X and 4 (polymorphic). The Muller element classification of each chromosome is indicated in parentheses.

31 because different centromeric forms can contain varying levels of these proteins, meiotic drive can
32 favor alternate centromere forms (Chmátal *et al.* 2014).
33
34 Two closely related sister-species in the *Drosophila virilis* species group, *D. americana* and *D.*
35 *novamexicana*, present an excellent opportunity to study the influence of female meiotic drive on
36 chromosome evolution. All members of the *D. virilis* group, except *D. americana*, maintain the ancestral
37 *Drosophila* karyotype of 6 acrocentric chromosomes, or "Muller elements" (Figure 1). In contrast, *D.*
38 *americana* has evolved two different chromosomal fusions that join the centromeres of non-
39 homologous chromosomes (Figure 1). One centromeric fusion is between the 2nd and 3rd
40 chromosomes (Muller elements D and E), whereas the other is between the X and 4th chromosomes
41 (Muller elements A and B). The X-4 fusion exhibits a latitudinal frequency cline throughout its range in
42 North America (McAllister 2002; McAllister *et al.* 2008): northern populations show a high frequency of
43 the X-4 fusion. Natural selection appears to maintain the alternate chromosome forms across the
44 species range, where the X-4 fusion is favored in cooler, northern latitudes. This study investigates the
45 existence of an intrinsically biased transmission rate favoring the derived fusions present in *D.*
46 *americana*.
47
48 Chromosomal inversions can potentially influence chromosome segregation by influencing the
49 formation of dicentric or acentric chromatids following crossing over. For example, during female
50 meiosis, a meiocyte has the ability to pull dicentric or acentric chromatids—which arise from a cross-

bioRxiv preprint doi: https://doi.org/10.1101/638684; this version posted May 24, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

51 over event within a heterozygous inverted region—towards the polar body during meiosis. This can
52 ensure that the egg nucleus will inherit the functional monocentric chromatid (Sturtevant and Beadle
53 1936; Carson 1946). This mechanism could potentially affect segregation of chromosomes that differ in
54 inversion content and produce meiotic drive. In *D. americana*, multiple paracentric inversions reside
55 near the X-4 centromere. Two different inversions are observed together on the 4th chromosome of *D.*
56 *americana*: the smaller *In(4)b* inversion is always found nested within the larger *In(4)a* inversion.
57 However, not every fused X-4 chromosome has this inversion complex (Hsu 1952; Evans *et al.* 2007).
58 This inversion complex is also not present within the unfused 4th chromosome of *D. americana*. In
59 contrast, *D. novamexicana* is fixed for the *In(4)a* inversion and lacks the *In(4)b* inversion. A third
60 inversion, *In(X)c*, is present on the X chromosome and is perfectly associated with the X-4 fusion but is
61 not found on the unfused arrangement of *D. americana* (Hsu 1952). It is, however, present on the
62 unfused X chromosome of *D. novamexicana*.
63
64 Here we analyze the transmission dynamics of the derived metacentric chromosomes of *D.*
65 *americana*, both in within-species heterozygotes and in *D. americana*/*D. novamexicana* hybrids. We
66 use two methods to track the transmission of the two chromosomal types. First, we use a visible eye
67 color mutation on the 4th chromosome to track the inheritance of the X-4 fusion from heterozygous
68 females to adult sons. Second, we use microsatellite markers that are tightly associated with the
69 centromeres to track the inheritance of the X-4 and 2-3 fusions from heterozygous females to early-
70 stage embryos. These two approaches allow us to assess the impact of differential viability on
71 transmission ratios. Finally, we analyze transmission rates of several combinations of inversion
72 heterozygotes to assess the effects of the three associated inversions on the transmission of the X-4
73 fusion. Our results show that biased meiotic transmission favors the derived, fused arrangements
74 present in *D. americana* at an average rate of ~57%. This bias favors the X-4 fusion regardless of
75 inversion status, suggesting that centromeres are likely the causal factor influencing meiotic drive.
76 Furthermore, we find no difference between our assessment of meiotic drive in embryos and adults,
77 indicating that differential viability is not an important factor in the observed transmission bias.
78
79
80
81
82
83
84

bioRxiv preprint doi: https://doi.org/10.1101/638684; this version posted May 24, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

85 **Materials and Methods**

86

87 ***Measuring transmission of the fused X-4 chromosome using a visible marker:***

88

89 The transmission rate of the fused and
90 unfused chromosomes was measured in F1
91 females obtained in reciprocal crosses between
92 five *D. americana* strains with the X-4 fusion
93 (SB02.02, SB02.06, SB02.08, SB02.10, *red*)
94 and five strains with the unfused arrangement
95 (ML97.3, ML97.4, ML97.5, ML97.6, *pur*). (See
96 Table S1 for description of strains). In all
97 crosses, virgin flies were collected within 36
98 hours of eclosion and kept at 22°C until
99 sexually mature at ~7 days. We performed 45
100 out of 50 possible reciprocal combinations of
101 'fused by unfused' crosses. To generate
102 heterozygous females, four virgin adult females
103 from a fused (or unfused) strain were crossed
104 with four virgin adult males from an unfused (or
105 fused) strain. Approximately 30 crosses of
106 individual F1 females mated with *cardinal* (*cd*)
107 males that carry a recessive red eye color
108 mutation on the 4th chromosome were
109 established to enable segregation of the fused
110 and unfused arrangements. F2 male sires were
111 backcrossed to *cd* females, and their F3
112 progeny were evaluated for an association between eye color and sex (Figure 2). F2 males inherit the
113 fused X-4 chromosome if all F3 males show the *cd* phenotype and all females are wild-type. On the
114 other hand, F2 males inherit the unfused X and 4th chromosomes if the *cd* phenotype is present in
115 equal proportions to the wild-type in both F3 males and F3 females. We measured the F1 female
116 transmission ratio from ≥100 F2 males for each heterozygous genotype combination.

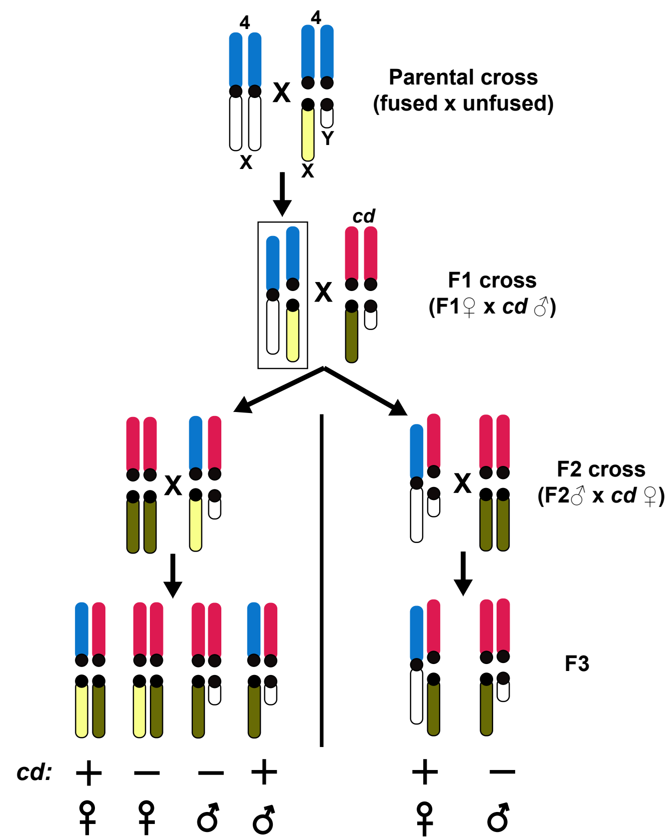

Figure 2. Crossing scheme used to track the transmission of the X-4 fused and unfused arrangements from F1 heterozygous females (rectangle) to F2 adult sons. Parental 4th chromosomes are shown in blue, and the *cd*-containing 4th chromosome is shown in red. The fused X, unfused X, and *cd*-derived X chromosomes are shown in white, yellow, and green, respectively.

117

118

bioRxiv preprint doi: https://doi.org/10.1101/638684; this version posted May 24, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

119 ***Measuring transmission of the fused X-4 chromosome using microsatellite markers:***

120

121 <u>Microsatellite marker design</u>: Microsatellite markers were developed near the centromeres of the X, 4th,
122 and 3rd chromosomes to track the transmission of the X-4 and 2-3 fused chromosomes from
123 heterozygous females to their offspring. Descriptions of twenty markers near the centromere of the X-4
124 fusion and nine markers near the centromere of chromosome 3 are provided in Table S2. The three *D.*
125 *virilis* genomic scaffolds we used for marker development were mapped previously to the centromere-
126 proximal euchromatin of *D. virilis* polytene chromosomes by Schaeffer *et al*. (2008). We identified 20-
127 50bp tandem repeats in the *D. virilis* genome sequence using RepeatMasker (Smit *et al.* 2013). We
128 chose repeats that were closest to the proximal end of the scaffold (closest to the centromere) for
129 further investigation. We designed primer pairs that flank the repeat regions using Primer3
130 (Untergasser *et al.* 2012). Primer sequences were blasted against the draft assembly of *D. americana*
131 (Reis *et al.* 2008) to check for sequence conservation and multiple annealing sites. We checked for
132 allelic differences among ten inbred *D. americana* and four *D. novamexicana* strains. We ultimately
133 obtained five informative microsatellite markers that can be
134 used to track transmission of the X-4 fusion, and two
135 informative markers to track transmission of the 2-3 fusion
136 (Table S2).

137

138 <u>Embryo collection</u>: Two different heterozygous female
139 genotypes were used in this experiment. The first genotype
140 was generated by crossing two inbred *D. americana* lines
141 (fused X-4: G96.23, unfused: HI99.12), and the second,
142 interspecific genotype was generated from a cross
143 between an inbred *D. americana* strain (G96.13) and an
144 iso-female *D. novamexicana* strain (1031.0). Parental
145 crosses were performed as described above. For the
146 intraspecific F1 cross, ~100 heterozygous F1 females were
147 crossed with males from a different unfused inbred *D.*
148 *americana* strain (ML97.5). For the interspecific F1 cross,
149 ~100 heterozygous F1 females were crossed with males
150 from a different *D. novamexicana* strain (1031.4). Females
151 were allowed to mate and lay eggs in 12-hour intervals on
152 grape agar plates, followed by an additional 10-12 hour

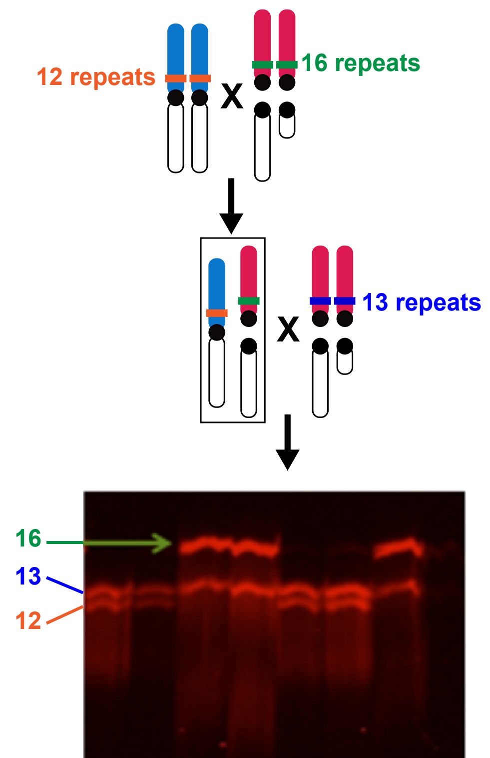

Figure 3. Crossing scheme to track transmission of the X-4 fusion from F1 females (rectangle) by microsatellite genotyping of three alleles in F2 embryos.

bioRxiv preprint doi: https://doi.org/10.1101/638684; this version posted May 24, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

153 incubation at 22°C to allow embryos to develop. Embryos were collected daily over an 8-day period to
154 ensure sampling from the females' entire sexual peak. Collected embryos were rinsed with distilled
155 water, washed with 50% bleach, and frozen at -20°C in 96-well plates. DNA was prepared from frozen
156 embryos using a standard squashing buffer (10mM Tris-HCl pH 8.2, 1mM EDTA, 25mM NaCl, 200μl/ml
157 Proteinase K).
158
159 *Microsatellite analysis*: Each microsatellite locus was amplified from an embryonic DNA sample using a
160 previously developed PCR method (Shimizu *et al.* 2002). This method entails three PCR primers at
161 differing concentrations: 26.7nM of a modified forward primer, 133.3nM of an IR-700 or IR-800
162 conjugated M13 forward primers, and 160nM of the reverse primer. The forward primer was
163 synthesized with an addition of the reverse complement of an M13 tail (5'-
164 GGATAACAATTTCACACAGG) at the 5' end. This modification introduces an M13 complement on the
165 PCR amplicon, which is used to produce dye labeled single-strand products from either the IR-700 or
166 IR-800 conjugated M13 primer (LI-COR, Lincoln, NE). Genotypes were determined following separation
167 on a 7% polyacrylamide gel and imaged using the LI-COR Odyssey infrared imager with Odyssey
168 application software v3.0 (Figure 3).
169
170 We determined the transmission of the fused X-4 chromosome from F1 G96.23/HI99.12 females by
171 genotyping embryos at the microsatellite locus of the 4th chromosome, ms1019560 (Table S2). Inbred
172 lines G96.23, HI99.12, and ML97.5 are each homozygous for different length alleles at ms1019560.
173 The F2 offspring each have the band corresponding to the paternal ML97.5 line and another band that
174 either corresponds to the G96.23 X-4 chromosome or the HI99.12 unfused 4th chromosome (Figure 3).
175 Transmission rates of the X-4 fused chromosome were calculated as the number of F2 embryos with
176 the G96.23 genotype divided by the total number of embryos genotyped.
177
178 To determine the transmission rate of the X-4 fused chromosome in interspecific F1 hybrid females
179 (G96.13/1031.0), F2 embryos were genotyped using three separate microsatellite loci on the 4th
180 chromosome: ms977861, ms1219821, and ms2825734 (Table S2). At each locus, G96.13 and 1031.0
181 had distinct microsatellite alleles. However, the strain that hybrid F1 females were crossed with
182 (1031.4) had the same allele at all three loci. Therefore, the F2 embryos from this cross were screened
183 for the G96.13 allele. Offspring that inherit the fused X-4 chromosome will carry both the 1031.0/1031.4
184 and G96.13 alleles, but those that inherit the unfused chromosome will only carry the 1031.0/1031.4
185 allele.
186

bioRxiv preprint doi: https://doi.org/10.1101/638684; this version posted May 24, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

187 ***Evaluating the effect of the chromosomal inversions in transmission ratio distortion:***
188
189 Inbred lines with previously confirmed inversion rearrangements (Mena 2009) were used to analyze the
190 effects of the different inversions on transmission bias. Two inbred fused X-4 lines that have *In(4)ab*
191 and *In(X)c* were used: G96.13 and HI99.34 (Table S1). Another two lines, G96.23 and OR01.50, have
192 the In(X)c inversion. The unfused X and 4th arrangement in *D. americana* lacks all three inversions
193 associated with X-4 fusion (inbred lines HI99.12 and *pur*). The two *D. novamexicana* lines contain both
194 *In(4a)* and *In(X)c*, but lack *In(4)b*.
195
196 These lines were systematically crossed to
197 generate three female genotypes that are
198 heterozygous for the X-4 fusion, but also contain
199 different heterozygous combinations of the three
200 inversions: (1) Females that are heterozygous for
201 all three inversions were generated by crossing
202 G96.13 females with *pur* males (Figure 4A), (2)
203 females heterozygous for *In(X)c* we generated
204 by crossing G96.23 or OR01.50 females with
205 HI99.12 males (Figure 4B), and (3) females
206 heterozygous for *In(4)b* but homozygous for the
207 other two inversions were generated by crossing
208 G96.13 or HI99.34 females with males from the *D.*
209 *novamexicana* strains, 1031.0 or 1031.4,
210 respectively (Figure 4C). Transmission of the fused
211 X-4 chromosome was tracked using the *cd* visible
212 eye marker, as outlined above and in Figure 2.

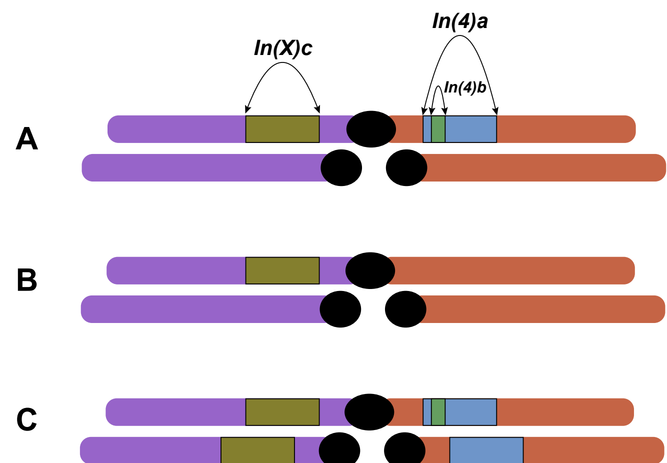

Figure 4. Visual representation of F1 females with the three different possible inversion states. A) Heterozygous for all three inversions. B) X-4 fused chromosome is lacking In(4)ab, therefore only In(X)c is heterozygous. Transmission ratio of the fused X-4 chromosome can be measured without the effects of In(4)ab. C) D. americana/D. novamexicana hybrid, In(4)a and In(X)c are homozygous and only In(4)b is heterozygous. Transmission ratio of the fused X-4 chromosome can be measured without the effects of In(X)c.

213
214
215 ***Assaying transmission of the fused 2-3 chromosome of D. americana:***
216
217 We tested whether meiotic drive causes transmission bias of another fused metacentric chromosome,
218 chromosome 2-3, by measuring transmission rates from *D. americana*/*D. novamexicana* hybrid females
219 to adult offspring. Heterozygous females were generated by crossing strain G96.13 (*D. ame*) with
220 1031.4 (*D. nov*), which were subsequently backcrossed to 1031.4 and her progeny frozen for DNA

bioRxiv preprint doi: https://doi.org/10.1101/638684; this version posted May 24, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

221 extraction. Whole fly DNA was prepared as described previously, and samples were analyzed at two
222 microsatellite loci (ms786503 and ms806741; Table S2) on the 3rd chromosome. F2 offspring were
223 screened for the presence of the G96.13 allele, which would indicate transmission of the fused 2-3
224 chromosome from the heterozygous F1 female. F2's from this cross were also genotyped at two
225 additional loci on the X and 4th chromosomes (ms1141205 and ms1219821, respectively; Table S2).
226
227 ***Statistical analysis:***
228
229 We used a logistic generalized linear model with maximum likelihood fitting to test for significant
230 deviations from Mendelian expectations of X-4 transmission (File S1). The model was used to examine
231 whether maternal or paternal inheritance of the X-4 fusion affected the transmission rate, and to obtain
232 transmission rate estimates and 95% confidence intervals for each cross (File S1). Analyses comparing
233 the transmission rates to embryos and adults from specific lines were tested against Mendelian
234 expectations using a χ^2 goodness-of-fit test: A 2x2 contingency table with χ^2 was used to compare
235 crosses to each other. The effects of different inversion rearrangements were analyzed using a χ^2
236 goodness-of-fit test against the Mendelian expectation of 50:50, and a 2x2 contingency table with a χ^2
237 test was used to compare inversion rearrangements against each other.
238
239
240 **Results**
241
242 ***The X-4 fusion exhibits a transmission advantage over its unfused homolog in D. americana:***
243
244 We performed reciprocal crosses between five parental unfused X and 4 lines and five parental fused
245 X-4 lines to produce F1 females that are heterozygous for the alternate arrangements of the X and 4th
246 chromosomes. Transmission rates from heterozygous F1 mothers to sons were measured by tracking
247 sex-linkage of a phenotypic marker on chromosome 4 (Figure 2). An average transmission rate of
248 56.6% (95% *C. I.* = 1.8%) for the X-4 fusion was observed among the F1 females produced from 45
249 different cross combinations. Introducing the X-4 fusion maternally or paternally does not significantly
250 influence the transmission rate (Figure 5A, Table S3, File S1); including this factor in a logistic
251 generalized linear model did not improve the fit of the model (χ^2=2.4, *p*=0.12, *d.f.*=1, File S1).
252

bioRxiv preprint doi: https://doi.org/10.1101/638684; this version posted May 24, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

253 Meiotic segregation of the alternative chromosome arrangements in these F1 females is expected to
254 have a 50:50 transmission ratio. The estimated 95% confidence intervals overlapped with the 50:50
255 Mendelian expectation for 28 out of the 45 heterozygotes produced from inter-strain crosses (Figure
256 5B, File S1). In contrast, the confidence intervals exceed 50:50 for the remaining 16 genotypes,
257 including instances where the X-4 chromosome was introduced maternally and paternally. In none of
258 the F1 genotypes examined was the estimated confidence interval for the transmission ratio below
259 50:50 (Figure 5B, File S1). Overall, these results show that the derived metacentric X-4 fusion
260 experiences a transmission bias over the ancestral acrocentric arrangement.
261
262 ***Meiotic drive is not caused by differential viability:***
263
264 To assess the effect of differential viability on meiotic drive, we measured transmission rates of the X-4
265 fusion from heterozygous females to adult sons and to embryonic offspring in two separate crosses.
266 We generated intraspecific (G96.23 x HI99.12) and interspecific hybrid (G96.13 x 1031.0) F1 females,

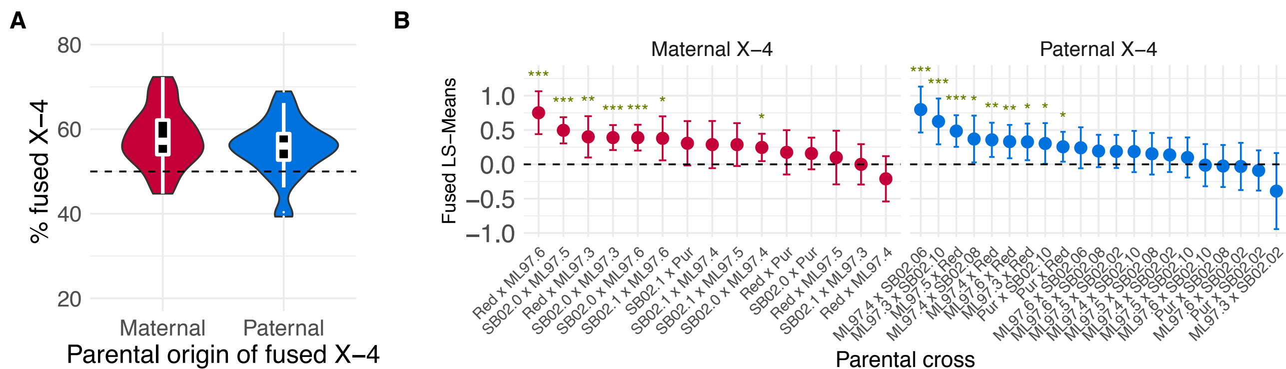

Figure 5. Transmission ratio of the fused X-4. (A) Females heterozygous for the arrangement of the X and 4th chromosomes were produced from pairwise reciprocal crosses between lines differing in chromosome arrangement, and transmission ratios are presented for females that inherited the X-4 fusion maternally or paternally. (B) Least squares means estimates of transmission ratios for the fused X-4 chromosome for each cross combination. The dotted line (LS-Means = 0) indicates 50:50 Mendelian segregation, and positive values indicate biased transmission favoring the X-4 fusion. Error bars represent 95% confidence intervals. Cross combinations with significant deviation from Mendelian segregation are indicated (*: $0.01 < p < 0.05$; **: $0.001 < p < 0.01$; ***: $p < 0.001$).

267 and subsequently genotyped their adult male progeny and embryo offspring at a molecular marker on
268 the 4th chromosome. Two separate trials of embryos were analyzed to ensure consistency of the
269 method.
270
271 Offspring from the conspecific F1 females showed a transmission bias for the fused X-4 chromosome in
272 both embryos and adult sons (Figure 6). One embryo trial showed a bias of 54.5% and the other a bias
273 of 58.9%; both statistically different from the predicted 50:50 (Table S4). These two embryo trials were

bioRxiv preprint doi: https://doi.org/10.1101/638684; this version posted May 24, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

274 not significantly different from each other with respect to transmission bias for the fused X-4
275 arrangement (χ^2= 1.03, *p*>0.05, *d.f.*=1), reflecting consistency in replicate trials. Transmission of the X-4
276 chromosome to adult sons (61.9%) is not significantly different from either embryo replicate (χ^2= 3.8,
277 *p*>0.05, *d.f.*=1; χ^2= 0.46, *p*>0.05, *d.f.*=1) (Table S4).
278
279 The assessment of transmission ratio was consistent between embryos and adult sons; however, this
280 consistency was attained with only 77% and 73% success genotyping individual embryos in the two
281 trials. The remaining embryos failed to produce amplified DNA at the microsatellite locus. To assess
282 whether our ability to successfully genotype an embryo was skewing the results in favor of the fused X-
283 4 arrangement, we crossed the parental unfused X and 4th line, HI99.12, to another unfused line,
284 ML97.5. F1 embryos were analyzed to
285 assess the success rate for genotyping the
286 unfused 4th chromosome: if the unfused
287 arrangement is less likely to produce
288 useable DNA for genotyping, it is expected
289 that the embryos from the parental unfused
290 line will produce fewer successfully
291 genotyped embryos. We compared our
292 embryo genotyping between offspring of the
293 parental unfused line and both trials of F1
294 heterozygous females. The genotyping
295 success rate for offspring originating from
296 the parental female was 75.1% (n=405), which was not significantly different in embryo trials of
297 embryos laid by F1 heterozygous females (2x3 contingency table chi squared; χ^2= 3.77, *p*>0.1, *d.f.*=2).
298 From this comparison we can conclude that this approach does not introduce a genotyping bias against
299 the unfused arrangement in embryos.

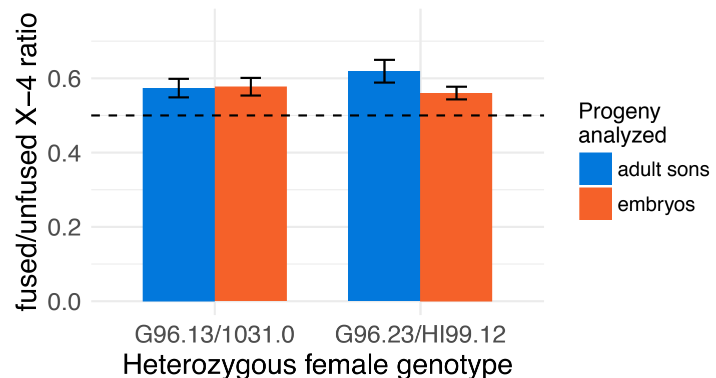

Figure 6. Transmission ratio of the X-4 fused chromosome from conspecific (right) and heterospecific (left) heterozygous females to adult sons and embryos. Error bars represent the binomial standard error.

300
301 F1 hybrids from the *D. americana*/*D. novamexicana* cross (G96.13 x 1031.0) were also assayed for
302 biased transmission of the X-4 fusion in embryos and adult sons by genotyping at three molecular
303 markers near the centromere of chromosome 4 (ms977861, ms1219821, and ms2825734; Table S2).
304 Marker ms977861 is most proximal to the centromere, roughly 1 Mb from the end of the scaffold, while
305 ms1219821 and ms2825734 are ~250kb and ~1.8Mb away from ms977661, respectively.
306

bioRxiv preprint doi: https://doi.org/10.1101/638684; this version posted May 24, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

307 The transmission rate of the fused X-4 chromosome was ~57% in both adult sons and embryos (Figure
308 6, Table S4). In this cross there was also no significant difference between the inheritance rates of the
309 fused X-4 to adult sons and embryo offspring (χ^2=0.01, p>0.05, *d.f.*=1). We observe distinct genotypes
310 between the most distal rearrangement (ms2825734) and the other 2 markers in 1.2% of embryos,
311 suggesting low levels of recombination between these loci. Only one recombinant genotype between
312 ms977861 and ms1219821 was observed, suggesting a recombination rate lower than 0.5%. All
313 samples that showed recombinant genotypes at any of the two loci were not included in the analysis.
314
315 ***Meiotic drive is not affected by centromere-associated inversions:***
316
317 We examined whether three inversions near the X and 4th chromosome centromeres influence
318 transmission of the X-4 fusion. We generated three combinations of heterozygous inversion genotypes
319 on an X-4 fusion heterozygous karyotype (Figure 4), and measured transmission ratios of the X-4 fused
320 chromosome using the crossing scheme described in Figure 2. First, we assayed meiotic drive in
321 females that are heterozygous for all three known inversions on the X and 4th chromosomes: *In(X)c*,
322 *In(4)a*, and *In(4)b* (Figure 4). In these females, the transmission rate of the fused X-4 chromosome was
323 57.8% (Figure 7, Table S5), which is significantly higher than Mendelian transmission (χ^2=7.46, p<0.01,
324 *d.f.*=1). Second, we examined the effect of a heterozygous *In(X)c* inversion genotype in an otherwise
325 collinear arrangement of the 4th chromosome—i.e. lacking the *In(4)ab* complex (Figure 4B). Here we
326 used two separate conspecific crosses to produce F1 females: G96.23xHI99.12 and OR01.50xHI99.12.
327 Heterozygous F1 females from both crosses showed a transmission bias for the X-4 fusion
328 (G96.23/HI99.12: χ^2=13.82, p<0.001, *d.f.*=1; OR01.50/HI99.12: χ^2=3.92, p<0.05, *d.f.*=1; Figure 7, Table
329 S5). F1 females from the G96.23/HI99.12 cross showed a higher transmission rate of the fused X-4
330 chromosome (61.9%) than the OR01.50/HI99.12 F1 females (54.9%), but these transmission rates are
331 not significantly different from each other (χ^2=3.18, p>0.05, *d.f.*=1). Furthermore, F1 females from these
332 two crosses showed no significant transmission difference from F1 females that are heterozygous for
333 *In(4)ab* (χ^2=3.19, p>0.05, *d.f.*=2), suggesting the linear arrangement of the 4th chromosome does not
334 affect meiotic drive for the fused X-4 chromosome.

bioRxiv preprint doi: https://doi.org/10.1101/638684; this version posted May 24, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

335 Finally, We assessed the effect of the smaller inversion on chromosome 4, *In(4b)*, in an otherwise
336 collinear genotype that is homozygous for *In(X)c* and *In(4)a*. Here, we utilized *D. novamexicana* to
337 generate heterospecific hybrid females, because it also has *In(X)c* but lacks *In(4)b*. We crossed *D.*
338 *americana* females that had *In(X)c* and *In(4)ab* with *D. novamexicana* males that had *In(X)c* and *In(4)a*
339 (Figure 4C). Two separate crosses (G96.13 x NOVA1031.0 and HI99.34 x NOVA1031.4) were
340 performed to generate F1 females. Again, transmission rates favored the X-4 fused chromosomes
341 (Figure 7). F1 G96.13/NOVA1031.0 females and F1 HI99.34/NOVA1031.4 females had a significant
342 transmission rate of 57.4% and 55.6%,
343 respectively, favoring the X-4 fusion
344 (G96.13/NOVA1031.0: χ^2=8.24, *p*<0.01, *d.f.*=1;
345 HI99.34/NOVA1031.4: χ^2=5.28, *p*<0.02, *d.f.*=1;
346 Figure 7, Table S5). These crosses were also
347 not significantly different from each other (χ^2=
348 0.26, *p*>0.05, *d.f.*=1). Furthermore,
349 transmission rates did not differ from those in
350 triple inversion heterozygotes (χ^2=0.426, *p*>0.8,
351 *d.f.*=2).
352
353 Taken together, these results indicate that the
354 three centromere-associated inversions on the
355 X and 4th chromosomes play no detectable
356 role in the observed transmission bias that
357 favors the X-4 fusion.

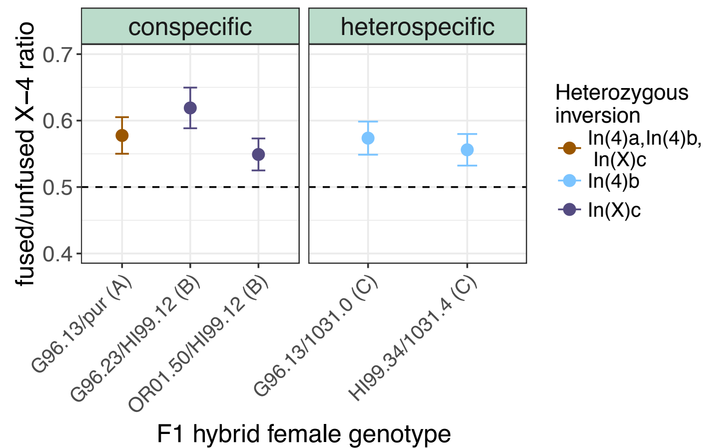

Figure 7. Transmission ratios of the X-4 fusion from heterozygous females to adult sons in crosses with differing inversion states. Point color represents heterozygous inversion genotype (legend on the right), which is also indicated by the letters in parenthesis next to the parental strain identification (see Figure 4). The left and right panels represent con- and heterospecific hybrid females, respectively. Error bars represent the binomial standard error, and a 50:50 segregation ratio is indicated by the dashed line.

358
359 ***The fused 2-3 chromosome shows biased transmission in D. americana/D. novamexicana hybrid***
360 ***females:***
361
362 We investigated whether female meiotic drive favors all fused metacentric chromosomes in *D.*
363 *americana* by measuring the transmission rates of the 2-3 fused chromosome from *D. americana*/*D.*
364 *novamexicana* hybrid females to adult offspring. F1 hybrid *D. americana*/*D. novamexicana* were
365 produced from a cross between G96.13 females and 1031.4 males, and were heterozygous for both
366 the X-4 and 2-3 fusions. The transmission rate of the 2-3 fused chromosome was determined by
367 microsatellite genotyping of adult offspring at two loci on the 3rd chromosome (ms786503 and
368 ms806741), which are ~200kb apart and show perfect co-segregation. The transmission of the X-4

bioRxiv preprint doi: https://doi.org/10.1101/638684; this version posted May 24, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

369 chromosome was measured by genotyping adult offspring at two loci: one on the X chromosome
370 (ms1141205) and another on the 4th chromosome (ms1219821). Genotypes at these two markers
371 were incongruent less that 1% of the time.
372
373 Adult offspring from F1 hybrid females inherited the 2-3 and X-4 fused chromosomes 62.6% and 55.4%
374 of the time, respectively (Table 1). The X-4 transmission rate is similar to the 57% ratio observed in
375 embryo and adult male offspring (χ^2= 0.72, *p*>0.3, *d.f.*=1). Furthermore, the transmission rate of the 2-3
376 fused chromosome is significantly greater than the X-4 fused chromosome (χ^2= 4.29, *p*<0.04, *d.f.*=1).
377
378 Finally, we analyzed co-segregation of the fused X-4 chromosome and the fused 2-3 chromosome
379 during meiosis in F1 hybrid females. We genotyped 333 flies for both the X/4th and 2nd/3rd
380 chromosome arrangements. We find no evidence of co-segregation between the X-4 fused
381 chromosome and the 2-3 fused chromosome (χ^2= 2.2, *p*>0.1, *d.f.*=1) (Table S6). Thus, the forces
382 biasing the transmission for the fused X-4 appear to act independently of the forces driving the fused 2-
383 3 chromosome.
384
385 **Table 1**. Transmission ratio of fused 2-3 and X-4 chromosomes in *D. amer*/*D. nov* hybrid females

Chr.	# fused	# unfused	Total	% fused	χ^2	*p*-value
2-3	221	132	353	62.6	21.9	2.87e-06
X-4	286	231	517	55.4	5.64	0.018

386
387
388 **Discussion:**
389
390 Here we presented evidence of meiotic drive favoring two derived metacentric chromosomes. The
391 fused X-4 chromosome in *D. americana*, which resulted from a fusion between two acrocentric
392 chromosomes, enjoys a transmission advantage of ~53-70% in heterozygous females. In addition, the
393 2-3 fused chromosome transmission rate is >60% over its unfused counterpart in heterospecific hybrid
394 females. The transmission bias for the fused X-4 chromosome is observed in both 24hr-old embryos
395 and adult sons, providing evidence that the observed meiotic drive is not an artifact of reduced survival
396 of the unfused genotype. Furthermore, the arrangements of the differing inversions do not play a
397 detectable role in the transmission bias, suggesting that the differing centromeres or a locus linked to
398 the centromere are impacting the observed bias. Finally, there is no difference in transmission bias
399 based on the parental origin of the fused X-4 chromosome, suggesting that maternally inherited

bioRxiv preprint doi: https://doi.org/10.1101/638684; this version posted May 24, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

400 components are not playing an important role in the transmission bias. We discuss these findings in
401 more detail below.
402
403 ***Meiotic drive vs. differential viability:***
404
405 We investigated two plausible explanations for the observed transmission bias between the karyotype
406 forms in *D. americana*: meiotic drive and differential viability. Observing the same biased ratio from
407 heterozygous females to embryos and to adult sons suggests that transmission bias takes place during
408 meiosis. However, we were unable to assay every embryo collected due to unsuccessful DNA
409 preparations for some embryos. This is likely due to the observation that some laid eggs are not
410 fertilized, and thus likely contain little DNA for microsatellite amplification. We have shown in previous
411 work that hatch rates within and between *D. americana* strains range from 70%-90%, and are primarily
412 due to lack of fertilization (Ahmed-Braimah and McAllister 2012). However, we found no correlation
413 between fertilization rates and presence/absence of the X-4 fusion in that study.
414
415 Another study of fertility/viability and early development between homozygotes of each arrangement at
416 a similar temperature that we maintain the lines in (22˚C) showed higher viability for the unfused lines
417 (Sillero *et al.* 2014). This suggests that if differential viability was playing a significant role, the
418 transmission bias would favor the unfused X and 4th arrangement rather than the fused X-4. Without
419 the ability to successfully assay every embryo collected from heterozygous females, we cannot
420 completely rule out the possibility that differential viability in early development between the two
421 chromosome arrangements is involved in biasing the inheritance pattern. However, if this were the
422 case, we would expect the unfused chromosomal arrangement to yield a lower successful embryo
423 genotyping percentage than the embryos of experimental heterozygous F1 females, but this was not
424 observed.
425
426 Recent studies have shown that meiotic drive likely contributes to the evolution of chromosomes with
427 differing centromeres such as the yellow monkeyflower, *Mimulus guttatus* (Fishman and Willis 2005;
428 Fishman and Kelly 2015), and the house mouse *Mus musculus* (LeMaire-Adkins and Hunt 2000;
429 Chmátal *et al.* 2014). Meiotic drive has also been hypothesized as a major contributor to mammalian
430 karyotype evolution (Pardo-Manuel de Villena and Sapienza 2001b; Yoshida and Kitano 2012). Taken
431 together, these studies and the findings presented here suggest that meiotic drive is a taxonomically
432 broad and pervasive force impacting karyotype evolution.
433

bioRxiv preprint doi: https://doi.org/10.1101/638684; this version posted May 24, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

434 ***Centromere vs. inversions:***

435

436 We did not observe a significant difference in segregation bias between different inversion states of the
437 fused chromosomes, and all chromosome arrangements showed a bias for fused chromosomes
438 regardless of inversion status. However, currently we are unable to create an F1 female that is
439 homozygous for all three inversions. Thus, it is possible that just the presence of an inversion could
440 cause meiotic drive between the differing centromere arrangements. The most plausible mechanism for
441 this inversion effect would arise when recombination occurs within the inversion break points. This
442 would generate dicentric and acentric chromosomes, which can be selectively eliminated by ensuring
443 transmission of the recombinant chromosome to the polar bodies (Carson 1946). If this were the case,
444 the size of the inversion would be expected to influence the magnitude of the drive as the
445 recombination events would have a greater likelihood of a recombination site inside a larger inversion.
446 Because *In(x)c* is much larger than *In(4)b*, we would expect G96.23/HI99.12 and OR01.50/HI99.12
447 lines to have greater drive than G96.13/Nova1031.0 and HI99.34/Nova1031.4. Yet, we observe no
448 difference between the lines that only differ at *In(4)b* and the lines that only differ at *In(x)c*, suggesting
449 that the centromeric fusion—or a locus closely linked to the centromere—is the driver of biased
450 segregation. In other systems, meiotic drive is also caused by differing centromere arrangements
451 (Fishman and Saunders 2008) or beta chromosomes that contain centromeric knobs (Buckler *et al.*
452 1999). Our results strongly implicate the centromere (or centromere-associated sequences) as the
453 target of biased segregation.

454

455 ***Transmission bias of the 2-3 chromosome vs. X-4 chromosome:***

456

457 We found meiotic drive favoring both the X-4 and the 2-3 chromosomes. Thus, in this system, meiotic
458 drive favors the derived metacentric chromosomes at the expense of the acrocentric homologs. Similar
459 mechanisms likely underlie the transmission bias for the X-4 and 2-3 fused chromosome arrangements.
460 However, the transmission bias for the 2-3 chromosomes appears to be greater than that of the X-4th
461 chromosome. In addition, the 2-3 and the X-4 chromosomes do not co-segregate within the same
462 meiosis. While both metacentric chromosomes are favored in female meiosis, the different magnitude
463 of drive and the lack of co-segregation indicates that the mechanism affecting meiotic drive in *D.*
464 *americana* are likely complex. Mechanisms of meiotic drive in *M. musculus* are affected by levels of
465 kinetochore proteins surrounding the differing arrangements (Chmátal *et al.* 2014). In populations that
466 have fixed metacentric chromosomes, the relative localization of the kinetochore proteins HEC-1
467 (Ndc80 in Drosophila) and CENP-A (CID in Drosophila) at metacentric centromeres is significantly

bioRxiv preprint doi: https://doi.org/10.1101/638684; this version posted May 24, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

468 higher. In contrast, populations that have all acrocentric chromosome have relatively higher amounts of
469 HEC-1 and CENP-A localizing to acrocentric chromosomes. In mouse populations that have closer to
470 half acrocentric chromosomes and half metacentric chromosomes, variation in relative localization of
471 HEC-1 and CENP-A between acrocentric and metacentric chromosomes is observed (Chmátal *et al.*
472 2014). Ndc80 in Drosophila (HEC-1) is a component of the ndc80 complex, which is a core component
473 of the kinetochore and is involved in many processes for both mitosis and meiosis, including
474 kinetochore assembly, congression of the chromosomes at the metaphase plate, and binding to the
475 spindle (Tooley and Stukenberg 2011). CID (CENP-A) is the centromere specific histone H3 protein
476 that plays a role in proper kinetochore recruitment and centromere formation (Blower and Karpen
477 2001). Differences in the observed transmission bias between the 2-3 and the X-4 could result from
478 differing centromere compositions, such that the 2-3 fused chromosome recruits higher levels of
479 kinetochore proteins than the 2nd and 3rd acrocentric chromosomes, and at a more consistent rate
480 than the X-4 over the X and 4th. A difference in centromere composition could be a factor in the
481 observation that the segregation of the X-4 chromosome does not affect segregation of the 2-3
482 chromosome.
483
484 ***Control of meiotic drive:***
485
486 Meiotic drive in excess of 50:50 segregation should facilitate driving the favored arrangement to fixation
487 in the absence of an opposing selective force. In nature the X-4 fused arrangement is not fixed in
488 populations, but rather exists in a latitudinal cline throughout the central United States (McAllister 2002;
489 McAllister *et al.* 2008). These contemporary population samples of adult flies from throughout the
490 species range, along with early surveys (Patterson and Stone 1952), reveal the widespread presence
491 and persistence of this chromosomal polymorphism where the derived X-4 fusion is rare in the south
492 and common in the north. The intrinsic segregation advantage for the metacentric chromosome is
493 difficult to reconcile in the context of this apparently stable cline. While stability of the cline has
494 previously been attributed to natural selection in the context of climatic variability, this variability may
495 also affect the mechanism causing meiotic drive. Recombination studies in *Drosophila melanogaster*
496 have suggested that transmission distortion may play a role in increased inheritance of chromatids that
497 have undergone crossing over in females which have been exposed to stress either by bacterial
498 infection or heat treatment (Singh *et al.* 2015; Jackson *et al.* 2015). Moreover, the latitudinal cline may
499 represent a balance between meiotic drive and forces of natural selection acting on the allelic content
500 of the alternative chromosome forms. This would suggest a strong selective advantage for the unfused
501 arrangement in the southern United States which decreases with increasing latitude, until the

bioRxiv preprint doi: https://doi.org/10.1101/638684; this version posted May 24, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

502 population is fixed for the fused X-4 chromosome. To further investigate these explanations,
503 transmission rates of heterozygous females exposed to differing stress inducers related to the northern
504 and southern United States should be examined.
505
506
507
508
509
510
511
512
513
514
515
516
517
518
519
520
521
522
523
524
525
526
527
528
529
530
531
532
533
534
535

bioRxiv preprint doi: https://doi.org/10.1101/638684; this version posted May 24, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

536 # References

537

538 Ahmed-Braimah Y. H., McAllister B. F., 2012 Rapid evolution of assortative fertilization between
539 recently allopatric species of *Drosophila*. International Journal of Evolutionary Biology **2012**: 1–9.

540 Bengtsson B. O., 1980 Rates of karyotype evolution in placental mammals. Hereditas **92**: 37–47.

541 Blower M. D., Karpen G. H., 2001 The role of *Drosophila* CID in kinetochore formation, cell-cycle
542 progression and heterochromatin interactions. Nat. Cell Biol. **3**: 730–739.

543 Buckler E. S., Phelps-Durr T. L., Buckler C. S., Dawe R. K., Doebley J. F., Holtsford T. P., 1999 Meiotic
544 drive of chromosomal knobs reshaped the maize genome. Genetics **153**: 415–426.

545 Carson H. L., 1946 The selective elimination of inversion dicentric chromatids during meiosis in the
546 eggs of *Sciara impatiens*. Genetics **31**: 95–113.

547 Chmátal L., Gabriel S. I., Mitsainas G. P., Martínez-Vargas J., Ventura J., Searle J. B., Schultz R. M.,
548 Lampson M. A., 2014 Centromere strength provides the cell biological basis for meiotic drive and
549 karyotype evolution in mice. **24**: 2295–2300.

550 Chmátal L., Schultz R. M., Black B. E., Lampson M. A., 2017 Cell biology of cheating-transmission of
551 centromeres and other selfish elements through asymmetric meiosis. Prog. Mol. Subcell. Biol. **56**:
552 377–396.

553 Coolon J. D., Stevenson K. R., McManus C. J., Graveley B. R., Wittkopp P. J., 2012 Genomic
554 imprinting absent in *Drosophila melanogaster* adult females. Cell Reports **2**: 69–75.

555 Evans A. L., Mena P. A., McAllister B. F., 2007 Positive selection near an inversion breakpoint on the
556 neo-X chromosome of *Drosophila americana*. Genetics **177**: 1303–1319.

557 Fishman L., Kelly J. K., 2015 Centromere-associated meiotic drive and female fitness variation in
558 *Mimulus*. Evolution **69**: 1208–1218.

559 Fishman L., Saunders A., 2008 Centromere-associated female meiotic drive entails male fitness costs
560 in monkeyflowers. Science **322**: 1559–1562.

561 Fishman L., Willis J. H., 2005 A novel meiotic drive locus almost completely distorts segregation in
562 mimulus (monkeyflower) hybrids. Genetics **169**: 347–353.

563 Henikoff S., Ahmad K., Malik H. S., 2001 The centromere paradox: stable inheritance with rapidly
564 evolving DNA. Science **293**: 1098–1102.

565 Hsu T. C., 1952 Chromosomal variation and evolution in the virilis group of *Drosophila*. In: Univ. Texas
566 Publ, pp. 35–72.

567 Jackson S., Nielsen D. M., Singh N. D., 2015 Increased exposure to acute thermal stress is associated
568 with a non-linear increase in recombination frequency and an independent linear decrease in
569 fitness in *Drosophila*. BMC Evolutionary Biology **15**: 175.

bioRxiv preprint doi: https://doi.org/10.1101/638684; this version posted May 24, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

570 Joanis V., Lloyd V. K., 2002 Genomic imprinting in *Drosophila* is maintained by the products of
571 Suppressor of variegation and trithorax group, but not Polycomb group, genes. Mol Gen Genomics
572 **268**: 103–112.

573 LeMaire-Adkins R., Hunt P. A., 2000 Nonrandom segregation of the mouse univalent X chromosome:
574 evidence of spindle-mediated meiotic drive. Genetics **156**: 775–783.

575 Lemos B., Branco A. T., Jiang P.-P., Hartl D. L., Meiklejohn C. D., 2014 Genome-wide gene expression
576 effects of sex chromosome imprinting in *Drosophila*. G3 **4**: 1–10.

577 Lloyd V., 2000 Parental imprinting in *Drosophila*. Genetica **109**: 35–44.

578 Maggert K. A., Golic K. G., 2002 The Y chromosome of *Drosophila melanogaster* exhibits
579 chromosome-wide imprinting. Genetics **162**: 1245–1258.

580 McAllister B. F., 2002 Chromosomal and allelic variation in *Drosophila americana*: selective
581 maintenance of a chromosomal cline. Genome **45**: 13–21.

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

584 Mena P. A., 2009 The role of chromosomal rearrangements in adaptation in *Drosophila americana*.
585 PhD thesis, University of Iowa.

586 Pardo-Manuel de Villena F., Sapienza C., 2001a Nonrandom segregation during meiosis: the
587 unfairness of females. Mamm. Genome **12**: 331–339.

588 Pardo-Manuel de Villena F., Sapienza C., 2001b Female meiosis drives karyotypic evolution in
589 mammals. Genetics **159**: 1179–1189.

590 Patterson J. T., Stone W. S., 1952 Evolution in the genus *Drosophila*. Macmillan, NY.

591 Reis M., Vieira C. P., Morales-Hojas R., Vieira J., 2008 An old bilbo-like non-LTR retroelement insertion
592 provides insight into the relationship of species of the virilis group. Gene **425**: 48–55.

593 Sandler L., Novitski E., 1957 Meiotic Drive as an Evolutionary Force. Am. Nat. **91**: 105–110.

594 Shimizu M., Kosaka N., Shimada T., Nagahata T., Iwasaki H., Nagai H., Shiba T., Emi M., 2002
595 Universal fluorescent labeling (UFL) method for automated microsatellite analysis. DNA Res. **9**:
596 173–178.

597 Sillero N., Reis M., Vieira C. P., Vieira J., Morales-Hojas R., 2014 Niche evolution and thermal
598 adaptation in the temperate species *Drosophila americana*. Journal of Evolutionary Biology **27**:
599 1549–1561.

600 Singh N. D., Criscoe D. R., Skolfield S., Kohl K. P., Keebaugh E. S., Schlenke T. A., 2015 Fruit flies
601 diversify their offspring in response to parasite infection. Science **349**: 747–750.

602 Smit A., Hubley R., Green P., 2013 *2013–2015. RepeatMasker Open-4.0*.

603 Sturtevant A. H., Beadle G. W., 1936 The relations of inversions in the X chromosome of *Drosophila*
604 *melanogaster* to crossing over and disjunction. Genetics **21**: 554–604.

bioRxiv preprint doi: https://doi.org/10.1101/638684; this version posted May 24, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

605 Tooley J., Stukenberg P. T., 2011 The Ndc80 complex: integrating the kinetochore's many movements.
606 Chromosome Res. **19**: 377–391.

607 Untergasser A., Cutcutache I., Koressaar T., Ye J., Faircloth B. C., Remm M., Rozen S. G., 2012
608 Primer3—new capabilities and interfaces. Nucl. Acids Res. **40**: e115–e115.

609 Wittkopp P. J., Haerum B. K., Clark A. G., 2006 Parent-of-origin effects on mRNA expression in
610 *Drosophila melanogaster* not caused by genomic imprinting. Genetics **173**: 1817–1821.

611 Yoshida K., Kitano J., 2012 The contribution of female meiotic drive to the evolution of new-sex
612 chromosomes. Evolution **66**: 3198-3208.