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Review

Eco-Evolutionary Genomics of Chromosomal Inversions

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Chromosomal inversions have long fascinated evolutionary biologists due to their suppression of recombination, which can protect co-adapted alleles. Emerging research documents that inversions are commonly linked to spectacular phenotypes and have a pervasive role in eco-evolutionary processes, from mating systems, social organisation, environmental adaptation, and reproductive isolation to speciation. Studies also reveal that inversions are taxonomically widespread, with many being old and large, and that balancing selection is commonly facilitating their maintenance. This challenges the traditional view that the role of balancing selection in maintaining variation is relatively minor. The ubiquitous importance of inversions in ecological and evolutionary processes suggests that structural variation should be better acknowledged and integrated in studies pertaining to the molecular basis of adaptation and speciation.

Highlights

Inversions are potent forces in local adaptation and diversification because they protect inverted sequences from recombination, allowing favourable allelic combination to be maintained in the face of gene flow.

High-throughput genomics methods have revived the popularity of research on inversions, and recent studies reveal that they are taxonomically more widespread than previously thought.

Inversions can be maintained over hundreds of thousands and even millions of years via various forms of balancing selection and often involve large genomic regions, each comprising hundreds of genes, together representing a significant portion of the genome.

The ubiquitous importance of inversions in ecological and evolutionary processes indicates that such structural variation must be better acknowledged and integrated in studies pertaining to the molecular basis of adaptation and speciation.

The Evolutionary Significance of Inversions: A Historical Perspective

Chromosomal inversions have long been implicated as drivers of evolutionary change. Indeed, beginning with the establishment of the modern synthesis, inversions have been used as privileged systems to study phylogenies, geographical clines, temporal cycles, and meiotic drive [1]. Inversions were first discovered during the early 1920s by Alfred Sturtevant while comparing the genetic maps of closely related *Drosophila* spp. [2]. He postulated that inversions segregate as distinct units by showing that they reduce the rate of transmission in recombinant chromosomes. His claims could be tested using the giant polytene salivary gland chromosomes in Diptera, which allowed the direct observation using simple microscopic analysis of inversions and their points of rearrangement. These cytological tests were able to compare the standard and inverted sequences in *Drosophila* spp. and proved what Sturtevant had deducted purely by genetic analysis [3].

Inversions subsequently became the first genetic markers used to build phylogenies [2,4] and to study the fixed differences between *Drosophila* spp. and other species [5]. During the 1970s, inversions lost popularity with the emergence of molecular evolutionary genetics [6]. In recent years, however, owing to high-throughput genomics methods, inversions have regained popularity with the growing awareness that they are taxonomically more widespread than previously thought and accumulate more genetic variation than collinear regions [7]. The studies confirmed some of the longstanding views, for example, the pronounced reduction of recombination within and around inversions [8]. These studies also showed that a limited amount of gene flux between inverted and non-inverted arrangements can occur via double crossovers and gene conversion. [9] Consequently, inversions typically leave a cryptic, chromosome-specific population substructure [10].

Inversions are now increasingly used to investigate major evolutionary processes; from mating systems to environmental adaptation, and, ultimately, speciation. Indeed, studies have repeatedly linked inversions to alternate reproductive strategies [11,12], adaptive divergence within

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species [13–18], the origin [19] and subsequent evolution of sex chromosomes [20], and speciation [21]. Recent studies have also uncovered the molecular underpinnings that allow inversions to be major contributors to evolutionary divergence, something that has only been recently possible [22]. Other insights have come from the dissection of the breakpoint structure, evolutionary age of inversions, and patterns of genetic variation associated with inversions in natural populations and, ultimately, the genes that selection is acting on [23,24] (Table 1). This has allowed researchers to move from the general description of inversion frequency shifts and associated patterns to a more detailed understanding of the targets of selection.

Here, we review recent genomics-enabled studies of inversion polymorphism in animals and plants to detail the causes and consequences that allow inversion polymorphisms to persist in nature. We begin by synthesising the emerging characteristics of inversion polymorphisms to summarise shared structural features and the predicted age, and to discuss selective processes involved in their maintenance. We then detail evidence for their involvement in phenotype–genotype associations, mating systems, and behaviour, as well as their role in environmental adaptation, reproductive isolation, and speciation. Lastly, we identify gaps in our knowledge, and suggest promising avenues for future research. In addition to summarising some of the basic characteristics of inversions (Box 1), we focus on recent empirical work rather than on theoretical evolutionary aspects pertaining to the origin, spread, and maintenance of inversions, since these have been thoroughly covered elsewhere [6,7,23–28].

Basic Characteristics of Inversions

Inversions Are Often Large and Contain Many Genes

While previous studies suggested that most inversions are small (e.g., reviewed in [6]), recent genomic-based studies revealed that large inversions are common. Table 1 represents a quasi-exhaustive coverage of recent genomic studies on inversions, and reveals that the average inversion size in both plants and animals is 8.4 megabases (mb), ranging from 130 kilobases (kb) to 100 mb. This supports the view that longer chromosomal inversions are favoured by selection relative to shorter inversions because they suppress recombination between a larger number of genetically distant loci [1,29]. The genome of several species also contains multiple large inversions, for instance eight inversions averaging 8.9 mb in the common fruitfly (*Drosophila melanogaster),* five inversions averaging 10.2 mb in the Atlantic cod (*Gadus morhua*), or four inversions averaging 26.8 mb in the zebra finch (*Taeniopygia guttata*) (Table 1). Consequently, such large inversions may represent a substantial proportion of the entire genome. For instance, the eight inversions in the *D. melanogaster* represent 71.25 mb or 43% of the genome. The five inversions in *G. morhua* represent 51 mb or over 6% of the genome. Single inversions, such as the 100-mb inversion in the white-throated sparrow (*Zonotrichia albicollis*), represent approximately 10% of the genome of this species, as does the large In(3L)P inversion in the *D. melanogaster*; in addition, the 63-mb inversion located on the Z chromosome constitutes 5% of the *T. guttata* genome. Thus, it is not surprising that the number of genes within any given inversion can be large, averaging 418 genes (Table 1 and references therein) and varying between 38 in the r7 inversion of the East African honeybee (*Apis mellifera*) and 1281 genes in the 2La inversion of the African malaria mosquito *Anopheles gambiae*. Admittedly, however, the recently reported inversion size estimates are affected by an ascertainment bias stemming from two sources. First, it is possible that only the most salient observations (e.g., strong associations between large inversion polymorphisms and striking phenotypic variation) were reported at the expense of neglecting smaller structural variants. It is also possible that genomic signatures of natural selection associated with adaptation are more easily detectable in large rather than small genomic targets, especially in species characterised by high recombination rates. Second, the extent of this bias is expected to vary with the

Table 1. Species Name, Common Name, Inversion Name and Size, Number of Genes Involved, Estimated Age (mya when in million years), Selective Processes Proposed to Maintain the Inversion in Nature and the Relevant References for each Species[a]

Species Name	Common Name	Name	Size (mb)	Number of Genes	Age in Years (Possible Range)	Selective Process	Refs
Invertebrates							
Timema cristinae	Stick insect	m, U, and S variants	13.0	na	13.5–8.0 mya (between m and U); 2.7–1.8 mya (between U and S)	Balancing selection (overdominance)	[35]
Drosophila melanogaster	Fruitfly	In(3L)P	17.396	1900	79 295 (59 790–126 990)	Balancing selection (spatially and temporally varying selection)	[60,81]
		In(2L)t	10.928	1754	69 398 (48 287–99 851)	Balancing selection (spatially varying selection)	
		In(2R)NS	4.885	962	178 886 (132 666–246 101)	Balancing selection (temporally varying selection)	
		In(3R)K	14.389	2223	68 247 (40 758–121 269)	Not determined	
		In(3R)M	7.624	1142	2861 (628–7817)	Balancing selection (spatially varying selection)	
		In(3R)P	8.311	1220	12 9481 (80 771–196 598)	Balancing selection (spatially varying selection)	
		In(1)A	5.953	764	25 870 (18 262–37 953)	Not determined	
		In(1)Be	1.764	225	33 (3–208)	Sex-ratio segregation distortion	
Drosophila pseudoobscura	Fruitfly	AR PP CH ST TL	5.928 12.068 4.877 3.155 6.695	786 1694 699 433 956	0.58 (0.51–0.65 mya) 0.99 (0.88–1.10 mya) 0.51 (0.46–0.57 mya) 1.38 (1.19–1.42 mya) 1.22 (1.09–1.34 mya)	Balancing selection (overdominance) but also directional and underdominant selection	[82,83]
Anopheles gambiae	Mosquito	2L 2Rj, -b, -c, -u	22.0 4.0–12.5	1281 2Rb(548)	3000–1100 383–10 600	Balancing selection (spatially and temporally varying selection)	[84,85]
Papilio polytes	Swallowtail butterfly	H-type, h-type	0.130	3	20–10 mya	Balancing selection (frequency-dependant selection?)	[30]
Heliconius numata	Numata longwing butterfly	Locus P	0.400	20	2.64 mya (2.15–2.94 mya)	Balancing selection (antagonistic frequency-dependent selection)	[44,86]
Apis mellifera	East African honeybee	r7h, r7l r9h, r9l	0.573 1.639	38 50	3.2 (2.8 ± 3.3) mya 1.28 (1.22 ± 1.30) mya	Balancing selection (spatially varying selection)	[32]

Table 1. (continued)

Species Name	Common Name	Name	Size (mb)	Number of Genes	Age in Years (Possible Range)	Selective Process	Refs
Solenopsis invicta	Fire ant	SB, Sb; both contain Gp9 on Chr. LG16	9.3	616	0.390 mya (0.35–0.42 mya)	Balancing selection (antagonistic selection between social phenotypes)	[55,56]
Ostrinia nubilalis	European corn borer moth	Inversion on sex chromosome	10.0	300	na	Divergent ecological and sexual divergence	[87]
Teleosts							
Gadus morhua	Atlantic cod	Inversion on LG1 Inversion on LG2 Inversion on LG7 Inversion on LG12 Inversion on LG23	17.4 ~6 ~10 ~13 ~3.5	763 293 324 419 97	0.6–2.0 mya >100 000 >100 000 >100 000 >100 000	Divergent selection Divergent selection Divergent selection Divergent selection Neutral divergence (?)	[17,50–52,88]
Oncorhynchus mykiss	Rainbow trout	MAR on LG Omy5	~55	1266	>1.0	Balancing selection (spatially and temporally varying selection)	[16,49,89]
Gasterosteus aculeatus	Threespine stickleback	LG1 I LG XI LG XXI	0.412 0.442 1.700	21 22 75	~3.5 mya na na	Divergent selection	[18]
Aves							
Calidris pugnax	Ruff	Independent Faeder Satellite (subsequent structural change)	4.5	125	3.8 mya 0.5 mya	Balancing selection (frequency-dependent selection)	[11,31]
		Chr. 11		125		Balancing selection (frequency-dependent selection)	[11]
Zonotrichia albicollis	White-throated sparrow	2m/2 on Chr. 2	100	1137	~1 mya	Disassortative mating	[12]
Taeniopygia guttata	Zebra finch	Chr. Tgu 5	15.54	325	na	Not determined but weak genotype–phenotype associations	[37]
		Chr. Tgu 11	12.20	250			
		Chr. Tgu 1	16.76	312			
		Chr. Tgu Z: A,B, and C karyotypes	62.92	619		Balancing selection (overdominance)	[38]
Phylloscopus trochilus	Willow warbler	Chr. 1,5 Chr. 3	11.6, 4.0 13.1	146, 53 135	0.75–1.6 mya 0.75–1.6 mya	Divergent selection Balancing selection (spatially varying selection)	[54]
Mammals							
Homo sapiens	Human	H1–H2 Chr. 17q21.31	0.900	56	3 mya	Positive selection (short term); balancing selection (long term)	[34,90]
Plants							
Mimulus guttatus	Monkeyflower	DIV1 on Chr. 8	6.0	362	na	Divergent selection	[14,15,91]
Zea mays	Maize	Inv1n-I, Inv1n-S	50	700	296 000 (221 000–398 000)	Divergent selection	[67]
Boechera stricta	Drummond's rockcress	Bsi 1	8.40	408	~2100–8800	Positive directional selection	[75]

Table 1. (continued)

Species Name	Common Name	Name	Size (mb)	Number of Genes	Age in Years (Possible Range)	Selective Process	Refs
Arabidopsis thaliana	Thale cress	Inversion on Chr. 4	1.17	186	5000	Divergent selection	[68]
Helianthus annuus/ *Helianthus argophyllus*	Common sunflower/ silverleaf sunflower	LG1 inversion LG2 inversion LG4 inversion LG5 inversion LG8 inversion LG10 inversion LG11 inversion LG12 translocation LG15 translocation LG16 translocation	38 85 139 235 1.4 139 169 7 103 221	198 574 734 1385 99 269 75 511 624 1387	~1.5 mya	Divergent selection	[77]

[a]Abbreviations: mb, megabase; mya, million years ago; na, areas that are unknown.

molecular techniques used. For instance, methods that generate a reduced genome representation (e.g., RAD genotyping) are more likely to detect large inversions. Therefore, it remains unclear whether large inversions represent the rule rather than the exception. Future sequencing work using long-read sequencing, which affords higher resolution, will help to develop a more nuanced view.

Inversions Are Generally Old and May Pre-Date Species Origin

Another prominent feature of inversions is that they have been segregated within species for hundreds of thousands or even millions of generations (Table 1). For instance, the 130-kb autosomal inversion controlling Batesian mimicry in *Papilio* butterflies likely originated 10–20 million years ago (mya) [30]. The 4.5-mb inversion controlling the expression of the satellite, independent, and faeder male reproductive phenotypes in the ruff (*Calidris pugnax*) is over 3 million years old [31], as is the r7 inversion in *A. mellifera* [32]. Some of these large inversions may even pre-date the origin of the species that carry them. A well-known example is the 900-kb inversion region on chromosome 17q21.31 in humans (*Homo sapiens*), which represents two distinct lineages, H1 and H2, that diverged 3 mya [33]. This pre-dates the emergence of anatomically modern *H. sapiens* and even the origin of the genus *Homo* [34]. Similarly, the >100-mb inversion-based supergene of *Z. albicollis* originated before the split from its sister species 1 mya, and may be polymorphic due to a hybridisation event with a now extinct species [12].

A Major Role for Balancing Selection in Maintaining Inversion Polymorphism

Long retention of within-species inversion polymorphisms will be facilitated if some form of balancing selection is involved. Indeed, for 21 of the 40 inversions in Table 1, one of the several forms of balancing selection (e.g., frequency-dependent selection, antagonistic pleiotropy, disassortative mating, overdominance, or spatially and temporally variable selection) was proposed as a likely evolutionary process for inversion maintenance. For instance, in the walking stick insect *Timema cristinae*, cryptic colour phenotypes appear to be linked to inversions that diverged millions of generations ago [35]. Inversion frequencies show an excess of heterokaryotypes, and the authors proposed that this may have been caused by overdominance, a form of balancing selection. This example supports the emerging view that balancing selection has an important role in the maintenance of genetic variation, even over extended time frames [36]. In *T. guttata*, Knief *et al.* [37] showed that a sex chromosome

rearrangement explains nearly all of the genetic and 40% of the sperm morphological variation. In this example, heterozygous males have the fastest and most successful sperm, allowing inversion maintenance via strong overdominance [38]. In many of the studies reported in Table 1, spatially varying selection, whereby a balanced polymorphism is maintained by local environmental selection acting in different directions within an otherwise panmictic or quasi-panmictic species, was also invoked as the main force explaining observed patterns of variation (see the section 'Environmental Adaptation'). Interestingly, genetic variation preserved by spatially balancing selection has been proposed to be more useful in allowing the population to respond to new environmental challenges compared with any other mechanisms that can maintain genetic variation [39]. As such, the long-term segregation of inversion polymorphisms due to balancing selection challenges the traditional view that the overall role of balancing selection in maintaining variation is relatively minor [40]. Nevertheless, not all studies have

Box 1. Paracentric and Pericentric Inversions

Genetic consequences of meiosis inversions can be broadly categorised into either paracentric or pericentric inversions [92] (Figure I). Paracentric ('away from the centre') inversions occur when breakpoints fall on one side of the centromere. After meiotic chromatid duplication, the paired inverted chromosome forms a loop for the loci to pair with their homologous counterparts on the uninverted chromosome. Crossover events inside the loop produce four types of product: a dicentric bridge; an acentric fragment (a fragment without a centromere); and two chromosomes with the standard and inverted gene orders. The acentric fragment lacks a centromere and, thus, is lost because it cannot be drawn to either cell pole. The two centromeres of the dicentric bridge are drawn to opposite poles and, as a consequence, the bridge breaks at a random position, leaving behind two deletion products (where one or more loci have been deleted, according to where breakage of the bridge occurred). Inheritance of one of these latter products will result in segmental aneuploidy. Hence, a crossover event, which normally generates the recombinant class of meiotic products, produces lethal products instead. The origin of paracentric inversions may be related to the homologous recombination of repetitive elements [27] because transposable elements have been found at high densities near breakpoints of paracentric inversions in both *Drosophila* [93] and *Anopheles* [94].

Pericentric ('around the centre') inversions appear less common than paracentric inversions [95], yet their genetic effect is the same as that of a paracentric one: crossover products are not recovered, but the reasons differ (Figure I). In a pericentric inversion, a crossover event inside the loop produces four types of product in the gametes: two chromosomes with the standard and inverted gene order; and two duplication and/or deletion products, in which one or more loci have been duplicated or deleted, depending on where the crossover took place, which leads to the generation of inviable gametes. Again, the result is the selective recovery of non-crossover chromosomes in viable progeny, leading to selection against the inversion (underdominance). Unlike a paracentric inversion, all loci are represented in the final products, and only the order of loci is changed.

The fact that chromosomal inversions alter recombination in heterozygotes represents an efficient way to facilitate the capture of favourable combinations of locally adapted alleles by preserving linkage between them. As such, an inversion can effectively function as a supergene, which was recently redefined by Thompson and Jiggins [26] as 'a genetic architecture involving multiple linked functional genetic elements that allows switching between discrete, complex phenotypes maintained in a stable polymorphism within a population'. However, this definition does not explicitly require suppression of recombination, which is implicit for inversions, because a supergene could also arise from initially very tightly linked elements, as found in the self-incompatibility (SI) and heterostyly systems in some flowering plants. Therefore, inversions form a subset of supergenes. At the same time, inversions also depart from Thompson and Jiggins' strict criterion of stable polymorphism within population. Namely, studies reviewed here revealed many cases of pronounced differences in inversion frequency among populations driven by local selection, and even alternate fixation.

Given that suppressed recombination allows mutational differences to accumulate between their variants, chromosomal inversions may also create 'genomic islands of divergence', which were first defined by Wu [96] as any gene region that exhibits significantly greater differentiation than expected under neutrality. In population genomics or speciation studies, such genomic regions are most often interpreted as reflecting the effect of divergent selection either acting on specific loci and those physically linked to them or promoting reproductive isolation that causes barriers to gene flow [97]. While inversions may indeed be under the effect of divergent selection, it may often not be the case and, consequently, care should be taken to verify whether 'genomic islands of divergence' identified in such studies are associated with inversions to avoid making erroneous interpretations regarding the role of divergent selection in shaping heterogeneous genomic landscapes.

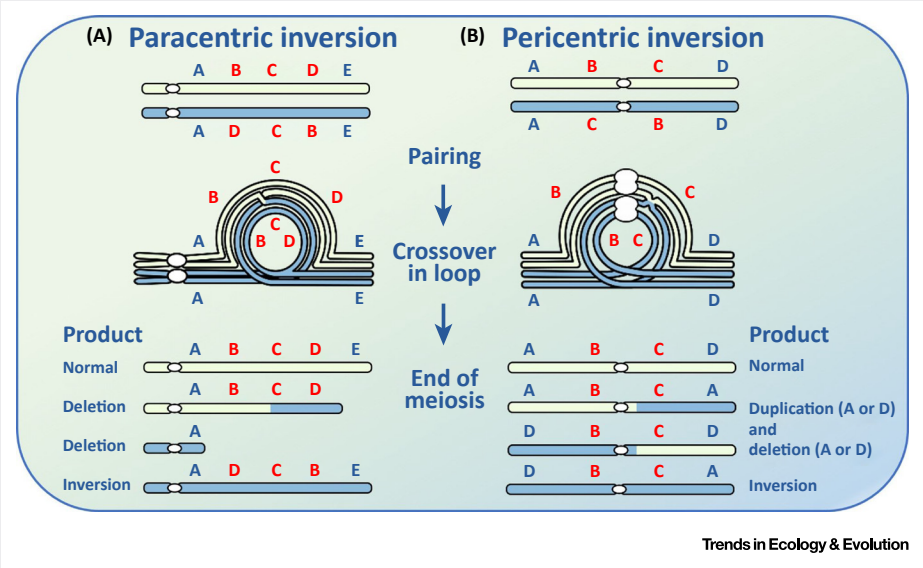


Figure I. Inversions can be either (A) Paracentric or (B) Pericentric. Pairing in paracentric inversion heterozygote produces four products: a dicentric bridge; an acentric fragment; and two chromosomes with the standard and inverted gene orders. The acentric fragment lacks a centromere and is lost because it cannot be drawn to either cell pole. The two centromeres of the dicentric bridge are drawn to opposite poles and, as a consequence, the bridge breaks, leaving behind two deletion products. Hence, a crossover event in paracentric heterozygotes produces lethal products. Pairing in pericentric inversions also produces four products: two chromosomes with the standard and inverted gene order; and two duplication and/or deletion products, in which one or more loci have been duplicated or deleted, leading to the generation of inviable gametes. Again, the result is the selective recovery of non-cross-over chromosomes in viable progeny. Reproduced from Bérénice Bougas.

inferred a role for balancing selection; divergent selection has also frequently been identified, for example in migratory phenotypes of *G. morhua*. In most of these cases, the inversion polymorphism within a given species varies either latitudinally or locally, suggesting that a balance between divergent selection and migration has contributed to maintain inversion polymorphism in the long-term [37].

Eco-Evolutionary Processes Involved in the Generation and Maintenance of Inversions

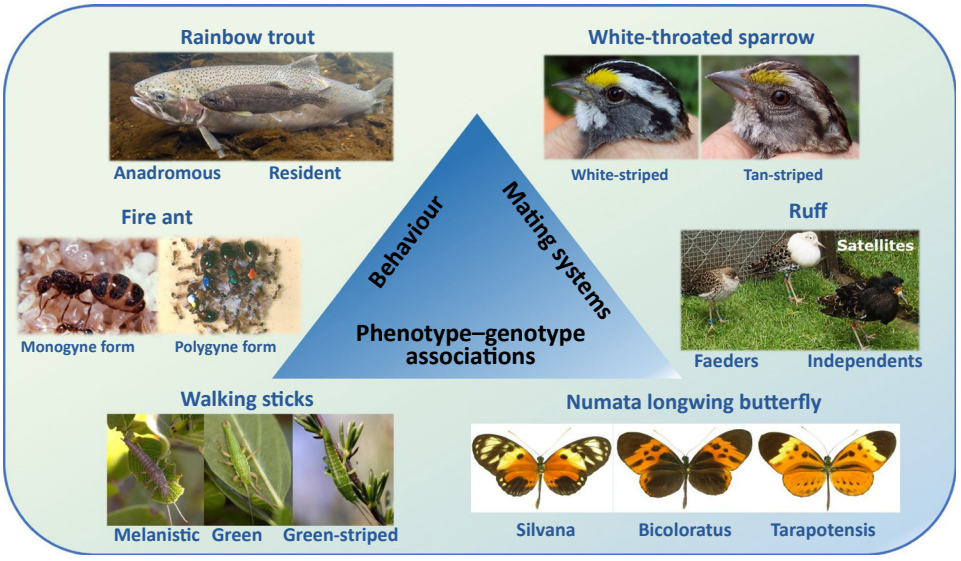
In this section, we list empirical evidence showing that inversions are directly linked to phenotype–genotype associations, mating systems and behaviour, environmental adaptation, and, ultimately, reproductive isolation and speciation.

Phenotype–Genotype Associations, Mating Systems, and Behaviour

Current work highlights several spectacular cases where inversions are directly linked to the coexistence of complex phenotypes. One of the most striking examples comes from mimetic butterfly species, where inversions have been shown to control colour. For example, female-limited mimicry in the Asian swallowtail butterfly (*Papilio polytes*) is caused by an inverted region that includes the gene *doublesex* (*dsx*), a key autosomal gene in insect sexual dimorphism [41,42]. Inversion of *dsx* has facilitated diverse sequence differences that modulate wing patterns, colours, and structures [43]. Experimental knockdown of the mimetic *dsx* allele produced a switch to a nonmimetic pattern, whereas knockdown of the nonmimetic allele in heterozygous individuals, which express the mimetic pattern because of dominance,

showed no phenotypic effect [30]. These results suggest that changes in *dsx* expression alone are insufficient to change colour, and that some regulatory component prevents the mimetic *dsx* allele from affecting the male phenotype. In another mimetic butterfly, the brush-footed butterfly (*Heliconius numata*), genomic rearrangements at the supergene locus *P* tighten the genetic linkage between at least two colour-pattern loci known to recombine in closely related species [44], with corresponding haplotype clades and inversion breakpoints being in complete association with wing-pattern morphs (Figure 1).

Distinct mating behaviours can also be the outcome of inverted chromosome parts. In *C. pugnax*, a chromosomal rearrangement encodes three alternative mating strategies; aggressive 'independents'; white submissive 'satellites'; and female-mimic 'faeders'. These alternative types not only differ in mating behaviour, but also in size, plumage, aggression, testis size, and steroid metabolism. Independents are homozygous for the ancestral sequence, whereas the development into satellites and faeders is determined by divergent alternative and dominant inversion haplotypes, of which one breakpoint disrupts the essential *CENP-N* gene, making inversion homokaryotypes lethal [11]. This resembles the situation in *Z. albicollis*, in which an approximately 100-mb pericentric inversion is associated with white-striped and tan-striped plumage phenotypes that exhibit divergent territorial and parental behaviour [45,46]: white-striped phenotypes almost exclusively mate with tan-striped birds, a pattern that results in four effective 'sexes' [47]. The inversion carries 1137 genes, which are correlated with territorial song, aggression, and plumage colour [48].



Trends in Ecology & Evolution

Figure 1. Inversions Dramatically Effect Phenotype–Genotype Associations, Mating Behaviour, and Behavioural Forms within Species. Representative examples for divergent behavioural forms include the steelhead and anadromous ecotypes of the rainbow trout *Oncorhynchus mykiss* (photo credit: Morgan Bond) and monogyne and polygyne social forms (polygyne queens are marked with coloured paint) of the fire ant *Solenopsis invicta* (photo credit: Kenneth Ross). Inversion effects on mating systems have been well described in the two colour types of the white-throated sparrow *Zonotrichia albicollis* (photo credit: Elaina Tuttle) and the three alternative male mating types of the ruff *Calidris pugnax* (photo credit: Susan Mc Rae). Phenotype–genotype associations can also be strongly driven by inversion polymorphisms, as documented by the mimetic wing forms of the numata longwing butterfly *Heliconius numata* (photo credit: Mathieu Joron) and green and melanic forms of walking stick insects *Timema cristinae* (photo credit: Aaron Comeault).

Inversions are also linked to migratory phenotypes. Life-history strategies of anadromous (steelhead) and resident rainbow trout (*Oncorhynchus mykiss*), such as spawning time, smoltification, development rate, and early maturation, have been linked to a large inverted region located on chromosome *Omy5* [49]. In *G. morhua*, two widely distributed migratory and stationary ecotypes are linked to inversions [50–52]. Berg *et al.* [51] described three inversions associated with migratory behaviour in this species, of which the one on LG1 was investigated by Kirubakaran *et al.* [50] in greater detail, revealing it to comprise two adjacent inversions. Comparative genomic analyses determined that the Northeast Arctic *G. morhua* ecotype is the derived state and dated the LG1 inversion to approximately 1.6–2.0 mya [50]. The inversion haplotype block harbours 763 genes, including candidate genes for regulating swim bladder pressure, haem synthesis, and skeletal muscle organisation [52]. Interestingly, this LG1 inversion was recently associated with parallel patterns of divergence between migratory and nonmigratory ecotypes on both sides of the Atlantic Ocean, providing further support for its role in local adaptation [53]. Likewise, in the willow warbler (*Phylloscopus trochilus*), two migratory ecotypes appear to be linked to three genomic regions located on chromosomes 1, 3, and 5 [54]. Genomic patterns of these three haplotype blocks and the rest of the genome indicate that recombination within haplotypes is rare or absent, consistent with the presence of inversions in those three genomic regions [54].

Finally, inversions have also been linked to social organisation, as documented by the two variants of the nonrecombining 'social chromosome' in the Alpine silver ant (*Formica selysi*). The two social forms differ in terms of whether the workers tolerate a single fertile queen (monogyne social form) or several fertile queens (polygyne social form) in their colony. The social chromosome in this species shares a similar genetic architecture with the fire ant (*Solenopsis invicta*), yet no homology in gene content [55]. Such convergence at the phenotype level and the genetic architecture associated with alternative social forms indicates general genetic mechanisms underlying social transitions [55]. Wang *et al.* [56] further showed that the two divergent social forms are part of heteromorphic chromosomes that share many key properties of sex chromosomes, and are characterised by a large 9.3-mb inverted, nonrecombining region, with homokaryotypes being nonviable. Importantly, most genes with demonstrated expression differences reside within the nonrecombining region. More recent genomic analyses show that the inverted and non-inverted region differ in gene content, with a high proportion of nonsynonymous gene substitutions between haplotypes [57]. These findings highlight how genomic rearrangements can maintain adaptive social phenotypes, involving many genes acting together like a supergene, by locally limiting recombination [58].

Environmental Adaptation

Strong evidence that inversions are involved in environmental adaptation comes from geographical clines. The classic example is the In(3R)Payne (3RP) inversion in *D. melanogaster*, with parallel environmental clines on three continents [1] that change in parallel in response to climate [59]. In North American *D. melanogaster*, the 3RP cline has remained stable for >40 years and frequencies are strongly correlated with climatic factors, independent of population structure [60]. Work on the same species by Rane *et al.* [61], following its invasion in Australia, found that the same region showed strong differentiation between tropical and temperate areas, and identified clinal inversion-associated single nucleotide polymorphisms (SNPs) located in genes associated with fitness-related traits that exhibit parallel differentiation along the North American cline. In their work on clines of the related fruitfly *Drosophila pseudoobscura*, Fuller *et al.* [62] found that inverted regions harbour multiple differentially expressed genes. This has also been documented in yeast [63], thus supporting a role of differential gene expression associated with local adaptation. Fuller *et al.* [64] studied six chromosomal

rearrangements on the third chromosome of 54 strains of *D. pseudoobscura* and found that they are likely maintained through suppressed recombination, allowing covariation of many small effect genes. Finally, studies on *Drosophila mojavensis* found several gene alterations at the breakpoints with putative adaptive consequences that directly imply natural selection as the cause of rapid chromosomal evolution [65].

Looking outside *Drosophila*, comparative analyses of within-inversion variation associated with strong environmental gradients among different anopheline mosquitoes adds further support for a role of inversions in local adaptation. Ayala *et al.* [66] investigated 23 chromosome inversions in the adaptation of the four major malaria mosquito species (*A. gambiae*, *Anopheles coluzzii*, *Anopheles arabiensis*, and *Anopheles funestus*) to local environments in Africa. Spatially explicit modelling to investigate distribution patterns of inversions showed that most inversions are environmentally structured, suggestive of a causal role in adaptation. Moreover, some inversions exhibited parallel ecological associations, providing strong evidence that local adaptation evolved similarly and independently. In *A. mellifera*, populations inhabiting mountain forests of East Africa differ in behaviour and morphology from those in lowland savannahs, despite forming a single panmictic population. Here, phenotype–habitat association correspond to the presence of nonrecombining haplotypes on chromosomes 7 and 9, with the chromosome 7 haplotype harbouring nearly all octopamine receptor genes, which are candidates for adaptation to highlands due to their role in learning and foraging [32]. Likewise, in *H. sapiens*, an inversion on chromosome 17q21.31 that comprises the H1 and H2 lineages shows no evidence of recombination. The H2 lineage is rare in Africans and East Asians but found at a frequency of 20% in Europeans, and is consistent with a history of positive selection. Another study showed that the H2 lineage is at a selective advantage in the Icelandic population, where carrier females have more children and higher recombination rates compared with noncarriers [34]. In maize (*Zea mays*), an inversion on chromosome 1 shows a strong altitudinal signature, a statistical association with environmental variables and phenotypic traits, and a skewed haplotype frequency spectrum for inverted alleles [67]. Likewise, in *Arabidopsis thaliana*, the 1.17-mb inversion on chromosome 4 shows a robust association with fecundity under drought [68]. Moreover, a review of rearrangement polymorphisms in eukaryotes demonstrated that inversions are correlated with phenotypic differences, consistent with varying fitness in different habitats. This review also suggested that some rearrangement polymorphisms are under positive selection, perhaps because they either trap favourable allelic combinations or alter the expression of nearby genes [69].

Compared with the number of studies documenting patterns of clinal variation, explicit tests for local adaptation are rare. Lowry and Willis [14] showed that alternative chromosomal inversions in the yellow monkeyflower (*Mimulus guttatus*) are associated with flowering time and morphological traits in annual and perennial ecotypes of this species (Figure 2). The authors conducted a reciprocal transplant experiment involving outbred lines, where alternative arrangements of the inversion were reciprocally introgressed into the genetic backgrounds of each ecotype. With this, they could demonstrate that the inversion contributes directly to adaptation, an annual–perennial life-history shift, and multiple reproductive isolating barriers. The seaweed fly *Coelopa frigida* inhabits wrackbeds along the coastlines of northern Europe and North America and forms a cline in Scandinavia. Reciprocal transplant experiments demonstrated that populations at clinal extremes had higher survival in their own habitat, consistent with the view that inversions can have direct and strong effects on fitness [70]. The implication of this inversion in local adaptation is further supported by the parallel patterns of inversion–environment associations between European and North American populations [71].

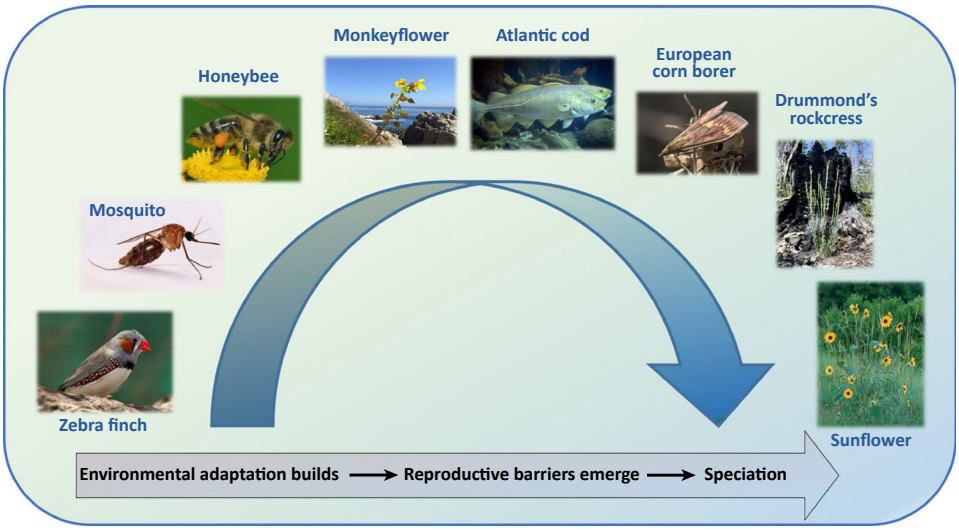


Figure 2. Continuum of Variation in Intraspecific Variation of Inversions in Plants and Animals. Recent genomic-enabled studies illustrate the whole continuum of variation in inversions from stabilised within-population polymorphism at similar frequency with small effects sizes and no heterozygote advantage in zebra finch *Taeniopygia guttata* (photo credit: Peripitus), to frequency shifts among populations associated with clinal latitudinal variation in the mosquito Anopheles gambiae (photo credit: Muhammad Mahdi Kari) and altitudinal variation in East African honeybee *Apis mellifera* (photo credit: Andreas Trepte), quasi-alternate fixation between migratory and stationary ecotypes of the Atlantic cod *Gadus morhua* (photo credit: Cecilia Helmerson), and locally adapted annual–perennial life-history shifts in monkey-flower *Mimulus guttatus* (photo credit: David Lowry), to inversions that capture quantitative trait loci (QTLs) acting as mechanisms of prezygotic reproductive isolation, for instance by controlling mate preference in the European corn borer moth *Ostrinia nubilalis* (pheromone mating preferences; photo credit: Sarah Gregg) or flowering differences expected to increase reproductive isolation between nascent species in the Drummond's rockcress *Boechera stricta* (photo credit: Tom Mitchell-Olds), and, finally, alternate fixation between the sunflower species *Helianthus annuus* and *Helianthus argophyllus* (photo credit: Jason Rick). Deciphering the molecular and evolutionary mechanisms underlying such striking variation in patterns of inversions should be a research priority.

Reproductive Isolation and Speciation

Inversions have long been known to be implicated in the reproductive isolation of species either via the creation of linkage groups that cause sterility or by facilitating the maintenance of co-adapted gene complexes [72,73]. In *A. funestus*, an inversion is implicated in strong assortative mating between ecotypes and accounts for up to 92% of reproductive isolation, indicating that this inversion can generate most of the genetic barriers needed for speciation [13]. Mating preferences of European corn borer moths (*Ostrinia nubilalis*) are driven by pheromone olfactory receptor genes located within a nonrecombining unit on the Z chromosome, the frequency of which is modulated by a combination of sexual and ecological divergence [74]. Chromosome painting, mapping, and sequencing work in the hybrid zone of Drummond's rockcress (*Boechera stricta*) showed that locally adapted quantitative trait loci (QTLs) reside in a young inversion, including flowering differences that are expected to increase reproductive isolation between subspecies [75]. With this, Lee *et al.* [75] provided the first direct evidence of linked, locally adapted QTLs being captured by young inversions during incipient speciation [76]. However, this interpretation was recently challenged. Charlesworth and Barton argued that, given the high selfing rate (and, therefore, high inbreeding coefficient), a scenario in which an inversion had spread to an intermediate frequency by drift and then picked up by an advantageous mutation and subsequently driven to a high frequency by hitchhiking, was equally likely.

A study in sympatric *Helianthus* sunflowers (*Helianthus annuus* and *Helianthus argophyllus*) revealed significantly elevated genetic structure in rearranged portions of the genome, indicating that such rearrangements are associated with restricted gene flow [77]. In *Drosophila* spp., seminal work by Noor and coworkers showed that most sympatric *Drosophila* species pairs differ by one or more inversions, whereas allopatric pairs are almost all homosequential [21]. Similarly, many sympatric sister species of rodents have more autosomal karyotypic differences compared with allopatric sister species [78]. Likewise, in passerine birds, sympatric sister species are significantly more likely to differ by an inversion than are allopatric sister species, with the number of inversion differences best explained by the level of geographical overlap [79].

Other work focussed on species that inhabit heterogeneous environments has documented links between environmental adaptation and the build-up of inversions related to reproductive isolation. One example comes from the comparative linkage mapping in the sister monkey-flowers *Mimulus lewisii* and *Mimulus cardinalis*, a textbook case of ecological speciation. Mapping detected two inversions specific to *M. cardinalis* [80] and identified that both floral QTLs as well as QTLs related to environmental adaptation clustered in putatively rearranged regions, and that all QTLs for male sterility, including two underdominant loci, mapped to regions with recombination suppression [80]. This provides strong evidence for a role of inversions in generating and consolidating ecological barriers to gene flow between these two taxa.

Concluding Remarks

Mounting evidence shows that inversions in a range of taxonomic groups are associated with complex phenotypes, environmental adaptation, and, ultimately, speciation. Many, if not most, inversions are large, often representing a substantial proportion of the genome, and have existed for extended periods of time. Some even pre-date species barriers and have moved across species barriers via introgressive hybridisation. The maintenance of these inversions across generations is governed by several processes, particularly one of the several forms of balancing selection. However, knowledge gaps for many inversion systems remain, including knowledge of the inversion breakpoints and the identification of causal genes under selection and how these genes covary and affect the expression of phenotypes (see Outstanding Questions). Filling these gaps is essential to test whether inversions are favoured because of their gene content (including gene interactions) or because they generate mutations or gene disruptions at breakpoints. Part of this knowledge gap is because the development of efficient assays to accurately detect breakpoints is challenging, and the accuracy of detection often relies on well-assembled genomes, something that is still only available for a few species. The ubiquitous importance of inversions in ecological and evolutionary processes highlighted here demonstrates that the analysis of inversions (as well as other structural variants) must be better integrated in studies pertaining to the molecular basis of adaptation and speciation, something that is becoming increasingly amenable owing to the fast development of improved genomic tools, such as long-read sequencing and gene-editing techniques.

Outstanding Questions

Even though genomics-enabled studies have made significant progress in understanding the origins, causes, and consequences of inversions, many areas are still little understood. Here, we propose five areas that require increased attention in future studies.

What are the causal genes and/or mutations that facilitate inversion maintenance? This is crucial for understanding whether inversions are favoured because of their content or because they generate mutations or gene disruptions at breakpoints, as seen in some *Drosophila* and *C. pugnax* [11,59]. Detection of causal genes is difficult because alleles within the inverted region are in linkage disequilibrium and show an equally strong association with phenotype. Several reviewed studies suggested candidate genes, but rigorous experimental tests to determine causal genes are lacking. One way to experimentally investigating the effect of putative causal genes would be to perform gene knockdown to document their phenotypic effect (or lack thereof). Alternatively, gene-editing methods, such as CRISPR/Cas9 genome editing, are becoming increasingly amenable to study non-model species [98] and could be apply, for instance, to test the effects of total gene knockout or alternative allelic variants associated with the different inversions on phenotypic expression.

What are the mechanisms by which causal genes control expression of alternative phenotypes? Here, it will be of particular interest to investigate whether epigenetic modifications underlying pure phenotypic plasticity or polymorphic regulatory elements are affecting the expression of one or several causal genes or, alternatively, if structural variation within the genes is responsible for expression differences.

Does the expression of causal genes within an inversion covary? Evidence for that would support the traditional hypothesis that co-adapted alleles at loci within the inversion have epistatic fitness effects [4,5]. Alternatively, lack of evidence would support the hypothesis that inversions are favoured because they bring locally adapted alleles together without inferring

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epistatic interactions [7]. Studies could also test Nijhout's hypothesis [99], whereby switching of multiple inversion phenotypes could be explained by a single transcription factor or by the alternative splicing of a gene with pleiotropic effects on downstream targets.

What are the ancestral inversions elements that were responsible for the initial spread? One way forward is to determine the ancestral population, and then to compare the contemporary with the ancestral inversion sequence to fine-map alleles associated with alternative arrangements. Another potential avenue is to use comparative phylogenomics to investigate systems where the same inversion occurs among related species or not [55]. Future studies on genome-wide patterns of nucleotide diversity and haplotype structures can also help to clarify the role of hybridisation in the spread of inversions.

What are the mechanisms that can explain why some inversions are maintained at stable and others at varying frequencies? Studies illustrate the whole continuum of variation, from the alternate fixation between closely yet reproductively isolated species (e.g., *Drosophila*), pronounced frequency differences among adapted populations (e.g., *Gadus morhua* and *Mimulus*), less pronounced frequency shifts among populations associated with clinal variation, to the maintenance of stabilised within-population polymorphism, either at similar (e.g., *Taeniopygia guttata*) or strikingly different frequencies (e.g., *Calidris pugnax*). Future studies should identify which mechanisms, including frequency-dependent (disruptive) selection, antagonistic pleiotropy, recessivity of deleterious mutations, overdominance, associative overdominance, underdominance, or segregation distortion [6,7,25,26,92,100], can explain these inversion frequency differences.

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