Structural Variants and Speciation: Multiple Processes at Play

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Abstract

Research on the genomic architecture of speciation has increasingly revealed the importance of structural variants (SVs) that affect the presence, abundance, position, and/or direction of a nucleotide sequence. SVs include large chromosomal rearrangements such as fusion/fissions, inversions and translocations, as well as smaller variants such as duplications, insertions, and deletions (CNVs). Although we have ample evidence that SVs play a key role in speciation, the underlying mechanisms differ depending on the type and length of the SV, as well as the ecological, demographic and historical context. We review predictions and empirical evidence for classic processes such as underdominance due to meiotic aberrations and the coupling effect of recombination suppression before exploring how recent sequencing methodologies illuminate the prevalence and diversity of SVs. We discuss specific properties of SVs and their impact throughout the genome, highlighting that multiple processes are at play, and possibly interacting, in the relationship between SVs and speciation.

Introduction

Intraspecific genetic variation represents the raw substrate shaped by evolutionary forces generating populations recognizable as species (Mayr 1942; Mallet 1995). Since gene flow and recombination oppose genetic differentiation, factors that impede the exchange and shuffling of genetic material are of seminal interest to researchers studying speciation. Hence, genetic variants may contribute to speciation by leading to non-random mating, by reducing recombination, and by preventing admixed genomes from contributing to subsequent generations.

Structural variants (SVs) are genetic variants encompassing changes in presence, abundance, position or direction of a sequence of significant length (Fig. 1). Because we focus on mechanisms associated with structural changes, we utilize a definition that does not impose an arbitrary length limit (Mérot et al. 2020) although length is an important property of SVs that we discuss in section 4. In this chapter, "SVs" include large chromosomal rearrangements (CRs) such as fusions, translocations, and inversions, insertions/deletions (indels), CNVs (copy number variants), as well as gains and losses of sequences due to transposable elements (TEs). All such SVs have long been considered important for speciation (see also chapter by Lucek et al, this volume).

It was the observation that chromosome numbers and structure often differ between closely related species across a wide taxonomic range from vertebrates to plants that first led researchers to the concept of "chromosomal speciation" (Sturtevant 1938; Stebbins 1950; White 1969; King 1995). Originally, this concept focused almost exclusively on the fact that meiosis in heterokaryotypes (i.e., individuals heterozygous for a CR) could result in pairing failure or the creation of unbalanced gametes thus generating underdominance (i.e., lower fitness of heterokaryotypes) and reducing gene flow. However, there was skepticism of chromosomal speciation models because they relied on genetic drift to establish the initial SV differences between populations (Lande 1979, 1985; Hedrick 1981; Walsh 1982; Coyne and Orr 2004; Potter et al. 2017). In contrast, over the last two decades, there has been a rebirth of interest in SVs due to their role as recombination modifiers. By locally reducing

recombination, SVs may facilitate both the buildup and maintenance of divergence in the face of the homogenizing effects of gene flow (Navarro and Barton 2003; Kirkpatrick and Barton 2006; Hoffmann and Rieseberg 2008; Faria and Navarro 2010; Feder et al. 2011; Guerrero and Kirkpatrick 2014). This mechanism has been viewed favorably because speciation usually involves divergence at multiple loci (Coyne and Orr 2004), and reproductive isolation is strengthened when these loci remain in association, i.e. "coupled" (Smadja and Butlin 2011; Flaxman et al. 2014; Nosil et al. 2021 but see chapter by Aubier et al. and by Dopman et al, this volume).

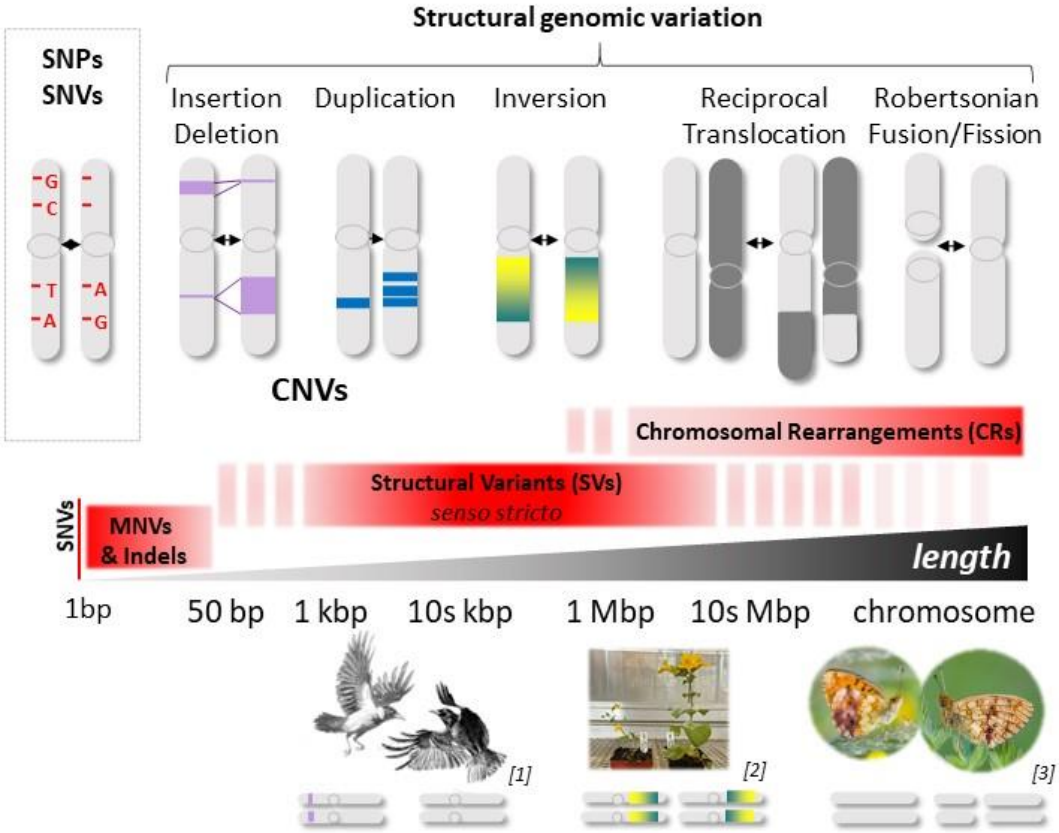

Figure 1: The diversity in type and length of structural variants involved in speciation.
The minimal and maximal length designated for "SVs" has varied a lot over the last ten years (e.g. 30-500bp, 1kb-3Mb, 50bp-100s Mb; Feuk et al. 2006; Escaramís et al. 2015; Ho et al. 2019), with the most common operational definition being above 50bp. Variants that encompass a large portion of a chromosome are more commonly called "chromosomal rearrangements" (CRs) while variants below 50bp are frequently called "indels" for insertions and deletions and/or MNVs (Multi Nucleotide Variants). The insert shows SNVs (single nucleotide variants), which include both 1bp indels and SNPs (Single nucleotide polymorphisms). SVs of different types and lengths have been pinpointed for their role in speciation: [1] A short insertion (2.25kb) which modulates plumage color, is involved in pre-mating reproductive isolation between two crow subspecies, *Corvus corone cornix* and *C. c. corone* (Drawing courtesy K. Fraune), (Weissensteiner et al. 2020). [2] *Mimulus guttatus* (Photo courtesy D. Lowry) is a species complex with partially isolated annual and perennial ecotypes that differ at Mb-long chromosomal inversions associated with different life-history traits underlying temporal changes in blooming and ecological adaptation (Lowry and Willis 2010; Coughlan and Willis 2019; Coughlan et al. 2021). [3] Extensive chromosomal fusions reduce gene flow in hybridizing fritillary butterflies, *Brenthis daphne* and *B. ino* (Mackintosh et al. 2023). Photo courtesy V. Dinca.

This shift in models coincided with a shift from cytological and marker-based research to genomics. Although the wide availability of short-read sequencing techniques led to an initial focus on single-nucleotide polymorphisms (SNPs) in the search for putative "speciation genes", long-read sequencing methods have revived interest in genome structure. Progress in genome sequencing has revealed that SVs are orders of magnitude more common than previously thought and cover 3 to 10 times more bases of the genome than SNPs (Catanach et al. 2019; Zhou et al. 2019; Abel et al. 2020; Mérot et al. 2023). Most critically, SVs also have different properties from SNPs that can impact their evolutionary trajectories and thus their role in speciation (Berdan et al. 2021b). The effect on recombination has been extensively studied for inversions but may also emerge in other types of SVs such as indels and CNVs (Sjödin and Jakobsson 2012; Rowan et al. 2019). Due to their length and secondary characteristics, the distribution of fitness effects (DFE) of SVs likely skews toward larger effect sizes (both positive and negative). Thus, SVs may have a disproportionate impact on population divergence and hence the tempo of speciation (Katju and Bergthorsson 2013; Berdan et al. 2021b). Furthermore, SVs may have indirect effects elsewhere in the genome than at their position by affecting chromatin structure and other epigenetic marks, translating into a putative widespread genomic impact leading to species divergence (O'Neill et al. 1998; Vara et al. 2021). Altogether, SVs have great potential to be key players in speciation but until recently the emphasis has either been on SNPs or biased towards a few large CRs, limiting our understanding of this class of genetic variants.

In this chapter, we review current empirical evidence and theory about the different mechanisms by which SVs may impact reproductive isolation and contribute to speciation. We highlight how considering the spectrum of structural genomic variation and their properties will help lead to a more comprehensive understanding of speciation. We emphasize how integrating different properties of SVs into theory is changing the way we view speciation, and how increasing the scope of empirical work to include all genetic variants is bringing new insights into the genetic basis of species differentiation. While we consider the whole of structural variation here, we note that different SVs are not interchangeable and encourage researchers to consider the totality of the different effects for each type of SV.

1 - Direct meiotic impacts: SVs can lower fitness in hybrids (heterokaryotypes)

Reduced hybrid fitness is a major reproductive barrier for many species pairs (see chapter by Reifova et al, this volume). Certain SVs (specifically CRs), when heterozygotes, disrupt (1) chromosomal pairing, (2) crossing-over or (3) segregation at meiosis, and thus may be important players in speciation because fitness is reduced in heterokaryotypes (underdominance).

In the first category of SV disruption, homologs can fail to pair properly early in meiosis (Fig. 2). Such unpairing may be associated with germ cell death (Searle 1993; He et al. 2016). In mammals, heterozygotes for Robertsonian fusions, reciprocal translocations, and inversions can show partial or complete sterility due to this effect on germ cells (Chandley et al. 1986,

1987; Searle 1993). In the second type of SV disruption, a crossover event can lead to unbalanced gametes. For example, in inversion heterozygotes, if homologous pairing occurs via an inversion loop (Fig. 2), crossing-over within the loop can generate gametes with duplications and deficiencies, reducing fertility (White 1954; Kaiser 1984; Madan 1995). Finally, heterokaryotypes for specific forms of CR (fusions, fissions, reciprocal translocations) can produce unbalanced gametes through missegregation of chromosomes at meiosis, sometimes strongly reducing fitness (Fig. 2) (Long 1988; Searle 1993; Morel et al. 2004; Stathos and Fishman 2014; Dobigny et al. 2017; Bozdag and Ono 2022).

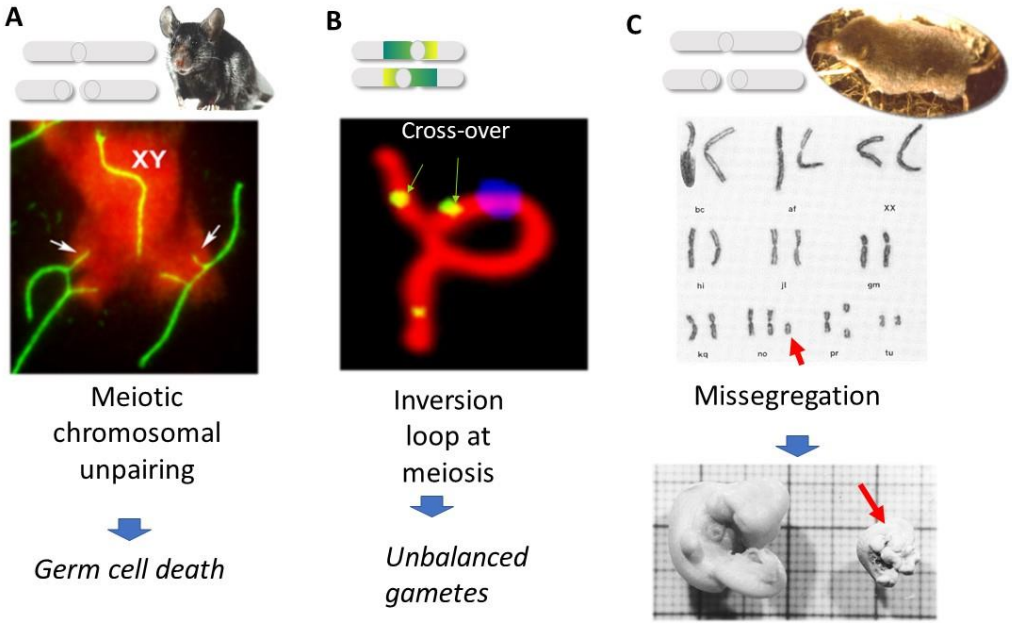

Figure 2: Examples of three mechanisms by which chromosomal rearrangements disrupt meiosis and lead to underdominance.
A) Unpaired chromosomal regions (arrows) at pachytene, an early stage of meiosis, in a house mouse (photo courtesy J.B. Searle) heterozygous for two Robertsonian fusions (the fusion of two acrocentric chromosomes at the centromere; Fig. 1) - (synaptonemal complexes: green immunostaining). Chromosome unpairing of this sort is associated with germ cell death, particularly when interacting with the XY bivalent in males (epigenetic inactivation: red immunostaining). (From Garagna et al. (2014), with permission).
B) Pairing of heterozygous chromosomes differing by a large inversion in the domestic pig. The chromosomes are paired homologously, as revealed with immunostained synaptonemal complexes (red) that show an inversion loop with the centromeres (blue) within the loop. Three recombination foci have been detected with immunostaining (in yellow/green) including one within the inversion loop that will lead to duplication and deficiency of chromosomal material in the gametes. (From Massip et al. (2010), with permission).
C) Trisomy (arrow) resulting from missegregation at meiosis in a wild common shrew (*Sorex araneus* - photo courtesy J.B. Searle) attributable to heterozygosity of a Robertsonian fusion in the mother. Below, the moribund trisomic fetus (arrow) in comparison to a normal fetus in the same pregnancy (From Searle (1984), with permission).

The underdominance model for chromosomal speciation (White 1978; King 1995) was based on the empirical foundation described above. However, fertility reductions associated with naturally occurring CRs have not matched these expectations. Detailed studies of single heterozygotes for Robertsonian fusions in house mice and common shrews show negligible germ cell death and meiotic missegregation (Searle 1993; Borodin et al. 2019). Additionally, some organisms like Lepidoptera have holocentric chromosomes that greatly reduce the risk of meiotic malfunction (Lukhtanov et al. 2018; Lucek et al. 2022). With regards to inversions, mechanisms can prevent the generation of unbalanced gametes. Unbalanced recombination products can be relegated to degenerate polar bodies rather than gametes, such as in *Drosophila* (Fuller et al. 2019). Recombination itself can also be bypassed altogether by, non-homologous pairing in heterokaryotypes, which has been demonstrated cytologically in a variety of taxa (Haines et al. 1978; Hale 1986; Torgasheva and Borodin 2010). These results fit the theoretical expectation that an inversion is unlikely to establish if there is substantial underdominance although small effect underdominance can evolve (Kirkpatrick and Barton 2006; Schluter and Rieseberg 2022). While individual CRs may not generate strong underdominance, the accumulation of multiple CRs in differentiating populations may create a situation with additive underdominance, of relevance to speciation. In mammals, multiple Robertsonian fusions fixed between populations or species can lead to hybrids with long multivalent chain or ring configurations at meiosis, resulting in substantial germ cell death and/or production of unbalanced gametes (Searle 1993; Garagna et al. 2014; Borodin et al. 2019). *Helianthus* sunflowers differ by multiple rearranged chromosomes and low pollen fertility is associated with several QTLs located near breakpoints (Lai et al. 2005).

2 - Indirect effects of recombination reduction: some SVs can increase linkage disequilibrium between isolating loci

The suppressed recombination associated with some SVs (including but not limited to inversions) is a powerful mechanism for establishing and maintaining coupling through strong linkage disequilibrium (LD) between loci involved in reproductive isolation (Rieseberg 2001; Noor et al. 2001a; Butlin 2005; Feder and Nosil 2009; see chapter by Dopman et al, this volume). Several models propose that the main role of SVs in speciation is that of recombination modifiers (Noor et al. 2001c; Rieseberg 2001; Faria and Navarro 2010). SVs may strengthen LD between loci underlying a single reproductive isolation barrier (e.g., male traits and female choice loci) (Trickett and Butlin 1994) or loci underlying multiple different reproductive isolating barriers, including genetic incompatibilities (Noor et al. 2001b; Navarro and Barton 2003; Butlin 2005; Smadja and Butlin 2011). While other regions of low recombination, as found near centromeres, may also facilitate speciation (Nachman and Payseur 2012), SVs differ from these in that their effects are conditional on karyotype. SVs suppress recombination only when heterozygous and behave as collinear regions when homozygous. When taxa are fixed for alternate karyotypes, this results in reduced recombination for a chromosomal region in hybrids, potentially limiting the rate of introgression and facilitating the evolution of additional genetic differences contributing to speciation. The persistence of recombination within rearrangements when homozygous in the parental populations also allows for the purging of deleterious mutations in the incipient species. Therefore, suppressed recombination in heterokaryotypes could have a strong impact on both the establishment and maintenance of species differences during primary or

secondary contact, and in parapatry or sympatry, in the face of gene flow (Kirkpatrick and Barton 2006; Feder et al. 2011). However, the majority of the work quantifying the extent of recombination reduction in heterokaryotypes has focused on inversions. Quantifying the direct effect of different SVs on recombination will be a necessary step to understanding their role in speciation.

The role of recombination suppressors during secondary contact is nevertheless debated. For chromosomal inversions, double crossovers are not the only way that gene flux (i.e., genetic exchange between arrangements; Navarro et al. 1997) can occur in heterokaryotypes. Non-crossover gene conversion can also move up to 100s of bp of DNA between arrangements. Korunes and Noor (2019) showed that gene conversion can be pervasive in chromosomal inversion heterozygotes in experimental crosses of *Drosophila pseudoobscura* and *D. persimilis* (1×10^{-5} to 2.5×10^{-5} converted sites per bp per generation). Given this high rate, gene conversion has the potential to reduce the efficacy of inversions as barriers to recombination over evolutionary time (Korunes and Noor 2019). Gene conversion may thus homogenize genetic differences between the inverted and standard arrangements unless segregating SNPs are associated with strong divergent selection between populations (Feder and Nosil 2009). Gene conversion and double recombination may also fairly rapidly eliminate SNPs causing negative epistatic fitness interactions in hybrids following secondary contact (Feder and Nosil 2009). Such elimination can homogenize the content of inversions and their association with reproductive isolation although this process might be slow enough to allow additional barriers to evolve (Rafajlović et al. 2021).

Another hotly debated topic is the order of events in which SVs contribute to speciation (Fig. 3). Some models assume that the alleles underlying reproductive isolation evolve after a SV originates ('gaining-inversion' scenario), while others rely on the SV capturing isolation loci already segregating in the population ('capturing-inversion' scenario)(e.g Kirkpatrick and Barton 2006; Charlesworth and Barton 2018). The capturing-inversion scenario is supported in monkeyflower *Mimulus guttatus*, where there is strong evidence that an inversion played an important role in adaptation and reproductive isolation between annual and perennial ecotypes in inland and coastal environments (Lowry and Willis 2010). The presence of the same distinctive QTLs in some related collinear perennial species (as *M. tilingii*) suggests the association among loci contributing to local adaptation evolved first and then was captured by an inversion that predated the evolution of the perennial /annual species in the *M. guttatus* species complex (Coughlan and Willis 2019). On the contrary, recent evidence from *Drosophila pseudoobscura* and *D. persimilis* suggests that the inversions distinguishing the two species originated before the evolution of incompatibilities located within the inverted regions (Fuller et al. 2018, but see also Noor et al. 2001c). Because many of the inversions involved in species divergence are relatively old, distinguishing the two scenarios remains nevertheless often difficult. This matter may be further addressed with phylogenetic comparative approaches aiming at reconstructing the history of the SV and/or thanks to emerging genome engineering techniques allowing to reverse the SV (Schmidt et al. 2019; Stern et al. 2023). What we observe in nature is also possibly a combination of both the gaining-inversion and capturing-inversion models (Faria et al. 2019b), as suggested by a recent theoretical study on the role of inversions in local adaptation (Schaal et al. 2022).

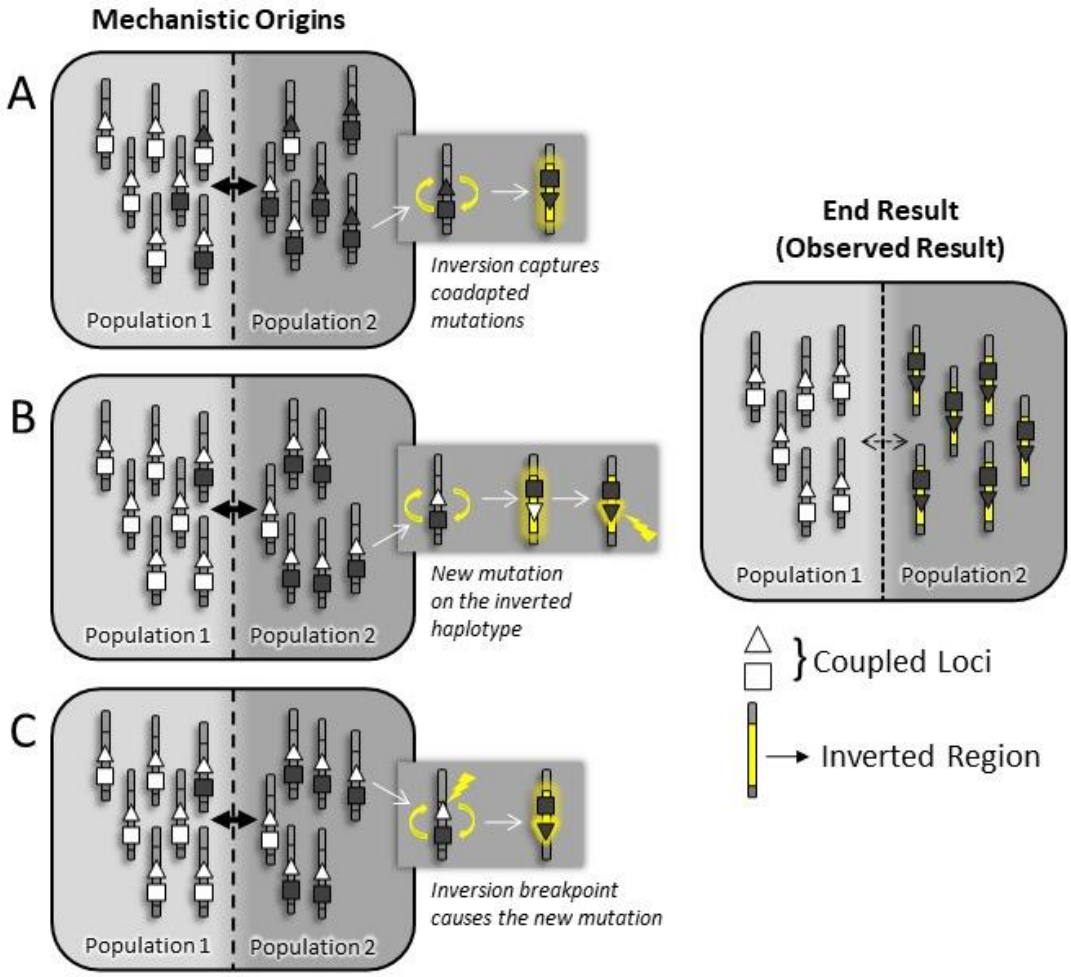

Figure 3. Different mechanisms leading to observed coupling.
In all scenarios, two populations occupying different environments (light and dark background) are initially connected by gene flow (double black arrow in the panel). Two loci, square and triangle, coding for two traits (e.g., leaf size and flowering time, respectively) may be involved in local adaptation, each with a light or dark variant conferring a benefit in the respective light or dark environment. All mechanistic origins (left) lead to the same observation (coupled loci, right). ***A) Capturing-inversion.*** The two alleles at both loci segregate in the two populations. Migration and recombination impose a fitness cost. An inversion occurs in population 2 that captures the haplotype with the locally adapted alleles at both loci (black) in that local environment. Alleles within the inversion stop recombining with those present in the standard chromosome. ***B) Gaining-inversion.*** The initial situation is a one-locus adaptation to environmental variation (square locus here). An inversion occurs in population 2 and is presumed neutral relative to the standard arrangement in that population, and so may drift to some frequency. A new mutation then occurs (gain) on a second gene (triangle) within the inversion, forming a haplotype with both locally-adapted alleles. ***C) Breakpoint model.*** As in B, the initial situation is a one-locus adaptation to environmental variation (square locus). An inversion containing the locally adapted allele at this locus occurs in population 2, and the breakpoint itself functionally modifies another locus (triangle) at or near the breakpoint. This forms, in a single step, an inverted haplotype with both locally-adapted alleles. In all three scenarios, recombination suppression brings an advantage to the inversion coupling locally-adapted alleles in population 2, causing it to invade population 2 and strengthen reproductive isolation.

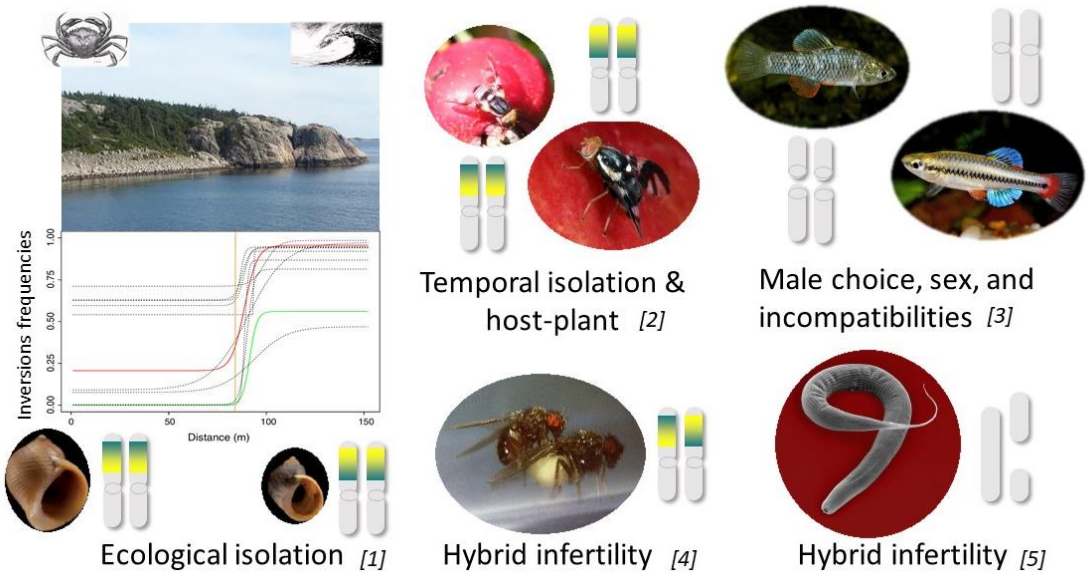

Figure 4: Examples of partially-isolated taxa in which SVs strengthen reproductive isolation via effects of recombination reduction.
[1] The periwinkle *Littorina saxatilis* ecotypes are partially reproductively isolated by adaptation to wave-exposed vs. crab-sheltered environments (Photo of the habitats and snails courtesy of R. Butlin and F. Pleijel). They differ by several inversions, some of which show a clinal frequency distribution (From Faria et al. (2019a), with permission) across the two habitats and are associated with divergently adaptive phenotypic traits (Koch et al. 2021).
[2] The apple maggot *Rhagoletis pomonella* (Photo courtesy J. Feder) includes two partially-isolated host races, one that parasitizes the hawthorn, its native plant, and one that parasitizes apple trees. Frequency of chromosomal inversions vary between hosts and underlie temporal variation in the reproductive period (Feder et al. 2003; Calvert et al. 2022).
[3] *Lucania parva* and *L. goodei* (Photo courtesy T. Terceira) are sister species that differ by a large chromosomal fusion that includes QTLs associated with sex determination, incompatibilities and mate choice (Berdan et al. 2021c).
[4] *Drosophila persimilis* and *D. pseudoobscura (*Photo courtesy M. Noor) occasionally hybridize but they show high hybrid infertility which has been associated with a large chromosomal inversion (Noor et al. 2001c).
[5] The androdioecious (hermaphroditic) *Pristionchus pacificus* (Photo courtesy R. Sommer) and its dioecious sister species *P. exspectatus* differ by chromosomal fusions which impact the recombination landscape. Male sterility was associated with a break of linkage in hybrids (Yoshida et al. 2023).

Despite a large amount of theoretical work, unequivocal empirical evidence for the role of SVs in linking critical loci for reproductive isolation is limited but increasing (Fig. 4). With advances in genomics, empirical support for the widespread presence of SVs and their evolutionary significance has been growing across a wide taxonomic range (Wellenreuther and Bernatchez 2018; Huang and Rieseberg 2020; Mérot et al. 2020 and references therein). However, most of these studies have focused on the intraspecific level and support a role for SVs in adaptation. Although this can result in ecological speciation, evidence for the role of SVs in strengthening reproductive isolation in nature has been limited to a few systems and a few SVs until recently. Empirical examples include inversions in *Drosophila pseudoobscura* and *D. persimilis* (Noor et al. 2001c but see also Fuller et al. 2018), *Helianthus* sunflowers (Rieseberg et al. 1999; Todesco et al. 2020), *Mimulus guttatus* monkeyflowers (Lowry and Willis 2010)

and *Rhagoletis* fruit flies (Feder et al. 2005). Evidence from other systems (e.g. *Littorina saxatilis* (Faria et al. 2019a; Koch et al. 2021) is emerging, including from fusions in *Pristionchus* nematodes (Yoshida et al. 2023), *Lucania* killifish (Berdan et al. 2021c) and *Brenthis* butterflies (Mackintosh et al. 2023). However, we are still far from having a taxonomically comprehensive view about the role of SVs in speciation across the tree of life (see chapter by Lucek et al. this volume).

3 - Elsewhere in the genome: SVs can have a widespread indirect impact influencing reproductive isolation

Beyond the mutated region itself, SVs may have impacts elsewhere in the genome which can influence speciation. The effects of many SVs extend outside of their breakpoints. Empirical studies in *Drosophila,* other insects and sunflowers have shown that recombination suppression can extend beyond the inverted region (Stevison et al. 2011). The suppression likely reflects reduced homologous synapsis in the vicinity of the chromosomal breakpoints (Pegueroles et al. 2010). There is also evidence that recombination is reduced in the vicinity of the breakpoints of chromosomal fusions, probably for the same reason (Davisson and Akeson 1993; Gimenez et al. 2013; Mackintosh et al. 2023; Yoshida et al. 2023). The impact on the recombination landscape may also extend genome-wide (Lucchesi and Suzuki 1968). In particular, in *Drosophila*, heterozygotes for paracentric inversions show an increased recombination rate in regions of the genome outside of rearrangements and breakpoint regions, a phenomenon known as the "interchromosomal effect" (Fig. 5)(Miller 2020). The precise mechanism is unclear, but it appears that there is a monitoring process that delays the pachytene phase of meiosis until the number of crossing-over events reaches that needed for a normal oocyte (Joyce and McKim 2010; Crown et al. 2018).

SVs are also associated with changes to the epigenome and gene regulatory landscape. In many organisms, the genome is organized into megabase-sized chromatin interaction domains named TADs (topologically associated domains) (Dixon et al. 2012, 2016; Wright and Schaeffer 2022). Interestingly, in an analysis comparing the human and gibbon genomes, which differ by multiple CRs, there was a very strong tendency for the breakpoints to be located at TAD boundaries, such that TADs are maintained in rearrangements that become fixed and persist (Lazar et al. 2018). This would suggest that, at least at the local scale, gene interactions and expression, as well as epigenetic processes are not necessarily perturbed by CRs. At a broader scale, there have also been studies examining the impact of SVs on chromosomal territories (CTs), the cell-type specific regions of the nucleus occupied by particular chromosomes (Croft et al. 1999), whose positioning may influence gene expression and gene interactions (Avelar et al. 2013; Harewood and Fraser 2014). Once again, analysis involving wide phylogenetic comparisons of primates suggests that SVs do not alter positioning of CTs, at least in terms of expectations based on gene density (Tanabe et al. 2002). However, recent studies addressing chromatin conformation (Hi-C) and accessibility (ATAC-seq) are pointing towards important changes due to SVs, which could affect gene expression (Vara and Ruiz-Herrera 2022). For example, in recently-diverged populations of house mice (Vara et al. 2021), Robertsonian fusions have a strong impact on the positioning of chromosomes in somatic cells and male germ cells, on TAD reorganization and a widespread effect on the recombinational landscape in germ cells (Fig. 5). Wright and Schaeffer (2022)

also found breakpoints within TADs in *Drosophila pseudoobscura* inversions, with implications for gene expression, and a possible involvement of position effects in establishment of the inversions. Similarly, between different species of *Heliconius* butterflies, 30% of differences in chromatin accessibility were related to SVs distributed across the genome, and in particular TEs (Fig. 5) (Ruggieri et al. 2022). Considered together, these various complex "side effects" of SVs have the potential to impact reproductive isolation. While direct evidence remains scarce, testing this hypothesis is now possible with the emergence of new techniques (Hi-C, ATAC-seq) which may unveil additional mechanisms by which SV contribute to speciation (see e.g. Li et al. 2023).

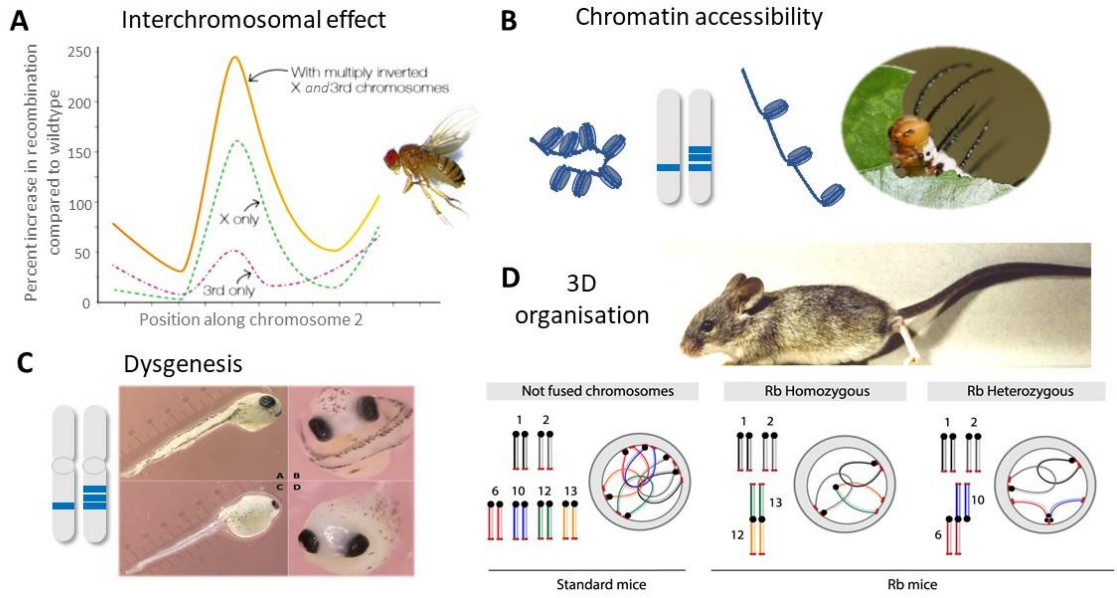

Figure 5: Examples of SVs impacts elsewhere in the genome.
A) The interchromosomal effect, *i.e.* an increase in recombination rate in collinear regions of the genome (i.e., non-rearranged areas), illustrated in inversion heterozygotes in *Drosophila melanogaster* (Photo courtesy A.E. Douglas) (From Miller (2020), with permission.) **B)** SVs can impact chromatin accessibility, as observed in *Heliconius melpomene* (Photo courtesy G. Vernade) (Ruggieri et al. 2022). **C)** Hybrid dysgenesis may result from widespread TE insertions and their deregulation in hybrids, as exemplified in the Lake Whitefish (Dion-Côté et al. 2014; Laporte et al. 2019). Viable (above) and non-viable (below) hybrids between dwarf and normal species of *Coregonus clupeaformis* (Photo courtesy L. Bernatchez). **D)** Chromosome organization is impacted by the presence of Robertsonian fusions. Schematic representation of the house mice *M. musculus*. (From Vara et al. (2021),with permission; photo courtesy J.B. Searle).

SVs may not always be isolated mutational events - when an SV occurs it may be one of many interrelated disruptive events happening in the genome. For example, CRs such as fusions and translocations may disrupt nuclear organization, predisposing the formation of additional rearrangements (Branco and Pombo 2006; Vara et al. 2021). Similarly, SVs formed by the insertion, deletion or duplication of TEs can emerge together during a burst of activity in specific TE families (Wells and Feschotte 2020). If such SV-generating processes occur in isolated populations, or involve different TE families, it could result in rapid genetic differentiation and associated reproductive isolation, as shown in a theoretical model

(Ginzburg et al. 1984). Such a process is supported by recently-diverged species that differ in the frequency and abundance of TE insertions (Ungerer et al. 2006; Weissensteiner et al. 2020; Mérot et al. 2023). TE activity is also suspected to have caused CRs contributing to rapid speciation in Antarctic fish (Auvinet et al. 2018). Hybridization between genetically distinct populations may lead to dysgenesis (genetic shock), leading to hybrid breakdown associated with TEs, a process that has been particularly well-studied in *Drosophila* (Sved 1979; Khurana et al. 2011). Dysgenesis due to the overexpression of TEs is also observed in hybrids between forms of whitefish that diverged sympatrically in the last 12,000 years and may be explained by a difference in epigenetic TE regulation, such as differential methylation between the parental forms (Fig. 5) (Dion-Côté et al. 2014; Laporte et al. 2019). Another example of the dysgenesis syndrome appears to involve genome-wide undermethylation, retroviral amplification and CRs in hybrid kangaroos (O'Neill et al. 1998). Although the occurrence of CRs and TE activity is likely associated, their relationship is complex (McClintock 1984) as epitomized in maize, where TEs may suddenly mobilize in response to chromosomal breakage during the rearrangement process.

4- Other important properties that influence the evolutionary dynamics of SVs

There are other properties of SVs aside from their impact on meiosis and recombination that may influence reproductive isolation. Here, we detail how the intertwined effects of length, mutation rate, and fitness effects may matter for the emerging evolutionary dynamics of SVs and the buildup of reproductive isolation.

SVs can encompass a large fraction of the genome (Fig. 6) (Conrad and Hurles 2007; Feulner et al. 2013; Catanach et al. 2019; Abel et al. 2020). For instance, deletions account for a substantial proportion of genomic variation in maize and mussels, with less than 75% of genes being present in all (sequenced) individuals (Gerdol et al. 2020; Haberer et al. 2020). As new sequencing technology allows us to examine the breadth of SVs beyond duplicated genes and large CRs, we are seeing a more comprehensive picture of genetic differentiation between species (Ho et al. 2019; Mérot et al. 2020). For example, in closely-related species of Lake Whitefish, genetic differentiation associated with deletions, insertions, duplications and inversions encompass a proportion of the genome five times larger than that of SNPs (Mérot et al. 2023). In cichlid fish, SVs between recently-diverged species contain genes regulating behavior, immunity and morphology, a set of traits that are highly diversified in this group and involved in reproductive isolation (Penso-Dolfin et al. 2020).

Length also has significant consequences for the functional impact of a single SV. Internal regions of SVs bracketed by breakpoints will generally span many potentially important genes affecting adaptation and reproductive isolation (Kirkpatrick and Barton 2006). When SVs reduce recombination, breakpoints remain in LD with variants present within the SVs. This can generate indirect selection owing to the presence of beneficial or deleterious alleles which are now in LD with the breakpoints. More generally, as explained in section 2, indirect selection can arise due to the reduction in recombination which can allow groups of co-adapted alleles to remain in LD. All these forms of indirect selection are expected to scale with

length, as longer SVs are more likely to contain variants under selection. Both the higher levels and further reach of LD means that the indirect effects of linked genes will be amplified and augment the direct effects of selection acting on a site within a SV more than it will be for an average SNP in equilibrium with surrounding sequences.

However, the relationship between SV length and divergence may not always be as straightforward as portrayed above. For example, longer SVs are more likely to harbor more deleterious mutations (i.e., have a larger mutational load) than shorter SVs, making them less likely to establish initially in populations (Nei et al. 1967; Jay et al. 2021). Furthermore, crossovers are more likely to occur in longer SVs and higher rates of gene flux can make it more difficult to assemble and keep suites of co-adapted alleles together. It is also not clear how the relationship between SV length and gene flux will affect the rate of deleterious recessive mutation accumulation in polymorphic CRs, which can affect their long-term fate and retention in populations (Berdan et al. 2021a). The consequences of SV length for speciation is therefore a topic requiring further study and is one that will benefit greatly from the increased resolution of long-read sequencing to detect and characterize the length distributions in a systematic manner.

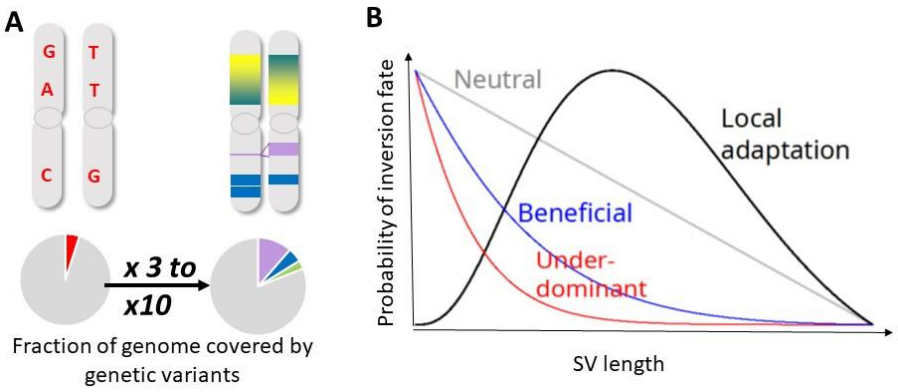

Figure 6: Relevance of the length of SVs
A) SV characterization and genotyping suggest that the total fraction of genome covered by SVs is higher than the fraction covered by SNPs by a factor of 3 to 10 (Catanach et al. 2019; Zhou et al. 2019; Abel et al. 2020; Mérot et al. 2023). **B)** The likelihoods of different evolutionary outcomes of an inversion vary depending on its length. Simulated data, adapted from Connallon & Olito (2021) with permission.

Large SVs can also have a large effect size if they influence gene expression by altering gene sequences, gene copy number, or regulatory elements (Harewood et al. 2010; Harewood and Fraser 2014; Berdan et al. 2021b; Lato et al. 2022; Lye et al. 2022). While changes can facilitate local adaptation (Colson et al. 2004; Avelar et al. 2013; Weetman et al. 2018), we know little about how these effects may scale with the type and length of SVs (Scott et al. 2021). As data on the distribution of SV lengths becomes increasingly available, we need clear theoretical predictions about how SV length relates to effect size, and ultimately impacts the speciation process. For instance, using formal theory, Connallon and Olito (2022) showed that the length distribution of inversions relates to their establishment (Fig. 6). Similar theoretical studies are

needed to understand the implication of such length distributions in terms of the evolution of reproductive isolation.

The DFE, as well as the mutation rate (*i.e.* the rate at which SVs arise), influence the evolutionary dynamics of SVs, possibly affecting the speed of the build-up of genetic differentiation and/or speciation. SVs may have similar mutation rates to SNPs. However, some studies have observed mutation rates for SVs 1-2 orders of magnitude lower than for SNPs (Berdan et al. 2021b), while other studies suggest a possibly faster rate, particularly for CNVs and SVs due to TEs (Katju and Bergthorsson 2013; Stapley et al. 2015). Because short-read methodologies are biased towards SNP detection, the true mutation rates of SVs and how these vary within and across taxa remain largely unknown. Newer technologies such as linked read sequencing that allows us to genotype SVs in large datasets (e.g. Meier et al. 2020) are needed to fill this knowledge gap. Similarly, we still do not understand much about the DFE of many SVs (Berdan et al. 2021b). The majority of the work done so far on *de novo* SVs indicates that many of them are deleterious and are removed by selection (Elena et al. 1998; Hollister and Gaut 2009; Katju and Bergthorsson 2013; Choi and Lee 2020). However, adaptive SVs have been discovered (e.g. Joron et al. 2006; Podrabsky 2009; Van't Hof et al. 2016; Lindtke et al. 2017) and many closely related species have different karyotypes (White 1978), meaning that at least a fraction of SVs can spread and persist. Therefore, more studies on the DFEs of SVs will be critical to allow researchers to infer the evolutionary dynamics of the different types of SVs and better predict their contribution to the buildup of reproductive isolation.

Several lines of evidence suggest that SVs can arise and/or spread quickly, fueling rapid divergence between populations and species. Duplications may underlie rapid evolution because of their larger impacts on expression dosage and new functions (Zhou et al. 2011; Katju and Bergthorsson 2013; Ohno 2013; Rogers et al. 2017). For example, duplications (and inversions) are involved in the rapid emergence of insecticide resistance (Weetman et al. 2018), as well as traits relevant for reproductive isolation such as hybrid sterility (Ting et al. 2004) or chemical communication (Horth 2007). By duplicating and changing position across the genome, TEs may also generate SVs at a rapid rate (Bourgeois and Boissinot 2019). TEs even display bursts of activity resulting in many insertions of the same age (de Boer et al. 2007; Rech et al. 2022) and their activity can be affected by environmental stress, which may favor rapid genetic differentiation in a species shifting to a new area (McClintock 1950; Stapley et al. 2015). Because TEs are associated with multiple processes leading to reproductive isolation, such as ecological differentiation, isolating mating traits, post-zygotic genomic shock and incompatibilities (reviewed by Serrato-Capuchina and Matute, 2018), their rapid dynamics may make them a key factor promoting fast speciation. Some studies also report accelerated evolutionary rate in large CRs, such as in shrews where Robertsonian fusions have been associated with rapid emergence of reproductive isolation (Basset et al. 2019).

Concluding remarks

Past discussions have focused on one of the three main ways that SVs may contribute to speciation differently than SNPs: (1) meiotic irregularities in heterokaryotypes affecting hybrid fitness and generating reproductive isolation, (2) reductions in recombination enabling

the buildup of divergent co-adapted allele complexes between populations impervious to gene flow, (3) mutations and changes in gene expression associated with the creation of SVs (e.g., breakpoint effects). Each of these hypotheses has largely been considered independently as standalone processes accounting for the initial establishment and role for SVs in speciation. However, it is far more likely that a combination of these different processes determines the role of SVs in speciation. A single SV may be involved in multiple processes or different SVs segregating between populations may contribute to reproductive isolation in different ways. Furthermore, the majority of work to date has focused solely on large CRs ignoring other SVs. However, we are now starting to discover the diversity and the extent of structural polymorphism and their ensuing impacts throughout the genome. Here we highlight a few points and indicate remaining open questions (See box).

Nuance is vital to the debate comparing underdominance and recombination suppression. It is challenging to determine which mechanism or combination of mechanisms has led to reproductive isolation because many genetic differences may have accumulated since the initiation of species divergence. However, in certain systems the number of SVs differentiating taxa seems to be a good predictor of an underdominance effect. For example, the low fitness cost associated with heterozygosity for single Robertsonian fusions means that these CRs may readily become fixed in populations, especially given that meiotic drive might promote this (Chmátal et al. 2014). Thus, different populations may accumulate multiple different Robertsonian fusions (all deriving from an ancestral set of acrocentrics), and when these populations come into contact they produce hybrids with long multivalent chain or ring configurations at meiosis (Searle 1993; Hauffe et al. 2012; Borodin et al. 2019). The degree of genetic isolation of karyotypically distinct populations likely reflects a combination of underdominance, breakpoint effects, and recombination reduction (Mackintosh et al. 2023; Yoshida et al. 2023). Disregarding one effect in favor of the others may lead to incorrect conclusions and we encourage researchers to examine multiple effects including the poorly studied interplay of SVs with their genetic background (Everett et al. 1996; Hauffe et al. 2012).

Furthermore, most studies have focused on the role of a single type of SV in promoting speciation. For example, inversions are classic recombination modifiers (Sturtevant 1917), TEs can drive rapid adaptation and divergence (McClintock 1950; Stapley et al. 2015), and fusions can easily fix within populations and can cause extreme underdominance upon secondary contact (Searle 1993; Garagna et al. 2014; Borodin et al. 2019). This points towards the idea that different types of SVs are involved in different types of isolating mechanisms. However, this hypothesis has yet to be truly tested. Examining the breadth of SVs within a system and the different reproductive isolating barriers they underlie will help us better understand the role of SVs in speciation.

In conclusion, species can be considered to represent diverged sets of genes evolving along different evolutionary trajectories. SVs have the potential to be of greater significance to the speciation process than SNPs because they represent variants that encompass and package regions of the genome into modules that can be differentially aligned between taxa. As such, SVs may create an intermediate level between single genes and whole genomes dampening the conflict highlighted by Wu (2001) about what is the unit of speciation. In effect, SVs can harness the effects of the variants they capture and/or gain by extending their joint barrier

effects across larger genomic regions, strengthening reproductive isolation between populations, which ultimately can restrict genetic exchange genome-wide upon the completion of speciation. A variety of different hypotheses concerning SVs and speciation have accumulated over the years with variable degrees of empirical support and contradictory evidence (see also the chapter by Lucek et al, this Volume). We suggest that insights may emerge from considering the possible synergistic effects of these mechanisms operating in concert rather than separately. Much may be gained by investigating if different SVs are associated with different types of isolating barriers (e.g., divergent ecological adaptations and genomic incompatibilities) and if this happens in combination with other SVs. Long-read sequencing is now making the identification and characterization of different types and length SVs methodologically tractable and cost effective for non-model species. Genotyping SVs in large datasets gives us the opportunity to apply approaches from speciation research (population genetics, experiments, comparative genomics, etc) previously reserved for SNPs to overlooked genetic variants. Alongside the development of more complex theoretical models and simulations accounting for SVs properties such as length, mutation rate, recombination impact, we are entering exciting times when answers to long-standing questions about SVs and speciation may finally be at hand.

Outstanding questions

- How do polymorphic SVs, often segregating under balancing selection within population (which can oppose speciation), end up contributing to speciation?
- Do new SVs frequently 'capture' loci involved in reproductive isolation or do these loci preferentially evolve in regions of low recombination (i.e., 'gain')?
- How do the properties of SVs such as type and length modulate their likelihood to contribute to speciation?
- What is the mutation rate and distribution of fitness effects (DFE) of SVs, and how do they influence the emergence of reproductive isolation and the dynamics of species divergence?
- Do the impacts of SVs that extend beyond the SV itself (i.e., epigenetic changes) affect reproductive isolation?
- How does the meiotic impact of CRs, and the putative resulting underdominance, vary across taxa?
- To what extent does structural variation contribute to the genomic divergence between closely related species?
- To what extent does underdominance of SVs, including many weak additive underdominant effects, may contribute to reproductive isolation?

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