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The Impact of Chromosomal Rearrangements in Speciation: From Micro- to Macroevolution

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Chromosomal rearrangements (CRs) have been known since almost the beginning of genetics. While an important role for CRs in speciation has been suggested, evidence primarily stems from theoretical and empirical studies focusing on the microevolutionary level (i.e., on taxon pairs where speciation is often incomplete). Although the role of CRs in eukaryotic speciation at a macroevolutionary level has been supported by associations between species diversity and rates of evolution of CRs across phylogenies, these findings are limited to a restricted range of CRs and taxa. Now that more broadly applicable and precise CR detection approaches have become available, we address the challenges in filling some of the conceptual and empirical gaps between micro- and macroevolutionary studies on the role of CRs in speciation. We synthesize what is known about the macroevolutionary impact of CRs and suggest new research avenues to overcome the pitfalls of previous studies to gain a more comprehensive understanding of the evolutionary significance of CRs in speciation across the tree of life.

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In eukaryotes, genetic information is arranged into chromosomes (i.e., discrete physical units with a complex three-dimensional structure and a specific distribution in the nucleus). Within individuals, mutations can alter their number (chromosomal fusions, fissions, polyploidization), their composition (deletions, duplications, translocations), the order of the genetic material within them (inversions), and their interactions with other chromosomes (Fig. 1). These rearranged chromosomes can impact organismal phenotype and reshape the evolutionary trajectories of populations, potentially resulting in speciation (see Berdan et al. 2023).

Various theoretical models have explored how chromosomal rearrangements (CRs) can be involved in speciation by reducing gene flow between populations and contributing to reproductive isolation (RI), and these have garnered empirical support (see Berdan et al. 2023). For instance, CRs can contribute to phenotypic adaptive divergence and/or lead to the accumulation of genetic incompatibilities, reducing gene flow between populations (e.g., Navarro and Barton 2003; Lowry and Willis 2010). However, most studies have focused at a microevolutionary scale on population divergence. Consequently, the contributions of CRs to the completion of the speciation process or evolution above the species level have remained unclear. A crucial question is, therefore, how frequently do CRs contribute to speciation events across the eukaryotic tree of life?

Among the first identified CRs were Robertsonian fusions in orthopterans (Robertson 1916) and inversions in *Drosophila* fruit flies (Dobzhansky and Sturtevant 1938; Dobzhansky 1970), which could easily be visualized applying cytogenetic techniques and microscopy. For a long period, CRs were used as genetic markers to distinguish species and for phylogenetic reconstruction (Stebbins 1971; White 1973; Krimbas and Powell 1992). Despite being largely descriptive, cytotaxonomy inspired the idea that CRs could have a causal role in speciation. However, the study of CRs was limited to those that could be visually detected (i.e., large translocations and inversions as well as chromosomal fusions and fissions). This resulted in a focus on some types and sizes of CRs, limited to some taxonomic groups where CRs are more common and are associated with different phenotypes. We are now moving away from this classical cytotaxonomy, thanks to new genomic methodologies (see below), which can complement cytogenetics in more inclusive macroevolutionary analyses.

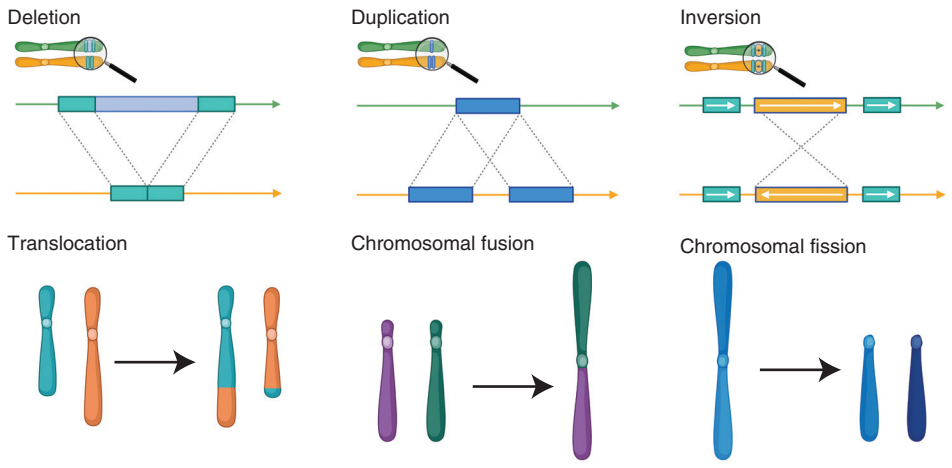

Figure 1. Summary of commonly studied chromosomal rearrangements (CRs), including the deletion of small segments along a chromosome, as well as the duplication or the inversion of such chromosomal material, respectively. Larger CRs include the reciprocal translocation of chromosomal regions between chromosomes, as well as the fusion or the fission of entire chromosomes. The fusion and fission illustrated are Robertsonian rearrangements (i.e., they involve breakage at the centromere). (Figure created with BioRender.com.)

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Even with better data on CRs across taxa, it is still challenging to establish a link between micro- and macroevolution (Harvey et al. 2019). Microevolutionary studies focus on processes driving divergence between populations that may or may not result in different species. In contrast, macroevolutionary studies usually compare differences among taxonomic groups above the species level. These differences may or may not have been instrumental in the evolution of RI, and could have evolved after speciation was completed. Nevertheless, if CRs play a role in speciation as suggested by different theoretical models and supported by empirical microevolutionary studies (see Berdan et al. 2023), we would expect a positive correlation between chromosomal change and rates of speciation (see below), even though this relationship might be hard to detect due to the influence of various other factors. Conversely, the absence of such a correlation could highlight that CRs are primarily involved in intraspecific divergence, but rarely lead to different species and increased speciation rates. Although correlations between chromosomal change and rates of speciation have been found for some CR types, including fusions and fissions (e.g., Bush et al. 1977; de Vos et al. 2020), it is unknown whether they apply for other CRs. Extracting the biological meaning of such macroevolutionary patterns or correlations requires a good understanding of the drivers of chromosomal evolution and how they influence speciation. Here, we aim to bridge the gap between micro- and macroevolutionary approaches toward the role of CRs in speciation—addressing technical, conceptual, and empirical aspects in the following sections.

THE TECHNICAL GAP: HOW TO ENSURE RELIABLE CR DETECTION

Studying the role of CRs in speciation requires their reliable detection. Phylogenetic studies on chromosome evolution often still rely on information from databases reporting chromosome numbers inferred using cytogenetic approaches limited to a subset of CRs and taxa (Jackson et al. 2016). Molecular cytogenetic approaches, such as fluorescent in situ hybridization (FISH), allow CR detection at a finer scale throughout the entire genome (comparative mapping) but often have a limited taxonomic scope (e.g., in mammals; Ferguson-Smith and Trifonov 2007). This is because suitable probes have to be developed de novo (Lysak et al. 2006; Provazníková et al. 2021). New approaches based on high-throughput and/or long-fragment sequencing technologies now offer an effective alternative for detecting CRs across the entire genome at a resolution close to base pair level, allowing a higher sensitivity in CR discovery (Huang and Rieseberg 2020; Mérot 2020). This will provide novel insights on the diversity and abundance of CRs across taxa, including nonmodel organisms. For instance, genomics has revealed that small CRs, such as short inversions or duplications, are abundant, yet only a fraction of them may play a role in RI (Lucek et al. 2019; Damas et al. 2022). Effectively, our understanding of the role played by small CRs is incomplete as current methods are still biased toward the detection of larger CRs (Mahmoud et al. 2019).

When high-quality reference genomes are available, CRs can be detected in a set of related taxa by mapping sequencing reads to a single reference genome. Inversions, duplications, deletions, and translocations (Fig. 1) can then be discovered based on the read mapping patterns they generate. For example, split reads approaches, where reads split in two parts, map to distant regions and can indicate the presence of an inversion, deletion, translocation, or fusion in the sequenced individual relative to the reference genome, depending on the mapping locations of the read fragments and their orientation (Ho et al. 2020). However, the efficiency of read mapping drops with increasing divergence to the reference genome, constraining its application at broader macroevolutionary scales. CR breakpoints are also difficult to resolve using short-read sequencing, notably when repetitive sequences obscure the pattern. Long-range structural information allows this difficulty to be overcome, for instance using linked-read approaches (Zheng et al. 2016). Nonetheless, long-read sequencing is becoming the standard approach for accurate breakpoint detection even in the presence of long repeat regions (Ho et al. 2020).

As an alternative to read mapping approaches, de novo assemblies of genomes from different

taxa can be aligned and compared to check for synteny. This approach enables the detection of CRs where read mapping becomes unreliable due to sequence divergence, or where regions are missing from a reference genome. Here, a series of whole-genome alignments between closely related species might be sufficient to reveal CRs across the entire phylogeny (e.g., Damas et al. 2022). Short- and long-read sequencing approaches may be combined, as shown, for example, for crow species (Weissensteiner et al. 2020), where read mapping and de novo assembly enabled the discovery of deletions, duplications, and inversions.

Generating many high-quality, contiguous assemblies requires long-read data, often combined with other recently developed tools such as chromosome conformation capture techniques (Hi-C) and optical mapping that allow for chromosome-level assembly (Mérot 2020), as well as large-scale bioinformatic and computational resources. Several international initiatives are currently generating chromosome-scale reference genomes for a substantial fraction of the extant biodiversity, such as the Darwin Tree of Life (Darwin Tree of Life Project Consortium et al. 2022), or the European Reference Genome Atlas (Formenti et al. 2022), which should substantially reduce the taxonomic bias in CR detection. Macroevolutionary studies of CRs will benefit from those initiatives. The availability of long-fragment sequencing also facilitates the detection of intraspecific diversity of CRs through pan-genome approaches (Sun et al. 2022). Although the recent deluge of genomic data highlights that CRs are diverse and ubiquitous both within and among species, we still have a long way to go to map this diversity across the tree of life and to establish their roles in species diversification.

THE CONCEPTUAL GAP: RECONCILING MICROEVOLUTIONARY THEORY WITH MACROEVOLUTIONARY INFERENCES

Predictions from Microevolutionary-Based Theory

Within and across many taxonomic groups, species display tremendous variation in the number of chromosomes, ranging up to three orders of magnitude of difference even within a taxonomic order (Rice et al. 2015; Román-Palacios et al. 2021). Concepts and models on how cytogenetically detectable CRs such as chromosomal fusions and fissions may contribute to speciation have been developed (White 1978; King 1995; Faria and Navarro 2010). Two major lines of theory exist (Rieseberg 2001; Faria and Navarro 2010). The classic line of theory assumes that alternative CRs fixed in differentiating species cause meiotic defects in heterozygotes, resulting in hybrid dysfunction and/or sterility (White 1978). However, if CRs cause strong reduction of hybrid fitness (underdominance), they would be selected against, especially when at low frequency (Navarro and Barton 2003). Novel, strongly underdominant CRs are therefore unlikely to spread to fixation. However, factors such as meiotic drive, a selective advantage of the rearranged karyotype, or inbreeding with strong genetic drift can counteract negative selection and facilitate the fixation of novel, yet underdominant CRs (Hedrick 1981). While weak underdominance would make fixation more likely, this would result in weaker RI, thus making such CRs unlikely to significantly contribute to speciation (Rieseberg 2001; Faria and Navarro 2010).

A more recent line of theory focuses on the effects of CRs on recombination, especially in scenarios where speciation occurs in the presence of gene flow (Rieseberg 2001; Navarro and Barton 2003; Faria and Navarro 2010; Guerrero and Kirkpatrick 2014). Under these models, the frequency of a new CR may increase when recombination suppression strengthens linkage disequilibrium (LD) between two or more locally advantageous alleles located within a rearranged genomic region (see Berdan et al. 2023). This will reduce effective recombination within the rearranged region in heterokaryotypes, whereby additional barrier loci (e.g., alleles involved in incompatibilities) can accumulate, enhancing RI (Lande 1985; Navarro and Barton 2003; Kirkpatrick and Barton 2006; Guerrero and Kirkpatrick 2014; Connallon and Olito 2022). The suppression of recombination within CRs can also contribute to the main-

tenance of differentiation between diverging populations or species after secondary contact (Noor et al. 2001; Rafajlović et al. 2021). Thus, CRs may have a role both in species divergence and in species persistence.

Although the aforementioned classes of models are often presented as alternatives, they are not mutually exclusive, as some CRs can to some extent be simultaneously underdominant and suppress recombination (Garagna et al. 2014; Yoshida et al. 2023). Both models predict that differences in CRs between populations contribute to RI and, therefore, that higher rates of chromosomal evolution should be associated with higher speciation rates (Fig. 2). While the prediction that CRs between populations contribute to RI is supported by many empirical studies (see Berdan et al. 2023), the association of rates of chromosomal evolution and speciation has been tested less frequently but is a focus of this work.

Macroevolutionary Inference

Different macroevolutionary approaches focusing on patterns of karyotype evolution and associated lineage splits in phylogenetic trees have been developed (Fig. 2). Probabilistic models for the evolution of CRs along phylogenies are currently restricted to CRs affecting chromosome numbers (Mayrose et al. 2010; Glick and Mayrose 2014). Early phylogenetic studies used comparative analyses to test for an association between karyotypic changes and speciation (e.g., Kandul et al. 2007). Two distinct likelihood-based models have since been developed that allow a more thorough estimation of diversification (ChromEvol; Mayrose et al. 2010; Glick and Mayrose 2014) and extinction (ChromoSSE; Freyman and Höhna 2018) related to chromosome number changes (reviewed in Mayrose and Lysak 2021). State-dependent speciation and extinction (SSE) models further allow researchers to estimate whether changes in chromosome number occur along branches of a phylogeny (i.e., anagenetic change) or at speciation events (i.e., cladogenetic change). SSE models may provide insights on how CRs may impact speciation (i.e., resulting in rapid phylogenetic splits [cladogenesis] or potentially by building up RI [anagenesis]) (Lucek et al. 2022). The relative contribution of CRs to ana- versus cladogenetic changes varies among taxonomic groups (Freyman and Höhna 2018; de Vos et al. 2020; Augustijnen et al. 2023) and can provide insights into the potential evolutionary mechanisms underlying the influence of CRs in speciation (underdominance, divergent selection, drift, etc.). For instance, strongly underdominant CRs have been suggested to contribute to cladogenesis (Lucek et al. 2022). However, evaluating the relative contribution of CRs to anagenesis and cladogenesis can be challenging. Microevolutionary studies have shown that inversion polymorphisms under balancing selection tend to be maintained for long periods of time before playing a role in speciation (Wellenreuther and Bernatchez 2018; Westram et al. 2022). Thus, although some CRs may influence cladogenesis, their overall role depends on their evolution within the ancestor population (anagenetic changes). CRs such as inversions may remain polymorphic through the divergence process but may still contribute to RI and facilitate the accumulation of additional reproductive barriers that may ultimately lead to the completion of speciation. Alternative arrangements can eventually fix within daughter lineages, or one may get fixed globally (i.e., it ends up in both lineages). However, the possibility of secondary loss of polymorphic CRs after speciation presents a particular challenge for inferring their role in speciation from a macroevolutionary perspective.

THE EMPIRICAL GAP: UNDERSTANDING THE ORIGIN AND FIXATION OF CRs

If CRs affect speciation rates, it is important to identify the factors that influence the evolution of CRs in the first place (Fig. 2). The genomic features underlying CR formation are not fully resolved. Rearranged regions are often enriched for repetitive DNA such as transposable elements (Ahola et al. 2014; Serrato-Capuchina and Matute 2018; Escudero et al. 2023) and segmental duplications (Coulibaly et al. 2007; Kidd et al. 2008; Catacchio et al. 2018), in agreement with a nonrandom occurrence of CRs across the genome (Eldridge and Johnston 1993). Rates of CR formation are expected to vary according to CR type and

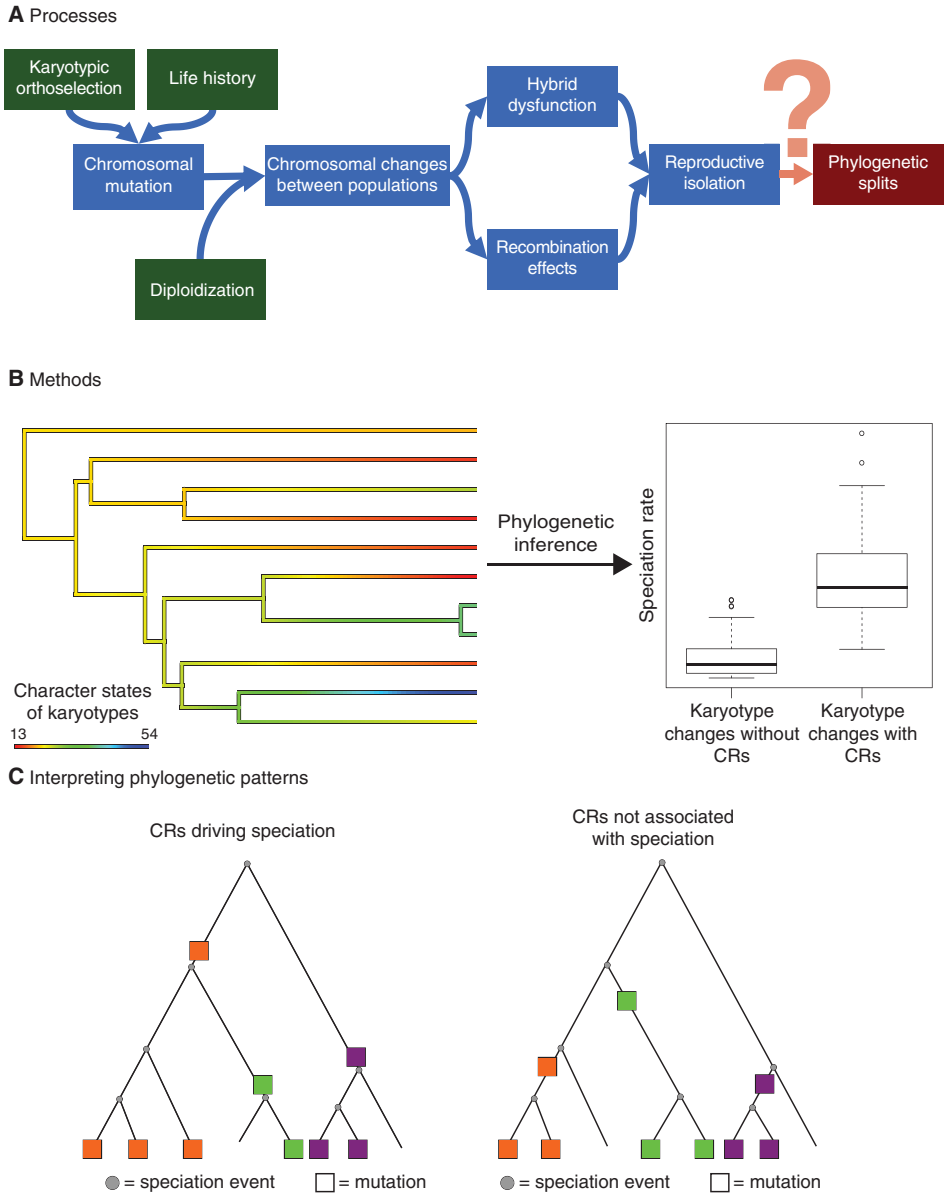

Figure 2. From patterns to processes studying chromosomal rearrangements (CRs) in speciation. (*A*) The three different components discussed in this article: microevolutionary processes result in reproductive isolation (RI) (blue), which potentially lead to phylogenetic splits, increasing speciation rates on the macroevolutionary scale (red). The probability of this happening depends on different factors that influence the emergence of chromosomal mutations in the first place (green). (*B*) Methods to study the macroevolutionary implications of CRs in speciation primarily involve phylogenetic approaches. Shown is an example for character states such as the number of chromosomes that are inferred along a phylogeny (*left*) and boxplots summarizing the phylogenetic inferences to assess the impact of CR-associated karyotypic changes on, for example, rates of speciation (*right*). The example was modified from de Vos et al. (2020) and is based on ChromoSSE. (*C*) Interpreting the phylogenetic signal of CRs at the tip level is often not trivial as a direct implication of CRs during speciation may not be given. A link between CRs and speciation can be inferred if a mutation that leads to a CR is frequently followed by a speciation event within a short amount of time.

among lineages. Although mutation rates of CRs are rarely known, some studies suggest that they can be lower than those of single-nucleotide polymorphisms (SNPs) (Berdan et al. 2021), yet there is much uncertainty (see Berdan et al. 2023).

As for other genetic variants, the probability of establishment and fixation of CRs is determined by both neutral and selective processes. Initially found at low frequency, CRs can be lost via genetic drift unless they occur in small populations or have a selective advantage (Spirito 1998; Jackson et al. 2016). However, the probability of loss is generally enhanced for underdominant CRs for instance, due to the production of unbalanced gametes or to direct deleterious effects at breakpoints. Conversely, CRs will increase in frequency and eventually reach fixation if they capture a haplotype with higher fitness or by meiotic drive (Faria et al. 2019a). Theoretical work showed that inversions can spread and get fixed in a population if they capture locally adapted alleles at two or more loci despite gene flow between diverging populations (Kirkpatrick and Barton 2006). Even if this may only confer a small selective advantage (Charlesworth and Barton 2018), a scenario of successive periods of allopatry and secondary contact can increase the fixation probability of CRs (Feder et al. 2011). Underdominance and some of the aforementioned processes affect the various types of CRs in a different manner (Huang and Rieseberg 2020), perhaps explaining differences in prevalence among types of CRs. The role of those processes on the probability of CRs being associated with speciation is still unclear.

Karyotypic Orthoselection

Chromosomal phylogenies often show an overwhelming predominance of particular types of CRs within a lineage. White (1973) was among the first to notice these patterns, and coined the term "karyotypic orthoselection" to describe the process that underlies them, although an actual selective basis for the repeated fixation of a particular type of mutation cannot be assumed. Striking examples for the co-occurrence of speciation events and fixation of types of CRs mainly come from mammals (e.g., in deer mice [*Peromyscus* spp.] inversions very frequently differentiate species) (Robbins and Baker 1981; Stangl and Baker 1984), and also occur substantially intraspecifically (Harringmeyer and Hoekstra 2022). Karyotypic orthoselection occurs in non-mammalian taxa as well (e.g., Molina et al. 2014; Srikulnath et al. 2019; Timm et al. 2021).

For Robertsonian fusions, single fusions may cause little underdominance, but taxa that accumulate multiple different fusions may experience substantial underdominance (Searle 1993; Garagna et al. 2014). This is the basis of particular models of chromosomal speciation (Rieseberg 2001), notably the monobrachial fusion model (Baker and Bickham 1986) and chain/cascade model (White 1978). Therefore, an underlying prediction of karyotypic orthoselection at the macroevolutionary level is that lineages with a high prevalence of Robertsonian fusions are likely to have unusually high speciation rates. Examples of mammalian lineages with many Robertsonian fusions are the rock-wallabies *Petrogale* (Fig. 3; Eldridge and Johnston 1993; Potter et al. 2017) and *Sorex* shrews (Bulatova et al. 2019). Muntjacs *Muntiacus* differ by multiple tandem fusions (Huang et al. 2006; Yin et al. 2021) and the associated underdominance has likely contributed to RI (White 1973; Gustavsson and Johansson 1980). Generally, the heterogeneous, clade-specific distribution of CR types at the macroevolutionary level, combined with varying propensities of CR types to be involved in RI, leads to different phylogenetic patterns of association between CR evolution and speciation rates. However, whenever multiple CRs have occurred in a lineage over time, it is more difficult to determine the order in which they have accumulated or infer their causality for speciation.

Different processes may explain the repeated evolution of CRs within one lineage. High mutation rates of a particular CR type, for example, by nonhomologous recombination of similar sequences on different chromosomes (Garagna et al. 2014) or promoted by multiple insertions of specific transposable elements (Lönnig and Saedler 2002), may increase the chance of repeated fixation of that CR type. Meiotic drive may play an important role in karyotypic orthoselection. Indeed, a bimodal distribution of karyotypes

was found in mammals, tending toward either all acrocentric or all metacentric chromosomes (de Villena and Sapienza 2001). This was attributed to meiotic drive favoring either the metacentric or acrocentric condition within lineages, according to strength of centromeres (Searle and de Villena 2022), and therefore the repeated fixation of either Robertsonian fusions or fissions. This received support by mechanistic studies of house mice (Chmátal et al. 2014) and comparative studies on mammals (Blackmon et al. 2019). A bimodal distribution of either the metacentric or acrocentric condition (Molina et al. 2014) also occurs in actinopterygian fish, suggesting that meiotic drive could also cause karyotypic orthoselection in non-mammalian groups.

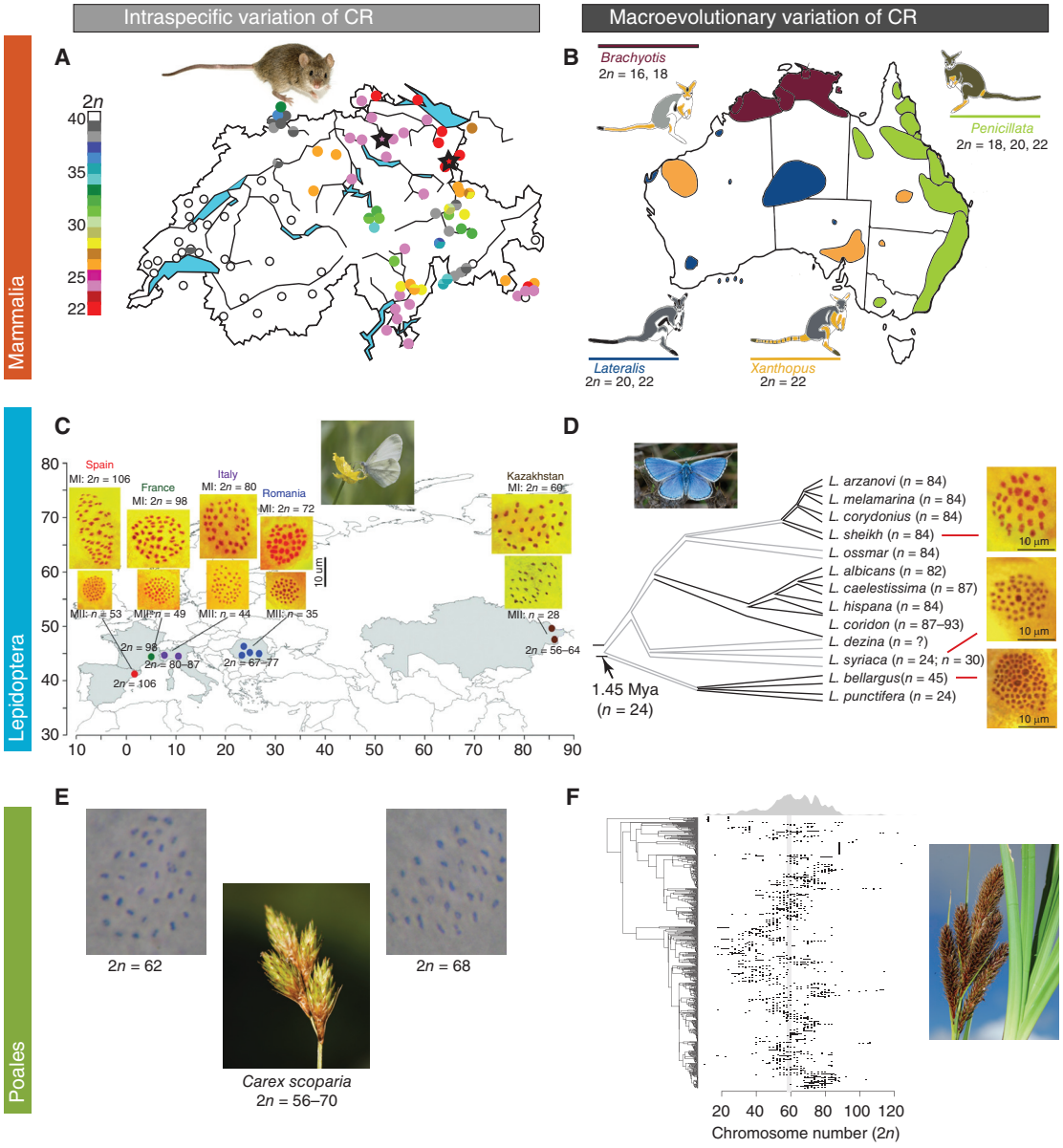

Figure 3. (*See following page for legend.*)

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THE EMPIRICAL GAP: UNDERSTANDING ALREADY OBSERVED MACROEVOLUTIONARY PATTERNS

Empirical support for a macroevolutionary role of CRs in speciation has been indirectly provided by phylogenetic work showing positive correlations between species diversity (Fig. 3) and CRs in several taxonomic groups, including plants (Levin and Wilson 1976; Carta and Escudero 2023), insects (de Vos et al. 2020), mammals (Bush et al. 1977), and reptiles (Olmo 2005). However, the underlying mechanisms among these are likely to be different, and the studies tend to be restricted to chromosomal fusions and fissions with limited taxonomic sampling. Despite these caveats, we review some illustrative macroevolutionary examples here, highlighting how CRs differ depending on the genomic regions they occur in or the genomic architecture of the species. Finally, we discuss how life history and biogeography could have shaped the evolution of various CRs among species.

What Can We Learn from Holocentric Chromosomes?

While most eukaryotes have chromosomes with centromeres, ~20% of extant animals and plants are holocentric, with small centromere-like features dispersed along the entire length of their chromosomes rather than a single large centromeric region (Melters et al. 2012; Mandrioli and Manicardi 2020). Because rearranged segments do not depend on distant centromeres for meiotic segregation, segmental rearrangements may not cause the same segregation defects during cell divisions as in monocentric species (Lukhtanov et al. 2018). A fission event in a monocentric species may, for example, result in a segment not attached to a centromere and liable to be lost, while the equivalent product in a holocentric species is more likely to segregate properly. Several holocentric groups show tremendous karyotypic variation at the highest taxonomic level (Ruckman et al. 2020), but is often restricted to few taxonomic subgroups. For example, in Lepidoptera, only some genera show variation in chromosome numbers, when most others have retained the putatively modal karyotype ($n = 31$), suggesting that chromosomal fusions and fissions are not always driving speciation (Robinson 1971; de Vos et al. 2020). Evidence for an association between CRs and rates of speciation in holocentric taxa is mixed and specific to each taxonomic group. Moreover, no difference in rates of chromosomal change or rates of species diversification was found at the broadest scale comparing mono- and holocentric groups (Márquez-Corro et al. 2018; Ruckman et al. 2020). By contrast, at the genus level, butterfly genera with higher variation

Figure 3. The role of chromosomal rearrangements (CRs) during speciation at both a micro- and macroevolutionary level exemplified by some animal and plant systems. (*A*) For the house mouse (*Mus musculus*), chromosomal races are common and can cause strong, yet not complete reproductive isolation. (Panel *A* is modified from Grize et al. 2019 under the terms of the Creative Commons Attribution 4.0 International License.) (*B*) At a macroevolutionary level, CRs have been suggested to have contributed to the diversification in rock-wallabies of the genus *Petrogale*. (Panel *B* is modified from Potter et al. 2017 under the terms of the Creative Commons Attribution License (CC BY) and the authors, © 2017 Potter, Bragg, Blom, Deakin, Kirkpatrick, Eldridge, and Moritz.) (*C*) Similar patterns are observed in the order Lepidoptera, where, for example, the wood-white butterfly *Leptidea sinapsis* shows a high variation in chromosome numbers across a Eurasian geographic scale but does not appear to cause strong reproductive isolation. (MI) Metaphase I of meiosis, (MII) Metaphase II of meiosis. (Panel *C* is modified from Lukhtanov et al. 2011 under the terms of the Creative Commons Attribution License 2.0, © 2011 by Lukhtanov et al., licensee BioMed Central.) (*D*) However, in other genera, chromosomal changes occur primarily among species and have been suggested as a driver for speciation. (Panel *D* based on data in Talavera et al. 2013.) (*E*) Intraspecific variation in chromosome numbers also occurs in plants (e.g., in panel *E*—*Carex scoparia*) where the degree of reproductive isolation scales with the number of CRs (see Escudero et al. 2016). (Image in Panel *E* kindly provided by Marcial Escudero.) (*F*) The overall diversity in chromosome numbers has been similarly attributed to have driven diversification in this genus. (Panel *F* is reprinted from Marques-Corro et al. 2021, with permission from the Institute of Botany, Chinese Academy of Sciences © 2021.) Pictures of Carex by Modesto Luceño Garces, pictures of Carex methaphases by Marcial Escudero.

in chromosome numbers tend to diversify faster (de Vos et al. 2020), but differences in chromosome numbers between closely related species or lineages may not always result in strong RI (Fig. 3; Lukhtanov et al. 2015, 2020). Changes in chromosome numbers are likewise suggested to drive diversification at the macroevolutionary level in the holocentric sedges (Cyperaceae; Escudero et al. 2012; Márquez-Corro et al. 2019). Experimental evidence at the microevolutionary scale draws a mixed picture, though, where CRs may cause hybrid dysfunction in some (Escudero et al. 2016) but not all studied *Carex* systems (Escudero et al. 2018). Both underdominance and changes in the recombination landscape are at play in some holocentric nematode species, where fusions seem to have played an important role in speciation (Yoshida et al. 2023). Current knowledge on holocentric species highlights the necessity of studying different taxonomic levels to fully understand the macroevolutionary influence of CRs.

CRs in Sex Chromosomes and Speciation

CRs may show distinct evolutionary dynamics between sex chromosomes and autosomes, which differ in effective population size, patterns of recombination, and selection. Supported by theory, several empirical studies suggest that genetic changes in sex chromosomes have a more substantial role in speciation than changes in autosomes (Payseur et al. 2018) (e.g., representing a large-X effect [Coyne and Orr 1989a] or Haldane's rule [Haldane 1922]). Although this is often mentioned in a context of genetic incompatibilities at the SNP or genic level, the same seems to hold true for CRs. Examining more than 400 species, a macroevolutionary study on cytologically identified pericentric inversions in passerine birds found that the fixation of such inversions occurs more frequently on sex chromosomes than on autosomes (Hooper and Price 2017). Although a causal role for CRs in speciation remained open, it was suggested that these inversions likely played a role in the accumulation of incompatibilities, contributing to RI. More generally, cytological comparisons of sex chromosomes between species provide insight into their roles in speciation (Nguyen and Carabajal Paladino 2016). This is shown by "sex chromosome turnover" in fishes, whereby new sex chromosomes arise through sex chromosome–autosome fusions, such as in stickleback (Kitano and Peichel 2012). At a microevolutionary level, there are indications that such CRs are important in speciation. However, it is important to distinguish whether empirical macroevolutionary patterns simply reflect the role of CRs in the evolution of neo-sex chromosomes (Jay et al. 2022), suggesting only an indirect role of CRs in speciation or because they facilitate the accumulation and/or maintenance of incompatibilities owing to recombination suppression, thereby contributing to RI (Rafajlović et al. 2021).

CRs and Speciation after Diploidization in Plants

Plants have diversification rates around twice as high as those of animals (Scholl and Wiens 2016). In particular, angiosperms, and among them, many eudicot families show high speciation rates (Puttick et al. 2015). This has long been associated with the high prevalence of whole-genome duplication in these lineages (Soltis et al. 2009); however, newly formed polyploids exhibit low diversification rates (Mayrose et al. 2011). The apparent diversification success of polyploids may instead be linked to genome downsizing following duplication (i.e., post-polyploidization diploidization) (Meudt et al. 2015; Dodsworth et al. 2016), which involves CRs such as translocations and the loss of entire chromosomes leading to dysploidy (Mandáková and Lysak 2018). When this process occurs independently in allopatric populations, it can result in different karyotypes and genome arrangements (as well as allelic content) that are incompatible in hybrids. Speciation rates among plant families are indeed positively correlated with rates of genome size evolution (Puttick et al. 2015), and shifts in diversification rate seem to occur some million years after inferred paleopolyploidizations (Schranz et al. 2012).

Chromosome Number and Speciation Potential

It has been suggested that diploid chromosome number has an evolutionary significance for

mammals (Qumsiyeh 1994; Qumsiyeh and Handal 2022): the more chromosomes, the greater the number of segregating units at meiosis, with dramatic differences between large and small diploid numbers. Also, the number of chiasmata can be related to the number of chromosome arms (Dutrillaux 1986). Thus, an increase in the number of chromosomes and number of chromosome arms should result in increased recombination, which often opposes speciation (Felsenstein 1981) and increased genetic variability. According to Qumsiyeh (1994), species with high diploid numbers should be able to exploit more niches but be less likely to diversify. Based on this argument, there should be an inverse relationship between chromosome or chromosome arm number and speciation potential. There has been some headway in examining this in reptiles, which show a positive association between the rate of chromosome evolution and the number of extant species, albeit with varying trends among different orders and suborders (Olmo 2005). Karyotypic variability was higher in lizards and snakes, which also tend to have more and smaller chromosomes than turtles and crocodiles. However, similar studies across other taxonomic groups, also taking into account factors such as genome size and composition, different types of CRs, and controlling for effective population size (see below), are needed before general conclusions can be made.

Macroevolutionary Associations between Life History, Biogeography, and CRs

Some of the earliest thorough macroevolutionary studies relating chromosomal evolution and speciation were implemented by Wilson et al. (1975) and Bush et al. (1977). The latter cytologically identified fusions, fissions, and pericentric inversions across 225 genera of vertebrates and found that both chromosomal evolution and speciation were fastest in those mammalian genera with small deme size such as horses or with limited dispersal and patchy distributions such as rodents. A more recent comparative phylogenetic study for mammals found that more subdivided geographic distributions correlated with a higher probability of fixation of CRs (Martinez et al. 2017). Also, taxa with more litters per year, lower longevity, and younger age of sexual maturity showed more karyotypic diversification, as expected for small species such as rodents, with highly subdivided distributions (Martinez et al. 2017). This may imply that a subdivided distribution promotes fixation of CRs, which in turn promotes speciation (e.g., Bush et al. 1977). However, because all these variables are often correlated, it is difficult to disentangle the main cause from its effects, especially when distantly related species are compared. A meta-analysis in finches focusing on pericentric inversions revealed that their fixation was faster in clades within continuous continental distributions compared to those with more discontinuous island distributions (Hooper and Price 2015). Taxa in the continental distributions had larger range sizes, and range size varied positively with inversion fixation rate. However, the ranges in continental distributions also had more overlap, and a broader analysis of passerine birds found a correlation of range overlap and inversion differences (Hooper and Price 2017). Extending on previous surveys of *Drosophila* (Coyne and Orr 1989b, 1997), Noor et al. (2001) showed that whereas sympatric or parapatric species pairs frequently differed by one to several cytologically detectable inversions, allopatric species pairs generally had collinear genomes. These patterns can be explained by inversions containing incompatibility alleles that cannot recombine out of the inversion contributing to the maintenance of species upon secondary contact, and eventually to reinforcement (Noor et al. 2001; Hooper and Price 2017). However, if most inversions get fixed according to the model of Kirkpatrick and Barton (2006), the number of inversion differences is also expected to be higher when gene flow plays a role in speciation.

MIND THE GAP! PITFALLS IN INTERPRETATION

Macroevolutionary inferences are necessary to advance our knowledge on the importance of CRs in speciation. However, commonly used ap-

proaches such as comparative phylogenetics are essentially testing for correlations between chromosomal change and speciation rates. Without any additional source of information, it is hard, if not impossible, to infer causation. Thus, although macroevolutionary patterns are important to reveal sister species pairs or entire clades where CRs could play a role in speciation, additional studies at the microevolutionary level are needed to investigate the causal links between the two. Another pitfall of macroevolutionary inferences is that despite phylogenetic advances that allow both speciation and to some degree extinction events to be taken into account, current methods cannot account for cases of parallel evolution of CRs in sister species or broad-scale introgression. Theoretical work is also typically focused on the microevolutionary scale but is generally unable to predict the macroevolutionary patterns resulting from RI driven by CRs.

The examples discussed in the sections above highlight that a major pitfall of these macroevolutionary approaches is the focus on taxonomic groups where the frequency of CRs is known to be high. Although these are a fertile ground where we expect CRs to play a role in diversification, a less limited taxonomic "sampling" to investigate these evolutionary questions is needed, including also taxa where relatively large CRs or different chromosome numbers were previously unknown (e.g., *Littorina* marine snails; Faria et al. 2019b).

Another important gap is that most macroevolutionary studies trying to evaluate the role of CRs in speciation to date mostly focus on changes in chromosomal numbers. This limitation is to some extent overcome by a new model incorporating chromosome arm number (Yoshida and Kitano 2021). However, information about other types of CRs have remained less used, except when they are large (e.g., pericentric inversions; Hooper and Price 2017). Although we did not cover the effect of CR size on macroevolutionary patterns of speciation, it is clear that variation in their length has important evolutionary implications (see Berdan et al. 2023). Even when using more powerful detection tools, it is important that the same criteria (e.g., in terms of minimum size) are used to allow a fair comparison when evaluating the most frequent type of CRs in a data set (Huang and Rieseberg 2020).

Although there is evidence for an association between chromosomal evolution and speciation rates across different taxonomic groups (see the previous section), this pattern is not universal. For instance, in *Saccharomyces* yeasts, no correlation between number of translocations and the degree and genetic distance was found, although they may play an important role in RI (Delneri et al. 2003; Hou et al. 2014). Cases like this not only highlight the importance of avoiding a bias toward reporting only positive correlations, but suggest that other factors are likely to play an important role in the macroevolutionary patterns of CRs (Hou et al. 2016).

CLOSING THE GAP: UNANSWERED QUESTIONS TO ADDRESS

By reviewing current knowledge on the role of CRs in speciation from a micro- to a macroevolutionary perspective, we identified several key interconnected questions that remain unanswered:

1. Which types of CRs are most commonly associated with speciation rates, and are larger CRs more likely to be involved in speciation?
2. At what phylogenetic scale do CRs have implications for macroevolution (i.e., between sister species or across genera or families)? Also do these scales differ for different types of CRs?
3. How can we test for a causal role of CRs during speciation?

The routine generation of reference genomes will allow the identification of the entire repertoire of CRs within and between species (pangenomes), including small CRs and less studied taxonomic groups. This will provide great opportunities to map the diversity of CRs across species, helping us to address questions (1) and (2), ultimately allowing for a more general view of the relationship between chromosomal evolution and speciation across the tree of life and its links to the biology of a species,

and to potentially bridge the gap between micro- and macroevolution.

To better understand the impact of CRs at the macroevolutionary level, similar phylogenetic frameworks that exist so far for large-scale rearrangements need to be adopted and developed for other rearrangement types and to include information on covariation. Phylogenetic reconstruction should take the information about pangenomes into account, together with sequencing data, morphological characters, and others.

To go from patterns to processes, we need to gather more information about the influence of the interplay between mutation, selection, and recombination in the evolution of CRs, as well as to infer their evolutionary history using model-based tools. Further theoretical work is especially required, for instance, by simulations, which realistically reflect observed patterns on the contemporary effects of CRs on RI. In addition, progress is needed to improve application of approaches such as convolutional neural networks that incorporate genomic data (Flagel et al. 2019). These approaches might be able to cost-effectively forecast likely evolutionary outcomes based on the contemporary chromosomal patterns, thereby providing critical insights into macroevolutionary patterns.

Finally, technological advancements may enable question (3) to be answered and for a more functional role of CRs during speciation to be tested. This becomes possible as direct experimental manipulations become feasible (Ansai and Kitano 2022). For example, karyotypic changes may be experimentally recreated through laser nanosurgery (Blázquez-Castro et al. 2020) or by generating artificial chromosomes (Lin et al. 2021). Similarly, inversions can be reinverted and their impact on RI assessed (Ansai and Kitano 2022). Applied to many species that are more or less closely related, such approaches could yield direct information on the macroevolutionary implications of different CRs.

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