The adaptive significance of chromosomal inversion polymorphisms in *Drosophila melanogaster*

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Abstract

Chromosomal inversions, structural mutations that reverse a segment of a chromosome, cause suppression of recombination in the heterozygous state. Several studies have shown that inversion polymorphisms can form clines or fluctuate predictably in frequency over seasonal time spans. These observations prompted the hypothesis that chromosomal rearrangements might be subject to spatially and/or temporally varying selection. Here, we review what has been learned about the adaptive significance of inversion polymorphisms in the vinegar fly *Drosophila melanogaster*, the species in which they were first discovered by Sturtevant in 1917. A large body of work provides compelling evidence that several inversions in this system are adaptive; however, the precise selective mechanisms that maintain them polymorphic in natural populations remain poorly understood. Recent advances in population genomics, modelling and functional genetics promise to greatly improve our understanding of this long-standing and fundamental problem in the near future.

KEYWORDS
chromosomal inversions, clines, *Drosophila melanogaster*, fitness, recombination, selection

1 | INTRODUCTION

At the beginning of the 20th century, when investigating crossing-over frequencies in the vinegar fly *Drosophila melanogaster*, Alfred H. Sturtevant, a former PhD student of Thomas H. Morgan, observed unusually low recombination frequencies among visual markers on the second chromosome (Sturtevant, 1917). He correctly speculated that a previously unknown chromosomal factor caused strong suppression of recombination in that genomic region (Payne, 1924; Roberts, 1976). It soon became clear that these factors were chromosomal inversions, structural mutations that result in the reversal of gene order in the affected region relative to the noninverted ("standard") chromosomal arrangement (Sturtevant, 1919, 1921; see Box 1).

In contrast to inversion or standard arrangement homozygotes, inversion heterozygotes have major problems with proper chromatid pairing in the inverted region, causing a dramatic reduction in the frequency of crossing-over and recombination (Griffiths, Miller, Suzuki, Lewontin, & Gelbart, 2000; Kirkpatrick, 2010; Box 1). To maximize homologous pairing of the chromatids during mitosis and meiosis inversion, heterozygotes form chromosomal loops ("inversion loops"; Griffiths et al., 2000; Torgasheva & Borodin, 2010). Such inversion loops can be detected by microscopy in giant polytene chromosomes, which represent thousandfold amplified daughter chromatids in the interphase nuclei of larval salivary glands or other tissues of various dipterans (Cooper, 1938) such as *Drosophila* (Alanen, 1986; Bridges, 1935; Coluzzi, Sabatini, Della-Torre, Di Deco, & Petrarca, 2002; Morales-Hojas, Päällysaho, Vieira, Hoikkala, & Vieira, 2006; Schaeffer et al., 2008).

Because polytene chromosomes can be easily examined with light microscopy, inversions were among the first genetic polymorphisms that could be studied in natural populations in the early days of population genetics (Ashburner & Lemeunier, 1976; Bridges & Bridges, 1938; Charlesworth & Charlesworth, 2010, 2017; Dobzhansky, 1937; Dobzhansky & Sturtevant, 1938; Krimbas & Powell, 1992; Lewontin, 1974; Payne, 1924; Wellenreuther & Bernatchez, 2018). The historical discovery of Dobzhansky and colleagues that selection is acting on chromosomal inversions in *Drosophila pseudoobscura* gave a first glimpse into how balancing selection can maintain polymorphisms and

Box 1 The origin and nature of inversions

Chromosomal inversions are rare structural mutations in which an entire segment of a chromosome is removed, flipped around and reinserted in the same genomic location; most of them are either deleterious or neutral. They can be small (<1 kb) but sometimes also very large (≫1 Mb) and either include or exclude the centromere (pericentric vs. paracentric inversions) (Griffiths et al., 2000; Kirkpatrick, 2010).

Despite examples of parallel evolution of cytologically identical inversions (Caccone, Min, & Powell, 1998; Goidts et al., 2005), most inversions result from unique mutation events (Krimbas & Powell, 1992; Sharakhov et al., 2006). However, certain genomic regions are more susceptible to inversions than others (Corbett-Detig, 2016), and breakpoints of inversions often cluster locally or are reused (González, Casals, & Ruiz, 2007; Pevzner & Tesler, 2003). Many of these regions are characterized by a surplus of "weak spots" prone to breakage, for example due to an excess of repetitive sequences such as transposable elements (TEs). Thus, the prevailing view of the origin of inversions is that they result from ectopic recombination between repetitive sequences in tRNAs, ribosomal genes (Kellis, Patterson, Endrizzi, Birren, & Lander, 2003; Szankasi et al., 1986), segmental duplications (Goidts, Szamalek, Hameister, & Kehrer-Sawatzki, 2004; Locke et al., 2003) or TEs (Cáceres, Ranz, Barbadilla, Long, & Ruiz, 1999; Daveran-Mingot, Campo, Ritzenthaler, & Le Bourgeois, 1998; Richards et al., 2005; Sharakhov et al., 2006).

Yet, comparative genomic analysis of the genus *Drosophila* has uncovered another mechanism for inversion origin, which is based on two staggered double-strand breaks around the future inversion breakpoints, followed by the reinsertion of the inverted segment and repair of the staggered breaks (Ranz et al., 2007). In contrast to ectopic recombination—leading to a "cut-and-paste" reinsertion of the inverted sequence—this "staggered break" mechanism results in duplications around the breakpoints, as found in several inversions of *D. melanogaster* (Corbett-Detig, Cardeno, & Langley, 2012; Matzkin et al., 2005) and *D. subobscura* (Puerma, Orengo, & Aguadé, 2016, 2017).

Interestingly, species within the genus *Drosophila* vary in their tolerance of inversion polymorphisms. For example, *D. simulans*, *D. mauritiana* and *D. sechellia*, which are sister species of *D. melanogaster*, are practically inversion-free, with only very few observations of unique inversion polymorphisms at very low frequencies in natural populations (e.g., Ashburner & Lemeunier, 1976; Aulard, Monti, Chaminade, & Lemeunier, 2004; Capy, Gibert, & Boussy, 2004; Krimbas & Powell, 1992; Lemeunier & Aulard, 1992; Ranz et al., 2007). The lack of inversions in these species might potentially be due to lower numbers of TEs, which can play an important role in generating inversions, and/or to larger population sizes as compared to *D. melanogaster* (see references above), which may result in contrasting patterns of genetic variation (Aquadro, Lado, & Noon, 1988).

Inversions can have various genetic effects. For example, inversions can alter gene expression by disrupting genes at the breakpoints, via positional effects that change the relative chromosomal position of the genes, or by rearranging regulatory domains (Lavington & Kern, 2017; Matzkin et al., 2005; Said et al., 2018; Salm et al., 2012; Wargent & Hartmann-Goldstein, 1974). Similarly, the breakpoints can result in gene duplications due to staggered breaks, often causing gene dosage effects (Mattei, Mattei, Ardissone, Taramasco, & Giraud, 1980; Puerma et al., 2016). Recent evidence suggests that in *D. melanogaster*, inversions have both local and genome-wide regulatory impacts on gene expression, both in *cis* and *trans*, and that these effects are not simply a consequence of altered genome structure (Said et al., 2018; also see Lavington & Kern, 2017). Moreover, inversions can have a pervasive impact on recombination rates elsewhere in the genome, that is increasing crossing-over frequency on other chromosomes, the so-called "interchromosomal effect" (Crown, Miller, Sekelsky, & Hawley, 2018; Ramel, 1966; Steinberg, 1936; Steinberg & Fraser, 1944).

In contrast to other structural mutations such as insertions/deletions (indels), translocations or copy number variants, inversions do not affect the genic content of the affected regions. Their main impact is the strong inhibition of recombination in heterozygotes (so-called heterokaryotypes or heterokaryons) because crossing-over within the inverted region results in abnormal chromatids (Dobzhansky & Epling, 1948; Dobzhansky & Sturtevant, 1938; Garcia & Valente, 2018). Recombination in paracentric inversions results in acentric and dicentric gametes, while crossing-over in pericentric inversions leads to duplications and deletions in the recombination products. Although the gene content of inverted and noninverted chromosomes is identical, the suppression of recombination in heterokaryotypes, together with other evolutionary forces (see Section 2), causes the allelic content of inverted and noninverted karyotypes to diverge.

However, suppression of recombination in heterokaryotypes is not complete. Two mechanisms, namely gene conversion and double crossovers, can result in a limited amount of genetic exchange ("gene flux") among inverted and noninverted karyotypes: gene flux is maximal close to the centre of the inversion but virtually absent in the proximity of the breakpoints where recombination is lowest (Chovnick, 1966, 1973; Guerrero et al., 2012; Navarro et al., 1997; Payne, 1924; Pegueroles, Aquadro, Mestres, & Pascual, 2013; Rozas & Aguadé, 1994; Schaeffer & Anderson, 2005; Stevison, Hoehn, & Noor, 2011).

For general reviews of the role of inversions in evolution see, for example, Hoffmann et al. (2004), Hoffmann and Rieseberg (2008), Kirkpatrick (2010), and Wellenreuther and Bernatchez (2018).

was a major impetus to the "modern synthesis" and the development of population and ecological genetics (Dobzhansky, 1937, 1943, 1955, 1970; Ford, 1975; Krimbas & Powell, 1992; Lewontin, Moore, Provine, & Wallace, 1981; Schaeffer et al., 2003; Wright & Dobzhansky, 1946).

Today, it is well established that inversion polymorphisms are widespread and can have a major impact on evolutionary change in natural populations, from plants and *Drosophila* to humans (Hoffmann & Rieseberg, 2008; Hoffmann, Sgrò, & Weeks, 2004; Kirkpatrick, 2010, 2017; Kirkpatrick & Barton, 2006; Kirkpatrick & Kern, 2012; Lowry & Willis, 2010; Rieseberg, 2001; Schaeffer et al., 2003; Stefansson et al., 2005; Wellenreuther & Bernatchez, 2018). However, despite 100 years of research on inversions, many fundamental questions about their adaptive nature remain incompletely understood (Kapun, Fabian, Goudet, & Flatt, 2016; Kirkpatrick, 2017; Kirkpatrick & Kern, 2012): What is the precise nature of the selective forces acting on inversions? What are the ecological factors underlying selection on inversions? How do inversions affect fitness-related traits on which selection acts? What are the genic targets of selection inside inversions?

Here, we review the adaptive significance of inversion polymorphisms in *Drosophila melanogaster*, the organism in which they were first discovered by Sturtevant (1917, 1921) (for the role of inversions in adaptation and speciation in *D. pseudoobscura* and *Drosophila persimilis* see Fuller, Koury, Phadnis, & Schaeffer, 2018; this issue). The edited volume by Krimbas and Powell (1992) gives a comprehensive treatment of *Drosophila* inversions; the chapter by Lemeunier and Aulard (1992) remains the most complete review of *D. melanogaster* inversions to date—here, we focus mainly on discussing newer findings.

We first give a general overview of the different types of selection that might explain the spread and maintenance of adaptive inversion polymorphisms. Next, we summarize the effects of *D. melanogaster* inversions on patterns of genetic variation, especially drawing on recent population genomic analyses. We then discuss multiple lines of specific evidence suggesting that several common cosmopolitan inversion polymorphisms in this species are maintained by positive selection. In particular, we present a comprehensive meta-analysis of inversion frequency clines in *D. melanogaster*, based on 34 data sets spanning >50 years of observations. Based on recent progress in genomics, modelling and functional genetics, we conclude that many of the major questions mentioned above might be in reach of being solvable.

2 | SELECTIVE FORCES AFFECTING THE SPREAD AND MAINTENANCE OF INVERSIONS

Before specifically discussing inversion polymorphisms in *Drosophila melanogaster*, we provide a summary of the selective forces that can act on inversions (Hoffmann & Rieseberg, 2008; Kirkpatrick, 2010, 2017; Wellenreuther & Bernatchez, 2018). Many new inversions likely have no fitness consequences, for example if they are very small and/or occur in intergenic regions, and are thus expected to evolve neutrally by random genetic drift (Kirkpatrick, 2010). Another large fraction of novel inversions is predicted to have deleterious effects, as is often the case for inversions that cause human diseases, for instance when the breakpoints of an inversion disrupt genes and/or perturb gene expression (Castermans et al., 2007; Feuk, 2010; Puig, Casillas, Villatoro, & Cáceres, 2015). Such inversions are selected against by purifying selection; yet, in some cases it is thought that inversions with negative fitness effects (e.g., underdominant inversions) can become fixed by drift if effective population sizes are small for a long period of time and/or when selection against the inversion heterozygotes is weak (Kirkpatrick, 2010; Kirkpatrick & Barton, 2006; Lande, 1984). Interestingly, many inversions that are fixed between species exhibit such underdominant fitness effects when they appear as heterokaryons in interspecies hybrids and might thus play an important role in postzygotic isolation (e.g., Navarro & Barton, 2003; White, 1973, 1978; also see discussion in Kirkpatrick, 2010; Kirkpatrick & Barton, 2006).

In this review, we are specifically concerned with adaptive inversions and thus with positive selection acting on inversions. Several theoretical models have been developed to explain the establishment and maintenance of such inversions under selection (Charlesworth & Barton, 2018; Charlesworth & Charlesworth, 1973; Dobzhansky, 1937, 1970; Hoffmann & Rieseberg, 2008; Kirkpatrick, 2010; Kirkpatrick & Barton, 2006). Several types of positive selection might be distinguished that can lead to the spread of an inversion (Connallon et al., 2018; Hoffmann & Rieseberg, 2008; Kirkpatrick, 2010, 2017; Kirkpatrick & Barton, 2006).

The first type of positive selection is local adaptation (i.e., adaptation due to local, differential selection pressures acting on populations from different environments) whereby a new inversion captures an advantageous haplotype (i.e., two or more locally adapted loci that are in initial linkage disequilibrium [LD]) (Charlesworth & Barton, 2018; Kirkpatrick & Barton, 2006). Such an inversion might spread to near fixation because it protects locally adapted loci from maladaptive gene flow, a mechanism that can work with or without epistasis among the selected loci (the "local adaptation" or "Kirkpatrick-Barton" model; Charlesworth & Barton, 2018; Connallon et al., 2018; Kirkpatrick & Barton, 2006). Thus, the inversion is favoured because it prevents the breakdown of LD caused by migration. Importantly, this mechanism requires neither drift nor epistasis ("coadaptation"; see below; Kirkpatrick & Barton, 2006). If there is no counteracting force, this mechanism can drive an inversion to high frequency, and this could potentially lead to "global," fixed differences among populations and species (see Kirkpatrick, 2010; Kirkpatrick & Barton, 2006). Connallon et al. (2018) have recently extended the Kirkpatrick–Barton local adaptation model, showing that the probability of establishment of fixed, locally adapted inversions is higher for X (or Z) chromosomes than for autosomes, presumably because the efficiency of purifying selection against locally maladaptive alleles is greater on sex chromosomes as compared to autosomes.

A second type of positive selection involves epistatic combinations of beneficial alleles. Under this model, an inversion might spread and be selectively maintained because suppression of crossing-over in the inversion heterozygotes reduces the probability that recombination breaks up locally adapted, epistatically interacting loci, so-called "coadapted gene complexes" (Dobzhansky's "coadaptation" model; Charlesworth & Charlesworth, 1973; Dobzhansky,

1937, 1970; Feldman, Otto, & Christiansen, 1997; Schaeffer et al., 2003). In this scenario, we might expect the inversion to spread to fixation (Hoffmann & Rieseberg, 2008; Kirkpatrick & Barton, 2006).

These two types of selection therefore represent indirect positive selection due to linkage, without the inversion being beneficial itself. Thus, either with (local adaptation) or without migration (coadaptation), the main condition for the spread of a new inversion is that LD must be present among the selected loci (e.g., Charlesworth & Barton, 2018; Charlesworth & Charlesworth, 1973; Charlesworth & Meagher, 1983). Under both types, we might expect to observe pronounced peaks of divergence between the noninverted and inverted karyotype that are centred on adapted loci, away from the breakpoints (Figure 1), whenever the selected loci are not exclusively located within or in close proximity to the breakpoints. This is due to an interplay of selection and gene flux between noninverted and inverted chromosomes. Gene flux occurs at a rate of ~10^{-2} to 10^{-8} per nucleotide and generation (Andolfatto, Depaulis, & Navarro, 2001; Navarro, Betrán, Barbadilla, & Ruiz, 1997); thus, given enough time (~10^4 to 10^6 generations or more), gene flux might tend to break up LD and homogenize differences between noninverted and inverted chromosomes, causing reduced levels of neutral divergence towards the centre of the inversion, except at the breakpoints where recombination is completely suppressed and in those regions where selection opposes such homogenization (Guerrero, Rousset, & Kirkpatrick, 2012; Kirkpatrick, 2017). In practice, the local adaptation and coadaptation mechanisms might be difficult to distinguish, mainly because the latter requires demonstrating that the adaptive alleles captured by the inversion exhibit positive fitness epistasis. Population genomic data from clinal inversions in *Anopheles gambiae* mosquitos (Cheng et al., 2012) and *D. melanogaster* (e.g., Kapun, Fabian et al., 2016) are qualitatively consistent with either mechanism (also see Kirkpatrick & Kern, 2012); however, because the conditions under which epistatic selection leads to the spread of an inversion are fairly restrictive, the local adaptation mechanism without epistasis might be a more parsimonious for the observed patterns of divergence between inverted and noninverted arrangements (cf. Kapun, Fabian et al., 2016; Kirkpatrick & Barton, 2006).

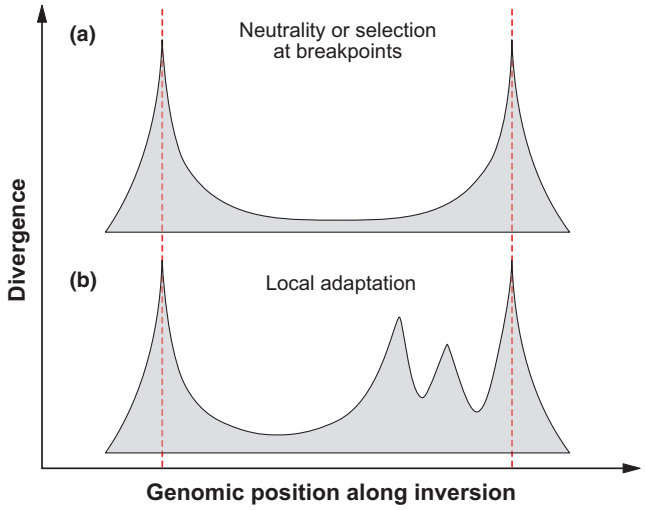

FIGURE 1 Patterns of divergence between inverted and noninverted chromosomes (as measured by F_{ST} or d_{xy}) under neutrality or when selection acts on the breakpoints themselves (a) or under local adaptation (b) (for a discussion see Guerrero et al., 2012; Kirkpatrick, 2010, 2017; Kirkpatrick & Kern, 2012). The chromosomal breakpoint positions are marked with dashed red lines. Under neutrality, or when selection acts directly on the breakpoints, we expect a pattern that resembles a suspension bridge, with maximal divergence at the breakpoints where recombination is maximally suppressed (a; e.g., see the pattern for *In(3L)P* in Kapun, Fabian et al., 2016). In contrast, under local adaptation we might expect additional peaks of divergence away from the breakpoints that are shaped by the interplay between selection and gene flux (b; for a potential example see the pattern for *In(3R)Payne* in Kapun, Fabian et al., 2016). These predictions have been corroborated by coalescent simulations (Guerrero et al., 2012) [Colour figure can be viewed at wileyonlinelibrary.com]

A third possibility is that the inversion breakpoints generate a beneficial mutation or cause favourable position effects; under this mechanism, the inversion might be maintained polymorphic or become fixed (Kirkpatrick & Barton, 2006). In terms of patterns of genetic divergence, this scenario can be difficult to distinguish from the pattern expected under neutrality (see Figure 1; Guerrero et al., 2012; Kirkpatrick, 2017). If selection is operating on the breakpoints, one would expect to observe alterations of gene expression or a disruption of gene structure at or near the breakpoints.

Fourth, locally adapted alleles might accumulate within the inversion after it has become established by some other mechanism, for instance random drift (Charlesworth & Barton, 2018; Guerrero et al., 2012; Kirkpatrick, 2017; Kirkpatrick & Barton, 2006; Noor, Grams, Bertucci, & Reiland, 2001). For example, an inversion might spread to intermediate frequency by drift and then fortuitously pick up a beneficial mutation, with the inversion spreading to high frequency due to hitchhiking with the positively selected site (Charlesworth & Barton, 2018; Kirkpatrick & Barton, 2006). Inferring this "inversion first" scenario would require the identification of the adaptive alleles within the inversion, dating their ages relative to the origin of the inversion, and showing that the inversion is older than the adaptive alleles (Kirkpatrick, 2010).

Once established, how are adaptive inversion polymorphisms maintained? Under some conditions, the above mechanisms can lead to the maintenance of an inversion polymorphism by balancing selection (Dobzhansky, 1954; Hoffmann & Rieseberg, 2008; Kirkpatrick & Barton, 2006; Wallace, 1968; Wellenreuther & Bernatchez, 2018). Indeed, several inversion polymorphisms in *Drosophila* seem to be maintained by some type of balancing selection, for example by overdominance (see Section 4.1; Dobzhansky, 1970; Kirkpatrick & Barton, 2006; Krimbas & Powell, 1992). True overdominance can, however, be difficult to distinguish from so-called "associative overdominance": a neutral locus can exhibit "apparent heterozygote advantage" because it is linked to one or more loci subject to true heterozygote advantage or to recessive deleterious mutations (Charlesworth & Charlesworth, 2010, 2018; Kirkpatrick & Barton, 2006; Zhao & Charlesworth, 2016). For example, if the locally adapted loci inside the inversion are linked to fully recessive deleterious loci, then an initially rare adaptive inversion can spread until the homokaryotypes become sufficiently frequent for

these deleterious mutations to be exposed to selection; this might in turn prevent the inversion from becoming fixed and stabilize it at some intermediate frequency (Kirkpatrick & Barton, 2006).

Other types of balancing selection that might maintain inversion polymorphisms are frequency-dependent selection, spatially varying (clinal) selection or temporally varying (fluctuating) selection (Alvarez-Castro & Alvarez, 2005; Charlesworth & Charlesworth, 2010; Dobzhansky, 1943; Haldane, 1948; Haldane & Jayakar, 1963; Kapun, Fabian et al., 2016; Kirkpatrick & Barton, 2006; Schaeffer, 2008; Wittmann, Bergland, Feldman, Schmidt, & Petrov, 2017; Wright & Dobzhansky, 1946). For example, Schaeffer (2008) used karyotype frequency data and a model of selection-migration balance to estimate fitness sets for 15 gene arrangements in six niches in *Drosophila pseudoobscura* and showed that "protected" inversion polymorphisms can be stably maintained through selection in heterogeneous environments (cf. Levene, 1953), that is by selection acting across all niches, not in a single niche. However, the conventional view has been that balancing selection generally plays only a relatively minor role in maintaining genetic variation in natural populations (Fijarczyk & Babik, 2015; also see Wellenreuther & Bernatchez, 2018). For example, the conditions for an inversion polymorphism to be maintained by frequency-dependent selection might be restrictive because over longer periods of time "gene flux" can break down LD between the selected "balanced" locus and the inversion (Kirkpatrick & Barton, 2006). An additional complication is that "apparent" frequency-dependent selection on an inversion can also result from constant fitness values (Charlesworth, 1974). Similarly, it has been widely thought that temporally varying selection is probably of limited relevance for maintaining genetic variation (Hedrick, Ginevan, & Ewing, 1976).

Yet, as we shall see in Section 4.1, the fact that many inversion polymorphisms in *Drosophila* are maintained at intermediate frequencies, form stable spatial clines and/or fluctuate predictably in frequency over time strongly suggests that they are maintained by some sort of balancing selection. This view is also consistent with a recent theoretical analysis (albeit independent of inversions) showing that the large amount of genetic variation for fitness components in *Drosophila* populations cannot be explained by mutation-selection balance and must reflect some form of balancing selection (Charlesworth, 2015). Moreover, recent theory indicates that the conditions for temporally varying selection to maintain balanced polymorphisms might be less restrictive than previously thought (Wittmann et al., 2017).

In summary, different forms of selection can be invoked to explain the spread and maintenance of adaptive inversion polymorphisms; a major challenge is to distinguish between these mechanisms in empirical data.

3 | EFFECTS OF *DROSOPHILA MELANOGASTER* INVERSIONS ON GENETIC VARIATION

Together with phenotypically visible colour and mimetic polymorphisms (Charlesworth & Charlesworth, 2010, 2017; Ford, 1975; Sheppard, 1975), inversions were among the first polymorphisms that allowed investigating the amount and distribution of genetic variation in natural populations (Dobzhansky, 1937, 1943; Krimbas & Powell, 1992; Lewontin, 1974; Wright & Dobzhansky, 1946). Around the same time as Dobzhansky's famous work in *Drosophila pseudoobscura*, several investigators began to use careful cytological studies to investigate inversions in natural populations of *Drosophila melanogaster* (Dubinin, Sokolov, & Tiniakov, 1937; Sturtevant, 1931; Warters, 1944; also see Ashburner & Lemeunier, 1976; Lemeunier & Aulard, 1992; and references therein) (Box 2). In the following, we discuss genetic polymorphisms in *D. melanogaster* inversions, the effects of inversions on patterns of genetic variation, and the demographic and phylogenetic history of inversion polymorphisms, including recent advances using population genomics.

Drosophila melanogaster is polymorphic for numerous naturally occurring inversions that are primarily found on the two major autosomes (chromosomes 2 and 3), but only in small numbers on the *X* chromosome (Ashburner & Lemeunier, 1976; Lemeunier & Aulard, 1992). The vast majority of these inversions (n = 339) are paracentric and do not span the centromere (Lemeunier & Aulard, 1992). In contrast, only 18 pericentric inversions have been identified to date (Lemeunier & Aulard, 1992), perhaps due to the fact that many pericentric inversions are underdominant and thus selected against (Kirkpatrick, 2010). Unlike many inversions in other *Drosophila* species, which are characterized by a complex evolutionary history

Box 2 How to identify inversions?

Chromosomal inversions are commonly studied cytologically in polytene (Kennison, 2008) or mitotic metaphase chromosomes (Pimpinelli, Bonaccorsi, Fanti, & Gatti, 2010; Roberts, 1998) and can be identified based on the characteristic inversion loops seen in heterozygotes (Ashburner & Lemeunier, 1976; Dobzhansky & Sturtevant, 1938; Kunze-Mühl & Müller, 1957). While chromosome preparations and their analyses are laborious and require experience, cytological screens still dominate the analysis of inversions in many drosophilids and other organisms. However, the genetic characterization of the breakpoint structure of many inversions in *D. melanogaster* has greatly facilitated the development of polymerase chain reaction (PCR) markers (Andolfatto et al., 1999; Corbett-Detig et al., 2012; Matzkin et al., 2005; Wesley & Eanes, 1994) which make it possible to unambiguously karyotype flies with simple molecular techniques. More recently, the combination of cytological karyotyping and whole-genome sequencing has allowed the identification of diagnostic SNPs in tight LD with *D. melanogaster* inversions, a powerful and efficient method for reliably estimating inversion frequencies from single individual- and pool-sequencing data (Kapun et al., 2014; Kapun, Fabian et al., 2016; also see Navarro & Faria, 2014).

and are often nested within each other (Dobzhansky & Sturtevant, 1938), most inversions in *D. melanogaster* have evolved uniquely from a standard (non-inverted) chromosome.

Depending on their frequency and geographic distribution, inversions in *D. melanogaster* can been categorized into four different classes (Lemeunier & Aulard, 1992; Mettler, Voelker, & Mukai, 1977): (a) Four chromosomal polymorphisms, one on each major autosomal arm, namely *In(2L)t*, *In(2R)NS*, *In(3L)P* and *In(3R)Payne*, are considered to be "common cosmopolitan" and found at frequencies ≥5% in most populations (in our review, we focus mainly on this group). (b) "Rare cosmopolitan" inversions, including *In(2L)NS*, *In(3L)M*, *In(3R)C*, *In(3R)K*, *In(3R)Mo* and *In(3R)M*; these are widespread but below 5% frequency in most populations. (c) With >330 described polymorphisms, "unique endemics" are the largest group; they are found in restricted geographic areas and only occur at low frequencies. (d) The most heterogeneous group are "recurrent endemics," occurring in more than one population but at low frequency. Lemeunier and Aulard (1992) give a comprehensive treatment of this classification.

It took, however, until the development of allozyme analysis, microsatellite and other molecular markers and—more recently—sequencing before the effects of *D. melanogaster* inversions on genetic variation and LD could be studied systematically (Box 2; for early studies associating inversions and allozymes see Lemeunier & Aulard, 1992; also see Voelker et al., 1978).

For example, Mukai and Voelker (1977), Inoue, Tobari, Tsuno, and Watanabe (1984), van Delden and Kamping (1989, Kamping and van Delden (1995), and Van 't Land, Van Putten, Villarroel, Kamping, and van Delden (2000) found evidence for linkage and possible epistatic interactions between *In(2L)t* and allozymes encoded by *alcohol dehydrogenase* (*Adh*) and α-*glycero-phosphate-dehydrogenase* (α-*Gpdh*), located outside the inversion breakpoints. Similarly, using restriction digestion of PCR products, Benassi, Aulard, Mazeau, and Veuille (1993) identified strong linkage between genetic variants in *Adh* and *In(2L)t* as well as with *In(2R)NS* in an African population. However, these authors failed to find similar patterns for the *P6* gene, even though it is also located within *In(2L)t*. These results provided a first hint that inverted chromosomes might be highly polymorphic and that they might harbour different amounts of genetic variation within the inverted genomic segment.

Subsequently, several studies used Sanger sequencing to investigate genetic variation around the breakpoints of the common cosmopolitan inversions *In(2L)t* (Andolfatto, Wall, & Kreitman, 1999), *In(3L)P* (Hasson & Eanes, 1996; Wesley & Eanes, 1994) and *In(3R)Payne* (Matzkin, Merritt, Zhu, & Eanes, 2005). Consistent with theoretical predictions, these analyses revealed reduced genetic and haplotype variation in the proximity of the breakpoints (Navarro, Barbadilla, & Ruiz, 2000; Navarro et al., 1997). Strongly suppressed recombination among karyotypes close to the inversion boundaries prevents the rapid re-establishment of genetic variation in initially monomorphic inverted chromosomes through genetic exchange among chromosomes of different orientation (Andolfatto et al., 2001). The low levels of nucleotide polymorphism suggest a relatively recent origin of *In(2L)t*, *In(3L)P* and *In(3R)Payne*, assuming that only novel mutations contribute to reconstituting genetic variation (Corbett-Detig & Hartl, 2012). The heterogeneous haplotype structure at the breakpoints of *In(2L)t* indicates, however, that low haplotype diversity might be the product of selection rather than of demography (Andolfatto et al., 2001; Navarro et al., 2000).

To learn more about the distribution and amount of variation and differentiation across genomic regions spanned by inversions, two studies analysed microsatellite markers within and in close proximity to *In(2L)t* (Kennington & Hoffmann, 2013) and *In(3R)Payne* (Kennington, Hoffmann, & Partridge, 2007) in Australian populations. These data showed that variation was overall lower in inverted as compared to standard chromosomes. At the same time, markers located within the inversions showed elevated levels of differentiation among karyotypes, in particular for *In(2L)t*. These findings are at odds with the neutral expectation that genetic differentiation should decay towards the centre of the inversion (Box 1; Navarro et al., 1997). However, even though these patterns are consistent with selection on haplotypes in tight LD with the inversion, simulations suggest that the observed differences could also reflect demographic effects due to putatively low numbers of inverted founders that initially colonized Australia (Kennington & Hoffmann, 2013).

While these studies provided major insights into patterns of genetic variation associated with inversions, only the development of whole-genome sequencing at the beginning of this century (Glenn, 2011; Harismendy et al., 2009; Mardis, 2008; Shendure & Ji, 2008) made it possible to comprehensively investigate variation and differentiation associated with inversions. For example, genomic analyses of fully sequenced lines from a single population in Raleigh (North Carolina, USA) (Huang et al., 2014; Mackay et al., 2012) and from several locations in Africa and Europe (Corbett-Detig & Hartl, 2012; Kapopoulou et al., 2018; Kapun, van Schalkwyk, McAllister, Flatt, & Schlötterer, 2014) showed that inversions make a major contribution to population substructure and genome-wide patterns of genetic diversity. This also led to growing awareness that the strong substructure caused by inversions can confound population genetic inferences when inversions are not being accounted for (Kapopoulou et al., 2018).

Consistent with analyses using Sanger sequencing, next-generation sequencing analyses confirmed that genetic variation is strongly reduced around the inversion breakpoints. However, the extent of this reduction is highly dependent upon the specific inversion, its age, frequency and size (Andolfatto et al., 2001). For example, Corbett-Detig and Hartl (2012) and Kapun et al. (2014) found that differences in genetic diversity among karyotypes vanished within a few hundred kbp around the breakpoints for *In(2)t* and *In(3L)P*. In contrast, for the rare cosmopolitan inversion *In(3R)Mo*, variation was almost completely absent even within a distance of ~1 million bp from the breakpoints (Kapun et al., 2014). Reductions in diversity beyond the breakpoints have also been found for other inversions on *3R* (*In(3R)C*, *In(3R)K*, *In(3R)Payne*) which all partially overlap with *In(3R)Mo* and each other (Corbett-Detig & Hartl, 2012). Because several of them occur at intermediate frequency, recombination might be strongly suppressed beyond their breakpoints. *3R* could thus be

particularly affected by substructure, resulting in reduced effective populations sizes relative to the rest of the genome and representing a combined effect of elevated drift within and strong suppression of recombination among karyotypes.

Karyotype-specific differences in diversity depend strongly on geography. Corbett-Detig and Hartl (2012) investigated how inversions affect variation by comparing chromosome-wide pairwise nucleotide diversity (π) in populations from Africa and Europe. While inversions had only a modest effect on variation in African populations, they resulted in a 30% increase in diversity in a French population, indicating that inverted haplotypes exhibit pronounced genetic differentiation. It remains unclear, however, why levels of differentiation among karyotypes often vary among continents. Corbett-Detig and Hartl (2012) speculate that this might reflect variation in migration rates of inverted and noninverted chromosomes among populations. If so, standard chromosomes in Europe might have had more time to diverge from their African ancestors by a combination of founder effects, novel mutations and spatially varying selection, while later arriving inverted chromosomes were largely of African identity. Alternatively, this pattern might be the result of local adaptation, whereby selection favours haplotypes in strong LD with the inversion.

Several genomic studies have compared patterns of genetic divergence among karyotypes to neutral expectations and searched for signals of selection. Corbett-Detig and Hartl (2012) identified strong differentiation at the breakpoints that rapidly decays towards the centre of the inversion for *In(2L)t*, *In(3L)P* and *In(3R)Payne* in African populations. These findings are qualitatively consistent with neutral evolution or with selection acting within or very close to the breakpoints (see Section 2, Figure 1). In contrast, the endemic African inversion *In(1)Be* as well as *In(3R)Mo* were characterized by strong differentiation between arrangements together with reduced polymorphism, suggesting that inverted haplotypes might be subject to selection.

Kapun, Fabian et al. (2016) took an indirect approach to investigate variation associated with inversions in North America. Based on a panel of inversion-specific SNP markers (Kapun et al., 2014), they employed a GWAS approach to search for SNP-wise associations between allele and inversion frequencies, thereby identifying SNPs in strong LD with specific inversions. Similar to Corbett-Detig and Hartl (2012), they found major differentiation around the breakpoints of *In(2L)t* and *In(3L)P*. However, patterns of divergence for the 8-mB large *In(3R)Payne* inversion were more complex than in African populations. Several localized and strongly differentiated regions inside the inversion and away from the breakpoints showed tight LD with *In(3R)Payne*, potentially consistent with local peaks of adaptive divergence between the inverted and noninverted karyotype (see Section 2, Figure 1; Guerrero et al., 2012). Moreover, the fact that this inversion is at intermediate frequencies in low-latitude populations (see Section 4.1) and that nucleotide diversity is strongly increased within the region spanned by *In(3R)Payne* might be consistent with balancing selection (Fabian et al., 2012). In contrast, Rane, Rako, Kapun, Lee, and Hoffmann (2015) did not observe major differentiation between inverted and noninverted *In(3R)Payne* chromosomes from the same sampling site in Australia; yet, because this study was based on RAD sequencing of a limited number of lines it remains unclear how these results compare to data from other continents.

The findings reviewed above thus suggest that patterns of genetic variation vary strongly among different inversions and across geography, indicating that various adaptive and neutral evolutionary forces are at play in affecting these patterns.

Comparative genomic analyses have also illuminated the history of *In(2L)t*, *In(2R)NS*, *In(3LP)* and *In(3R)Payne*: they are of sub-Saharan African origin and predate the out-of-Africa migration and subsequent colonization of the rest of the world (Corbett-Detig & Hartl, 2012). Models of variation around the breakpoints based on Approximate Bayesian computation further show that these inversions are relatively young. Compared to previous estimates based on pairwise differences among karyotypes in the breakpoints regions of *In(2L)t* (~160 kyr; Andolfatto et al., 1999), *In(3L)P* (~360 kyr; Hasson & Eanes, 1996) and *In(3R)Payne* (~330 kyr; Matzkin et al., 2005), the refined estimates of Corbett-Detig and Hartl (2012) point to a more recent origin of all four inversions, ranging from ~75 kyr ago for *In(2L)t*, ~130–150 kyr for *In(2R)Payne* and up to ~240 kyr ago for *In(2R)NS*. For *In(3L)P*, the situation is a bit unusual because the proximal and distal breakpoints result in different age estimates of the inversion (Corbett-Detig & Hartl, 2012).

Despite an increasing amount of genomic data for inversions in *D. melanogaster*, we still lack fundamental knowledge about the demographic history of inversions on the different continents, about the details of haplotype structure and LD inside inversions, and about the patterns and rates of recombination and gene flux among heterokaryotypes. This currently limits our ability to unambiguously distinguish between neutral and adaptive hypotheses of inversion evolution. In the near future, it will thus be important to combine fine-grained analyses of phased sequencing data with theoretical modelling (e.g., coalescent-based models) to test hypotheses about the forces shaping inversion evolution (e.g., Guerrero et al., 2012; Peischl, Koch, Guerrero, & Kirkpatrick, 2013; Rousset, Kirkpatrick, & Guerrero, 2014). However, one challenge of such approaches is that, while demography is expected to affect all regions of the genome equally, inversions of different ages will create genomic regions with different demographic histories. Combined with the effects of selection, this can lead to a large number of alternative evolutionary scenarios that might be difficult to distinguish.

4 | EVIDENCE FOR ADAPTIVE INVERSIONS IN *DROSOPHILA MELANOGASTER*

In addition to patterns of genetic differentiation that are potentially consistent with selection (see above), multiple lines of evidence reviewed below suggest that several inversion polymorphisms are adaptive in *Drosophila melanogaster*, including clines, predictable temporal fluctuations, changes in inversion frequencies in population cage or experimental evolution experiments, and phenotypic

effects of inversions upon fitness components (for reviews also see Lemeunier & Aulard, 1992; Hoffmann et al., 2004; Hoffmann & Rieseberg, 2008; Kapun, Fabian et al., 2016, and references therein).

4.1 | Spatio-temporal patterns of inversion frequencies

The spatio-temporal distribution of inversion frequencies has been extensively studied in *D. melanogaster*, either using direct cytological karyotyping or using molecular markers, including microsatellites, PCR markers or—most recently—inversion-specific SNP markers (Box 2; see Lemeunier & Aulard, 1992 for a review of the older literature; also cf. Kapun et al., 2014; Kapun, Fabian et al., 2016). Importantly, spatio-temporal changes of inversion frequencies in natural or laboratory populations might carry signals of selection. We first discuss spatial clines and their temporal stability before reviewing seasonal changes in inversion frequencies.

The *In(2L)t*, *In(2R)NS*, *In(3LP)* and *In(3R)P* polymorphisms have received particular attention because they exhibit a cosmopolitan distribution—the fact that they are common and geographically widespread might be a first—albeit inconclusive—hint that they might be maintained by selection. Beginning in the 1970s, many studies performed comprehensive surveys of the frequencies of these inversions in North America (Fabian et al., 2012; Kapun, Fabian et al., 2016; Knibb, 1982; Machado et al., 2018; Mettler et al., 1977; Sezgin et al., 2004; Stalker, 1976, 1980; Voelker et al., 1978), Australia (Anderson, Knibb, & Oakeshott, 1987; Knibb, 1982, 1986; Knibb, Oakeshott, & Gibson, 1981), Southern and Eastern Asia (Das & Singh, 1990, 1991; Glinka, Stephan, & Das, 2005; Inoue & Igarashi, 1994; Inoue & Watanabe, 1979; Inoue, Watanabe, & Watanabe, 1984; Singh & Das, 1992) and—to a lesser extent—in Africa and Europe (Aguadé & Serra, 1980; Aulard, David, & Lemeunier, 2002; Aulard & Lemeunier, 1985; Kapun et al., 2018; Pool, Braun, & Lack, 2017; Taberner & González, 1991; Zacharopoulou & Pelecanos, 1980).

Overall, these studies reveal that these inversions are typically more common in low-latitude populations from subtropical/tropical climates than in high-latitude populations from temperate regions where they are at low frequency or absent (see the meta-analysis below; Figure 2, Supporting Information Tables S1 and S2; also cf. Lemeunier & Aulard, 1992; Kapun, Fabian et al., 2016). In particular, several inversions—especially *In(3R)Payne*—exhibit steep negative frequency gradients across latitude (so-called "clines"; Endler, 1977) on multiple continents and subcontinents, for example along the North American and Australian east coasts (Kapun, Fabian et al.,

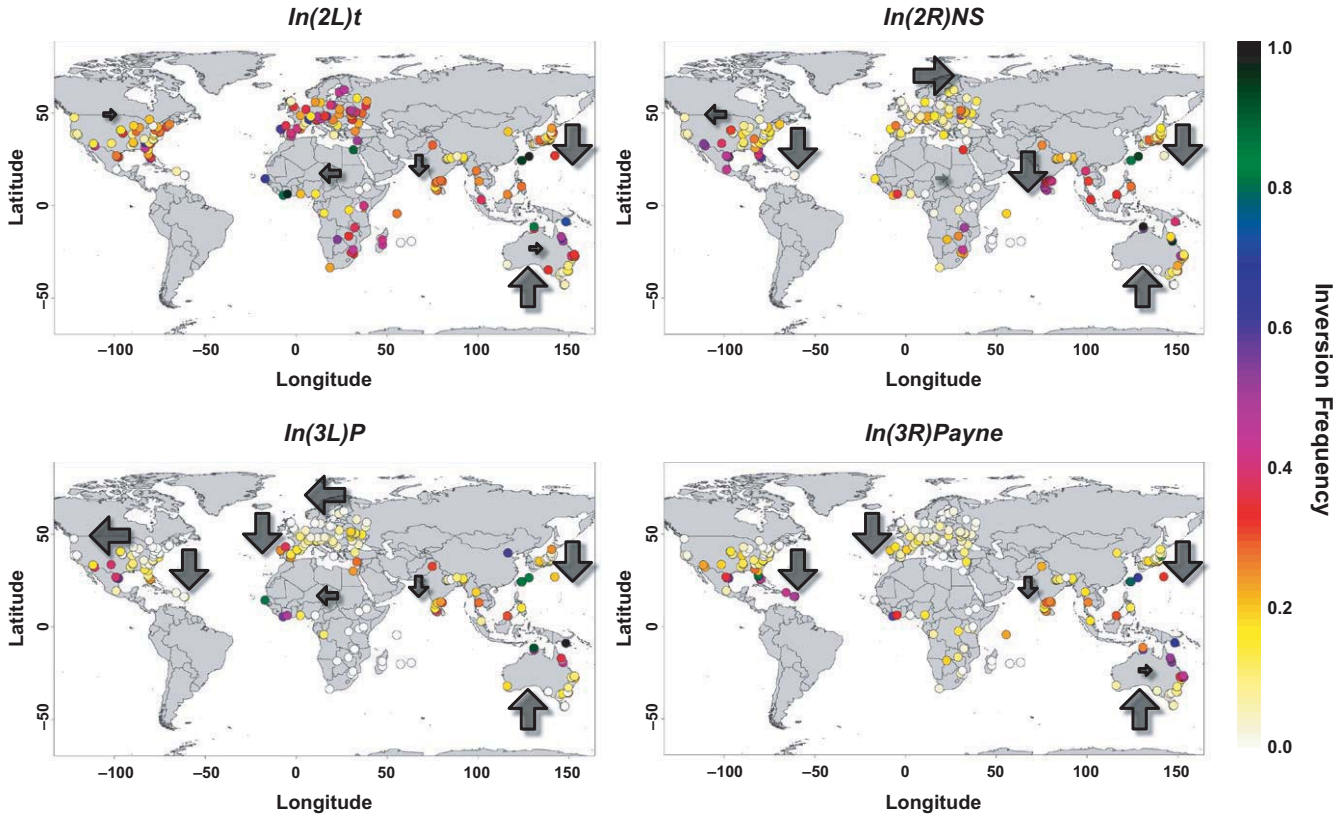

FIGURE 2 Meta-analysis of estimates of inversion frequencies in *D. melanogaster* from across the world. The four common inversion polymorphisms, *In(2L)t*, *In(2R)NS*, *In(3L)P* and *In(3R)Payne*, exhibit significant latitudinal and/or longitudinal clinality on multiple continents and subcontinents. The arrows highlight significant clinal patterns along latitudinal (vertical arrows) and longitudinal (horizontal arrows) axes in North America, Africa, Europe, India, Japan and Australia—the arrowheads point in the direction of increasing inversion frequencies. The different sizes of the arrows correspond to different significance levels: small arrows indicate $p < 0.05$; intermediate arrows $p < 0.01$; and large arrows $p < 0.001$. See Table S1 for raw data and Table S2 for further statistical analyses [Colour figure can be viewed at wileyonlinelibrary.com]

2016), but also in Australasia (Knibb, 1982; Knibb et al., 1981) and Europe (Kapun et al., 2018). Latitudinal clines have also been reported for *In(2L)t*, *In(2R)NS* and *In(3L)P*, as well as for *In(3R)Mo* and *In(3R)C*, but the extent of their clinality varies quite strongly among geographic regions and sampling decades (Knibb, 1982; Kapun, Fabian et al., 2016; also see below). Interestingly, a study by Glinka et al. (2005) suggests that most common inversion polymorphisms are not clinal in Southeast Asia, for reasons that are not entirely clear yet (see Figure 2, Supporting Information Table S2).

Drosophila inversions can also exhibit clines across altitude, as first observed by Dobzhansky in *Drosophila pseudoobscura* (Dobzhansky, 1948; Krimbas & Powell, 1992), and across longitude. For example, in African populations of *D. melanogaster* the four common polymorphisms tend to be more frequent in tropical lowland sites as compared to high-altitude locations, perhaps suggesting that the continent-wide latitudinal clines are locally mirrored by altitudinal clines (Pool et al., 2017; also see discussion in Klepsatel, Gáliková, Huber, & Flatt, 2014; and Fabian et al., 2015). In addition, there is evidence that several inversions, especially *In(2L)t* and *In(3L)P*, exhibit longitudinal clinality, although such clines tend to be less pronounced and often covary with latitudinal patterns (see Figure 2, Supporting Information Table S2; Aulard et al., 2002; Kapun et al., 2018; Kapopoulou et al., 2018; Knibb, 1982).

The clinal distribution of inversions, observed in a parallel fashion on multiple continents, is particularly interesting in view of the notion that clines are often shaped by spatially varying (clinal) selection (Charlesworth & Charlesworth, 2010; Dobzhansky, 1970; Endler, 1977, 1986; Fabian et al., 2012; Haldane, 1948; Kapun, Fabian et al., 2016; Mayr, 1963). For example, if clines are maintained in the face of extensive migration (gene flow), spatially varying selection might counteract the homogenizing effects of gene flow, as seems to be the case for several clinal inversion polymorphisms in *D. melanogaster* where gene flow along the North American cline seems to be strong and isolation by distance to be weak (e.g., Kapun, Fabian et al., 2016). However, because clines can also result from population structure and demography, clinality per se cannot be taken as *prima facie* evidence for spatially varying selection (Flatt, 2016). For example, in North American and Australian *D. melanogaster*, clinal patterns in the genome can be confounded by admixture and secondary contact with ancestral populations: low-latitude populations exhibit a high proportion of admixture with African genotypes, whereas high-latitude populations have a high proportion of admixture with European genotypes, thus generating an "ancestry" or "admixture" cline (Bergland, Tobler, González, Schmidt, & Petrov, 2016). It is therefore important to distinguish between demographic and selective causes of clinality (Bergland et al., 2016; Flatt, 2016). This has recently been attempted, for example, by Kapun, Fabian et al. (2016) who examined inversion clines in 10 populations along the North American east coast by comparing them to the clinality of a genome-wide panel of ~10,000 presumably neutral SNPs in short introns outside inversions, by accounting for population structure using latent factor mixed models and by contrasting the clinal behaviour of the inversions to that expected under admixture. These analyses suggest that the strong latitudinal clinality of *In(2L)t* and *In(3R)Payne* along the North American east coast is likely due to spatially varying selection and not caused by neutrality and/or demography (Kapun, Fabian et al., 2016).

Clinal patterns of inversion frequencies are underpinned by, or correlated with, environmental factors that covary with geography (e.g., latitude). For example, Knibb (1982) found significant correlations between the frequencies of the common cosmopolitan inversions and annual maximum temperature, minimum temperature and minimum rainfall in Asian, Australian and North American populations that predict the clinality of these inversions. More recently, Kapun, Fabian et al. (2016) used principal components (PCs) analysis of 19 climatic variables from the WorldClim data set (Hijmans, Cameron, Parra, Jones, & Jarvis, 2005), followed by regression analysis of inversion frequencies against the PCs. This analysis showed that the clinal frequencies of *In(2L)t*, *In(3L)P* and *In(3R)Payne* along the North American east coast are positively correlated with most measures of temperature and precipitation, while temperature dispersion (range) and seasonality predict higher frequencies of the noninverted arrangements that prevail at higher latitudes. The observation by Pool et al. (2017) that frequencies of five common inversions (*In(2L)t*, *In(2R)NS*, *In(3L)P*, *In(3R)K*, *In(3R)Payne*) are higher in African lowland as compared to highland populations suggests parallelism between altitudinal and latitudinal clines and that temperature might be the major common determinant of inversion frequencies. Generally, however, the specific climatic (and selective) factors that causally underlie clinal patterns of inversion frequencies remain unknown, in part because most climatic predictors are highly intercorrelated with each other.

Another important question about inversion clines is the extent to which they remain stable over time because stability might indicate that they are being maintained by selection. The best data come from 48 populations of *D. pseudoobscura*, where the clines of several inversions have remained stable for over 40 years (Anderson et al., 1991). Similarly, data from Australasian *D. melanogaster* populations show that the clines of *In(2L)t* and *In(3L)P* have remained invariant across several decades (Anderson et al., 1987; Kennington & Hoffmann, 2013; Knibb, 1982; Knibb et al., 1981; Umina, Weeks, Kearney, McKechnie, & Hoffmann, 2005), and in North American populations, the latitudinal cline of *In(3R)Payne* has been stably maintained for at least ~40 years (Kapun et al., 2014; Kapun, Fabian et al., 2016). On the other hand, several studies have found cases in which inversion clines have changed over time. For example, in contrast to the situation in North America, the intercept of the latitudinal cline of *In(3R)Payne* in Australia has shifted southwards over a time span of 20 years, presumably due to climate change (Anderson, Hoffmann, McKechnie, Umina, & Weeks, 2005; Umina et al., 2005). Likewise, while the Australian cline of *In(2L)t* appears to have remained stable (Anderson et al., 2005; Umina et al., 2005), the cline of this inversion in North America has shifted northwards over the last 40 years (Kapun, Fabian et al., 2016). Yet, whether this heterogeneity among continents reflects differential patterns of climate adaptation or demography (e.g., caused by founder effects as, for example, found for

the 8p23 inversion in humans; Salm et al., 2012) remains unclear. Another example of the dynamic behaviour of inversion polymorphisms is the rare cosmopolitan inversion *In(3R)Mo* in North America: while previous data showed that this inversion is nonclinal (Mettler et al., 1977), recent analyses have found a positive latitudinal cline that must have evolved over the last 40 years (Kapun et al., 2014; Kapun, Fabian et al., 2016).

In Figure 2 and Supporting Information Table S2, we provide a comprehensive meta-analysis of patterns of clinality for the four common cosmopolitan inversion polymorphisms (*In(2L)t*, *In(2R)NS*, *In(3L)P*, *In(3R)Payne*), based on 34 data sets and spanning half a century of data collected from across the world (see Supporting Information Table S1). Whenever possible, we tested for clinality along latitudinal, longitudinal and altitudinal axes and for temporal stability across sampling decades for each inversion and continent separately. Within the Asian continent, we distinguished between India, Japan and Southeast Asia, given the unequal sampling in these areas. Data were analysed by applying multifactorial general linear models (GLMs) to arcsine square root transformed inversion frequency estimates, including all (or—in the case of missing information—on a subset) of the four predictor variables mentioned above as well as all possible interaction terms in *R* (R Development Core Team, 2009). Consistent with previous studies, our meta-analysis confirms that the four common cosmopolitan inversions are typically much more frequent in subtropical/tropical areas than in temperate, seasonal environments; most of them exhibit significant and stable latitudinal clines on multiple continents. A notable exception is *In(2L)t* for which we failed to identify—in contrast to earlier data and contrary to the pattern in Australia—significant latitudinal clines in North American and Europe (also see Kapun, Fabian et al., 2016), suggesting that patterns of clinality of this inversion have changed over time. This is supported by the significant effect of sampling decade for this inversion, which indicates pronounced temporal frequency shifts in recent years. We also found evidence for longitudinal and altitudinal clines for multiple inversions, even though these patterns were not as pronounced as the latitudinal clines.

Overall, the data available to date strongly suggest that clines of several inversion polymorphisms in *D. melanogaster* might be stably maintained by spatially varying selection, a notion that is consistent with other evidence reviewed further below. However, the extent to which demography (e.g., population structure, founder effects, range expansion, admixture) contributes to the observed clines remains poorly understood (cf. Flatt, 2016). In some cases, demography might likely be sufficient to explain the clinal distribution of a given inversion polymorphism, whereas in other cases, inversion clines clearly deviate from neutrality and are unlikely to be explained by demography alone (e.g., *In(3R)Payne*; see Kapun, Fabian et al., 2016).

Inversion frequencies can also change seasonally. Beginning with the seminal observations of predictable seasonal fluctuations of inversion frequencies in *D. pseudoobscura* by Dobzhansky (Dobzhansky, 1943, 1948, 1970; Dobzhansky & Ayala, 1973), several studies have detected seasonal changes in the abundance of inversions in other *Drosophila* species, including in *D. melanogaster* (Krimbas & Powell, 1992; Lemeunier & Aulard, 1992; Rodríguez-Trelles, Alvarez, & Zapata, 1996; Sperlich & Pfriem, 1986; Stalker, 1980). For example, local seasonal fluctuations have been found in independent studies of *In(3R)Payne* in Japan, Egypt, Spain and North America (Inoue, 1979a; Kapun, Fabian et al., 2016; Masry, 1981; Sanchez-Refusta, Santiago, & Rubio, 1990) and of *In(2R)NS* in Japanese, Australian and North American populations (Inoue, 1979a; Kapun, Fabian et al., 2016; Knibb, 1986). In a North American orchard population from Pennsylvania, for instance, the frequencies of *In(3R)Payne* and *In(2R)NS* increased from summer-to-fall but declined from fall-to-summer in a predictable fashion over a 4-year time span (Kapun, Fabian et al., 2016). Notably, the temporal changes in the frequency of *In(2R)NS* were in almost perfect antiphase relative to changes in temperature (Kapun, Fabian et al., 2016). In some cases, inversion polymorphisms (e.g., *In(2R)NS*) also seem to exhibit "seasonal phase clines" (Rhomberg & Singh, 1988) whereby populations along the cline differ in the onset of their seasonal cycle depending on their latitude (Kapun, Fabian et al., 2016). However, two important caveats are that (a) temporal changes of *D. melanogaster* inversion frequencies are mostly very small and (b) whether these changes are driven by demography (e.g., drift due to cyclic population "booms" and "busts" or migration from neighbouring populations) or temporally varying selection (Behrman, Watson, O'Brien, Heschel, & Schmidt, 2015; Bergland, Behrman, O'Brien, Schmidt, & Petrov, 2014; Wittmann et al., 2017) remains—in the absence of better long-term data and experimental evidence—unknown.

Thus, while Dobzhansky's case for temporally varying selection acting on *D. pseudoobscura* inversions is quite strong (see discussion in Powell, 1992), the evidence that this form of selection acts on *D. melanogaster* inversions is rather weak—an issue that deserves more study.

4.2 | Evidence from population cage and experimental evolution experiments

Investigations of the adaptive nature of inversions using population cages and/or experimental evolution approaches have a long tradition in *Drosophila*. Dobzhansky was the first to investigate the temporal dynamics of inversion frequency changes under controlled conditions (reviewed in Krimbas & Powell, 1992). His landmark experiments in *D. pseudoobscura* showed that certain inversions are subject to balancing selection (Dobzhansky, 1948; Wright & Dobzhansky, 1946): they consistently returned to specific intermediate equilibrium frequencies within a few generations after the starting frequencies had been perturbed away from equilibrium at beginning of the experiment. One major caveat of these and similar experiments is that when such cage experiments were initiated with starting inversion frequencies similar to those seen in natural populations, the chromosomal arrangements in the cages attained equilibrium frequencies that were different from those observed in nature (e.g., see Powell, 1997 for a discussion).

About thirty years after Dobzhansky's efforts, in the 1970s, similar experiments were begun to be performed in *D. melanogaster* (Lemeunier & Aulard, 1992). Nassar, Muhs, and Cook (1973),

for example, concluded from manipulations of inversion frequencies that *In(3R)Payne* is under frequency-dependent selection, however, their study relied on a single inverted line, thus limiting the generality of the inference. In contrast, Barnes (1983), in the context of a long-term laboratory selection experiment for DDT resistance, documented overdominant selection on *In(3R)Payne* but failed to find evidence for frequency-dependent selection. This might imply that, depending on the context, this inversion is subject to different forms of balancing selection, an issue that deserves more investigation. Inoue (1979b) carried out experimental evolution experiments for adaptation to laboratory conditions with natural populations collected in Japan. Interestingly, all polymorphic inversions decreased in frequency and vanished after ~20 months of laboratory maintenance, suggesting that these inversions are somehow disadvantageous under laboratory conditions as compared to standard chromosomes. Using a multiyear survey of inversion frequencies in a tropical greenhouse as well as laboratory experiments, van Delden and Kamping (1989) showed that *In(2L)t* is selectively favoured under warmer conditions (also see van Delden & Kamping, 1991). More recently, Kapun et al. (2014) found that *In(2R)NS*, *In(3L)P* and *In(3R)Payne* originating from an outbred Portuguese population rapidly decreased in frequency across independent replicates exposed to two thermal ("cold" vs. "warm") evolution regimes. In marked contrast, *In(3R)C* increased in frequency under warm conditions but decreased under cold conditions, whereas the opposite pattern was observed for *In(3R)Mo*. The result that *In(3R)Mo* is selectively favoured under cool conditions is particularly noteworthy given that its frequency along the North American cline increases from low to high latitudes (Kapun, Fabian, et al., 2016; Kapun et al., 2014).

These findings thus suggest that *In(3R)C*, *In(3R)Mo* and *In(2L)t* respond to thermal selection and indicate that *In(3R)Payne* might be subject to balancing selection. However, as mentioned above, results from population cage experiments are difficult to extrapolate to the situation in the wild: while homogeneous laboratory conditions do not closely resemble natural conditions, experimentally simulating realistic natural conditions in the laboratory is very difficult. There is clearly much room for future work in this area, using larger and better controlled experiments, in conjunction with precise manipulations of environmental factors, and optimally coupled with genomic and phenotypic analyses.

4.3 | Effects of inversions on fitness components

Many studies have reported associations between chromosomal inversions and phenotypic traits in *Drosophila* (e.g., Battaglia & Smith, 1961; De Jong & Bochdanovits, 2003; Dobzhansky & Pavlovsky, 1961; Dobzhansky & Spassky, 1962; Durmaz, Benson, Kapun, Schmidt, & Flatt, 2018; Etges, 1989; García-Vázquez & Sánchez-Refusta, 1988; Hoffmann & Rieseberg, 2008; Hoffmann & Weeks, 2007; Hoffmann et al., 2004; Kapun, Schmidt, Durmaz, Schmidt, & Flatt, 2016; Krimbas & Powell, 1992; Lemeunier & Aulard, 1992; Sperlich & Pfriem, 1986). However, only few studies have isolated and phenotyped a large number of chromosomal lines to examine the effects of inverted vs. noninverted chromosomes on fitness components (i.e., life history traits), the major phenotypic targets of selection (Durmaz et al., 2018; Kapun, Schmidt et al., 2016). Thus, still little is known about how inversions impact fitness-related traits (for a notable exception in monkey flowers see Lowry & Willis, 2010). Yet, knowledge about how inversions affect fitness components is critical for our understanding of how selection acts on adaptive inversion polymorphisms.

Because several inversions exhibit strong clinality (see Section 4.1), and because many fitness traits also vary clinally (Adrion, Hahn, & Cooper, 2015; De Jong & Bochdanovits, 2003; Durmaz et al., 2018; Fabian et al., 2012, 2015; Hoffmann, Anderson, & Hallas, 2002; Hoffmann, Shirriffs, & Scott, 2005; Hoffmann & Weeks, 2007; Kapun, Schmidt et al., 2016), an attractive hypothesis is that inversions—for example by capturing adaptive alleles at multiple loci—are an important causal determinant of clinal variation in these traits. Given that phenotypic components of fitness represent highly polygenic traits, inversions might act as "supergenes," that is cluster of tightly linked loci affecting multiple complex phenotypes (cf. Schwander, Libbrecht, & Keller, 2014). Along the North American latitudinal cline, for example, high-latitude populations of *D. melanogaster* exhibit larger body size, reduced fecundity, increased stress resistance, and longer lifespan and can undergo reproductive dormancy, whereas flies from low-latitude populations possess the opposite combination of phenotypes (Coyne & Beecham, 1987; Mathur & Schmidt, 2017; Paaby, Bergland, Behrman, & Schmidt, 2014; Paaby, Blacket, Hoffmann, & Schmidt, 2012; Schmidt & Conde, 2006; Schmidt, Matzkin, Ippolito, & Eanes, 2005; Schmidt & Paaby, 2008; Schmidt, Paaby, & Heschel, 2005), and it thus interesting to ask whether and how clinally varying inversions contribute to these patterns.

In the context of phenotypic clines, the best—and practically the only—investigated inversion polymorphism in *D. melanogaster* is *In(3R)Payne*. For example, several studies suggest that this inversion polymorphism underlies latitudinal clines in body size on multiple continents. Consistent with this idea, two QTL studies observed that chromosome arm *3R* accounts for a major proportion of size variation between the endpoints of the Australian and South American clines (Calboli, Kennington, & Partridge, 2003; Gockel, Robinson, Kennington, Goldstein, & Partridge, 2002). A more direct connection was established by Weeks, McKechnie, and Hoffmann(2002) and Kennington et al. (2007) who identified multiple indel and microsatellite polymorphisms that are associated with size variation among Australian populations and which are in strong LD with *In(3R)Payne*. Importantly, Rako, Anderson, Sgrò, Stocker, and Hoffmann (2006), for the Australian cline, and Kapun, Schmidt et al. (2016), for the North American cline, established a direct causal effect of *In(3R)Payne* on body size by isolating inverted and noninverted lines from natural populations and showing that the inverted arrangement confers reduced size. Takahashi and Takano-Shimizu (2011) found that *In(3R)Payne* is in LD with an enhancer of *ebony* which causes lighter trident

coloration on the thorax; this is interesting because trident pigmentation exhibits latitudinal and altitudinal clinality and because genetic variation in pigmentation can have pleiotropic effects upon several fitness components, including fecundity and lifespan (Bastide, Yassin, Johanning, & Pool, 2014; Pool & Aquadro, 2007; Rajpurohit et al., 2016). Similarly, Endler, Gibert, Nolte, and Schlötterer (2018) have independently confirmed the association between lighter trident pigmentation and *In(3R)Payne* found by Takahashi and Takano-Shimizu (2011). A negative association between *In(3R)Payne* and susceptibility to cold has been found by Anderson, Collinge, Hoffmann, Kellett, and McKechnie (2003) in Australian populations. More recently, Durmaz et al. (2018) have shown that *In(3R)Payne* contributes to the latitudinal clinality of lifespan, starvation resistance and survival upon cold shock along the North American east coast: inverted karyotypes live shorter and are less stress resistant than noninverted karyotypes. Thus, in sum, the *In(3R)Payne* inversion confers reduced body size, lighter pigmentation, increased susceptibility to cold, decreased starvation resistance and shortened lifespan, whereas the noninverted arrangement has the opposite effects. However, why these alternative trait combinations are selectively advantageous in warmer vs. cooler climates remains unclear (Durmaz et al., 2018; Kapun, Schmidt et al., 2016). While it is possible that the effects of the Payne inversion on these traits are caused by a single pleiotropic locus (e.g., at the breakpoint), it might be more parsimonious to assume that *In(3R)Payne* represents a clinally varying "life history" supergene (Durmaz et al., 2018). Together with the evidence reviewed in Sections 3, 4.1 and 4.2, these data clearly show that *In(3R)Payne* affects multiple fitness components and is subject to spatially varying selection on multiple continents.

Unfortunately, despite the experimental tractability of the *D. melanogaster* model, almost nothing is known about the phenotypic effects of other inversions in this species (Lemeunier & Aulard, 1992). One exception is the finding that the frequency of *In(3R)C* is correlated with bristle number and that artificial selection for increased bristle number increases the frequency of this inversion (García-Vázquez & Sánchez-Refusta, 1988; García-Vázquez, Sanchez-Refusta, & Rubio, 1989; Izquierdo, García-Vázquez, & Villar, 1991). Moreover, a series of studies showed that *In(2L)t* heterokaryotypes exhibit overdominance with regard to fecundity and fertility in Japanese populations (Watanabe, 1969; Watanabe & Watanabe, 1973; Watanabe, Watanabe, & Oshima, 1976). van Delden and Kamping (1991) also found effects of *In(2L)t* on fitness traits, showing that inverted homokaryotypes had longer development time and lower body weight than the heterokaryotypes and noninverted standard homokaryotypes. Weeks et al. (2002) found that the frequency of *In(3L)P* is negatively associated with cold resistance—an observation consistent with the findings of Anderson et al. (2003) and Durmaz et al. (2018) for *In(3R)Payne* and those of Pool et al. (2017) showing that African highland populations are more cold-tolerant but have lower frequencies of common inversion polymorphisms. Given the tropical African origin of common cosmopolitan inversions, and their typically higher frequency in warmer climates, the negative relationship between their frequency and cold tolerance is intriguing and strongly points to a direct causal involvement of these chromosomal rearrangements in climate adaptation.

4.4 | Candidate genic targets of selection

What are the genic targets of selection within adaptive *D. melanogaster* inversions? As Hoffmann and Rieseberg (2008, p. 32) state: "Genes underlying traits may eventually be identified from microarray analyses, high-resolution mapping, mutagenesis, RNAi, and other approaches, ideally supported by manipulations through techniques like homologous recombination to test the effects of specific alleles on traits in the same genetic background. We are unaware of these approaches successfully identifying allele-trait associations segregating with inversions…." Although this situation is largely unchanged today, genomic and transcriptomic studies are beginning to provide a first glimpse into potentially adaptive loci associated with *D. melanogaster* inversions.

Using a GWAS approach with inversion-specific SNP markers, Kapun, Fabian et al. (2016) identified many biologically important candidate genes containing inversion-linked SNPs for *In(3R)Payne*, *In(2L)t*, *In(2R)NS*, *In(3L)P*, *In(3R)C*, *In(3R)K* and *In(3R)Mo*. For instance, given the effects of *In(3R)Payne* on size, lifespan and stress resistance (Durmaz et al., 2018; Kapun, Schmidt et al., 2016), it is noteworthy that this inversion contains many candidate genes that are known from studies of laboratory mutants and transgenes to affect these fitness-related traits, including several major loci of the insulin/insulin-like growth factor signalling (IIS)/target of rapamycin (TOR) pathway (for details of candidate loci see Kapun, Fabian et al., 2016; Kapun, Schmidt et al., 2016; and Durmaz et al., 2018; also see De Jong & Bochdanovits, 2003; Fabian et al., 2012). Several of the candidates identified by Kapun, Fabian et al. (2016) overlap with the highly localized centre peaks of divergence in *In(3R)Payne* and thus represent promising candidate targets of selection (cf. Figure 1). The breakpoint region of *In(3R)Payne* itself might also have important effects: variation at the *Dca* (*Drosophila cold acclimation*; also known as *smp-30*) locus, a gene located near the proximal breakpoint of *In(3R)Payne*, is associated with wing size variation (McKechnie et al., 2010), and a clinally varying polymorphism in the promoter of this gene reduces wing size (Lee et al., 2011; McKechnie et al., 2010).

More recently, Lavington and Kern (2017) analysed gene expression of *In(2L)t* and *In(3R)Mo* homokaryotypes and observed that these inversions affect the abundance of hundreds of transcripts genome-wide. Similarly, Said et al. (2018) found pervasive genome-wide effects of *In(2L)t* and *In(3R)K* on gene expression; notably, they found that differentially expressed loci for both inversions were enriched for genes involved in the immune response. Importantly, the authors generated synthetic inversions whose breakpoints closely matched the natural inversion as controls and showed that simply reversing the corresponding DNA segments did not result in the large-scale gene expression differences seen in the natural inversions. The analyses of Kapun, Fabian et al. (2016), Lavington and Kern (2017) and Said et al. (2018)

Box 3 Some major unresolved questions about inversion polymorphisms

- How do demography and selection interact to shape inversion polymorphisms? Approaches: for example, fitting population genetic models, with explicit demography, to genomic data.
- What types of selection act on inversions (e.g., additive, epistatic, overdominant, frequency-dependent selection)? Approaches: for example, population cage experiments to monitor inversion frequency and genotype trajectories; fitness assays of homo- vs. heterokaryons; assays of adaptive inversion-associated loci to distinguish between additivity and epistasis.
- What are the ecological factors that cause selection on inversions? Approaches: for example, ecological field studies and surveys, monitoring of environmental variables, outdoor population cage experiments and reciprocal transplantation experiments.
- Do different inversions interact to affect adaptation and, if so, how? Approaches: for example, comprehensive phenotyping of fitness components, population cage experiments using different combinations and frequencies of distinct inversions.
- What is the identity of adaptive loci associated with inversions? Approaches: for example, fitting coalescent models to phased sequencing data, genetic mapping approaches such as deficiency complementation mapping, CRISPR/Cas9.
- Do adaptive inversion polymorphisms represent "coadapted gene complexes" or do they harbour independent loci kept together by strong LD? Approaches: for example, functional tests of fitness epistasis among adaptive loci, for example using CRISPR/Cas9.
- How and why do inversions affect gene expression (e.g., within the inversion body, within short distance to the breakpoints and/or genome-wide)? Approaches: for example, RNA-seq, ATAC-seq.
- What are the effects of inversion polymorphisms, and the adaptive loci contained within them, on phenotypic components of fitness (life history traits)? Approaches: for example, comprehensive phenotyping assays, estimates of total fitness effects, competition assays, RNAi, CRISPR/Cas9.

provide useful, genome-wide lists of candidate loci associated with inversions (see Fuller, Haynes, Richards, & Schaeffer, 2016, for a transcriptomic analysis of *D. pseudoobscura* inversions)—importantly, such lists can be used to formulate novel, experimentally testable hypotheses, such as "the effects of *In(3R)Payne* on body size are due to genetic variation in the insulin signalling pathway," or "*In(2L)t* and *In(3R)K* are maintained by frequency-dependent selection on immune function.".

Because the loci inside inversions are subject to strong linkage, the perhaps greatest challenge lying ahead will be to distinguish between causative adaptive sites and noncausative sites subject to "hitchhiking." This might be technically feasible for evolutionarily relatively old inversions for which gene conversion and double crossover events have had sufficient opportunity to break up associations except for the targets of selection (see Figure 1). In the future, locally adapted loci inside inversions might be identified statistically by fitting coalescent models to phased sequencing data for inversions (Guerrero et al., 2012; Kirkpatrick, 2017); while the basic theoretical framework is in place, high-quality phased data required for model fitting have so far largely been lacking. Ultimately, however, identifying causal targets of selection associated with inversions, either those in the breakpoints or within the inversion body itself, will require functional genetic testing, for example using homologous allele replacement with CRISPR/Cas9 (Turner, 2014).

5 | CONCLUSIONS

For 100 years, since the early studies of Sturtevant and Dobzhansky, generations of evolutionary biologists have been fascinated by how inversions impact evolutionary change. Here, we have reviewed the adaptive role of inversion polymorphisms in *Drosophila melanogaster*. As our survey of the literature shows, excellent progress has been made in demonstrating that several inversion polymorphisms in this species are shaped by selection. Yet, despite a century of work, how selection does so remains still relatively poorly understood (cf. Kirkpatrick & Kern, 2012): What types of positive selection lead to the spread of inversions? What types of balancing selection are acting to maintain a given polymorphism once it has spread and how? And what are the genic targets of selection associated with inversions? Given recent advances in genomics, gene editing and theoretical modelling, we are hopeful that major progress towards addressing these fundamental questions can be made in the near future (see Box 3).

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DATA ACCESSIBILITY

The inversion frequency data shown in Figure 2 are available in Supporting Information Table S1 and have been deposited at Dryad under accession: https://doi.org/10.5061/dryad.3gq3gc6.

CONFLICT OF INTEREST

The authors of this manuscript have declared no competing interests.

AUTHOR CONTRIBUTION

M.K. analysed inversion frequency data, and M.K. and T.F. wrote the manuscript.

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