

## Opinion

## Evolving Inversions

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**Empirical data suggest that inversions in many species contain genes important for intraspecific divergence and speciation, yet mechanisms of evolution remain unclear. While genes inside an inversion are tightly linked, inversions are not static but evolve separately from the rest of the genome by new mutations, recombination within arrangements, and gene flux between arrangements. Inversion polymorphisms are maintained by different processes, for example, divergent or balancing selection, or a mix of multiple processes. Moreover, the relative roles of selection, drift, mutation, and recombination will change over the lifetime of an inversion and within its area of distribution. We believe inversions are central to the evolution of many species, but we need many more data and new models to understand the complex mechanisms involved.**

### The Paradox of Inversions and Felsenstein's Dilemma

Early studies of inversions were restricted to species with easily visualised chromosomes (e.g., flies). Today, inferring the presence of inversions is technically possible in many species as reference genomes, genetic maps, and extensive sequencing data become available. Classical work has suggested that inversions are important in local adaptation and speciation [1,2], and later studies have emphasised that they offer a potential solution to **Felsenstein's dilemma** [3] (see [Glossary](#)). Suppressed recombination among genes inside the inversion, in **heterokaryotype** individuals, results in largely independent genome evolution of **derived and ancestral arrangements** and opportunities for divergence and speciation [4–9]. Yet, inversions are commonly polymorphic within populations [10]. This is a paradox that current models cannot resolve, because **balancing selection** (which maintains polymorphism within populations) typically opposes divergence (needed for speciation). However, the evolution of inversions is multifaceted and variable over space and time. Using a life-history framework that describes the possible fates of new inversions, we highlight the need for a deeper understanding of the evolution of inversions by making connections among existing ideas and identifying gaps in our knowledge.

### A Life-History Perspective on Inversions

Many authors have considered the conditions for the initial spread of a new inversion [4,8,11], while the subsequent evolution of the inversion has been studied less, especially the changing allelic contents of the ancestral and derived arrangements. The life history of an inversion embraces evolutionary change from its appearance by mutation of a single, flipped **haplotype**, to its loss or fixation. Importantly, a new derived arrangement has no genetic variation at the start, while the ancestral arrangement is variable (in common with collinear regions of the genome). Over time, the derived arrangement tends to become increasingly variable (unless selective sweeps are frequent), and recombination among haplotypes increases as **homokaryotypic** individuals become more common. Thus, the dynamics of inversion polymorphisms change over time, and there are also many possible interactions between the derived and the ancestral arrangement with implications for the fate of the inversion and its role in the evolution of the population ([Box 1](#)).

### Highlights

Empirical data suggest that many inversions are maintained polymorphic within populations by balancing selection, which impedes divergence and speciation.

Contrary to earlier beliefs, we here argue that balancing and divergent selection may act together shaping the frequencies of inversions, maintaining many of them polymorphic, and having important consequences for adaptation and speciation.

Inversions are not static but the derived and ancestral arrangements of an inversion continue to evolve, partly separately from each other and from the collinear genome, until lost or fixed. However, the evolution of inversions after their establishment is often neglected.

New modelling approaches and data from additional taxa are needed to understand how inversions evolve over time and space, and what roles they play in adaptation, divergence, and speciation.

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The rates of origin of new inversions or new mutations inside inversions are rarely recorded, but most new, derived arrangements are lost by genetic drift soon after they appear, as the initial frequency is low ( $\sim 1/2N$ ). Deleterious effects at breakpoints or fitness reduction in heterokaryotypes due to the elimination of recombinant gametes increase the probability of rapid loss [4]. In contrast, inversions with positive fitness effects in heterokaryotypes occur less frequently but are more likely to become established, that is, to be maintained long enough in the population for other evolutionary processes to influence their fate. Rarely, a new inversion might capture a universally favoured haplotype, for example, one with a low load of deleterious mutations [12] or favoured by meiotic drive [13], and spread rapidly to fixation.

An inversion polymorphism can establish in one of two ways. The derived arrangement might spread to fixation by drift or selection in some populations while being absent or lost in others, potentially with some local polymorphism maintained by gene flow (we refer to this as Type I inversion polymorphism). Alternatively, a balanced polymorphism can be supported within one or more populations, for example, by **overdominance** or frequency dependence (Type II inversion polymorphism) (Figure 1). Several different mechanisms influence these alternatives. Genetic drift in small and isolated populations can fix a new arrangement locally, even with some **underdominance** [1], leading to Type I polymorphisms. Selection for local adaptation, even in the presence of gene flow [6], can also generate Type I polymorphisms. However, balancing selection is needed to maintain Type II polymorphisms. This can arise from **epistatic interactions** among alleles at different loci [14], from **associative overdominance** [15] or, on rare occasions, when the inversion captures a locus that is, itself, under balancing selection (Box 1).

Local adaptation with gene flow is common, and has been suggested as a likely driver for the establishment of new inversions [6]. If locally fit alleles at two or more loci on the same chromosome are captured by an inversion, their association is conserved for extensive periods of time and the haplotype within the inversion is favoured over recombining haplotypes. Importantly, the rate of spread of the inversion is proportional to the migration rate between populations [6], and larger inversions that capture more locally adapted alleles are more likely to spread. Furthermore, populations that cycle between stages of isolation and migration promote the spread of inversions under even broader sets of conditions [16].

Critically, the types of alleles that drive these processes continue to arise by mutation, on both the ancestral and derived arrangements, with potentially profound consequences for the life history of the inversion.

### The Evolutionary Processes Following Establishment

The relative roles of selection, drift, mutation, and recombination change over the lifetime of an inversion. For example, the derived and ancestral arrangements diverge by new mutations, but converge by occasional **gene flux** (see Figure I in Box 1). As the contents of both the ancestral and derived arrangements change over time, the opportunities for selection also change. Recombination is infrequent in a low-frequency arrangement, because **homokaryotypes** are rare, and this also shifts the balance between drift and selection towards drift [5]. Further inversion mutations in the same genomic region might reduce gene flux or extend the genomic region of suppressed recombination [17]. The result can be a complex and changing pattern of differentiation between arrangements, as seen in the Payne inversion in the vinegar fly *Drosophila melanogaster* [18], analogous to the patterns of differentiation among populations generated by selection–migration balance.

To maintain a Type II (balanced) inversion polymorphism requires either **heterosis** or negative frequency-dependent selection (including selection in temporally or spatially heterogeneous

### Glossary

**Associative overdominance:**

overdominance caused by recessive deleterious alleles or dominant advantageous alleles each present in only one inversion arrangement such that homokaryotypes for the inversion suffer reduced fitness but heterokaryotypes do not.

**Balancing selection:**

selection favouring the maintenance of polymorphism, for two or more alleles at a locus or arrangements in the case of inversions.

**Breakpoint effects:**

positive or negative effects of the disruption of the genome caused by the break and later repair when an inversion arises.

**Coadaptation:**

adaptation at two or more loci to the same environment. Coadaptation can involve, but does not require epistatic interaction between loci (see below).

**Collinear genome:**

the part of the genome that is outside regions polymorphic for inversions.

**Derived and ancestral arrangements:**

respectively, the reversed and original gene order for the part of the genome that contains an inversion (see Figure 2Figure I in Box 1).

**Dobzhansky–Müller incompatibilities:**

negative epistatic interactions between alleles when brought together in the same individual following their independent evolutionary origin. Dobzhansky–Müller incompatibility is thought to be a major source of barriers to gene flow between closely related species.

**Epistatic interactions:**

positive or negative interactions between alleles at two different loci affecting fitness.

**Felsenstein's dilemma:**

that recombination facilitates adaptive evolution but breaks up associations among coadapted genes and in this way counteracts divergence and speciation. Felsenstein [4] considered this a major reason why speciation is not more frequent.

**Gene flux:**

the exchange of genes between two inversion arrangements in a heterokaryotype by double crossover and gene conversion. Gene flux is less likely than exchange between haplotypes due to recombination in the collinear genome or in inversion homokaryotypes.

environments), while divergent selection maintains Type I polymorphism. In either case, the alternative arrangements receive different types of mutations (see Figure II in Box 1). At the same time, the fates of these mutations depend on the state of the inversion polymorphism. For example, in the absence of homokaryotypes, **recessive deleterious mutations** are not exposed to selection and tend to accumulate on the rarer arrangement (initially the derived arrangement). Importantly, recent modelling (Berdan *et al.* personal communication) shows that the ancestral arrangement also accumulates deleterious recessive alleles slowly, but at a higher rate than the **collinear genome**. This is because there is also a lower recombination rate in the ancestral arrangement compared to the collinear genome. These processes might result in fitness loss in both homokaryotypes and increased heterokaryotype advantage due to associative overdominance (see Figure II in Box 1). This accumulation of deleterious recessive alleles is more likely under Type II polymorphism than Type I polymorphism where each arrangement has a large local effective population size (Figure 1).

In contrast, inversions that differentiate populations are likely to accumulate further locally adapted alleles and can also acquire alleles that promote assortative mating. However, the advantage provided by suppressed recombination is available only in populations that are influenced by gene flow. Divergent selection can also create and maintain among-population variation for underdominant inversions [6], which are less likely to persist within populations (although this is possible with some forms of frequency dependence [19]).

**Dobzhansky–Müller incompatibilities** are most likely to become associated with inversions that are fixed different between isolated populations, due to the independent spread of mutations under drift or selection. These incompatibilities might be expressed on secondary contact and be important in maintaining reproductive barriers [20]. They can also accumulate within inversions in the presence of gene flow [21]. However, it is unlikely that alleles causing incompatibilities could spread within inversions that are maintained as balanced polymorphisms within populations (Type II inversion polymorphisms), unless the same alleles are advantageous within their own genomic background.

Generally, the conditions for inversion polymorphisms seem broader than those for single-locus balanced polymorphisms. This is for the simple reason that an inversion contains many, potentially interacting loci resulting in many possible allele combinations that could drive the balance. For example, a new inversion that captures both locally favoured or epistatic alleles and deleterious recessive alleles will increase in frequency but rarely reach fixation as the homokaryotype for the inversion expresses the deleterious alleles and is selected against. However, a central point here is that the mechanisms maintaining inversion polymorphisms are not static. If an inversion polymorphism is initially established by, for example, one overdominant locus inside the inversion or by frequency-dependent selection, over time, increasing stability might evolve by the accumulation of different recessive deleterious mutations in the derived and ancestral arrangements building up associative overdominance. Alternatively, increasing divergence between populations might occur as locally adapted alleles and, later, Dobzhansky–Müller incompatibilities accumulate within the inversion. Frequency-dependent selection or heterosis could maintain both balanced polymorphism and divergence between populations at the same time. Equilibrium frequencies of arrangements differ between populations either because the same sets of alleles confer different fitness, or because the same arrangements carry different alleles in each local population, as observed in *D. melanogaster* [22,23]. Thus the end point need not be either among-population divergence (Type I) or balanced polymorphism (Type II); it can be a combination of both (Box 2).

**Haplotype:** a set of alleles, up to the size of the whole haploid genome, that is inherited together until disrupted by recombination or mutation. Genome sequences belonging to the same haplotype are genetically identical. A mutation causing an inversion will produce a derived arrangement (the reversed gene order) which initially only occurs as a single haplotype (see Figure 2Figure I in Box 1).

**Heterokaryotype:** an individual carrying one copy of each of the two arrangements (the ancestral and the derived) of an inversion.

**Heterosis:** classically hybrid vigour; used here for selection favouring heterokaryotypes for the inversion due to associative overdominance, overdominance at individual loci or epistatic interactions.

**Homokaryotype:** an individual carrying two copies of the same arrangement of an inversion. Note that these individuals might be heterozygous at individual loci within the inversion, that is, they carry two distinct haplotypes in this region.

**Introgression:** mixing of genomes of two earlier separated lineages (e. g., species, subspecies, or ecotypes). Introgression is often a result of secondary contact between lineages that have earlier been separated in allopatry.

**Overdominance:** selection favouring heterozygote individuals over homozygote individuals.

**Recessive deleterious mutations:** mutations causing deleterious effects in homozygotes but not heterozygotes.

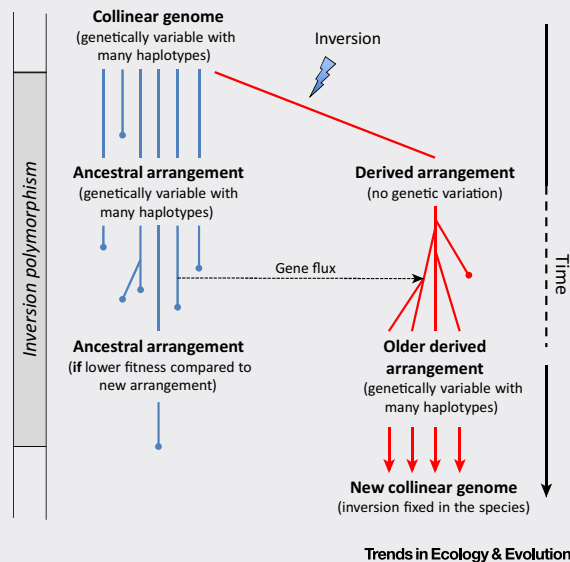
**Reinforcement:** evolution of increased barriers to gene exchange due to selection against hybrids.

**Supergene:** a single locus, containing genes inherited together which influences multiple phenotypic traits.

**Underdominance:** selection favouring homozygote individuals over heterozygote individuals.

## Box 1. Concepts and Mechanisms

When a chromosomal mutation forms an inversion, the derived arrangement has no variation (Figure 1) but accumulates new genetic variation over time by mutation, or by rare gene flux (double crossovers and gene conversion, [44]) in heterozygotes for the inversion (heterokaryotypes). Within the pool of derived haplotypes, recombination is possible in homozygotes for the inversion (homokaryotypes). If the derived arrangement is favourable and finally fixed, it becomes the new collinear region and is only distinguishable by comparing the sequence order with other populations or species (Figure 1).



**Figure 1. Simplified Life History of an Inversion.** A mutation generating a new inversion results in one derived and one ancestral arrangement; the former initially without variation. Over time, point mutations and gene flux add new variation, and selection and drift reduce variation in both arrangements. Eventually, one of the arrangements (in this illustration the ancestral one) might be lost and the remaining arrangement (here the derived one) is the new collinear genome in this genomic position. Note that the single gene flux event shown goes in one direction and while gene conversion is unidirectional, double crossover goes in both directions.

The initial stages in the life history of an inversion are governed by its direct effects (positive or negative **breakpoint effects**), its allelic content, and/or its effects on neighbouring genes. Meiotic problems, including the loss of unbalanced recombinant gametes, might cause underdominance [1] (Figure IIA1,2). Underdominance usually causes loss of the derived arrangement, unless drift or other fitness effects bring it to high frequency.

The inversion might capture different types of alleles that increase or decrease the fitness of the derived arrangement and, critically, mutations subsequently introduce new variation at random into the derived and ancestral arrangements. Some of these mutations tend towards fixing one arrangement locally, thus generating divergence among populations and progress towards speciation (Figure IIB). Others tend to promote polymorphism within populations by generating balancing selection (Figure IIC).

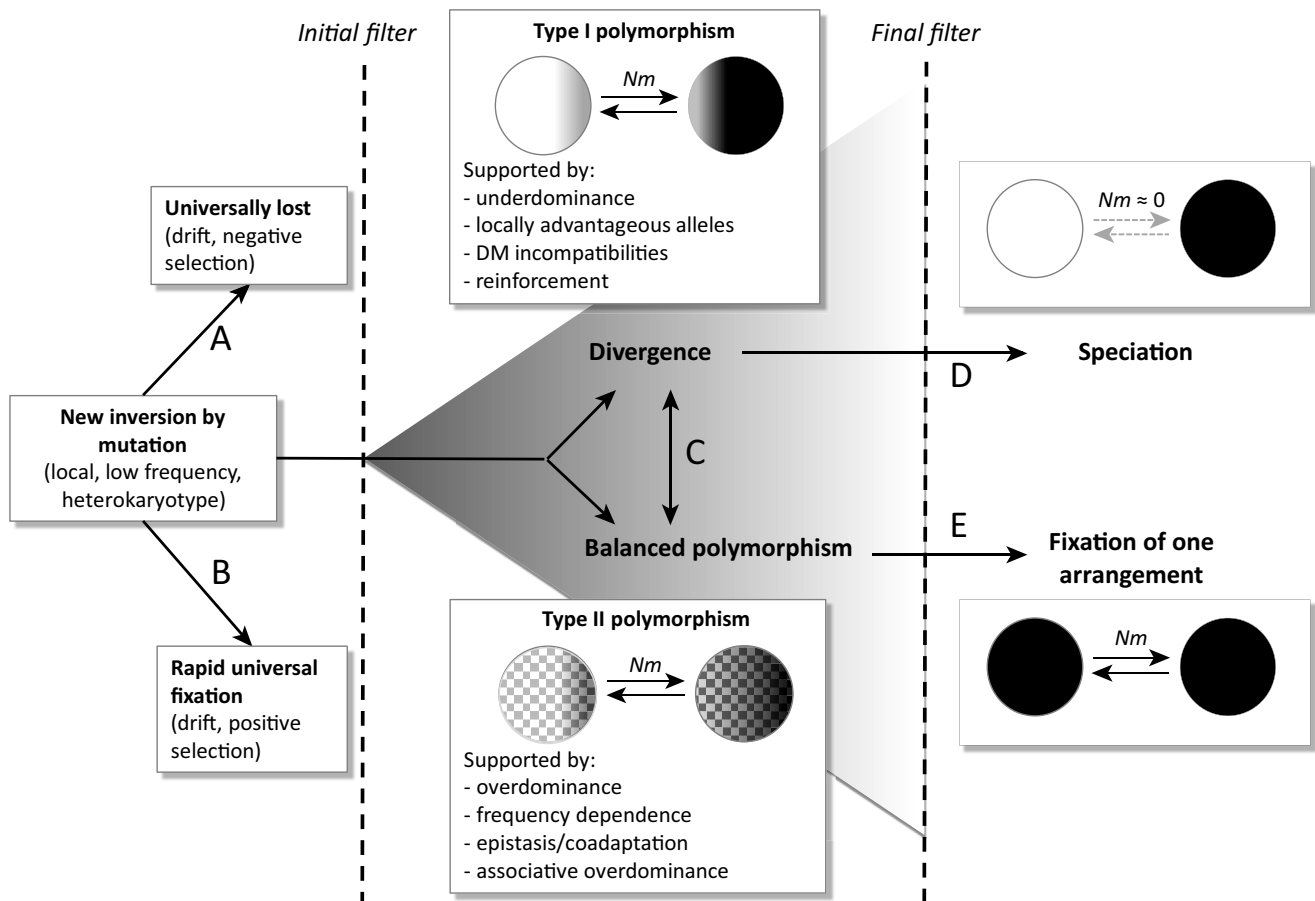
Multiple and locally advantageous alleles within the inverted region, in the presence of gene flow, can favour establishment of the derived arrangement [6], and further locally advantageous alleles can accumulate subsequently (Figure IIB3). Dobzhansky–Müller incompatibilities might accumulate within the inverted region [21], and the inversion helps to maintain them following secondary contact [20] (Figure IIB4). High linkage disequilibrium within the inversion favours the spread of alleles causing nonrandom mating in response to low fitness of heterokaryotypes (i.e., reinforcement [27]) (Figure IIB5).

Individual loci within the inversion might show overdominance or be under negative frequency-dependent selection (including frequency dependence due to environmental heterogeneity; e.g., [19]), generating balancing selection within populations (Figure IIC6). Overdominance for the inversion can result from epistatic interactions among alleles at different loci [14], and new alleles that contribute to this **coadaptation** are subsequently favoured [2] (Figure IIC7). Finally, associations maintained by suppressed recombination between recessive deleterious alleles at different loci, dominant advantageous alleles at different loci, or both, can make the heterokaryotype more fit than either homokaryotype (associative overdominance [15]) (Figure IIC8,9).

(A) Direct inversion effects	
1. Breakpoint disrupts gene or modifies expression ('position effect')	
2. Underdominance due to problems in meiosis or loss of unbalanced gametes	
(B) Alleles favouring divergence	
3. Locally advantageous alleles favour initial spread, accumulate subsequently, or both	
4. Dobzhansky–Müller incompatibilities accumulate in allopatry (or perhaps with gene flow)	
5. Reinforcement by spread of assortment alleles under linkage disequilibrium	
(C) Alleles favouring balanced polymorphism	
6. Individual overdominant loci, frequency dependent loci or both	
7. Overdominance or frequency dependence due to epistasis	
8. Associative overdominance due to recessive deleterious alleles	
9. Associative overdominance due to dominant advantageous alleles	

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**Figure II. Evolutionary Effects of Inversions and the Loci within Them.** The genetic mechanisms of direct (A) and indirect (B, C) effects of an inversion are illustrated, including those generating divergence (B) and polymorphism (C). Ancestral alleles are in black, upper case indicates dominance, derived alleles are green if advantageous, red if deleterious, and blue if neutral but generating assortment. An allele that causes overdominance is indicated by an asterisk. Arrows indicate epistatic interactions.



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**Figure 1. Lifetime Evolution of an Inversion.** Initial filtering removes a majority of all new inversions (A), while some fix throughout the species range (B). The few that remain polymorphic are polymorphic among populations due to divergent selection and migration (Type I polymorphism) or within populations due to balancing selection (Type II polymorphism). Interactions between processes favouring Type I and II polymorphisms either promote continued balanced polymorphism, or continued divergence, or both (C), and potentially lead to speciation (D) through an array of different mechanisms. Alternatively, one of the arrangements will eventually be fixed throughout the species (E). Shades of grey in populations (circles) represent different frequencies of the ancestral and derived arrangements from fixation of one (white) to fixation of the other (black). Abbreviation: DM, Dobzhansky–Müller.

Establishment of an inversion polymorphism is also a first step from which there are several possible scenarios for further evolution along other trajectories. For example, the early stages of evolution of sex chromosomes and **supergenes** often involve inversions [24,25]. In all cases, the initial mechanism spreading a suppressor of recombination is later combined with other processes that can come to dominate subsequent evolution.

### Inversion Polymorphisms and Speciation

For a Type I inversion polymorphism established by divergent selection and migration, recombination in the heterokaryotype is strongly reduced [6]. Although limited to a part of the genome, this situation is similar to allopatry where a physical barrier impedes gene flow. Under these conditions, locally favourable alleles are trapped in the different arrangements and, if incompatible under epistatic interactions, heterokaryotypes are selected against in a way that is similar to hybrids between populations in a classical Dobzhansky–Müller mechanism [21,26]. This might promote **reinforcement** by evolution of premating mechanisms, and eventually



completion of speciation [27,28]. Thus, suppression of recombination by inversions can promote the evolution of species barriers in the presence of gene flow [29]. If the barrier is completed, the two incipient species will be fixed for different chromosomal arrangements.

However, speciation is not an inevitable outcome. If, for example, habitat choice or assortative mating reduces gene flow at an early stage in the process (as a consequence of local adaptation), the mechanisms that promote establishment of an inversion are weakened. That is, with little gene flow and thus a low rate of effective recombination, the selective advantage of the inversion is strongly reduced [6,9]. In a similar way, the spatial pattern of populations across an environmental cline is important as it affects migration, and the impact is greater for demes close to the habitat transition than for those further away. Consequently the effective recombination rate among loci underlying local adaptation is reduced and selection favouring an inversion less strong. Multideme models that extend the classical two-deme models to clines are needed to sort out the more specific conditions under which inversions are established and form strong barriers to gene flow.

Polymorphic inversions might also hinder speciation if heterosis or other processes prevent fixation. Indeed, empirical examples show that the derived and ancestral arrangements can be far from fixation in the habitat where they are positively selected [30–35]. This is not what we expect from current models. Over time, accumulation of Dobzhansky–Müller incompatibilities is

### Box 2. What Happens When Balancing Selection and Local Adaptation Both Influence Inversion Frequencies?

We constructed a simple simulation model to show how balancing selection interacts with divergent selection when they both impact on the same inversion. We simulated a linear chain of 150 demes, each of width 1, with a habitat transition after deme 75 and with dispersal of 1.5. Random mating was followed by viability selection, dispersal and then drift (deme size 20). The allele frequency was initially equal for all demes in one environment but different between the two environments (randomly chosen from the range 0–1 for each environment independently), treating the inversion as a single locus, and simulations were run for 1000 generations.

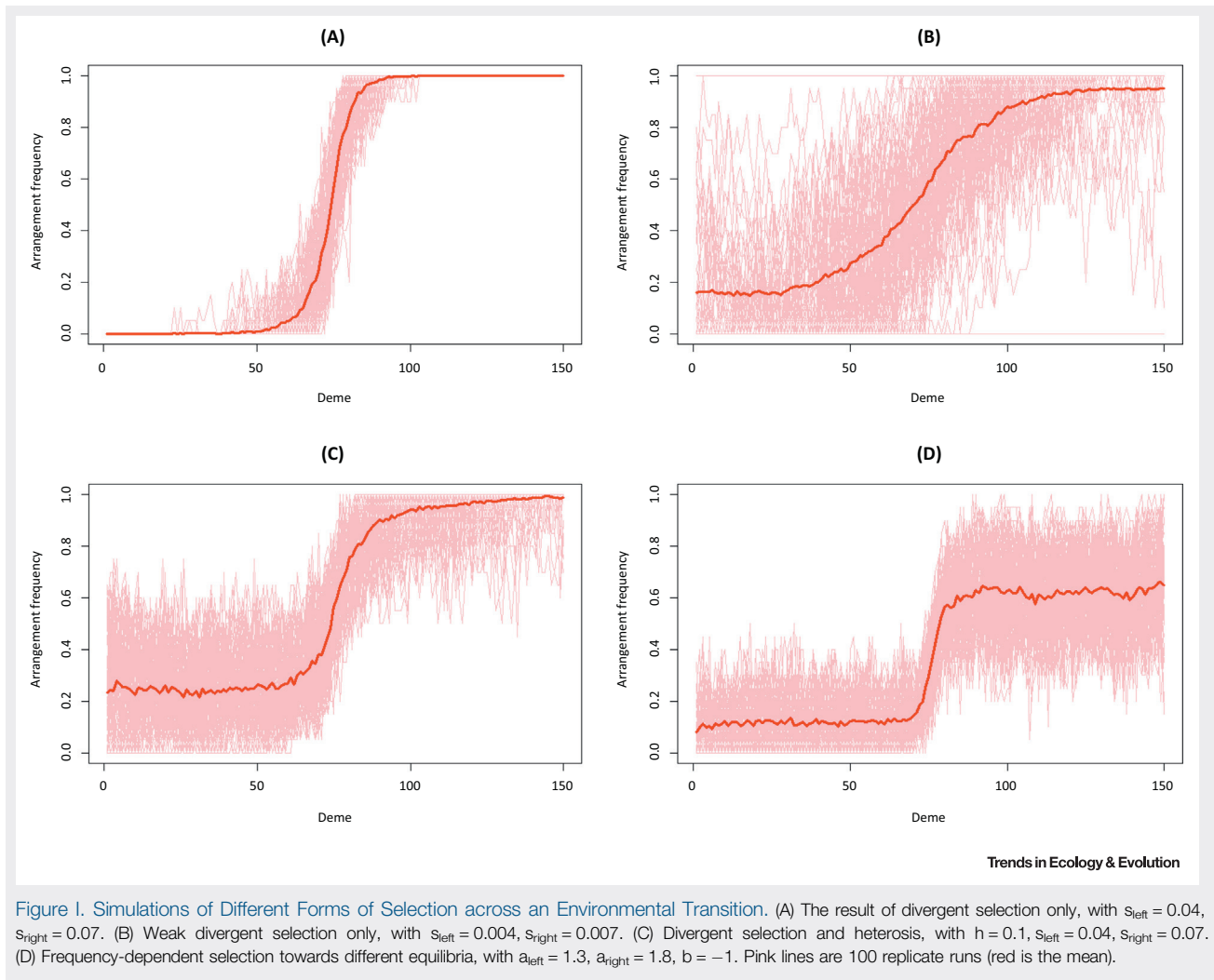
We considered three forms of selection: divergent only, divergent with heterosis, and frequency-dependent with different equilibria in the two habitats. Fitness is indicated in Table 1.

As expected, we find that divergent selection alone generates clines that approach fixation in populations distant from the habitat transition (Figure 1). When divergent selection is weak, clines are wide and noisy. In contrast, balancing selection can result in different equilibrium frequencies in the two habitats, with steep clines close to the habitat transition.

Table 1. Fitness of the Three Karyotypes in Each of Two Habitats (Left and Right) for Three Different Model Simulations

		Left habitat	Right habitat
Divergent only	Ancestral homokaryotype	$1-2s_{\text{left}}$	1
	Heterokaryotype	$1-s_{\text{left}}$	$1-s_{\text{right}}$
	Derived homokaryotype	1	$1-2s_{\text{right}}$
Divergent plus heterosis	Ancestral homokaryotype	$1-2s_{\text{left}}$	1
	Heterokaryotype	$1-s_{\text{left}} + h$	$1-s_{\text{right}} + h$
	Derived homokaryotype	1	$1-2s_{\text{right}}$
Frequency dependent <sup>a</sup>	Dominant phenotype	$a_{\text{left}} + bP$	$a_{\text{right}} + bP$
	Recessive phenotype	1	1

<sup>a</sup>Phenotype frequency  $P = p_D^2 + 2 p_D p_A$ , where  $p_A$  is the ancestral arrangement frequency and  $p_D$  is the derived arrangement frequency.



**Figure 1. Simulations of Different Forms of Selection across an Environmental Transition.** (A) The result of divergent selection only, with  $s_{\text{left}} = 0.04$ ,  $s_{\text{right}} = 0.07$ . (B) Weak divergent selection only, with  $s_{\text{left}} = 0.004$ ,  $s_{\text{right}} = 0.007$ . (C) Divergent selection and heterosis, with  $h = 0.1$ ,  $s_{\text{left}} = 0.04$ ,  $s_{\text{right}} = 0.07$ . (D) Frequency-dependent selection towards different equilibria, with  $a_{\text{left}} = 1.3$ ,  $a_{\text{right}} = 1.8$ ,  $b = -1$ . Pink lines are 100 replicate runs (red is the mean).

expected to decrease fitness of the heterokaryotypes [21], and new adaptive mutations increase local fitness of homokaryotypes [6]. Along with reinforcement mechanisms [27], this would tend to push the locally beneficial arrangements towards fixation at each end of an environmental gradient. This paradox suggests that some counteracting force, such as heterosis or frequency-dependent selection, is maintaining these balanced polymorphisms, in line with our general argument that multiple processes are likely to contribute over the lifetime of inversions (Figure 1). Combining divergent selection with a mechanism of balanced polymorphism (heterosis or frequency dependence) can explain inversions that remain polymorphic at cline ends under various conditions (Box 2). Indeed, early empirical data promoted heterosis as a model to explain inversion polymorphisms in *D. melanogaster* [2,36], and a recent review suggests that it is widespread among taxa [10]. A model with heterosis is attractive because under the same environmental conditions, the strength of heterosis increases by the continuous accumulation of new deleterious recessive alleles. A similar effect is achieved with accumulation of universally advantageous alleles in each arrangement, or alleles advantageous in the background of each arrangement that will be expressed jointly only in the heterokaryotype [5].



### Parallel Evolution of Ecotypes

Importantly, a balanced inversion polymorphism containing alleles differentially adapted to local environments provides an operational pool of standing genetic variation allowing rapid and repeated colonisation and establishment of different ecotypes or subspecies in a heterogeneous landscape. Rapid and repeated establishment of freshwater populations of three-spined stickleback (*Gasterosteus aculeatus*) from marine founders involving inversions important for freshwater adaptation [30,37] is a famous example, although it remains uncertain how the inversions persist in marine populations. Similar processes of parallel ecotype formation are also found in, for example, the monkey flower *Mimulus guttatus* [38], and the marine snail *Littorina saxatilis* [34,39,40]. These examples also illustrate the fact that inversions might be old and considerably predate more recent ecotype divergence events. We speculate that an old inversion can promote local adaptation in several different ways. First, an ancestral inversion polymorphism that is older than the split in locally adapted ecotypes provides useful genetic variation that seeds the system from the start [41]. Second, an old and widespread inversion is also a platform that allows accumulation of locally adapted alleles specific for a geographic area. Finally, the inversion might be the 'ready-to-use kit' that contributes to the genetic variation needed for local adaptation within species, in particular, if it is introduced by **introgression** from a related species [42,43]. However, we emphasise that old inversions are also variable, within both arrangements. More empirical data covering variation among arrangement haplotypes in different geographic areas, and comparative studies that trace the ancestry of inversions, are needed to distinguish between these alternatives.

### Concluding Remarks

When a new inversion has become established in an early polymorphic state, it might already have captured either coadapted or epistatic alleles that are universally favourable for one phenotype of a polymorphic species, or alleles that are locally favourable in a specific habitat, or a mix of these possibilities. Whichever is the strongest effect on fitness of individuals is likely to drive the role of the inversion in further evolution. If the inversion evolves towards a polymorphism with alternative rearrangements favoured in different environments, it will approach a second crossroads. Here, the new path is determined by the opposing forces of Dobzhansky–Müller incompatibilities selecting against heterokaryotype individuals and recessive deleterious alleles favouring them. An inversion that stays polymorphic everywhere contributes to standing variation and potentially parallel ecotype formation but not speciation. In contrast, where incompatibilities dominate over local adaptation or heterosis, polymorphism is only present in a narrow hybrid zone and the inversion mostly contributes to the final stages of speciation. Coupling between early and late inversion lifetime events, inversion and collinear genome relationships, and potential interactions with other inversions are among the issues that need to be addressed (see Outstanding Questions). Furthermore, the roles of gene flux between arrangements and reinforcement of barriers (including addition of new inversions) need consideration. Thus, many different processes appear entangled, some in synergy, others in opposition, and the long-term outcome is currently difficult to predict. We conclude that there is plenty of room for new modelling initiatives and many more empirical data to solve the many ambiguities of inversion evolution.

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### Outstanding Questions

How can we distinguish among the potential mechanisms maintaining inversion polymorphisms, both within and between populations?

How can we identify the role of individual loci within inversions that contribute to polymorphism (within or between populations)?

What is the relative importance of overdominance (e.g., due to recessive deleterious alleles) and underdominance (e.g., due to Dobzhansky–Müller incompatibilities) under different conditions, and how do the two interact? How does this affect the probability of speciation?

Are there evolutionary paths more probable than others during the lifetime of an inversion, and to what extent is this affected by the allelic content when the inversion first emerged?

How important are gene conversion and double crossover in determining evolution of an inversion?

What interactions occur between inversions, and between inversions and collinear parts of the genome?

How important are inversions in promoting parallel evolution, compared to collinear regions of the genome?

## References

- White, M.J.D. (1973) *Animal Cytology and Evolution*, Cambridge University Press
- Dobzhansky, T.G. (1970) *Genetics of the Evolutionary Process*, Columbia University Press
- Felsenstein, J. (1981) Skepticism towards Santa Rosalia, or why are there so few kinds of animals? *Evolution* 35, 124–138
- Kirkpatrick, M. (2010) How and why chromosome inversions evolve. *PLoS Biol.* 8, e1000501
- Butlin, R.K. (2005) Recombination and speciation. *Mol. Ecol.* 14, 2621–2635
- Kirkpatrick, M. and Barton, N. (2006) Chromosome inversions, local adaptation and speciation. *Genetics* 173, 419–434
- Hoffmann, A.A. and Rieseberg, L.H. (2008) Revisiting the impact of inversions in evolution: from population genetic markers to drivers of adaptive shifts and speciation? *Annu. Rev. Ecol. Syst.* 39, 21–42
- Feder, J.L. *et al.* (2014) Assessing when chromosomal rearrangements affect the dynamics of speciation: implications from computer simulations. *Front. Genet.* 5, 295
- Charlesworth, B. and Barton, N.H. (2018) The spread of an inversion with migration and selection. *Genetics* 208, 377–382
- Wellenreuther, M. and Bernatchez, L. (2018) Eco-evolutionary genomics of chromosomal inversions. *Trends Ecol. Evol.* 33, 427–440
- Connallon, T. *et al.* (2018) Local adaptation and the evolution of inversions on sex chromosomes and autosomes. *Phil. Trans. R. Soc. B* 373, 20170423
- Nei, M. *et al.* (1967) Frequency changes of new inversions in populations under mutation-selection equilibria. *Genetics* 57, 741–750
- Coyne, J.A. (1989) A test of the role of meiotic drive in fixing a pericentric inversion. *Genetics* 123, 241–243
- Charlesworth, B. and Charlesworth, D. (1973) Selection on new inversions in multi-locus genetic systems. *Genet. Res.* 21, 167–183
- Ohta, T. (1971) Associative overdominance caused by linked detrimental mutations. *Genet. Res.* 18, 277–286
- Feder, J.L. *et al.* (2011) Adaptive chromosomal divergence driven by mixed geographic mode of evolution. *Evolution* 65, 2157–2170
- Navarro, A. *et al.* (1997) Recombination and gene flux caused by gene conversion and crossing over in inversion heterokaryotypes. *Genetics* 146, 695–709
- Kennington, W.J. *et al.* (2006) Patterns of diversity and linkage disequilibrium within the cosmopolitan inversion In(3R)Payne in *Drosophila melanogaster* are indicative of coadaptation. *Genetics* 172, 1655–1663
- Wilson, D.S. and Turelli, M. (1986) Stable underdominance and the evolutionary invasion of empty niches. *Am. Nat.* 127, 835–850
- Noor, M.A.F. *et al.* (2001) Chromosomal inversions and the reproductive isolation of species. *Proc. Natl. Acad. Sci. U. S. A.* 98, 12084–12088
- Navarro, A. and Barton, N.H. (2003) Accumulating postzygotic isolation genes in parapatry: a new twist on chromosomal speciation. *Evolution* 57, 447–459
- Fryenberg, J. *et al.* (2003) DNA sequence variation and latitudinal associations in hsp23, hsp26 and hsp27 from natural populations of *Drosophila melanogaster*. *Mol. Ecol.* 12, 2025–2032
- Kennington, W.J. *et al.* (2007) Mapping regions within cosmopolitan inversion In(3R)Payne associated with natural variation in body size in *Drosophila melanogaster*. *Genetics* 177, 549–556
- Charlesworth, D. (2016) The status of supergenes in the 21st century: recombination suppression in Batesian mimicry and sex chromosomes and other complex adaptations. *Evol. Appl.* 9, 74–90
- Thompson, M.J. and Jiggins, C.D. (2014) Supergenes and their role in evolution. *Heredity* 113, 1–8
- Faria, R. and Navarro, A. (2010) Chromosomal speciation revisited: rearranging theory with pieces of evidence. *Trends Ecol. Evol.* 25, 660–669
- Trickett, A.J. and Butlin, R.K. (1994) Recombination suppressors and the evolution of new species. *Heredity* 73, 339–345
- Servedio, M.R. and Noor, M.A.F. (2003) The role of reinforcement in speciation: theory and data. *Annu. Rev. Ecol. Syst.* 34, 339–364
- Smadja, C.M. and Butlin, R.K. (2011) A framework for comparing processes of speciation in the presence of gene flow. *Mol. Ecol.* 20, 5123–5140
- Jones, F.C. *et al.* (2012) The genomic basis of adaptive evolution in three spine sticklebacks. *Nature* 484, 55–61
- Kapun, M. *et al.* (2016) Genomic evidence for adaptive inversion clines in *Drosophila melanogaster*. *Mol. Biol. Evol.* 33, 1317–1336
- Kapun, M. and Flatt, T. (2018) The adaptive significance of chromosomal inversion polymorphism in *Drosophila melanogaster*. *Mol. Ecol.* Published online September 19, 2018. <http://dx.doi.org/10.1111/mec.14871>
- Barth, J.M. *et al.* (2017) Genome architecture enables local adaptation of Atlantic cod despite high connectivity. *Mol. Ecol.* 26, 4452–4466
- Westram, A.M. *et al.* (2018) Clines on the seashore: the genomic architecture underlying rapid divergence in the face of gene flow. *Evol. Lett.* 2, 297–309
- Faria, R. *et al.* (2018) Multiple chromosomal rearrangements in a hybrid zone between *Littorina saxatilis* ecotypes. *Mol. Ecol.* Published online December 8, 2018. <http://dx.doi.org/10.1111/mec.14972>
- Dobzhansky, T. (1949) Genetic of natural populations IX: temporal changes in the composition of populations of *Drosophila pseudoobscura*. *Genetics* 28, 162–186
- Lescaek, E.A. *et al.* (2015) Evolution of stickleback in 50 years on earthquake-uplifted islands. *Proc. Natl. Acad. Sci. U. S. A.* 112, E7204–E7212
- Twyford, A.D. and Friedman, J. (2015) Adaptive divergence in the monkey flower *Mimulus guttatus* is maintained by a chromosomal inversion. *Evolution* 69, 1476–1486
- Johannesson, K. *et al.* (2010) Repeated evolution of reproductive isolation in a marine snail — unveiling mechanisms of speciation. *Phil. Trans. R. Soc. B* 365, 1735–1747
- Butlin, R.K. *et al.* (2014) Parallel evolution of local adaptation and reproductive isolation in the face of gene flow. *Evolution* 68, 935–949
- Guerrero, R.F. and Hahn, M.W. (2017) Speciation as a sieve for ancestral polymorphism. *Mol. Ecol.* 26, 5362–5368
- Kirkpatrick, M. and Barrett, B. (2015) Chromosome inversions, adaptive cassettes and the evolution of species' ranges. *Mol. Ecol.* 24, 2046–2055
- Jay, P. *et al.* (2018) Supergene evolution triggered by the introgression of a chromosomal inversion. *Curr. Biol.* 28, 1839–1845
- Pegueroles, C. *et al.* (2013) Gene flow and gene flux shape evolutionary patterns of variation in *Drosophila subobscura*. *Heredity* 110, 520–529