Title: Novitski's Distal Shift in Paracentric Inversion Evolution

Author Name: Spencer A. Koury

Affiliation: Department of Ecology and Evolution, Stony Brook University

Address: 650 Life Sciences Building

100 Nichols Road, Stony Brook, NY 11794

Abstract: In *Drosophila pseudoobscura* younger chromosomal inversions tend to be found distal to older inversions. By examining phylogenetic series of overlapping inversions for 134 gene arrangements of 13 chromosomes this pattern was extended to five additional *Drosophila* species. This distinct pattern arose repeatedly and independently in all six species and likely reflects an underlying principle of chromosome evolution. In this study it is illustrated how transmission of distal inversions is *always* favored in female meiosis when crossing over in homosequential regions of overlapping inversions generates asymmetric dyads. This cytogenetic mechanism for female meiotic drive is described in detail and advanced as an explanation for the distal shift in phylogenetic series of overlapping inversions as well as several better known patterns in the evolution of serially inverted chromosomes.

Keywords: Chromosome Evolution, Meiotic Drive, Nonrandom Disjunction, Inversion

INTRODUCTION

The study of *Drosophila* chromosomal inversion polymorphism emerged as model system for evolutionary genetics in the 1930's and played a major role in the Modern Synthesis (Krimbas and Powell, 1992). Interest in inversions started to wane in the 1960's with the appearance of genetic markers, such as allozymes, that could be more readily applied to many other organisms. However, beginning in the 1990's, application of sequencing technology in natural population genetics led to the discovery of inversions in several non-model organisms and a renaissance in both empirical and theoretical research on inversion polymorphism and its role in adaptation and speciation (Jones *et al.*, 2012; Kirkpatrick and Barton, 2006; Le Poul *et al.*, 2014; Lowry and Willis, 2010). Clearly, inversion polymorphism is an aspect of structural genome evolution in more than just *Drosophila*. However, for the investigation of nuanced patterns and mechanisms in chromosome evolution the knowledge base developed in *Drosophila* cytogenetics remains indispensable (Corbett-Detig and Hartl, 2012; Schaeffer *et al.*, 2008). As information on inversion polymorphism accumulates in the post-genomic era, early studies of chromosomal rearrangements in model systems will play a fundamental role in organizing these abundant data to uncover the principles of chromosome evolution (Bhutkar *et al.*, 2008; Gong *et al.*, 2005; Ranz *et al.*, 2007).

Inversion polymorphism in *Drosophila* was the first genetic marker system studied for phylogenetic inference and natural population genetics. Central to this research was Alfred Sturtevant, who first discovered chromosomal inversions, their effects in transmission genetics, and described natural variation in several species (Sturtevant and Novitski, 1941; Sturtevant, 1917; Sturtevant, 1921; Sturtevant and Beadle, 1936; Sturtevant and Dobzhansky, 1936). In 1936, Sturtevant proposed to make inversion polymorphism in *D. pseudoobscura* a model

system for evolutionary biology through collaboration with Theodosius Dobzhansky and Sewall Wright (Provine, 1981). From this collaboration came the first phylogenetic series of overlapping inversions (figure 1), and would for the next four decades be expanded by Dobzhansky *et alia* into the Genetics of Natural Populations I-XLIII (Dobzhansky and Sturtevant, 1938; Lewontin, 1981).

Despite this impressive scientific history, the mechanisms governing inversion origin, establishment, and maintenance remain obscure. The experimental study of spontaneous mutation of inversions and their invasion in populations is logistically difficult, if not impossible, and thus remains underexplored (Krimbas and Powell, 1992; Yamaguchi and Mukai, 1974). Balancing selection (associative overdominance, multiple niche, *etc*.) has long been favored in the maintenance of inversion polymorphism and has been variously supported by sampling natural populations as well as population cage and field experiments (Dobzhansky, 1948; Levitan and Etges, 2009; Schaeffer, 2008; Wright and Dobzhansky, 1946). However, experimental efforts on all fronts are intrinsically biased by the idiosyncrasies of chromosomal breakages and linked genetic backgrounds for the small handful of common inversions that provide the genetic material for analysis (*e.g.* Dobzhansky, 1950; Levitan, 1962; Yamaguchi and Mukai, 1974).

In contrast, non-experimental methods such as direct sequencing of inversion breakpoints and surveys of molecular diversity in inverted regions have provided a historical perspective on the origin of the common inversions. Common cosmopolitan inversions of *D. melanogaster* have reduced levels of polymorphism and originated relatively recently, on the order of 100,000 years ago (Andolfatto *et al.*, 1999; Corbett-Detig and Hartl, 2012; Hasson and Eanes, 1996; Matzkin *et al.*, 2005; Wesley and Eanes, 1994). Similar patterns are observed for

the serially inverted third chromosome of *D. pseudoobscura*, where the long and complex history of local adaptation and selection of epistatic effects have shaped the molecular diversity among gene arrangements (Fuller *et al.*, 2016; Schaeffer, 2008; Schaeffer *et al.*, 2003; Wallace *et al.*, 2011; Wallace *et al.*, 2013). However, the discovery that common inversions from several species exhibit long range linkage disequilibrium, epistatic fitness effects, and are associated with meiotic drive renews concerns about drawing inferences from the exclusive study of this relatively small sample of inversion polymorphism (Corbett-Detig and Hartl, 2012; Houle and Márquez, 2015; Schaeffer *et al.*, 2003).

A long standing hypothesis that inversions result from ectopic recombination of transposable elements or other repetitive sequence found little support in the first polymorphic inversion breakpoints to be directly sequenced, *D. melanogaster*'s *In(3L)P*, *In(2L)t*, and *In(3R)P* (Andolfatto *et al.*, 1999; Matzkin *et al.*, 2005; Wesley and Eanes, 1994). Outside of *D. melanogaster*, there is both direct (Cáceres *et al.*, 1999b) and indirect (Orengo *et al.*, 2015) support of this mechanism. However, in 29 fixed inversions in the *melanogaster* group, Ranz *et al.* (2007) found only two instances of inverted repetitive sequences that would even allow this mechanism to operate. Complicating this historical analysis is the tendency for transposable elements to accumulate on the minority arrangements (Eanes *et al.*, 1992; Sniegowski and Charlesworth, 1994), and the possibility of transposable element remnants eroding over time (Puerma *et al.*, 2014; Ranz *et al.*, 2007). No unified characterization of inversion breakpoints, or the mechanisms governing the process, has emerged as the breakpoints studied have ranged from simple "cut and paste" to complex rearrangements including small inverted duplications and deletions (Cáceres *et al.*, 1999a; Ranz *et al.*, 2007; Wesley and Eanes, 1994).

Beyond the molecular characterization, the cytological study of inversion breakpoints along chromosome arms can also provide information about mechanisms controlling inversion polymorphism. The standard null distribution would assume inversions result from a rejoining of two chromosomal breakages that occur with uniform probability along the chromosome (Van Valen and Levins, 1968). When compared to this null distribution, breakpoints of a given inversion are observed to be further apart than expected, creating a deficiency of small inversions (*e.g.* Brehm and Krimbas, 1991). When comparing breakpoints *among* inversions, the breakpoints tend to be grouped closer together than expected creating extensive overlap of chromosomal inversions (Novitski, 1946). Finally, rather than the expected uniform distribution, serially inverted chromosomes tend to have inversion breakpoints clustered in distal regions of chromosome arms (Novitski, 1946).

Using the direct ancestor-descendant relationships of gene arrangements within species to generate phylogenetic series, it can be shown that breakpoints of derived inversions tend to lie distal of the corresponding inversion breakpoints in the ancestral arrangement (Novitski, 1946; Sturtevant and Dobzhansky, 1936). The telomeric progression of overlapping rearrangements for serially inverted chromosomes is called Novitiski's distal shift, because this little-known rule of chromosome evolution was first described by Ed Novitski with cytogenetic evidence from Muller element C of *Drosophila pseudoobscura* (Krimbas and Powell, 1992; Novitski, 1946). To extend the observation of Novitski's distal shift, I examined published data from 28 phylogenetic series for 13 different chromosomes in six *Drosophila* species of the *obscura* group. Each phylogenetic series arose independently in each species and represents the direct ancestor-descendent relationship in paracentric inversion evolution. Having validated the distal shift empirically, I propose a meiotic drive mechanism for the evolution of overlapping

chromosomal inversions that explains the progressive distal shift towards the telomere. I discuss difficulties in extending this mechanism to serially inverted chromosomes and suggest the same mechanism is the cause of other notable patterns in paracentric inversion evolution.

MATERIALS AND METHODS

The cytogenetics for sixteen *Drosophila* species in the *obscura* group have been published. Although most species in this group have extensive inversion polymorphism, only six species had sufficient data (multiple overlapping rearrangements) to allow the construction of phylogenies for overlapping paracentric inversions. Inversion phylogenies are unrooted trees. Incorporating information on arrangement frequency, geographical distribution, and the karyotype of interspecific hybrids, consensus ancestral arrangements (often designated "Standard") are used to polarize inversion phylogenies. Note, arrangements other than the Standard may have been ancestral, as proposed for two chromosomes in *D. subobscura* and demonstrated with molecular variation for *D. pseudoobscura* (Krimbas, 1992; Wallace *et al.*, 2011). The first gene rearrangement in a phylogenetic series is designated as the "series originating inversion." An inversion's rank in a phylogenetic series was recorded as the minimal number of inversion back steps required to obtain the ancestral arrangement. Species and chromosome information for the 28 phylogenetic series of overlapping inversions used in this paper are listed in table 1 with a sample series illustrated in figure 1 (see supplementary figure 1 for dataset from all series)

To quantify the distal shift, the cytogenetic location of breakpoints were converted to a numerical value where the centromere position was zero and each successive cytogenetically discernable region is considered one unit distal. Where ambiguity exists for an inversion

breakpoint location, the midpoint of the range indicated was taken. To facilitate comparison across chromosomes and species, the scale for each chromosome was standardized to 100 unit lengths. This quantification does not necessarily correspond to a linear function of physical distance (bp) or genetic distance (cM), but it does assign a value between 0-100 for every inversion breakpoint and represents its cytogenetic location on the chromosome relative to the centromere. Inversion breakpoints location on this scale must be determined based on the arrangement upon which that new inversion first arose, not necessarily on the standard arrangement scale that is often reported in the literature. Similarly, inversion size was estimated as the distance between proximal and distal inversion breakpoints on the gene arrangement upon which it first occurred. Thus breakpoint location, inversion size, and phylogenetic rank for a given inversion are not obvious from casual observation and these data are provided in supplemental table 1.

To test the distal shift, inversion breakpoint location and size must be adjusted for the position and size of each respective phylogenetic series. Inversion breakpoint locations were therefore expressed as a deviation from the midpoint of the series originating inversion and inversion size was expressed as a deviation from the average inversion size for each phylogenetic series. On the standardized adjusted scale, zero represented the location of the originating inversion for each series, positive values represented a distal shift, and negative values indicated proximal movement of the derived inversion. Similarly, positive values indicates an above average size and negative values represent a smaller than average size after standardization and adjustment for each phylogenetic series.

Statistical analysis of the distal shift was performed by least squares regression of standardized inversion breakpoint location upon the inversion's rank in a phylogenetic series.

For each phylogenetic series the Muller element and species were incorporated as nested categorical variables in the regression analysis. Phylogenetic correction for the species term (incorporating non-independence of observations due to shared ancestry) is statistically inappropriate when analyzing polymorphisms unique to each species, precisely because there can be no covariation due to coancestry. Separate analyses were performed for proximal and distal inversion breakpoints with statistical significance of the regression coefficients assessed by two sided *t*-test for $\beta = 0$. A statistical test for equality of slopes was performed using *F*-statistics for the null hypothesis $\beta_{proximal} = \beta_{distal}$ (Sokal and Rohlf, 1995). Statistical significance of inversion size reduction was assessed by two sided *t*-test for $\beta = 0$ after regression of standardized inversion size on phylogenetic series.

RESULTS

The quantitative analysis of 134 inversions in 28 phylogenetic series on 13 chromosomes from six *obscura* group species provided strong statistical support of the telomeric progression of sequentially derived inversion breakpoints (figure 2). The regression of proximal inversion breakpoint location on phylogenetic rank yielded a statistically significant regression coefficient ($\beta=10.00$, $t=4.54$, $df=1$, $p< 0.001$) (table 2). Distal inversion breakpoint location when regressed on phylogenetic rank was also statistically significant ($\beta=7.07$, $t=2.98$, $df=1$ $p=0.004$) (table 3). Variance components and the occasional statistical significance associated with nested categorical variables (species, element, series) is likely a product of standardization across chromosomes with heterogeneous map resolution and genetic length. Again phylogenetic correction for these tests is both logically and statistically inappropriate as the inversion polymorphism analyzed is unique to and arose independently in each species.

The telomeric progression was stronger for the proximal breakpoints than the distal breakpoints (figure 2), suggesting a reduction in size for inversions of high rank (figure 3). However, the slopes of these two regressions do not differ with statistical significance ($F_{1,4}=0.37$, $p=0.496$) (table 4), and the apparent trend towards size reduction was not statistically significant ($\beta=-2.93$, $t=-1.25$, $df=1$ $p=0.22$) (table 5). Empirically, the phylogenetic series from just six species provides strong evidence for distal shift but insufficient data to demonstrate the trend toward size reduction with statistical significance.

DISCUSSION

Novitski's Distal Shift: The telomeric progression of overlapping inversions for serially inverted chromosomes was previously known only as qualitative pattern from a single Muller element. The distal shift was confirmed here by a quantitative analysis of 28 phylogenetic series of 134 paracentric inversions from six *Drosophila* species of the *obscura* group. Each phylogenetic series arose independently in every species and represents the direct ancestor-descendent relationships in paracentric inversion evolution. Therefore, each of the 28 phylogenetic series is a unique, independent realization of a distinct directional pattern in chromosome evolution. To what degree the distal shift represents a general rule of inversion evolution, as opposed to a chromosomal anomaly of the *D. obscura* group, is the subject of a forthcoming publication.

One feature of the *obscura* group sex chromosomes proves an exception to this rule. "Sex ratio" (SR) chromosomes are coadapted gene complexes that cause strong unequal transmission of X and Y chromosomes and are found in all six species (Jaenike, 2001). SR chromosomes in all six species carry inverted gene arrangements, but these inversions tend to be non-overlapping

and rarely form phylogenetic series. When SR chromosomes do form phylogenetic series (Muller element A of *D. subobscura,* element D of *D. athabasca*), they tend to be smaller series and do not exhibit the distal shift or size reduction. It is likely that the very strong selection on sex ratios and recombination suppression associated with the strong transmission bias of SR chromosomes overwhelms the statistical signal produced the relatively weak force that drives the distal shift.

If the distal shift observed for autosomes in these species is just an extreme illustration of some underlying principle common to all paracentric inversion evolution, then a cytogenetic mechanism is required for this pattern. Novitski himself presented a biased mutational model after observing the non-uniform distribution of inversion breakpoints in the phylogenetic series of *D. pseudoobscura* (Bernstein and Goldschmidt, 1961; Novitski, 1946; Novitski, 1961). This ingenious model invokes a bias of spontaneous chromosome breakage in inversion heterozygotes and remains a viable explanation awaiting experimental examination. However, as Novitski noted, this model is limited to explaining the clumped distribution of inversion breakpoints and alone is insufficient to explain the distal shift, size reduction, or variability of inversion abundance (Novitski, 1946). Below I present an alternative mechanism with well-validated assumptions that addresses all these aspects of inversion polymorphism, and does not invoke the logistically untestable mutational bias assumption.

Meiotic Drive Mechanism: In *Drosophila*, recombination between different gene arrangements is effectively suppressed because crossing over produces acentric and dicentric meiotic products that for mechanical reasons are relegated to the polar body nuclei and never included in the functional egg (Hinton and Lucchesi, 1960; Sturtevant and Beadle, 1936). However, as illustrated in figure 4A, heterozygotes for overlapping inversions have a

homosequential region, where if crossing over were to occur, all four meiotic products would be monocentric and could in principle be included in the functional egg (figure 4B). Because of the figure eight pairing pattern (figure 4C), crossing over in meiosis I generates large deletions and duplications resulting in dyad asymmetry (figure 4B) (Sturtevant and Beadle, 1936). In *Drosophila* females, it is a thoroughly established fact that the shorter chromatid of an asymmetric dyad in meiosis II has a higher probability of being included in the functional egg (Lindsley and Sandler, 1965; Novitski and Sandler, 1956; Zimmering, 1955). The phenomenon of unequal recovery from asymmetric dyads is known as nonrandom disjunction and is a well-known form of female meiotic drive (reviewed in Novitski, 1951; Novitski, 1967).

For any overlapping paracentric inversions that are two steps apart in a phylogenetic series, a homosequential region exists where crossing over produces asymmetric dyads (Sturtevant and Beadle, 1936). Furthermore, for any such inversions, a single crossover event will create dyads pairing the distal inversion with the duplications and the proximal inversion with the deletions. The resulting asymmetric dyads will favor the inclusion of the chromatids carrying the distal inversion and the deletions in the functional egg. Because large chromosomal deletions and duplications generally form inviable zygotes, the final result is an over-representation of distal inversions in the viable progeny of females heterozygous for overlapping inversions.

This meiotic drive mechanism applies equally to included inversions (supplemental figure 2), where the asymmetric dyads generated from crossing over consist of a proximal duplication and distal deletion (or vice versa). The mechanism operates for inversions in repulsion phase (as illustrated in figure 4) or coupling phase as observed for serially inverted chromosomes (supplemental figure 3 and 4). In the latter case, the serially inverted chromosome

will drive against the standard arrangement only if the second inversion is distal to the first. Therefore, single crossover events in homosequential regions of any two inversions (overlapping or included, in repulsion or coupling phase) will unequivocally create a bias favoring the inclusion of distal inversions in the functional egg. I propose that it is this intrinsic bias in female meiosis that generates the distal shift in a phylogenetic series of serially inverted chromosomes. This hypothesis also explains the weak tendency towards size reduction as a by-product of favoring evermore distal inversions in subtelomeric regions where large inversions are precluded by the position of the telomere.

Challenges of Drive Theory: This meiotic drive theory of Novitski's distal shift presents several difficulties from both transmission genetic and population genetic perspectives. Although crossing over in homosequential regions of overlapping inversions has been directly observed, experimental investigation has been limited to X chromosomes and requires the use of compound chromosomes or translocation stocks to recover recombinant products (Grell, 1962; Novitski and Braver, 1954; Sturtevant and Beadle, 1936). Crossing over in shared inverted regions has not yet been demonstrated for inversions of autosomes segregating in natural populations. Formal genetic analysis for the common inversions of Muller element E in *D. melanogaster* and Muller element C in *D. pseudoobscura* could not detect nonrandom disjunction (Meisel and Schaeffer, 2007, Koury unpublished). However, using realistic parameters for crossing over and nonrandom disjunction, expected transmission ratios do not exceed $k = 0.513$ (Koury unpublished), a deviation that is on the same order of magnitude of viability effects of phenotypic markers used and well within the measurement error of both experiments. Nonetheless, it is not uncommon for effects below the threshold of experimental detection (*e.g.* codon bias) to have major evolutionary significance. Furthermore, there are

276 experimental refinements possible with *D. melanogaster* model system currently being pursued
277 to enhance the ability to experimentally detect drive in this scenario.

278 More difficulty is encountered when considering the population genetics of the distal
279 shift. The relative rarity of inversion mutations, overlapping inversion heterozygotes, and
280 crossing over in homosequential regions (each a precondition for the next) paired with the
281 relatively weak strength of drive and the underdominance of overlapping inversion heterozygotes
282 (due to dominant lethal deletion and duplications), suggests a small role for nonrandom
283 disjunction in paracentric inversion evolution. In considering the extension to serially inverted
284 chromosomes, the later gene rearrangements can only drive at the expense of the earlier steps.
285 So this force, while of plausible importance in the first, second, or third steps of a phylogenetic
286 series, quickly becomes vanishingly small in later steps. These population genetic questions
287 require rigorous quantitative analysis beyond the scope of this paper and are the subject of a
288 forthcoming publication.

289 Interestingly, the challenges outlined in this section generate several predictions which
290 are consistent with the *obscura* group data. First, the relatively weak female meiotic drive for
291 overlapping inversions (maximum $k = 0.513$) does not generate a distal shift for SR
292 chromosomes where stronger forces are expected to prevail. Second, for the most extensive and
293 best resolved phylogenetic series (Muller element C of *D. athabasca, D. pseudoobscura,* and *D.*
294 *subobscura*), the ancestral arrangements and early steps tend to be rare or absent. Finally, for
295 autosomes no series greater than four inversion steps was observed (supplement table 1) and the
296 distal shift is relatively weak for the few inversions of rank four (figure 3).

297 **Patterns in Inversion Evolution:** It is very encouraging to note that the proposed
298 meiotic drive mechanism bears on several other patterns in inversion polymorphism. To justify

299 experimental investigation and population genetic simulations for this scenario I enumerate some
300 of these observations. The patterns of paracentric inversion variability mentioned in the
301 introduction suggest this form of structural heterozygosity has autocatalytic properties (Bernstein
302 and Goldschmidt, 1961; Novitski, 1961). The meiotic drive mechanism predicts serial inverted
303 chromosomes, especially distally placed second inversions, have intrinsic advantages in invading
304 a population already segregating for chromosomal rearrangements in the same genomic region.
305 And although this advantage does not hold for advanced stages in the phylogenetic series, the
306 approach to complete recombination suppression by favoring inversions in later stages has the
307 second order effect of reducing genetic load due to this particular form of meiotic drive (Crow
308 and Kimura, 1970).

309 In considering just three consecutive steps of a phylogenetic series (ancestral,
310 intermediate, and derived), Wallace observed that the intermediate arrangement is often absent in
311 a given population (Wallace, 1953). Wallace's "Rule of Triads" is immediately comprehensible
312 on the view that the intermediate and derived arrangements have similar gene contents and
313 fitness; however, the derived arrangement has the added benefit of driving against the ancestral
314 state and thus outcompetes the intermediate arrangement. Along these same lines, the commonly
315 observed local extirpation of arrangements of low rank in a long phylogenetic series may be
316 related to being commonly driven against.

317 Finally, although Novitski's (1961) biased mutational model explains the clustered
318 pattern of inversion breakpoints generating extensive inversion overlap, the meiotic drive theory
319 offers an equally viable alternative. The drive mechanism predicts, even with uniform
320 distribution of spontaneous inversion breakpoints, that the inversions with greatest overlap and
321 thus greatest opportunity for nonrandom disjunction, would invade natural populations. The

clumped distribution of observed breakpoints would therefore be the result of biases during the establishment phase of inversion not from any spontaneous mutational bias.

Rates of spontaneous chromosomal inversion, and any biases thereof, are outside the scope of reasonable experimental investigation (*cf.* Yamaguchi and Mukai, 1974 for an unreasonable attempt). As a consequence it is unclear how to practically differentiate alternative theories of chromosome evolution based solely on patterns of natural inversion polymorphism. The meiotic drive theory of paracentric inversion evolution introduced here has the potential to explain with a single mechanism a number of different chromosome patterns that were previously thought to be unrelated. Furthermore, the meiotic drive theory is based on a cytogenetic mechanism that is amenable to direct experimentation, thereby conferring a high degree of testability to this model of chromosome evolution.

Conclusion: In a phylogenetic series of overlapping inversions in the *Drosophila* species of the *obscura* group derived arrangements tend to have distally shifted breakpoints resulting in smaller inversions. The distal shift, viewed *in extremis* for the hypervariable Muller element C of *D. pseudoobscura* and *D. athabsaca*, likely reflects a fundamental mechanism of paracentric inversion evolution, while the size reduction is simply a byproduct of the distal shift in subtelomeric regions. Nonrandom disjunction of overlapping inversions was demonstrated to always favor transmission of distal inversions and is hypothesized here to favor the evolution of serially inverted chromosomes. Therefore, far from being selectively beneficial, inversion polymorphism, serially inverted chromosomes, and the associated distal shift result from intrinsic biases in meiosis and generate a substantial genetic load. This novel hypothesis requires further investigation along both experimental and theoretical lines. The meiotic drive mechanism

proposed should be of considerable interest as it can explain Novitski's distal shift as well as several related patterns of breakpoint distribution and paracentric inversion evolution.

REFERENCES

Andolfatto P, Wall JD, Kreitman M. 1999. Unusual haplotype structure at the proximal breakpoint of *In(2L)t* in a natural population of *Drosophila melanogaster*. Genetics 153(3):1297-1311.

Bernstein N, Goldschmidt E. 1961. Chromosome Breakage in Structural Heterozygotes. Am Nat 95(885):53.

Bhutkar A, Schaeffer SW, Russo SM, Xu M, Smith TF, Gelbart WM. 2008. Chromosomal rearrangement inferred from comparisons of 12 *Drosophila* genomes. Genetics 179(3):1657-1680.

Brehm A, Krimbas CB. 1991. Inversion Polymorphism in *Drosophila obscura*. J Hered 82(2):110-117.

Cáceres M, Ranz JMa, Barbadilla A, Long M, Ruiz A. 1999a. Generation of a Widespread *Drosophila* Inversion by a Transposable Element. Science 285(5426):415-418.

Cáceres M, Ranz JMa, Barbadilla A, Long M, Ruiz A. 1999b. Generation of a widespread *Drosophila* inversion by a transposable element. Science 285(5426):415-418.

389 Corbett-Detig RB, Hartl DL. 2012. Population Genomics of Inversion Polymorphisms in
390 *Drosophila melanogaster*. PLOS Genetics 8(12):e1003056.
391
392 Crow JF, Kimura M. 1970. An introduction to population genetics theory. Harper & Row, New
393 York, NY.
394
395 Dobzhansky T. 1948. Genetics of natural populations. XVI. Altitudinal and seasonal changes
396 produced by natural selection in certain populations of *Drosophila pseudoobscura* and
397 *Drosophila persimilis*. Genetics 33(2):158.
398
399 Dobzhansky T. 1950. Genetics of Natural Populations .19. Origin of Heterosis through Natural
400 Selection in Populations of *Drosophila pseudoobscura*. Genetics 35(3):288-302.
401
402 Dobzhansky T, Sturtevant AH. 1938. Inversions in the chromosomes of *Drosophila*
403 *pseudoobscura*. Genetics 23(1):28-64.
404
405 Dobzhansky TG, Epling C. 1944. Contributions to the genetics, taxonomy, and ecology of
406 *Drosophila pseudoobscura* and its relatives. Publs Carnegie Instn, Washington, D.C.
407
408 Eanes WF, Wesley C, Charlesworth B. 1992. Accumulation of P elements in minority inversions
409 in natural populations of *Drosophila melanogaster*. Genetical Research 59(1):1-9.
410

411 Fuller ZL, Haynes GD, Richards S, Schaeffer SW. 2016. Genomics of natural populations: how
412 differentially expressed genes shape the evolution of chromosomal inversions in
413 *Drosophila pseudoobscura*. Genetics 204(1):287-301.
414
415 Gong WJ, McKim KS, Hawley RS. 2005. All paired up with no place to go: pairing, synapsis,
416 and DSB formation in a balancer heterozygote. PLoS Genet 1(5):e67.
417
418 Grell RF. 1962. A new model for secondary nondisjunction: the role of distributive pairing.
419 Genetics 47(12):1737.
420
421 Hasson E, Eanes WF. 1996. Contrasting histories of three gene regions associated with
422 *In(3L)Payne* of *Drosophila melanogaster*. Genetics 144(4):1565-1575.
423
424 Hinton CW, Lucchesi JC. 1960. A cytogenetic study of crossing over in inversion heterozygotes
425 of *Drosophila melanogaster*. Genetics 45(1):87.
426
427 Houle D, Márquez EJ. 2015. Linkage disequilibrium and inversion-typing of the *Drosophila*
428 *melanogaster* Genome Reference Panel. G3: Genes| Genomes| Genetics 5(8):1695-1701.
429
430 Jaenike J. 2001. Sex chromosome meiotic drive. Annual Review of Ecology and Systematics
431 32(1):25-49.
432

433 Jones FC, Grabherr MG, Chan YF, Russell P, Mauceli E, Johnson J, Swofford R, Pirun M, Zody
434 MC, White S. 2012. The genomic basis of adaptive evolution in threespine sticklebacks.
435 Nature 484(7392):55-61.
436
437 Kirkpatrick M, Barton N. 2006. Chromosome inversions, local adaptation and speciation.
438 Genetics 173(1):419-434.
439
440 Krimbas CB. 1992. The inversion polymorphism of *Drosophila subobscura*. In: Krimbas CB,
441 Powell JR, editors. *Drosophila* inversion polymorphism. CRC Press, Boca Raton, FL.
442
443 Krimbas CB, Powell JR. 1992. Introduction. In: Krimbas CB, Powell JR, editors. *Drosophila*
444 inversion polymorphism. CRC Press, Boca Raton, FL.
445
446 Le Poul Y, Whibley A, Chouteau M, Prunier F, Llaurens V, Joron M. 2014. Evolution of
447 dominance mechanisms at a butterfly mimicry supergene. Nature Communications
448 5:5644.
449
450 Levitan M. 1962. Spontaneous chromosome aberrations in *Drosophila robusta*. Proceedings of
451 the National Academy of Sciences 48(6):930-937.
452
453 Levitan M, Etges WJ. 2009. Rapid response to perturbation of chromosome frequencies in
454 natural populations of *Drosophila robusta*. Genetica 137(1):1.
455

456 Lewontin RC. 1981. The scientific work of Theodosius Dobzhansky. In: Lewontin RC, Moore
457 JA, Provine WB, Wallace B, editors. Dobzhansky's genetics of natural populations I-
458 XLIII. Columbia University Press, New York, NY.
459
460 Lindsley D, Sandler L. 1965. Meiotic behavior of tandem metacentric compound X
461 chromosomes in *Drosophila melanogaster*. Genetics 51(2):223.
462
463 Lowry DB, Willis JH. 2010. A widespread chromosomal inversion polymorphism contributes to
464 a major life-history transition, local adaptation, and reproductive isolation. PLoS Biol
465 8(9):e1000500.
466
467 Matzkin LM, Merritt TJS, Zhu CT, Eanes WF. 2005. The structure and population genetics of
468 the breakpoints associated with the cosmopolitan chromosomal inversion *In(3R)Payne* in
469 *Drosophila melanogaster*. Genetics 170(3):1143-1152.
470
471 Meisel RP, Schaeffer SW. 2007. Meiotic transmission of *Drosophila pseudoobscura*
472 chromosomal arrangements. PloS one 2(6):e530.
473
474 Novitski E. 1946. Chromosome Variation in *Drosophila athabasca*. Genetics 31(5):508-524.
475
476 Novitski E. 1951. Non-random disjunction in *Drosophila*. Genetics 36(3):267.
477
478 Novitski E. 1961. Chromosome Breakage in Inversion Heterozygotes. Am Nat 95(885):250.

Novitski E. 1967. Nonrandom disjunction in *Drosophila*. Annual review of genetics 1(1):71-86.

Novitski E, Braver G. 1954. An analysis of crossing over within a heterozygous inversion in *Drosophila melanogaster*. Genetics 39(2):197-209.

Novitski E, Sandler L. 1956. Further notes on the nature of non-random disjunction in *Drosophila melanogaster*. Genetics 41(2):194.

Orengo D, Puerma E, Papaceit M, Segarra C, Aguadé M. 2015. A molecular perspective on a complex polymorphic inversion system with cytological evidence of multiply reused breakpoints. Heredity 114(6):610-618.

Provine WB. 1981. Origins of the genetics of natural populations series. In: Lewontin RC, Moore JA, Provine WB, Wallace B, editors. Dobzhansky's genetics of natural populations I-XLIII. Columbia University Press, New York, NY.

Puerma E, Orengo DJ, Salguero D, Papaceit M, Segarra C, Aguadé M. 2014. Characterization of the breakpoints of a polymorphic inversion complex detects strict and broad breakpoint reuse at the molecular level. Molecular biology and evolution:msu177.

500 Ranz JM, Maurin D, Chan YS, Von Grotthuss M, Hillier LW, Roote J, Ashburner M, Bergman
501 CM. 2007. Principles of genome evolution in the *Drosophila melanogaster* species
502 group. PLoS Biol 5(6):e152.
503
504 Schaeffer SW. 2008. Selection in heterogeneous environments maintains the gene arrangement
505 polymorphism of *Drosophila pseudoobscura*. Evolution 62(12):3082-3099.
506
507 Schaeffer SW, Bhutkar A, McAllister BF, Matsuda M, Matzkin LM, O'Grady PM, Rohde C,
508 Valente VL, Aguadé M, Anderson WW. 2008. Polytene chromosomal maps of 11
509 *Drosophila* species: the order of genomic scaffolds inferred from genetic and physical
510 maps. Genetics 179(3):1601-1655.
511
512 Schaeffer SW, Goetting-Minesky MP, Kovacevic M, Peoples JR, Graybill JL, Miller JM, Kim
513 K, Nelson JG, Anderson WW. 2003. Evolutionary genomics of inversions in *Drosophila*
514 *pseudoobscura*: evidence for epistasis. Proceedings of the National Academy of Sciences
515 100(14):8319-8324.
516
517 Sniegowski PD, Charlesworth B. 1994. Transposable element numbers in cosmopolitan
518 inversions from a natural population of *Drosophila melanogaster*. Genetics 137(3):815-
519 827.
520
521 Sokal RR, Rohlf FJ. 1995. Biometry : the principles and practice of statistics in biological
522 research.W. H. Freeman and Company, New York, NY.

Sturtevant A, Novitski E. 1941. The homologies of the chromosome elements in the genus *Drosophila*. Genetics 26(5):517.

Sturtevant AH. 1917. Genetic Factors Affecting the Strength of Linkage in *Drosophila*. Proc Natl Acad Sci U S A 3(9):555-558.

Sturtevant AH. 1921. The North American species of *Drosophila*. Publs Carnegie Instn, Washington, D.C.

Sturtevant AH, Beadle GW. 1936. The Relations of Inversions in the X Chromosome of *Drosophila melanogaster* to Crossing over and Disjunction. Genetics 21(5):554-604.

Sturtevant AH, Dobzhansky T. 1936. Inversions in the third chromosome of wild races of *Drosophila pseudoobscura*, and their use in the study of the history of the species. P Natl Acad Sci USA 22:448-450.

Van Valen L, Levins R. 1968. Origins of Inversion Polymorphisms. Am Nat 102(923p):5-&.

Wallace AG, Detweiler D, Schaeffer SW. 2011. Evolutionary history of the third chromosome gene arrangements of *Drosophila pseudoobscura* inferred from inversion breakpoints. Molecular Biology and Evolution 28(8):2219-29.

546 Wallace AG, Detweiler D, Schaeffer SW. 2013. Molecular population genetics of inversion
547 breakpoint regions in *Drosophila pseudoobscura*. G3: Genes| Genomes| Genetics
548 3(7):1151-1163.
549
550 Wallace B. 1953. On coadaptation in *Drosophila*. The American Naturalist 87(837):343-358.
551
552 Wesley CS, Eanes WF. 1994. Isolation and Analysis of the Breakpoint Sequences of
553 Chromosome Inversion *In(3L)Payne* in *Drosophila melanogaster*. P Natl Acad Sci USA
554 91(8):3132-3136.
555
556 Wright S, Dobzhansky T. 1946. Genetics of natural populations. XII. Experimental reproduction
557 of some of the changes caused by natural selection in certain populations of *Drosophila*
558 *pseudoobscura*. Genetics 31(2):125.
559
560 Yamaguchi O, Mukai T. 1974. Variation of Spontaneous Occurrence Rates of Chromosomal-
561 Aberrations in Second Chromosomes of *Drosophila melanogaster*. Genetics 78(4):1209-
562 1221.
563
564 Zimmering S. 1955. A genetic study of segregation in a translocation heterozygote in
565 *Drosophila*. Genetics 40(6):809.
566
567
568

569 Table 1. The list of 28 phylogenetic series used in this study. Note that the 14 different
570 Muller elements listed constitute only 13 chromosomes as elements A and D were fused
571 to form the metacentric X chromosome in *D. athabasca.*

Species	Muller Element	Number of Series	Number of Inversions
D. subobscura	A	2	4
D. subobscura	B	2	7
D. subobscura	C	1	8
D. subobscura	D	1	2
D. subobscura	E	2	7
D. athabasca	A	2	6
D. athabasca	B	1	3
D. athabasca	C	5	30
D. athabasca	D	4	8
D. azteca	C	1	5
D. azteca	E	1	3
D. obscura	C	2	7
D. persimilis	C	1	10
D. pseudoobscura	C	3	34
Total 6	14	28	134

572
573
574
575 Table 2. ANOVA table for regression of proximal inversion breakpoint location on
576 phylogenetic rank. Data corresponds to the open symbols in figure 2.

Source of Variation	*df*	*SS*	*MS*	*F ratio*	*p*
Regression on Rank	1	5149.37	5149.37	20.58	< 0.001
Species	5	2806.09	561.22	2.24	0.055
Element within Species	8	2620.44	327.56	1.31	0.247
Series within Element	14	6609.16	472.08	1.89	0.036
Error	105	26270.46	250.19		
Total	133	53987.24			

Table 3. ANOVA table for regression of distal inversion breakpoint location on phylogenetic rank. Data corresponds to the shaded symbols in figure 2

Source of Variation	*df*	*SS*	*MS*	*F ratio*	*p*
Regression on Rank	1	2574.71	2574.71	8.86	0.004
Species	5	992.29	198.46	0.68	0.637
Element within Species	8	3008.67	376.08	1.29	0.254
Series within Element	14	5927.14	423.37	1.46	0.140
Error	105	30499.63	290.47		
Total	133	49107.05			

Table 4. ANOVA table testing equality of slopes from regression of proximal and distal inversion breakpoints against phylogenetic rank. The weighted average deviation from regression ($\bar{s}^2_{Y \cdot X}$) was calculated as described in Sokal and Rohlf (1995) using summary statistics from tables 2 and 3

Source of Variation	*df*	*SS*	*MS*	*F ratio*	*p*
Variation among regressions	1	398.02	398.02	0.37	0.496
Average variation within regressions	4	4328.72	1082.18		

Table 5. ANOVA table for regression of inversion size on phylogenetic rank. Data corresponds to figure 3.

Source of Variation	*df*	*SS*	*MS*	*F ratio*	*p*
Regression on Rank	1	441.62	441.62	1.55	0.216
Species	5	16.63	3.33	0.01	> 0.999
Element within Species	8	92.43	11.55	0.04	> 0.999
Series within Element	14	94.32	6.74	0.02	> 0.999
Error	105	29886.67	284.63		
Total	133	30355.41			

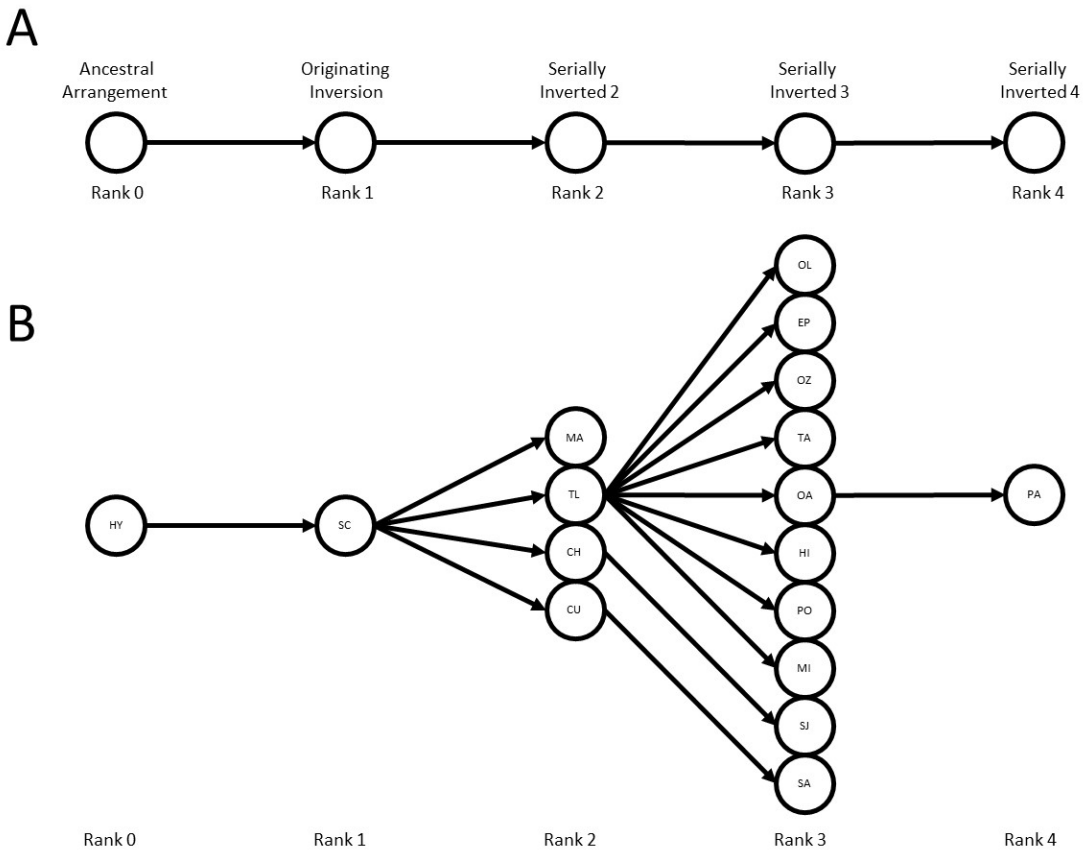

Figure 1. Schematics of phylogenetic series. A) A simple series from ancestral arrangement (rank 0), to the series originating inversion (rank 1), and then serially inverted chromosomes of rank 2 through 4. B) The "Santa Cruz" phylogenetic series of *D. pseudoobscura* chromosome III (illustrating only arrangements repeatedly observed in natural populations). Two letter codes refer to names of each gene arrangement (*cf.* Dobzhansky and Epling, 1944).

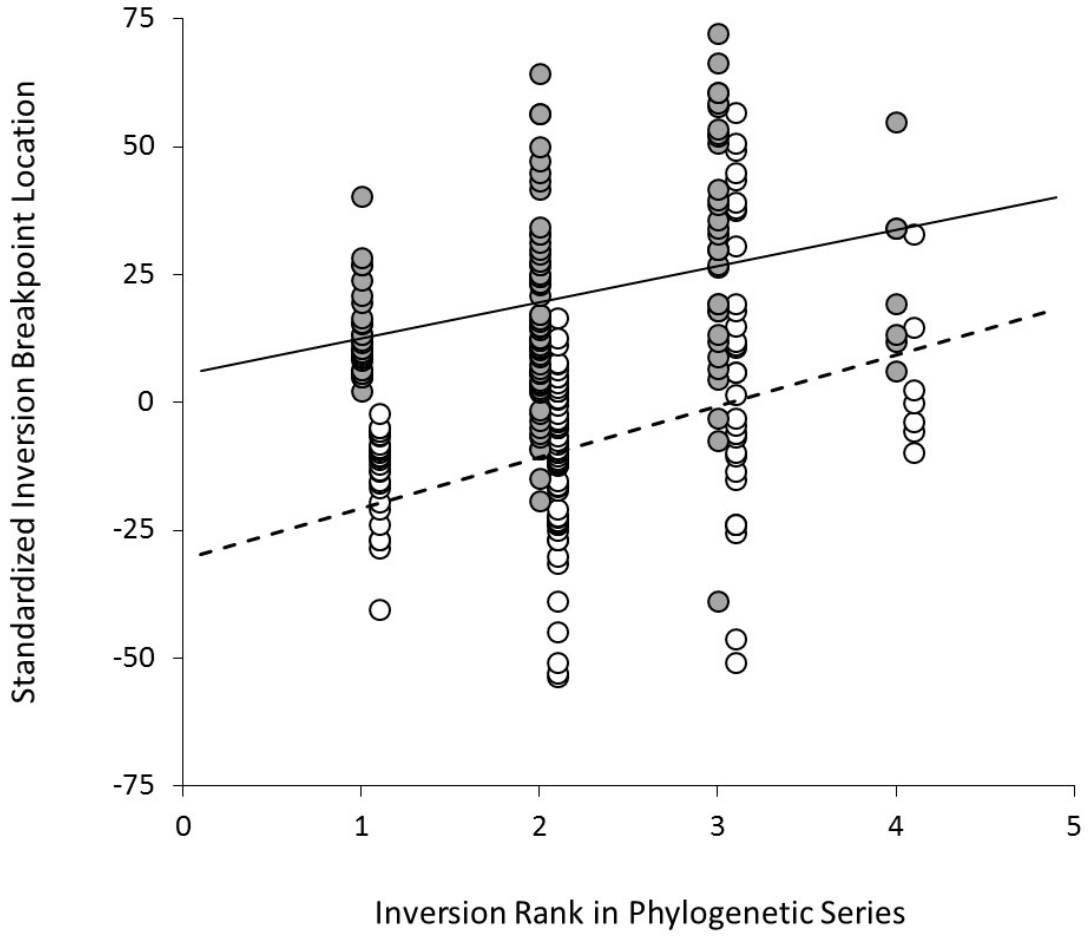

Figure 2. Standardized inversion breakpoint location regressed on phylogenetic rank. Movement in the positive direction on the standardized scale is a movement toward the telomere (a distal shift). Closed circles are the distal breakpoints, open circles are proximal breakpoints (displaced by +0.1 units on the x-axis for ease of visualization). Statistically significant linear regression is depicted by solid line for distal breakpoints and dotted line for proximal breakpoints (table 2 and 3).

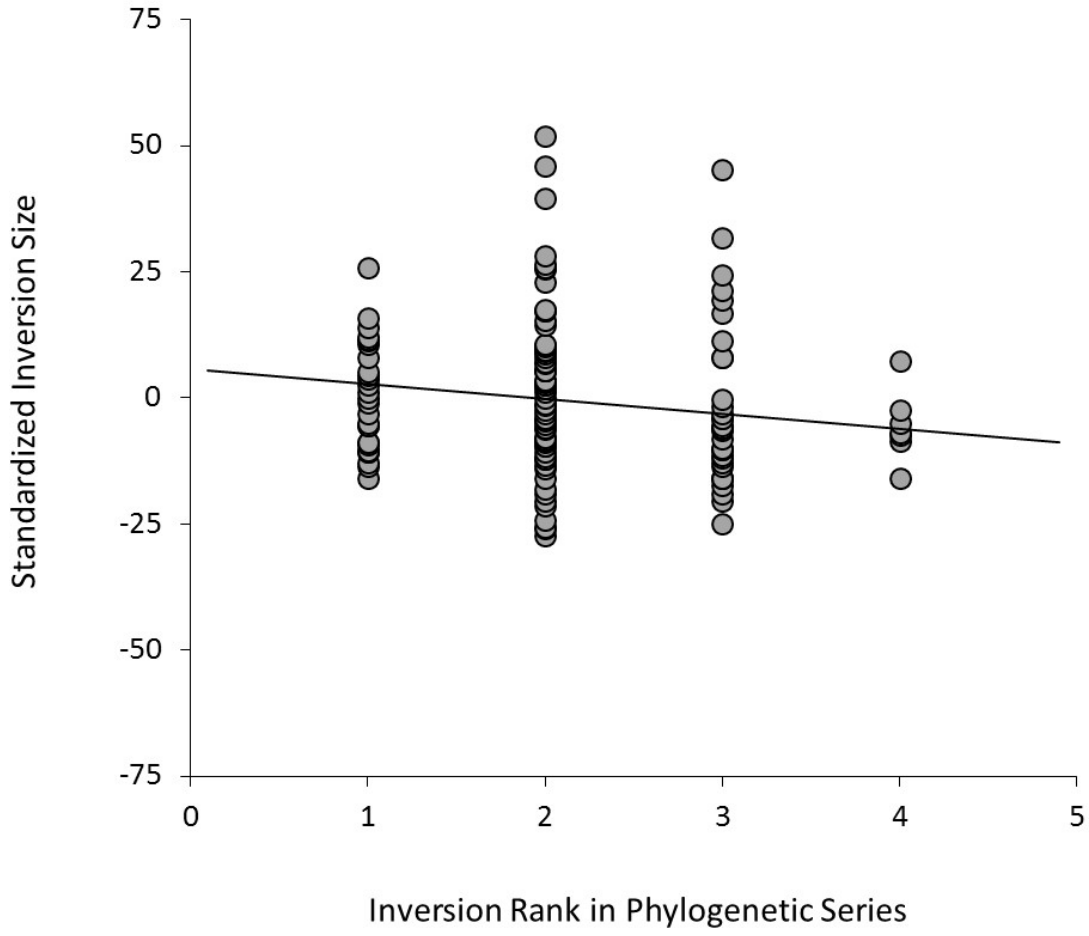

Figure 3. Progressive trend towards inversion size reduction in phylogenetic series. The line of best fit for inversion size regressed on phylogenetic rank is depicted by solid line (table 5). Regression coefficient of this line does not differ from zero with statistical significance.

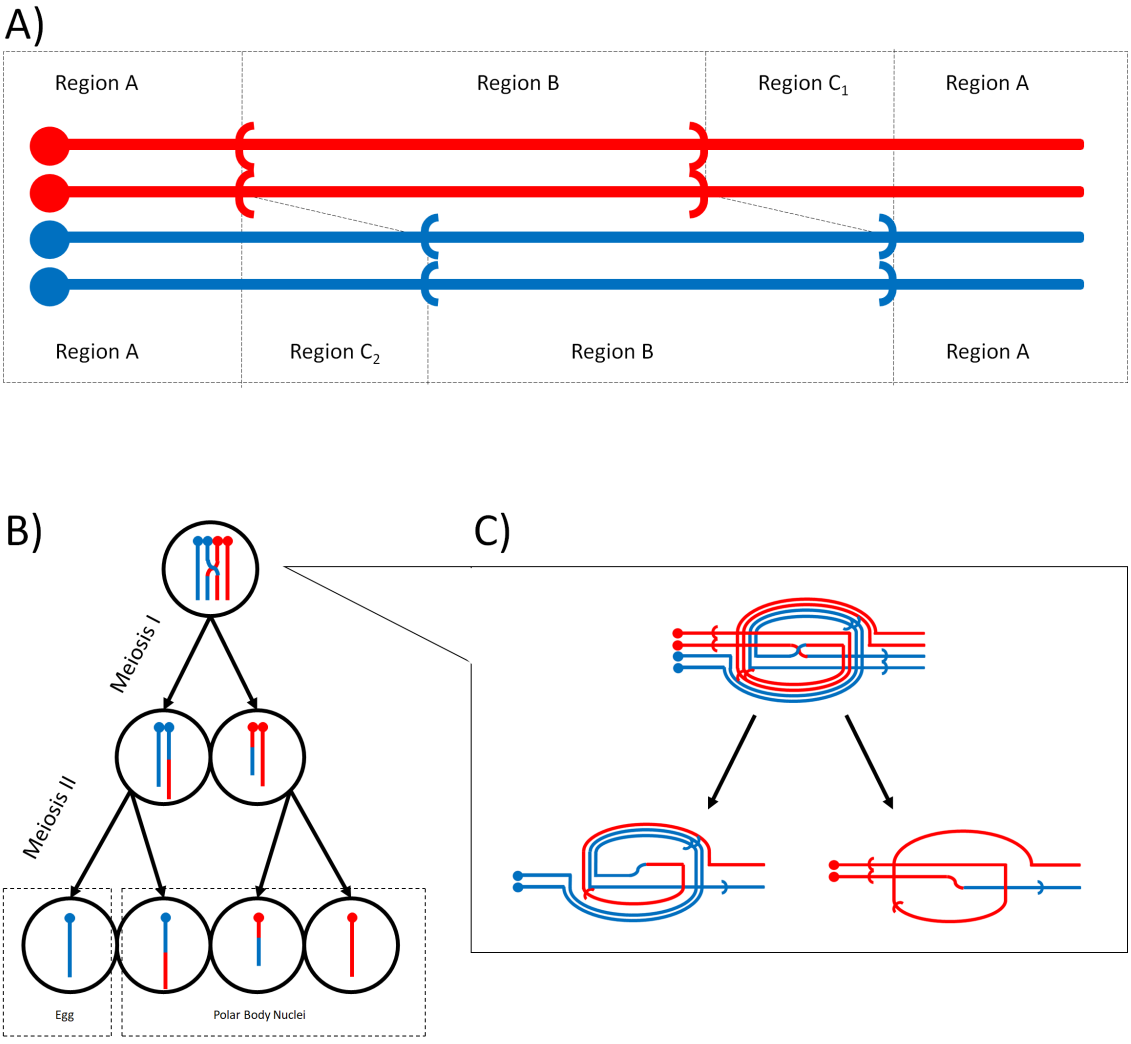

Figure 4. Schematic for nonrandom disjunction of overlapping inversion. A) Four strand bundle diagram divided into homosequential regions external to inversions (Region A), regions inverted relative to one another (Region C_1 and C_2), and the homosequential region internal to inversions (Region B). B) Progression of asymmetric products from a crossover in Region B through meiosis I and II, migration of chromatids to the egg pole is probabilistic but always favors the transmission of distal inversion (blue non-recombinant chromatid). C) Figure eight pairing of overlapping inversions and the resulting asymmetric dyads resulting from crossing over in Region B, illustrating all four possible meiotic products and their relative sizes.