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Opinion

Beyond supergenes: the diverse roles of inversions in trait evolution

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Chromosomal inversions are ubiquitous across the Tree of Life, with genome-wide studies revealing a bias toward smaller inversions, yet research has disproportionately focused on large, supergene-like inversions linked to discrete phenotypes. This limits our understanding of inversions' roles in trait evolution, as their size affects their potential functional impact. Investigation of smaller inversions and multi-inversion genotypes is crucial to elucidate their role in shaping continuous traits and evolutionary adaptation. Addressing this requires a shift towards a systematic study of smaller inversions and the use of experimental assays and functional annotation to identify the evolutionary forces driving different genomic trait architectures.

Highlights

Recent genome-wide studies show that most chromosomal inversions in eukaryotic genomes are small, often spanning only a few hundred base pairs.

Research has predominantly focused on large, supergene-like inversions, limiting our understanding of inversions that are smaller or contribute to continuous traits, whose functional and evolutionary roles – particularly in influencing continuous traits and eco-evolutionary dynamics – remain poorly understood.

The length of an inversion affects the number of genes and genetic elements it captures, influencing functional effects, genome reorganisation, and the likelihood of an inversion containing co-adapted gene complexes.

Improving our understanding of the role of inversions in trait evolution will require the study of small inversions, coupled with experimental assays and functional annotation to go beyond observational data and clarify their role in ecoevolutionary dynamics.

Structural variation across the Tree of Life

The advent of chromosome-level genome assemblies, pangenome graphs, and the integration of long-read sequencing data resulted in a surge of studies on **structural variants (SVs)** (see Glossary), which affect the presence, abundance, position, or orientation of nucleotide sequences [1]. SVs are now being systematically catalogued across the Tree of Life, enriching population studies and advancing our understanding of interspecific genomic diversity [2]. As SVs frequently affect large regions of the genome and collectively span more base pairs than single-nucleotide polymorphisms (SNPs) (e.g., [3,4]), they likely contribute substantially to genomic variation

One type of SV that has received particular attention is the chromosomal inversion, which reverses the orientation of a genomic region ranging in size from a few base pairs (bps) to several megabases (Mbs) [5]. The suppression of **recombination** in large, inverted regions (Box 1) has garnered significant attention due to its role in preserving co-adapted gene complexes. This phenomenon is closely linked to key evolutionary processes such as speciation, local adaptation, and changes in genome architecture. These large, inverted regions are often referred to as **supergenes** in cases when the combination of inverted linked loci controls complex phenotypes [6–8]. Such gene complexes are often implicated in the development of alternative phenotypes, as is the case in wing colour of mimetic butterfly species [9,10]. Inversion supergenes can also prevent local maladaptation in nearby populations that are connected by gene flow but have divergent optimal phenotypes (a **Type I polymorphism** sensu [11]), such as in seaweed flies [12] and ants [13]. Alternatively, they can enable multiple optimal phenotypes in a single population that experiences fluctuating environments (a **Type II polymorphism** sensu [11]), as in yellow monkeyflowers (*Mimulus* spp.) [14] and *Drosophila* spp. [15]. Due to these characteristics, inversions are scrutinized in many population genomic research programmes addressing adaptive trait evolution and species diversification [6,7].

Inversion–phenotype associations are typically identified through one of two approaches. In a phenotype-first approach, a visible polymorphism reveals the underlying inversion-associated

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Box 1. Mechanisms and impacts of inversions on genome function and evolution

Inversions are commonly generated by **non-allelic homologous recombination involving inverted repeats [**17]. They can also result from double-strand break repair mechanisms, such as **non-homologous end joining, or replication-associated processes like fork stalling and template switching mediated by microhomology [**17]. Inversions are particularly intriguing due to their dual direct and indirect effects on genome function and organization [19]. They can directly influence genome function by altering genes intersecting with inversion boundaries ('breakpoints') or by disrupting *cis-regulatory elements that control gene transcription [*37,54]. Another direct impact arises from the reversal of sequence orientation, which can influence gene expression through the '**position effect variegation' phenomenon. This occurs when a euchromatic gene is repositioned adjacent to heterochromatin, resulting in altered gene activity [**55]. Inversions also directly inhibit recombination in heterozygotes because inviable gametes are produced when crossovers occur within inverted regions, unless there is a double-crossover event or gene conversion [56].

Notably, when a new inversion arises, the derived arrangement originates from a single copy. Being a single mutational event, it bears zero within-arrangement variation compared to the ancestral haploblock. While inversions can, in principle, be aged by the accumulation of these private mutations since their origin [11], this approach is complicated by linked selection, reduced effective population size, and the lack of recombination – all of which violate molecular clock assumptions [56]. As a result, flanking recombining regions are often used as proxies for dating inversions. Reduced recombination between inverted and non-inverted arrangements leads to genetic isolation, allowing sequence divergence to accumulate over time [57]. This generates distinct haplotypes in linkage disequilibrium (LD) within each arrangement, which can maintain combinations of alleles that work together towards a complex phenotype or are favourable under certain environmental conditions.

Inversions can persist when they are maintained by **balancing selection [**58,59]. Polymorphic inversions can confer a heterozygote advantage, a phenomenon often attributed to associative overdominance or pseudo-overdominance. In these cases, different inversion arrangements may accumulate distinct deleterious mutations, which are mitigated when present in a heterozygous state, allowing these inversions to persist and shape evolutionary trajectories [9].

In this opinion article, we define large inversions as >1 Mb and small inversions as <1 Mb. We acknowledge that these categories are simplifications and that the functional impact of an inversion generally scales to some extent with length. These thresholds are not meant as absolute biological boundaries but rather as practical definitions to aid discussion and interpretation throughout the text.

architecture. Alternatively, if the inversion is large, it can be detected using genetic karyotyping technologies [16]. Both approaches tend to bias inversion discovery toward larger variants that produce obvious, discrete phenotypes, while overlooking smaller inversions or those involved in more subtle, continuous traits. The genome-first approach, enabled by high-throughput sequencing, has revealed a high prevalence and wide size range of inversions but with a notable skew toward smaller inversions [3,4]. These genome-wide studies show that many inversions lack clear phenotypic associations. The ubiquitous presence of small inversions in genomes, coupled with their notably higher frequency compared to large inversions, suggests a significantly underappreciated role of small inversions in trait evolution. Inversions are difficult to functionally annotate precisely because the genes contained within are in tight linkage. Even for many of the better studied inversions, we often have only observational or correlative data that hints at an ecological or functional role.

Here, we highlight the diverse role of inversions in trait evolution. First, we propose how inversions can contribute to continuous trait variation. Second, we examine how inversion length affects their influence on trait architecture, arguing that inversions range from large, supergene-like variants to small ones resembling SNPs. This continuum – especially the understudied small-to-medium range – may include variants with distinct regulatory or structural effects not well captured by current models. Third, we highlight the need for rigorous ecological and functional annotation of inversions, ideally supported by experimental validation.

Inversions can play a role in diverse genomic architectures

Inversions are known for discrete polymorphisms

The 'supergene' concept was introduced to explain the inheritance of balanced polymorphisms in multiple traits controlled by a single Mendelian factor [17]. Fisher proposed that Batesian mimicry in butterflies could be explained by genes in linkage disequilibrium due to suppressed recombination [18]. Accordingly, the discovery of widespread inversion polymorphism in *Drosophila* spp. offered a plausible mechanism for recombination suppression [19].

Today, a 'supergene' describes a genomic region with closely linked loci with little recombination, resulting in sets of alleles at these loci being inherited together as haploblocks [20]. Most known supergenes are controlled by inversions, though exceptions exist [5]. Notable examples of supergenes include the inversion-mediated polymorphism in the red imported fire ant, *Solenopsis invicta*, which determines colony structure (polygynous or monogynous) [13], and the supergene inversion in the ruff, *Calidris pugnax*, which controls distinct male reproductive morphs [21]. In both cases, alternative karyotypes correspond closely to multi-trait phenotypic polymorphisms [22].

Inversions can contribute to continuous phenotypic variation

While small and large inversions have been linked to discrete traits – as in medical examples such as haemophilia A [23] and Hunter syndrome [24] – they can also underlie continuous trait variation by modulating quantitative traits in a more subtle, polygenic fashion [8]. Indeed, some supergene-type inversions do not correspond with discrete phenotypes or the haplotype–trait associations are context-dependent [3,25–27]. This is coupled with numerous observations of gradual environmental clines in inversion haplotype frequencies [28,29]. In follow-up studies, these clines have been linked to individual-level variation in continuous traits, such as flowering time [30] or thermal tolerance [31], supporting a role for inversions in shaping variation in continuous traits.

Inversion-encoded continuous trait variation can be produced in several ways (Figure 1). For example, a trait architecture consisting of one major-effect inversion locus and many unlinked loci of small effect would be expected to produce continuous trait variation and inversion haplotype-trait or haplotype-environment associations. Likewise, multiple, interacting inversions could produce similar patterns. Even if an inversion is the sole locus underlying a trait, phenotypic plasticity could result in a continuous phenotypic spectrum. Altogether, this suggests that understanding the broader role of inversions in evolutionary processes requires considering them in the context of their genomic and environmental backgrounds, rather than as isolated supergenes.

The impact of inversions is intimately tied to their nucleotide length

Bias in inversion research: overlooking the small and ubiquitous

Technological advances in detecting inversions have shown a wide length spectrum of segments spanning a few bps to several Mb. We compiled studies in animal and plant species that conducted genome-wide scans for inversions, focusing on those that did not apply minimum length cutoffs (Figure 2; see also the supplemental information online). We found a notable bias towards animal studies and a strongly skewed distribution of inversion lengths unrelated to genome size (Figure 2). A clear predominance of shorter inversions was detected across studies, typically with a median length of less than 5 kb (see Table S1 in the supplemental information online). In contrast, many functional studies target large inversions with a minimum size of 1 Mb (Figure 2 and the supplemental information online), introducing a significant bias likely due to past technological constraints and the frequent association of large inversions with visible phenotypic effects [6] (Box 2).

Glossary

Associative overdominance: apparent overdominance caused by recessive deleterious alleles or dominant advantageous alleles linked within different chromosomal arrangements (e.g., inversions), resulting in reduced fitness of homokaryotypes and a fitness advantage of heterokaryotypes.
Balancing selection: selection that maintains multiple alleles at a locus or chromosomal arrangements in a population due to mechanisms such as heterozygote advantage, frequency-dependent selection, or spatial/temporal environmental variation.
Epigenetic: refers to heritable changes in gene expression or phenotype that do not involve alterations in the DNA sequence, often mediated by DNA methylation, histone modifications, or non-coding RNAs.
Fork stalling: a transient interruption of replication fork progression due to obstacles such as DNA damage, secondary structures, tightly bound proteins, or transcription-replication conflicts.
Gene conversion: a process by which one DNA sequence replaces a homologous sequence such that the sequences become identical after the conversion event.
Haploblock (block of differentiation): a region of reduced recombination, characterized by high linkage disequilibrium (LD), and often associated with high local differentiation between genetic groups.
Heterokaryotypes/homokaryotypes: individuals which are heterozygous/homozygous for a structural variant considered as a single locus, such as an inversion; the alleles are distinct haplotypes (e.g., inverted versus non-inverted).
Heterozygote advantage: a form of balancing selection in which heterozygous individuals have higher fitness than either homozygote, maintaining genetic diversity at a locus.
Inversion polymorphism: a chromosomal inversion in which a DNA segment is reversed end-to-end; it varies in frequency within or among populations. Inversions can suppress recombination and help maintain linked adaptive alleles, affecting large or small genomic regions.
Inversion Type I polymorphism: a form of inversion polymorphism in which alternative chromosomal arrangements

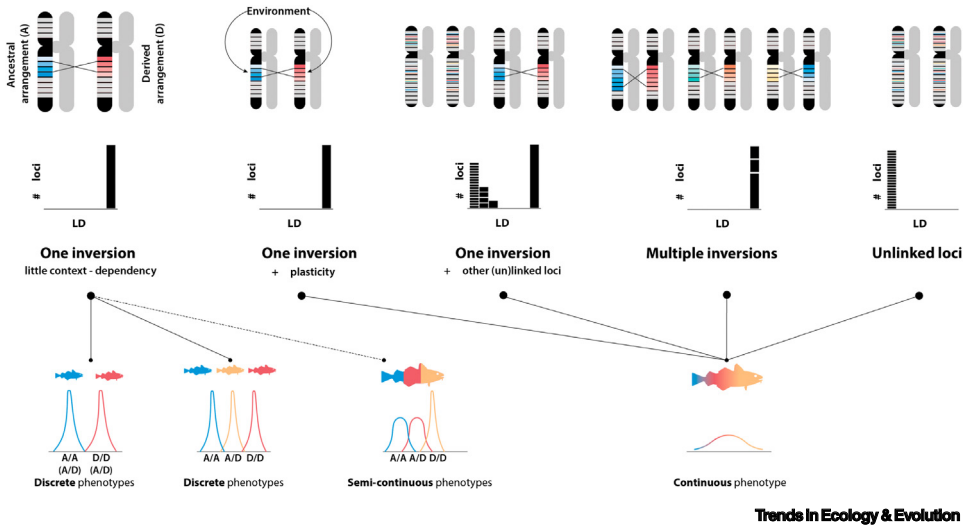

Figure 1. Different scenarios of how inversion polymorphisms can influence phenotypic traits, using fish colour as an example. These scenarios are not exhaustive but are intended to highlight the various roles inversions can play in shaping phenotypic outcomes. From left to right, the first example shows a large inversion in high linkage disequilibrium influencing the phenotype by coding for two or three discrete fish colour morphs, or even continuous colour morphs. The next example introduces plasticity, resulting in a more variable phenotype with a continuous distribution. In the following scenario, one large inversion interacts with many other genetic polymorphisms, such as single-nucleotide polymorphisms (SNPs) or indels, leading to continuous phenotypic variation. The remaining scenarios showcase the interaction of multiple inversions and, lastly, the interaction of many unlinked genetic polymorphisms. Abbreviation: LD, linkage disequilibrium.

By focusing mainly on large inversions, we may overlook how inversion length itself can impact genome function, fitness, and broader evolutionary patterns. Understanding the full spectrum of inversion sizes is key to grasping their diverse roles in shaping traits.

How length impacts function

The length of an inversion has profound effects on evolution [32], as the direction and intensity of selection varies predictably with length [33,34]. Inversions >1 Mb in length can dramatically rearrange the physical proximity of genes and regulatory elements (Box 1). This rearrangement can move genes closer or further from heterochromatic regions, thereby influencing gene expression [35]. In addition, long inversions cover larger segments of the chromosome, often involving multiple genes and regulatory elements simultaneously. This can lead to chromosome-wide reshuffling of enhancers controlling multiple linked genes, affecting bidirectional promoters while altering promoters of operons, including their pleiotropic interactions. This pleiotropic interplay is particularly important for the genetic architecture of complex traits, which often require the coordinated interaction of multiple genes [36].

By contrast, the effects of smaller inversions have been less thoroughly investigated. Evidently, their smaller size involves fewer genetic elements that have less impact on the chromosomal landscape and more subtle phenotypic effects. Their effect on recombination is also expected to be significantly reduced. Additionally, their limited genetic content makes smaller inversions less likely to harbour co-adapted gene complexes or induce large-scale phenotypic changes across multiple traits.

are maintained among populations; it is typically due to divergent selection and restricted gene flow, contributing to local adaptation and population differentiation.

Inversion Type II polymorphism: a form of inversion polymorphism maintained within populations by balancing selection, such as heterozygote advantage, frequency-dependent selection, or temporally/spatially varying selection, promoting within-population diversity.

Linkage disequilibrium (LD): non-random association of alleles at different loci.

Maternal effects: influences of the mother on offspring traits that are not due to the offspring's own genes, often via egg nutrients, hormones, or the early environment.

Non-allelic homologous recombination (NAHR): a form of homologous recombination occurring between DNA sequences with high similarity that are not alleles, such as repeated transposable element copies, often leading to structural variants.

Non-homologous end joining (NHEJ): an error-prone repair mechanism for DNA double-strand breaks that directly ligates DNA ends without the need for sequence homology.

Position effect variegation (PEV): mosaic gene expression resulting from epigenetic silencing when a gene is relocated near heterochromatin due to a chromosomal rearrangement.

Recombination: the exchange of genetic material between homologous DNA molecules, typically during meiosis, generating new allele combinations.

Structural variant (SV): genomic variation among individuals affecting the presence, position, orientation, or copy number of DNA segments, including inversions, duplications, deletions, and translocations.

Supergene: a genomic region comprising tightly linked loci that control complex adaptive traits and are inherited together due to suppressed recombination.

Template switching: a replication error in which the DNA polymerase jumps to a different template strand, often mediated by microhomology, generating structural variants or gene conversions.

Translocation: a structural variant in which a DNA segment changes position within the genome. It can be reciprocal

Inversion length also affects neutral evolutionary dynamics. With increasing inversion age, stochastic mutations accumulate due to the suppression of recombination, often referred to as Muller's Ratchet [37]. Because inversion segments experience reduced effective population sizes compared to the rest of the genome, the effectiveness of purifying selection is reduced, while the influence of genetic drift is enhanced. Conversely, the latter effect is predicted to be weak when an arrangement is at intermediate or high frequency, and becomes pronounced when it is rare at the population level [37]. While the length of the inversion does not qualitatively alter this process, larger inversions tend to accumulate slightly more mutations per kilobase (kb), particularly in the absence of **gene conversion** [37]. As mutations accumulate, the resulting inversion supergene can diversify within the lineage of each arrangement (or **haploblock**) [11]. A greater diversity of genotypes within each arrangement can generate a wider spectrum of phenotypic variation, which is increasingly likely to overlap and appear as semi-continuous phenotypes rather than distinct alternatives (Figure 1). Larger inversions presumably have greater potential for diversification because the mutational target is larger and they have a slightly higher mutation rate (Figure 1) [37].

or non-reciprocal, affecting chromosome number via fusions or fissions, including Robertsonian translocations at centromeres.

Annotating inversions: moving from observations to ecological and environmental functions

We highlight indirect methods for studying eco-environmental associations of inversions and direct approaches for identifying inversion fitness effects that depend on environmental contexts (Figure 3).

Indirect evidence: observational annotation of inversions

Eco-environmental correlations and clinal patterns. Inversion frequency clines along environmental gradients are used to correlate inversion patterns across populations with environmental variables [31] (Figure 3). These studies help to identify conditions for manipulative experiments and guide the search for causal genes. Many well-described inversions were first identified through correlations with environmental factors [38,39], later confirmed by **linkage disequilibrium (LD)** scans showing recombination suppression [29]. An example is ecological divergence in marine snails of the genus *Littorina*, where inversions underlie 'wave' and 'crab' ecotypes despite spatial proximity. By integrating empirical cline data with modelling, drift was distinguished from divergent selection in maintaining inversion clines. **Translocation** studies confirmed selection acting on these inversions [40,41]. However, observational studies often face limitations due to their correlative nature, despite methodological attempts to correct influences of drift, demographic history, and other population processes [42,43].

Changes to genome structure and function. Inversions can disrupt genes and affect regulation but identifying impacted genes and predicting regulatory effects remains challenging. Identifying inversion-associated changes in gene expression resulting from the disruption of breakpoints, alterations in gene position, order, orientation, or chromatin architecture reveals mechanisms underlying phenotypic impacts (e.g., [44,45]). **Epigenetic** modifications influence recombination frequency and distribution through chromatin and histone changes [8], with affected sites scaling with inversion size. Unlike structural changes that suppress recombination, epigenetic mechanisms can target specific loci. Understanding how epigenetic variation relates to inversion length and evolution will improve insights into methylation's role in stress responses (Box 3). A productive approach to systematically organize this information is to develop a gene-environment ontology framework linking gene functions and genomic features to phenotypes and ecological factors [46]. Such a framework would create a comprehensive repository of gene-environment associations, facilitating future research.

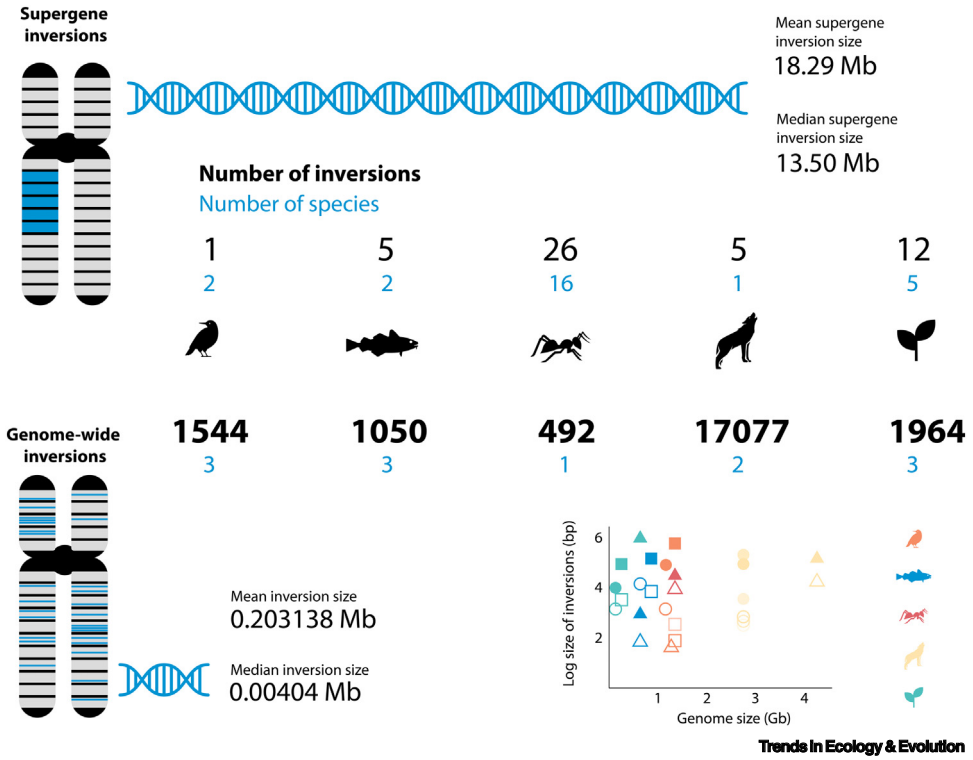

Figure 2. Summary of inversion sizes from studies focusing on supergenes (top; Table S2 in the supplemental information online) compared to studies cataloguing inversion sizes and their frequencies across the entire genome of the species (bottom; Table S1 in the supplemental information online). The mean and median inversion sizes are larger for studies focusing on inversion supergenes, a pattern observed in genome-wide studies across species in multiple clades, including fish (in blue: Australasian snapper *Chrysophrys auratus* depicted by triangles, three-spined stickleback *Gasterosteus aculeatus* depicted by circles, and Atlantic silverside *Menidia menidia* is the depicted by squares), birds (in orange: Common starling *Sturnus vulgaris* depicted by triangles, House finch *Haemorhous mexicanus* depicted by circles, and House sparrow *Passer domesticus* depicted by squares), mammals (in yellow: Deer mouse *Peromyscus maniculatus* depicted by triangles and grey wolf *Canis lupus* depicted by circles), insects (in red: Cristina's walkingstick *Timema cristinae* depicted by triangles), and plants (in green: *Eucalyptus* spp. depicted by triangles, *Oryza* spp. depicted by squares, and *Arabidopsis thaliana Ler* depicted by circles). In studies of *Passer domesticus* and *Canis lupus* the different translucencies of shapes depict the different filters used. The scatterplot (bottom left) shows no clear correlation between mean (solid shapes) or median (outlined shapes) inversion size (bp) and genome size (Gb) across these studies.

Direct evidence: experimental annotation of inversions

Experiments. Common garden, reciprocal transplant, and stressor experiments (Box 3) help to assess inversions' eco-environmental impacts. Where inversions are associated with multivariate environmental gradients, experiments can interrogate of these are influencing traits and are driving selection (Box 3). Though resource-intensive, these tests can provide evidence of adaptation, clarifying whether inversions enhance fitness or persist due to factors like recessive costs, behaviour, or gene flow. In the insect pest *Thrips palmi* [47], clinal patterns in thermal physiology were first established in common garden experiments, revealing strong associations to polymorphic inversions. Likely owing to logistical challenges of experiments, relatively few studies have thoroughly investigated the relationship between genetic inversions and phenotypic traits [8].

Box 2. Advancement and bias in identifying inversions and their roles: insights from Atlantic cod

Atlantic cod (*Gadus morhua*) is an ecologically important species, spanning oceanic gradients in temperature, salinity, and oxygen levels. With high dispersal potential, fecundity, and population sizes, it has become a prime system to study the genomic basis of local adaptation. Past population genomic studies revealed large genomic haploblocks with significant linkage disequilibrium (LD) across four linkage groups (LG1, LG2, LG7, and LG12; Figure I) [25,26,60,61]. Crosses of different migratory ecotypes were used to further describe the haploblocks on LG1 [60] and long-read assemblies spanning breakpoints later validated the haploblocks as inversions [62].

Inversion polymorphisms in cod have been associated with ecological and behavioural traits including migratory behaviour, such as the inversion on LG1, which is strongly linked to migratory and stationary life histories. Migratory cod undertake long-distance seasonal migrations, while stationary cod remain in coastal or fjord regions. One inversion arrangement is nearly fixed in stationary ecotypes, suggesting a significant role in local adaptation [25,63,64]. Similarly, there are strong correlations between LG2 and low salinity and LG12 and cool temperatures, and weak correlations between LG2, LG7, and LG12, and temperature, salinity, and oxygen [25,65–67]. However, these associations remain correlative, and the functional consequences of most inversions are unknown. This highlights the need for experimental approaches to validate the adaptive roles of inversions.

Recently, genome-wide sequencing data enabled identification of smaller inversions. These include inversions on LG6, 11, and 21 (0.5–1.78 Mb) [62], as well as preliminary evidence for inversions on LG4, 10, 11, 17, and 19 (0.5–10 Mb) [62], which can potentially be identified through whole genome synteny comparisons and LD patterns [68]. This expanding catalogue includes previously overlooked smaller inversions and reveals a complex genome architecture with many inversions of varying size and potential significance. The abundance and partial environmental overlap of these inversions complicate efforts to link them directly to phenotypic traits or adaptive functions. Disentangling their evolutionary roles will require comprehensive genome scans across the spectrum of inversion sizes paired with functional investigations – ideally using long-read population datasets and controlled experimental validations.

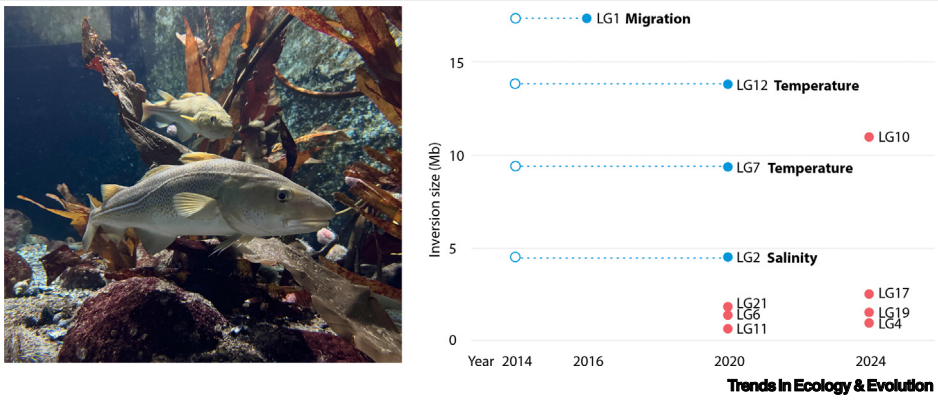

Figure I. Inversions in Atlantic cod. (A) An adult Atlantic cod (*Gadus morhua*, credit to *Noah Breuninger*). (B) A plot of inversions detected in Atlantic cod over time, with the year of discovery on the x-axis and inversion size on the y-axis. Filled points indicate inversions identified through sequence comparisons, while empty points represent inversions based on linkage disequilibrium patterns from earlier studies, where the inversion status were tentative. Blue filled points denote inversions that have been studied for eco-environmental associations, whereas red filled points represent inversions that have not been investigated for such associations.

Gene editing and chromosomal engineering. Isolating and manipulating inversions [48] can directly assess their effects on phenotype, fitness, and recombination rates [49], providing a powerful tool for studying function and adaptive significance. Clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPR-associated protein (Cas) (CRISPR-Cas) and site-specific recombinases enable targeted genome modifications, allowing functional analysis of loci, genes, and mutations [36]. This approach would be particularly effective for dissecting

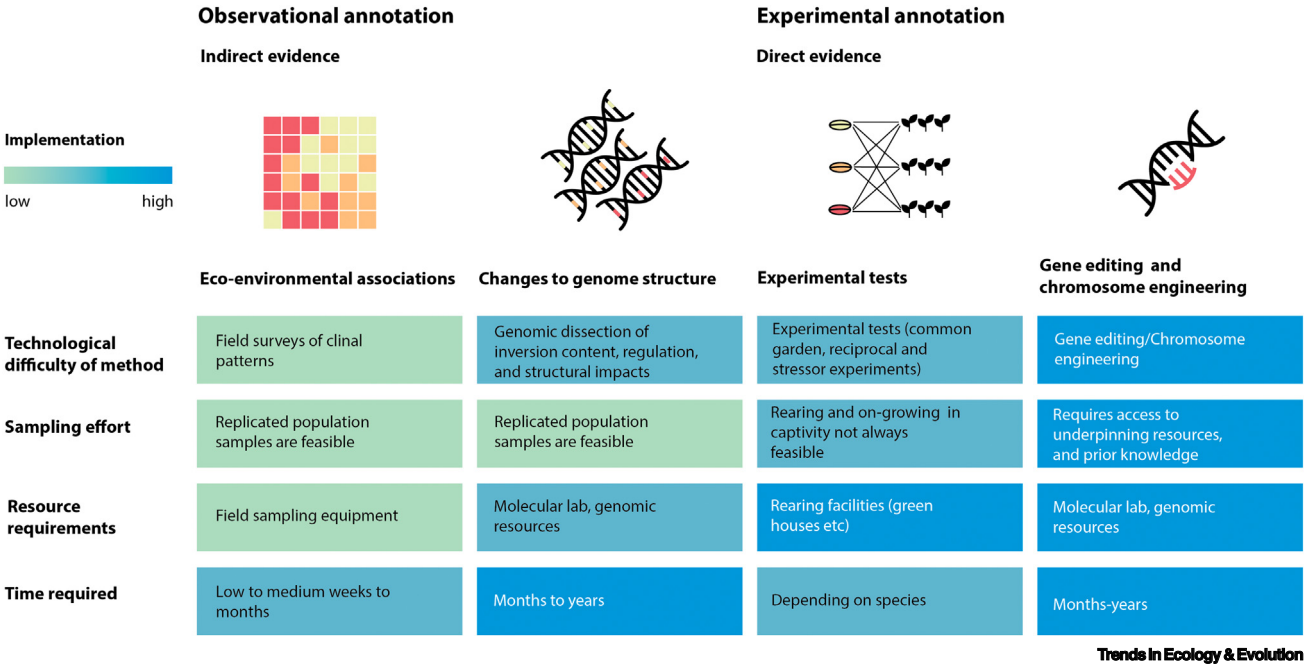

Figure 3. A simplified workflow for annotating inversions and investigating their role in trait evolution. Four key steps: (i) gathering observational evidence from field studies to identify inversion-mediated effects, (ii) evaluation inversion mediated changes to genome structure and function, (iii) designing experiments to evaluate fitness impacts, and (iv) using targeted gene editing and chromosomal engineering. The required resources: (i) addressing technical challenges, (ii) sampling efforts across individuals or populations, (iii) infrastructure and genomic tool needs, and (iv) time considerations for completing the process.

large inversions containing multiple genes. A key challenge is generating multiple single-gene edits within the same inversion.

Chromosomal engineering, through the targeted induction of chromosomal rearrangements or the reversal of existing ones to their ancestral orientation [48,50], reveals direct effects on genome structure and function [51]. This approach allows precise analysis of how inversions affect gene expression, regulation, and genome architecture but remains underutilized in non-model species due to the need for extensive genomic resources.

Box 3. Stressor experiments reveal the role of inversions in adaptation and trait evolution

Stressor studies are one way to gain insights into the role of inversions in trait evolution. They employ factorial experiments to investigate how inversions affect fitness under various environmental conditions [69]. These experiments manipulate stressors individually and in combination, which are powerful approaches for disentangling the complex interactions between inversions and environmental factors [70]. They can reveal whether inversions control discrete or continuous phenotypes by testing phenotypes across environmental gradients. Through statistical or methodology control, stressor studies can reveal which inversion(s), big or small, drive adaptation when multiple inversions are correlated with the same environmental condition. However, they require balancing trade-offs among ecological realism, replication, and temporal and spatial scales.

Careful selection of the type, intensity, sequence, and timing of stressors, guided by observational correlations between inversions and environmental conditions, optimizes experimental design (Figure I) [71]. Additionally, attention must be given to confounding factors, such as **maternal effects** – which can alter offspring responses [72]. Inversions may also co-vary with genetic background, complicating the interpretation of phenotypic effects and genetic interactions. These confounding effects can be disentangled through optimal breeding designs or large sample sizes from multiple families combined with robust statistical analysis.

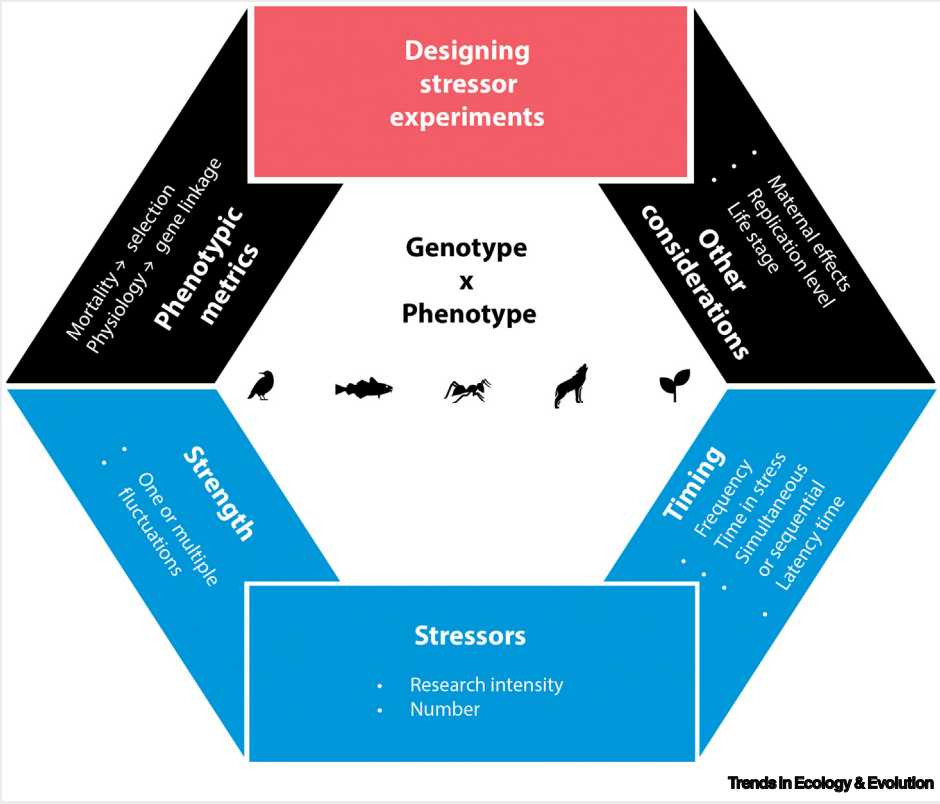

Figure I. Overview of key considerations when designing stressor experiments to investigate the role of inversions. Stressor experiments assess the response of different inversion genotypes to phenotypic changes and can be conducted across various animal and plant species. Before designing such experiments, it is essential to decide on the phenotypic metrics to be measured, as well as the desired stressor strength. Considerations related to potential maternal effects, replication levels, and sample sizes required for statistically rigorous results must also be addressed. Additionally, the life stages to be studied should be determined beforehand. When testing stressors, the strength of the stressor(s) must be carefully considered, along with whether to test a single or multiple stressors. The application method is also important, including whether the stressor is applied continuously or intermittently (e.g., fluctuating). Furthermore, the timing and frequency of stressor application, the duration of exposure, and the latency period between stressor application and response need to be carefully planned.

Concluding remarks and future directions

The study of large inversions and supergenes has long intrigued evolutionary biologists [1,6,20] because they behave fundamentally differently than standard genetic polymorphisms. Inversions suppress recombination, preserving co-adapted gene complexes across generations. While attention has historically focused on large inversions, advances in chromosome-level assemblies, pangenomes, long-read sequencing, and machine learning now reveal that small chromosomal inversions are far more prevalent than previously recognized (Figure 2). This raises the fundamental question of whether findings from large inversions can be extrapolated to smaller ones [52]. Because inversion size influences the number of genes and regulatory elements affected, the functional and evolutionary impacts of large and small inversions are expected to differ, underscoring the need for a more comprehensive approach to studying their roles in genome evolution (see Outstanding questions). Paradoxically, ecological adaptation – a process associated with the generation of diversity – often seems to benefit from mechanisms that reduce

Outstanding questions

How do smaller inversions influence recombination rates and phenotypic variation compared to larger inversions, and what are their evolutionary consequences across different taxa? Investigating smaller inversions and their impact on phenotypic traits and recombination suppression remains an important yet underexplored area that could reveal nuanced mechanisms of adaptation and genome evolution.

Which experimental approaches are best suited for identifying the causal role of inversions in adaptation? Experimental approaches, such as mapping inversions to phenotypes using regular trait measurements, experimental approaches, and genome-wide association studies (GWAS), can shed light on their contribution to trait variation.

How do polymorphic inversions interact with other genetic elements, such as regulatory variants, transposable elements, and epigenetic marks, to influence gene expression and individual fitness? Disentangling these interactions is crucial for understanding how inversions integrate within the genomic and environmental context, shaping both fitness and ecoevolutionary trajectories.

What role do small inversions play in long-term evolutionary processes, such as genome size evolution and the emergence of new species? Investigating inversion dynamics across evolutionary timescales can reveal their contribution to structural genomic variation and speciation.

What mechanisms maintain small polymorphic inversions in populations, and how do these vary between adaptive and non-adaptive scenarios? Theoretical, experimental and observational studies can help determine whether selection, genetic drift, or other evolutionary forces primarily drive the persistence of such inversions.

To what extent do environmental conditions modulate the evolutionary dynamics and phenotypic effects of chromosomal inversions, particularly smaller inversions? Systematic studies assessing inversion frequency and persistence across diverse

recombination. This suggests that some constraint on recombination plays an important factor for local adaptation to proceed efficiently and at pace. The interplay between inversion size and the number of inversions involved in adaptation may also be critical to understanding how recombination suppression contributes to adaptation and divergence.

To understand the mechanisms maintaining polymorphic inversions and their phenotypic effects, we argue for a transformative research approach including studying the multitude of small inversions, with experimental functional validation. This approach involves examining small inversions' interactions with other inversions, genetic variants, regulatory elements, and epigenetic influences, as well as studying their effects on gene expression, regulatory networks, and genomic stability. Identifying the functional role of small inversions is particularly challenging, as many may be quasi-neutral. Dissecting these interactions requires analysing multiple genomic regions under varying environmental conditions alongside comprehensive phenotypic assays. Additionally, examining population-level dynamics of inversions – such as their frequency, distribution, and persistence – can illuminate their potential deleterious consequences. Bridging these knowledge gaps will provide a more nuanced understanding of the overall impact of inversions on organismal fitness and their role in evolution.

In conclusion, integrating both observational and experimental approaches will better elucidate how inversions contribute to trait evolution, adaptation, and speciation. Experimental validation – through techniques such as reciprocal transplant experiments, gene editing, and stressor studies – will be crucial for uncovering the (non)adaptive significance of inversions and understanding their role in population dynamics [53]. This knowledge is not only vital for advancing evolutionary biology it also holds practical implications for conservation efforts, where the potential for translocating adaptive inversion haplotypes could help bolster species' resilience in the face of environmental change.

ecological settings can identify the role of environmental heterogeneity in inversion-mediated adaptation.

How can advances in genome editing (e.g., CRISPR) be leveraged to experimentally validate the effects of inversions on traits? Genome-editing tools provide opportunities to test causative links between inversions and phenotypic traits by creating or reverting inversions in model systems.

What are the implications of maintaining specific inversion haplotypes for conservation strategies, particularly in populations facing environmental stressors or habitat fragmentation? Exploring the potential of translocating individuals with adaptive inversion haplotypes to bolster population resilience can provide actionable insights for biodiversity management and conservation efforts.

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Declaration of interests

No interests are declared.

Supplemental information

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