Title

A holocentric twist to chromosomal speciation?

Authors

Kay Lucek[1]*, Hannah Augustijnen[1], Marcial Escudero[2]

[1] Department of Environmental Sciences, University of Basel, Schönbeinstrasse 6, 4056 Basel, Switzerland

[2] Department of Plant Biology and Ecology, University of Seville, Reina Mercedes, ES-41012 Seville, Spain

* Corresponding Author kay.lucek@unibas.ch

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Abstract

Chromosomal rearrangements trigger speciation by acting as barriers to gene flow. However, the underlying theory was developed with monocentric chromosomes in mind. Holocentric chromosomes lacking a centromeric region have repeatedly evolved and account for a significant fraction of extant biodiversity. Because chromosomal rearrangements may be more likely retained in holocentric species, holocentricity could provide a twist to chromosomal speciation. Here we discuss how the abundance of chromosome-scale genomes combined with novel analytical tools offer the opportunity to assess the impacts of chromosomal rearrangements on rates of speciation by outlining a phylogenetic framework that aligns with the two major lines of chromosomal speciation theory. We further highlight how holocentric species could help to test for causal roles of chromosomal rearrangements in speciation.

A chromosomal view on speciation

While most taxonomic groups have chromosomes with centromeric regions, **holocentric** (see Glossary) chromosomes that lack such regions have repeatedly evolved in animals and plants [1,2]. Across the tree of life there is moreover a tremendous variation in the number of chromosomes that mono- and holocentric species have, ranging up to three magnitudes of difference within a taxonomic order [3,4]. The evolutionary significance of this variation has gathered much attention over the decades [5–7], and the interest in the evolution of chromosomal changes is currently undergoing a renaissance [8–10]. This is because novel technologies make it possible to obtain chromosome-scale genomes even for non-model organisms (*e.g.* [11,12]). Together with the emergence of new analytical approaches, this allows tackling the evolutionary impact of chromosomal variation, *e.g.* on rates of speciation [13,14] or gene flow [15]. Variation in chromosome numbers may evolve through very different processes. Large-scale changes in chromosome numbers can for example result from hybridization events [16] or genome duplications through **polyploidization**, the latter being particularly common in plants [17,18]. Other common processes include the fusion of two chromosomes into a single one or the fission of a chromosome into two, resulting in **dysploidy** [6].

Rearrangements that produce variation in chromosome numbers may eventually result in chromosomal speciation, whereby divergent rearrangements directly or indirectly cause reproductive isolation [5,6]. However, intraspecific karyological variation may also persist and result in only limited levels of reproductive isolation [19–22]. Two major lines of theoretical models exist that outline how chromosomal rearrangements could cause chromosomal speciation (reviewed in [7,23]). The first line comprises many of the classic models, which are based on hybrid dysfunction and assume that differentially fixed chromosomal rearrangements between closely related species cause problems during meiosis in hybrids and therefore act as **Dobzhansky-Muller incompatibilities** (DMIs, [5,6,24,25]). The problem with these types of models is that they require chromosomal rearrangements to be fixed in order to be of major effect. This is because newly arising chromosomal rearrangements would typically be **underdominant**, *i.e.*, they would lead to reduced fitness of hybrid individuals (Fig. 1), either within or between species or populations. While strong underdominance makes

it unlikely that novel chromosomal rearrangements spread to fixation, weak underdominance may allow for fixation, but would ensure that chromosomal rearrangements represent only shallow barriers, and are therefore unlikely to cause speciation [7,26]. Nevertheless, empirical evidence for such chromosomal speciation exists, and has been primarily found in mammals [27], including mice [28] and wallabies [8]. Here, **monobrachial homology**, *i.e.*, multiple chromosomal fusions with one or more common chromosome arms in different fusion arrangements that are fixed between populations or species, has been suggested to result in reproductive isolation [25]. Explanations on how such species may have overcome the underdominance paradox vary, and include **genetic drift**, genetic bottlenecks and founder effects [29,30]. Indeed, chromosomal speciation may initially result in a reduction of the effective population size (N_e), which could in turn affect rates of speciation and change the fixation probabilities of new karyotypes in allopatry [31]. This has been suggested for mammals, where families with large geographic distributions but whose species have restricted geographic ranges showed a greater probability for fixing different karyotypes [32]. Shifts in mating system, *e.g.*, from outcrossing to selfing [33] or **meiotic drive**, whereby some alleles or associated rearrangements are more likely to be transmitted [34] have similarly been suggested to overcome underdominance. All these scenarios have received much criticism in the past, however, and formal experiments for a causal association between chromosomal rearrangements and speciation are lacking [7,24].

The second major line of theoretical models was developed more recently and has attempted to overcome the underdominance paradox by focusing on changes in recombination associated with chromosomal rearrangements [7,23,26,35]. In essence, under these suppression of recombination type models, rearranged chromosomes can become fixed by drift but also by selection, *e.g.*, when two or more adaptive loci become physically coupled or by locally reducing recombination, both enhancing existing reproductive isolation [26,35]. Such rearranged regions of reduced recombination may act as barrier loci and promote further differentiation, which may eventually lead to postzygotic isolation through the buildup of genetic incompatibilities [7,26,35]. Reproductive isolation associated with chromosomal rearrangements may be further enhanced by sexual selection or **reinforcement** and may thus promote speciation upon secondary contact. If chromosomal rearrangements contain physically

linked clusters of genes they may themselves represent genomic islands of differentiation, or **supergenes** [36,37]. Albeit such supergenes have been suggested to promote speciation [38], their actual contribution towards reproductive isolation remains controversial [39].

The current theory on chromosomal speciation has an important gap – it is based on the assumption that chromosomes are monocentric and have a centromeric region that concentrates all **kinetochores** for the attachment of the spindle tubules during mitosis and meiosis [5–7] (Fig. 2). However, holocentric chromosomes that lack a centromeric region have evolved in very distinct taxonomic groups (Fig. 3), comprising some of the most diverse branches of the tree of life such as the sedge family Cyperaceae with ~5'500 species [40], the order Lepidoptera with ~160'000 butterfly and moth species [41], as well as the nematode model organism *Caenorhabditis elegans* [2]. In contrast to monocentric chromosomes, holocentric chromosomes have molecular features that allow kinetochore proteins to bind along the entire chromosome, permitting microtubules to attach broadly [1] (Fig. 2). As a consequence, rearranged parts of the genome may not cause segregation problems during cell divisions. Holocentricity could therefore provide a twist to chromosomal speciation theory. Indeed White already highlighted in his classic work on chromosomal speciation [5] that "*The laws and principles of chromosomal rearrangements in these* [holocentric] *organisms are not yet fully understood, but certainly they differ in some respects from those governing chromosomal rearrangements in species with the more usual monocentric chromosome*." However, despite a recent increase in interest in the evolutionary implications of holocentric chromosomes, the potential effects of holocentricity on chromosomal speciation have remained unclear [2,9,10]. Holocentricity may for example help to overcome the initial underdominance paradox of the classic chromosomal speciation theory (Fig 1). This is because large-scale rearrangements through chromosomal fusions as well as fissions may be more likely to be retained as rearranged chromosomes maintain kinetochore function [1]. This contrasts to most scenarios in monocentric species, where fission events result in chromosomal segments that are not attached to a centromere and may therefore be lost during meiosis (Fig. 2) or where fusion events result in dicentric chromosomes with two centromeres and similarly cause problems during meiosis [42]. Monocentric chromosomal fusions may not always result in segregation problems though, *e.g.* when

two chromosomes with terminal centromeres are involved and both chromosomal arms are retain in the fused chromosome [25]. This scenario applies, however, only when nearly complete chromosomes become rearranged and excludes fission events. In addition, intraspecific crosses between holocentric chromosomal races or closely related species may not necessarily cause a significant immediate reduction in offspring fitness [19–22], suggesting that suppression of recombination could also be an important driver of chromosome associated speciation in taxa with holocentric chromosomes.

Mono- and holocentric species further differ in several aspects of their meiotic cell division that may affect the potential for chromosomal speciation. In holocentric species the recombination and segregation functions interfere during meiosis, restricting the potential number of **chiasmata** in bivalents [43]. In this way, some holocentric groups have evolved an inverted meiosis, where, opposite to monocentric groups, the first meiotic division separates the sister chromatids and the second division the chromosomal homologs [19,21]. This inverted meiosis has been suggested to promote the evolution of new karyotypes and possibly chromosomal speciation by facilitating a correct chromosome segregation in hybrids between populations or species that differ in their karyotype [21]. Other holocentric groups, like the nematode *C. elegans*, have evolved a monokinetic-like meiosis as they only keep kinetochore activity in the telomeres [44] avoiding potential interference between chiasmata and spindles. While these mechanisms may help to establish novel karyotypes, their impact on meiotic recombination remains unclear [45], also because comparatively few recombination maps exist so far for holocentric species [45–47]. Importantly, because recombination is often, but not always [47], reduced close to centromeres in monocentrics [46], patterns of recombination are likely to differ across holocentric chromosomes. Also, while holocentric chromosomes lack a centromere, their kinetochores may not be equally distributed [48]. The latter is true for *C. elegans* [44], where recombination increases towards the telomeric regions in contrast to the postman butterfly *Heliconius melpomene*, where recombination is similar across chromosomes [47]. Processes similar to meiotic drive in monocentric species may consequently be at play for holocentric species as has been found for sedges, rushes (*Juncus sp.*) and other holocentric lineages [49]. However, given the repeated evolution of holocentricity, it

remains to show to which degree such **holokinetic drive** may be common among holocentric groups.

The causality between chromosomal rearrangements and species diversification has remained contentious [7]. Phylogenetic inferences suggest that rates of chromosome evolution might be similar between holo- and monocentric species in insects [9], and that rates of diversification are similar between holo- and monocentric clades when comparing sister holo- and monocentric taxonomic orders across eukaryotes [50]. However, there is often substantial variation in chromosome numbers between genera or families within orders, that are moreover associated with different rates of speciation [8,10,51]. The relative contribution of chromosomal fusion and fission on phylogenetic species diversification varies similarly among taxonomic groups and thus likely impacts rates of diversification differently [10,51]. Empirical evidence for chromosomal speciation is rare, either because speciation is already complete or not, often precluding causal implications of one or multiple rearrangements [7,19]. The few examples for holocentric species suggest that intrinsic postzygotic reproductive isolation between species with different karyotype seems to be limited for Lepidoptera [16,19,22,52] and sedges [53,54]. Experimental hybrids between cytogenetic races of the same sedge species showed that hybrid dysfunction is very limited between populations that differ in few chromosome rearrangements but increases as the number of chromosome rearrangements increase [53,54]. These few empirical examples contrast the vast diversity of the taxonomic groups that have evolved holocentric chromosomes and karyotypic diversity [1,2,9]. Here, novel phylogenetic approaches [13,14] could help to assess the macroevolutionary implications of changes in chromosome numbers more generally and provide a framework for comparative analyses between holo- and monocentric groups.

A phylogenetic framework of chromosomal speciation

Recent advances allow to disentangle models of chromosomal evolution in a phylogenetic framework and to distinguish if a phylogenetic event is rather associated with **ana**- or **cladogenesis** [13,14] (Fig. 4). Under cladogenesis, karyotype evolution occurs at a speciation event, while under anagenesis karyotypes evolve along a branch and speciation happens later. Ana- and cladogenesis are compatible with the two aforementioned major lines of chromosomal speciation models, where cladogenesis

198 resembles the classic hybrid dysfunction type models and anagenesis the recombination
199 suppression type models. Importantly, ana- and cladogenetic processes may not be
200 exclusive and may similarly result in a phylogenetic event when they occur together.
201
202 The phylogenetic framework outlined in (Fig. 4) allows to quantify how
203 common changes in karyotype numbers might be associated with speciation events at
204 a macroevolutionary scale and to compare between mono- and holocentric clades [9].
205 Current limitations are primarily given by the availability of dense phylogenies
206 associated with associated karyotype data, often only allowing to study chromosomal
207 speciation at a lower taxonomic level [10]. As this framework allows identifying
208 branching events that are more likely to have resulted in ana- or cladogenetic events
209 respectively, such species pairs could be used to perform in-depth comparative genomic
210 analyses to identify which rearrangements are more likely to result in one or the other
211 phylogenetic event.
212
213 Because a correct segregation of chromosomes may initially be often possible
214 in hybrids of holocentric parental species with different karyotypes [21], holocentricity
215 provides an excellent system to experimentally study chromosomal speciation. The
216 outlined phylogenetic analyses (Fig. 4) combined with crossing experiments could for
217 example quantify the impact of chromosomal rearrangements on reproductive isolation
218 in relation to *e.g.*, the respective evolutionary distance among distinct species pairs.
219 Irradiation based experiments on holocentric plants moreover suggest that
220 holocentricity and a fast formation of new telomeres at breakpoints enables rapid
221 karyotype evolution in holocentric species, though the impact on reproductive isolation
222 was not tested [55]. As direct experimental manipulations of individual chromosomes
223 become technically feasible through novel laser nanosurgery approaches [56] or by
224 generating **artificial chromosomes** [57], the outcome of specific artificial fusion or
225 fission events can now be experimentally studied, enabling to recreate karyotypic
226 changes between sibling species and to assess their direct impact on reproductive
227 isolation.
228
229 *Concluding remarks*
230 Although some of the most diverse taxonomic groups of animals and plants have
231 evolved holocentric chromosomes [1], the potential evolutionary implications of

holocentricity remain elusive. As we outlined, holocentricity could provide a new twist to chromosomal speciation but further research is required. Studying holocentric species could help to advance our understanding on chromosomal speciation (see Outstanding Questions). In addition, we suggest future theoretical explorations, for example, to assess the potential for chromosomal speciation in holocentric taxa, where novel rearrangements may not immediately result in hybrid dysfunction but include a lag time during which heterozygous rearrangements may be tolerated [19]. While chromosomal rearrangements could be an important driver of speciation in cases where they contribute to reproductive isolation, additional pre-zygotic barriers may need to subsequently evolve to complete the speciation process [7]. Comparisons between evolutionary young sibling species that coexist or form zones of secondary contact are thus needed to assess the contribution of chromosomal rearrangements on reproductive isolation in relation to other barriers [58]. To further gain a better understanding on the macroevolutionary impact of karyotype evolution and holocentricity more in-depth and comparative analyses are required to first identify the genomic mechanisms underlying chromosomal fusion and fission sites and their (non-)parallelism across holocentric groups. This would then allow us to identify why for example in Lepidoptera only some genera show tremendous karyotypic variation whereas other genera show none [10]. Lastly, the increased availability of genomic resources for non-model species combined with recently developed models for chromosome evolution [13,14] allow for large-scale macroevolutionary studies both within and across taxonomic orders to decipher the evolutionary consequences of holocentricity.

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Figure legends

Fig. 1: The underdominance paradox. Depicted are hypothetical fitness landscapes for species with different karyotypes and their F1 hybrids with the effects of strong and weak underdominance as predicted by theory for monocentric species [7,26]. While no such theory exists for holocentric species, predictions are given based on empirical findings, which suggest that F1 hybrids in holocentric species may not necessarily suffer from the underdominance paradox [19,21].

Fig. 2: Comparison of the outcomes of fission events during cell division for mono- and holocentric species. If fission occurs during anaphase, the fragment that is not attached to a centromere is lost for monocentric species. In contrast, fragmented chromosome sections of holocentric species can maintain kinetochore function due to the distribution of centromere-like structures along the chromosome, and may so be retained.

Fig. 3: Examples of the diversity of holocentric species and their haploid karyotypes. A – *Carex esenbeckiana* (n = 13). B – *C. fischeri* (n = 36). C – *Polyommatus atlantica* (n = 224), adapted from the Natural History Museum London & [59]. D – *P. aroaniensis* (n = 47), adapted from [60], [61]. Pictures in A, B – courtesy of Modesto Luceño Garces. Scale bars represent a length of 10 μm.

Fig. 4: Contrasting phylogenetic models of karyotype evolution with their putative counterparts of major lines of chromosomal speciation models. The outcome of the different models of karyotype evolution are outlined along a hypothetical phylogeny, with cladо- and/or anagenetic karyotypical changes being indicated. Colors of branches indicate changes in haploid chromosome numbers, while color gradients indicate that the process of karyotype fixation may occur more slowly after anagenetic changes.

Glossary

Anagenesis: Type of speciation in which an ancestral species gradually evolves into another by accumulating changes within a single lineage over time.

Artificial chromosomes: Artificially created chromosomes that have the necessary properties (e.g. centromeres, telomeres and origins of replication) to be self-replicating and stable.

Dobzhansky-Muller incompatibilities: Negative epistatic interactions or incompatibilities that occur between loci with different evolutionary histories. Populations may diverge in allopatry and accumulate such incompatibilities through drift and/or through mutations that prevent hybridization upon secondary contact.

Dysploidy: Process that increases or decreases the number of chromosomes within a species through chromosomal rearrangements with no significant changes in DNA content.

Chiasma: Point of contact between chromatids from two homologous chromosomes during meiotic divisions that allows recombination through chromosomal crossovers between both chromatids.

Cladogenesis: Type of speciation in which an ancestral species splits into two or more species.

Genetic drift: A stochastic evolutionary process that results in changes of allele frequencies by sampling a finite number of individuals each generation.

Holocentric / holokinetic chromosome: Chromosomes with non-localized centromere-like structures. The kinetochore activity is distributed along the whole chromosome.

Holokinetic drive: Perturbation of the normal meiotic process so that a particular allele is preferentially transmitted to the progeny over another allele caused by variation in kinetochore distribution along the holocentric chromosomes or the size of holocentric chromosomes.

Kinetochores: Protein structures located on the chromosomes. Microtubules of the mito- or meiotic spindles are anchored to this structure during cell division. For

monocentric species, kinetochores are located in the centromere whereas for holocentric species, they occur throughout the chromosomes.

Meiotic drive: Perturbation of the normal meiotic process so that a particular allele is preferentially transmitted to the progeny over another allele. The centromere, its location and size are factors that can result in meiotic drive.

Monobrachial homology: Homology between two bi-armed chromosomes that is restricted to only one of the two chromosome arms.

Polyploidy: Chromosome multiplication entailing the addition of complete chromosome sets.

Reinforcement: Evolutionary process whereby pre- or postzygotic mechanisms increase reproductive isolation between two closely related lineages upon secondary contact.

Supergene: A set of genes in strong linkage that segregate together during meiotic divisions because there is a mechanism that impedes recombination within the supergene, such as chromosomal rearrangements, like inversions.

Underdominance: Strong selection against heterozygotes. For chromosomally diverging populations, chromosomal hybrids have low fitness and there is a strong selection against them.

Highlights

Chromosomal speciation, whereby major chromosomal rearrangements trigger reproductive isolation, is a classic evolutionary concept.

The underlying theory was developed for chromosomes with centromeres when holocentric chromosomes that lack centromeres have repeatedly evolved across the tree of life.

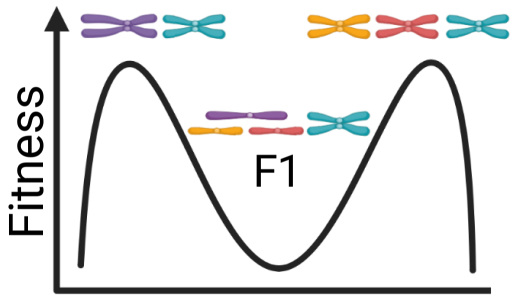
We argue that holocentricity may help to overcome problems associated with classic chromosomal speciation theory and that the special characteristics of holocentric chromosomes vastly expand the potential for experimental research on chromosomal speciation.

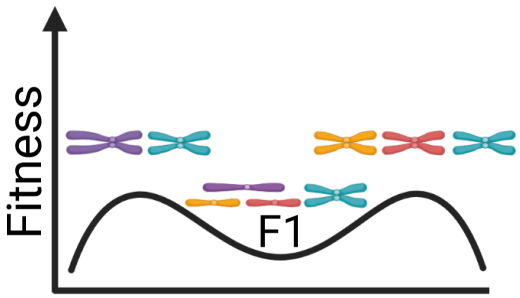
We outline how new approaches allow to quantify the macroevolutionary impact of chromosomal speciation and to distinguish the associated evolutionary mechanisms.

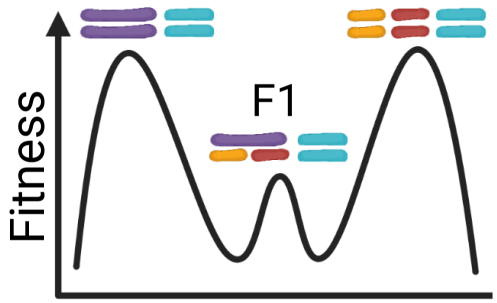
Outstanding questions

- What are the genomic features underlying chromosomal fusion and fission sites and did they evolve repeatedly across the tree of life? Are there common rearrangement hotspots?
- How do chromosomal rearrangements affect gene flow and does it differ between mono- and holocentric species?
- If rearranged chromosomes act as barrier loci, how does reproductive isolation buildup in the rest of the genome? Are rearranged regions enriched for functional genes?
- How does recombination differ between mono- and holocentric species and what are the implications of fusion and fission on recombination?
- To which degree do ana- and cladogenic phylogenetic events reflect the two lines of chromosomal speciation theory?
- What is the macroevolutionary impact of chromosomal rearrangements between mono- and holocentric species and what are the predominant underlying mechanisms? (see Box 1)
- Is chromosomal speciation more likely to occur in holocentric species?

Figure 1 (PDF)







	Monocentric	**Monocentric**	**Holocentric**
Underdominance	strong	weak	*weak*
Establishment of new karyotype	hard	easy	*easy*
Reproductive barrier strength	strong	weak	*weak*
F1 fitness	low	normal	*normal*

Figure 2 (PDF)

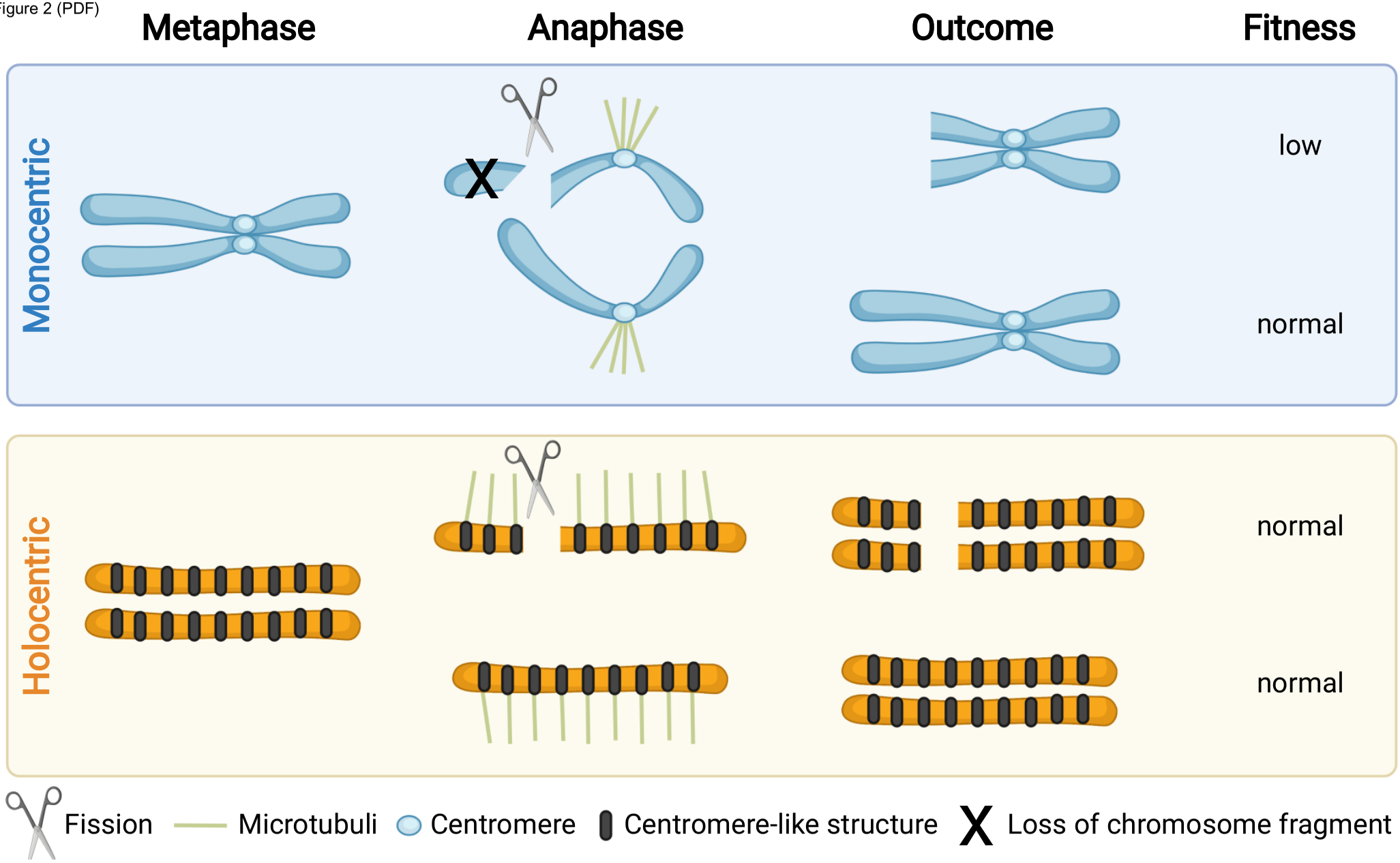

Metaphase
Anaphase
Outcome
Fitness
Monocentric
low
normal
Holocentric
normal
normal
Fission
Microtubuli
Centromere
Centromere-like structure
Loss of chromosome fragment

Figure 3 (PDF)

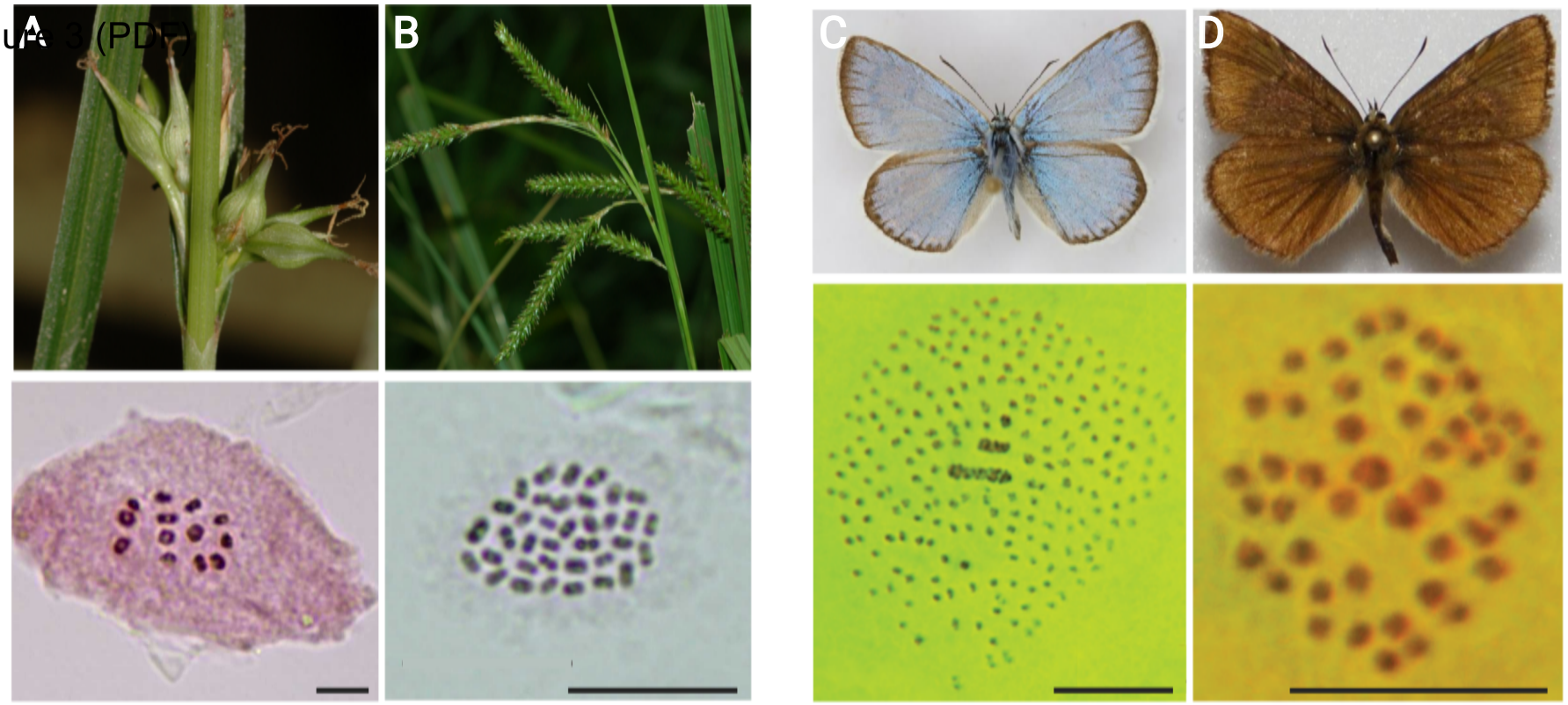
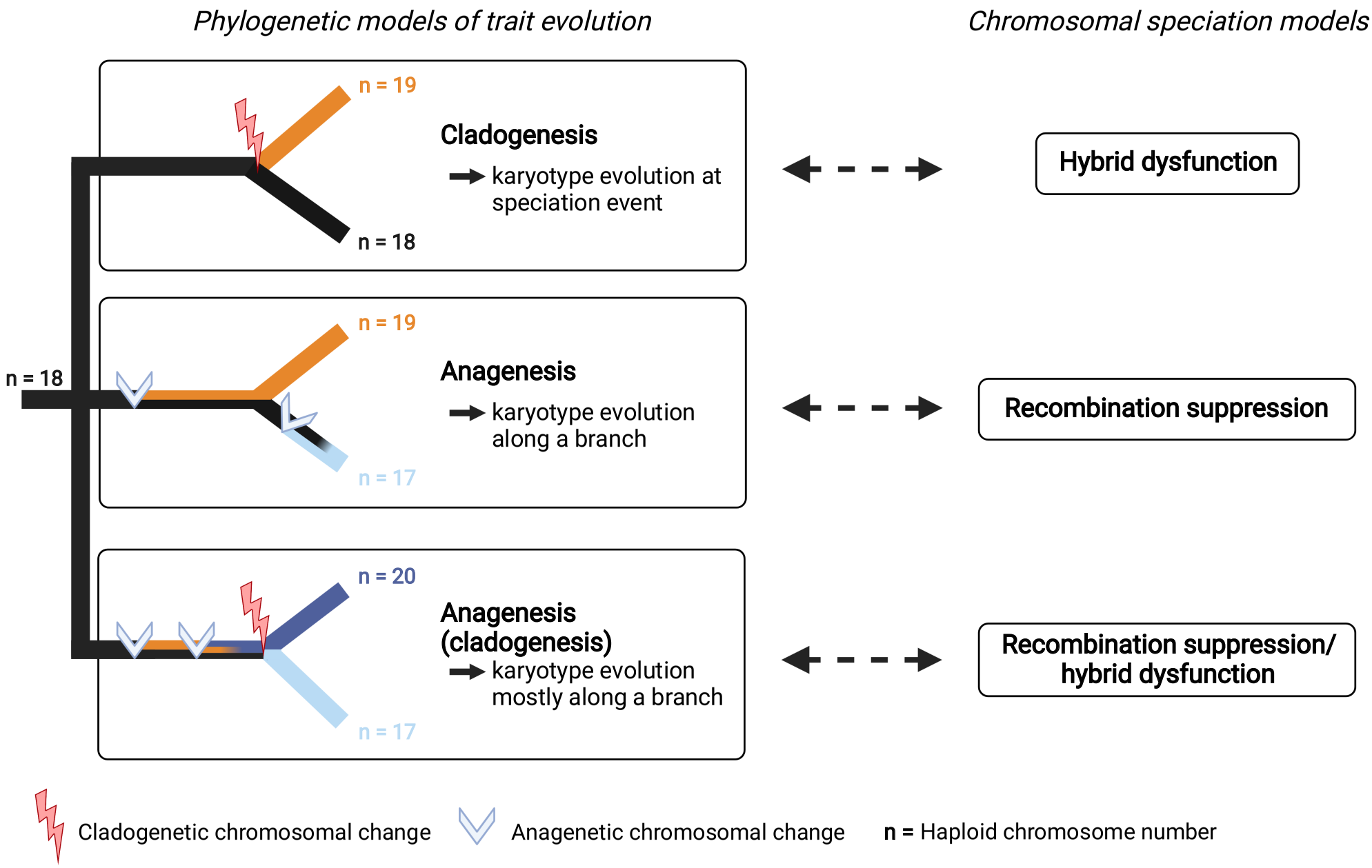


Figure 4 (PDF)


Phylogenetic models of trait evolution
Chromosomal speciation models
n = 19
Cladogenesis
karyotype evolution at speciation event
n = 18
Hybrid dysfunction
n = 18
n = 19
Anagenesis
karyotype evolution along a branch
n = 17
Recombination suppression
n = 20
Anagenesis (cladogenesis)
karyotype evolution mostly along a branch
n = 17
Recombination suppression/ hybrid dysfunction
Cladogenetic chromosomal change
Anagenetic chromosomal change
n = Haploid chromosome number