Chromosomal speciation through holocentric lenses

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Abstract

Chromosomal rearrangements (CRs) can trigger speciation by acting as barriers to gene flow. Two main theoretical frameworks were developed on how they contribute to speciation: causing infertility in heterokaryotypes (hybrid dysfunction) or creating islands of divergence through recombination suppression. However, these theories were developed with monocentric chromosomes in mind. Species with holocentric chromosomes, which lack a localized centromere, account for 15-20 % of the eukaryote biodiversity in terms of species richness. These organisms have a higher tolerance for large CRs but the number of chiasmata (recombination) is more restricted, defying assumptions for both types of chromosomal speciation models. This highlights the need to look at chromosomal speciation through holocentric lenses.

Here, we synthesize how holocentricity redefines these frameworks across diverse lineages, shedding light into the diverse patterns in both karyotypic diversity and their impact on speciation. We further show that the effects of CRs depend on interactions among meiotic mechanisms, genome organization, reproductive barriers, and demographic history. Rather than producing a single evolutionary outcome, holocentricity generates a diversity of pathways through which chromosome evolution can influence speciation.

Introduction:

Chromosome evolution is one of the major drivers of diversification in eukaryotes, where chromosome architecture plays a fundamental role in shaping genome stability and evolutionary trajectories (Coghlan et al., 2005). The structural organization at the chromosomal level influences how the genetic material is transmitted and recombined, ultimately impacting macroevolutionary processes such as adaptation and speciation (Huang & Rieseberg, 2020). Among the various features contributing to chromosomal diversity, variation in centromere organization represents one of the most intriguing, with important consequences for karyotype evolution and the establishment of reproductive barriers (Lucek et al., 2022; Steckenborn & Marques, 2025). Two types of chromosomes can be distinguished depending on centromere organization: monocentric and holocentric. In monocentric chromosomes, a single region, the centromere, serves as the organizing centre for Mendelian partitioning of homologues during cell divisions. While in holocentrics, centromeric functions can be found throughout the chromosome (Benítez-Benítez et al., 2025; Hipp et al., 2009; Mayrose & Lysak, 2021). Holocentric chromosomes have evolved multiple times across eukaryotes and species with holocentric chromosomes account for 15-20% of the species diversity in this domain (Escudero et al., 2016; Márquez-Corro et al., 2018).

The diffuse centromere organization of holocentric chromosomes represents a fundamental shift in genomic stability and karyotype evolution. Disperse kinetochoric activity increases the likelihood of chromosomal fragments and fused chromosomes to be correctly segregated, facilitating rapid karyotypic shifts through gains (chromosome fission) or losses (chromosome fussion) of single chromosomes (Escudero et al., 2014; Mayrose et al., 2010). In addition, inversions could theoretically be larger and occur through a wider set of areas within the chromosome in holocentric organisms, as they are not limited by a localized centromere. In monocentric organisms, contrary to holocentrics, inversions where the centromere is involved (pericentric inversions) can be quite underdominant as they alter symmetry between homologs, resulting in impaired chromosome pairing (Coyne et al., 1991; Kaiser, 1984).

However, in holocentrics, meiosis remains challenging because of the spatial interference between recombination and segregation functions, which can lead to negative outcomes such as chromosome missegregation (Nambiar & Smith, 2016). Monocentric organisms solve this problem by separating these functions in space: recombination is suppressed in centromeres and their flanking areas where segregation takes place (Fernandes et al., 2019). In holocentric

organisms, spatial separation is much more compromised as kinetochore proteins assemble along the entire chromosome length. These additional constraints are reflected by the number of crossing-over (CO) events per chromosome, as holocentric organisms seem to not tolerate more than two COs per chromosome (Castellani et al., 2024; Nokkala et al., 2004; Zedek et al., 2026) while monocentric chromosomes routinely reach 3 or 4 CO events (Lian et al., 2023; Zedek et al., 2026). This reduction likely reflects structural constraints during meiosis, as holocentric bivalents retain a parallel, flat configuration through metaphase I rather than forming the perpendicular chiasma arrangements typical of monocentric chromosomes (Figure 1). As a result, chiasmata remain effectively interlocked, generating complex three-dimensional structures that can hinder proper bipolar orientation and increase the risk of segregation errors (Nokkala et al., 2004). Several holocentric lineages have evolved modified meiotic processes to overcome these challenges (Figure 2). A remarkable solution is inverted meiosis (Figure 2), in which sister chromatids segregate during the first meiotic division, followed by homologous chromosomes undergoing reductional segregation, reducing the complexity of the process (Mandrioli & Manicardi, 2020). Inverted meiosis has been reported in insects belonging to Hemiptera and Lepidoptera and some flowering plants such as sedges and genus *Luzula* within rushes (Bogdanov, 2016). Other holocentric groups seem to maintain canonical meiosis restricting the number of crossovers (Márquez-Corro et al., 2018), with some Lepidoptera avoiding COs altogether in female meiosis (Lukhtanov *et al.*, 2018), while nematodes limit cross-overs to one, which happens typically in the terminal region, forming a perpendicular chiasma arrangement in meiosis I similar to the typical segregation of monocentric organisms (Rockman & Kruglyak, 2009; Carlton et al., 2022). However, the knowledge on meiosis across holocentric groups remains limited to a few species.

Figure 1: Comparison of chromosome segregation for different types of centromeres and microtubule attachment modes.

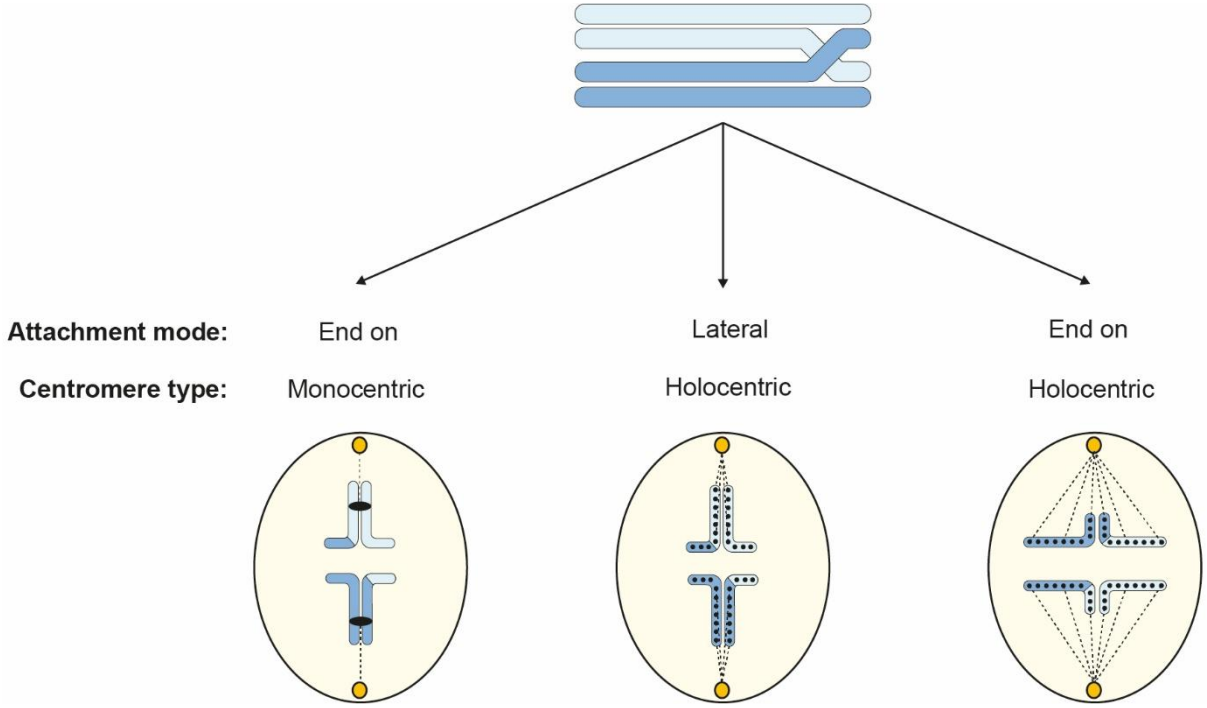
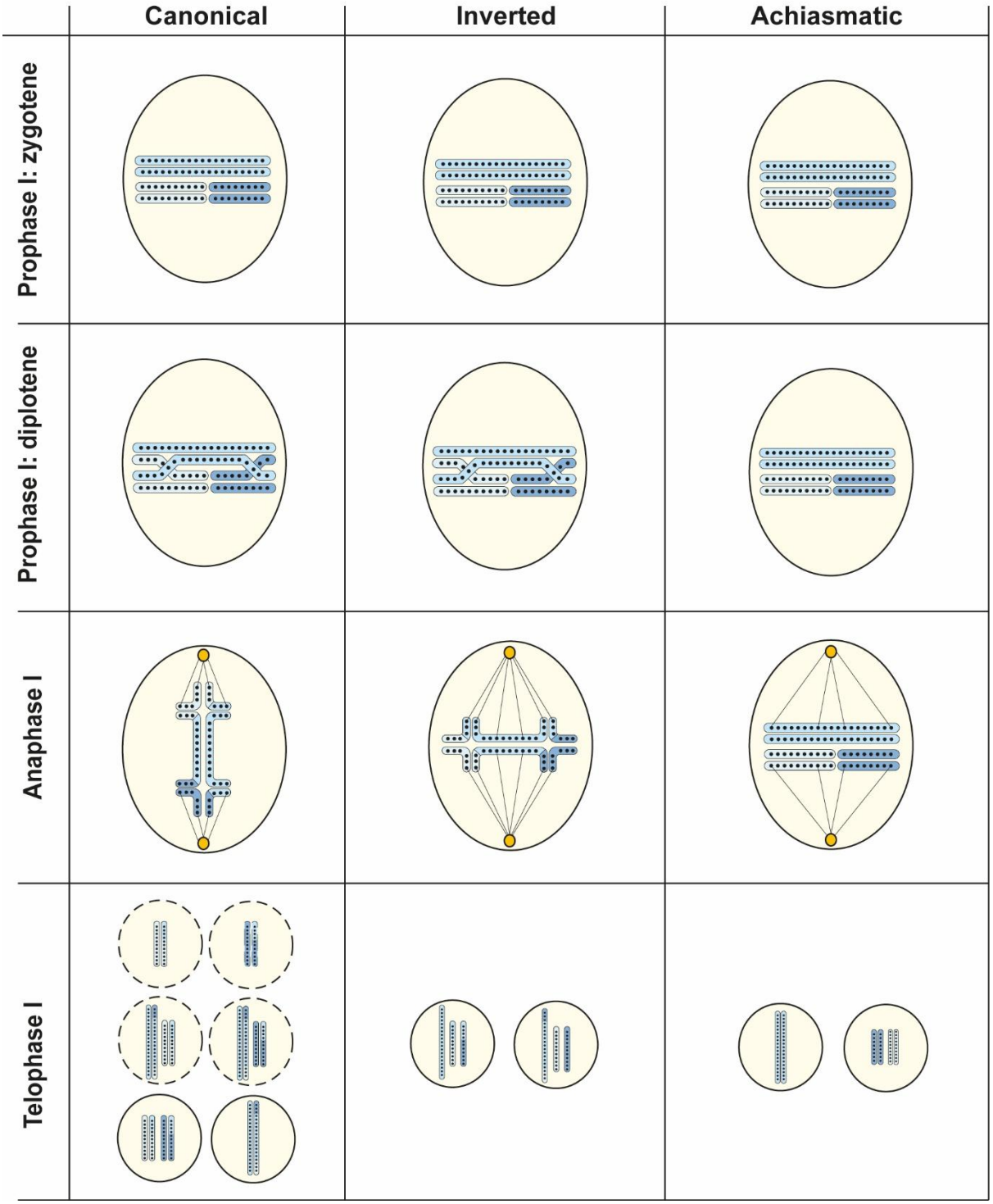


Figure 2: Types of meiosis in holocentric organisms throughout different meiosis phases, represented for heterokaryotypes: canonical (left), inverted (center) and achiasmatic (right). Dashed cell boundaries in Telophase 1 of the canonical meiosis indicate unbalanced meiotic products.



The gain or loss of single chromosomes without changing DNA content, also known as dysploidy, is common across eukaryotic lineages (Benítez-Benítez et al., 2025; Escudero et al., 2014). However, it is especially frequent among groups with holocentric chromosomes (Benítez-Benítez et al., 2025; Escudero et al., 2014), including some of the most species-rich and diverse lineages: nematodes (25,000 sp; Hodda, 2011), true bugs (80,000 sp; Forero, 2008), sedges (5,500 sp; Martín-Bravo *et al.*, 2019) and butterflies and moths (160,000 sp; Nieukerken *et al.*, 2011). The role of chromosomal rearrangements (CRs) on speciation has been debated for decades (Coyne et al., 2004; King, 1993; Rieseberg, 2001; White, 1978). Different theories have been conceptualized to explain how CRs can create barriers to gene flow (Faria & Navarro, 2010; Rieseberg, 2001). The literature traditionally distinguishes two main theoretical frameworks: the Hybrid Dysfunction and the Recombination Suppression models. The former emphasizes the immediate post-zygotic fitness costs of CRs (White, 1973). Hybrids between distinct chromosomal cytotypes often carry mismatched chromosomes, which will fail to pair or segregate correctly during meiosis, leading to the production of aneuploid gametes and reduced fertility (King, 1993; White, 1978). The latter framework suggests that CRs promote speciation not by reducing hybrid viability, but by preserving advantageous gene combinations. Rearrangements such as inversions, fissions/fusions or translocations suppress recombination in specific genomic regions, allowing sets of co-adapted alleles ("supergenes") to be inherited together (Ayala & Coluzzi, 2005). By acting as barrier loci, these CRs can enable populations to adapt to different environments even in the presence of some gene flow. While hybrid dysfunction is often linked to cladogenetic events, i.e. rapid branching along a phylogeny, recombination suppression is more consistent with gradual change along a lineage (anagenesis) (Lucek et al., 2022). Both theoretical frameworks propose that variation in CRs among populations can facilitate reproductive isolation, thereby increasing the potential for speciation (Lucek et al., 2023). However, all these models were developed with monocentric organisms in mind.

Here, we synthesize the current knowledge on the role of centromeric architecture in shaping the mechanisms and evolutionary consequences (i.e. speciation) of chromosome evolution. By integrating insights from hybrid dysfunction and recombination suppression models, we seek to evaluate how holocentric chromosomes defy these models assumptions exposing new conditions and outcomes.

1. Hybrid dysfunction and holocentricity

Hybrid dysfunction models of chromosomal speciation were the first to be conceptualized (reviewed in King, 1993; White, 1978), based on observations that hybrids between two chromosomal races (heterokaryotypes) often exhibit abnormal chromosome pairing during meiosis. The broader hybrid dysfunction concept was already previously applied for individual genes: Bateson-Dobzhansky-Muller incompatibilities (BDMi) (Bateson, 1909; Dobzhansky, 1936; Muller, 1942), where negative epistasis between genes with different evolutionary histories act as gene flow barriers between divergent populations.

Both hybrid dysfunction models, chromosomal and genic, present the same well-known problem: the underdominance paradox. If an allele or CR is highly underdominant, it is unlikely to become established or fixed within a population, and therefore would not constitute an effective barrier to gene flow or contribute to speciation (Lande, 1985; Walsh, 1982). Some models assume high individual rearrangement underdominance (Chromosomal Transilience Model, Table 1), and present the conditions necessary for the new underdominant karyotype to become fixed. Almost all of them rely on small and inbred populations (low effective population size), where drift is stronger than selection, facilitating the establishment of a new karyotype in spite of its underdominance. Also, the majority of these models assume some kind of geographic isolation of the new karyotype, facilitating its fixation. For example, the Quantum speciation model (Grant, 1981; Table 1) assumes that the new karyotype occupies peripheral areas of the distribution with a different environment. This way, while isolation and founder effects facilitate the fixation of the new karyotype, genetic incompatibilities and local adaptation could also be contributing to speciation.

Nonetheless, the most important criticism concerning high underdominance hybrid dysfunction models was the discovery that highly underdominant rearrangements were rarer than previously expected, especially those that are not completely deleterious (Coyne et al., 1993; John, 1981; Lande, 1985; Nachman & Myers, 1989). Strong underdominant rearrangements are expected to be even rarer in holocentric species even for large scale CRs such as chromosomal fusions and fissions (Lucek et al., 2022): while in monocentric organisms, fissions lead to acentric chromosomal segments that are lost during meiosis, in holocentric organisms these segments retain centromeric activity and can segregate normally (Melters et al., 2012). In the same way, fusions do not lead to dicentric chromosomes in

holocentric organisms, which typically lead to unstable meiosis, chromosome rearrangement and breakage (Hill & Bloom, 1987; McClintock, 1939). Lower underdominance and/or deleteriousness of large CRs would be reflected in higher establishment rates of new structural variants and therefore, higher intra- and interspecific karyotypic diversity. Such exceptional karyotypic diversity has been found in some holocentric clades (Table 2) but a general higher karyotypic diversity in holocentric clades compared to monocentric ones is contentious (Table 2). CR tolerance seems to vary between holocentric organisms, mainly in the ability of rearranged fragments to segregate correctly through meiosis with multivalents and other aberrant formations, which depends on their type of meiosis (Figure 2). Available data suggest that inverted meiosis is the mechanism that results in proper segregation with higher number of heterozygous rearrangements (Lukhtanov et al., 2018; Mandrioli & Manicardi, 2020), while allowing more than one cross-over. In fact, in species capable of both canonical and inverted meiosis, the latter is preferred in face of heterokaryotypes (Murakami & Imai, 1974; Yoshida, in press). It is not surprising that in groups with highest karyological diversity, i.e. sedges (Cabral et al., 2014), aphids (Viera et al., 2008) and butterflies from the family Lycaenidae (Lukhtanov & Dantchenko, 2017) and Pieridae (Lukhtanov et al., 2018), inverted meiosis has been reported.

In some karyotypically highly diverse holocentric clades, a significant part of the variation is intraspecific (Table 2). Such intraspecific variation and the inherent tolerance for rearrangements in holocentric organisms challenge high-underdominance models; instead, they support low underdominance hybrid dysfunction models. These frameworks rely on the gradual accumulation of rearrangements with minimal fitness costs, which establish an effective gene flow barrier in a step-by-step manner—as described by the Chain Cascade Model (Table 1)—paralleling the dynamics of Bateson-Dobzhansky-Muller incompatibilities (BDMIs). For BDMIs, there are expansions of the speciation models where the incompatibility does not even need to be fixed within its deme in order to contribute to speciation (Cutter, 2012; Fierst & Hansen, 2010). Therefore, one can conceptualize the same for CRs, further broadening the applicability of the models to clades with intrapopulation CR diversity. The Chain Cascade Model has been proposed to contribute to the diversification of sedges (Escudero et al., 2016; Tribble et al., 2025). Apart from the speed at which new karyotypes are established in *Carex* L., there is empirical evidence showing that F1 viability decreases with higher number of rearrangements in *Carex scoparia* Schkuhr ex Willd. (Escudero et al., 2016), leading to a

positive correlation between the number of CRs and genetic distance (Hipp et al., 2010; Whitkus, 1988). At a higher evolutionary scale, a significant correlation between time of coalescence of each species and their chromosome variation has been inferred in the *Carex* clade Spirostachyae (Escudero, Hipp, et al., 2010). Sedges present reproductive and demographic characteristics that favor the establishment of new karyotypes: high selfing rates and inbreeding (Escudero, Valcárcel, et al., 2010; Friedman & Barrett, 2009) together with high dispersal capability (Escudero et al., 2009; Escudero, Valcárcel, et al., 2010; Jiménez-Mejías et al., 2011) promoting small and highly homozygotic populations (Escudero et al., 2016). Chromosomal speciation in *Carex* has been explored at a higher evolutionary level through a phylogenetic framework (Tribble et al., 2025). Theoretically it has been suggested that in holocentrics, cladogenic events with changes in chromosome number are consistent with classic hybrid dysfunction models (high underdominance), while anagenic ones are more consistent with chromosome changes that lead to recombination suppression models (Lucek et al., 2022). The mixture of anagenic and cladogenic events support the Chain Cascade Model, where CRs accumulate until one leads to a speciation event ("last straw hypothesis") (Tribble et al., 2025). Aphids have similar demographic characteristics, as they reproduce mainly asexually through parthenogenesis and winged individuals have important dispersal capability being able to create a new population on their own (Lombaert et al., 2006). Similarly, aphids show high rates of CR across the whole clade, and the most diverse clades are also those with the highest chromosome rearrangement rates (Huang et al., 2025). However, studies at lower taxonomic scales, testing key evidence of hybrid dysfunction—such as heterokaryotype infertility and the association between genetic distance and chromosome number—would be necessary to determine chromosomal hybrid dysfunction role in their speciation.

Patterns of chromosome evolution across Lepidoptera provide additional context for these hypotheses. The majority of Lepidoptera species maintain a chromosomal number close to the ancestral state (n=31), which are known as the Merian elements (Wright et al., 2024). Chromosome fissions are rarer than fusions and occur mainly in families with high karyological diversity (Wright et al., 2024), where fissions often coincide with cladogenic events linked to hybrid dysfunction chromosomal speciation models (Augustijnen et al., 2024; de Vos et al., 2020). Reproductive isolation in butterflies is often prezygotic through mate choice (Kandul et al., 2007; Lukhtanov et al., 2005), so CRs may contribute mostly as introgression barriers between recently isolated species (de Vos et al., 2020). However, alternative mechanisms have been reported where CRs directly drive speciation. Specifically, a recombinational hybrid

dysfunction model—also conceptualized as the chromosomal variant of Hybrid Homoploid Speciation (HHS; Table 2)—has been proposed for certain butterfly groups (Lukhtanov et al., 2015). In this framework, hybridization between two parental species with different fixed rearrangements lead to a homozygous recombinant F2 with a new combination of rearrangements. If the recombinant progeny is isolated through post-zygotic barriers from the parents, it can be considered as a chromosomal speciation event through hybrid dysfunction (Lukhtanov et al., 2015). High karyotypic diversity has only led to speciation in one (family Lycaenidae) of the two clades where inverted meiosis has been reported (families Pieridae and Lycaenidae; Table 2). Correlation between genetic distance and the number of CRs points towards chromosomal speciation in genus *Polyommatus* (family Lycaenidae; Lukhtanov et al., 2005), but hybrid fertility measures would be necessary to confirm hybrid dysfunction. While in the family Pieridae, *Leptidea sinapis* did not show this pattern (Lukhtanov et al., 2011), and high hybrid fertility was found between parents differing in 24 fusions/fissions (Lukhtanov et al., 2018). The main differences between these two clades lie in their demographic dynamics: *L. sinapis* is a generalist with a wide distribution area, leading very low population structure (Lukhtanov et al., 2011), while *Polyommatus* populations are smaller and geographically isolated, allowing allopatric speciation to occur (Lukhtanov et al., 2005).

The Stasipatric Model considers the role of centromere drive in the establishment and/or fixation of a highly underdominant karyotype within a continuously distributed population (Table 1). According to this meiotic drive model, centromeres make a selfish effort to exploit an asymmetric meiosis, where only one of the four meiotic products survives, to secure their preferential transmission at the expense of their homologous counterparts (Henikoff et al., 2001; Malik & Henikoff, 2009). Centromere size is typically the determinant factor by which centromeres will be favoured (Burrack et al., 2011). A similar phenomenon, the holokinetic drive (Bureš & Zedek, 2014a), has been suggested for some holocentric species, where the preferential transmission of chromosomes depends on chromosome size, as kinetochore assembly occurs along their entire length. The most compelling evidence for holokinetic drive comes from sedges, as it would be expected considering that both meiosis in spermatogenesis and oogenesis are asymmetric (Furness & Rudall, 1999). A negative correlation between genome size and chromosome number is found across different evolutionary scales, including in the holocentric Cyperaceae and Juncaceae families (Elliott et al., 2022; Roalson et al., 2007). However, at lower evolutionary levels, the opposite pattern, a positive correlation, has been reported (K.-S. Chung et al., 2012), while within individual

Carex species, no relationship or only a weak positive relationship has been found (Chung et al., 2011; Escudero et al., 2015). The role of holokinetic drive in karyologically dynamic holocentric animals seems to be more clade dependent, with only one of the meiosis being asymmetric in aphids (Yan et al., 2020) and butterflies (Boman et al., 2024). Among aphids, the pattern previously described is also found (Bureš & Zedek, 2014a). In Lepidoptera, evidence of holokinetic drive is so far restricted to karyologically dynamic clades with high chromosome numbers (Beuret, 1957; Boman et al., 2024; Nagaraju & Jolly, 1986) and its effect is unidirectional and conservative—contrasting with aphids and sedges—as it opposes the transmission of derived fused chromosomes (Boman et al., 2024). Nematodes do not show consistent patterns with holokinetic drive likely due to their segregation pattern in meiosis which share some similarities with monocentric organisms (Bureš & Zedek, 2014a) (Figure 2, Table 2).

One of the most diverse and well-studied holocentric groups exhibiting surprisingly low interchromosomal CR rate is nematodes (Carlton et al., 2022). This pattern is reflected in their unusually stable karyotype (Carlton et al., 2022) and a conserved macrosynteny across distantly related species (Cotton et al., 2016; Rödelsperger, 2024). Their unique chromosome organization is one possible explanation: chromosome centers are enriched in conserved fundamental genes with strong linkage disequilibrium and their smaller and DNA repetitive chromosome arms with non essential genes (Carlton et al., 2022; The *C. elegans* Sequencing Consortium, 1998). Interchromosomal rearrangements that disturb these centers are strongly selected against as they contain coadapted genes involved in essential metabolic pathways (Gonzalez de la Rosa et al., 2021; Rödelsperger, 2024). Also, selection against underdominant karyotypes is facilitated by large effective population sizes (Carlton et al., 2022; Hillier et al., 2007). Another possible explanation is that their unique subtelomeric sequence motifs, which define pairing centers, are important for initiation of synapsis of homologous chromosomes and may be disrupted by chromosome fusions or fissions (Carlton et al., 2022; Hillier et al., 2007). The ultimate reasons behind this karyotype stability remain unknown (Carlton et al., 2022), but are likely nematode specific rather than incorrect chromosome segregation during meiosis as traditional hybrid dysfunction chromosomal speciation models hypothesize. Nevertheless, there is one example for the nematode genus *Pristionchus*, which contains closely related species that underwent independent chromosome fusions and whose hybrids suffer from lower fertility due to aneuploidy (Yoshida et al., 2023), fitting a strong underdominance model. Interestingly, chromosomes involved in fusions are the same across

different clades (Rödelsperger, 2024), suggesting that they have unique characteristics that relax the tight regulation of interchromosomal rearrangements in nematodes.

Table 1: Overview of major hybrid dysfunction chromosomal speciation models, summarizing the level of underdominance, the evolutionary forces involved, the requirement for geographic isolation, and their likelihood in holocentric organisms.

Hybrid Dysfunction Model	Underdominance level	Evolutionary forces	Need for geographic isolation?	Likelihood and examples in holocentric organisms
Chain Cascade Model (White, 1978)	Weak/ moderate	Drift and positive selection (if the new karyotype is located in an area with different environmental characteristics)	Yes	High, reported mainly in sedges (Whitkus, 1988; Escudero et al., 2016; Gómez-Ramos et al., 2026a)
Chromosomal Transilience Model (Templeton, 1981)	Strong	High drift	Yes	Low
Monobrachial Fusion Model (Baker & Bickham, 1986)	Weak between ancestral and derived populations. Strong between two derived populations (simplest case of Chain Cascade Model)	Drift	Yes	Not applicable, as the model depends on centromere position
Recombinational Model (also referred as Chromosomal Homoploid Hybrid Speciation model) (Grant, 1981)	Strong	Hybridization	Maybe	Reported in butterflies (Lukhtanov et al., 2015).
Quantum speciation model (Grant, 1981)	Strong	Drift and positive selection	Yes (peripheral populations)	Low if the original model is considered, high if we expand it to include low underdominant rearrangements (Whitkus, 1988; Escudero et al., 2016; Gómez-Ramos et al., 2026b)
Stasipatric model (White, 1978)	Strong	Drift and meiotic drive	No	Yes, with the particularities of holokinetic drive (Escudero et al., 2016; Bureš & Zedek, 2014)

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368 Table 2. Empirical support for key predictions on how holocentricity and other related traits can impact hybrid
369 dysfunction chromosomal speciation models.

Prediction	**Empirical support**
(i) Holocentric organisms have higher tolerance for chromosomal rearrangements leading to high karyological diversity caused by dysploidy in holocentric clades	✓Sedges: (E. H. Roalson, 2008) ✓Woodrushes (Bozek et al., 2012) ~Butterflies (only two families) (de Vos et al., 2020) ~ Hemiptera (only for scale insects (Cook, 2000) and aphids (C. Huang et al., 2025) ~Scorpions (one family (Schneider et al., 2009)) ~Six eyed spiders (one family Dysderidae) ✗ Nematodes (Blaxter, 2024)
(ii) The most karyotypically diverse clades in holocentrics have inverted meiosis	✓ Inverted meiosis in sedges and woodrushes (Cabral et al., 2014) ~ Inverted meiosis only in sex chromosomes during spermatogenesis in Hemiptera (Viera et al., 2008) ✓ Inverted meiosis in two butterfly families with high karyotypical diversity (Lukhtanov et al., 2018; Lukhtanov & Dantchenko, 2017)
(iii) Holocentric clades consistently show higher chromosomal rearrangement rates than monocentric ones	✗ Insects (Ruckman et al., 2020) ~ Plants (Valdés-Florido et al., 2026)
(iv) Part of these karyological diversity remains intraspecific or even intrapopulational in highly karyotypically diverse clades	✓ True sedges (genus *Carex* ~40% sp with intraspecific variation) (Escudero et al., 2013, 2024; Luceño & Castroviejo, 1991) ~ Butterflies (*Leptidea sinapis* (Lukhtanov et al., 2018)

(v) The increase of heterozygous rearrangements in a heterokaryotype increases hybrid inviability and/or infertility	✓ True sedges: *Carex scoparia* (Escudero et al., 2016) ~Butterflies: ✗ *Leptidea sinapis* (Lukhtanov et al., 2018) ✓ *Polyommatus* (Lukhtanov & Dantchenko, 2017) ✓ ✓ Nematodes: *Pristionchus exspecatus-P.pacificus* (Yoshida et al., 2023)
(vi) Genetic distance/time of coalescence between populations/species is correlated with their difference in the number of rearrangements	✓ *Carex scoparia* (Escudero et al., 2016) ✓ *Carex* clade Spirostachyae (Escudero et al., 2010) ✓ Butterflies: *Polyommatus* (Lukhtanov et al., 2005) *Leptidea sinapis* (Lukhtanov et al., 2011)

2. Recombination suppression models and holocentricity

For hybrid dysfunction models, the main difference between holocentric and monocentric organisms is clear: large CRs exhibit lower underdominance (Lucek et al., 2022). This distinction is less evident in recombination suppression models, as there are no substantial available data to determine whether these models behave differently in holocentric organisms. The increasing availability of recombination data from a wider range of organisms, driven by the reduced costs of sequencing technologies, has revealed certain promising and particularities of recombination patterns in holocentric species.

The compromised spatial separation between segregation and recombination functions in holocentric chromosomes translates into a lower number of CO events per chromosome: with no more than two CO per chromosome and most chromosomes only having one, while monocentric chromosomes routinely reach three or four CO events (Castellani et al., 2024; Lian et al., 2023; Nokkala et al., 2004; Zedek et al., 2026). In addition to the absence of a single centromere, which constrain patterns of recombination in monocentrics, the limitation to only one CO in the majority of holocentric chromosomes leads to a more constant recombination rate within chromosomes, which is not observed when there is more than one CO as they must maintain a certain distance between each other (Hunter, 2015). In this way, larger holocentric chromosomes that are able to fit two COs during meiosis, do show a bimodal pattern of recombination density as the two COs must maintain a certain distance with each other (Table

4). The mechanisms underlying segregation differ among holocentric organisms, consequently influencing recombination. For instance, sedges possess satellite-based holocentromeres where the kinetochore is recruited similarly to monocentrics (Marques et al., 2015). In contrast, Lepidoptera assemble kinetochores wherever chromatin is permissive (Senaratne et al., 2021). Finally, nematodes exhibit a form of meiosis in which a single subtelomeric crossover per chromosome pair forms cruciform bivalents composed of short and long bivalent arms (Nabeshima et al., 2004; Rockman & Kruglyak, 2009), where the long bivalent arms are directed toward the segregation poles, as in canonical meiosis, rather than in inverted meiosis (Figure 1c).

According to traditional recombination suppression models, the evolutionary potential of inversions lies in how much they change the recombination landscape and the number of genes they bring into linkage disequilibrium. One of the main determinants of an inversion's evolutionary potential is its size: the larger the more impact it can have. In monocentric organisms, inversions are typically restricted within the same chromosome arm (Coyne et al., 1991). Inversions where the centromere is affected (pericentric inversions) impact chromosome pairing as they alter symmetry between homologs (Coyne et al., 1991; Kaiser, 1984). This limitation does not exist in holocentric chromosomes, so a wider range of genes can be linked together through inversions. While inversions typically emerge due to ectopic recombination between repetitive elements (Gray, 2000; Kent et al., 2017), this seems to be more evolutionary impactful when inverted genes are located in subtelomeric areas with high recombination rates (Fernandes et al., 2019; Muller et al., 2019). Similarly to recombination, the lack of a centralized centromere leads to a more homogenous distribution of genes and repetitive elements in holocentric organisms, with the exception of nematodes and mites (Table 4). Overall, we can hypothesize that evolutionary relevant inversions can appear in a wider range of locations throughout the genome in holocentric organisms, potentially linking together a more diverse set of gene groups. While there are some examples for the role of inversions in speciation for holocentric organisms (Gómez-Ramos et al., 2026b; Hillier et al., 2097; Joron et al., 2011), the lack of in-depth studies on the impact of inversions on genes across a wider set of holocentric organisms impedes testing this hypothesis. As sequencing technologies become more accessible, examples of evolutionary relevant inversions will increase and comparing the size, location and redundancy of these inversions between mono- and holocentric will become possible, shedding some light into their unique evolutionary patterns.

Previous authors (Sánchez-Villegas et al., 2026; (Benítez-Benítez et al., 2025; Berdan et al., 2024; Lucek et al., 2023) have called for increased attention to CRs other than inversions as recombination-suppressors, as these have received far less attention even with emerging evidence of their impact on recombination (Guerrero & Kirkpatrick, 2014; Vara et al., 2021). In addition to local suppression recombination near the breakpoints, translocations and fusions can create new combinations of genes in linkage disequilibrium. Because reciprocal translocation, fissions and fusions generally exhibit lower underdominance in holocentric organisms than in monocentrics, particularly in those with inverted meiosis (Lukhtanov et al., 2018), their role in speciation may arise primarily through their effects on recombination rather than through direct reduction in hybrid fitness. Chromosomal fusions are more common and widespread in Lepidoptera than fissions (Wright et al., 2024) and are mostly associated with anagenic chromosomal speciation events (Augustijnen et al., 2024; Mackintosh et al., 2024), supporting the hypothesis that they contribute to chromosomal speciation as recombination suppressors. This is further supported by empirical evidence of reduced introgression (from reduced recombination) in fused chromosomes—as a whole and near breakproints—facilitating the appearance of genic incompatibilities that eventually lead to prezygotic reproductive barriers (Edelman et al., 2019; Mackintosh et al., 2023). This is also an alternative hypothesis to explain the correlation between karyological diversity and species richness in aphids, which could be more likely considering the importance of plant-host relation for their speciation (Peccoud et al., 2014). An example of the role of chromosome fusions and fissions as recombination architects is found within the nematode genus Pristionchus, an exception to the karyological nematode stability, who has undergone frequent chromosome fusions and fissions along with their diversification (Yoshida et al., 2024). Recombination analyses of closely related Pristionchus species with independent chromosome fusions indicated that these CRs extensively reshaped the recombination landscape, restoring a single crossover limited in new subtelomeric regions (i.e. chromosome arms; Yoshida et al., 2023). Because holocentric chromosomes have highly restricted crossover formation, genes located on ancestral chromosome arms, which now lie in chromosome centers, have undergone strong recombination suppression and have become tightly linked as a consequence of chromosome fusions.

Many CRs involved in local adaptation can remain polymorphic for a long time before leading to speciation (Wellenreuther & Bernatchez, 2018). However, if one of the coadapted genes is involved in mate choice, the CR is more likely to result in speciation (Servedio et al.,

2011; Trickett & Butlin, 1994). Sex chromosomes are enriched in reproductive functions, accounting for a disproportionately high fraction of reported BDMi (Coyne, 1992; Haldane, 1922). One particular form of chromosomal speciation through recombination suppression is sex chromosome-autosome fusions, as they bring reproductive genes in linkage with a new set of autosomal genes. These fusions can be common across whole clades (Wright et al., 2024) and they also tend to be the first CRs to appear when species diverge (Carabajal Paladino et al., 2019; Nguyen et al., 2013; Nilsson et al., 1988). The higher turnover rate of sex chromosomes over autosomes appears to remain true no matter the CR rate of the clade (Gonzalez de la Rosa et al., 2021; Yoshida et al., 2024). Aphids offer an exception to this rule, with sex chromosomes being more conserved than autosomes, but these exceptions are likely associated with their particular life cycle where multiple asexual generations are followed by a sexual one (C. Huang et al., 2025). CRs involving sex chromosomes are not limited to holocentric species as sex chromosome turnover is common in fishes (Kitano et al., 2009; Kitano & Peichel, 2012) and mammals (Graves, 2016), contributing to their diversification. However, they involve other mechanisms rather than large interchromosomal rearrangements, such as small translocations and duplications of key genes involved in gonad-differentiation changing the sex chromosome identity (Graves, 2016).

While large interchromosomal rearrangements are rare in most nematode clades, smaller rearrangements within, or even between, subtelomeric regions (i.e. chromosome arms) are common as they are enriched for repeats (Hillier et al., 2007; Stein et al., 2003). These chromosome arms have high recombination rates and higher proportion of non-essential genes such as lineage specific duplicates and taxonomically restricted orphan genes (Prabh & Rödelsperger, 2022; Thomas, 2006). Small CRs contribute to genic innovation in chromosome arms in nematodes (Rödelsperger, 2024) either directly by interfering with genes or indirectly by suppressing recombination. In Caenorhabditis elegans, genes with functions related to environmental interactions (i.e. chemoreceptors, antimicrobial defenses), which likely promote local adaptation, are enriched in chromosome arms (Prabh & Rödelsperger, 2022; Thomas, 2006).

Decreased flexibility in CO events per chromosome affects how CRs impact recombination rate outside of the local inverted areas and near breakpoints (Senaratne et al., 2021). While mostly ignored by recombination suppression models (RSM) (Table 3), compensatory increase of recombination within the rearranged chromosome or even across

other chromosomes has been known since the first experiments of inversions in Drosophila (Sturtevant, 1921; Sturtevant & Beadle, 1936). The higher prevalence of fissions and fusions in certain holocentric groups and the higher recombination rate dependency on the number of chromosomes in holocentric organisms suggests a role of fusions/fissions as recombination rate regulators (Box S1), where holokinetic drive selects different chromosome sizes depending on the level of recombination favourable in different environments (see Box S1). CRs may be selected not depending on their impact on genes but because of their impact on recombination rate presenting an alternative scenario for intraspecific chromosome number clines. An example is Carex laevigata, where in populations in northern and higher areas, fusions may be selected as they reduce recombination which is advantageous in less favorable environments, where immediate fitness may be required (see Box S1).

Table 3: Overview of major recombination suppression chromosomal speciation models, summarizing the evolutionary forces involved, the scope of influence achromosomal rearrangement (CR), their requirement for geographic isolation, and the way the CR may contribute to speciation.

Recombinant suppression model	**Evolutionary forces**	**Scope of influence of CRs**	**Need for (partial) geographic isolation?**	**How does CRs contribute to speciation?**
Coluzzi's model (Coluzzi, 1982)	Local adaptation	Restricted to CRs	Yes	Not specified
Trickett and Butlin's model (Trickett & Butlin, 1994)	Local adaptation + Sexual selection and reinforcement	Restricted to CRs	Yes	Not specified
Rieseberg's model (2001)	Local adaptation + Sexual selection and reinforcement	CRs + Epistasis with BDMi's in other genomic areas	Yes	Not specified
Chromosomal Homoploid Hybrid Speciation (incompatibility is genic and inside CR) (Lukhtanov et al., 2015)	Local adaptation + Sexual selection and reinforcement +Hybridization	Restricted to CRs	No	Not specified
Kirkpatrick–Barton model (2006)	Local adaptation + Sexual selection and reinforcement	Restricted to CRs	Yes	CRs protect BDMi's from introgression

Navarro-Barton model (2003)	Local adaptation + Sexual selection and reinforcement	Restricted to CRs	Yes	CRs protect BDMi's from introgression +accelerate the appearance of new BDMi's

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515 Table 4. Empirical support for key predictions on how holocentricity itself and other related traits impact
516 recombination suppression chromosomal speciation models.

Prediction	**Empirical support**
(i) Holocentric organisms have a more homogenous chromatin organization and/or TE's throughout the genome	✓ Sedges: true sedges (Gómez-Ramos et al., 2026a), break-sedges (Hofstatter et al., 2022) ✓ Aphids (Grbić et al., 2011) ✓ Butterflies (Mandrioli & Manicardi, 2012) ✗ Nematodes: gene-rich center and repeat-rich ends (Carlton et al., 2022; The C. elegans Sequencing Consortium, 1998) ✗ Mites: gene-rich center and repeat-rich ends (Chmielewski et al., 2025)
(ii) Holocentric chromosomes represent a constant recombination landscape in small chromosomes. In larger chromosomes, recombination is suppressed towards the center due to the interference between the two cross-overs.	✓ Butterflies: (Haenel et al., 2018; Palahí i Torres et al., 2023; Shipilina et al., 2022) ✓ Sedges: beaksedges (Castellani *et al.,* 2024), true sedges (Gómez-Ramos et al, in prep; Sánchez-Villegas et al., 2026) ✗ Nematodes: always reduced recombination in the chromosome center with one CO (Rockman & Kruglyak, 2009; Rödelsperger et al., 2017; Yoshida et al., 2023) ✗ Mites: reduced recombination in chromosome centers (Chmielewski et al., 2025)
(iii) Sex chromosomes are more likely to be involved in inter-chromosomal rearrangements that lead to chromosomal speciation compared to autosomes	✓ Butterflies (Wright et al., 2024) ✓ Nematodes (Gonzalez de la Rosa et al., 2021) ✗ Aphids (Huang et al., 2025)
(iv) Holocentric organisms have fewer	✓ Plants (Cabral et al., 2014; Cuacos et al., 2015;

crossing-over per chromosome, mostly one, sometimes two per chromosome.	Hofstatter et al., 2021) ✓ Nematodes (Schvarzstein et al., 2010) ✓ Hemiptera (Marques & Pedrosa-Harand, 2016; Melters et al., 2012; Nokkala et al., 2004) ✓ Butterflies (Marques & Pedrosa-Harand, 2016; Melters et al., 2012)

4. Final remarks

The advent of high-throughput sequencing technologies has triggered a conceptual renaissance for classical chromosomal speciation models (Lucek et al., 2023). As cytological studies and genome assemblies are available for a wider set of organisms, their role in diversification and speciation has been explored in a broader context. Holocentric chromosomes have gained interest over the recent decades as they have been discovered among highly diverse groups such as sedges, butterflies, true bugs and nematodes (Melters et al., 2012), with some of them exhibiting a great karyological diversity. However, holocentricity per se does not appear to lead to greater diversification rate in general (Márquez-Corro et al., 2018). We have reevaluated classic chromosomal speciation models to see how holocentric groups may defy their assumptions and the particularities of the main diverse groups.

Holocentric chromosomes offer a unique tolerance towards large CRs such as fissions and fusions (Lucek et al., 2022), but only in certain groups it has resulted in a higher karyotypic diversity. Inverted meiosis appears to be a necessary, but not sufficient, condition for increased CR rates (Lukhtanov et al., 2018; Mandrioli & Manicardi, 2020). Similarly, a heightened CR rate does not always increase diversification rates, depending on the species demographic and reproductive characteristics.

In theory, holocentric species would best adhere to the low underdominance hybrid dysfunction models, such as Chain Cascade Models (White, 1978) where reproductive isolation builds up step-by-step through the accumulation of different CRs. The efficacy of these models depend on: (i) population structure being fragmented (small populations with weak gene flow between them) and (ii) reproductive isolation arises primarily from chromosomal mispairing. As the genus *Carex* mostly follows these conditions, pointing to their diversification adhering to chain cascade models (Escudero et al., 2016; Tribble et al., 2025). These models potentially

explain diversification in other groups with similar demographic and reproductive characteristics such as aphids (C. Huang et al., 2025; Lombaert et al., 2006).

While Hybrid dysfunction models have already been regarded through holocentric lenses (Lucek et al., 2022), how recombination suppression models may work differently in holocentric organisms has not been previously addressed. Heightened sequencing availability has allowed the discovery of recombination particularities in holocentric organisms: (i) inversions are not limited in size and location by centromeres, (ii) recombination rate and gene distribution is more homogenous among chromosomes, so evolutionarily relevant CR can occur along wider genome areas and (iii) these organisms display higher tolerance for interchromosomal rearrangements highlight their role as recombination suppressors. Differences on how inversions lead to chromosomal speciation in holocentric species are speculative and yet to be proven. Nevertheless, chromosomal fusions and fissions as recombination suppressors have played a key role in diversification of Lepidoptera (Augustijnen et al., 2024; Mackintosh et al., 2024; Wright et al., 2024), especially those involving sex chromosomes as they tend to contain mate choice functions: a key reproductive barrier in many Lepidoptera clades (Carabajal Paladino et al., 2019; Nguyen et al., 2013). The structural constraint in the number of crossovers (Zedek et al., 2026) point to fissions/fusions' role as recombination rate architects regulating recombination in response to ecological demands.

Nematodes appear as a stable karyotype likely due to their unique segregation mechanism and genomic features linked with intra-chromosomal position (Carlton et al., 2022; Rödelsperger, 2024). Intra-chromosomal CRs play an important role in adaptation and speciation as they contribute to innovation of gene regulation through recombination around chromosome arms (Prabh & Rödelsperger, 2022; Thomas, 2006). However, within nematodes some groups have undergone frequent inter-chromosomal CR. In the case of *Pristionchus,* CRs have promoted hybrid dysfunction and reshaped recombination landscape under their strict chromosome-wide regulation (Yoshida et al., 2023, 2024). Taken together, nematodes show a different side of holocentric chromosomal speciation.

Holocentric lenses look more like a prism as holocentricity leads to different speciation and diversification patterns depending on an array of other factors, including the type of meiosis, genomic features distribution, main reproductive barriers and demographic patterns, affecting both the frequency of CR and how they impact differentiation. However, most of the

knowledge of holocentric organisms is mainly driven from a few clades and more studies are therefore required to understand the (non)-parallelism of holocentric species across the Tree of Life.

Acknowledgements:

Acknowledgements:

The authors wish to thank Dr. Alexander Mackintosh for his contribution in the reunion where the idea for this review was conceived.

Funding:

Project PID2024-157198NB-I00 financed by MICIU/AEI /10.13039/501100011033 and by

FEDER, UE. IG-R was supported by the Andalusian Regional Ministry of Economy, Knowledge, Business, and University (PREDOC_00632). RS-V was supported by the Andalusian regional government (Spain) through project ProyExcel_00125 and by AEI-MICIU through project PID2023-147332NB-I00. KL was supported by the Swiss National Science Foundation grants PCEFP3_202869 and 220868 as well as the Fondation Pierre Mercier pour la Science and the Fondation du Jardin Botanique de Neuchâtel.

AI use statement:

The authors used Artificial Intelligence (AI) to assist with language editing and improve the clarity of the manuscript.

Conflict of interest statement

I/We declare we have no competing interests.

Data Availability Statement

598 Data sharing is not applicable to this article as no new data were created or analysed in this
599 study.

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Supplementary materials

Box S1: The proxies and the evolution of recombination rates in holocentrics

The ecological and evolutionary role of recombination

Recombination plays a central, albeit dual, role in the diversification of eukaryotes: it acts as a powerful force for adaptation, yet risks breaking apart beneficial allele combinations (Stapley et al., 2017a). Despite its fundamental importance, genome-wide recombination rates are highly flexible, varying by more than tenfold across taxa due to differences in genome architecture and selective pressures (Stapley et al., 2017b). Historically, a disconnect has existed between theoretical models explaining the evolutionary advantages of recombination and empirical data describing its natural variation (Dapper & Payseur, 2017). From an ecological perspective, theories such as sib-competition suggest that specific recombination rates are maintained primarily to reduce the intensity of competition among offspring in varying environments (Koella, 1993).

Chromosome number as a proxy

Chromosome number has served as a proxy for recombination rates, initially finding correlations with species vegetative and morphological traits in the holocentric genus *Carex* (Bell, 1982). As outlined, the additional constraint in cross-over location in holocentric organisms make chromosome number a better predictor for recombination rate in holocentric organisms compared to monocentric ones (Zedek et al., 2026), while still being adequate proxies in the latter. Investigating this within a phylogenetic framework, Escudero et al. (2012) concluded that low recombination rates are optimal when immediate fitness is required, whereas stable environments favor high recombination rates to allow for evolutionary innovation. Broadening the scope to the entire clade of angiosperms (Carta et al., 2018) supported this evolutionary role, concluding that environmental stability favors higher recombination rates compared to unstable environments. Focusing specifically on holocentric lineages, subsequent studies in Cyperaceae have also found significant correlations between chromosome number, bioclimatic variables, and morphology (Márquez-Corro et al., 2021). A recent study by Zedek et al. (2026) has further clarified these dynamics. First, the authors confirmed the long-standing hypothesis that monocentric species have significantly more crossovers per chromosome as holocentric species. Second, they also confirmed recombination rate increase with chromosome number, and, surprisingly they found that recombination rates decrease with mean chromosome size. Because of the crossover constraints previously described in holocentrics, chromosome number serves as a better proxy for recombination rates in holocentric lineages than in monocentrics. However, overall mean chromosome size emerges as a significantly better predictor for both groups (and specially good for holocentrics). Interestingly, this aligns with earlier observations in

the cyperoid clade, where mean chromosome size was already found to be a stronger predictor of ecological and biogeographical traits—such as distribution range size—than either chromosome number or overall genome size (Elliott et al., 2022).

The mechanism of genomic shift: holokinetic drive

Given that chromosome size and number so effectively predict recombination rates, understanding the mechanistic drivers underlying these genomic changes is essential. In holocentric lineages, this rapid chromosomal evolution can be explained by the model of holokinetic drive (Bureš & Zedek, 2014a). If natural selection favors specific recombination rates based on environmental stability, holokinetic drive could act as the primary evolutionary engine facilitating these adaptive shifts. By promoting size-preferential inheritance during meiosis—thereby driving chromosomal fission, fusion, and overall changes in mean chromosome size—holokinetic drive effectively provides the mechanism through which holocentric organisms tune their recombination potential to meet ecological demands. Crucially, as diverging populations independently tune their recombination landscapes via holokinetic drive to suit local environments, these rapid structural shifts (fissions and fusions) inherently generate the genomic incompatibilities and recombination-suppressed regions necessary to restrict gene flow. In this way, the ecological pressure to optimize recombination acts as a direct catalyst for chromosomal speciation.