1   Title: Interference hypothesis for recombination suppression in chromosomal inversion

2   heterozygotes: A formal genetics analysis in *Drosophila melanogaster*

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47   ABSTRACT

48   Suppressed recombination in chromosomal inversion heterozygotes is a well-known but

49   poorly understood phenomenon. Recombination suppression is the result of at least four different

50   regional effects of inversion heterozygosity; surprisingly, this includes areas outside inversions

51   where there are no barriers to normal pairing, synapsis, double strand break formation, or

52   recovery of crossover products. The interference hypothesis of recombination suppression

53   proposes heterozygous inversion breakpoints possess chiasma-like properties such that

54   recombination suppression extends from breakpoints in a process analogous to crossover

55   interference. This hypothesis is qualitatively consistent with chromosome-wide patterns of

56   recombination suppression, unifying the various regional effects under a single mechanism. The

57   present study generates quantitative predictions for this hypothesis using a probabilistic model of

58   crossover interference as a stationary renewal point process with gamma-distributed interarrival

59   distances. These predictions were tested with formal genetic data (>40,000 meioses) on crossing-

60   over in intervals external and adjacent to four cosmopolitan inversions of *Drosophila*

61   *melanogaster*. The decay of recombination suppression differs for each of the four cosmopolitan

62   inversions, with a strong dependence on proximity to the centromere. Counterintuitively, greater

63   than expected recombination was observed in centromeric regions for the most proximally

64   placed inversions. Structural features of chromosomes are discussed as potentially confounding

65   variables in modelling recombination suppression. The true form of the decay function is not

66   currently known and will require high-resolution mapping of rare crossover events in regions

67   immediately adjacent to inversion breakpoints. A simple extension of the present experimental

68   system can be conducted as a genetic screen for those rare crossover events.

69

70   INTRODUCTION

71   Based solely on formal genetic mapping data Alfred Sturtevant predicted the existence of

72   chromosomal inversions. This hypothesis was based on four discoveries also made by

73   Sturtevant; first, crossover data could be used to create genetic maps of linear chromosomes

74   (STURTEVANT 1913), second, chromosomes segregating in *Drosophila melanogaster* populations

75   carried crossover modifiers (STURTEVANT 1917), third, complementation tests show *D. simulans*

76   and *D. melanogaster* share genes (STURTEVANT 1920), and fourth, those shared genes were not

77   necessarily in the same order (STURTEVANT 1921). These experiments suggested the elegant

78   hypothesis that the polymorphic crossover modifiers in *D. melanogaster* were in fact inverted

79   gene orders. Cytological confirmation of this farsighted hypothesis came over a decade later

80   (PAINTER 1933), and the evolutionary analysis of inversion polymorphism quickly followed,

81   again pioneered by Sturtevant (STURTEVANT AND DOBZHANSKY 1936; STURTEVANT AND

82   MATHER 1938; STURTEVANT AND NOVITSKI 1941). Although scientific consensus is not

83   complete, it is likely that the evolutionary persistence of inversions is either a direct or indirect

84   effect of their ability to suppress recombination in the heterozygous state (KIRKPATRICK AND

85   BARTON 2006; HOFFMANN AND RIESEBERG 2008). Recombination suppression was Sturtevant's

86   first clue to the existence of inversions, but more than a century later the dimensions of this

87   complex meiotic phenotype are still poorly understood. In the present paper, the reasons for an

88   incomplete understanding of the fundamental property of inversions are briefly summarized and

89   then an interference model of recombination suppression is introduced as a potentially unifying

90   hypothesis.

91   In *D. melanogaster* recombination suppression is the combined effect of at least four

92   phenomena which occur in several distinct regions relative to an inversion breakpoint (figure

4

93   1A). Suppression effects have been extensively documented, but the use of heterogeneous live

94   material (in terms of genetic backgrounds, inversions, markers, and species) to study reduction in

95   recombination rates complicates integration and interpretation of a sizeable literature (KRIMBAS

96   AND POWELL 1992). Classical work focused on structural features like size and position of

97   inverted regions as the major determinants of recombination suppression effects, but recent

98   experimental population genetic research has demonstrated there is substantial genic variation

99   for the genomic rate and chromosomal distribution of recombination events segregating in many

100   species (BAUER *et al.* 2013; KONG *et al.* 2014; JOHNSTON *et al.* 2016), including *D. melanogaster*

101   (COMERON *et al.* 2012; HUNTER *et al.* 2016). It is currently unknown to what degree an

102   inversion's recombination profile is a function of its structural features (size and position of

103   inverted regions), as opposed to the genetic elements that are in strong linkage disequilibrium

104   with inverted regions. However, several generalizations do apply to this diverse set of

105   experiments; first, the strongest effects of recombination suppression are experienced in the local

106   vicinity of inversions and second, diffuse recombination effects are experienced throughout the

107   genome, even in physically unlinked regions (KRIMBAS AND POWELL 1992).

108   In the region immediately adjacent to inversion breakpoints (figure 1A region A, at least

109   2 Mb in *D. pseudoobscura* (STEVISON *et al.* 2011)), crossing-over has not been observed and

110   thus recombination suppression is assumed to be complete. Historically researchers thought

111   failure of homologous chromosomes to synapse in region A caused this complete suppression

112   (DOBZHANSKY 1931; STURTEVANT AND BEADLE 1936; NOVITSKI AND BRAVER 1954). More

113   recently, cytological analysis of multiply inverted *D. melanogaster* X chromosomes revealed that

114   pairing, synapsis, and the formation of double strand breaks are approximately normal (GONG *et*

115   *al.* 2005). The weak effect of failed pairing or synapse immediately at inversion breakpoints is

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116   insufficient to explain complete suppression of recombination in region A. GONG *et al.'s* (2005)

117   findings indicate crossovers are initiated at normal rates, although they are not resolved at

118   normal rates. Thus, the mechanism by which complete suppression in region A occurs is

119   unknown.

120   The most well-known effect of inversion heterozygosity is recombination suppression in

121   the region internal to inversion breakpoints (figure 1A region B). This is because a single

122   crossover event generates acentric and dicentric recombinant products that are never included in

123   the functional egg *via* a meiotic drive mechanism unique to asymmetric meiosis in females and

124   only known from studies in *Diptera* (figure 1B) (STURTEVANT AND BEADLE 1936; MCCLINTOCK

125   1941; CARSON 1946; MADAN *et al.* 1984). Recombination can and does occur in region B as

126   demonstrated by the recovery of double crossover events under normal conditions and the ability

127   to recover single crossover events under special experimental conditions (*e.g.,* using compound

128   X chromosomes) (STURTEVANT AND BEADLE 1936). Using experimental constructs to avoid the

129   mechanical difficulties associated with recombinants, or using viability reduction with

130   pericentric inversions as an indirect measure, the recombination rate in region B is estimated to

131   be about 25% of wildtype (NOVITSKI AND BRAVER 1954; COYNE *et al.* 1993; NAVARRO AND

132   RUIZ 1997). Despite the clear understanding of the selective elimination of aberrant products

133   from single crossover events (figure 1B), the mechanism for the ~75% partial suppression of

134   crossing-over in region B remains unknown.

135   In the regions external to inversion breakpoints (figure 1A regions C and D)

136   recombination occurs, but generates a genetic map strongly influenced by proximity to inversion

137   breakpoints. In region C there are no apparent barriers to normal pairing, crossing-over, and

138   recovery of all meiotic products; nonetheless, recombination is still reduced in areas near the

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139   inversion (STURTEVANT 1931; STURTEVANT AND BEADLE 1936; GRELL 1962). In regions very

140   distant from inversion breakpoints (figure 1A region D) recombination has sometimes been

141   observed to increase and this is termed the intrachromosomal effect (DOBZHANSKY AND

142   STURTEVANT 1931; DOBZHANSKY 1933). A similar observation of increased recombination on

143   non-homologous chromosomes is termed the interchromosomal effect (SCHULTZ AND REDFIELD

144   1951). The mechanisms for the partial suppression in region C, the intrachromosomal effect in

145   region D, and interchromosomal effect on other chromosomes is unknown (LUCCHESI AND

146   SUZUKI 1968). Further complicating matters, the relationship amongst, and the transition

147   between the mechanisms responsible for the complete, partial, and negative suppression (regions

148   A, C, and D respectively) remains obscure.

149   Clearly, recombination suppression *sensu lato* is a complex phenotype consisting of

150   several types of crossover modification at different distances from inversion breakpoints which

151   vary in both magnitude and extent. Most of these effects were described in the pre-molecular era

152   of biology. The application of molecular genetics methods in the multiply inverted chromosome

153   of *D. melanogaster* has shown older assumptions about pairing, synapsis, and formation of

154   double strand breaks near inversion breakpoints to be incorrect (GONG *et al.* 2005; MILLER *et al.*

155   2016a; MILLER *et al.* 2016b). To explain the lack of recombination despite normal rates of

156   crossover initiation GONG *et al.* (2005) suggest heterozygous inversion breakpoints possess the

157   interference-like properties of chiasmata. The present paper provides a mathematical elaboration

158   of the interference hypothesis for recombination suppression that has the potential to unify the

159   disparate regional effects of inversion heterozygosity under a single mechanism.

160   Similar to recombination suppression, interference is a well-described phenomenon

161   whose causal basis remains poorly understood (SPEED 1996; MILLER *et al.* 2016c). Interference

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162   is the non-independence of crossover events and is traditionally described by a coefficient of

163   coincidence. Formally, interference is the conditional probability of crossing-over in a focal

164   interval, given a crossover event elsewhere in the genome, scaled by the unconditional

165   probability of crossing-over for the focal interval. Practically, quantification of interference

166   requires multi-locus data and the coefficient of coincidence is expressed as the ratio of observed

167   double crossovers to the expected number under crossover independence (*i.e.,* joint probability /

168   product of marginal probabilities) (MULLER 1916; BAILEY 1961).

169   Interference was first observed in *D. melanogaster* (STURTEVANT 1915; MULLER 1916),

170   and much of the mathematical development of interference theory has been based on single spore

171   data generated from the *D. melanogaster* model system (HALDANE 1919; KOSAMBI 1943;

172   BAILEY 1961). Modelling the underlying point process for the gold standard multi-locus datasets

173   (WEINSTEIN 1936; MORGAN *et al.* 1938), multiple groups have all arrived at the same

174   conclusion: interference in *D. melanogaster* is most accurately modeled as a stationary renewal

175   process where interarrival times follow a gamma distribution with shape parameter (γ) of

176   approximately 5 and a rate parameter (μ) scaled to cumulative interval distances (FOSS *et al.*

177   1993; MCPEEK AND SPEED 1995; ZHAO *et al.* 1995). When the shape parameter is an integer, the

178   counting model of FOSS *et al.* (1993), the chi-square model of ZHAO *et al.* (1995), and gamma

179   model of MCPEEK AND SPEED (1995) are equivalent. Following MCPEEK AND SPEED (1995), the

180   coefficient of coincidence (*C*) as a function of genetic distance (*z*) from the previous crossover

181   event is

$$C(z) = \frac{\gamma}{\mu} \sum_{n=1}^{\infty} \frac{1}{\Gamma(\gamma n)} \mu^{\gamma n} z^{\gamma n - 1} e^{-\mu z}$$

182   equation 1

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185   where γ is the shape parameter, μ is the rate parameter, and n is an auxiliary variable. The *D.*

186   *melanogaster* specific parameterization of the coincidence function above suggests negligible

187   recombination (< 0.0001) will occur within 5 map units of inversion breakpoints, recombination

188   suppression will decay over approximately 35 map units, and a weak elevation of recombination

189   rates above wildtype will be observed in regions 40-60 map units distant from the inversion

190   breakpoints (where map units refers to cM for the standard arrangement genetic map of *D.*

191   *melanogaster*). These predictions are qualitatively consistent with the effects described for the

192   regions A, C, and D in *D. melanogaster* (STURTEVANT 1931; STURTEVANT AND BEADLE 1936;

193   GRELL 1962; MILLER *et al.* 2016b)

194   The interference model also allows quantitative prediction of the magnitude and spatial

195   extent of recombination suppression for any inversion, and is graphically illustrated for a

196   hypothetical case in figure 2. The predictions are generated by using the MCPEEK AND SPEED

197   (1995) stationary renewal model of interference, where probability density of interarrival

198   distances are gamma distributed with shape parameter $\gamma = 5$ and intensity parameter $\mu = 2\gamma d =$

199   *10*, and where d is the cumulative interval distances. This intensity parameter reflects the

200   expected number of crossovers for the whole chromosome, $d = 1$ for a chromosome

201   approximately 1 Morgan in length, such that it encompasses the absolute suppression of the

202   inverted region and any intrachromosomal effects. To adapt this model to the present study of

203   recombination suppression, heterozygous inversion breakpoints were treated as chiasmata and

204   suppression effects were allowed to extend across the centromere. With these assumptions the

205   predicted recombination fraction in inversion heterozygotes is equal to the area under the curve

206   for the coincidence function (equation 1, figure 2).

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207   The present study characterizes the recombination suppression effects of

208   heterokaryotypes for four different cosmopolitan paracentric inversions of Muller element E in

209   *D. melanogaster*. Formal genetic data is presented on recombination fractions for two adjacent

210   chromosomal intervals flanked by dominant phenotypic markers. The balanced design of

211   experiments incorporating both markers and inversions in all possible *cis* and *trans* arrangements

212   allows the simultaneous estimation of viability effects and the strength of recombination

213   suppression (BAILEY 1961). This is a critical aspect of experimental design as both viability and

214   suppression effects in each of the five gene arrangements differed in statistical significance. A χ^2

215   goodness-of-fit test was applied to the viability-corrected recombination fractions to evaluate the

216   interference hypothesis. Viability-corrected counts of crossover events did not fit the

217   expectations from the interference model, with the counterintuitive result that the decay of

218   recombination suppression is faster for inversions closer to the centromere (*i.e.* recombination

219   rate are *higher*, not lower, than expected near the centromere). The description of position

220   dependence of interference effects identifies the necessary theoretical developments and

221   experimental controls for future tests of the interference hypothesis of recombination

222   suppression. Finally, a simple extension of the current experimental system to conduct genetic

223   screens for those rare crossover events necessary for fitting the suppression decay function is

224   described.

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226   MATERIALS AND METHODS

227   **Stock Construction:** Inbred lines carrying the standard arrangement (*St*) and cosmopolitan

228   inversions *In(3R)C*, *In(3R)K*, *In(3R)Mo*, and *In(3R)P* were drawn from the *Drosophila*

229   *melanogaster* Genetic Reference Panel (DGRP) (table 1) (figure 3). Inversions were identified

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230   by polytene chromosome squashes of third instar larva salivary glands and confirmed with PCR

231   amplification of inversion breakpoints where possible. The inversion *In(3R)C* was found in a

232   single DGRP stock only (*DGRP 907*). In this stock *In(3R)C* was maintained in the heterozygous

233   state (despite 20 generation of full sib mating) due to recessive viability and fertility effects

234   segregating in the line (KOURY Unpublished). All other inversions were selected from lines free

235   of heterogenic regions, residual heterozygosity, or major fitness defects (LANGLEY *et al.* 2012;

236   MACKAY *et al.* 2012; KOURY Unpublished).

237   Focal chromosomes were isolated by balancer chromosome assisted extraction and

238   placed on a common, standard arrangement genetic background for the X, Y, mitochondrial, and

239   second chromosome. The non-recombining heterochromatin-rich fourth chromosome underwent

240   repeated backcrossing to the standard genetic background giving a >75% chance of full

241   background replacement, a probability that increases with each generation subsequent to stock

242   formation. The necessary crossing scheme required introduction of balancers and a marked

243   translocation into the standard genetic background (*DGRP 399*) and is illustrated in supplemental

244   figure 1. This procedure was repeated for all five third chromosome gene arrangements.

245   Three dominant phenotypic markers (*Gl^1*, *Sb^1*, and *Dr^1*) were selected for this study

246   because of their relative position to inversion breakpoints (figure 3) (Locus *Gl* has recently been

247   renamed *DCTN1- p150* in Flybase). Notably, the three mutations causing dominant phenotypic

248   effects are also recessive lethals. These mutations were recombined onto standard and inverted

249   arrangements followed by repeated backcrossing for a minimum of ten generations after

250   confirmation of inversion presence by chromosome squash and PCR. Two exceptions were

251   made, *Dr^1* on *In(3R)Mo* and *Sb^1* on *In(3R)P*, because the marker loci were experimentally

252   determined to exist in a region of near complete recombination suppression, as >10,000 meioses

253   failed to produce desired recombinants (Koury unpublished).

254   All three dominant markers (Gl^1, Sb^1, and Dr^1) were also introgressed into the common

255   tester stock *Canton-S* (Bloomington Drosophila Stock Center #1) which has the standard

256   arrangement on all chromosome arms. Sometime prior to 2011, *Canton-S* strain was

257   contaminated at the Bloomington Drosophila Stock Center with a y^-w^- transgene that is also

258   present in this study. *Canton-S* contributes equally to all experimental crosses and thus any

259   defects, if present, cannot explain variation observed among these experiments. Finally, the

260   isogenic stock *w*; 6326; 6326* was used for outcrossing the F_1 experimental females. A full list

261   of stocks used in experiments is provided in supplemental table 1.

262

263   **Crossing Design:** To generate F_1 experimental genotypes, three virgin females of genotype

264   *Canton-S* were crossed to three males homozygous for a given gene arrangement *Standard*,

265   *In(3R)C*, *In(3R)Mo*, *In(3R)P*, or *In(3R)K*, and hereafter collectively referred to as *In(3R)x*.

266   Desired F_1 experimental genotypes were selected based on the presence of dominant phenotypic

267   markers and outcrossed to male *w*; 6326; 6326*. The progeny of this cross (F_2) were scored for

268   recombination *via* dominant markers and non-disjunction *via* white eyed patroclinous exceptions

269   (figure 4a).

270   A balanced design was employed to estimate genetic distances and simultaneously

271   control for viability effects of dominant phenotypic markers, as well as direct fitness effects of

272   the genetic backgrounds and inversions themselves. This balanced design was achieved by

273   conducting both "marker switching" and "*cis-trans*" recombination experiments with all possible

274   marker-inversion combinations on a common genetic background. Four different crosses were

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275   performed to generate F_1 females with all markers and inversions in a full factorial array. In the

276   P generation, virgin *Canton-S* females with marker genotypes $Sb^+ Dr^+$, $Sb^1 Dr^+$, $Sb^+ Dr^1$, or Sb^1

277   Dr^1 were mated with males homokaryotypic for one of the five gene arrangements carrying

278   either $Sb^1 Dr^1$, $Sb^+ Dr^1$, $Sb^1 Dr^+$, or $Sb^+ Dr^+$, respectively. Thus, the females selected from the

279   progeny as the F_1 experimental genotypes carried Dr^1, Sb^1, and $In(3R)x$, all in heterozygous state

280   and in all possible linkage arrays on a common genetic background (figure 4B). For each gene

281   arrangement a second experiment was conducted independently following the same methods, but

282   using Gl^1, Sb^1, and $In(3R)x$ in all possible combinations.

283

284   **Experimental Conditions:** Experimental conditions follow the standard methods for mapping

285   established by BRIDGES AND BREHME (1944). To generate the F_1 experimental genotype three

286   virgin females with the background *Canton-S* were crossed to three males for a given gene

287   arrangement $In(3R)x$. Virgins of the desired F_1 genotype were collected over a three day period,

288   aged an additional three days, then outcrossed to isogenic stock *6326*, which had the standard

289   arrangement on all chromosome arms and the X-linked mutation w^{1118}. Five F_1 experimental

290   genotype virgin females were crossed with five *6326* males using light CO_2 anesthesia, and after

291   allowing 24 hours for recovery the mated group of ten individuals were tap transferred into half

292   pint bottles with 30-40 ml of standard cornmeal-agar *Drosophila* food. Three replicate bottles

293   were set for each cross. After five days of egg laying the F_1 adults were removed from bottles. A

294   2.5 inch x 2.5 inch blotting paper square was added to provide ample pupation sites with 0.05%

295   v/v propionic acid added as needed to hydrate food. Emerging progeny (F_2) were then scored

296   daily for recombination (*via* dominant markers) and non-disjunction (*via* white-eyed patroclinous

297   exceptions) for 15 days after the last eggs were laid (20 days after first eggs were laid). All vials

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298   and bottles were held at 25° C, greater than 50% relative humidity, under 24 hour light in a

299   Percival incubator.

300

301   **Statistical Analysis:** The crossing scheme outlined above yields a 2 x 2 x 2 full factorial design

302   with respect to markers and inversions. With knowledge of the initial *P* genotypes, the frequency

303   of each progeny phenotypic class can be further decomposed into the effect of recombination in

304   addition to the viability effects of the dominant phenotypic markers and genetic background (*i.e.,*

305   2 x 2 x 2 x 2 full factorial design). The angular transformation $\left(\theta = \sin^{-1}\sqrt{p}\right)$ was applied to

306   the observed frequency of each phenotypic class to fit ANOVA's error term normality

307   assumption (SOKAL AND ROHLF 1995). Data for each arrangement and was independently fit to

308   the following model:

309

310    $y_{ijklm} = \mu + r_i + Dr_j + Sb_k + Arr_l + (DrSb)_{jk} + (DrArr)_{jl} + (SbArr)_{kl} + (DrSbArr)_{jkl} + \epsilon_{ijklm}$

311   equation 2A

312

313   Where y_{ijklm} is the angular transformed frequency of a phenotypic class, r_i denotes whether the

314   class is recombinant or nonrecombinant, $Dr_j + Sb_k + Arr_l$ are effects of markers Dr^1, Sb^1 and

315   *In(3R)x* for that phenotypic class. The analogous model for the *Gl - Sb* interval is:

316

317    $y_{ijklm} = \mu + r_i + Sb_j + Gl_k + Arr_l + (SbGl)_{jk} + (GlArr)_{kl} + (ArrSb)_{lm} + (SbGlArr)_{jkl} + \epsilon_{ijklm}$

318   equation 2B

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320   Thus for each of the 102 bottles in this study there were four data points each corresponding to a

321   count for a unique phenotypic class. Each count was converted into a proportion and arcsine

322   square root transformed for an analysis of variance corresponding to the linear model described

323   above.

324   Effects of markers and gene arrangements were reported as a proportional viability of

325   wildtype or the standard gene arrangement of *Canton-S*. Using the angular back-transformation

326    $(p = (\sin\theta)^2)$, mean (μ), and fixed effects estimates (*e.g., Dr*) the proportional viability of

327   phenotypic marker Dr^1 would be:

328

329    $v_{Dr} = \frac{(\sin(\mu - Dr))^2}{(\sin(\mu + Dr))^2}$ equation 3

330

331   Similarly, the viability-corrected recombination fractions were calculated by estimating the

332   effect of recombinant class from respective ANOVAs, back-transforming, and then pooling

333   across both recombinant classes.

334

335    $rf_{corrected} = 2 * (\sin(\mu - r))^2$ equation 4

336

337   In the results sections both the statistically inferred viability corrected recombination fraction and

338   the raw per bottle recombination fractions (+/- 95% confidence intervals) were shown to

339   illustrate the low repeatability, large uncertainty, and importance of a balanced design that allows

340   statistical correction in recombination experiment.

341   Finally, one-fourth of X chromosome nondisjunction events in the F_1 experimental

342   genotypes are detectable as male white-eyed patroclinous exceptions. The rate of nondisjunction

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343   was estimated as four times the number white-eyed males observed per meiosis. Non-disjunction

344   for all arrangements were analyzed jointly after angular transformation with the linear model

345

346   $y_{ij} = \mu + Arr_i + \epsilon_{ij}$ equation 5

347

348   **Testing the Interference Hypothesis:** Under the interference hypothesis the recombination

349   intensity will be an increasing function of distance to inversion breakpoint, representing the

350   decay of recombination suppression with increasing genetic distance. This function takes the

351   value zero at the inversion breakpoint indicating fully suppressed recombination, and will

352   increase with a limit of one (wildtype recombination intensity *i.e.* no suppression) in regions

353   most distant from inversion breakpoints. The form of this function and the instantaneous

354   recombination intensity for every intermediate distance is predicted by the coincidence function

355   based on the gamma model of MCPEEK AND SPEED (1995) with *D. melanogaster*-specific

356   parameterization ($\gamma = 5$, $\mu = 10$). The expected recombination fraction is calculated by

357   integration of this function for the intervals *Gl – Sb*, proximal breakpoint *In(3R)x - Sb*, and distal

358   breakpoint *In(3R)x – Dr*. Equations 6A,B give the exact expression for predicted recombination

359   fractions, which are also illustrated in figure 6 and are listed in table 2.

361   $$E[rf_{Gl-Sb}] = \int_{Sb}^{Gl} \frac{5}{10} \sum_{n=1}^{\infty} \frac{1}{\Gamma(5n)} 10^{5n} z^{5n-1} e^{-10z}$$

360   equation 6A

363   $$E[rf_{Sb-Dr}] = \int_{Sb}^{Prox\ BP} \frac{5}{10} \sum_{n=1}^{\infty} \frac{1}{\Gamma(5n)} 10^{5n} z^{5n-1} e^{-10z} + \int_{Dist\ BP}^{Dr} \frac{5}{10} \sum_{n=1}^{\infty} \frac{1}{\Gamma(5n)} 10^{5n} z^{5n-1} e^{-10z}$$

362   equation 6B

364

365   The interference hypothesis was tested with a χ^2 goodness-of-fit test by comparing the observed

366   count of crossover events corrected for viability effects and the theoretical expectations based on

367   recombination fractions adjusted to sample sizes of the corresponding experiments.

368   All stocks constructed for these experiments are listed in supplemental table 1 and are

369   available upon request. The author affirms that all data necessary for confirming the conclusions

370   of the article are present within the article, figures, and tables. Full ANOVA tables for every

371   experiment conducted are included as supplemental tables 2-7, and raw count data are available

372   upon request.

373

374   RESULTS

375   The full experiment scored the product of 44,230 meioses for crossing-over in two

376   intervals for five gene arrangements, consisting of 34 parental crosses and 102 experimental F_1

377   bottles. Each gene arrangement *St*, *In(3R)C*, *In(3R)K*, *In(3R)Mo*, and *In(3R)P* was analyzed

378   separately. Full ANOVA tables for each of the ten experiments (two intervals for five gene

379   arrangements) are included in the supplemental material (supplemental tables 2-6), table 3 in the

380   main text provides a summary of viability main effects, their statistical significance, and the

381   corresponding viability corrected recombination fraction. Under the described experimental

382   conditions the viability-corrected recombination fraction for standard arrangement

383   homokaryotypes interval *Sb - Dr* was 0.413 and for the interval *Gl – Sb* was 0.186, both of which

384   are consistent with the standard FlyBase genetic map and COMERON *et al.* (2012) estimates of the

385   genetic distances (table 3, supplemental table 2). This result validates the experimental

386   methodology, choice of phenotypic markers, and construction of genetic stocks. Furthermore, it

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387   justifies the use of the cytological band-genetic map conversion based on COMERON *et al.* (2012)

388   to generate the expected recombination fraction between inversion breakpoints and phenotypic

389   markers.

390   The rare cosmopolitan inversion *In(3R)K* is the most proximally placed inversion studied

391   and spans 39% of third chromosome standard genetic map. For the interval *Sb - Dr*, all 15

392   observed crossovers are assumed to occur distal to the inversion due to *Sb[1]*'s position internal to

393   inverted region. For the interval *Sb - Dr*, accounting for viability effects, back-transforming, and

394   summing over complementary recombinant classes, the viability corrected recombination

395   fraction was estimated as 0.00152 (supplemental table 3A). Between markers *Gl - Sb* crossing-

396   over was again assumed to be limited to the proximal region due to *Sb[1]*'s placement inside the

397   inversion. Statistically significant viability effects of *Gl[1]*, the genetic background of *In(3R)K*, and

398   the interaction of these terms were detected (supplemental table 3B). The balanced design allows

399   statistical control of these effects and an estimation of viability-corrected recombination fraction

400   of 0.140.

401   Common cosmopolitan inversion *In(3R)P* is a medially placed inversion of 26% of the

402   standard genetic map of the third chromosome. The proximal breakpoint of *In(3R)P* is within 4

403   Mb of *Sb[1]*, disallowing full factorial design (supplemental table 4A). Despite this obstacle, a

404   viability-corrected recombination fraction for interval *Sb - Dr* is estimated as 0.00449 by

405   controlling for the effects of *Dr[1]* and the combined effect of *Sb[1]* and the genetic background of

406   *In(3R)P*. A similar procedure based on the partially confounded design for interval *Gl - Sb*

407   estimated a viability-corrected recombination fraction of 0.113 (supplemental table 4B).

408   The rare cosmopolitan inversion *In(3R)C* covers 28% of the standard genetic map and is

409   a terminal inversion (*i.e.,* the distal breakpoint is at the telomere). Therefore, marker *Dr[1]* is

410   internal to the inversion and all crossing-over is assumed to occur proximal to the inversion. The

411   viability-corrected recombination fraction of 0.00195 for the interval *Sb - Dr* accounts for the

412   statistically significant effects of marker Sb^1 (supplemental table 5A). The estimate of viability-

413   corrected recombination fraction for region *Gl - Sb* was 0.094 after controlling for statistically

414   significant effects of Gl^1, Sb^1, and the interaction of Gl^1 and genetic background associated with

415   *In(3R)C* (supplemental table 5B).

416   The smallest and most distally positioned inversion in this study, rare cosmopolitan

417   *In(3R)Mo*, is a subtelomeric rearrangement of 21% of the third chromosome standard genetic

418   map. The distal breakpoint of *In(3R)Mo* is within 5 Mb of marker Dr^1, disallowing a full

419   factorial analysis of interval *Sb - Dr* but not affecting the design of experiments for interval *Gl -*

420   *Sb*. The viability-corrected recombination fraction for region between Sb^1 and the proximal

421   breakpoint of *In(3R)Mo* is 0.00871 (supplemental table 6A). The viability-corrected fraction for

422   interval *Gl - Sb* of 0.110 controls for the statistically significant effects of Gl^1, Sb^1, and $Gl^1 * Sb^1$

423   (supplemental table 6B)

424   Analysis of variance for angular transformed rate of non-disjunction revealed no

425   detectable effect of arrangement ($F_{4,97} = 0.537$, $p = 0.709$) (supplemental table 7). Non-

426   disjunction rates were on average slightly higher, although without statistical significance, for the

427   standard arrangement homokaryotypes than for chromosomal inversion heterokaryotypes.

428   A summary of the estimated viability effects and the viability-corrected recombination

429   fractions from all ten experiment are provided in table 3. These are calculated as the fixed effect

430   of marker mutations or inverted gene arrangements extracted from the respective ANOVAs,

431   back-transformed, and then expressed as a proportional reduction in viability assuming wildtype

432   for markers and standard gene arrangement have no viability defects. Inspection of table 3

433   reveals viability reductions are common (19 of 27), often with statistical significance (10 of 27),

434   but not of uniform magnitude across experiments. Thus, table 3 highlights the importance of

435   correcting for these viability effects in each given experiment estimating recombination rates, the

436   variability due to the linkage array of viability factors for a given cross can be visualize with

437   figure 7C and 7D introduced below. In contrast to the viability effects, the sufficiency of the

438   experimental design and sample size is confirmed by the uniform finding of high statistical

439   significance ($p \ll 0.001$) for the effect of recombinant versus non-recombinant classes in all

440   experiments (supplemental tables 2-6). To account for viability effects of markers and

441   backgrounds, a corrected recombination rate was estimated by back-transforming the estimated

442   effect of recombinant class from the respective ANOVAs and summing across the two

443   complementary recombinant classes. These corrected recombination rates are reported in the

444   final column of table 3.

445   Table 4 provides a summary of the goodness-of-fit test including expectations,

446   observations, and viability-corrected estimates of crossover events for experiments on all four

447   cosmopolitan inversions. The predictions of the interference hypothesis were rejected ($\chi^2 =$

448   14621.41, $p \ll 0.001$). As illustrated in figure 7A and 7B, after viability correction the

449   interference hypothesis under-predicts recombination suppression (*i.e.,* observed recombination

450   is less than expected) in both intervals for the two distal inversions *In(3R)C* and *In(3R)Mo*. In

451   contrast, recombination suppression was severely over-predicted (*i.e.,* observed recombination is

452   much greater than expected) for the two proximal inversions *In(3R)K* and *In(3R)P*.

453   Approximately 50% of all observed deviation from expectations is attributable to high rates of

454   crossing-over in the centromeric region of the most proximally located inversion *In(3R)K*. The

455   rejection of the interference model due to poor fit in centromere adjacent regions for *In(3R)K* and

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456   *In(3R)P* is particularly noticeable upon inspection of the 95% confidence intervals for the

457   recombination fractions calculated on a per experimental bottle basis (figure 7C and 7D).

458

459   DISCUSSION

460   Perhaps the least studied effect of chromosomal inversion heterozygosity is the reduction

461   in crossing-over for regions external to inversion breakpoints but still on the same chromosome

462   arm (figure 1A region C). Recombination suppression in this region is enigmatic as it is *not* due

463   to selective elimination of acentric or dicentric chromosomes *nor* is it due to failed pairing,

464   synapsis, or formation of double strand breaks. In the broadest sense, recombination suppression

465   in this region is an interference effect simply because there is an altered probability of crossing-

466   over conditional on structural heterozygosity elsewhere in the genome. Based on cytological

467   analysis, GONG *et al.* (2005) make the more explicit hypothesis that inversion breakpoints

468   possess chiasma-like properties and exert *crossover* interference. Qualitatively, the interference

469   hypothesis is consistent with the general crossover modifying effects observed both internal and

470   external to inverted regions as well as intrachromosomal effects from a wide range of

471   experimental designs and model systems. However, the precise quantitative predictions of this

472   hypothesis systematically fail (*i.e.* consistently under-predicting recombination suppression for

473   distal inversions and consistently over-predicting recombination suppression for proximal

474   inversions) to describe the formal genetic data in the present study.

475

476   **Modelling Recombination Suppression:** The interference hypothesis is a major development in

477   the study of recombination suppression for inversion heterozygotes because it is the *only*

478   hypothesis to make explicit predictions for the magnitude and extent of recombination

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479   suppression external to inverted regions. This is largely based on the pre-existing theoretical

480   treatment of crossover interference, therefore, critical assessment of the current modeling of

481   interference is warranted. There are three possible reasons for the observed deviation from

482   expectations under the interference hypothesis:

483   1) Incorrect model of interference,

484   2) Incorrect parameterization of model, or

485   3) Missing effects in the interference model.

486   As previously mentioned, recombination suppression external to the inverted region is by

487   definition a form of interference, however, there is no necessary reason the form of this effect

488   must be the same as that for crossover interference. MCPEEK AND SPEED (1995) evaluate six

489   alternative models for interference, and although they conclude the gamma model used in the

490   present study is the best mathematical description of crossover interference, it is possible other

491   models may capture the process of recombination suppression more accurately.

492   Importantly, the form and parameterization of the interference model used in this study

493   was a convergence of several different modeling efforts with different motivations and

494   assumptions (FOSS *et al.* 1993; MCPEEK AND SPEED 1995; ZHAO *et al.* 1995). In addition to *D.*

495   *melanogaster*, the counting model of FOSS *et al.* (1993) has successfully described interference

496   in *Neurosproa crassa*, *Sacchromyces cerevisiae*, *Arabidopsis thaliana*, and *Homo sapiens* model

497   systems (COPENHAVER *et al.* 2002; HOUSWORTH AND STAHL 2003; STAHL *et al.* 2004;

498   BERCHOWITZ AND COPENHAVER 2010, but see FOSS AND STAHL 1995). The *D. melanogaster*

499   specific parameterization used in this study is derived independently from two gold standard

500   multi-locus recombination datasets (WEINSTEIN 1936; MORGAN *et al.* 1938). Notably, the shape

501   parameter inferred for the counting model is in agreement with maximum likelihood estimated

502   from chi-square and gamma models (FOSS *et al.* 1993; MCPEEK AND SPEED 1995; ZHAO *et al.*

503   1995). Thus, while theoretical developments may improve models and parameterization of

504   crossover interference (*e.g.*, the beam-film model of KLECKNER *et al.* 2004; ZHANG *et al.* 2014),

505   the explicit predictions of the gamma model of MCPEEK AND SPEED (1995) are the appropriate

506   null model for the experimentalist.

507   Evidence for the third possibility, missing effects in the interference model, can be found

508   in patterns of systematic deviations from expectations. The current interference model over-

509   predicts recombination suppression for *In(3R)K* and *In(3R)P* in both intervals (*i.e.,* observed

510   recombination greater than expected), and under-predicts suppression for *In(3R)C* and *In(3R)Mo*

511   (*i.e.,* observed recombination less than expected). There are two major factors unaccounted for in

512   the current model that likely influence interference effects; inversion size and position of

513   inverted regions.

514   Inversion size would alter conditional probability of crossing-over external to inverted

515   regions only if chiasmata leading to non-crossover gene conversion events in inverted regions

516   exert interference effects, or alternately if there is strong crossover homeostasis. Inversion size

517   does not explain the observed deviations from expectations in these experiments ($r^2 = 0.39$). The

518   relative unimportance of inversion size in the current experiment is consistent with the recent

519   finding that in standard arrangement homokaryotypes non-crossover gene conversion events do

520   not exhibit interference (MILLER *et al.* 2016c). Crossover interference and crossover homeostasis

521   have been proposed to be the result of a common patterning process described in the beam-film

522   model (WANG *et al.* 2015). However, crossover homeostasis has not been demonstrated in *D.*

523   *melanogaster*, and if present is likely to be a weak force in determining the distribution of

524   crossover events (MEHROTRA AND MCKIM 2006).

23

525   Inversion position along a chromosome would alter conditional probability of crossing-

526   over external to inverted regions if there is a centromere effect on interference. This effect is

527   easily envisioned given the well-known centromere effect on the *unconditional* probability of

528   crossing-over per base pair (COMERON *et al.* 2012; MILLER *et al.* 2016c), and the comparative

529   method generalization that distal inversions are stronger suppressors of recombination (KRIMBAS

530   AND POWELL 1992). Models of interference, including the gamma model, often assume the

531   effects of interference are uniform with respect to the genetic map (MCPEEK AND SPEED 1995).

532   This is the same as assuming either no centromere effect on conditional probabilities scaled to

533   genetic distances, or alternately, centromere effects on conditional and unconditional

534   probabilities are proportional when scaled to physical distance. The observation that

535   recombination suppression is over-predicted for the two proximally placed inversions while

536   under-predicted for the two distally placed inversions, with ~50% of total deviations being

537   attributed to the inversion with breakpoints closest to the centromere, strongly suggests the

538   uniform interference assumption is not valid. Furthermore, under-prediction of recombination

539   suppression by the gamma model for proximal inversions would be expected if conditional

540   probabilities are uniform in physical distance (as in the beam-film model) while unconditional

541   probability of crossing-over per base pair decreases at the centromere. Mathematical evaluation

542   of the hypothesis that crossover interference (conditional probability) scales according to

543   physical distance rather than genetic distance at the centromere is straightforward, but requires

544   higher resolution data than the formal genetic data for two large interval in the present paper.

545   Confounding the positional effect based on proximity to centromeres is the potential for

546   discontinuities in the decay function introduced by "boundary sites" (SHERIZEN *et al.* 2005).

547   Formerly thought to be required pairing sites, these chromosomal regions mark a boundary for

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548   the recombination suppression effects of translocation heterozygotes used to map their position

549   (ROBERTS 1970; ROBERTS 1972; HAWLEY 1980; SHERIZEN *et al.* 2005). All four cosmopolitan

550   inversion's proximal breakpoints lie between the 85A-C and 91A-93D boundary sites and all

551   distal breakpoints lie beyond the last confirmed boundary sites for Muller element E (SHERIZEN

552   *et al.* 2005). However, the effect of relative proximity of inversion breakpoints to boundary sites

553   within these intervals is unknown. The rough equivalency of observed crossing-over between *Gl*

554   - *Sb* for all four cosmopolitan inversions (figure 7B) could be due to the elimination of

555   interference effects by the 85A-C boundary site centrally placed in this interval. Assuming

556   boundary sites completely inhibit the propagation of interference effects would account 75% of

557   the deviation observed for interval *Gl - Sb* of proximally placed inversions *In(3R)K* and *In(3R)P*.

558   Boundary sites should not be confused with "sensitive sites" (*sensu* COYNE *et al.* 1993)

559   which were not mapped, but chromosomal regions statistically inferred to have the greatest

560   recombination suppression effect based on fertility reduction in pericentric inversion

561   heterozygotes. The proximal breakpoints of *In(3R)C* and *In(3R)Mo* closely coincide with a

562   sensitive site at cytological division 92, which is also the mapped location of a boundary site

563   (*sensu* SHERIZEN *et al.* 2005). It is, therefore, possible that under-prediction of recombination

564   suppression for the two distal inversions, *In(3R)C* and *In(3R)Mo,* was due to the reduction of the

565   *unconditional* probability of crossing-over for these specific gene arrangements which has not

566   been incorporated into the current predictions. However, under this "sensitive sites"

567   interpretation the over-prediction for distal inversions is unrelated to the under-prediction for the

568   two proximal inversions, which leaves >50% of deviation in these experiments unexplained.

569   Although this discussion highlight a number of paths forward, higher resolution data for a wider

570   range of chromosomal inversions is needed to describe even the most basic patterns of

571   recombination suppression.

572

573   **Future Directions:** As stated earlier, both crossover interference and recombination suppression

574   by structural heterozygosity are well-known but poorly understood phenomena. The interference

575   hypothesis for recombination suppression in chromosomal inversion heterozygotes and

576   accompanying quantitative predictions establishes the theoretical framework of a null model for

577   testing positional effects associated with boundary sites and centromeres. However, experimental

578   tests manipulating position of inverted regions while rigorously controlling for breakpoint

579   microstructure and genetic background cannot be achieved with X-ray induced rearrangements

580   or inversions drawn from natural populations. Fortunately, development of the *FLP/FRT*

581   recombination system in *D. melanogaster* can be used to synthesize inversions of desired size

582   and position (GOLIC AND GOLIC 1996). Similarly, advances in whole genome sequencing allows

583   rapid detection of global crossover modifying effects, however, the collection of the all-

584   important rare crossover events in close proximity to inverted regions still presents a logistical

585   problem.

586   A genetic screen for crossover events adjacent to inversion breakpoints can be conducted

587   by simply following same experimental design described in this study where the F_1 virgins with

588   Sb^1 and Dr^1 in *trans*, are outcrossed to a male double balancer line w^{1118}; 6326; TM3, Sb^1 e^1 /

589   *TM6B, Tb^1 e^1 Dr^{Mio}* (supplemental figure 2). Because the dominant markers used for scoring

590   recombination are lethal recessive and the same as the balancer markers, the result is the

591   selective elimination of 50% of all non-recombinant chromosomes. Viable zygotes with the other

592   50% of non-recombinant chromosome are easily selected against as they will all be double

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593   heterozygotes for *Sb* and *Dr* (either *Dr^1 / TM3, Sb^1 e^1* or *Sb^1 / TM6B, Tb^1 e^1 Dr^{Mio}*). In addition to

594   elimination of undesirable meiotic products, the viable recombinant chromosome will be marker

595   free (*Sb$^+$* and *Dr$^+$*) and automatically preserved in a balanced state in the first generation

596   (heterozygous with either *TM3* or *TM6B* balancer chromosomes).

597   In the current study, crossovers between dominant phenotypic markers and inversions

598   occurred at an experimentally tractable rate of 10^{-2} to 10^{-3} per meiosis for *Sb^1* and proximal

599   breakpoints of *In(3R)Mo* or *In(3R)C*. Rates of recombination were similar for *Dr^1* and the distal

600   breakpoints of *In(3R)P* and *In(3R)K*, but recombination rates below 10^{-4} are indistinguishable

601   from absolute suppression of recombination. In all the preparatory and pilot phases of the

602   experiment, crossing-over was never observed between *Dr^1* and distal breakpoints of *In(3R)Mo*

603   or *Sb^1* and the proximal breakpoints of *In(3R)P* out of >10,000 meioses each. Likewise, the

604   double crossover events to introgress *Dr^1* into *In(3R)C* and *Sb^1* into *In(3R)K* were only observed

605   once and only after scoring the product of >10,000 meioses combined. Given the logistics of

606   generating these dominant marker-inversion associations *via* a selective screen the proposed

607   genetic screen represents a >75% reduction in effort by eliminating half of non-recombinants and

608   selecting against a single phenotypic class. Employing this method would allow a definitive

609   mapping of the near complete suppression adjacent to inversion breakpoints (figure 1A region A)

610   and provide a means to collect and preserve ample genetic material to fit the true form of the

611   decay with distance function for suppression external to inverted regions (figure 1A region C).

612

613   **Conclusion:** The discussion highlights a large number of potentially confounding factors,

614   including but not limited to inversion size, inversion position (proximity to boundary sites,

615   sensitive sites, centromeres, and telomeres), breakpoint microstructure, and genetic background.

616   Further complicating matters are the comparative method generalizations based on experiments

617   using heterogeneous live material in terms of genetic backgrounds, inversions, markers, and

618   species. As a result, the magnitude and extent of recombination suppression external to inverted

619   regions is often considered an idiosyncratic behavior of chromosomal inversions, but this lack of

620   understanding is likely due to the absence of mechanistic expectations. The interference

621   hypothesis of recombination suppression for inversions heterozygotes proposed by GONG *et al.*

622   (2005) provides the necessary mechanism to clarify this behavior. Mathematical predictions for

623   this mechanism were generated from a probabilistic model of crossover interference as a

624   stationary renewal point process with gamma distributed interarrival distances. This interference

625   model was tested and rejected based on formal genetic data for crossing-over in two intervals

626   external to inverted regions of four cosmopolitan inversions of *D. melanogaster* Muller element

627   E. Systematic deviation from predictions revealed crossing-over in the centromeric interval of

628   proximally located inversions is much higher than expected. Interestingly, this result suggests the

629   well-known decrease in unconditional probability of crossing-over at centromeres is reversed

630   when considering conditional probabilities. Greater than expected crossing-over in regions near

631   the centromeres when heterozygous for an paracentric inversion can only be detected and

632   evaluated once explicit predictions, such as those derived from the interference hypothesis, are

633   generated for recombination suppression external to inverted regions. Finally, a simple extension

634   of the current experimental system is presented as a genetic screen for the rare crossover events

635   necessary to fit the function describing the decay with distance of recombination suppression

636   external to inverted regions.

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661   Table 1.   Cytological and genetic map location of gene arrangements used in this study.

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Gene Arrangement	Inbred Line	Cytological Location				Genetic Map Location	
		Proximal Breakpoint		Distal Breakpoint		Proximal Breakpoint	Distal Breakpoint
Standard	DGRP 399	---------		---------		---------	---------
In(3R)K	DGRP 105	86F1	- 86F11	96F11	- 96F14	48.8	87.5
In(3R)P	DGRP 786	89C2	- 89C3	96A18	- 96A19	58.7	84.5
In(3R)C	DGRP 907*	92D1	- 92D9	100F2	- 100F3	71.0	99.1
In(3R)Mo	DGRP 555	93D	- 93D	98F2	- 98F3	74.1	94.5

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679   Table 2.   Expected recombination fractions for five alternative third chromosome

680   arrangements based on genetic maps from COMERON *et al.* (2012).

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Gene Arrangement	*Gl-Sb*	*Sb-Dr*
Standard	0.227	0.410
In(3R)K	0.010	0.005
In(3R)P	0.044	0.013
In(3R)C	0.140	0.016
In(3R)Mo	0.164	0.030

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697   Table 3.   Summary table of the proportional viability of markers or gene arrangement, and

698   the corresponding viability corrected recombination fractions for intervals A) *Sb-*

699   *Dr* and B) *Gl-Sb*. Asterisks denote statistical significance, confer tables 3-7.

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701   A

Arrangement	Stubble		Drop	Inversion	Recombination Fraction
Standard	0.935	*	0.954	0.998	0.413
In(3R)K	0.933	*	1.026	0.987	1.52E-03
In(3R)P	------		1.017	0.996	4.49E-03
In(3R)C	0.911	*	0.978	0.950	1.95E-03
In(3R)Mo	0.943		------	0.991	8.71E-03

           B

Arrangement	Glued		Stubble		Inversion		Recombination Fraction
Standard	1.013		0.812	***	0.933		0.186
In(3R)K	0.923		0.904	*	1.178	**	0.140
In(3R)P	0.961		-----		0.932		0.113
In(3R)C	0.888	*	0.883	*	0.995		0.094
In(3R)Mo	1.066	*	0.898	***	0.942		0.110

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709   Table 4.   Summary table for the χ^2 goodness of fit test of the interference hypothesis

710   including observed, expected, and viability corrected number of crossover events

711   per interval. Asterisks denote statistical significance.

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Arrangement	Interval	Sample Size	Expected	Observed	Viability Corrected	Deviation
In(3R)K	*Sb-Dr*	4960	5.56	15	7.55	0.72
In(3R)P	*Sb-Dr*	2389	8.96	17	10.73	0.35
In(3R)C	*Sb-Dr*	8364	43.32	24	16.34	16.80
In(3R)Mo	*Sb-Dr*	2985	35.40	31	25.99	2.50
In(3R)K	*Gl-Sb*	4774	28.73	644	668.36	14242.99
In(3R)P	*Gl-Sb*	1771	77.50	196	200.12	194.03
In(3R)C	*Gl-Sb*	4324	604.07	400	404.73	65.78
In(3R)Mo	*Gl-Sb*	5463	897.93	578	600.93	98.23

$$\chi^2_{[7]} = 14621.41 \; ***$$

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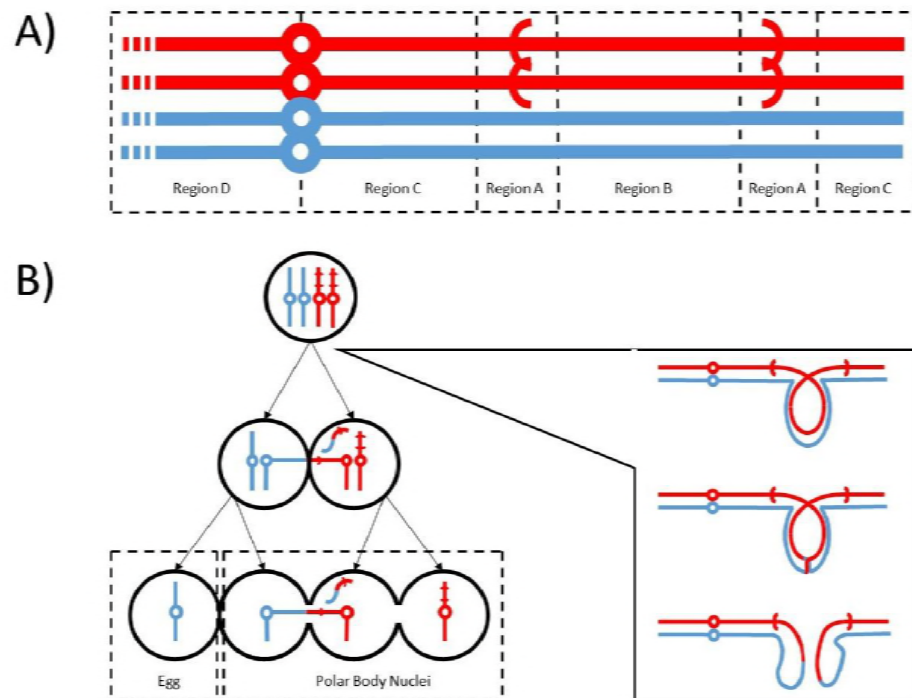
727   Figure 1.   A) Chromosomes of an inversion heterozygote as a four strand bundle

728   highlighting the regions of different recombination suppression effects relative to

729   inversion breakpoints (indicated by parentheses). B) The formation of acentric

730   and dicentric chromosomes as a consequence of crossing-over in the inversion

731   loop, illustrated to the right, and selective elimination of dicentric chromosomes

732   by confinement to internal positions and therefore polar bodies in the linear array

733   of meiotic products.

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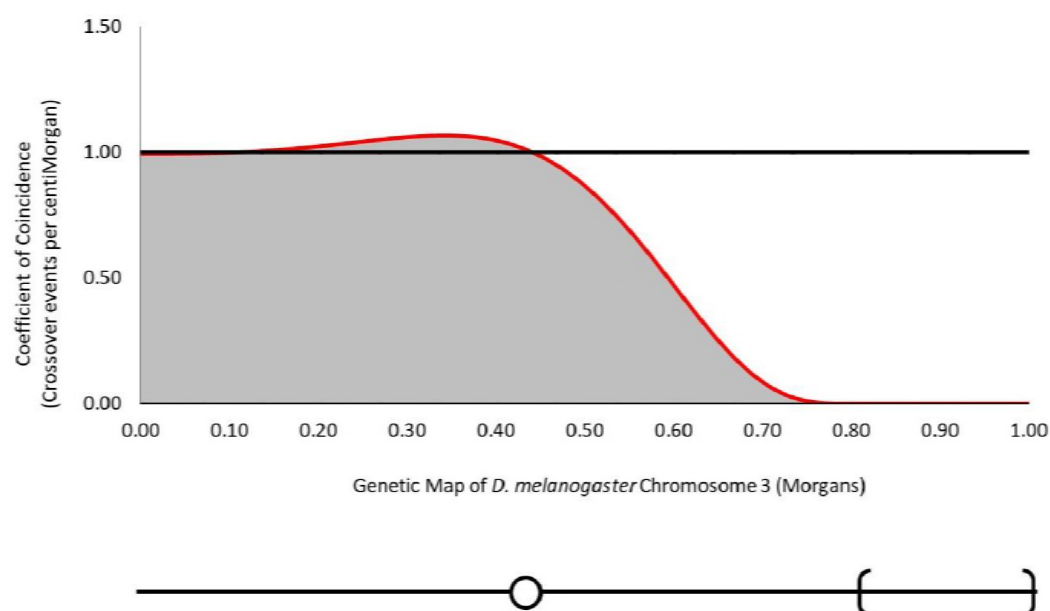
741   Figure 2.   The coincidence function for a hypothetical terminal inversion based on the

742   gamma model of (MCPEEK AND SPEED 1995). The black line represents

743   unconditional probability of crossing-over for homokaryotypes and the red line is

744   the conditional probability under the interference hypothesis. The expected

745   recombination fraction for a heterokaryotype is equal to the gray shaded area

746   under the curve.

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755   Figure 3.   The relative position of phenotypic markers and the four cosmopolitan inversions

756   of *D. melanogaster* Muller element E. The centromere is drawn as a circle and

757   inversion breakpoints are denoted by parentheses. Diagram is to scaled based on

758   the standard genetic map. For cytological and genetic map locations see table 1,

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A)

P ♀♀ $\dfrac{Canton\text{-}S}{Canton\text{-}S}$ $\dfrac{Canton\text{-}S}{Canton\text{-}S}$ $\dfrac{Canton\text{-}S, + Dr^1}{Canton\text{-}S, + +}$ x $\dfrac{DGRP\ 399}{DGRP\ 399}$ $\dfrac{DGRP\ 399}{DGRP\ 399}$ $\dfrac{In(3R)X, Sb^1 +}{In(3R)X, + +}$ ♂♂

F₁ ♀♀ $\dfrac{DGRP\ 399}{Canton\text{-}S}$ $\dfrac{DGRP\ 399}{Canton\text{-}S}$ $\dfrac{In(3R)X, Sb^1 +}{Canton\text{-}S, + Dr^1}$ x $\dfrac{w^{1118}}{}$ $\dfrac{6326}{6326}$ $\dfrac{6326, + +}{6326, + +}$ ♂♂

F₂ $\dfrac{Canton\text{-}S, + +}{6326, + +}$ $\dfrac{Canton\text{-}S, + Dr^1}{6326, + +}$ $\dfrac{In(3R)X, Sb^1 +}{6326, + +}$ $\dfrac{In(3R)X, Sb^1 Dr^1}{6326, + +}$

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B)

F₁ ♀♀ $\dfrac{DGRP\ 399}{Canton\text{-}S}$ $\dfrac{DGRP\ 399}{Canton\text{-}S}$ $\dfrac{In(3R)X, + +}{Canton\text{-}S, Sb^1 Dr^1}$

F₁ ♀♀ $\dfrac{DGRP\ 399}{Canton\text{-}S}$ $\dfrac{DGRP\ 399}{Canton\text{-}S}$ $\dfrac{In(3R)X, Sb^1 +}{Canton\text{-}S, + Dr^1}$

F₁ ♀♀ $\dfrac{DGRP\ 399}{Canton\text{-}S}$ $\dfrac{DGRP\ 399}{Canton\text{-}S}$ $\dfrac{In(3R)X, + Dr^1}{Canton\text{-}S, Sb^1 +}$

F₁ ♀♀ $\dfrac{DGRP\ 399}{Canton\text{-}S}$ $\dfrac{DGRP\ 399}{Canton\text{-}S}$ $\dfrac{In(3R)X, Sb^1 Dr^1}{Canton\text{-}S, + +}$

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769 Figure 4.   A) General crossing scheme of this experiment. B) The four alternative F_1

770   experimental genotypes used to perform both cis-trans and marker switching

771   experiments.

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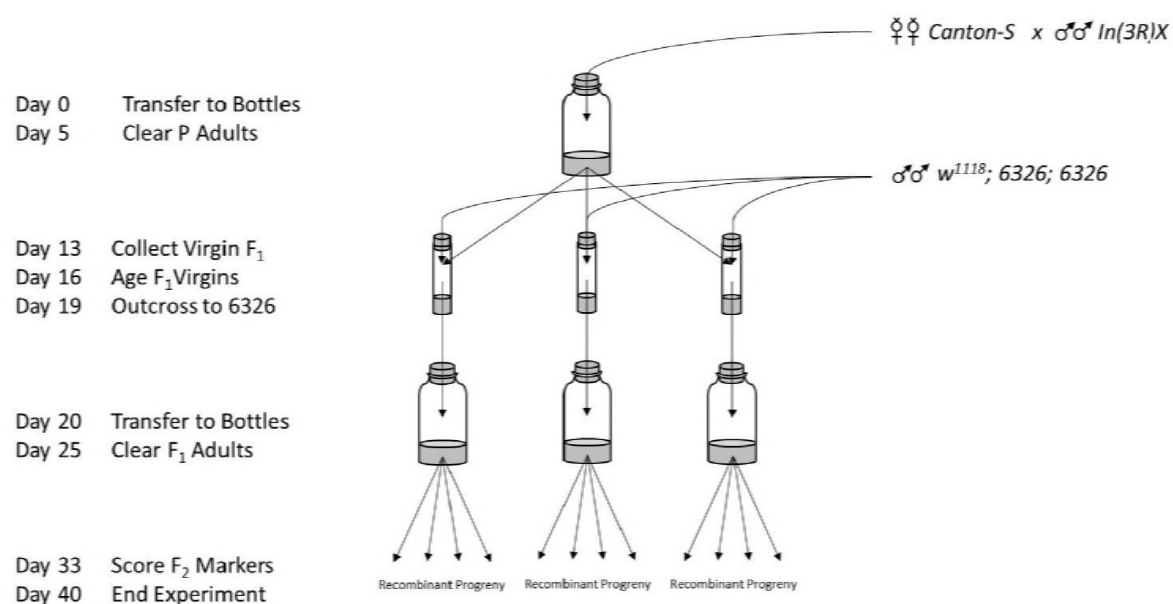
774   Figure 5.   General experimental setup and daily schedule for recombination experiments.

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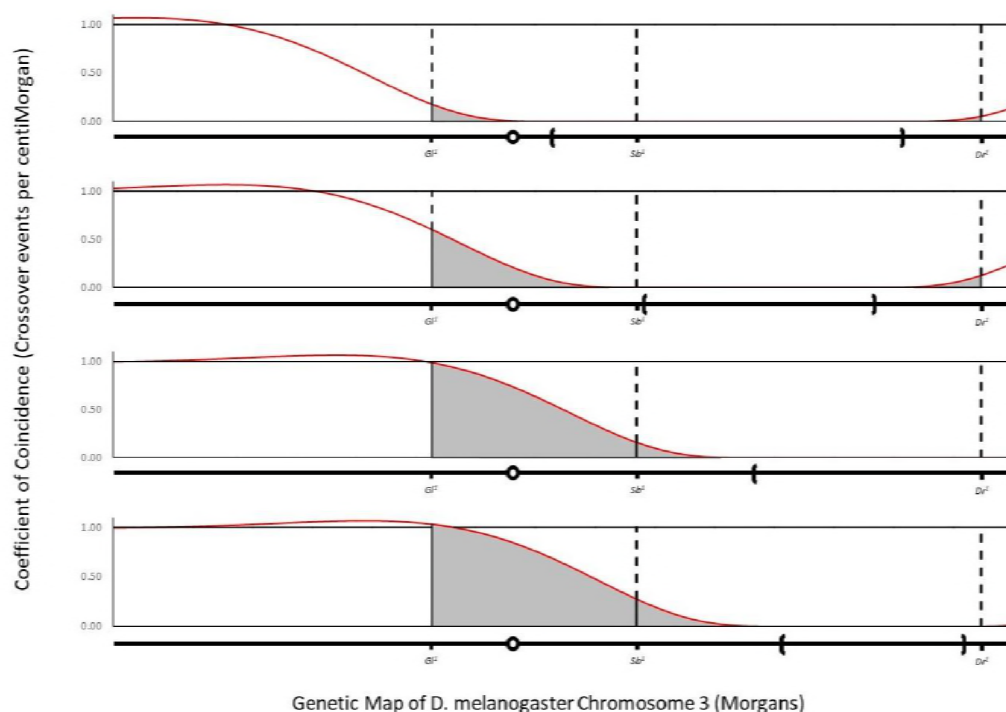
788   Figure 6.   Graphical predictions for the interference hypothesis. Black line is unconditional

789   probability of crossing-over, the red line is the conditional probability for

790   inversion heterozygotes. Expected recombination fraction for a given interval is

791   the gray shaded area under the curve. Position of chromosomal inversions are

792   illustrated below predictions and drawn to scale for the standard genetic map.

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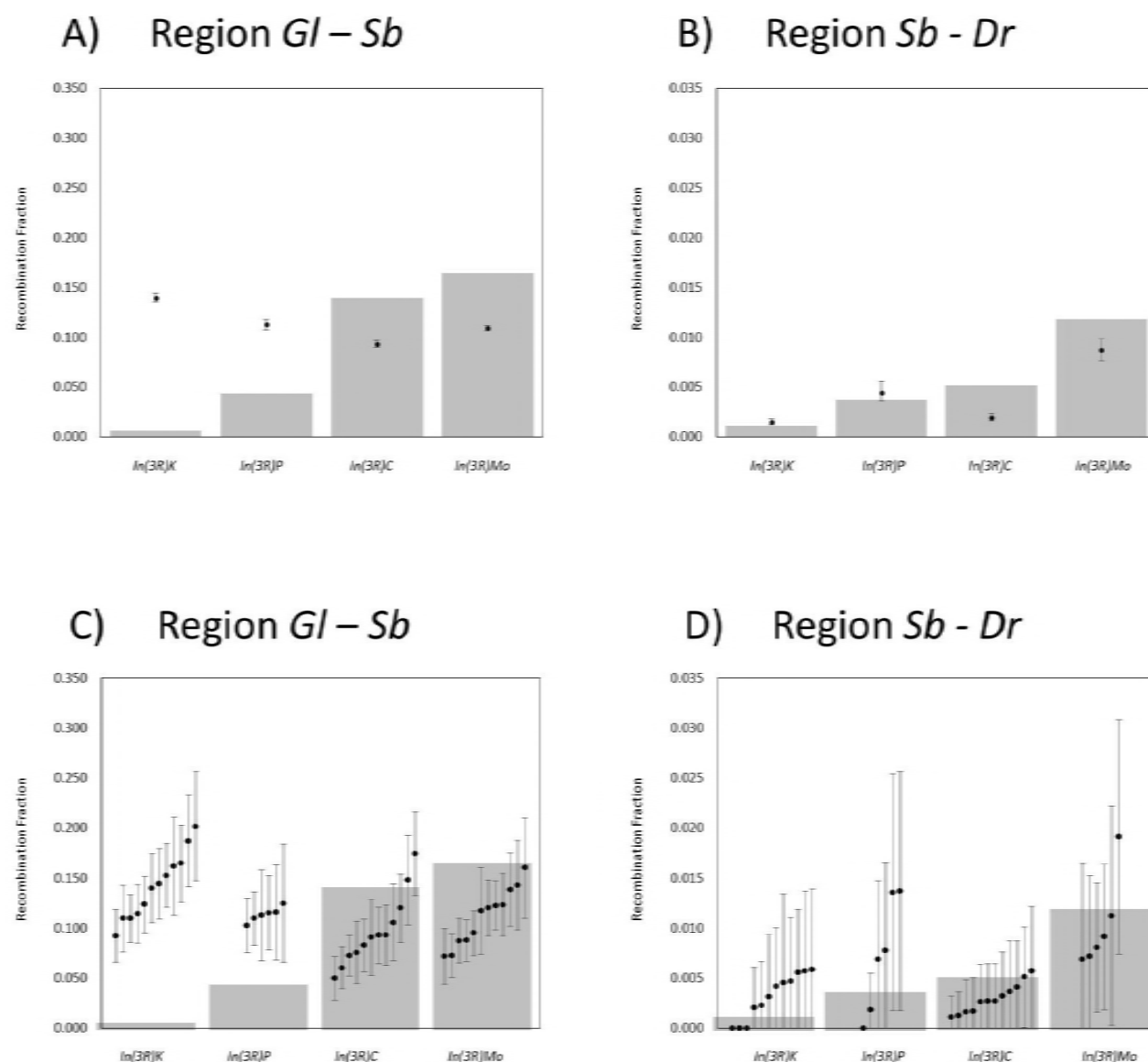
Figure 7.   Graphical illustration of the χ^2 goodness of fit test. Gray bars are expected

recombination fractions under the interference hypothesis (equation 6A,B and

area under the curve figure 6). Black points are viability corrected recombination

fractions +/- one standard error (A and B), and the corresponding raw per bottle

recombination fractions with 95% CI (C and D).

810   LITERATURE CITED

811

812   Bailey, N. T., 1961 *Introduction to the mathematical theory of genetic linkage*. Claredon Press, Oxford.

813

814   Bauer, E., M. Falque, H. Walter, C. Bauland, C. Camisan *et al.*, 2013 Intraspecific variation of

815   recombination rate in maize. Genome Biology 14**:** R103.

816

817   Berchowitz, L. E., and G. P. Copenhaver 2010 Genetic interference: Don't stand so close to me. Current

818   Genomics 11**:** 91-102.

819

820   Bridges, C. B., and K. S. Brehme, 1944 *The mutants of Drosophila melanogaster*. Pub. Carnegie Instn.

821   Washington 552: 257.

822

823   Carson, H. L., 1946 The selective elimination of inversion dicentric chromatids during meiosis in the eggs

824   of *Sciara impatiens*. Genetics 31**:** 95-113.

825

826   Comeron, J. M., R. Ratnappan and S. Bailin, 2012 The many landscapes of recombination in *Drosophila*

827   *melanogaster*. PLOS Genetics 8**:** e1002905.

828

829   Copenhaver, G., E. Housworth and F. Stahl, 2002 Crossover interference in *Arabidopsis*. Genetics 160**:**

830   1631-1639.

831

832   Coyne, J. A., W. Meyers, A. P. Crittenden and P. Sniegowski, 1993 The fertility effects of pericentric

833   inversions in *Drosophila melanogaster*. Genetics 134**:** 487-496.

834

835   Dobzhansky, T., 1931 The decrease of crossing-over observed in translocations, and its probable

836   explanation. The American Naturalist 65**:** 214-232.

837

838   Dobzhansky, T., 1933 Studies on chromosome conjugation. Molecular and General Genetics MGG 64**:**

839   269-309.

840

841   Dobzhansky, T., and A. Sturtevant, 1931 Translocations between the second and third chromosomes of

842   Drosophila and their bearing on *Oenothera* problems. Pub. Carnegie Instn. Washington 421**:** 39-

843   59.

844

845   Foss, E., R. Lande, F. W. Stahl and C. M. Steinberg, 1993 Chiasma interference as a function of genetic

846   distance. Genetics 133**:** 681-691.

847

848   Foss, E. J., and F. W. Stahl, 1995 A test of a counting model for chiasma interference. Genetics 139**:**

849   1201-1209.

850

851   Golic, K. G., and M. M. Golic, 1996 Engineering the *Drosophila* genome: chromosome rearrangements

852   by design. Genetics 144**:** 1693-1711.

853

854   Gong, W. J., K. S. McKim and R. S. Hawley, 2005 All paired up with no place to go: pairing, synapsis,

855   and DSB formation in a balancer heterozygote. PLoS Genet 1**:** e67.

856

857   Grell, R. F., 1962a A new model for secondary nondisjunction: the role of distributive pairing. Genetics

858   47**:** 1737-1754.

859

860   Haldane, J., 1919 The combination of linkage values and the calculation of distances between the loci of

861   linked factors. J Genet 8**:** 299-309.

862

863   Hawley, R. S., 1980 Chromosomal sites necessary for normal levels of meiotic recombination in

864   *Drosophila melanogaster*. I. Evidence for and mapping of the sites. Genetics 94**:** 625-646.

865

866   Hoffmann, A. A., and L. H. Rieseberg, 2008 Revisiting the impact of inversions in evolution: from

867   population genetic markers to drivers of adaptive shifts and speciation? Annual Review of

868   Ecology, Evolution, and Systematics 39**:** 21-42.

869

870   Housworth, E., and F. Stahl, 2003 Crossover interference in humans. The American Journal of Human

871   Genetics 73**:** 188-197.

872

873   Hunter, C. M., W. Huang, T. F. Mackay and N. D. Singh, 2016 The genetic architecture of natural

874   variation in recombination rate in *Drosophila melanogaster*. PLoS Genet 12**:** e1005951.

875

876   Johnston, S. E., C. Bérénos, J. Slate and J. M. Pemberton, 2016 Conserved genetic architecture

877   underlying individual recombination rate variation in a wild population of Soay sheep (*Ovis*

878   *aries*). Genetics 203**:** 583-598.

879

880   Kirkpatrick, M., and N. Barton, 2006 Chromosome inversions, local adaptation and speciation. Genetics

881   173**:** 419-434.

882

883   Kleckner, N., D. Zickler, G. H. Jones, J. Dekker, R. Padmore *et al.*, 2004 A mechanical basis for

884   chromosome function. Proc. Nat. Acad. Sci. 101**:** 12592-12597.

885

886   Kong, A., G. Thorleifsson, M. L. Frigge, G. Masson, D. F. Gudbjartsson *et al.*, 2014 Common and low-

887   frequency variants associated with genome-wide recombination rate. Nat Genet 46**:** 11-16.

888

889   Kosambi, D. D., 1943 The estimation of map distances from recombination values. Annals of Eugenics

890   12**:** 172-175.

891

892   Krimbas, C. B., and J. R. Powell, 1992 Introduction, pp. 1-52 in *Drosophila inversion polymorphism*,

893   edited by C. B. Krimbas and J. R. Powell. CRC Press, Boca Raton, FL.

894

895   Langley, C. H., K. Stevens, C. Cardeno, Y. C. G. Lee, D. R. Schrider *et al.*, 2012 Genomic variation in

896   natural populations of *Drosophila melanogaster*. Genetics**:** 192: 533-598.

897

898   Lucchesi, J. C., and D. T. Suzuki, 1968 The interchromosomal control of recombination. Annual Review

899   of Genetics 2**:** 53-86.

900

901   Mackay, T. F. C., S. Richards, E. A. Stone, A. Barbadilla, J. F. Ayroles *et al.*, 2012 The *Drosophila*

902   *melanogaster* genetic reference panel. Nature 482**:** 173-178.

903

904   Madan, K., M. Seabright, R. Lindenbaum and M. Bobrow, 1984 Paracentric inversions in man. Journal of

905   Medical Genetics 21**:** 407-412.

906

907   McClintock, B., 1941 The stability of broken ends of chromosomes in *Zea mays*. Genetics 26**:** 234-282.

908

909   McPeek, M. S., and T. P. Speed, 1995 Modeling interference in genetic recombination. Genetics 139**:**

910   1031-1044.

911

912   Mehrotra, S., and K. McKim, 2006 Temporal analysis of meiotic DNA double-strand break formation and

913   repair in *Drosophila* females. PLoS Genet 2**:** e200.

914

915   Miller, D. E., K. R. Cook, A. V. Arvanitakis and R. S. Hawley, 2016a Third chromosome balancer

916   inversions disrupt protein-coding genes and influence distal recombination events in *Drosophila*

917   *melanogaster*. G3: Genes| Genomes| Genetics 6**:** 1959-1967.

918

919   Miller, D. E., K. R. Cook, N. Y. Kazemi, C. B. Smith, A. J. Cockrell *et al.*, 2016b Rare recombination

920   events generate sequence diversity among balancer chromosomes in *Drosophila melanogaster*.

921   Proc. Nat. Acad. Sci. 113**:** E1352-E1361.

922

923   Miller, D. E., C. B. Smith, N. Y. Kazemi, A. J. Cockrell, A. V. Arvanitakis *et al.*, 2016c Whole-genome

924   analysis of individual meiotic events in *Drosophila melanogaster* reveals that noncrossover gene

925   conversions are insensitive to interference and the centromere effect. Genetics 203**:** 159-171.

926

927   Morgan, T. H., C. Bridges and J. Schultz, 1938 Constitution of the germinal material in relation to

928   heredity. Pub. Carnegie Instn. Washington 37**:** 304-309.

929

930   Muller, H. J., 1916 The mechanism of crossing-over. The American Naturalist 50**:** 193-221.

931

932   Navarro, A., and A. Ruiz, 1997 On the fertility effects of pericentric inversions. Genetics 147**:** 931-933.

933

934   Novitski, E., and G. Braver, 1954 An analysis of crossing over within a heterozygous inversion in

935   *Drosophila melanogaster*. Genetics 39**:** 197-209.

936

937   Painter, T. S., 1933 A new method for the study of chromosome rearrangements and the plotting of

938   chromosome maps. Science 78**:** 585-586.

939

940   Roberts, P., 1972 Differences in synaptic affinity of chromosome arms of *Drosophila melanogaster*

941   revealed by differential sensitivity to translocation heterozygosity. Genetics 71: 401-415.

942

943   Roberts, P. A., 1970 Screening for x-ray-induced crossover suppressors in *Drosophila melanogaster*:

944   prevalence and effectiveness of translocations. Genetics 65**:** 429-448.

945

946   Schultz, J., and H. Redfield, 1951 Interchromosomal effects on crossing over in *Drosophila*, pp. 175-197

947   in *Cold Spring Harbor symposia on quantitative biology*. Cold Spring Harbor Laboratory Press.

948

949   Sherizen, D., J. K. Jang, R. Bhagat, N. Kato and K. S. McKim, 2005 Meiotic recombination in

950   *Drosophila* females depends on chromosome continuity between genetically defined boundaries.

951   Genetics 169**:** 767-787.

952

953   Sokal, R. R., and F. J. Rohlf, 1995 *Biometry : the principles and practice of statistics in biological*

954   *research*. W. H. Freeman and Company, New York.

955

956   Speed, T. P., 1996 What is a genetic map function?, pp. 65-88 in *Genetic Mapping and DNA sequencing*

957   edited by T. Speed and M. Waterman. Springer, New York.

958

959   Stahl, F. W., H. M. Foss, L. S. Young, R. H. Borts, M. F. Abdullah *et al.*, 2004 Does crossover

960   interference count in *Saccharomyces cerevisiae*? Genetics 168**:** 35-48.

961

962   Stevison, L. S., K. B. Hoehn and M. A. F. Noor, 2011 Effects of inversions on within- and between-

963   species recombination and divergence. Genome Biology and Evolution 3**:** 830-841.

964

965   Sturtevant, A., 1920 Genetic studies on *Drosophila simulans*. I. Introduction. Hybrids with *Drosophila*

966   *melanogaster*. Genetics 5**:** 488-500.

967

968   Sturtevant, A., 1921 A case of rearrangement of genes in *Drosophila*. Proc. Nat. Acad. Sci. 7**:** 235-237.

969

970   Sturtevant, A., 1931 Known and probable inverted sections of the autosomes of *Drosophila*

971   *melanogaster*. Pub. Carnegie Instn 421**:** 1-27.

972

973   Sturtevant, A., and E. Novitski, 1941 The homologies of the chromosome elements in the genus

974   *Drosophila*. Genetics 26**:** 517-541.

975

976   Sturtevant, A. H., 1913 The linear arrangement of six sex-linked factors in *Drosophila*, as shown by their

977   mode of association. Journal of Exper. Zoo. A: Ecol. Genet. and Phys. 14**:** 43-59.

978

979   Sturtevant, A. H., 1915 The behavior of the chromosomes as studied through linkage. Molecular and

980   General Genetics MGG 13**:** 234-287.

981

982   Sturtevant, A. H., 1917 Genetic factors affecting the strength of linkage in *Drosophila*. Proc. Natl. Acad.

983   Sci. 3**:** 555-558.

984

985   Sturtevant, A. H., and G. W. Beadle, 1936 The relations of inversions in the X chromosome of

986   *Drosophila melanogaster* to crossing over and disjunction. Genetics 21**:** 554-604.

987

988   Sturtevant, A. H., and T. Dobzhansky, 1936 Inversions in the third chromosome of wild races of

989   *Drosophila pseudoobscura*, and their use in the study of the history of the species. Proc. Natl.

990   Acad. Sci.22**:** 448-450.

991

992   Sturtevant, A. H., and K. Mather, 1938 The interrelations of inversions, heterosis and recombination. The

993   American Naturalist 72**:** 447-452.

994

995   Wang, S., D. Zickler, N. Kleckner and L. Zhang, 2015 Meiotic crossover patterns: obligatory crossover,

996   interference and homeostasis in a single process. Cell Cycle 14**:** 305-314.

997

998   Weinstein, A., 1936 The theory of multiple-strand crossing over. Genetics 21**:** 155-199.

999

1000   Zhang, L., Z. Liang, J. Hutchinson and N. Kleckner, 2014 Crossover patterning by the beam-film model:

1001   analysis and implications. PLoS Genet 10**:** e1004042.

1002

1003   Zhao, H., T. P. Speed and M. S. McPeek, 1995 Statistical analysis of crossover interference using the chi-

1004   square model. Genetics 139**:** 1045-1056.

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1014   Supplemental Table 1. A full list of stocks used in these experiments.

X chromosome		2nd chromosome		3rd chromosomes		
w^{1118}	;	6326	;	6326, Standard, +	/	6326, Standard, +
399	;	CyO	;	TM3, Sb^1, e^1	/	T(2;3) ap
399	;	399	;	TM3, Sb^1, e^1	/	TM6B, Tb^1, e^1, Dr^{Mio}
399	;	399	;	399, Standard, +	/	399, Standard, +
399	;	399	;	399, Standard, Dr^1	/	TM3, Sb^1, e^1
399	;	399	;	399, Standard, Sb^1	/	TM6B, Tb^1, e^1, Dr^{Mio}
399	;	399	;	399, Standard, Gl^1	/	TM3, Sb^1, e^1
	;		;		/	
399	;	399	;	105, In(3R)K, +	/	105, In(3R)K, +
399	;	399	;	105, In(3R)K Dr^1	/	TM3, Sb^1, e^1
399	;	399	;	105, In(3R)K Sb^1	/	TM6B, Tb^1, e^1, Dr^{Mio}
399	;	399	;	105, In(3R)K Gl^1	/	TM3, Sb^1, e^1
	;		;		/	
399	;	399	;	786, In(3R)P, +	/	786, In(3R)P, +
399	;	399	;	786, In(3R)P Dr^1	/	TM3, Sb^1, e^1
399	;	399	;	786, In(3R)P Gl^1	/	TM3, Sb^1, e^1
399	;	399	;	907, In(3R)C, +	/	907, In(3R)C, +
399	;	399	;	907, In(3R)C Dr^1	/	TM3, Sb^1, e^1
399	;	399	;	907, In(3R)C Sb^1	/	TM6B, Tb^1, e^1, Dr^{Mio}
399	;	399	;	907, In(3R)C Gl^1	/	TM3, Sb^1, e^1
399	;	399	;	555, In(3R)Mo, +	/	555, In(3R)Mo, +
399	;	399	;	555, In(3R)Mo Dr^1	/	TM3, Sb^1, e^1
399	;	399	;	555, In(3R)Mo Sb^1	/	TM6B, Tb^1, e^1, Dr^{Mio}
399	;	399	;	555, In(3R)Mo Gl^1	/	TM3, Sb^1, e^1
Canton-S	;	Canton-S	;	Canton-S, Standard, +	/	Canton-S, Standard, +
Canton-S	;	Canton-S	;	Canton-S, Standard, Dr^1	/	TM3, Sb^1, e^1
Canton-S	;	Canton-S	;	Canton-S, Standard, Sb^1	/	TM6B, Tb^1, e^1, Dr^{Mio}
Canton-S	;	Canton-S	;	Canton-S, Standard, Gl^1	/	TM3, Sb^1, e^1

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1021   Supplemental Table 2.   ANOVA table for standard arrangement recombination

1022   experiments A) for interval *Sb-Dr* and B) for interval *Gl-Sb*.

1023   A

Source of Variation	df	SS	MS	F	p	
Recombination	1	397.480	397.480	197.004	0.000	***
Drop	1	7.307	7.307	3.622	0.064	
Stubble	1	14.794	14.794	7.332	0.010	*
Arrangement	1	0.011	0.011	0.005	0.942	
Dr*Sb	1	0.974	0.974	0.483	0.491	
Dr*Arr	1	0.079	0.079	0.039	0.844	
Sb*Arr	1	0.006	0.006	0.003	0.957	
Dr*Sb*Arr	1	1.055	1.055	0.523	0.474	
Residual Error	39	78.688	2.018			
Total	47	500.394				

B

Source of Variation	df	SS	MS	F	p	
Recombination	1	5701.128	5701.128	1244.567	0.000	***
Glued	1	0.466	0.466	0.102	0.751	
Stubble	1	126.221	126.221	27.554	0.000	***
Arrangement	1	14.321	14.321	3.126	0.085	
Gl*Sb	1	4.240	4.240	0.926	0.342	
Gl*Arr	1	20.506	20.506	4.477	0.041	*
Sb*Arr	1	0.272	0.272	0.059	0.809	
Gl*Sb*Arr	1	0.097	0.097	0.021	0.885	
Residual Error	39	178.652	4.581			
Total	47	6045.903				

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1027 Supplemental Table 3.   ANOVA table for *In(3R)K* recombination experiments A) *Sb-Dr*

1028   and B) *Gl-Sb*.

1029 A

Source of Variation	df	SS	MS	F	p	
Recombination	1	22528.649	22528.649	11077.574	0.000	***
Drop	1	1.155	1.155	0.568	0.456	
Stubble	1	8.715	8.715	4.285	0.045	*
Arrangement	1	0.332	0.332	0.163	0.688	
Dr*Sb	1	3.842	3.842	1.889	0.177	
Dr*Arr	1	1.510	1.510	0.742	0.394	
Sb*Arr	1	0.724	0.724	0.356	0.554	
Dr*Sb*Arr	1	3.309	3.309	1.627	0.210	
Residual Error	39	79.315	2.034			
Total	47	22627.551				

B

Source of Variation	df	SS	MS	F	p	
Recombination	1	7837.628	7837.628	1308.166	0.000	***
Glued	1	17.803	17.803	2.971	0.093	
Stubble	1	28.362	28.362	4.734	0.036	*
Arrangement	1	75.644	75.644	12.626	0.001	**
Gl*Sb	1	1.360	1.360	0.227	0.636	
Gl*Arr	1	46.094	46.094	7.693	0.008	**
Sb*Arr	1	0.172	0.172	0.029	0.866	
Gl*Sb*Arr	1	0.158	0.158	0.026	0.872	
Residual Error	39	233.661	5.991			
Total	47	8240.882				

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1032   Supplemental Table 4.   ANOVA table for *In(3R)P* recombination experiments A) *Sb-Dr*

1033   and B) *Gl-Sb*.

1034   A

Source of Variation	df	SS	MS	F	p	
Recombination	1	10620.987	10620.987	2376.222	0.000	***
Drop	1	0.266	0.266	0.059	0.810	
Stubble	-----	-----	-----	-----	-----	
Arrangement	1	0.013	0.013	0.003	0.958	
Dr*Sb	-----	-----	-----	-----	-----	
Dr*Arr	1	3.752	3.752	0.839	0.371	
Sb*Arr	-----	-----	-----	-----	-----	
Dr*Sb*Arr	-----	-----	-----	-----	-----	
Residual Error	19	84.924	4.470			
Total	23	10709.941				

B

Source of Variation	df	SS	MS	F	p	
Recombination	1	4697.739	4697.739	979.216	0.000	***
Glued	1	2.169	2.169	0.452	0.509	
Stubble	-----	-----	-----	-----	-----	
Arrangement	1	6.805	6.805	1.419	0.248	
Gl*Sb	-----	-----	-----	-----	-----	
Gl*Arr	1	0.927	0.927	0.193	0.665	
Sb*Arr	-----	-----	-----	-----	-----	
Gl*Sb*Arr	-----	-----	-----	-----	-----	
Residual Error	19	91.152	4.797			
Total	23	4798.791				

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1037   Supplemental Table 5.   ANOVA table for *In(3R)C* recombination experiments A) *Sb-Dr*

1038   and B) *Gl-Sb*.

1039   A

Source of Variation	df	SS	MS	F	p	
Recombination	1	22316.274	22316.274	7638.438	0.000	***
Drop	1	0.879	0.879	0.301	0.586	
Stubble	1	15.941	15.941	5.456	0.025	*
Arrangement	1	4.873	4.873	1.668	0.204	
Dr*Sb	1	0.130	0.130	0.044	0.834	
Dr*Arr	1	1.874	1.874	0.641	0.428	
Sb*Arr	1	0.233	0.233	0.080	0.779	
Dr*Sb*Arr	1	2.704	2.704	0.926	0.342	
Residual Error	39	113.941	2.922			
Total	47	22456.849				

B

Source of Variation	df	SS	MS	F	p	
Recombination	1	10584.022	10584.022	1770.025	0.000	***
Glued	1	37.274	37.274	6.234	0.017	*
Stubble	1	40.744	40.744	6.814	0.013	*
Arrangement	1	0.061	0.061	0.010	0.920	
Gl*Sb	1	2.778	2.778	0.465	0.500	
Gl*Arr	1	30.347	30.347	5.075	0.030	*
Sb*Arr	1	7.206	7.206	1.205	0.279	
Gl*Sb*Arr	1	12.099	12.099	2.023	0.163	
Residual Error	39	233.204	5.980			
Total	47	10947.735				

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1043 Supplemental Table 6. ANOVA table for *In(3R)Mo* recombination experiments A) *Sb-Dr*

1044 and B) *Gl-Sb*.

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1046 A

Source of Variation	df	SS	MS	F	p	
Recombination	1	10047.766	10047.766	3818.301	0.000	***
Drop	-----	-----	-----	-----	-----	
Stubble	1	3.475	3.475	1.321	0.265	
Arrangement	1	0.089	0.089	0.034	0.856	
Dr*Sb	-----	-----	-----	-----	-----	
Dr*Arr	-----	-----	-----	-----	-----	
Sb*Arr	1	1.672	1.672	0.635	0.435	
Dr*Sb*Arr	-----	-----	-----	-----	-----	
Residual Error	19	49.998	2.631			
Total	23	10102.999				

B

Source of Variation	df	SS	MS	F	p	
Recombination	1	9580.159	9580.159	3989.876	0.000	***
Glued	1	11.003	11.003	4.582	0.039	*
Stubble	1	31.083	31.083	12.945	0.001	***
Arrangement	1	9.712	9.712	4.045	0.051	
Gl*Sb	1	21.620	21.620	9.004	0.005	**
Gl*Arr	1	0.030	0.030	0.013	0.911	
Sb*Arr	1	2.674	2.674	1.114	0.298	
Gl*Sb*Arr	1	1.419	1.419	0.591	0.447	
Residual Error	39	93.644	2.401			
Total	47	9751.343				

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1049   Supplemental Table 7. ANOVA table for inferred rates of non-disjunction.

Source of Variation	df	SS	MS	F	p
Arrangement	4	3.323	0.831	0.537	0.709
Residual Error	97	150.090	1.547		
Total	101	153.413			

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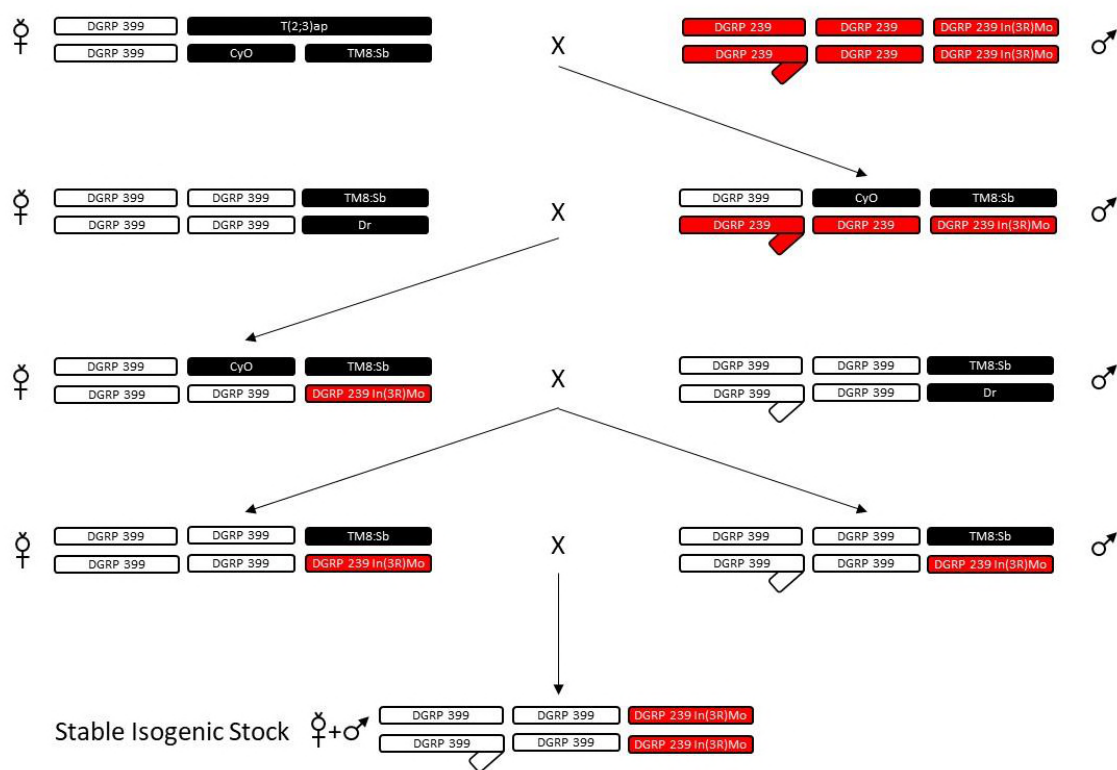
1071   Supplemental Figure 1. General crossing scheme for background replacement.

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♀♀ $\dfrac{Standard, \ + \ Dr}{Standard, \ + \ +}$ x $\dfrac{In(3R)Mo, \ Sb \ +}{In(3R)Mo, \ + \ +}$ ♂♂

♀♀ $\dfrac{In(3R)Mo, \ Sb \ +}{Standard, \ + \ Dr}$ x $\dfrac{TM3, \ Sb}{TM6B, \ Tb, \ Dr}$ ♂♂

$\dfrac{Recombinant}{Balancer}$ $\dfrac{Non\text{-}Recombinant}{Balancer}$ $\dfrac{Non\text{-}Recombinant}{Balancer}$ $\dfrac{Recombinant}{Balancer}$

Viable Recombinant
Selectable single dominant marker

Viable Sb/Dr

Inviable Sb/Sb or Dr/Dr

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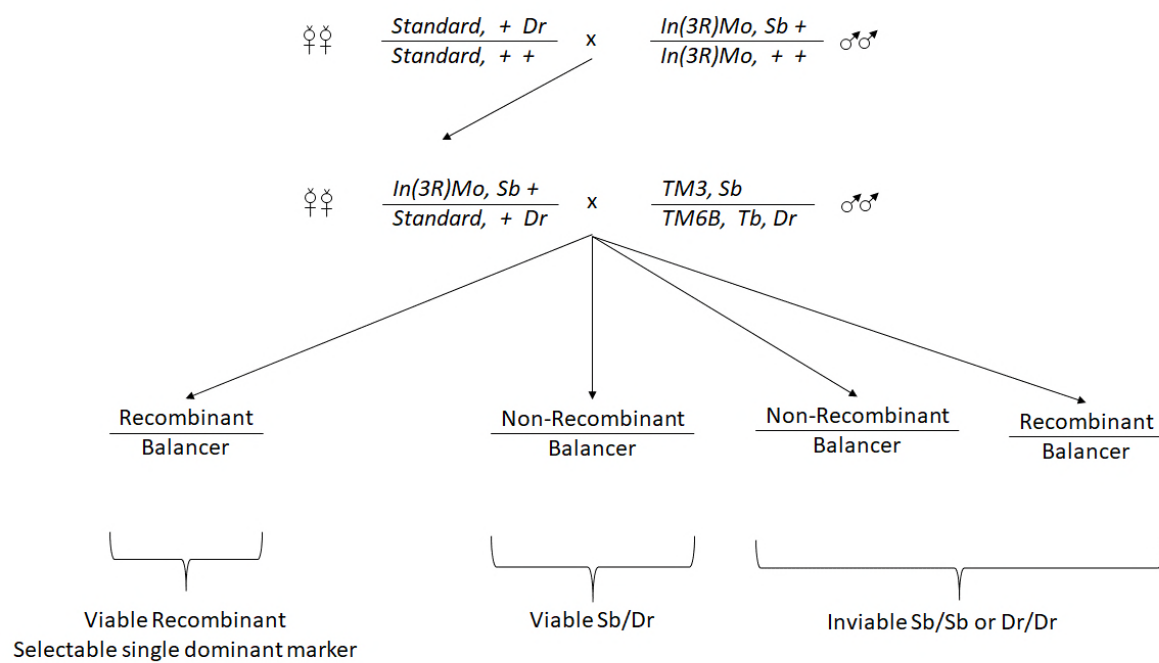
1080   Supplemental Figure 2. Proposed genetic screen for rare crossovers adjacent to inversions.

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