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Editorial

A genomic update on the evolutionary impact of chromosomal rearrangements

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The field of evolutionary and speciation genomics has been revolutionized by the ubiquity and availability of genomic data even for non-model organisms. The capability to sequence long-fragment DNA has particularly spurred trans-national initiatives to generate publicly available chromosome-resolved reference genomes across the Tree of Life. Initiatives such as the Darwin Tree of Life (The Darwin Tree of Life Project Consortium et al., 2022) or the European Genome Atlas (ERGA; Mazzoni et al., 2023) enable researchers around the globe to address unresolved questions and pursue novel lines of research. The role of chromosomal rearrangements (CRs) in driving evolution has been a long-standing question in evolutionary biology (Berdan et al., 2023; Dobzhansky & Sturtevant, 1938; King, 1995; Robertson, 1916; Wellenreuther & Bernatchez, 2018; White 1978). CRs comprise an array of rearrangements that reorganize the linear sequence of the genome, ranging from local structural variants (SVs) such as inversions or duplications to large-scale karyological changes, including chromosomal fusions and fissions (Berdan et al., 2023; Lucek et al., 2023). Current genomic data has already highlighted that CRs are much more common and diverse across taxa than previously thought (e.g. Damas et al., 2022; Weissensteiner et al., 2020). While CRs can now more easily be mapped within and across taxa, their potential role for evolution and species diversification has yet often remained enigmatic, and theoretical explorations exist for only a few types of CR (Berdan et al., 2023). At the dawn of broad genome availability for model and non-model organisms alike, our special issue aims to provide a genomic update on the evolutionary impact of various types of chromosomal rearrangements. Specifically, our special issue asks what causes the evolution and establishment of CRs and whether these differ among taxa? Are CRs randomly distributed across the genome, and do they cause other chromosomal structures? How can CRs promote diversification and how do they potentially lead to speciation? Is the evolutionary impact of CRs the same among different types of CRs? Finally, do chromosomal rearrangements have a different evolutionary impact depending on whether autosomes or sex chromosomes are involved?

Identifying CRs in the genomics era

Whether CRs are directly or indirectly involved in adaptation and diversification, detecting them and understanding the factors influencing their appearance in the first place are key to start shedding light on the evolutionary processes linked to CRs. Traditionally, CRs were detected either directly, with cytogenetic screenings (Krimbas & Powell, 1992; Stebbins, 1971; White, 1973), or indirectly, by looking at the consequences of the rearrangements (e.g. Dobzhansky & Sturtevant, 1938). However, the resolution of these techniques and/or the limitation of cross-species comparisons have restricted the study of the role of CRs for evolution. In this special issue, several contributions have successfully used chromosome-level assemblies and long-read sequencing to directly analyze CRs in a broad range of systems and linked these to evolutionary processes at different scales.

Focusing on holocentric sedges of the genus *Carex*, Escudero et al. (this issue) estimated an overall high rate of CRs across the genus. Using a dual approach, the authors then combined genome assemblies and linkage maps to identify CRs between species, to later refine the CRs using only *Carex* genome assemblies and an outgroup to define conserved and rearranged genomic regions. Interestingly, despite high rates of chromosome fission and fusion, longer than randomly expected syntenic blocks remained between species. Comparing the distribution of transposable elements (TEs) and genes with the two types of genomic regions, they further showed that conserved syntenic regions correlate with gene dense areas, while rearranged regions correlate with TEs, potentially pointing to hotspots of chromosome reshuffling.

Going one step further, Cornet et al. (this issue) compared the distribution of repetitive elements between species of two independently evolved holocentric clades, i.e. in *Carex* sedges and *Erebia* butterflies in relation to CRs that result in karyotype changes in these clades. First, using low-coverage short-read sequencing data they characterized the species-specific repeat landscape, highlighting that different repeat classes occur between the two clades. Focusing on four *Erebia* species, the authors collected repeat information for several populations and showed that changes in repeat landscapes are common between populations, and scale with the degree of genetic differentiation, suggesting that similar evolutionary processes may affect repeat landscapes and the rest of the genome. At a macroevolutionary scale, the clustering of species based on differences in the repeat landscape showed a general concordance with their gene-based phylogeny, although this was stronger in *Carex*. Moreover, using chromosome number as a proxy for inter-chromosome rearrangements, the authors found that certain types of repeats are more likely to be associated with karyotype changes. Overall, their results showed that chromosomal fusion and fission events are likely associated with different repetitive elements but the relative impact of specific repeats on karyotypic changes differs between independently evolved holocentric groups.

By analyzing chromosome-level genome assemblies of three medaka fishes representing three of their major karyotypes, i.e. metacentric or acrocentric chromosomes with larger and smaller arm numbers, respectively as well as large metacentric chromosomes, Ansai et al. (this issue) tested whether the karyotypic differences among the three species were caused by centric fusions or fissions, pericentric inversions, centromere repositioning, or tandem fusions. They identified putative centromeric repeats using continuous long reads polished with short reads, assessed tandem repeats using unassembled short reads, and used phylogenetic analyses to compare these centromere-associated repeats across the different species. In their study, they not only identified multiple different repeats enriched in centromeric and pericentromeric regions but also that some of these repetitive sequences were not conserved in all chromosomes, suggesting that centromeres on different chromosomes may have different repetitive landscapes. Moreover, they showed that these centromere-associated repeats are more likely caused by centromere repositioning, highlighting the role that this mechanism may have played in the karyotype evolution in medaka fishes, allowing evolutionary changes in chromosome shape without altering chromosome numbers.

Using a different approach, Arias-Sardá et al. (this issue) aligned genome assemblies of 26 ruminant species and reconstructed five ancestral karyotypes, ranging from the ancestor of all ruminant species to the most recent ancestor of bovids. In doing so, the authors identified CRs, evolutionary breakpoint regions (EBRs) and syntenic blocks. The authors showed that CRs are not randomly distributed across the genome, with some ruminant chromosomes maintaining synteny for more than 50 million years while others showed a high rate of rearrangement. Similarly to Escudero et al. (this issue), Arias-Sardá and colleagues looked into the distribution of TEs and genes within syntenic and rearranged genomic regions, showing that TEs are depleted in syntenic regions. Going one step further, they used gene expression data of at least nine tissues in four species to define orthologous housekeeping genes. They found that synteny breaks are depleted of genes, while housekeeping genes tend to be located in conserved syntenic blocks. These results, combined with previous publications on carnivores and rodents (Álvarez-González et al., 2022; Corbo et al., 2022; Damas et al., 2022) highlight that synteny breaks co-localize with boundaries of topologically associated domains, suggest that syntenic regions might be regulatory blocks and only CRs not disrupting functional and essential genes can become fixed in evolution.

But what happens when CRs do occur? Wang et al. (this issue) identified hotspot regions in *Neurospora* fungi that are located in the telomeres where CRs, gene duplication and further relocation led to the appearance and evolution of lineage-specific genes. They found that 78% of such genes are within telomeric regions and that most of them evolved through regional CRs and gene rebirth. To investigate the functions of these lineage-specific genes, the authors assembled 68 experiments from 14 transcriptomic studies, covering most of the morphological stages and distinct culturing conditions in

N. crassa. Although lineage-specific genes were involved as non-essential partners in various aspects of *N. crassa* biology, the knocked down gene *mas-1* increased resistance to toxins, suggesting that lineage-specific genes might contribute to new phenotypes under specific conditions. All in all, this study highlights CRs as a source of novelty in adaptation and speciation.

Chromosome rearrangements can also lead to the formation of novel chromosome structures, including neo-sex chromosomes, i.e. the fusion between an autosome and a sex chromosome. In this special issue, using butterflies of the tribe Danaini, Mora et al., (this issue) investigated the evolution of neo-sex chromosomes. These butterflies stand out for containing two prominent examples of fused neo-sex chromosomes: a recently evolved neo-*W* and another much older neo-*Z* chromosome. In this study, the authors combined Oxford Nanopore long reads, Illumina short reads and Illumina RNA-Seq libraries to assemble and annotate four different Danaini species. Moreover, they mapped the distribution and expression levels of genes on both sex chromosomes and autosomes, identifying sex-biased genes. They found a strong association between the presence of sex-biased genes and the likelihood of sex chromosome turnover, particularly affecting the *W* chromosome. Specifically, they identified clusters of genes with significantly biased expression towards either males or females, suggesting potential roles for sex chromosome evolution. This accumulation of sex-biased genes, likely influenced by mechanisms such as sexual antagonism, emerges as a critical factor contributing to CRs observed in sex chromosomes over evolutionary time scales.

Finally, from a macro-evolutionary perspective, genome evolution and geographic isolation are two of the major drivers of biodiversity. Márquez-Corro et al., (this issue) conducted an integrative study combining phylogeography, chromosomal evolution and ecological requirements in *Carex*, a plant species complex, distributed in the Western Euro-Mediterranean region. First, using RADseq data of 152 samples, the authors constructed a new phylogeny for the four studied *Carex* species. Combined with karyotypic data of all the samples, they showed that both molecular and cytogenetic evidence points to southern Iberia-north Africa as the evolutionary cradle for this *Carex* species group. In addition, landscape genomics analyses identified 74 loci correlated with variables related to local adaptation, such as temperature and precipitation. Interestingly, chromosome number was one of these potential variables correlated to climatic variables. Therefore, this study supports the hypothesis that karyotype variation, at least in species with holocentric chromosomes, could be selected toward different optima climatic regimes.

Ranging from sedges and butterflies with holocentric chromosomes to fungi, fishes and mammals with monocentric chromosomes, a clear picture emerges, chromosome rearrangements are not randomly distributed across genomes, instead they are paired with TEs and other repetitive elements. CRs are moreover hotspots for gene novelty related to new traits.

Establishment and fixation of chromosomal rearrangements

If CRs are selectively advantageous or involved in reproductive isolation, they may eventually become fixed (Faria & Navarro, 2010; Kirkpatrick & Barton, 2006). However, while empirical observations suggest that CRs could be involved in the diversification of several taxonomic groups (Berdan et al., 2023; Lucek et al., 2023), their fixation is not easy from a theoretical point of view: If CRs cause meiotic defects in heterozygotes, they would result in hybrid dysfunction and/or sterility and likely be underdominant, especially when at low frequency (Navarro & Barton, 2003). Consequently, new and strongly underdominant rearrangements are thus unlikely to spread to fixation. While weak underdominance would make fixation more likely, reproductive isolation would be weaker and rearrangements less likely to promote speciation (Faria et al., 2019; Faria & Navarro, 2010; Rieseberg, 2001). The ambiguous role of underdominance for the fixation of CRs has consequently been referred to as the "underdominance paradox" (Spirito, 1998). Fixation may occur if CRs have a selective advantage, for instance through overdominance, or through inbreeding with strong genetic drift, which could counteract negative selection and facilitate the fixation of novel, yet underdominant CRs (Guerrero & Kirkpatrick, 2014; Hedrick, 1981; Navarro & Barton, 2003). However, the processes and conditions under which CRs could become fixed are still elusive and have been addressed by two contributions to this special issue.

The first contribution by Jay et al. (this issue) implements a two-island population genetic model to explore the impact of local adaptation and gene flow on the establishment of overdominant CRs that act as supergenes. Supergenes represent a particular type of polymorphism that group linked functional genetic elements, which then segregate as a single Mendelian locus (Thompson & Jiggins, 2014) and are often associated with CRs, particularly inversions. Modeling inversions evolving under disruptive selection between populations resulted in an increased frequency of poorly adapted immigrant inversion haplotypes. In this model, supergenes could evolve and be maintained through balancing selection due to inversion overdominance, where inversions promote the maintenance of alternative haplotypes even when they bear unequal benefits for local adaptation. This is the case when the recombination load due to gene flow balances the inversion fitness load, such that the spread of an inversion could result in the loss of an existing locally adapted haplotype, which in turn would promote the maintenance of differentially adapted and non-recombining haplotypes. Overall, this model extends the idea that supergenes can be maintained through alternative environmental fitness optima (Schwander et al., 2014), as the study highlights that supergenes can evolve because of the intrinsic features of inversions themselves.

Using a forward-in-time simulation framework, Banse et al. (this issue) explore the evolutionary outcome of populations in the presence of substitutions, insertion-deletions (InDels) and CRs. Their models highlight that CRs promote gene duplications but also reduce epistasis, which together support long-term adaptation. Here, CRs that promote gene duplications have a much higher fitness benefit in simulations that started far off the optimum, highlighting that they could support adaptive evolution. Within the simulated framework, small InDels and CRs impact genome size evolution and the adaptive potential of a species differently. While CRs allow for gene duplications, InDels do not facilitate a de novo evolution of genes but instead promote the evolution of existing genes. Consequently, if only InDels were allowed to evolve, they would introduce random genetic material, leading to larger genomes with fewer genes than in the presence of CRs. Together, the theoretical explorations of Jay et al. (this issue) and Banse et al. (this issue) highlight that CRs can promote adaptive evolution, and therefore speciation, under various conditions, but also stress the need to develop more theoretical frameworks that go beyond inversion type CRs.

While CRs are widespread across the Tree of Life, it often remains unclear whether they have been established by chance, *e.g.* through drift, or by selection. Using chromosome level genome assemblies, Mackintosh et al. (this issue) explore this question in *Brenthis* butterflies. Based on the inferred demographic history of their focal species, they show that most CRs are likely selectively neutral or very weakly underdominant in their system. They suggest that drift is not strong enough to fix considerably underdominant rearrangements and that there is only weak evidence that chromosome fusions fixed through positive natural selection or meiotic drive. Indeed, only one chromosomal fusion event showed evidence for a strong recent selective sweep, though positive selection in the more distant past could not be excluded. Overall, Mackintosh et al. (this issue) provide a novel population genetic framework that combines genome assemblies and whole-genome sequence data to study the potential contribution of drift and selection to the fixation of CRs at macroevolutionary scale.

Chromosomal rearrangements, local adaptation, and speciation

Chromosomal rearrangements contribute to the diversification of species, e.g. through the suppression of recombination (Noor et al., 2001; Rieseberg, 2001) or by inducing heterozygote disadvantage (White, 1973), though the two mechanisms may also operate in tandem (e.g. Yoshida et al., 2023). Inversions (Kirkpatrick & Barton, 2006), and chromosomal fusions (Guerrero & Kirkpatrick, 2014), are expected to locally reduce recombination, thereby potentially capturing and linking together locally adaptive alleles, which can promote species divergence even in the face of gene flow. The role of inversions in diversification is comparatively well studied (Faria et al., 2019; Wellenreuther & Bernatchez, 2018), while fewer studies have focused on the impact of chromosomal fusions and fissions (but see Augustijnen et al., 2024; Escudero et al. this issue; Mackintosh et al. this issue; Mora et al. this issue), translocations (see Guerrero & Kirkpatrick, 2014), or other chromosomal rearrangements (see Wang et al. this issue). Several contributions in this special issue expand on the roles of CRs in local adaptation, species diversification, and on the maintenance of inversion polymorphisms.

For instance, Ravagni et al. (this issue), characterize the presence and absence of an inversion that acts as a supergene in the common quail *Coturnix coturnix* on the Azores. This inversion has previously been identified in quails from Europe and Africa, including the other Macaronesian archipelagos, and is associated with the loss of migratory behavior (Sanchez-Donoso et al., 2022). For the Azores, Ravagni and colleagues find genetic divergence among populations of quails containing the inverted and standard configuration to be minimal, although the two arrangements have coexisted for a long time in relatively large populations. The authors therefore suggest that balancing selection, rather than divergent selection or drift, maintains the frequencies of inversion polymorphism on the Azores. Indeed, migration is absent in Azorean quails whether the inversion was present or not, highlighting that the role of CRs can vary across geographical scales.

The degree to which inversions, particularly those underlying repeated ecotype formation, are polymorphic across a species' range is further explored by Reeve et al. (this issue). In the rough periwinkle *Littorina saxatilis,* as well as in the closely related *L. arcana,* inversion polymorphisms are common, and can often be tied to parallel evolution of the same ecotypes across vast geographical scales. Inversions related to the same ecotypes may nonetheless show location-dependent patterns, indicating that they may contain different sets of adaptive alleles across populations, or be influenced by different selective pressures. Altogether, this suggests a broad but complex role for inversion polymorphisms in the formation of ecotypes, given that the underlying genetic basis may not always be the same. Reeve and colleagues further develop a novel, complementary approach to detect inversions using shifts in heterozygosity, which may be particularly useful in cases when reference genomes are not fully resolved, and when CRs are relatively rare.

Mediterranean ecotypes of the long-snouted seahorses *Hippocampus guttulatus* studied by Meyer et al. (this issue), are similarly linked to two large, ancient polymorphic inversions: the first inversion is alternatively fixed between marine and lagoon environments, likely through divergent selection, and may play a role in speciation in this system. The second inversion is only polymorphic in lagoon populations of the Mediterranean Sea and may be governed by a combination of pseudo-overdominance and local adaptation. However, a possible breakdown of the link between the first inversion with the marine-lagoon environment for some populations, particularly those where the second inversion is not polymorphic, could point to the presence of epistatic interactions between the two inversions. Meyer and colleagues therefore bring up interesting questions concerning the interplay of multiple chromosomal rearrangements within segregating populations.

While many CRs that have been linked to adaptive divergence seem to involve large sections of a genome (Meyer et al. this issue; Ravagni et al. this issue; Schaal et al., 2022; Wellenreuther & Bernatchez, 2018), this does not need to be always the case. Using extensive, low-coverage whole-genome data for the sockeye salmon *Oncorhynchus nerka*, Euclide et al. (this issue) detect many small islands of divergence between ecotypes related to spawning ground. Most of these islands show divergence on a local scale and are not consistently associated with the same ecotype divergence everywhere. Only four islands of divergence are conserved across all studied populations, and three of them are likely linked to inversions, while the other seems to be a result of divergence hitchhiking.

Euclide and colleagues therefore suggest that the radiation of sockeye salmon into a multitude of locally adapted populations may result from a mosaic of unique allele combinations across small genomic regions of divergence. They further show that islands of divergence do not require underlying structural variation, and that small genomic regions can be of adaptive importance. They suggest that genetic architecture and the strength of selection pressures may further influence the size of adaptive islands in the genome.

Sex chromosomes can play a disproportionately large role in speciation compared to autosomes (Payseur et al., 2018) and their evolution is often linked to CRs (Mora et al. this issue; Wright et al., 2016). In this issue Viitaniemi et al. (this issue) study the effects of inversions on the Z-chromosome of the zebra finch, *Taeniopygia guttata* on sperm morphology. As inversions can disrupt gene regulation in a tissue-specific manner (*e.g.* Kraft et al., 2019), they analyzed gene expression in somatic tissue, the liver, and across several time points in testes development. The presence of the inversions on the Z-chromosome impacts expression in both testes and the liver, with heterokaryotypes showing intermediate expression or resembling one or the other homokaryotype in a gene-specific and tissue-specific manner. Taken together, the results of Viitaniemi et al. and colleagues suggest that the regulatory effects of inversions can be pleiotropic and vary between tissues.

Conclusions and Outlook

Technical limitations have for a long time restricted the study of CRs to few model organisms and few types of CR. Recent developments, especially long-fragment sequencing have overcome this obstacle and international sequence initiatives are democratizing the access to genomic resources. This special issue brings together examples of various types of CRs from across broad taxonomic diversity, studied both at a micro- and macroevolutionary level, as well as empirical and theoretical explorations, highlighting the distinct and complex nature of CRs across the Tree of Life. Going beyond classic detection methods that are based on short-read sequence data, several studies of this special issue use current state-of-the-art comparative genomic approaches to detect various CRs at genome scale. Contrasting classic predictions from gene homology maps (Nadeau & Sankoff, 1998), several studies suggest that CRs are not randomly distributed across the genome, where for example specific synteny blocks may be less likely to undergo rearrangements (Arias-Sardá et al. this issue; Escudero et al. this issue). However, the underlying genomic and evolutionary mechanisms as well as the generality of these observations require further investigation. Indeed, both neutral processes such as drift or demographic history (Cornet et al. this issue; Mackintosh et al. this issue) as well as selection (Wang et al. this issue) can shape the diversity and distribution of CRs across genomes. CRs can nevertheless have a profound impact on the genome structure, including centromere repositioning (Ansai et al., this issue) or the evolution of sex chromosomes (Mora et al. this issue; Viitaniemi et al. this issue; Wright et al., 2016).

Despite the ease at which genome-wide CRs can now be quantified, the evolutionary processes that promote their spread and potentially fixation as well as their role for speciation have often remained enigmatic. Overcoming the classic dominance paradox, Jay et al. (this issue) show that inversions can be maintained through balancing selection if they are linked to supergenes. However, the impact of such supergenes may be context dependent (Ravagni et al. this issue; Sanchez-Donoso et al., 2022). Other types of CRs such as InDels may similarly establish if they are adaptive, e.g. by promoting gene duplications Banse et al. (this issue). Macroevolutionary investigations have the potential to identify additional extrinsic agents of selection that could have promoted the fixation of CRs among species (Lucek et al., 2023), such as temperature or precipitation Márquez-Corro et al. (this issue) or demographic history (Potter et al., 2017). If and how CRs could promote local adaptation and speciation seems to be context dependent and differ among taxa, where intraspecific variation in CRs can promote repeated ecotype formation (Euclide et al. this issue; Reeve et al. this issue; Wellenreuther & Bernatchez, 2018).

Given that having reference genomes for all eukaryotic species is likely becoming a reality over the next decade, large-scale macroevolutionary inferences will become possible, allowing to map CRs across taxonomic genera and families and to test for their potential impact on the evolution and maintenance of biodiversity. Combining intra- and interspecific pan-genome approaches could hold the key to bridge between the micro- and macroevolutionary roles of CRs, shedding light on what CRs could promote speciation. Identifying putative target CRs further allows for experimental approaches such as CRISPR/CAS9 (Cheng et al., 2024; Yoshida et al., 2023) or nanosurgery (Blázquez-Castro et al., 2020) to go beyond correlational associations. Understanding the evolutionary impact of CRs also requires additional theoretical explorations, which have so far especially focused on inversions (Berdan et al., 2023; Faria et al., 2019) or changes in chromosome numbers (Faria & Navarro, 2010; White 1978). Here, Banse et al. (this issue) provide a framework that could test the impact of other CR types such as InDels. While the impact of CRs has been primarily studied along the linear genome, the latter is also folded in a complex three-dimensional structure which can be altered by CRs. Recent technical advances now allow to study this three-dimensional structure even for non-model organisms, allowing to study the interplay between CRs and chromosomal structure, also in the context of speciation (Mohan et al., 2024).

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