



REVIEW

Locking Adaptive Supergenes: How Chromosomal Inversions Buffer Climate Stress

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ABSTRACT: Anthropogenic climate change poses a critical threat to global plant diversity, necessitating rapid adaptive responses in both wild and cultivated lineages. Traditional landscape genomics primarily investigates single-nucleotide polymorphisms; however, polygenic climate-resilient traits, including phenological shifts, flowering time, and drought escape, depend on the coordinated inheritance of complex, multi-locus genotypes. Persistent gene flow and meiotic recombination disrupt these advantageous allelic combinations, resulting in significant migration load. Chromosomal inversions represent a primary mechanism for mitigating genetic homogenization by structurally suppressing recombination in heterokaryotypes. Although other genomic mechanisms may contribute to maintaining co-adapted alleles, inversions effectively lock these loci into “supergenes” that facilitate fitness along environmental gradients. Empirical evidence from diverse plant systems, including model annuals such as *Mimulus*, long-lived woody perennials, and major cereal crops, indicates that adaptive inversions are widespread and often sustain parallel latitudinal clines or distinct ecotypic boundaries. Nevertheless, landscape genomic scans are susceptible to false positives due to demographic history, background selection, or genetic hitchhiking, which creates a “correlation gap” between spatial distribution and functional causality. This review introduces a comprehensive, four-tiered hierarchical framework—the Causality Roadmap—to guide researchers from initial bioinformatic discovery to mechanistic validation through long-read pangenomics, multi-omics integration, 3D chromatin conformation and topologically associating domain (TAD) mapping, and CRISPR/Cas9-mediated structural genome engineering. The “first-event” dilemma is also addressed by contrasting the Alleles-First, Inversion-First, and Introgression-First evolutionary models to determine whether inversions actively construct or passively protect adaptive modules. By integrating evolutionary chronology with functional validation, this framework lays the foundation for identifying key structural variants, assessing population vulnerabilities, and providing biological insights for targeted germplasm conservation under shifting macroclimatic conditions.

KEYWORDS: Chromosomal inversions; supergenes; local adaptation; recombination suppression; landscape genomics; CRISPR/Cas9 engineering

1 The Role of Genomic Architecture in Climate Adaptation

Anthropogenic climate change threatens global plant diversity by exposing species to rapid changes in temperature and rainfall, as well as to extreme events. To survive, plant populations must migrate or adapt quickly. While most adaptation research has focused on point mutations such as single-nucleotide polymorphisms (SNPs), complex traits, such as drought or salinity tolerance, usually require coordinated

changes across multiple loci [1,2]. When adaptive alleles are scattered, recombination can break up beneficial gene combinations, especially with gene flow from maladapted populations [3]. Plants use genomic modifications, particularly chromosomal inversions, to preserve sets of co-adapted alleles, providing a structural mechanism for adapting to climate change [3,4].

A chromosomal inversion occurs when a chromosome segment breaks in two places, rotates 180°, and reinserts at the original site [5]. While the gene sequence remains unchanged, this rearrangement alters recombination. In heterokaryotypes, a loop forms during meiosis, and crossovers within this loop create unbalanced gametes that are usually inviable [6,7]. Thus, recombination is suppressed within the inverted region in heterozygotes but not in homozygotes [8]. This suppression links multiple alleles into a single block, called a “supergene” [9], protecting complex traits from recombination and preventing the loss of variants through genetic homogenization [7,10].

Kirkpatrick and Barton [11] provide key theoretical models explaining how recombination suppression aids adaptation. Their work shows that, under divergent selection, gene flow can break up favorable allele combinations, but chromosomal inversions can preserve them by forming protected linkage blocks (i.e., supergenes) maintained by selection. This allows populations to keep co-adapted gene complexes that boost fitness in specific environments. As a result, inversions expand the range of conditions a population can withstand and support local adaptation or even ecological speciation [12–16].

Inversions help maintain distinct ecotypes across environmental gradients despite gene flow. For instance, a single inversion can link genes for early flowering, deep roots, and efficient stomatal function, supporting climate resilience [12,17–19]. As climates shift, inversion haplotype distributions can move within a species’ range, providing genetic variation that buffers populations from sudden change [20,21]. Understanding how these structural variants (SVs) relate to stress responses is vital for explaining plant resilience. This review surveys current approaches to studying inversion-mediated adaptation and the potential of genome engineering to clarify their roles.

2 Genetic Architecture and the Conflict between Selection and Gene Flow

In local adaptation, plant populations often live along environmental gradients with varying selective pressures over short distances. Gene flow between these populations creates conflict, as maladapted migrants and recombination disrupt beneficial allele combinations, introduce disadvantageous alleles, and lower mean fitness, slowing local adaptation [22–24].

Chromosomal inversions are a structural solution to this evolutionary challenge. Yeaman [25] showed that local adaptation depends on genomic architecture; when adaptive alleles are spread out, recombination separates them, preventing their joint inheritance. However, inversions suppress recombination in heterokaryotypes, help accumulate co-adapted alleles (e.g., supergenes) for local environments, and protect them from the homogenizing effects of gene flow [12,19,26,27].

Inversions help reduce genetic load from environmental differences by preserving adaptive gene complexes. This allows populations to keep distinct ecotypes even with high migration, which might otherwise replace local genes with foreign ones [12,19]. Thus, inversions are favored when strong divergent selection and gene flow coexist, making them active drivers of rapid ecological divergence and local adaptation during environmental change (Fig. 1).

While inversions are known for aiding in adaptation, they can also form and persist through neutral evolutionary processes [28–31]. Genomic islands of differentiation, often seen as signs of selection, may actually result from background selection, recombination variation, or differences in gene density [32]. SVs can also accumulate through genetic drift in small populations, mimicking adaptive patterns [33].

Distinguishing adaptive from neutral inversions is difficult, so analyses must consider demographic history and mutation rates to rule out non-adaptive causes of genomic differentiation.

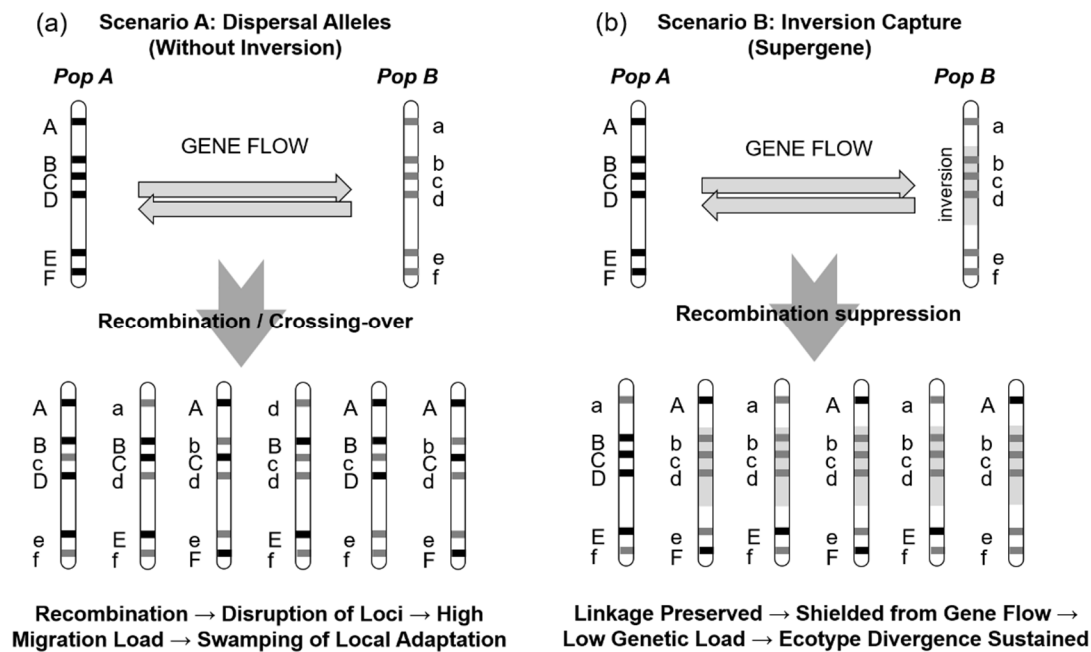


Figure 1: Mechanisms of local adaptation maintenance under gene flow: Comparison between dispersed alleles and inversion-mediated supergenes. This schematic illustrates two genetic architectures for maintaining local adaptation across environmental gradients in the presence of gene flow. **(a) Scenario A: Dispersed alleles without inversion.** When adaptive loci are scattered across the genome, migration between populations (Pop A and Pop B) facilitates recombination, which breaks up co-adapted allelic combinations (b-c-d). This results in high genetic load and genomic homogenization, hindering the persistence of local adaptation. **(b) Scenario B: Inversion capture and supergene formation.** Chromosomal inversions capture multiple co-adapted loci into a single non-recombining block. This structural variation effectively shields adaptive combinations (b-c-d) from recombination-mediated breakdown despite ongoing gene flow. Consequently, distinct ecotypes are maintained and migration load is reduced, facilitating stable local adaptation across environmental gradients. Different lowercase letters (a and b) designate distinct evolutionary scenarios, while letters within the schematics (b, c, d) represent specific loci. Uppercase and lowercase letters ('ABCDEF' and 'abcdef') denote distinct alleles of chromosomal loci.

3 Evidence Linking Inversions to Climate Adaptation

Empirical links between chromosomal inversions and ecological gradients strongly suggest their adaptive significance. Studies across many taxa show that SVs often align with environmental axes, supporting the idea that structural polymorphism is a widespread adaptive strategy [12,15,17,28,34,35].

3.1 Classic Evidence from *Drosophila*: The Evolutionary Baseline

The link between chromosomal inversions and macroclimatic adaptation began with studies in *Drosophila*. Dobzhansky showed that *Drosophila pseudoobscura* and *D. melanogaster* have clear geographic and latitudinal clines in inversion frequencies [36–40]. For example, the *In(3R)Payne* inversion in *D. melanogaster* forms parallel latitudinal clines across continents [41–44]. This SV is common in warm, subtropical areas and rare in cooler, high-latitude regions. Dobzhansky's work demonstrated that inversion polymorphisms change predictably with geography, matching selective pressures like temperature and

season length. These structures stably carry multiple fitness alleles, supporting the evolutionary principle that chromosomal rearrangements can track environmental variation across large areas [16,17].

3.2 Empirical Evidence from Plant Systems

Moving from animal to plant models shows that sessile organisms also use similar genomic strategies to handle environmental and climatic stress. Chromosomal inversions reduce recombination in heterokaryotypes, protecting adaptive gene complexes from genetic homogenization across environments [16,17].

- (1) **Model Systems and Herbaceous Plants:** The yellow monkeyflower (*Mimulus guttatus*) shows strong divergence between coastal perennials, which grow more and exhibit later flowering due to steady moisture, and inland annuals, which flower quickly to survive summer drought [45–48]. A major chromosomal inversion on chromosome 8 drives this divergence by capturing alleles for anthesis time, morphology, and herbivore resistance in a non-recombining block, promoting adaptation and reproductive isolation [12,17,49]. In *Boechera stricta*, multiple inversions separate meadow from subalpine populations, maintaining flowering and water-use differences [19]. In *Arabidopsis thaliana*, localized inversions are linked with cold hardiness and overwintering [50–52].
- (2) **Wild Relatives and Outcrossing Lineages:** Recent pan-genomic studies show that multiple large inversions often drive complex ecotype differentiation over wide regions. In wild sunflowers (*Helianthus annuus*, *H. petiolaris*, *H. argophyllus*), resequencing has found many large, non-recombining haplotype blocks—some up to 100 megabases (Mb) [53,54]. These supergenes are linked to key traits like seed size, flowering time, and physiological responses to soil and climate. In *H. argophyllus*, a single large block causes a 77-day flowering time difference between coastal and inland ecotypes, aligning reproduction with local moisture [53]. Many adaptive inversions are ancient, sometimes arising via introgression from related species before local selection favored them [54].
- (3) **Cereal Crops and Agricultural Landraces:** Inversions are key for crop adaptation to different environments. In maize (*Zea mays*), the 13-Mb Inv4m inversion on chromosome 4 enables highland landraces to survive cold and intense UV radiation, distinguishing them from lowland types [13,55]. In barley (*Hordeum vulgare*), a large inversion on chromosome 1H separates northern European and Mediterranean landraces by maintaining photoperiod sensitivity and drought-escape flowering, supporting crop success across latitudes [18,56].
- (4) **Legumes and Tree Species:** Narrow-leafed lupin (*Lupinus angustifolius*) has a major inversion on chromosome 6 (*NLL-06*) that maintains structural diversity between wild and domesticated lines [57]. In long-lived trees, preserving adaptive gene complexes is crucial for climate resilience. Scots pine (*Pinus sylvestris*) features a large macro-inversion linked to latitudinal adaptation in phenology [58]. In silver birch (*Betula pendula*), an inversion on chromosome 1 separates northern and southern populations, corresponding to growing season and cold tolerance [59].

3.3 Convergence of Phenological Trait Capture: A Macro-Evolutionary Pattern

Empirical evidence across plant lineages shows a clear macro-evolutionary pattern in the traits captured by adaptive inversions. Most documented cases involve traits like life-history transitions, flowering time, photoperiod sensitivity, and drought escape. In contrast, core physiological processes, such as metabolism, electron transport, and photosynthesis, are mostly absent or only peripheral in these regions (Table 1; see Table 2 for details). This pattern cannot be explained by sampling bias, but reflects the genetic architecture and evolutionary forces that favor recombination suppression in plant adaptation.

Table 1: Summary of key trait categories captured by adaptive plant inversions.

Functional Trait Category	Estimated Prevalence/Overlap	Key Genetic Elements & Mechanistic Focus	Representative Plant Lineages
Flowering Phenology & Photoperiod	Extensive/Pervasive	Master regulatory switches (FT, CO), circadian clock components, vernalization controls	<i>Mimulus</i> , <i>Helianthus</i> , <i>Zea</i> , <i>Hordeum</i> , <i>Boechera</i> , <i>Medicago</i> , <i>Lupinus</i> , <i>Betula</i>
Abiotic Stress & Drought Escape	Frequent	Morphological allocation, stomatal patterning, drought-escape phenological triggers	<i>Mimulus</i> , <i>Boechera</i> , <i>Zea</i> , <i>Hordeum</i> , <i>Medicago</i>
Macroclimatic & Altitudinal Adaptation	Moderate	UV-B tolerance, cold hardiness, frost resistance, growing season synchronization	<i>Zea</i> , <i>Pinus</i> , <i>Betula</i> , <i>Hordeum</i>
Microenvironmental & Tissue Defenses	Localized/Specific	Localized trichome density, herbivore-defense secondary metabolites, soil chemistry tolerance	<i>Mimulus</i> , <i>Helianthus</i> , <i>Boechera</i> , <i>Medicago</i>
Core Metabolism & Photosynthesis	Rare/Negligible	Highly diffused polygenic networks; rarely captured as primary drivers within standard inversions	<i>Arabidopsis</i> , <i>Solanum</i> , <i>Oryza</i>

Qualitative synthesis based on published plant inversion studies summarized in Table 2.

Table 2: Summary of empirically validated adaptive inversions in plants.

Taxon/Study System	Inversion Scale & Chromosome	Divergent Ecotypes/Phenotypes	Preserved Climate-Resilient Traits	Key Mechanisms/ Verification Methods	Primary References
<i>Mimulus guttatus</i> (Yellow monkeyflower)	Chr 8A, 2.22 Mb, nested inversions within inv_chr8A	Coastal Perennial vs. Inland Annual; Drought Avoidance vs. Salt Tolerance	Flowering time, vegetative growth allocation, and localized herbivore resistance under severe summer drought.	ONT, QTL mapping, reciprocal transplants, field fitness assays.	Lowry and Willis [12], Kollar, Stanley [17], Coughlan and Willis [49]
<i>Helianthus argophyllus</i> (Silverleaf sunflower)	Chr 3, 6, 10, 16; 25 Mb in arg06.01, others 3–47 Mb	Coastal Sand-Dune vs. Inland Heat-Resistant	Severe shift in flowering time (77-day divergence) to synchronize reproduction with local seasonal moisture.	Hi-C, Population resequencing, pan-genomic scaffolding, GEA.	Todesco, Owens [53], Soudi, Jahani [54]
<i>Zea mays</i> (Maize/Teosinte)	Chr 4: 13–14 Mb; Chr 9: 3 Mb; CRISPR/Cas9 induces an inversion of 75.5 Mbp	Lowland vs. Highland landraces	Altitudinal adaptation, managing low temperature stress, heavy UV radiation, preserving macrohairs in the leaf blade, and modified flowering time.	High-density SNP genotyping, GEA, karyotype visualization.	Crow, Ta [13], Calfee, Gates [55], Pyhäjärvi, Hufford [60], Romero Navarro, Willcox [61]

Table 2: *Cont.*

Taxon/Study System	Inversion Scale & Chromosome	Divergent Ecotypes/Phenotypes	Preserved Climate-Resilient Traits	Key Mechanisms/ Verification Methods	Primary References
<i>Hordeum vulgare</i> (Wild and cultivated Barley)	Chr 2H: 10 Mb; Chr 3H: 36.6 Mb; Chr 4H: 37.2 Mb; Chr 7H: 141 Mb	Northern European vs. Mediterranean landraces; Wild Type vs. Cultivated; Drought Tolerance vs. Early Maturity	Latitudinal and macroclimate adaptation, managing photoperiod sensitivity and drought-escape flowering timing.	Hi-C, pangenomics, RILs, macroclimatic geographic clines, expression profiling of circadian genes.	Zhang, Tan [18], Jayakodi, Padmarasu [56], Russell, Mascher [62], Monat, Padmarasu [63]
<i>Boechera stricta</i> (Drummond's rockcress)	Ch1 (LG1): 8.4 Mb	Low-elevation Meadow vs. High-elevation Subalpine	Flowering phenology, water-use efficiency under drought, and defensive glucosinolate production.	Fine QTL mapping, Chromosome Painting, RILs, longitudinal common garden experiments, fitness trade-off evaluation.	Lee, Wang [19], Prasad, Song [64]
<i>Arabidopsis thaliana</i> (Model system)	Chr 1, 2, 4, 5: Varies (engineered lines from kb to Mb)	Local Ecotypes around the World; Synthetic/ Experimental lines	Potential preservation of multi-genic abiotic stress complexes.	Dual-guide RNA CRISPR/Cas9 site-directed chromosome surgery, Junction-specific PCR, Droplet digital PCR.	Schmidt, Pacher [51], Rönspies, Schindele [65]
<i>Solanum lycopersicum</i> (Tomato/crop)	Chr 6: 300 kb; Chr 11: 200 kb; engineered lines: 37.5 Mb in Chr 6	Domesticated vs. Wild relatives	Target crop resilience traits (e.g., fruit quality or cold tolerance linkages); disease-resistant genes from the wild (Mi-1 insect-resistant and Ty-2 virus-resistant regions).	PacBio long-read sequencing, crystal digital PCR, CRISPR/Cas9-induced rearrangements in protoplasts to test structural heredity.	Grubben, Bijsterbosch [66]
<i>Pinus sylvestris</i> (Scots pine)	~300 Mbp (Putative)	Environmental gradients (clinal)	Clinal adaptation to photoperiod and temperature for controlling bud set and germination, growth cessation, and frost hardiness.	Targeted sequence capture, LD heatmap, F_{ST} outliers, spatial PCA, conStruct.	Tyrmi, Vuosku [58], Savolainen, Bokma [67]
<i>Medicago truncatula</i> (Barrel medic)	Chr 1, 2, 4, 8; ~2.5 Mb in Chr 2	Different Ecotypes (e.g., A17 & R108)	Adaptation to climate through interactions between flowering phenology and soil environment, leaf marking, salt tolerance, and nitrogen fixation capacity; Aluminum toxicity and sensitivity to iron-deficient environments.	Hi-C, Synthetic analysis; pcadapt, X^TX statistic, LD heatmap on 150 samples of Exome capture.	Yoder, Stanton-Geddes [68], Li, Liu [69]

Table 2: *Cont.*

Taxon/Study System	Inversion Scale & Chromosome	Divergent Ecotypes/Phenotypes	Preserved Climate-Resilient Traits	Key Mechanisms/ Verification Methods	Primary References
<i>Betula pendula</i> (Silver birch)	Chr 1: 9 Mb	Latitudinal clines (South vs. North)	Adaptation to growing season length and cold hardiness; Temperature-related bud-break and apical-meristem development	Population genomics along environmental gradients, contact zone analysis, LD heatmap.	Herrera-Egoavil, Leal [59]
<i>Lupinus angustifolius</i> (Narrow-leaved lupin)	Chr 6 (NLL-06): large fragment of paracentric inversion	Wild vs. Domesticated (Ecotypes)	Genomic diversity preservation and adaptation to domestication environments: Impermeable seeds, early flowering, and indehiscent pods.	Oligo-painting FISH, BAC-FISH, Pan-genome assembly, chromosome-length reference mapping by Hi-C, and structural variant analysis.	Bielski, Książkiewicz [57], Garg, Kamphuis [70]

Abbreviations ONT denotes Oxford Nanopore Technologies; FISH denotes fluorescence *in situ* hybridization; BAC-FISH denotes Bacterial Artificial Chromosome-FISH; PCR denotes polymerase chain reaction; QTL denotes quantitative trait locus/loci; Hi-C denotes high-throughput chromosome conformation capture; GEA denotes Genome-Environment Association; RIL denotes recombinant inbred line; SNP denotes single-nucleotide polymorphisms; LD denotes linkage disequilibrium; F_{ST} denotes fixation index.

This uneven distribution stems from differences between master regulatory networks and housekeeping genes. Traits like flowering time and photoperiod are usually controlled by a few major regulators, such as *FLOWERING LOCUS T* (*FT*), *CONSTANS* (*CO*), or MADS-box genes [71,72]. These act as evolutionary switches, allowing niche shifts [73], such as from a perennial to a drought-escaping annual life history [74]. Capturing a master regulator in an inversion can create a powerful supergene, consolidating adaptive traits, enabling rapid adaptation, and shielding the co-adapted complex from gene flow [17].

In contrast, physiological traits like photosynthesis, carbon fixation, and respiration are controlled by thousands of minor-effect genes spread across the genome [75–77]. Because these housekeeping traits lack a central control point, even large inversions rarely capture more than a few needed loci [78,79]. Metabolic genes are usually stabilized by selection to maintain homeostasis [80–82], while phenological traits face strong, variable selection across climates [83–85]. Thus, plant genome evolution tends to favor inversions that capture developmental switches for timing environmental responses rather than those that alter core metabolism [86]. Locking in these phenological switches provides a genomic buffer, helping plants adapt ecotypically to climate change without disrupting basic metabolic functions (see Tables 1 and 2).

These examples show that chromosomal inversions are common and effective structural mechanisms, not rare anomalies. Across diverse plants, several macro-evolutionary patterns recur: (1) Clinal and ecotypic distribution: inversions often follow environmental gradients or mark sharp ecotype boundaries [87]; (2) The genomic buffer: inversions keep co-adapted alleles together, supporting plasticity and rapid divergence even with gene flow, buffering against climate change [35,88]; (3) The correlation gap: most studies focus on genome-environment correlations, not functional causality [13,15–17,89,90] (Table 2).

4 When Inversions Are Not Adaptive: Alternative Explanations

4.1 Demographic Processes and Neutral Outliers

While chromosomal inversions aligned with environmental gradients often suggest climate adaptation, similar genomic patterns can arise from non-adaptive demographic processes, such as bottlenecks, founder effects, and range expansions [91–93]. Secondary contact between previously isolated populations can create hybrid zones where neutral structural differences appear as statistical outliers. Analyses such as local principal component analysis (local PCA, e.g., Lostruct) [94–96] or window-based fixation index (F_{ST}) [97–99] frequently highlight these regions due to deep divergence. However, these genomic islands usually reflect historical structure and limited gene flow rather than recent ecological selection.

4.2 Recombination Suppression and Background Selection

False positives in landscape genomics often result from the interplay of structural variation, recombination suppression, and background selection [30,100]. In inverted regions, reduced crossing-over creates large blocks with low recombination. These areas are subject to background selection, where purifying selection removes harmful mutations, also reducing neutral diversity (π) at the inversion locus [101,102]. Standard divergence scans may then yield artificially high F_{ST} values, leading researchers to misinterpret regions under purifying selection as adaptive islands.

4.3 Genetic Hitchhiking and Selective Sweeps outside Inversions

Even with clear evidence of positive selection, a chromosomal inversion may not be the direct target but rather an evolutionary hitchhiker [100,103]. If a nearby advantageous mutation sweeps rapidly, strong linkage disequilibrium (LD) can cause the whole inversion block to rise in frequency. This genetic hitchhiking

can mask the true target of selection, creating a large region of high differentiation and low diversity around the inversion [104].

4.4 Inversions as Evolutionary Byproducts

Many chromosomal inversions persist as neutral or slightly deleterious byproducts. Structural changes, such as those caused by transposable elements or non-allelic homologous recombination, occur randomly [105–107]. Most new inversions do not affect gene function and are neutral. Through genetic drift, especially in species with small populations, these variants can reach high frequency or fixation. Slightly deleterious inversions may also persist if they occur in regions of low recombination. Thus, not all SVs are adaptive; genetic drift [108,109] and molecular noise [110] also play key roles in maintaining them as passive genomic features.

Common statistical signals, like high F_{ST} or elevated LD, can detect inversions but are sensitive to demographic history and background selection [97–99]. To determine whether a rearrangement is adaptive or due to drift, we propose a diagnostic framework that integrates multiple layers of genomic evidence (Table 3).

Table 3: Diagnostic framework for distinguishing adaptive from neutral inversions. This table outlines key genomic and ecological indicators used to differentiate structural rearrangements caused by natural selection from those resulting from genetic drift or demographic stochasticity. Consistent alignment across these five criteria is necessary to advance from correlative findings to functional validation.

Evaluation Criterion	Adaptive Inversion (Evidence of Selection)	Non-Adaptive/Neutral Inversion (Neutral/Drift)
Geographic Pattern	Significant correlation with environmental gradients (e.g., climate clines)	Randomized distribution or isolation-by-distance only
Frequency Dynamics	Stable, repeated parallel clines across independent lineages	Erratic fluctuations; lack of environmental predictability
Gene Composition	Significant enrichment of stress-response or phenological genes	Random gene distribution; no clear functional enrichment
Linkage Disequilibrium (LD)	High LD restricted to the inverted segment	Low LD or LD decaying at background genomic rates
Coalescent Signature	Selective sweep signals; reduced diversity within the inversion	Neutral coalescent signatures; diversity consistent with flanking regions

4.5 Bridging the Correlation Gap: Dissecting Causal Drivers within Inversion Blocks

A key challenge in landscape genomics is the “correlation gap,” which separates strong statistical associations with environmental variables from evidence of true functional causality. Chromosomal inversions often cover large genomic regions with many linked genes. Even when data show that an inversion improves fitness in certain environments, this broad observation does not reveal the specific molecular drivers of adaptation. In these non-recombining regions, high LD, selective sweeps, and hitchhiking effects make it difficult to distinguish neutral mutations from true causal variants.

To address this gap, evolutionary genomics should move beyond spatial allele frequency clines and adopt multi-layered, integrative methods. Analyzing the functional architecture of inversion blocks requires advanced molecular tools such as allele-specific expression assays, expression Quantitative Trait Locus (eQTL) mapping, and high-resolution fine-mapping near inversion breakpoints. Integrating epigenomic

profiling, 3D chromatin conformation capture (such as high-throughput chromosome conformation capture [Hi-C]), and single-cell transcriptomics can help determine whether adaptive traits result from gene disruption at breakpoints or changes in regulatory networks. A structured, stepwise experimental framework is essential to move from observational correlations to clear, mechanistic evidence of causality.

5 The Causality Roadmap: From Genomic Correlation to Functional Validation

To prove that chromosomal inversions are adaptive, researchers must go beyond genomic data and provide experimental validation. While SVs have often been linked to climate adaptation through spatial correlations [9,12,15], demonstrating that an inversion is actively selected rather than just the result of demographic history or drift requires a clear framework. We present a four-tiered “Causality Roadmap” to help move from genomic associations to mechanistic proof (Fig. 2).

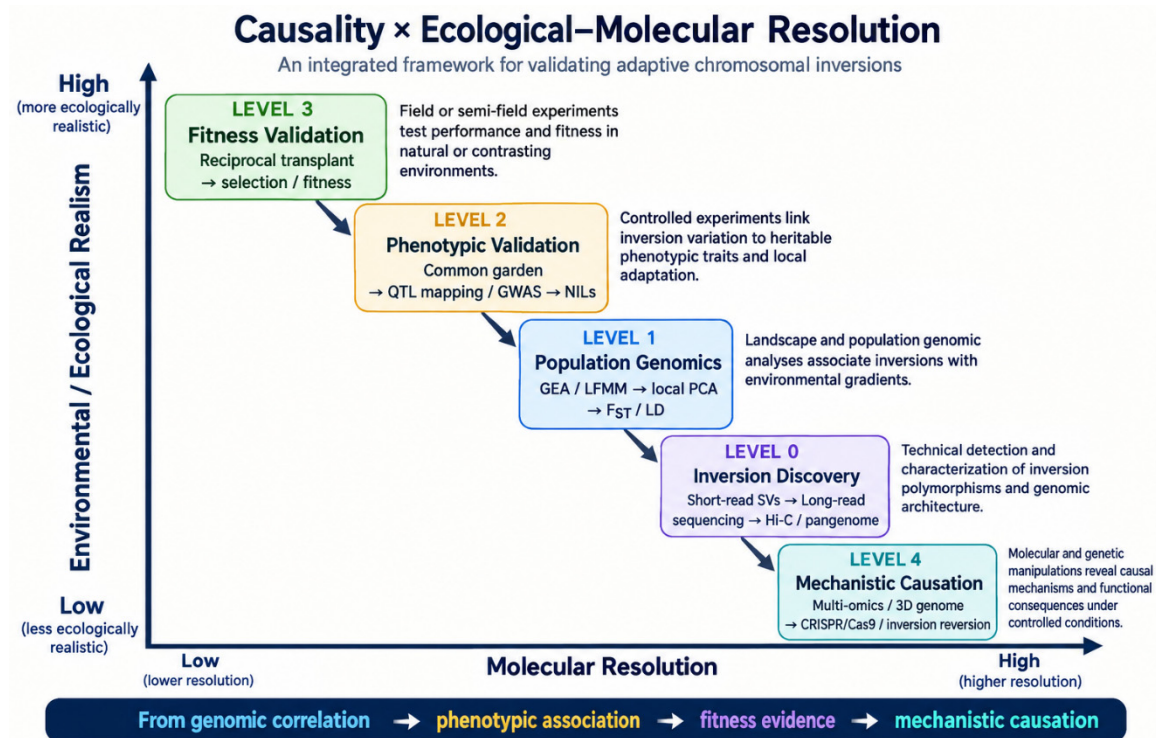


Figure 2: An integrated framework for validating adaptive chromosomal inversions across ecological realism and molecular resolution. The framework positions five complementary levels of evidence along two orthogonal axes: ecological realism (environmental relevance) and molecular resolution (technical detail). Level 3 (fitness validation) captures the highest ecological realism because it directly tests performance and fitness in natural or contrasting environments. Level 2 (phenotypic validation) uses controlled experiments to link inversions to heritable traits. Level 1 (population genomics) identifies statistical associations between inversions and environmental gradients at the population scale. Level 0 (inversion discovery) focuses on detecting and characterizing inversion polymorphisms and genomic architecture, providing essential foundational information but limited evidence about adaptation. Level 4 (mechanistic causation) provides the highest molecular resolution by revealing causal mechanisms through multi-omics, 3D genome analyses, and genetic manipulations (e.g., CRISPR/Cas9 or inversion reversion), typically in highly controlled conditions. Integrating evidence across levels strengthens inference from genomic correlation to mechanistic causation. This visualization was refined using AI technologies to improve graphical clarity and layout; the original raw data and schematics are fully provided in Supplementary Figs. S1 and S2.

5.1 Discovery: Navigating Structural Genomic Architecture

High-throughput sequencing allows researchers to identify and map chromosomal inversions, providing a genomic basis for study. Before testing adaptive significance, scientists must detect these rearrangements and their breakpoints. Earlier work relied on indirect genomic signals, like high F_{ST} , extended LD, and suppressed recombination [5,42,97–99,111]. Recent advances now make direct genomic characterization of inversions possible, eliminating the need for purely inference-based approaches.

Short-read resequencing remains common for finding inversions in non-model plants [112,113]. Tools like Delly [114], Lumpy [115], and Manta [116] use discordant read mapping, split-read alignment, and abnormal insert sizes to detect inversions. These cost-effective methods work for large studies, but often miss large inversions in repetitive regions or fail to pinpoint breakpoints [117].

Long-read sequencing platforms, such as PacBio HiFi and Oxford Nanopore, have greatly advanced inversion discovery [118–120]. Tools such as Sniffles [121] and SVIM [122] detect SVs from kilobases to megabases by spanning inversion breakpoints. These methods resolve repetitive regions and provide nearly complete inversion maps.

Chromosome conformation capture methods, such as Hi-C, provide additional evidence for inversions by revealing unique interaction patterns [123,124]. Inversions change chromosome structure, so Hi-C contact maps often show distinct “bowtie” or disrupted signatures that help pinpoint and confirm breakpoints, especially in large-scale rearrangements [125,126].

Graph-based pangenomes are powerful tools for SV discovery [127,128]. Unlike linear genomes, they capture alternative haplotypes and rearrangements in a single graph, allowing accurate detection of inversion polymorphisms across populations [129–131]. This is especially useful for plants with extensive structural variation, where a single reference can miss adaptive inversions. Table 4 summarizes each method’s strengths and limitations by evidence tier to guide tool selection.

Table 4: Methods for inversion discovery and validation.

Method	Data Requirement	Applicability	Strength	Cost/Effort	Key Limitation
F_{ST} /PCA	WGS (SNPs)	Population screening	High-throughput scalability; broad population screening	Low	Indirect; confounding by demography
Local PCA	WGS (SNPs)	Detecting discordance	Captures local genealogical discordance	Low	Requires good sampling design
Delly/Lumpy	Short-read WGS	Large-scale cohorts	Computational efficiency for large-scale cohorts	Moderate	Breakpoint imprecision; repetitive areas
Sniffles/SVIM	Long-read (HiFi/ONT)	Accurate assembly	Base-pair level breakpoint resolution	High	Sequencing cost; depth requirements
Hi-C	Chromatin capture	Large SVs/Topology	Resolves complex 3D chromosomal topology	High	Expensive; specialized library prep
Pangenome Graph	Multi-assembly	High-diversity pops	Captures full structural variation spectrum	Very High	Computationally intensive; complex pipelines

Abbreviations WGS, HiFi, and ONT denote whole-genome sequencing, High Fidelity, and Oxford Nanopore Technologies, respectively.

5.2 Level 1 & 2: Establishing Evolutionary Significance and Phenotypic Linkage

After finding SVs, we use **Level 1** population-genomic methods to assess their evolutionary importance (Fig. 2; Table 4). Local PCA, using tools like Lostruct, helps spot inversion-associated regions by visual-

izing discordance across chromosomes [96]. Elevated LD and F_{ST} peaks also indicate inversions [97–99]. Landscape genomics and genotype-environment association tools, such as Latent Factor Mixed Models (LFMM) [132], can then test if inversion frequencies track environmental gradients.

When crossbreeding is not possible, population genomics offers computational ways to infer selection across regions. Researchers use advanced pipelines to scan SNP arrays or whole-genome data for local genomic anomalies. Landscape genomics and genotype-environment association (GEA) analyses correlate SV allele frequencies with climate variables, revealing clinal patterns along environmental gradients [133]. Divergence-based methods track F_{ST} outliers and genomic clines to spot regions of high differentiation despite gene flow [134]. While these models effectively identify candidate inversions in large wild populations or trees, they can only infer historical selection and cannot demonstrate the functional or physiological mechanisms linking inversions to climate resilience [100,135].

Level 2 moves beyond spatial patterns by linking inversions to adaptive traits. Common garden experiments grow different inversion karyotypes in uniform conditions to separate genetic effects from environmental plasticity [136,137]. This tests whether the inversion genetically determines traits such as flowering time and grain size [137]. Quantitative Trait Locus (QTL) mapping in controlled crosses checks if climate-resilient traits map to the inversion, confirming its role as a supergene. RNA-sequencing transcriptomics (RNA-seq) then reveals if the inversion changes gene expression under stress, connecting structural variation to macroclimatic stress tolerance [138,139].

To test if adaptive traits co-localize with SVs, researchers use QTL mapping and Genome-Wide Association Study (GWAS) [140]. Crossing divergent ecotypes creates F2 or recombinant inbred line (RIL) populations for mapping climate-resilient traits to chromosomal regions [141,142]. However, mapping inversions is challenging: they suppress crossing over in heterokaryotypes, thereby increasing LD and causing all genes within the inversion to segregate as a single large locus [143]. Standard GWAS can reveal strong associations between an inversion haplotype and traits like transpiration or flowering time, but recombination suppression prevents fine-mapping the causal locus [144]. This results in a cluster of fully linked markers, requiring alternative validation strategies.

To separate genetic effects from environmental plasticity, researchers use common garden experiments in controlled settings, such as phytotrons or greenhouses. Exposing different SVs to simulated stress (e.g., heat or drought) tests if specific genomic architectures consistently give better physiological performance. This allows precise tracking of photosynthetic efficiency, stomatal conductance, and leaf water potential under set conditions [145,146]. Adding comparative metabolomics helps reveal whether inversion-linked genes drive coordinated changes, such as increased levels of osmoprotectants or antioxidants [147]. While these experiments lack field complexity, they are key for demonstrating whether an inversion variant provides a reproducible physiological advantage against certain climate stressors.

5.3 Level 3 & 4: Fitness Quantification and Mechanistic Causation

Level 3 focuses on fitness, measuring the selective advantage of inversions under specific conditions (Fig. 2). Reciprocal transplant experiments are the gold standard for estimating selection in natural environments [12]. By growing populations with different inversion arrangements in both native and non-native habitats [148], researchers can track life-history traits across seasons [12]. By tracking life-history traits and fitness proxies, such as seed output, germination, seedling survival, biomass, phenology, and fecundity across microclimates, researchers can assess home-site advantage and estimate selection on inversion haplotypes [149,150]. While this approach offers high ecological realism and strong evidence of natural selection, it remains labor-intensive and sensitive to environmental variability, which can obscure specific

climate drivers [151]. Additionally, experimental evolution studies can also track changes in karyotype frequencies under stress, providing real-time evidence of selection against maladaptive arrangements [135].

Level 4 of the roadmap focuses on pinpointing the genetic mechanisms within an inversion, rather than the whole block (Fig. 2). While this is the frontier of functional genomics, it is not necessary for every study. Many strong cases of adaptive inversions in *Helianthus* [3,9,16,27,53,54] and *Mimulus* [15,17,34,49,152] rely on converging evidence from Levels 1–3, without CRISPR manipulation. Definitive proof comes from using clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPR-associated protein 9 (Cas9) to create or reverse chromosomal inversions *in vivo* [50,51], and from reverting an adaptive inversion in the same genetic background to test whether architecture or linked mutations drive adaptation. This distinguishes structural from sequence effects. Combining CRISPR with gene knockouts or base editing within the inversion clarifies the molecular mechanisms of plant adaptation in changing environments [153].

5.4 Mechanistic Dissection of Inversion Functions

Genetic confounding complicates structural variation genomics, as natural inversions harbor many background variants from diverse histories. Near-isogenic lines (NILs) address this by backcrossing inversions into a uniform genetic background via marker-assisted selection [154], yielding lines that differ mainly at the inversion and minimizing other variation [155,156]. Consequently, observed differences in phenology or drought tolerance can be attributed to the inversion, not background mutations. NILs are widely used to isolate inversion effects in crops like maize, rice, and wheat [157–159].

Demonstrating an inversion as a regulatory supergene—not just linked loci—requires a multi-omics approach. RNA-seq under stress identifies differentially expressed genes (DEGs) within the inversion, with spike-ins for normalization [160]. Allele-specific expression in heterokaryotypes distinguishes cis-from trans-effects [161]. Comparing gene expression in standard and inverted chromosomes reveals if breakpoints disrupt cis-elements or act through trans-factors [162,163]. eQTL mapping can show if the inversion is a regulatory hotspot [164]. Isoform sequencing (Iso-Seq) captures altered transcript architecture, such as alternative splicing and fusion transcripts, often missed by short reads [143,165,166]. These changes add post-transcriptional complexity, rarely documented in plant evolution [106,143].

High-resolution metabolomics links transcriptomic changes to phenotypic adaptation. Monitoring metabolite shifts from gene and transcript variants shows how inversions affect phenotypes [13,27,167]. Integrating epigenomics, transcript isoforms, regulatory hotspots, metabolic data, and 3D genome architecture clarifies how structural variation impacts fitness under climate stress [168]. Inversions rearrange DNA and nuclear topology, creating new regulatory landscapes and enabling rapid evolution. This involves reorganizing topologically associating domains (TADs) that restrict enhancer–promoter interactions in plants [169–171]. Inversion breakpoints can disrupt 3D boundaries, rewiring chromatin and genome configurations [170].

Inversions disrupt 3D genome architecture mainly via enhancer hijacking [172,173] and position effects [174,175]. Enhancer hijacking repositions genes near or away from enhancers, causing tissue-specific expression changes without altering coding sequences [176]. Position effects shift genes across heterochromatin-euchromatin boundaries, rapidly changing expression and plasticity [105,177]. Assays for Transposase-Accessible Chromatin using sequencing (ATAC-seq), Chromatin Immunoprecipitation sequencing (ChIP-seq), and Hi-C reveal how inversions reshape the epigenetic landscape and 3D gene regulation [178–180]. Despite these advances, direct evidence for inversion-induced enhancer hijacking in plants is limited [176].

5.5 Pragmatic Synthesis: A Weight-of-Evidence Approach

After connecting an inversion to an adaptive trait, the next challenge is distinguishing true causal factors from background variation. Population genomics can flag candidate regions but rarely separates neutral from functional changes. Combining NILs, multi-omics, and 3D epigenomics offers a stronger approach to resolve this complexity than any single method.

NILs help separate structural effects from demographic history. Multi-omics approaches, like RNA-seq and metabolomics, clarify functional consequences by linking transcript isoforms to metabolites. 3D epigenomics (Hi-C, ATAC-seq) offers deeper insight into chromatin rewiring and enhancer hijacking. This toolkit is a flexible framework, not a strict rule, and should be tailored to each study system when assessing an inversion as a unified regulatory supergene.

A key question in this roadmap is what counts as sufficient evidence to classify a chromosomal inversion as “adaptively validated.” While Level 4 mechanistic proof is the ideal, demanding it in all cases is unrealistic and exclusionary. For many non-model organisms, such as long-lived trees, marine macrophytes, or wild species, creating transgenics or multi-generation inbred lines is often unfeasible. Thus, we suggest adopting a context-dependent, weight-of-evidence approach, where validation comes from multiple converging lines of evidence rather than a strict requirement for the highest level of proof.

For species with complex life histories or transformation challenges, strong support for adaptation comes from combining Level 1, 2, and 3 evidence (Fig. 2). If landscape genomics shows parallel environmental clines (Level 1), common garden studies link karyotypes to distinct, fixed phenotypes (Level 2), and reciprocal transplants or demographic tracking reveal a home-site fitness advantage (Level 3), the inversion meets criteria for ecological validation. Such multi-layered alignment is very unlikely to result from drift or hitchhiking alone [181]. While Level 4 is key for dissecting mechanisms within the supergene, linking genomic, phenotypic, and fitness evidence offers a rigorous baseline for declaring an inversion functional and adaptive to climate change [35].

5.6 Practical Implementation Challenges in Non-Model Plant Species

While the proposed “Causality Roadmap” provides valuable conceptual guidance for linking genomic signals to adaptive phenotypes, its implementation in non-model plant species faces significant challenges. Non-model taxa often have complex genomic features, including high heterozygosity, extensive repetitive elements, and polyploidy, which complicate the detection, alignment, and assembly of large SVs such as chromosomal inversions. In contrast to model organisms with well-curated reference genomes, the complexity of non-model genomes often obscures variant breakpoints and evolutionary origins, making accurate genomic identification technically demanding.

Establishing functional causality from correlative genomic patterns requires robust experimental validation, such as gene editing, transcriptomic knockdowns, or creating NILs. These tools are often difficult or currently impossible to develop for ecological or forestry taxa due to long generation times, low transformation efficiencies, or the absence of stable transformation systems. Therefore, applying this roadmap to non-model systems will require a cautious, phased approach, starting with comparative multi-omics integration and population-scale resequencing before direct functional assays.

5.7 Critical Analysis of Methodological Trends

A review of 11 key plant systems (Table 2) reveals a clear methodological hierarchy and a notable “validation gap.” All studies use Level 1 genomic methods, such as LD detection, F_{ST} outliers, and environmental associations, to identify SVs. Yet, functional validation lags: 63% establish phenotypic links (Level 2),

27% use field-based fitness data (Level 3), and only 18% demonstrate mechanistic causation with Level 4 techniques such as CRISPR/Cas9 reversal (Table 5).

Table 5: Integrated quantitative synthesis and comparative evaluation of validation approaches across the chromosomal inversion causality roadmap. Evidence tiers (Levels 1–4) reflect the analytical continuum from observational genomic scans to functional causality tests. Literature frequencies quantify the percentage of empirical studies employing each tier ($N = 11$ systems), demonstrating a strong methodological bias toward correlative Level 1 approaches (100%) over experimental Level 3 fitness assessments (27%) and Level 4 causal validations (18%). Methodological categories are evaluated by primary technical strengths, operational limitations, and ideal biological applications, highlighting key bottlenecks in validating supergene functionality in non-model and long-lived taxa.

Evidence Tier & Focus	Literature Frequency	Representative Approaches	Primary Strengths	Key Limitations	Ideal Biological Context
Level 1: Genomic Association (Correlative)	100% (11/11)	Population resequencing, GEA, Lostruct, pyRho, Long-read pangenomics, LD heatmaps	Enables large-scale cohort screening and precise resolution of complex repetitive breakpoints	Susceptible to demographic noise; high sequencing costs for long-read assemblies	Initial evolutionary scans and reference-grade structural variant detection
Level 2: Phenotypic Linkage (Associative)	63% (7/11)	QTL mapping, Common garden experiments, RNA-seq, Hi-C/TAD mapping	Separates genetic effects from environmental plasticity; identifies 3D chromatin regulatory shifts	Broad QTL intervals due to suppressed recombination; requires fresh, high-quality tissue	Dissecting climate-resilient polygenic traits and regulatory domain reorganization
Level 3: Fitness Assessment (Selection-based)	27% (3/11)	Reciprocal transplants, <i>In-situ</i> lifetime fitness tracking, Fitness proxies	Quantifies home-site advantage and local adaptation directly under natural selection pressures	Multi-year commitment; logistically challenging for long-lived or large organisms	Measuring differential survival and fecundity along environmental gradients
Level 4: Mechanistic Causation (Causal)	18% (2/11)	CRISPR/Cas9 inversion engineering, Site-directed genomic surgery	Establishes direct functional causality while eliminating background genetic variation	Low transformation and editing efficiency in recalcitrant or non-model species	Functional verification of candidate breakpoints in transformable model systems

This distribution reveals that, despite advances in sequencing and pangenomics, most research still depends on correlative evidence. Limited validation at Levels 3 and 4, especially for long-lived species where transformation and fitness tracking are difficult, underscores the need for functional assays that do not require transgenics or tissue culture. Bridging this gap is key to shifting the view of inversions from mere ‘genomic artifacts’ to ‘mechanistic buffers’ against climate change.

6 Evolutionary Cause or Consequence: The “First-Event” Dilemma in Structural Genomic Evolution

6.1 Evolutionary Trajectories: Three Models of Inversion-Mediated Adaptation

Despite progress in understanding chromosomal rearrangements, a key debate remains: are inversions primary drivers of local adaptation or evolutionary byproducts? This question has led to two main models: “Alleles-First” (Model A) and “Inversion-First” (Model B). Recent pangenomic and divergence studies have added a third, “Introgression-First” (Model C) (Table 6). Distinguishing among these pathways is challenging but essential for understanding how genomic architecture shapes speciation and climate resilience.

Table 6: Chronological and mechanistic distinctions among the three main evolutionary models of inversion origin. The table compares the Alleles-First, Inversion-First, and Introgression-First frameworks by their evolutionary baselines, the sequence of structural and functional mutations, and the inversion’s primary role in supporting local adaptation.

Evolutionary Model	Initial State (Time 0)	The “First Event” Trigger	Role of the Inversion
Alleles-First	Dispersed adaptive mutations already segregated in the population.	A new inversion occurs and captures the pre-existing adaptive alleles.	Passive Shield: Acts as a container to protect existing combinations from recombination swamping.
Inversion-First	A neutral or underdominant inversion arises in a homogeneous genomic background.	Adaptive mutations subsequently accumulate inside the protected inverted region.	Active Incubator: Suppresses recombination first, creating a safe zone for localized adaptation.
Introgression-First	Adaptation or inversion evolves independently in an allopatric/sister lineage.	Interspecific hybridization and backcrossing (gene flow) introduce the inverted block.	Cassette Transporter: Imports a fully-formed, pre-packaged “supergene module” into the recipient gene pool.

Model A: Alleles-First (Consequence)

Adaptive Alleles Cross-Environment → Divergent Selection → New Inversion Arises → Captures Co-adapted Block

In Model A, the “Alleles-First” scenario, local adaptation happens before the SV forms. Here, unlinked loci across a chromosome adapt to different climates through divergent selection, but gene flow and recombination can break up these combinations. If a chromosomal inversion later arises that includes these adaptive alleles, recombination suppression is favored, preserving the adaptive trait set. Thus, the inversion acts as a secondary, reinforcing mechanism shaped by prior selection [19,182].

Model B: Inversion-First (Cause)

Neutral/Slightly Deleterious Inversion → Suppressed Recombination → Accumulates New Mutation Locally

Model B, the “Inversion-First” pathway, sees the structural rearrangement as the main driver of divergence. In this scenario, an SV initially appears neutral or slightly deleterious. If it persists via genetic drift, its suppression of recombination creates an insulated evolutionary zone. Over time, this region accumulates new, climate-resilient mutations. Thus, the rearrangement itself causes adaptation, setting the stage for a new supergene [111,182,183].

Model C: Introgression-First (Modular Translocation)

Ancient Adaptive Inversion in Species A → Hybridization/Introgression → Transferred Whole Module to Species B → Rapid Adaptation

Model C, the “Introgression-First” pathway, describes adaptation via horizontal transfer of a whole, pre-adapted genomic module rather than gradual local evolution. Here, an adaptive inversion and its supergene evolve in a donor species (Species A). After hybridization with a recipient species (Species B), the full structural block is transferred through adaptive introgression [184]. The inversion suppresses recombination in hybrids, protecting co-adapted alleles during backcrossing [8,185]. This allows Species B to acquire the climate-resilient module intact, enabling immediate ecological divergence and rapid adaptation without new mutations.

Empirical evidence from model systems strongly supports the Introgression-First paradigm. In wild sunflowers (*Helianthus*), multi-megabase supergenes controlling ecotype shifts often predate the current species, pointing to ancient introgression from related species [53]. Similar patterns are seen in *Mimulus* (monkeyflowers) [15], *Drosophila* [186–188], and marine *Littorina* ecotypes [189,190], where shared structural polymorphisms across species boundaries reflect trans-specific modules maintained by introgression and balancing selection.

6.2 Methodological Challenges in Disentangling Evolutionary Causality

Although frameworks for inversion-mediated adaptation exist, distinguishing these evolutionary paths empirically remains challenging. As summarized in Table 6, determining if an inversion is a primary architect (Model B), passive protector (Model A), or imported module (Model C) depends on overcoming key methodological limitations.

Adaptive introgression may explain the rapid spread of adaptive SVs, but distinguishing it from incomplete lineage sorting or complex demography is difficult [191]. *D*-statistics (ABBA-BABA) are widely used to detect gene flow [191,192], but can be confounded by mutation rate variation and population structure, which may mimic introgression [193]. Coalescent-based models also depend on assumptions about population size and divergence timing that are often unmet in non-model systems, thereby increasing false-positive rates [194,195]. Newer methods, such as Ancestral Recombination Graphs and simulation-based approaches, provide higher-resolution evolutionary histories [196,197], but require substantial computational resources and high-quality genomes [198,199]. Thus, identifying the sequence of events for an inversion requires combining topological discordance, branch-length analysis, and robust demographic modeling rather than relying on single-statistic tests.

To distinguish among these models, researchers should go beyond static fitness measures and focus on evolutionary chronology. Integrating graph-based pangenomics, multi-species coalescent simulations, and molecular clock analyses helps date structural breakpoints relative to allelic variation. Clarifying this sequence is key to understanding how structural variation shapes selection and genetic homogenization during climate adaptation.

7 Evolutionary Drivers and Methodological Considerations

Chromosomal inversions play a key role in maintaining co-adapted gene complexes and promoting local adaptation. However, most discussions emphasize their immediate adaptive benefits and often suggest they are optimal evolutionary solutions. A closer examination shows that inversions also introduce high evolutionary costs and constraints. Suppressed recombination in heterokaryotypes limits the effectiveness of natural selection, leading to the accumulation of harmful mutations and a substantial recessive load within inverted regions. As a result, not all inversion polymorphisms reflect perfect adaptation; many persist due to a balance between adaptive divergence and the risk of genomic degradation.

7.1 The Paradox of Recombination Suppression: Balancing Selection and Mutation Load

The adaptive value of inversions stems from recombination suppression, but this paradoxically traps deleterious mutations, thereby increasing overall mutation load [27,200]. Balancing selection can maintain inversion polymorphisms, but it may also prevent the removal of harmful variants [201]. It is crucial to determine whether climate resilience is due to beneficial alleles or to the persistence of mildly deleterious alleles. Distinguishing the effects of balancing selection from the costs of recombination suppression is a core challenge in understanding why inversion haplotypes persist.

The fitness effects of chromosomal inversions depend on environment, development, and sex, complicating selection and removal of harmful mutations [202–204]. While inversions are linked to adaptation and speciation, they can also retain deleterious mutations [4,28,205]. Balancing selection promotes diversity and adaptation but may weaken purifying selection, letting harmful mutations persist [201]. Thus, balancing selection maintains diversity and inversion polymorphisms, but also protects variants that lower fitness, highlighting the complex trade-off between adaptation and genetic load in evolution.

The theoretical framework for mutation load accumulation due to recombination suppression is well-established and supported by empirical models in animals, such as *Drosophila* supergenes. However, direct evidence from plant systems remains limited. The evolutionary consequences of this mutation load paradox likely differ by plant life history. Perennial woody plants, unlike short-lived annuals, have long generation times, large effective population sizes, and delayed reproduction. In long-lived perennials such as pine (*Pinus*) [58] and birch (*Betula*) [59], ongoing recombination suppression across large inversion blocks could accelerate the accumulation of deleterious mutations over time. At the same time, perennials may have unique buffering mechanisms, including meristematic redundancy or somatic mutation selection, which could affect their tolerance to recessive load compared to annuals. Future empirical studies should systematically assess how different life history strategies influence the balance between the adaptive benefits of inversions and long-term genomic degradation.

7.2 Distinguishing Adaptive Inversions from False-Positive “Islands”

A major challenge in structural genomics is the frequent occurrence of false-positive inversion islands. Artifacts like alignment bias, low sequencing depth, or complex demography can create selection-like signals, such as high LD or local F_{ST} peaks [206–208]. As noted in Section 4 and summarized in Table 3, these patterns are not unique to SVs, so finding a divergent region is not definitive proof of a functional inversion. Filtering SNPs by LD or using reference-agnostic analyses helps control for demographic biases [207,208]. Accurate classification requires high-resolution long-read assemblies integrated with topological data, such as Hi-C, Oxford Nanopore, and PacBio, rather than relying on summary statistics confounded by demography [206,209]. Studies should use stricter evidence standards and treat candidate inversion islands as provisional until physically confirmed.

Environmental selection is often used to explain geographic clines or population differences in inversion frequencies, and past interpretations have frequently attributed all SV patterns to local adaptation. However, non-adaptive and neutral evolutionary forces are equally important in shaping genomic architecture. Demographic events such as population bottlenecks, historical range expansions, and secondary contact between previously isolated lineages can significantly affect inversion frequencies through genetic drift. As a result, spatial variation in inversion polymorphisms may arise or persist without direct selection. Rigorous demographic modeling is essential to distinguish neutral historical effects from true adaptive signatures when interpreting inversion distributions.

7.3 The Life Cycle of Structural Variants: From Origin to Evolutionary Fate

Understanding the ‘life cycle’ of chromosomal inversions, from formation to dissolution, is crucial. An inversion’s fate depends on selection acting on its alleles and the chance of rare double-crossover events that can revert the arrangement, allowing some gene flow [210]. The age of an inversion shapes its adaptive significance: older inversions may be stable parts of the genome, while newer ones might reflect short-term adaptation. In *Mimulus guttatus*, older and younger nested inversions (e.g., inv_chr8A, inv_chr8B) illustrate this, with older ones as reservoirs of adaptive variation and younger ones capturing

new traits [17]. The persistence of inversions in plants results from historical constraints, selection, and environment. In *Mimulus guttatus*, inversions promote reproductive isolation and adaptation across environments, supporting their role in stable adaptation [12]. Future studies should integrate temporal evolutionary modeling (e.g., molecular clock calibrations) and structural genomics to view inversions as dynamic, rather than static, genomic features.

A more rigorous evaluation of methods for detecting inversions and inferring selection pressures is necessary. Many previous studies have relied on indirect genomic signatures or correlations with environmental gradients to suggest adaptation, but these approaches face technical limitations. Issues such as low sequencing coverage, difficulties in assembling complex SVs from short reads, and confounding gene flow can obscure true selective signals or generate false positives. Associations between inversion frequencies and environmental factors should be interpreted with caution. Without robust long-read validation or functional evidence, over-interpreting spatial correlations may conflate neutral demographic patterns with true adaptive significance.

8 Future Perspectives: Embracing Pangenomics, Transgenic Barriers, and Predictive Artificial Intelligence (AI)

Site-directed nucleases have revolutionized genetic validation. However, moving from single-gene edits to analyzing plant chromosomal inversions poses multidisciplinary challenges [4,9]. While future research aims to combine high-throughput gene editing with the complex dynamics of wild plant genomes under climate stress, achieving this integration will require substantial methodological breakthroughs (Fig. 3).

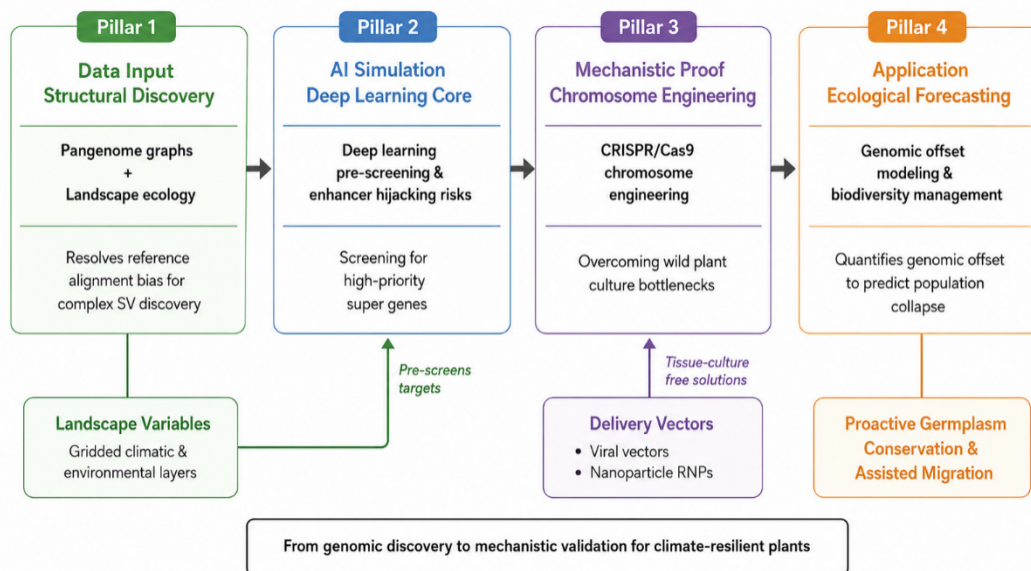


Figure 3: Proposed integrative workflow for structural variant discovery, validation, and evolutionary forecasting. This roadmap synthesizes structural genomic research to address climate stress over the next decade. The workflow includes: (1) Discovery, which captures complex inversion networks using graph-based plant pangenomics; (2) Simulation, which uses predictive AI to simulate regulatory effects and pre-screen target supergenes; (3) Validation, which overcomes traditional transformation bottlenecks with tissue-culture-independent clustered regularly interspaced short palindromic repeats (CRISPR)/Cas9 engineering; and (4) Application, which integrates empirical clinal shifts into genomic offset models to guide germplasm conservation and assisted migration. Abbreviations SV and RNPs denote structural variation and ribonucleoproteins, respectively. This illustration was optimized using AI-assisted tools for clarity, with foundational schematics and source data archived in Supplementary Fig. S3.

A key challenge is shifting from linear reference genomes to graph-based pangenomics [129–131]. Traditional pipelines often miss complex SVs due to single-reference alignment bias. With more long-read sequencing, building species-specific pangenome graphs is expected to help botanists catalog inversion polymorphisms across wild and cultivated populations. This resolution is especially vital for long-lived perennials and species with large, repetitive genomes, where nested inversions and transposons complicate breakpoint detection [127,211]. Integrating pangenomics and landscape data holds the potential to move the field beyond isolated inversions, ultimately enabling mapping of SV networks that help populations adapt to climate change once computational and assembly hurdles are fully overcome.

CRISPR/Cas9 dual-guide RNA methods can functionally validate pangenomic variants by mimicking or reverting chromosomal arrangements *in vivo* [66]. However, using these techniques in wild, non-model plants poses major hurdles in tissue culture and transformation. While targeted megabase-scale inversions have been generated in model species like *Arabidopsis* and tomato [51,65], validating them across diverse ecotypes, especially forest trees, remains currently difficult [212]. Addressing these challenges will require the future development of new tissue-culture-independent delivery systems, such as viral vectors [213] or nanotech-mediated ribonucleoprotein (RNP) delivery to floral meristems [214]. Until transformation becomes genotype-independent [215], validating mechanistic causality of inversions across taxa will remain severely limited.

Beyond functional validation, an important long-term objective is predicting evolutionary responses to rapid climate change. Integrating landscape genomics with models such as genomic offset, which measures mismatches between current genetics and future fitness landscapes [216–218], enables researchers to forecast population vulnerability using spatial shifts in inversion frequencies, although these models still require rigorous empirical calibration. Because adaptive inversions carry supergenes controlling key traits, tracking their clinal shifts is a sensitive genomic indicator. Modeling these patterns may eventually help predict if certain inversion haplotypes will rise in frequency, shift geographically, or fix under future warming scenarios. Including SVs in forecasting algorithms could better identify high-risk populations and support conservation and assisted migration strategies [219,220], provided that assumptions regarding selective neutrality and demographic stability are met.

Looking further ahead, frameworks are increasingly exploring the integration of AI and deep learning to integrate structural, transcriptomic, and 3D chromatin data [221]. Although currently in their infancy, predictive models are being conceptualized to simulate phenotypic and regulatory outcomes of chromosomal rearrangements, potentially helping forecast if an inversion will isolate a gene or cause unwanted regulatory effects [222,223]. In theory, future iterations of AI can pre-screen supergene candidates and pinpoint key loci within haplotype blocks, letting researchers prioritize impactful SVs for validation. If successfully developed and validated, this multi-layered strategy combines multi-omics, machine learning, and tissue-culture-free editing into a unified framework (Fig. 3) and may eventually improve prediction, management, and conservation of plant diversity as environments change.

9 Conclusion

Chromosomal inversions are a key mechanistic link between genomic variation and ecological adaptation. Past research has mainly described inversion patterns, but future work should focus on causality: showing how inversions reshape recombination, preserve adaptive alleles, and impact plant performance under changing environments. Inversions are not just SVs; they form a genomic framework for organizing and maintaining complex adaptations. Integrating long-read sequencing, functional genomics, ecological

experiments, and genome engineering can move inversion biology from description to prediction, offering insights into plant evolution and practical tools for climate resilience.

The future of plant inversion biology depends not on how many inversions we find, but on how well we prove their adaptive value. The field is moving from discovery to validation, treating SVs as testable links between genotype, phenotype, and fitness. The next frontier in inversion biology is no longer discovering inversions, but demonstrating why they matter. This shift calls for integrating genomics, ecological experiments, functional genetics, and predictive modeling into a unified framework. With this approach, inversions could be recognized as core genomic architectures that enable plants to adapt and persist amid climate change.

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