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How chromosomal rearrangements shape adaptation and speciation: Case studies in *Drosophila pseudoobscura* and its sibling species *D. persimilis*

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Abstract

The gene arrangements of *Drosophila* have played a prominent role in the history of evolutionary biology from the original quantification of genetic diversity to current studies of the mechanisms for the origin and establishment of new inversion mutations within populations and their subsequent fixation between species supporting reproductive barriers. This review examines the genetic causes and consequences of inversions as recombination suppressors and the role that recombination suppression plays in establishing inversions in populations as they are involved in adaptation within heterogeneous environments. This often results in the formation of clines of gene arrangement frequencies among populations. Recombination suppression leads to the differentiation of the gene arrangements which may accelerate the accumulation of fixed genetic differences among populations. If these fixed mutations cause incompatibilities, then inversions pose important reproductive barriers between species. This review uses the evolution of inversions in *Drosophila pseudoobscura* and *D. persimilis* as a case study for how inversions originate, establish, and contribute to the evolution of reproductive isolation.

Keywords

Drosophila; Chromosomal Inversions; Population Genomics; Recombination; Linkage Disequilibrium; Reproductive Isolation; Speciation

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Author Contributions

ZLF, SAK, NP, and SWS conceived the project. SWS analyzed the fitness set data. ZLF, SAK, NP, and SWS wrote the manuscript.

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Data Accessibility

Data and Computer Archive: Data was previously published (Fuller et al., 2016, 2017). The computer code is found in a collection within Scholarsphere (https://scholarsphere.psu.edu/collections/h44558f89j) entitled “Computer Code for “How chromosomal rearrangements shape adaptation and speciation in *Drosophila*” to appear in Molecular Ecology”

Introduction

Chromosomal inversions were first discovered in 1913 by Alfred Sturtevant as strong crossover modifiers segregating in natural populations of *Drosophila melanogaster* (Sturtevant, 1917). Because visible markers were required to demonstrate inverted gene order, the analysis of chromosomal inversions remained restricted for the next two decades to *D. melanogaster* and the closely related *D. simulans* (Sturtevant, 1921; Sturtevant & Plunkett, 1926). The development of cytogenetic methods that visualized the repeatable patterns of bands and puffs on *Drosophila* polytene chromosomes (Painter, 1934) opened up a more direct method of analysis of structural variation that exists within populations (Dobzhansky & Sturtevant, 1938) and between species (Sturtevant & Novitski, 1941; Sturtevant & Tan, 1937). Single chromosomal rearrangements generated by spontaneous inversions were already known to exist as fixed differences between closely related species of *Drosophila* (Sturtevant, 1921), but as more *Drosophila* species were examined, the degree of gene rearrangement was also found to vary among species., Some species have only one gene order on each chromosome, while have an extensive array of inversion polymorphisms segregating on some or all major chromosomal arms (Dobzhansky & Sturtevant, 1938; Dubinin, Sokolov, & Tiniakov, 1936; Sperlich & Pfriem, 1986).

Careful examination of polytene chromosomes and fixed inversion differences between closely related species were used to develop the first species phylogenies based on genetic characters (Ashburner & Lemeunier, 1976; Dobzhansky, 1944; Sturtevant & Dobzhansky, 1936; Wasserman, 1960). In this case, fixed inversion differences were assumed to be neutral taxonomic characters to discriminate among species. Maps of chromosomal banding patterns were also found to vary among individuals of the same species. Within species inversion polymorphisms were also initially considered neutral variants because only gene order and not gene content differs between arrangements, and thus the geographic distribution of arrangements were thought to be governed by non-selective mechanisms (Dobzhansky & Queal, 1938). The collaboration between Theodosius Dobzhansky and Sewall Wright (Dobzhansky & Wright, 1941, 1943; Provine, 1986; Wright & Dobzhansky, 1946; Wright, Dobzhansky, & Hovanitz, 1942) led to a selective paradigm that emerged from pioneering research from observation of the spatio-temporal distributions of inversions in nature to the subsequent use of population cage experiments to estimate fitnesses of karyotypess (Dobzhansky, 1955; Lewontin, 1974; Provine, 1986).

Despite extensive survey data that quantified levels of variation within and between populations as well as structural differences between species, no clear consensus emerged for the evolutionary genetic mechanisms responsible for (1) the origin of inversion mutations, (2) their subsequent establishment within populations, or (3) their ultimate fixation or elimination from populations (Dobzhansky, 1943, 1948b). This highly productive research program fell out of favor as new genetic markers, such as allozymes, restriction fragment length polymorphisms, nucleotide sequences, and microsatellites were used to address questions of genic and genomic evolutionary processes. Beginning in the 2000s, the application of DNA sequencing technology in natural populations led to the discovery of inversions in organisms other than *Drosophila* and a renaissance of both empirical and

theoretical research on inversion polymorphism ensued (Jones et al., 2012; Kirkpatrick & Barton, 2006; Le Poul et al., 2014; Lowry & Willis, 2010; Stefansson et al., 2005).

Inversion polymorphisms have been implicated in a wide variety of evolutionary processes including chromosomal evolution, complex trait evolution, local adaptation, and speciation. Inversions alter the structure of genomes at different rates, *e.g.,* (genome rearrangement in platyfish Amores et al., 2014; and mosquitos Neafsey et al., 2015). Inversions are observed to capture allelic combinations involved in polygenic traits (sperm morphology and swimming speed in songbirds Kim et al., 2017; reproductive morphotypes in ruffs Küpper et al., 2015; reproductive output in humans Stefansson et al., 2005). Inversions capture suites of genes underlying traits involved in local adaptation (life history traits in mosquitos Cheng et al., 2012; monkey flowers in this issue Coughlan & Willis; flowering time and annual/perennial life history strategies in monkey flowers Lowry & Willis, 2010; altitude and dispersal in teosinte Pyhäjärvi, Hufford, Mezmouk, & Ross-Ibarra, 2013; migratory behavior in Atlantic cod Sinclair-Waters et al., 2018; neurotransmitters in honey bees Wallberg, Schöning, Webster, & Hasselmann, 2017). Inversions have also been shown to capture genes involved in reproductive isolation between species (Cheng et al., 2012; rats Engelbrecht, Taylor, Daniels, & Rambau, 2011; monkey flowers Fishman, Stathos, Beardsley, Williams, & Hill, 2013; sticklebacks Jones et al., 2012; Drosophila Lohse, Clarke, Ritchie, & Etges, 2015; Lowry & Willis, 2010; Drosophila Noor, Grams, Bertucci, & Reiland, 2001; Pyhäjärvi et al., 2013; Sinclair-Waters et al., 2018; Stefansson et al., 2005; Wallberg et al., 2017).

Karyotypes and karyotypic frequencies cannot adequately test hypotheses about the common ancestor of arrangements, the age of arrangements, the role that selection plays in their maintenance, and the gene targets of selection; all of which are critical in defining how inversions arise, are established, maintained, and are fixed in populations. The advent of high throughput sequencing has inspired a renewed interest in testing hypotheses about the role genetic changes within inverted and non-inverted regions play in the evolution of populations and the divergence of species. Nucleotide and genome sequence data can better identify breakpoints, infer phylogenies, infer the history of different regions of the genome, and detect regions with signatures of selection. Recent genomic investigations of structural variation in the *Drosophila* genus have provided important new insights into how inversions shape adaptation and speciation, including that inversion breakpoints are influenced by selection (Corbett-Detig, 2016), inversions can capture multiple genes that are differentially expressed (Fuller, Haynes, Richards, & Schaeffer, 2016; Lavington & Kern, 2017; Said et al., 2018) and are potential targets of selection (Corbett-Detig & Hartl, 2012; Fuller, Haynes, Richards, & Schaeffer, 2017; Kapun, Fabian, Goudet, & Flatt, 2016; Pool, Braun, & Lack, 2017; Rane, Rako, Kapun, Lee, & Hoffmann, 2015; Simões & Pascual, 2018), rearrangements are fixed extensively between species (Bhutkar et al., 2008), and they can prevent the spread of reproductive isolation genes between sibling species (Kulathinal, Stevison, & Noor, 2009; Lohse et al., 2015; McGaugh & Noor, 2012; Noor, Grams, Bertucci, & Reiland, 2001; Sanchez-Flores et al., 2016).

The prevailing view prior to the availability of nucleotide and genome sequence data was that inversion polymorphisms were adaptive, but the mechanisms for the establishment and maintenance of the rearrangements were unclear. Stable clines of inversion frequencies

correlated with environmental gradients provided convincing albeit circumstantial evidence that inversion polymorphisms were adaptive, especially when latitudinal clines are recapitulated in the northern and southern hemispheres on multiple continents (Balanya, Huey, Gilchrist, & Serra, 2009; Hoffmann, Sgro, & Weeks, 2004; Kennington & Hoffmann, 2013). The long-term stability of such clines, and concomitant stable seasonal cycling of inversion frequencies (Dobzhansky, 1943; Knibb, 1982; Stalker, 1980), provides some of the strongest evidence of local adaptation to spatio-temporally varying selection (Cogni et al., 2017; Endler, 1977). In a particularly well-characterized example, the relative frequencies of third chromosome arrangements of *Drosophila pseudoobscura* form an east-west cline across the Southwestern United States, a distribution that has remained stable since its initial description by Dobzhansky nearly 80 years ago (240 generations) (Anderson et al., 1991; Dobzhansky, 1944), while loci on the other autosomes show no evidence for clinal variation (Kovacevic & Schaeffer, 2000; Table 26 in Lewontin, 1974; Riley, Hallas, & Lewontin, 1989; Schaeffer & Miller, 1992), suggesting that the clines are maintained despite the homogenizing effect of gene flow. Population cage experiments in the laboratory also aided in understanding the forces acting on gene arrangement polymorphisms (Dobzhansky, 1948c, 1950; Pavlovsky & Dobzhansky, 1966; Wright & Dobzhansky, 1946).

Population cage experiments showed that two inversions, say A and B, collected from population X reached stable equilibria over several generations (Wright & Dobzhansky, 1946), but the equilibrium points reached differed for the A and B arrangements drawn from localities X and Y (Dobzhansky, 1948c). Mixing pairs of arrangements from different geographic populations, say arrangement A from locality X (A^X) and arrangement B from locality Y (B^Y), failed to reach stable equilibria. Yet, stable equilibria were restored when homokaryotypes had chromosomes from different populations (A^X/A^Y) and the heterokaryotypes had chromosomes from the same population (A^X/B^X or A^Y/B^Y) (Dobzhansky, 1950). Dobzhansky favored overdominance being responsible for the stable equilibria, while Wright's calculations found that frequency dependent selection fit the frequency change data better (see pages 807–809 in Lewontin, Moore, Provine, & Wallace, 1981; Pavlovsky & Dobzhansky, 1966; Wright & Dobzhansky, 1946). Dobzhansky's overall model was that the inversions captured coadapted genes not only among the different arrangements, but also among the same arrangement from different populations, however, this hypothesis is inconsistent with nucleotide sequence data that fails to observe differentiation of homosequential inversions among localities (Fuller et al., 2017; Schaeffer et al., 2003).

Inversions are often thought to be locally adaptive because of their ability to maintain associations among multiple alleles that confer higher fitness in particular environments (Kirkpatrick & Barton, 2006). Heterozygosity for chromosomal inversions severely reduces the rate of recombination through multiple mechanisms reviewed below, thereby preventing multiple beneficial alleles and/or epistatically interacting loci from recombining. Polygenic adaptation to heterogeneous environments is sensitive to recombination rates. Typically, rates of adaptation are thought to increase in the presence of recombination (McDonald, Rice, & Desai, 2016) presumably because beneficial alleles can become unlinked from deleterious alleles and new positive epistatic combinations of alleles with respect to fitness can form more rapidly. On the other hand, recombination will also generate maladaptive

combinations and beneficial associations can be broken apart (Charlesworth & Barton, 1996). Although inversions are widely thought to be favored because they suppress recombination in the heterozygous state, the potential evolutionary forces that favor their establishment, then either maintain them at appreciable frequencies or drive them to fixation or loss remain unclear in many cases.

Here, we present a review of case studies from the obscura subgroup of *Drosophila* of how spontaneous inversions originated and were established in *D. pseudoobscura* populations, then once established how inversions contributed to adaptation, and ultimately what role inversions may have played in the formation of new species. Recent data from *Drosophila* support the hypothesis that inversions are established because of their indirect effects on fitness through recombination suppression that promotes local adaptation to heterogeneous environments. The reduction of recombination within inverted regions also leads to the accumulation of genetic differences within the inverted segments. We review recent findings about the role that inversions play in establishing species boundaries. We focus our analyses on *D. pseudoobscura* and *D. persimilis,* which are model systems for understanding the population genetics of inversions within species as well as the evolutionary genetics of differences between species.

The Nature of Recombination Suppression in Inversions

Chromosomal inversions alter gene order, but not gene content, such that the major genetic effect of inversion heterozygosity is strong recombination suppression. The direct fitness effects of inversion heterozygosity in *Drosophila* could be minimal and confined to the potential for gene disruption or gene expression alteration at breakpoints (Fuller et al., 2016, 2017). Alternatively, the population genetic consequences of forming large blocks of tightly linked allelic variants may confer indirect fitness effects on gene rearrangements that ultimately govern the establishment phase of chromosomal inversion evolution. Thus, in a population genetic framework, natural selection drives the evolution of common chromosomal inversions and recombination suppression is the trait selected upon (but not necessarily the trait selected for). There are three mechanisms that can suppress recombination in inversion heterozygotes, and the relative importance of these mechanisms can vary between taxonomic groups. In reverse chronological order they are: (1) a selective process where crossing-over inside of inverted regions causes gross gametic aneuploidy and therefore zygotic lethality, *e.g.,* as observed in maize and mice (Koehler et al., 2002; McClintock, 1941), (2) a mechanical process where crossing-over inside of inverted regions cause dicentric chromosomes that segregate to the polar bodies in female meiosis and are never transmitted, *e.g.,* as observed in *Drosophila* and *Sciara* (Carson, 1946; Sturtevant & Beadle, 1936), and (3) crossover modification processes where the distribution of crossover events is shifted away from inverted regions (Crown, Miller, Sekelsky, & Hawley, 2018; Lucchesi & Suzuki, 1968; Sturtevant & Beadle, 1936). Although the transmission genetic consequence of these three mechanisms is the same (namely suppressed recombination), the population genetic consequences are drastically different. It is therefore critical for evolutionary models to accurately reflect the different cause(s) of recombination suppression.

In the heterozygous state, pairing and synapsis of chromosomal inversions and the standard arrangement occur normally throughout the inverted region (Gong, McKim, & Hawley, 2005), to achieve this, a characteristic “inversion loop” is formed. The consequence of crossing-over inside this inversion loop is the duplication and deletion of large regions of the chromosome (Figure 1). If the inversion loop does not encompass the centromere, then in addition to the duplications and deletions the crossover products will either be acentric or dicentric (containing no or two centromeres) which causes segregation abnormalities. The consequences of these forms of gross gametic aneuploidy are almost invariably zygotic lethality, with any viable offspring being non-recombinants giving the population-level appearance of recombination suppression. When this mechanism operates it is a form of strong fitness underdominance (*i.e.*, where heterozygotes are less fit than either homozygote), with the basic population genetic expectation that inversions (as the minority allele) would rapidly be eliminated from the population (Wright, 1941). Although this form of recombination suppression was first observed in maize (McClintock, 1931, 1941) and operates in a wide range of organisms, there are some unique features of dipteran meiosis that make this mechanism largely irrelevant for the evolution of *Drosophila* inversion polymorphism.

All brachycerous Diptera have achiasmate male meiosis, which means crossing over is limited to females (see Gethmann, 1988 for rare exceptions). Female meiosis is asymmetric, that is for every one functional egg there are three polar body nuclei formed. In *Drosophila*, these form a linear array with one of the terminal nuclei always becoming the gamete (Huettner, 1924). In a process mathematically equivalent to gametic selection there can be competition among homologous chromosomes, or recombinant chromosomes, to be in a terminal position included in the functional egg; this process is termed true meiotic drive and is potentially a very strong evolutionary force (Lindholm et al., 2016; Sandler & Novitski, 1957). There are several forms of meiotic drive in female meiosis of *Drosophila melanogaster* (Ashburner, Golic, & Hawley, 2005), but perhaps the most important is the preferential segregation of dicentric products of crossing-over in inversion loops to the central position in the linear array of meiotic products. As a result of this meiotic drive, the aberrant chromosomes that would produce lethal zygotes are efficiently sequestered in polar bodies, such that there is no substantial underdominance realized for paracentric inversions in *Drosophila* species (Sturtevant & Beadle, 1936). Although this effectively eliminates direct fitness effects due to segregation problems, the mechanical process does not apply to pericentric inversions, which in turn is likely responsible for their rarity in natural populations of Drosophila (Coyne, Aulard, & Berry, 1991; Coyne, Meyers, Crittenden, & Sniegowski, 1993; Krimbas & Powell, 1992a; Navarro, Betrán, Barbadilla, & Ruiz, 1997). Even though the operation of the mechanical process appears simple at first, the molecular biology and biophysics for segregation and breakage of dicentric chromosomes is not completely understood and is an active area of research (Hill & Golic, 2015; Koehler et al., 2002; Lopez et al., 2015).

Although the transmission genetic and population genetic consequences of the chromosome mechanics have been well-studied and are at the theoretical core of inversion evolution, the preceding mechanisms are limited to explaining only one quarter of recombination suppression inside inverted regions (Novitski & Braver, 1954), which corresponds to 10–

20% of the total chromosome-wide recombination suppression observed in initial studies (Sturtevant & Beadle, 1936), later work (Grell, 1962; Roberts, 1962), and recent investigation of the interference hypothesis (Koury, 2017). Therefore, the vast majority (80–90%) of recombination suppression due to inversion heterozygosity is determined by a third mechanism that shifts the distribution of crossing-over away from inversions – collectively referred to as crossover modifying processes. Unfortunately, almost nothing is known about the causes of these processes and they remain poorly described despite being the major determinant of the selected effect of segregating inversions. In what follows, an outline of the basic components of these effects are described and the interference hypothesis for recombination suppression is introduced as a conceptual framework for future research into the crossover modifying processes.

Just like recombination rates, there are both genetic and environmental sources of variation for recombination suppression and it is a process affected by proximity to structural features of chromosomes (*e.g.*, centromeres, telomeres, boundary sites, heterochromatin, *etc.*) (Comeron, Ratnappan, & Bailin, 2012; Coyne et al., 1993; Hunter, Robinson, Aylor, & Singh, 2016; Koury, 2017). Recombination suppression, however, has an added degree of complexity because its relative strength is dependent on genetic distance to the heterozygous inversion breakpoints. Therefore, it is useful to conceptualize recombination suppression as an interference effect in the broad sense, *i.e.*, as an altered probability of crossing-over conditional on distance to a heterozygous inversion breakpoint. In this theoretical framework, it is possible to account for strong local and diffuse global suppression, both inside and outside the inverted region, as well as position dependent effects of inversion heterozygosity on recombination landscapes (Koury, 2017; McPeek & Speed, 1995; Navarro et al., 1997).

Evidence for interference effects also comes from the reduced rates of single and double crossover events inside the inversion loop (Krimbas & Powell, 1992b). Interestingly, the rates of gene conversion are not as strongly affected (Chovnick, 1973; Korunes & Noor, 2017; Schaeffer & Anderson, 2005), which is consistent with Gong *et al.*'s (2005) observation that the distribution of crossover precursors (such as double strand breaks) are approximately normal for inversion heterozygotes and the suppression is achieved by preventing the crossover maturation of chiasmata. Using experimental constructs to avoid the difficulties associated with dicentric chromosomes, or using viability reduction with pericentric inversions as an indirect measure, the crossover rate inside heterozygous inversions is estimated to be about 25% of wildtype (Coyne et al., 1993; Navarro & Ruiz, 1997; Novitski & Braver, 1954). Thus, the lack of recombination both internal and external to inverted regions is largely determined by the interference effects of heterozygous breakpoints as crossover modifiers.

In comparative studies of recombination suppression by heterozygous inversions, size and position dependent effects have often been noted (Krimbas & Powell, 1992b). Smaller inversions distally placed on chromosomes tend to suppress recombination stronger than larger or more proximal inversions near the centromere, although analysis of the relative importance of size versus position is limited by the irregular distribution of natural variants with incompletely known recombination profiles. The size effect, especially in terms of

double crossover events inside inversions, have long been known to be a result of crossover interference (Krimbas & Powell, 1992b; Navarro et al., 1997; Sturtevant & Beadle, 1936). More surprisingly, similar results in terms of interference and position effects have been shown for reciprocal translocations (Hawley, 1980; Roberts, 1970, 1972; Sherizen, Jang, Bhagat, Kato, & McKim, 2005), demonstrating these effects are not due to inversions *per se*, but rather due to breakpoint heterozygosity from any type of chromosomal rearrangement. Several evolutionary arguments for selection acting on inversion polymorphisms are based on the effects of suppressed recombination, and these have variously (and often conflictingly) claimed that distal inversions are favored (Novitski, 1946), smaller inversions are favored (Krimbas & Powell, 1992b; Olvera et al., 1979), larger inversions are favored (Caceres, Barbadilla, & Ruiz, 1999; van Valen & Levins, 1968), or that inversions disrupting boundary sites are necessary for normal crossing-over (Corbett-Detig, 2016). However, all of the selective explanations of inversion polymorphism make simplifying assumptions about recombination suppression (the selected character) based on the mechanical process. Clearly the majority of the phenotypic effect of heterozygous inversions is neither caused by this mechanism, nor is it limited to the inverted region of the chromosome (Fuller et al., 2016; Lavington & Kern, 2017).

Considering how little is mechanistically known in the model organism *D. melanogaster*, it is hard to speculate about other less studied species. Inversion heterozygosity suppresses recombination in the limited number of *Drosophila* species studied thus far (Carson, 1953; Dobzhansky & Epling, 1948; Singh & Singh, 1987), and does so *via* the same mechanisms, yet the relative strength and importance of these mechanisms is unknown (Krimbas & Powell, 1992b). Given that both recombination maps and crossover interference are known to vary between species, it is reasonable to expect the magnitude and extent of recombination suppression to differ as well (Brand, Cattani, Kingan, Landeen, & Presgraves, 2018; Cáceres, Ranz, Barbadilla, Long, & Ruiz, 1999; True, Mercer, & Laurie, 1996). While this expectation is fulfilled for rates of double crossovers inside inversions, interestingly work in *D. subobscura* (a species with a longer genetic map) shows the effect is in the opposite direction (lower) of expectations (Spurway & Philip, 1952). Indeed, interspecific variation in the distribution of chromosomal inversions, recombination rates, and crossover interference is likely to provide important evolutionary insight to the importance of meiotic phenotypes, and is particularly fruitful ground for further exploration of the interference hypothesis for recombination suppression (Caceres et al., 1999; Gong et al., 2005; Koury, 2017; True et al., 1996).

In summary, it is likely the indirect fitness effects of chromosomal inversions determine their evolutionary fate, and those fitnesses are the combined direct effects of alleles in strong linkage disequilibrium within inverted segments. This linkage is caused by recombination suppression observed for inversion heterozygotes. There are three possible causes of recombination suppression, two of which could generate strong fitness underdominance as a direct effect of inversion heterozygosity. However, the details of meiosis in higher Diptera allow *Drosophila* species to escape the negative consequences of crossover induced chromosomal aberrations (aneuploidy, dicentric chromosomes, *etc.*). In the mechanical process, single crossover events generate acentric and dicentric recombinant products that are never included in the functional egg *via* a meiotic drive mechanism unique to

asymmetric female meiosis only known from studies of Diptera and not other taxa (Carson, 1946; Madan, Seabright, Lindenbaum, & Bobrow, 1984; McClintock, 1941; Sturtevant & Beadle, 1936). Despite the mechanical process being more thoroughly studied than the poorly known interference effects, the former mechanism's operation is limited to the inverted regions and accounts for only 10 to 20 percent of total chromosome-wide recombination suppression (Grell, 1962; Koury, 2017; Roberts, 1962; Sturtevant & Beadle, 1936). In contrast, the crossover modifying process operates both inside and outside inversions, extends chromosome-wide, and accounts for 80 to 90 percent of recombination suppression. Future experimental work, empirical analysis, and evolutionary modelling should focus on the chromosome-wide interference effects caused by the crossover modifying process as the major determinant of recombination suppression for inversion heterozygotes.

The Evolutionary Mechanisms That Establish Inversions in *Drosophila* Populations- A case history in *D. pseudoobscura*

Different species of *Drosophila* offer increasing levels of complexity in the study of inversion polymorphisms in nature. The inversion polymorphisms of *D. melanogaster* are relatively simple in that two major cosmopolitan arrangements are segregating on the large chromosomal arms (X, 2L, 2R, 3L, and 3R) with a large number of minor rare endemic arrangements (Lemeunier & Aulard, 1992). Corbett-Detig and Hartl (2012) showed that the derived arrangements have putative selective targets distributed across the inverted regions. The inversion polymorphisms of *D. robusta* and *D. subobscura* are the most complex in that non-overlapping inversions are found to be in linkage disequilibrium within and between chromosomal arms (Carson, 1958; Krimbas, 1992; Levitan, 1958; Levitan, 1992), which require future studies to understand how each inversion arises, and why the combinations are non-randomly associated with each other. Here, we focus on the third chromosome gene arrangement polymorphism of *D. pseudoobscura*, a system slightly more complex than *D. melanogaster* where a single chromosomal arm segregates for more than 30 different overlapping inversions (Dobzhansky & Sturtevant, 1938), many reaching appreciable frequencies and wide geographic distributions.

D. pseudoobscura has served as model system for testing how the effects of recombination suppression lead to the establishment and maintenance of inversions in natural populations, as there is a wealth of well characterized gene arrangements on its third chromosome that were generated through overlapping inversion mutations that strongly suppress recombination (Dobzhansky & Epling, 1948; Dobzhansky & Sturtevant, 1938; Levine, 1956; Levine & Levine, 1954, 1955). *D. pseudoobscura* is native to the western United States from the Rocky Mountains in the east to the Pacific coast in the west and from British Columbia in the north to Guatemala in the south (Figure 2). A subspecies of *D. pseudoobscura* is found in the highlands near Bogota, Colombia (*D. pseudoobscura bogotana* not shown), that has served as a model for the early stages of speciation (Orr, 1989; Phadnis, 2011; Phadnis & Orr, 2009).

A large body of evidence suggests that the arrangements of the third chromosome are selected. First, the third chromosome's gene arrangement frequencies are found in a geographical cline that has been stable for at least eighty years (Anderson et al., 1991; Dobzhansky, 1944; Schaeffer et al., 2003) with the major transitions in frequencies occurring at the boundaries of major physiographic provinces (Lobeck, 1948) (Figure 2). The gradient of gene arrangement frequencies exists despite the homogenizing effect of extensive migration ($4N_em > 1$) among *D. pseudoobscura* populations (Fuller et al., 2017; Kovacevic & Schaeffer, 2000; Riley et al., 1989; Schaeffer et al., 2003; Schaeffer & Miller, 1992) suggesting that strong selection counters the effects of gene flow. In addition, the gene arrangements cycle in frequency over seasons (Dobzhansky, 1943).

Schaeffer (2008) used gene arrangement frequencies in natural populations to infer karyotypic fitnesses as opposed to the population cage approach of Wright and Dobzhansky (Dobzhansky, 1948c; Wright & Dobzhansky, 1946). The inversion frequency cline can be divided into six niches where the gene arrangement frequencies are similar within niche, but are different between niches. Schaeffer (2008) developed a model of migration-selection balance that estimated karyotypic fitnesses within each niche necessary to transform estimated migrant frequencies to the observed local frequencies. The karyotypic fitness estimates in the six niches do not predict the stable maintenance of all arrangements in a single niche, but instead that they are maintained as a stable protected polymorphism across all niches (Levene, 1953; Levins & MacArthur, 1966). The application of Levins and MacArthur (1966) fitness set analysis suggests that the environments of adjacent niches are similar, but that the climates of non-adjacent niches are different (see supplemental information). In addition, the gene arrangement frequencies that would maximize mean fitness in each niche differ from the observed gene arrangement frequencies suggesting that migration creates a genetic load in each niche (Figure 3).

Although the fitness of each arrangement can be estimated from their frequency distribution in space, the genetic basis of their origin, whether selection plays a role, and if so, what the genetic basis for the selection has remained elusive. Genomic and transcriptomic analysis of the *D. pseudoobscura* third chromosomal gene arrangements have provided clues about how inversions arose and the genetic forces that led to their establishment in *D. pseudoobscura* populations. The discovery of transposable elements (TEs) suggested that inversion mutations may occur through ectopic exchange between TEs in reverse orientation (Lim & Simmons, 1994). In *D. pseudoobscura*, no known TEs were found at inversion breakpoints (Richards et al., 2005). Instead, sets of small repeat sequences approximately 100 – 200 bp in length were found at the proximal and distal inversion breakpoints in reverse orientation suggesting that these repeats paired and generated the reversal of the region between the repeats (Figure 4). This differs from the structure of breakpoints in other *Drosophila* species, where random breaks have been typically observed (Wesley & Eanes, 1994) or staggered cuts in the breakpoint region results in duplication of genes adjacent to the breaks (Calvete, Gonzalez, Betran, & Ruiz, 2012; Matzkin, Merritt, Zhu, & Eanes, 2005; Puerma, Orengo, & Aguadé, 2016; Ranz et al., 2007). The *D. pseudoobscura* third chromosomal arrangements result from single mutational events based on the monophyly of the major inversions (Fuller et al., 2017; Wallace, Detweiler, & Schaeffer, 2011). A central question is how the *D. pseudoobscura* arrangements were established in populations after they arose.

Kirkpatrick and Barton (2006) summarized the major mechanisms that could establish new inversion mutations in populations. The first possibility is that inversions are neutral and their frequency increases or decreases via genetic drift (Kimura, 1968; Lande, 1984; Lande, 1985). Under the drift model, the current frequency cline in *D. pseudoobscura* represents a transient phase of gene arrangement polymorphism. This explanation is not likely, however, given the effective population sizes of *D. pseudoobscura* are estimated to be between 1.9 to 4.5×10^6 based on the mutation ($4N_e\mu$) and recombination ($4N_ec$) parameters (Schaeffer, 1995), which is much larger than drift models require (Lande, 1984). The second explanation is that a new inversion mutation is driven to higher frequency because it captured a single sweeping beneficial allele (Maynard Smith & Haigh, 1974).

The third possibility is that the breakpoints create variation that selection acts upon, which is a direct effect of the inversion mutation. This could take several forms such as disrupting the structure of genes (Puerma et al., 2016) or altering gene expression by separating the coding sequence from its upstream *cis* regulatory sequences (Puig, Caceres, & Ruiz, 2004). The disruptions caused by breakpoints could have more regional effects if they occur within regions of coordinated gene expression, *i.e.,* topologically associated domains (TADs) (Hou, Li, Qin, & Corces, 2012). Another direct effect of an inversion could be to set up conditions for meiotic drive where one arrangement has an advantage over another based on differences in length of non-sister chromatids (Novitski, 1951). Crossing-over in overlapping inversion heterozygotes generates asymmetry in non-sister chromatids in Meiosis II, which causes meiotic drive favoring the more distally positioned inversions (Koury, 2017). No evidence for meiotic drive was observed when comparing transmission of arrangements in heterokaryotypes in females versus males (chiasmate versus achiasmate segregation) (Meisel & Schaeffer, 2007). Interestingly, the historical sequence of inversions in *D. pseudoobscura* is consistent with a high fitness of distal inversions on the third chromosome (Koury, 2017). The success of particular gene arrangements could also result from the proximity of breakpoints to pairing sensitive sites that promote crossing over (Corbett-Detig, 2016).

A fourth mechanism to establish new inversions is an indirect effect, where gene rearrangements are established because of their ability to suppress recombination in heterozygotes (Sturtevant & Beadle, 1936). Inversions would be favored if the fitness of a particular gametic combination is greater than the mean fitness of the population (Charlesworth & Charlesworth, 1973). Inversions might also be favored to increase if they capture regions relatively devoid of deleterious recessive mutations (Nei, Kojima, & Schaffer, 1967). Dobzhansky suggested that inversions would increase in frequency if the mutation captured alleles at multiple loci that act epistatically together (Dobzhansky, 1950). Ohta and Kojima (1968) mathematically modeled this idea using a time heterogeneous branching process to incorporate the fitness decay of inversions as deleterious mutations accumulate, and were able to demonstrate that the ultimate fate of inversions is the selective elimination from populations unless there is unique epistasis associated with alleles inside the inversion. Kirkpatrick and Barton (2006) suggested that inversions could be favored to increase if they captured locally adapted alleles in a species where migration allowed maladapted gene combinations to be transported between habitats.

These different scenarios are expected to result in different signatures at the sequence level depending on the age of the inversion (Guerrero, Rousset, & Kirkpatrick, 2012). It is generally assumed that a new derived inversion mutation will occur in a single individual from an ancestral arrangement and increase in frequency either through neutral or selective forces. The derived arrangement will capture a multi-site genotype from one of the segregating ancestral chromosomes. If the new arrangement is neutral, then over time, genetic flux (gene conversion and double cross overs) among arrangements will homogenize variation within the central inverted regions with the most differentiated regions at the breakpoints (Navarro, Barbadilla, & Ruiz, 2000; Navarro et al., 1997). The degree of homogenization depends on the age of the arrangement, with older arrangements being more homogenized (see the dashed line in Figure 1 of Guerrero et al., 2012). On the other hand, if a new arrangement captures one or more selected genes, then genetic flux will homogenize variation in non-selected regions and as the inversion increases in age, selected genes will become increasingly differentiated (see the right hand side of Figure 1 in Guerrero et al., 2012). It should be noted that the Guerrero *et al.* (2012) do not incorporate new mutations in their model.

Vann (1966) showed that artificially induced inversions are able to establish within laboratory cultures in eight to nine generations, but the young age of these new arrangements would prevent one from distinguishing among establishment hypotheses with nucleotide sequence data. One would expect that all copies of these induced arrangements would be nearly identical in sequence.

The *D. pseudoobscura* inversion polymorphism is relatively old, with the different arrangements ranging in age from 0.51 to 1.38 million years or equivalently 1.5 to 4.14 million generations ago (Wallace et al., 2011) based on three generations per year (Aquadro, Weaver, Schaeffer, & Anderson, 1991). The majority of naturally occurring inversion heterozygotes differ by at least two inversion events such that most genetic flux in heterokaryotypes will be in the form of gene conversion, estimated to occur at a rate of 3.4×10^{-6} per generation, which is 100 times greater than the mutation rate in this species (Schaeffer & Anderson, 2005), but is 3 to 6 times lower than the single generation gene conversion estimate (Korunes & Noor, 2019). The frequency of heterokaryotypes among niches varies from 10 to 70%, but the mean frequency is expected to be near 40% across all niches based on the extensive migration observed among populations (Kovacevic & Schaeffer, 2000; Riley et al., 1989; Schaeffer & Miller, 1992). Given the age of the inversions and level of genetic flux among arrangements, differentiation is predicted to be significantly decreased in the central regions of the inversion (Guerrero et al., 2012).

Fuller et al. (2016, 2017) used evidence from genomic and transcriptomic analyses of the third chromosome of *D. pseudoobscura* to address these hypotheses. Fuller *et al.* (2017) mapped the locations of seven pairs of inversion breakpoints and found no evidence of direct position effect mutations that disrupted the structure of genes near the inversion breakpoints (Fuller et al., 2017) or altered gene expression of loci in the immediate proximity of the breakpoints (Fuller et al., 2016). The expression data, however, did not rule out position effects that disrupt transcriptional variation at a larger genomic scale due to alteration of TADs.

Fuller *et al.* (2017) sequenced the complete genomes of 54 *D. pseudoobscura* individuals, representing six different third chromosome arrangements, to detect loci with an abundance of fixed derived mutations (Derived Allele Frequency test - DAF) or significantly long arrangement specific branch lengths (Population Specific Branch Length test - PSBL). A total of 277 outlier loci were discovered with the DAF and PSBL tests which also harbored arrangement specific fixed amino acid changes.

Fuller *et al.* (2016) quantified gene expression levels among gene arrangements to test whether any loci contained within the inversions were differentially expressed in different life stages (Fuller et al., 2016). They detected 45, 45, and 187 genes on the third chromosome that were differentially expressed in larvae, females, or males, respectively, for a total of 205 genes. The vast majority of differentially expressed genes (95%) were located within the overlapping inverted regions. More recently, Lavington and Kern (2017) and Said *et al.* (2018) also demonstrated that gene arrangements in *D. melanogaster* captured differentially expressed genes within inverted regions. Said *et al.* (2018) ruled out position effects by showing in an elegant synthetic inversion experiment, that an inversion mutation by itself does not lead to widespread gene expression changes within an inversion, suggesting that linked genetic variation captured by the inversion is responsible for transcriptional differences rather than the direct structural effects introduced by the inversion. Their data suggest that the effects of suppressed recombination between inversions in natural *D. melanogaster* populations maintain arrangement specific patterns of gene expression with potential phenotypic differences and selectable variation. Functional genomic experiments are necessary to demonstrate that transcriptional differences lead to changes in phenotype and fitness, and thus are possible targets of selection. Moreover, similar synthetic inversions are yet to be tested in *D. pseudoobscura* to exclude the position effect hypothesis.

Taken together, multiple outlier loci are located within the inverted regions of chromosome three and these loci have potentially selectable variation at the amino acid or transcriptional levels. In the majority of cases, this variation exists as changes to encoded proteins rather than gene expression differences. If we assume that outlier loci have adaptive alleles, two non-mutually exclusive models could explain the multiple outlier loci: (1) the initial inversion captured a single sweeping allele and other adaptive alleles were added successively to the arrangement later or (2) the initial inversion events captured suites of adaptive alleles at the outset. The first model of successive addition of adaptive alleles seems inconsistent with the observed data especially when you consider that the Pikes Peak has 127 outlier loci. Successive sweeps would be expected to continually remove genetic variation from the inverted region making it difficult to recover genetic diversity across the inverted region. None of the *D. pseudoobscura* arrangements show low levels of genetic diversity. The second model suggests that the suite of outlier alleles existed prior to the inversion event. Once captured within the inversion, gene conversion in heterokaryotypes begins to homogenize regions within the inversion except for those that affect fitness. Over time, only the adaptive loci will be differentiated among arrangements. The *D. pseudoobscura* arrangements are 0.5 million years old or older (Wallace et al., 2011) and the rates of gene conversion in heterokaryotypes are sufficiently high (Korunes & Noor, 2019; Schaeffer & Anderson, 2005) to reveal signatures of adaptation if they exist. Functional

genetic studies are needed to rule out selective neutrality of the outlier alleles as an explanation for the inversion polymorphism. In addition, new theoretical models are needed to address what the pattern and organization of genetic diversity will be under these two models. Therefore, we conclude that the *D. pseudoobscura* inversions were most likely established and maintained due to the indirect effects of inversions as recombination suppressors in response to local adaptation or epistastic selection among loci. Potential functions of the outlier genes that may drive selection are sensory or detoxification activities (Fuller et al., 2016, 2017). An open question about the molecular genetics of inversions is: are there interactions among loci in inversion heterozygotes? Selection does not act on single inversions, but on the individual or inversion karyotype. Transcriptome data show that the majority of loci show additive effects in heterozygous individuals with only a few loci showing underdominant or overdominant expression (Fuller et al., 2016), which suggests that there are few interactions among loci at the expression level. Again, functional genetic experiments are needed to determine if these gene expression differences have effects on fitness.

We now know that selection in heterogeneous environments plays a prominent role in the establishment of the third chromosome inversions of *D. pseudoobscura*. In addition, selection in heterogeneous environments has led to clinal variation in the frequency of gene rearrangements in space. What happens if populations become isolated?

The Role of Fixed Inversions in the Speciation Process-A case history from *D. pseudoobscura* and *D. persimilis*

The establishment of inversion polymorphisms within heterogeneous environments may well set the stage for the formation of new species. Interest in speciation genetics traces its origins to the 1930s. At the time, *D. melanogaster* was the overwhelming favorite model species and its closest relative for genetic studies of reproductive isolation was *D. simulans*. *D. melanogaster* and *D. simulans* were not an idealas a model for speciation genetics because crosses between these species yielded only dead or sterile hybrids (Sturtevant, 1920). *D. obscura* Race A and B (Lancefield, 1929), later renamed *D. pseudoobscura* and *D. persimilis* (Dobzhansky & Epling, 1944; Frolowa & Astaurow, 1929), were found to be morphologically indistinguishable and yielded sterile males and fertile females in reciprocal genetic crosses. With fertile hybrid female offspring, crosses between the two species allowed Dobzhansky (1936) and later Orr (Orr, 1987) to attribute hybrid male sterility to at least nine genes distributed across the genome with genes on the X chromosome having the largest effect.

Figure 5 shows the genomic rearrangement differences on the six chromosome arms of *D. pseudoobscura* and *D. persimilis*. The X chromosome of the two species is comprised of two arms, XL and XR, which correspond to the A and D Muller (Muller, 1940) elements. XL has a fixed paracentric inversion between the two species, while XR is segregating for two gene arrangements within *D. persimilis*: Standard and Sex Ratio (SR). *D. persimilis* males that carry the SR arrangement produce over 95% daughters. The *D. persimilis* SR chromosome is homosequential and identical by descent with the *D. pseudoobscura* XR Standard

chromosome gene arrangement (Fuller, Leonard, Young, Schaeffer, & Phadnis, 2018). *D. pseudoobscura* is also segregating for an independent Sex Ratio chromosome, which differs from the Standard arrangement by three non-overlapping inversions. The second chromosome (Muller E) has a fixed inversion difference between the two species. The third chromosome (Muller C) is polymorphic for paracentric inversions in both species, but only one gene arrangement is shared in common, the relatively older Standard (note this naming refers to the third chromosome) arrangement (Figure 6). Meanwhile, chromosomes four (Muller B) and five (Muller F) have the same gene arrangement, *i.e.,* are collinear in both species.

Cytogenetic and molecular genetic data have suggested that *D. pseudoobscura* and *D. persimilis* hybridize (Dobzhansky, 1973; Machado & Hey, 2003; Machado, Kliman, Markert, & Hey, 2002; McGaugh & Noor, 2012; Noor, Garfield, Schaeffer, & Machado, 2007; Powell, 1983; Wang & Hey, 1996; Wang, Wakeley, & Hey, 1997). Dobzhansky (1973) observed a single female out of 27,099 examined that was heterozygous for third chromosome gene arrangements specific to either *D. pseudoobscura* or *D. persimilis* third chromosome (CH/MN, see Figure 6). Dobzhansky concluded that despite evidence for an interspecific hybrid, reproductive isolation is likely complete because of the absence of widespread sharing of third chromosome gene arrangements between species. Additionally, Powell (1983) observed an unambiguous hybrid rate of 3/30,000 in collected females. It is possible that both of these hybridization rates underestimate the true value, as the species are not equally abundant in sympatric locations, and hence the opportunity for hybridization may vary greatly depending on location. Thus, although hybrids have rarely been observed in nature or in the laboratory, a direct estimate of the level of present gene exchange between the two species is lacking.

Mitochondrial and nuclear genes have shown varying degrees of shared nucleotide polymorphisms or low genetic divergence between *D. pseudoobscura* and *D. persimilis*. Shared polymorphisms could result from the persistence of variation from the common ancestral population or they could indicate hybridization or gene flow between the species. Evidence from the study of mitochondrial DNA haplotypes has resulted in conflicting support for the existence of shared haplotypes between *D. pseudoobscura* and *D. persimilis* (Hale & Beckenbach, 1985; Powell, 1983; Wang & Hey, 1996). In the nuclear genome, the divergence population genetics approach analyzed nucleotide sequences from multiple loci from the two species and found differentiated as well as undifferentiated loci (Machado & Hey, 2003; Machado et al., 2002; McGaugh & Noor, 2012; Noor et al., 2007; Wang & Hey, 1996; Wang et al., 1997). The differentiated genes map within fixed inversions between the two species and the undifferentiated genes were located in collinear regions of the genome. Furthermore, Noor *et al.* (2001) showed that genomic regions associated with male hybrid sterility also largely map to the inverted regions of XL and chromosome two. Noor *et al.* (2001) concluded that inversions play an important role in the speciation process by preventing the spread of incompatibility genes between these two species, while allowing gene flow in collinear regions.

Linkage disequilibrium (LD) based tests of shared derived mutations have been used to identify loci that have experienced gene flow between the two species (Machado et al.,

2002), however, it is not clear how these statistics behave when shared polymorphisms are ancestral. Models based on Isolation with Migration (IM) are consistent with gene flow between the two species at loci in collinear regions of the genome, but not within inverted regions (Hey & Nielsen, 2004; Machado & Hey, 2003; Machado et al., 2002). The pattern that emerges is that genes within inverted regions show little evidence for gene flow while collinear regions suggest higher levels of genetic exchange between *D. pseudoobscura* and *D. persimilis* (Machado, Haselkorn, & Noor, 2007; Noor et al., 2007). Kulathinal et al. (2009) observed that shared polymorphisms are greater for sympatric (*D. pseudoobscura* vs. *D. persimilis*) as opposed to allopatric (*D. pseudoobscura pseudoobscura* vs. *D. pseudoobscura bogotana*) species pairs leading the authors to conclude that isolation with gene flow rather than ancestral polymorphism explained the shared polymorphism in collinear regions (Kulathinal et al., 2009). Thus, the IM model proposes that incompatibility genes accumulate throughout the genome, but are selectively removed from collinear regions by natural selection when they are shuffled between two species via gene flow, yet persist within inverted regions due to recombination suppression (Noor, Grams, Bertucci, & Reiland, 2001). However, the ancestral polymorphism hypothesis could not be definitively excluded from the Kulathinal et al. (2009) analysis. (Kulathinal et al., 2009)

When and how did the inversions get fixed between *D. pseudoobscura* and *D. persimilis* relative to when gene flow between these two species ceased? There are two contrasting views on this question. On the one hand, genome comparisons have suggested that both of the fixed X chromosome inversion between *D. pseudoobscura* and *D. persimilis* originated at the time of divergence from the outgroup species *D. miranda*, but with the XL inversion fixing prior to the XR inversion (Fuller et al., 2018; McGaugh & Noor, 2012). It is not clear from the IM model, or from invoking post-speciation gene flow, what forces fixed these inversions within isolated populations. Is it that inversions spread initially in a population through a local adaptation mechanism (e.g., see preceding section, Kirkpatrick and Barton (2006)) and subsequently accumulated mutations that generate speciation phenotypes?

On the other hand, a more recent analysis of the SR and Standard XR arrangements of *D. persimilis* and *D. pseudoobscura* revealed a pattern of phylogenetic discordance at inversion breakpoints that suggest the inversions arose in the ancestral population (Fuller et al., 2018). Moreover, by estimating sequence divergence between inverted and collinear regions, the evolutionary history of all fixed chromosome differences was reconstructed and provided evidence that, in fact, all inversions distinguishing the two species arose in the ancestral population and were freely segregating for a substantial period of time prior to the beginning of speciation. This model alternatively suggests that inversions may arise through similar mechanisms of local adaptation, yet in the ancestral population. While segregating in this ancestor, the genomic regions spanning the inversion would accumulate genetic divergence by the action of suppressed recombination in heterozygotes. If populations then become allopatric, there may be incomplete lineage sorting of the ancestrally segregating inversions. Here, at the onset of speciation, collinear regions would appear undifferentiated, yet inverted regions would already harbor high levels of divergence and perhaps be predisposed to the formation of incompatible alleles (Fuller et al., 2018). Importantly, these results did not rule out the presence of post-speciation gene flow (Hey & Nielsen, 2004; Kulathinal et al., 2009; Machado et al., 2002; Noor et al., 2007; Noor, Johnson, & Hey, 2000; Wang et al., 1997),

but demonstrated that exchange upon secondary contact was not the sole mechanism for observed patterns of increased divergence across fixed inversion differences in *D. pseudoobscura* and *D. persimilis*. (Fuller et al., 2017; Fuller et al., 2018; Kirkpatrick & Barton, 2006).

It is intriguing to speculate upon plausible scenarios responsible for the split between *D. pseudoobscura* and *D. persimilis*. For example, there is some evidence for an association between altitude and the frequency of the shared Standard (ST) arrangement on the third chromosome in both species in the Sierra Nevada mountains, where ST is found at frequencies of 10–30% (Figure 2)(Dobzhansky, 1944). Furthermore, Dobzhansky had suggested that *D. persimilis* inhabits higher elevations (>5,000 feet) than *D. pseudoobscura* (<5,000 feet) (page 14 in Dobzhansky & Epling, 1944). Perhaps the fixed inversion differences observed at present were initially under selection in the ancestral population for adaptation to higher elevation or colder climates. Other phenotypic differences between *D. pseudoobscura* and *D. persimilis* have been noted for a number of other ecological factors, including humidity, heat stress, and food availability, among others(Coyne, Bundgaard, & Prout, 1983; Matzkin, Watts, & Markow, 2009). However, without a detailed understanding and characterization of the ecology and life history of current *D. pseudoobscura* and *D. persimilis* populations, possible speciation scenarios remain little more than speculation (Dobzhansky, 1948b; Navarro & Barton, 2003).

Concluding remarks

The genus *Drosophila*, and particularly the sister species of *D. pseudoobscura* and *D. persimilis*, is an important model for the study of evolutionary genetics and genomics. Genes have been conserved on chromosomal arms, but gene order has been shuffled extensively both within and between species (Carson, 1992; Dobzhansky, 1944; Levitan, 1982; Throckmorton, 1982; Wasserman, 1982) through the mechanism of chromosomal inversion. While inversions were initially used as a neutral character to discriminate between species, their role as potent drivers of evolutionary change and adaptation in the genus has become increasingly appreciated. If, as we argue, inversions are selected as a result of their recombination suppression in heterozygotes, it is astounding to learn that only 10–20% of this phenotype can be explained by accepted cytogenetic mechanisms. Further research should focus on clarifying the mechanism(s) by which inversion heterozygosity causes recombination suppression, now known to be a form of crossover distribution modification. From empirical, experimental, and theoretical studies ranging from polytene chromosome analysis to high-throughput sequencing, a picture has emerged whereby inversions underlie adaptation across heterogeneous environments in *D. pseudoobscura* and *D. persimilis* through their indirect effects as recombination suppressors (Fuller et al., 2018). Further ecological studies are needed to better characterize the heterogeneous environments experienced by both species; for example, the separate challenges faced by a limited dispersal larval phase and an adult phase capable of extensive migration among diverse habitats (Coyne et al., 1982; Coyne, Bryant, & Turelli, 1987). One of the primary reasons that genomic and bioinformatic analysis has been pursued was to obtain valuable clues about the ecology of *D. pseudoobscura*, which has remained difficult to study. Despite its lengthy history in evolutionary genetics, many basic aspects of its life history remain unknown.

Genomic analyses have suggested that *D. pseudoobscura* may have meaningful interactions with plants in its environment based on detoxication proteins that are enriched among potential selective targets (Fuller et al., 2016, 2017). Adapting polygenic traits for one habitat may be maladaptive for another and evidence suggests that inversions are a potential evolutionary mechanism to both maintain sets of locally adapted loci, while preventing exchange with maladaptive migrant alleles. The ecological diversification of gene arrangements to particular habitats is followed by genetic diversification within inverted regions, which may provide the raw material for incompatibility genes to emerge and isolate populations forming new species. While we call for additional research in this pair of sister species, we also recognize and call attention to the cactophilic species of *Drosophila* as an additional important model system to understand inversion polymorphism because of the well-known ecology and the extensive inversion polymorphism present (Guillén et al., 2015; Lohse et al., 2015; Wasserman, 1982, 1992).

There is still much work to be done. While inversions present a powerful model for adaptation and speciation, recombination suppression presents challenges for mapping possible Dobzhansky-Muller Incompatibility genes and other loci involved in differences between species and arrangement type (.e.g, cold tolerance, cuticular hydrocarbons, courtship song, among other behavioral traits). It is intriguing to consider that the extent of gene arrangement variation seen within and between *Drosophila* species is reflective of an ecologically driven process of adaptation to heterogeneous environments and to speculate upon the role of chromosomal inversions in the formation of species.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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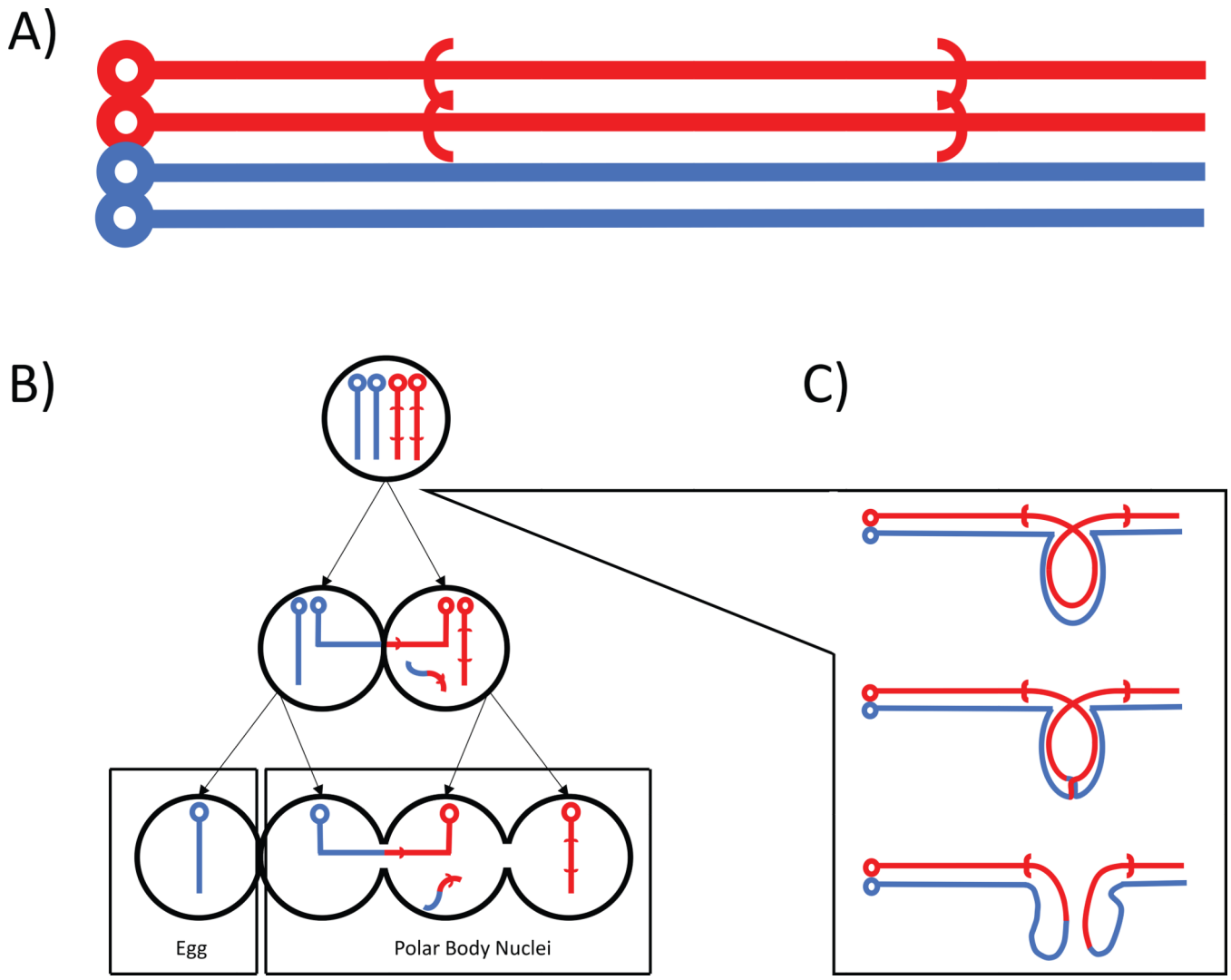

Figure 1.

Drosophila female meiosis in an inversion heterozygote. A) Chromosomes of an inversion heterozygote as an unpaired four strand bundle, red chromosome carry an inverted arrangement illustrating breakpoints with parentheses. B) The progression of asymmetric (female) meiosis contingent on a crossover event causing acentric/dicentric chromosomes. Dicentric chromosome is selectively eliminated by confinement to internal positions, and therefore polar bodies nuclei, in the linear array of meiotic products. C) Formation of the inversion loop illustrating the consequences of crossing-over in the inverted region, showing only two chromatids for simplicity.

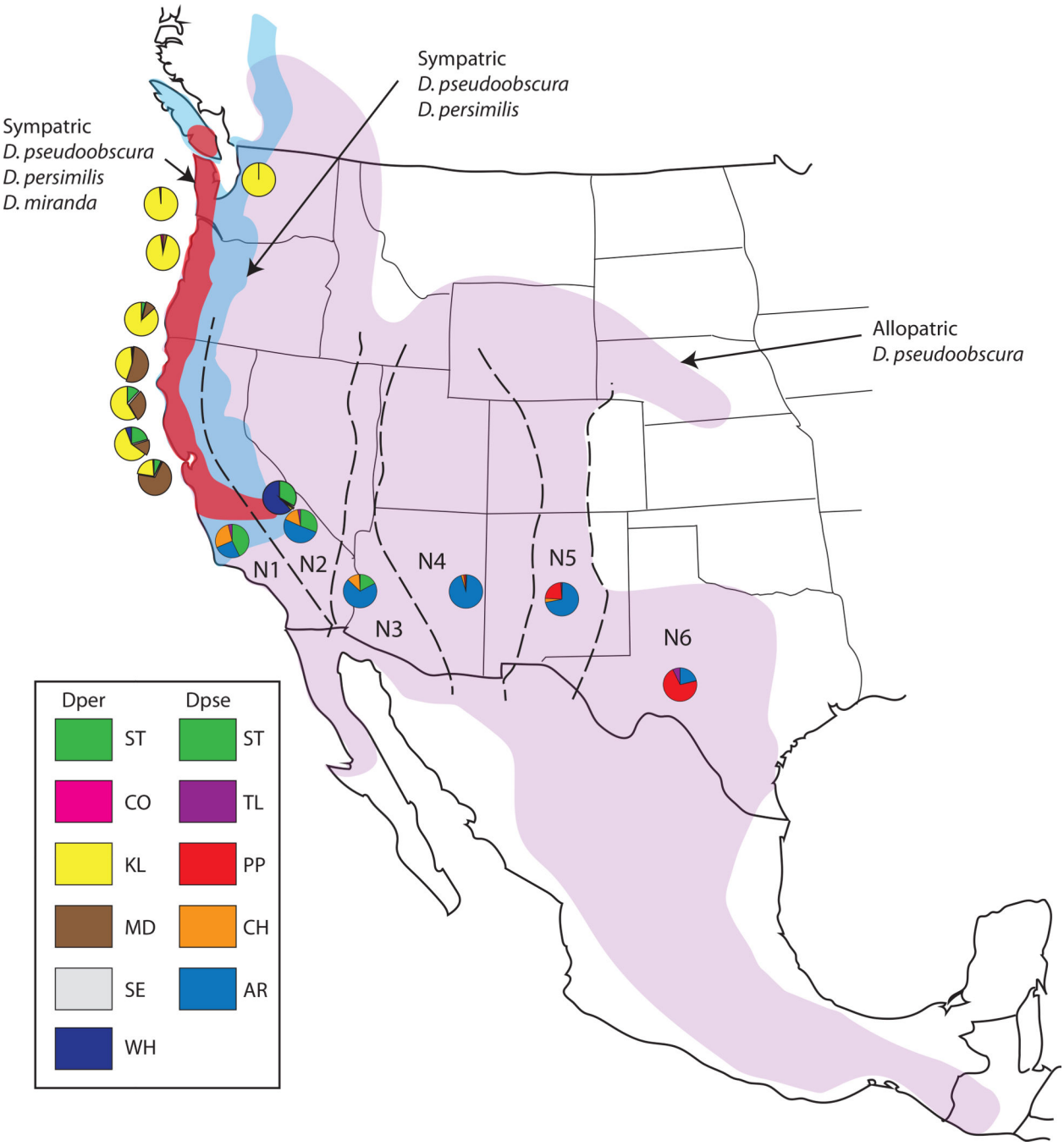

Figure 2.

Distribution of the members of the North American obscura group species, *Drosophila pseudoobscura*, *D. persimilis*, and *D. miranda*. The pie diagrams show the frequencies of the major gene arrangements of *D. pseudoobscura* and *D. persimilis.* The gene arrangement frequencies of *D. pseudoobscura* in six niches (N1 to N6, see Schaeffer, 2008) are indicated by the six pie diagrams at the bottom of the niche while all other pie diagrams shown above are for *D. persimilis*. Only the Standard (ST) gene arrangement is found in *D. pseudoobscura* and *D. persimilis*.

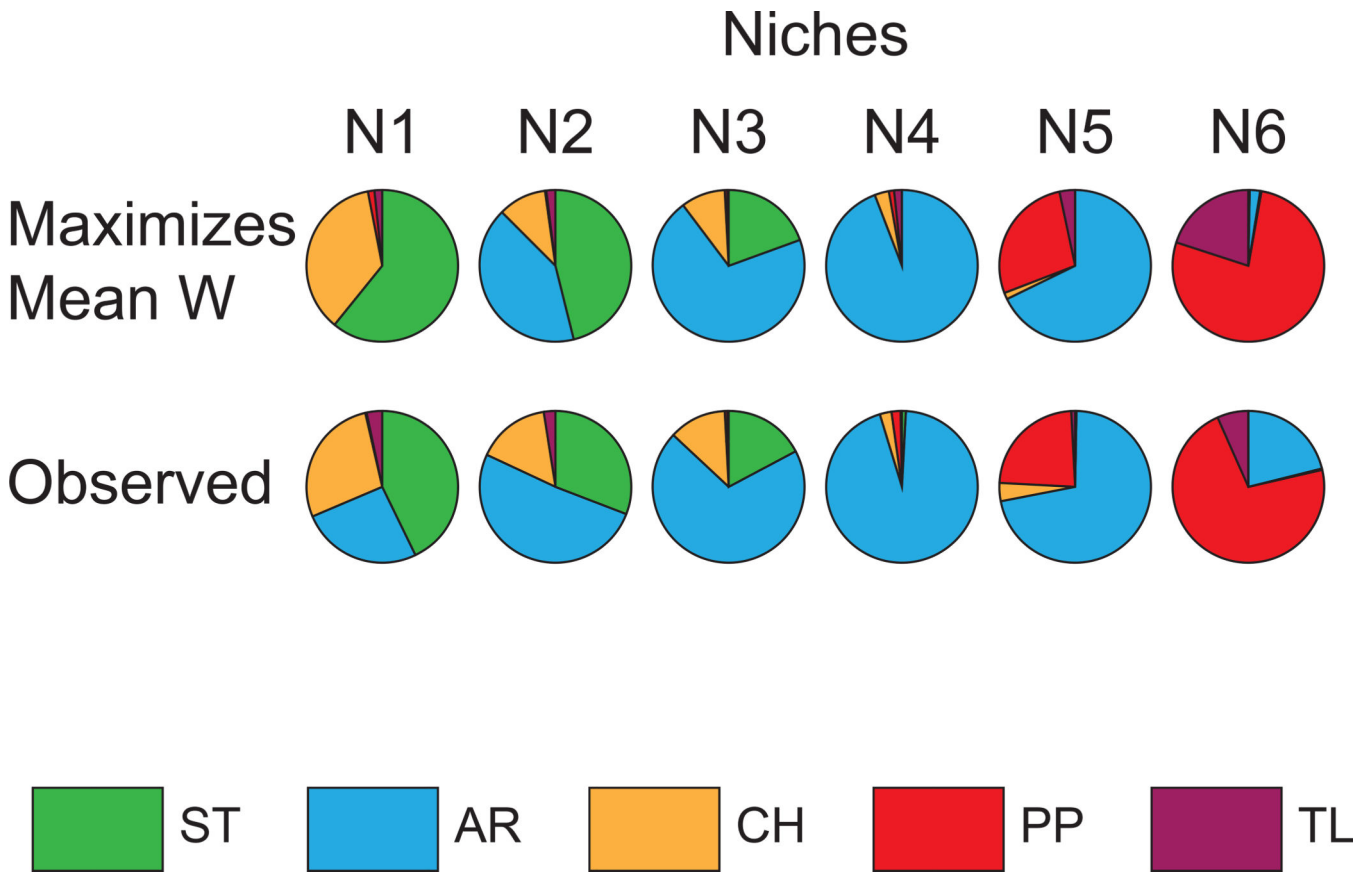

Figure 3.

Gene arrangements frequencies in the six different niches. The top line of pie diagrams shows the randomly chosen gene arrangement frequencies that maximize the mean fitness (W) within the niche. The bottom line shows the observed gene arrangement frequencies in the six different niches.

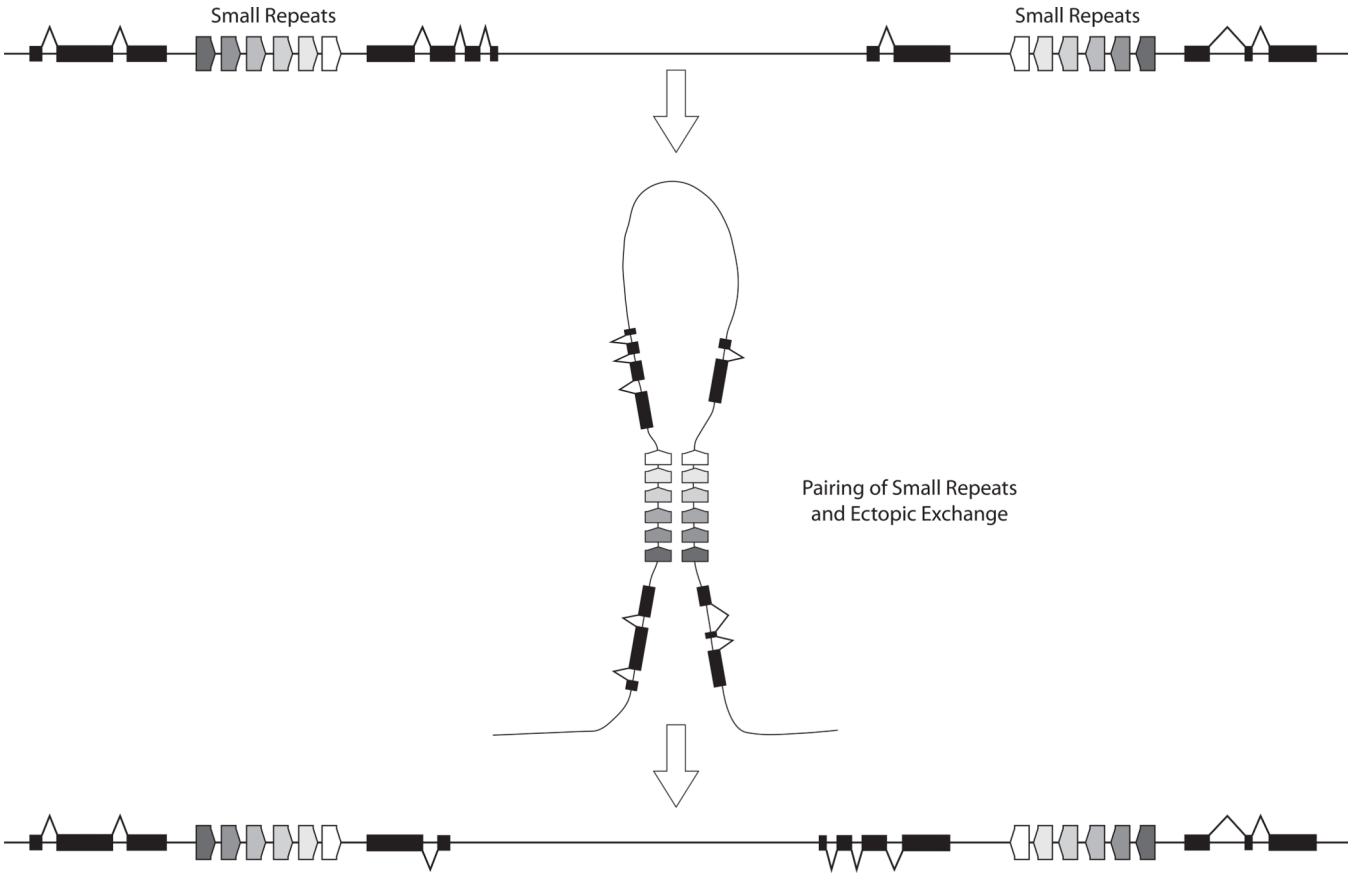

Figure 4.

Mechanism to invert chromosomes in *D. pseudoobscura* that uses small repeats sequences (100–200 bp) in reverse orientation that pair and undergo ectopic exchange (center). Repeats are located between coding regions (black filled in boxes separated by intron sequences).

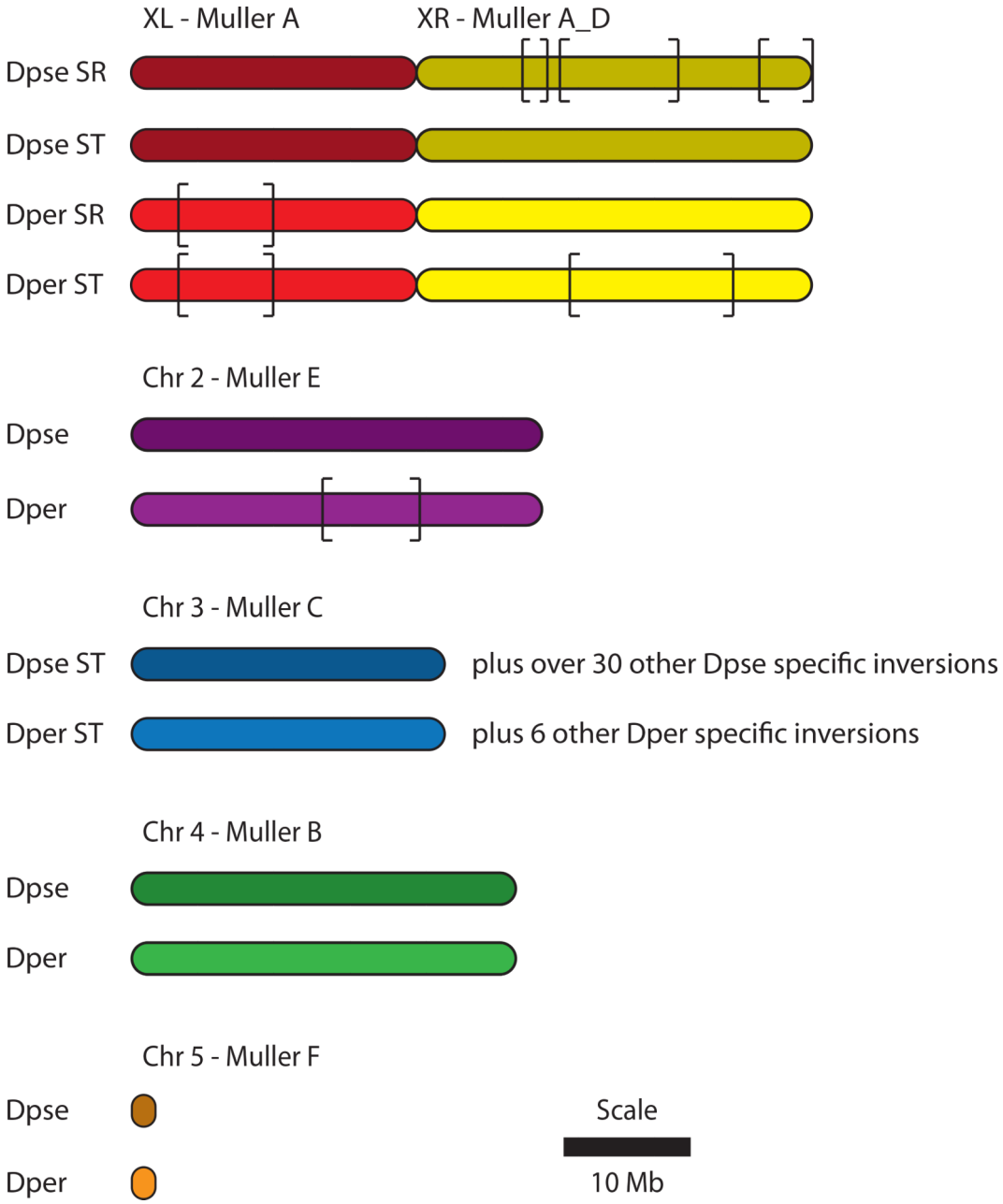

Figure 5.

Organization of the *D. pseudoobscura* and *D. persimilis* genomes. The brackets indicate inversion differences between the genomes as well as the inversions that are associated with the Sex ratio chromosome on the right arm of the X (XR) in the two species. The *D. pseudoobscura* chromosomes are colored slightly darker compared to their *D. persimilis* counterparts.

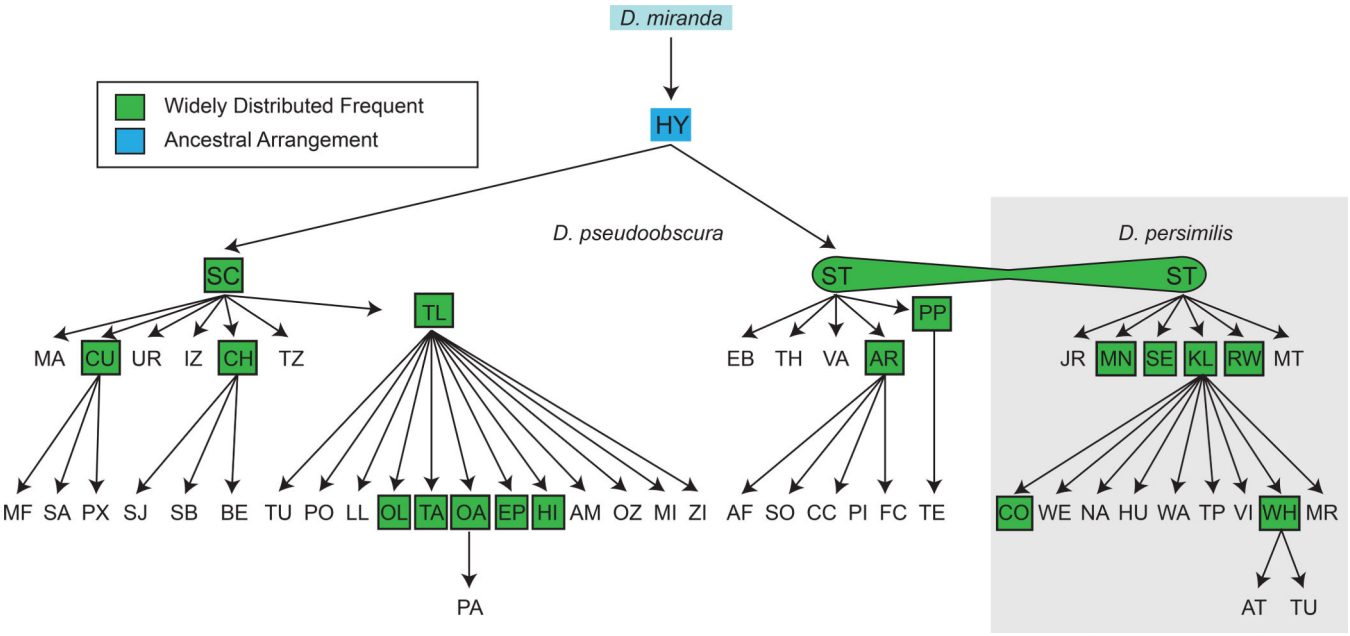

Figure 6.

Phylogeny of the third chromosome gene arrangements of *D. pseudoobscura* and *D. persimilis*. The *D. pseudoobscura* gene arrangements are: AM, Amecameca; AF, American Fork; AR, Arrowhead; BE, Berkeley; CH, Chiricahua; CC, Cochise; CU, Cuernavaca; EB, East Bay; EP, Estes Park; FC, Fort Collins; HI, Hidalgo; HY, Hypothetical; IZ, Iztaccihuatl; LL, Los Lirios; MA, Mammoth; MI, Michoacan; MF, Miraflores; OA, Oaxaca; OL, Olympic; OZ, Ozumba; PA, Patzcuaro; PX, Paxtepec; PP, Pikes Peak; PI, Pinon; PO, Popocatepetl; SA, San Antonio; SJ, San Jacinto; SB, Santa Barbara; SC, Santa Cruz; SO, Sonoita; ST, Standard; TA, Tarasco; TE, Texas; TH, Thomas; TL, Tree Line; TU, Tulancingo; TZ, Tzintzuntzan; UR, Uruapan; VA, Vandeventer; and ZI, Zirahuen (Anderson, Dobzhansky, Pavlovsky, Powell, & Yardley, 1975; Crumpacker & Kastritsis, 1967; Dobzhansky, 1944; Dobzhansky, 1948a; Dobzhansky et al., 1975; Epling & Lower, 1957; Espinoza-Velazquez & Salceda, 1981; Olvera et al., 1979; Olvera et al., 1985; Strickberger & Wills, 1966; Turner & Jeffery, 1977). The *D. persimilis* gene arrangements are: AT, Atwell Mill; CO, Cowichan; HU, Humboldt; JR, James Reserve; KL, Klamath; MR, Maple Ridge; MT, Mather; MN, Mendocino; NA, Nainaimo; RW, Redwoods; SE, Sequoia; ST, Standard; TP, Thetis Park; TU, Tuolumne; VI, Victoria; WA, Wawona; WE, Weott; and WH, Whitney (Anderson et al., 1975; Dobzhansky, 1944, 1948b; Dobzhansky & Sturtevant, 1938; Moore & Taylor, 1986; Spiess, 1950, 1965). The Hypothetical arrangement was demonstrated to be the ancestral arrangement based on adjacency information of genes that flank inversion breakpoints (see page 3 Supplemental Information, Table S3 and Figures S4-S23, Fuller et al., 2017)