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Chromosomal inversions and their impact on insect evolution

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Abstract

Insects can adapt quickly and effectively to rapid environmental change and maintain long-term adaptations, but the genetic mechanisms underlying this response are not fully understood. In this review, we summarize studies on the potential impact of chromosomal inversion polymorphisms on insect evolution at different spatial and temporal scales, ranging from long-term evolutionary stability to rapid emergence in response to emerging biotic and abiotic factors. The study of inversions has recently been advanced by comparative, population, and 3D genomics methods. The impact of inversions on insect genome evolution can be profound, including increased gene order rearrangements on sex chromosomes, accumulation of transposable elements, and facilitation of genome divergence. Understanding these processes provides critical insights into the evolutionary mechanisms shaping insect diversity.

Keywords

Chromosomal inversions; comparative genomics; cytogenetics; genome evolution; Hi-C method; insect adaptation; population genomics

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Declarations of interest

None.

Introduction

Chromosomal inversions are structural genomic rearrangements in which a portion of a chromosome is flipped 180 degrees, creating a reverse order of genetic material. As a result, the inverted region of the genome is protected from meiotic recombination with the original region, and large clusters of genes located within the inversion are inherited almost intact as a single supergene. Nevertheless, genetic flux between arrangements still can occur in the form of gene conversion [1,2]. Thus, inversions have provided a model system to identify gene conversion events because of the differentiation between arrangements [3]. Different sets of alleles captured by the alternative inversion arrangements can have dramatic effects on the ecological, behavioral, and physiological adaptations of their carriers. Polymorphic inversions can form co-adapted gene complexes within species, and fixed inversions can cause hybrid breakdown and reproductive isolation between species [4–6].

In addition to modifying recombination rates, inversions can alter gene structure and regulation. For example, chromosome breakage has led to the disruption of breakpoint region genes in the fire ant *Solenopsis invicta* [7], to the appearance of new chimeric genes in fruit fly *Drosophila mojavensis* [8], and to the origin of a new lncRNA gene U3X at the breakpoint of the doublesex mimicry supergene in *Papilio* butterflies [9]. Chromosome breakage has also led to position effects in *Drosophila* by altering gene expression patterns of the neighboring genes [10,11], or by altering three-dimensional nuclear organization through occasional disruption of compartments and topologically associated domains in *Drosophila* and *Anopheles* [12,13]. In the seaweed fly *Coelopa frigida*, a large chromosomal inversion has been associated with large differences in gene expression between inverted and standard genotypes, likely due to both *cis*-regulatory linked variation present within the inversion locus, and *trans*-regulatory effects of linked variation within the locus on genes throughout the genome [14].

Growing evidence for the prominent role of inversions in insect biology suggests that without chromosomal polymorphism, insect species would be less diverse, their populations would be smaller, and invasive pests would be less common. The most common mechanism by which inversions operate appears to be divergence of allelic sets between alternative arrangements due to suppression of recombination between arrangements. However, the available direct data on the precise mechanisms of the functional effects of inversions are still limited, and it is too early to make conclusive generalizations.

Discovery and detection of inversions

Different features are used to discover new inversions and detect known inversions by different methods. Here we briefly describe three main approaches to inversion discovery and detection: genetic, cytogenetic, and genomic.

Genetic approach

Chromosomal inversions were discovered in *Drosophila melanogaster* in genetic mapping experiments as strong reducers of crossing over [15,16]. Although genetic mapping is still being used to find new inversions in insects, this method is not precise in determining

the exact genomic locations of the inversions. For example, the development of a linkage map based on hybrids between two strains of the arboviral mosquito vector *Aedes aegypti* suggested the presence of four, one, and three inversions in chromosomes 1, 2, and 3, respectively [17]. A subsequent genomic study of *Ae. aegypti* confirmed the total number of inversions in these mosquito strains but provided different genomic locations for the inversions [18]. Thus, genetic mapping can inform researchers about the presence or absence of chromosomal inversions and can provide their approximate locations within chromosomes. The accuracy of this approach depends on genetic recombination rates and the availability of markers for genetic mapping.

Cytogenetic approach

A number of chromosomal inversions have been discovered and studied using cytogenetic mapping of polytene chromosomes found in dipteran species such as *Drosophila* [19–21], *Chironomus* [22], and *Anopheles* [23–25]. This method is based on the difference in orientation of the banding pattern between the standard and inverted chromosome arrangements identified by microscopy. It can be used to detect both polymorphic inversions within populations and fixed inversions between closely related species. The sensitivity of this method is limited by the size of the inversion (typically several megabases (Mb)) and the clarity of the banding pattern. The use of fluorescence *in situ* hybridization (FISH) increases the resolution of the cytogenetic approach and extends inversion detection to distantly related species and insect species without polytene chromosomes. Inversion is detected by the standard and reverse orders of two or more fluorescently labeled DNA probes hybridizing simultaneously to a chromosomal preparation. Physical mapping of the polytene X chromosome using FISH led to the discovery of two large nested inversions fixed between the sibling malaria mosquito species *An. atroparvus* and *An. messeae* [26]. FISH of cloned cDNA probes with mitotic chromosomes validated the presence of polymorphic inversions in natural populations of *Ae. aegypti* identified by the genomic approach [18].

Genomic approach

The increasing availability of chromosome-scale reference genome assemblies for insect species provides an excellent opportunity to apply genomics to the discovery and detection of chromosomal inversions. The genomic approach can be divided into different methods based on the characteristics of the inversions used. The comparative genomics method is based on direct comparison of sequenced and assembled genomes. This method allows researchers to detect all types of chromosomal rearrangements, including fusion, fission, translocations, inversions, duplications, deletions, and transpositions. Inversions of any size can be detected based on the difference in orientation of genomic segments between compared genomes. The first interspecies whole-genome sequence comparison was performed for *D. melanogaster* and *An. gambiae* [27]. More recently, rearrangements have been identified and their rates have been calculated among the genomes of five *Anopheles* species [13] as well as among the mosquito genera *Aedes*, *Culex*, and *Anopheles* [28] and among *Drosophila* species [29]. Comparative genomics provides the most accurate identification of breakpoint regions for both fixed and polymorphic inversions. Once the genomic location of the breakpoint regions has been identified, PCR-based methods can be

employed for the detection of inversions [30]. Such methods have been successfully used for molecular karyotyping of insect populations [31–33].

The population genomics inversion discovery method is based on the identification of regions of increased differentiation between genomes, e.g. measured as the highly accentuated fixation index (F_{ST}). These genomic regions typically correspond to inversions, as the latter reduce recombination leading to increased genomic differentiation. However, increased genomic differentiation may also be due to complex structural variation rather than an inversion. Using the population genomics method, a large block of genomic differentiation corresponding to an inversion was recently discovered in the *Timema* stick insect [34]. Because reduced recombination can lead to fixed nucleotide polymorphisms (SNPs) between arrangements, previously characterized inversions can be routinely detected using principal component analysis [35,36]. Tag SNP sets within inversions, in which the biallelic genotype is strongly correlated with the inversion genotype, have been used for high-throughput molecular genotyping of inversions in the African malaria mosquitoes *An. gambiae* and *An. coluzzii* [37,38]. An SNP-based method was used to detect two of the most common inversions, 1pA and 3qG, in a set of 393 individual whole-genome sequences obtained from *Ae. aegypti* populations across Africa [18].

The most recently developed 3D genomics method for inversion discovery employs the Hi-C technique [39,40]. This method detects chromosomal rearrangements by identifying high-frequency physical contacts between genomic loci in the 3D nuclear space [41–43]. A JuiceBox software [44,45] allows the visualization of chromatin interactions on Hi-C heat maps. Short-range interactions form the bright main diagonal line, while long-range interactions appear as bright spots outside the main diagonal when Hi-C reads are mapped to the reference genome. When an inversion occurs, the breakpoint regions that interact with each other in the nucleus appear on Hi-C maps as bright interacting loci of a ‘butterfly’ shape [13,28,46]. If the Hi-C experiment is performed on a heterozygote for an inversion, then sequencing reads originating from the two breakpoints will produce four different fragments resulting in the ‘butterfly’ contact pattern (Figure 1a). If the Hi-C experiment is performed on a homozygote for an arrangement, then the Hi-C reads must be mapped against an alternative gene order reference sequence to identify the inversion (Figure 1b). Since this method of inversion detection relies on the visualization of long-range interactions, Hi-C works well only for finding large and medium-sized inversions (>1 Mb). The accuracy of inversion identification is highly dependent on the quality of the reference genome assembly. New polymorphic inversions were detected by Hi-C in laboratory colonies of *An. merus* and *An. atroparvus* [13]. The Hi-C method was applied to 25 strains of *Ae. aegypti* and resulted in the discovery of 20 inversions ranging in size from 5.2 to 55 Mb [18]. This study clearly demonstrated that multiple inversions can be identified and studied in dipteran species that lack well-polytenized chromosomes. Given the high sensitivity and reproducibility of the Hi-C method, its application has the potential to uncover previously unrecognized chromosomal inversion polymorphisms in diverse insect species. Once new inversions are discovered by comparative, population, or 3D genomics methods, they can be detected in insect populations by less expensive and more rapid PCR- and SNP-based methods.

Inversions and insect adaptation

Some pest, medical, and beneficial insect species have the ability to adapt quickly and effectively to rapid changes in the environment. Chromosomal inversions have been shown to play an important role in the adaptation of insects to such changes. The association between inversion genotype and environment could be a result of natural selection, which can influence the fate, frequency, and distribution of inversion polymorphisms at different spatial and temporal scales. To ascertain whether inversions are subject to natural selection, it is essential to conduct experiments utilizing genomic manipulation techniques, including allelic knockouts and knockins.

Latitudinal and longitudinal geographic clines in the frequency of inversions have traditionally been observed using a cytogenetic method in dipteran insects with well-developed polytene chromosomes, notably *Drosophila* [47,48] and *Anopheles* [23,24,49]. More recently, a combination of cytogenetic and genomic approaches developed for inversion discovery and detection has facilitated studies of the adaptive role of inversions in diverse insect species.

A recent study of the *Timema* stick insect has shown that an inversion is responsible for the long-term maintenance of genetic variation that affects the growth and survival of these insects on alternative host plants [34]. Among several generalist species that can use different host plants, *T. knulli* lives on both redwood trees and flowering plants. A genotyping-by-sequencing approach performed on 138 field-collected individuals identified a large block of genomic differentiation on chromosome 11 called the *Perform* locus. A PCA of SNPs within this locus revealed three population genetic clusters segregating within this species. Alignment of the recently sequenced genome of the redwood-dwelling *T. knulli* with the genomes of two other *Timema* species revealed a large ~30 Mb inversion on the same chromosome. Most critically, the breakpoints of this inversion identified by comparative genomics coincided with the identified boundaries of the *Perform* locus discovered by population genomics analysis. Furthermore, the three population genetic clusters in the PCA of the SNPs correspond to two homozygous and one heterozygous inversion arrangements. The study found that this polymorphic inversion has been maintained in *T. knulli* populations for several million years and that multiple factors, including environmental heterogeneity and gene exchange, contribute to the persistence of this polymorphism [34].

Inversions may also help insects to adapt quickly to the new environmental conditions during invasions into different climatic regions. Using population genomic methods based on a local PCA of SNPs and linkage disequilibrium (LD) analyses, a recent study of a small insect pest, the melon thrips *Thrips palmi*, investigated the genetic basis of adaptations following its rapid contemporary expansion from subtropical to temperate regions [50]. In about 2 decades *T. palmi* has spread from its area of introduction in southern China to greenhouse environments in northern regions. Both field populations and greenhouse groups in this study exhibited clinal patterns in thermal tolerance as measured by critical thermal maximum. The study identified three inversions in chromosomes 3, 5, and 14 that accounted for 49.9%, 19.6%, and 8.6% of the variance in critical thermal maximum among

populations. This finding highlights the importance of inversions as carriers of large-effect allelic variation in rapid climate adaptation [50].

Chromosomal inversions can change their frequency not only in response to spatial adaptation but also in response to temporal variations in local climate. A recent study on the highly polymorphic species *D. subobscura* analyzed the response of inversions to global warming by comparing inversion frequencies in several populations in Serbia and Spain from 1995 to 2019–2022 [51]. The study has used a cytogenetic method for inversion detection and classified all inversions found in natural populations of *D. subobscura* as 'cold' or 'warm' adapted as well as 'non-thermal' inversions. A significant increase of the 'warm' adapted inversions was found during the study period, when a gradual increase in the annual temperature was also observed. This study of chromosomal inversion polymorphism in different climatic regions showed that several Balkan populations of *D. subobscura* have thermally adapted inversions [51]. A rapid shift in the frequency of temperature-related inversions in response to climate change has also been documented in malaria mosquitoes. A dramatic tenfold decrease in the frequency of the 'cold tolerance' inversion 2R1 was observed in two Siberian populations of the Eurasian species *An. messeae* between 1975 and 2013 [52].

A geographic pattern in inversion distribution within an invasive species may reflect migration history. A recent study has revealed specific geographic patterns in inversion distribution in the arboviral mosquito vector *Ae. aegypti* using a Hi-C inversion discovery method [18]. This study identified 20 multi-Mb polymorphic inversions in 25 natural populations of this species across its global distribution. Fifteen inversions were found in African strains, while only 3 inversions were identified in non-African strains. Among the 15 inversions, only inversions 1pA and 3qG have widespread distribution throughout the African continent, while most of the endemic chromosomal inversions (1qF, 1qD, 1qE, and 1qG) were found in West Africa. The higher inversion polymorphism in Africa is consistent with this continent being the ancestral range of *Ae. aegypti* populations and with non-African populations having spread around the globe within the last ~500 years. Interestingly, a significant association was found between the 3pA and 3pB inversions of *Ae. aegypti* and the 2Rj inversion of *An. gambiae.* Also, the 2pA and 3pD inversions of *Ae. aegypti* have significant overlap of orthologous genes with the 2La inversion and the multi-inversion region on the 2R chromosome arm of *An. gambiae*, respectively. The finding of common gene sets in polymorphic inversion regions of the distant mosquito species may explain their parallel evolution under similar environmental conditions in Africa [18].

Inversions and genome evolution

Structural genome variations are common within and between species, but its characterization depends on the quality of genome assemblies. The increasing availability of chromosome-scale genome assemblies allows researchers to study the impact of inversions on genome evolution in different insect genera. A recent study used new high-quality genome assemblies for *D. persimilis* and *D. pseudoobscura* to reveal a previously hidden landscape of structural divergence between fruit fly genomes [53]. Previously estimated genome sizes were 134.6 Mb (26.4% repetitive) for *D. pseudoobscura* and 145.5 Mb

(33.3% repetitive) for *D. persimilis* [54]. The divergence time between the species was estimated to be between 450,000 and 1 million years ago (mya) [55]. The study confirmed higher levels of nucleotide divergence between fixed inversions compared to collinear genomic regions, but also found a significant overrepresentation of insertions and deletions within the inversions. As regions of low recombination, inversions accumulate transposable elements (TEs) and structural variants. There was a strong association between the fixed chromosomal inversions and differentially expressed genes involved in neural development, spermatogenesis, and oocyte-to-embryo transition. Thus, this study highlights the importance of inversions in driving genomic and transcriptomic divergence between evolving species [53].

In another study, five genome assemblies were generated for three *D. virilis* group species to characterize sequence divergence, repetitive DNA evolution, and structural rearrangements [29]. The genome size in *D. virilis* is relatively large (~400 Mb) [56] and the genomes of *D. virilis* group species are rich in repetitive sequences (e.g., 40% of the genome is covered by the simple satellites) [57]. Divergence times have been estimated to be ~5 mya (*D. virilis* split) and ~1 mya (*D. americana* and *D. novamexicana* split) [58]. The study showed that *D. americana* and *D. novamexicana* differ from each other by only two fixed inversions located on chromosome 2. Most of the multiple fixed inversions between *D. virilis* and the other two species are located on the X chromosome including several nested inversions in the pericentromeric region. The X chromosome also showed elevated nucleotide diversity compared to the autosomes in *D. americana* and *D. novamexicana*, but not in *D. virilis*. In addition, the three *D. americana* strains differed by both fixed and polymorphic inversions. The DAIBAM TEs were enriched in breakpoint regions within *D. novamexicana* compared to the rest of the genome. However, the rapid evolution of the DAIBAM sequences makes it difficult to assess whether this element accumulated before or after the inversions. Inversion breakpoints are more likely to accumulate TEs because selection against deleterious TE insertion is weaker in regions of low recombination [59,60]. Thus, the causative role of DAIBAM in the origin of inversions remains unclear [29].

A recent work characterized genomic landscapes and chromosome rearrangements by comparing three mosquito genera that have evolved over long time scales. *Culex* and *Aedes* diverged ~68.6 mya, and *Anopheles* split from them ~134.6 mya [28]. The genome sizes of *An. albimanus*, *An. coluzzii*, *Cx. quinquefasciatus*, and *Ae. aegypti* are 173 Mb, 270 Mb, 579 Mb, and 1376 Mb, respectively [61–64]. This work found that chromosomal evolution in mosquitoes included: 1) numerous paracentric inversions and whole arm translocations in all three mosquito genera; 2) a large X-autosomal translocation during the split between Anophelinae and and Culicinae mosquitoes; 3) a chromosome 1 pericentric inversion between *Cx. quinquefasciatus* and *Ae. aegypti*. The study determined that, similarly to *Drosophila* species, the X chromosomes in *An. albimanus* and *An. coluzzii* and the sex-determining chromosome 1 in *Cx. quinquefasciatus* and *Ae. aegypti* have evolved significantly more rapidly than autosomes in each species, especially in *Anopheles*, highlighting the role of sex-determining chromosomes in the evolution of dipteran insects. TEs were enriched on the X chromosome of *An. albimanus* and *An. coluzzii* but not on the homomorphic sex chromosomes of *Cx. quinquefasciatus* and *Ae. aegypti* [28].

Thus, chromosomal inversions are a prominent part of dipteran insect genome evolution. Inversions substantially rearrange gene order and can lead to the accumulation of TEs and increased genomic divergence, affecting the evolution of insect species.

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Highlights

- Methods of comparative, population, and 3D genomics are becoming increasingly fruitful for the discovery of chromosomal inversions in diverse insect species.
- Inversion polymorphisms in insects exist at different spatial and temporal scales: they can be evolutionarily stable or arise rapidly in response to changing environments.
- Inversions can influence insect genome evolution by disproportionately increasing gene order rearrangements on sex chromosomes, accumulating transposable elements, and facilitating genomic divergence.

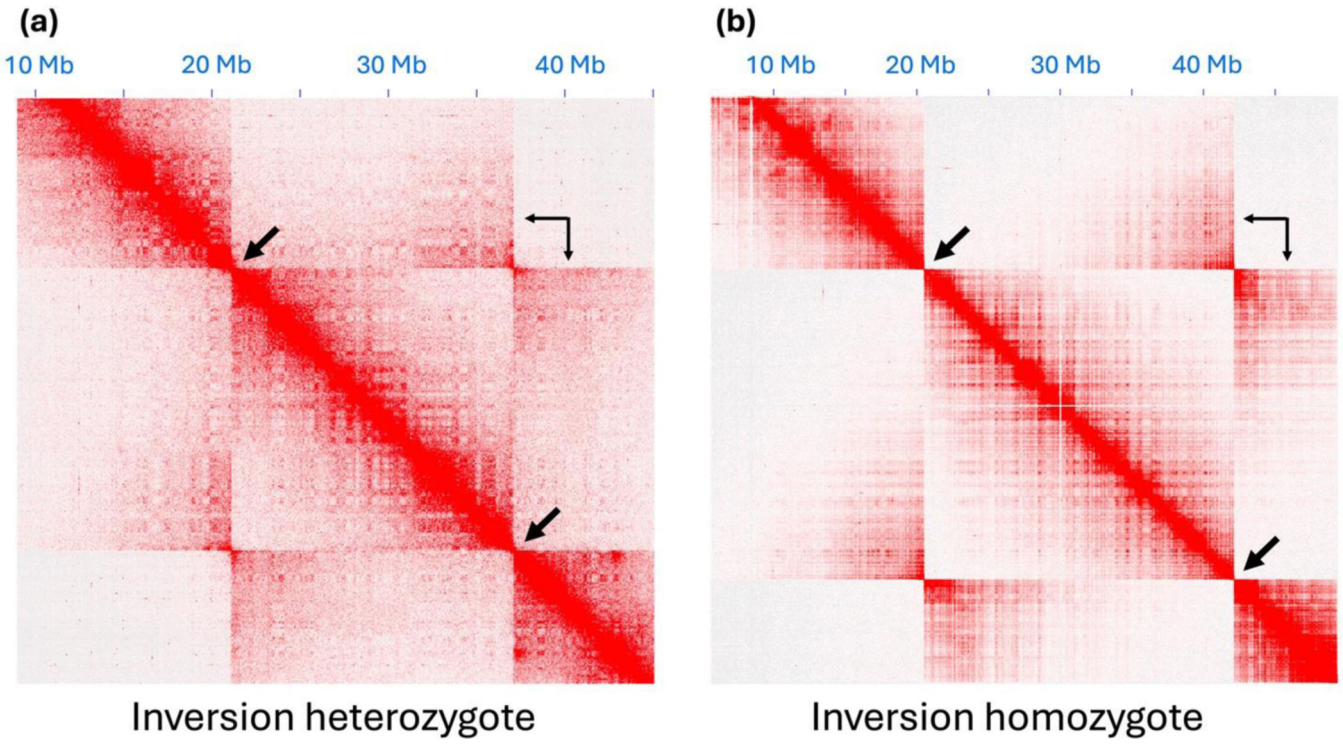

Figure 1.

Inversion identification by the Hi-C method. **(a)** Region of the *An. stephensi* Hi-C contact map with the 2Rb polymorphic inversion manifested as a distinct 'butterfly' pattern. The 'butterfly' wings (shown by thin arrows) connect at a point that marks the inversion breakpoints (shown by thick arrows). Chromatin interaction is also seen around the 'butterfly' and the breakpoints of the heterozygous 2Rb/+ arrangement. The red color intensity reflects the frequency of chromatin contacts. **(b)** Region of the *An. coluzzii* (the MOPTI strain) Hi-C contact map with the 2La fixed inversion mapped against a standard gene order of the *An. gambiae* PEST reference genome. No chromatin interaction is seen around the 'butterfly' (thin arrows) and the breakpoints (thick arrows) of the homozygous 2La/a arrangement. This is manifested by a complete disruption of the main diagonal at the breakpoints. Genomic coordinates are given in megabase pairs (Mb). The Hi-C data are from [13].