Insect Science

Insect Science (2024) 31, 328–353, DOI 10.1111/1744-7917.13262

REVIEW

Spatiotemporal dynamics in butterfly hybrid zones

Ananda R. Pereira Martins[1,2], Natalie B. Warren[1], W. Owen McMillan[2] and Rowan D. H. Barrett[1]

[1] *Redpath Museum, McGill University, 859 Sherbrooke Street West, Montreal, Quebec, Canada* and [2] *Smithsonian Tropical Research Institute, Gamboa, Panama City, Panama*

Abstract Evaluating whether hybrid zones are stable or mobile can provide novel insights for evolution and conservation biology. Butterflies exhibit high sensitivity to environmental changes and represent an important model system for the study of hybrid zone origins and maintenance. Here, we review the literature exploring butterfly hybrid zones, with a special focus on their spatiotemporal dynamics and the potential mechanisms that could lead to their movement or stability. We then compare different lines of evidence used to investigate hybrid zone dynamics and discuss the strengths and weaknesses of each approach. Our goal with this review is to reveal general conditions associated with the stability or mobility of butterfly hybrid zones by synthesizing evidence obtained using different types of data sampled across multiple regions and spatial scales. Finally, we discuss spatiotemporal dynamics in the context of a speciation/divergence continuum, the relevance of hybrid zones for conservation biology, and recommend key topics for future investigation.

Key words butterflies; human impacts; hybrid zones; movement; spatiotemporal dynamics; stability

Introduction

Hybrid zones (HZs) have long been considered natural laboratories for investigating evolutionary processes (Hewitt, 1988; Barton & Hewitt, 1989). HZs can be defined as regions where genetically distinct populations within a parapatric distribution mate and produce viable mixed offspring (Barton & Hewitt, 1985, 1989; Hewitt, 1988; Mallet, 2005). Studies on HZs have allowed us to explore the persistence of distinct lineages despite gene flow (Barton & Gale, 1993; Harrison & Larson, 2014; Larson *et al.*, 2014), the spread of advantageous alleles throughout populations (Arnold & Martin, 2009; Edelman & Mallet, 2021), the accumulation of incompatibilities between divergent populations (Jiggins & Mallet, 2000), the strengthening or weakening of reproductive isolation in the face of gene flow (Barton & Hewitt, 1985, 1989; Smadja & Butlin, 2011), as well as helping to identify candidate adaptive loci (Pardo-Diaz *et al.*, 2012).

HZs have commonly been described as a collection of clines using a single-locus model, where each cline represents the gradual frequency change of a trait or allele over a geographical range (Endler, 1977; Barton & Hewitt, 1985). In a *Heliconius erato* hybrid zone (HZ) in Panama, for example, the transition of distinct phenotypes characterized by the presence or absence of a yellow hindwing bar is controlled by a single locus and can be represented as a cline in which allele frequency change has the shape of a sigmoid curve (Fig. 1). However, more complex genetic architectures involving, for instance, epistasis (Cordell, 2002; Phillips, 2008) and linkage disequilibrium (LD) (Nordborg & Tavaré, 2002; Slatkin, 2008), required the development of more complex multilocus models (Barton, 1983; Szymura & Barton, 1986, 1991; Barton & Gale, 1993; Barton & Shpak, 2000;

Correspondence: Ananda R. Pereira Martins, Redpath Museum, McGill University, 859 Sherbrooke Street West, Montreal, H3A0C4 Quebec, Canada. Email: ananda.pereiramartins@mail.mcgill.ca

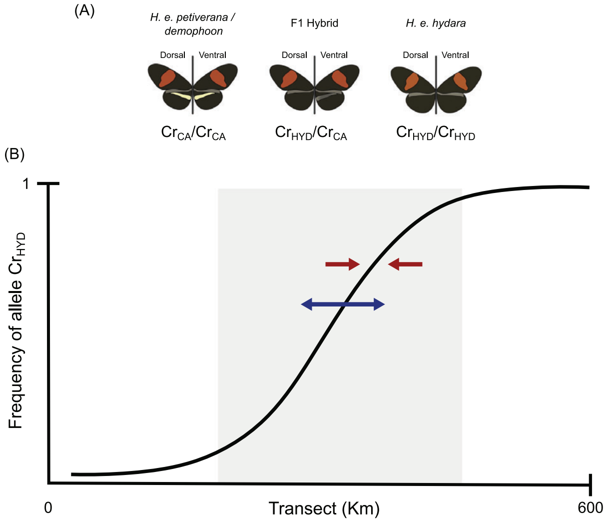


Fig. 1 Example of a *Heliconius erato* hybrid zone in Panama showing the transition of a phenotype characterized by the presence of a yellow hindwing bar, crossing hybrids presenting a faint bar (visible as a shadow on the ventral hindwing), to a phenotype without the bar (for more details about phenotypes, see Mallet, 1986 and Thurman *et al.*, 2019). (A) Wing patterns and genotypes of *H. e. petiverana* or *H. e. demophoon*, hybrid (heterozygous), and *H. e. hydara*. *H. e. petiverana* and *H. e. demophoon* are two *H. erato* subspecies presenting very similar phenotypes, and as both cooccur in Panama, it is difficult to classify a yellow bar individual based solely on color pattern. (B) Black sigmoid curve represents the Cr_{HYD} allele frequency change across a 600 km transect. The light gray area represents the cline width (i.e., width is determined by the strength of selection and dispersal), which tends to become wider (represented by a double blue arrow) with the increase of parental dispersal into the zone and narrower (represented by red arrows) as selection against hybrids gets stronger. This figure is adapted from the results of Thurman *et al.* (2019).

Gompert & Buerkle, 2009). In particular, in cases where several loci affect fitness, multiple clines are steeper and coincident at the center of the HZ where recombination is lower and the whole genome tends to be in LD. On the other hand, cline tails are discontinuous, with different loci experiencing distinct patterns of selection (Barton, 1983) (Fig. 2).

Clines are maintained in a stable position by a balance between dispersal and selection against hybrids, which can include endogenous (i.e., genetic incompatibilities) and/or exogenous (i.e., environmental) selective pressures (Barton, 1979; Barton & Hewitt, 1985, 1989) (Figs. 1 and 2). Nonetheless, various forces can disrupt stability and cause a HZ geographic movement (Barton, 1979; Barton & Hewitt, 1985; Buggs, 2007). For instance, if selection on distinct phenotypes results in parental populations adapted to different habitats, an environmental change can generate asymmetric selection favoring one population, increasing its frequency, and causing an asymmetric parental migration that leads to the HZ movement. Accordingly, the movement can cease if the HZ reaches a new dispersal-selection equilibrium (Barton, 1979; Barton & Hewitt, 1985; Mallet, 1993).

Butterflies are a well-studied group that have been used as a key model organism in HZ investigations, with a vast literature dating back to Henry Walter Bates (1862). There are a number of reasons that butterflies have been the focus of hybrid zone research. Foremost, they are easy to study in the field and have relatively short generation times, which facilitates collection of temporal data (Watt & Boggs, 2003; Kral *et al.*, 2018; Freitas *et al.*, 2021). In addition, their basic ecology is well understood, and selection pressures can often be determined and measured (Gilbert & Singer, 1975; Boggs *et al.*, 2003; Monteiro, 2015; Van Belleghem *et al.*, 2021). Moreover, many species can be kept under controlled conditions, making analysis of mating preferences, reproductive incompatibilities, and the genetic characterization of key phenotypic traits possible (Jiggins *et al.*, 2004; Concha *et al.*, 2019; Orteu & Jiggins, 2020; Rossi *et al.*, 2020; Livraghi *et al.*, 2021; Jay *et al.*, 2022). The result is that butterfly HZ studies have greatly contributed to our understanding of hybrid zone dynamics (Scriber *et al.*, 2014; Dupuis & Sperling, 2016; Gauthier *et al.*, 2020) and revealed evolutionary and ecological outcomes, including the discovery of adaptive loci and their effects on population dynamics (Mallet, 1993; Merrill *et al.*, 2015; Capblancq *et al.*, 2019; Morris *et al.*, 2019; Zhang *et al.*, 2021). Butterflies are also well represented in museum collections around the world (Brown, 1991; Watt & Boggs, 2003; Brooks *et al.*, 2014), which facilitates temporal and regional assessments (Parmesan, 2003; Thomas, 2005, 2016). Importantly, under the current global environmental change, the spatiotemporal dynamics of butterfly HZs stand out as an important indicator of the impacts of climate change due to butterflies' high sensitivity to environmental changes (Bonebrake *et al.*, 2010; Taylor *et al.*, 2015; Freitas *et al.*, 2021; Hill *et al.*, 2021), such that environmentally driven changes and movements of population ranges can be detected using standardized sampling methods.

Herein, we review published papers to investigate the spatiotemporal dynamics of butterfly HZs and the potential mechanisms that could lead to their movement or stability. We examine and compare different types of evidence obtained across multiple regions and spatial scales and evaluate their respective advantages and disadvantages. We then discuss the effects of HZ spatiotemporal dynamics on evolution and conservation biology. Finally, we discuss future directions for research

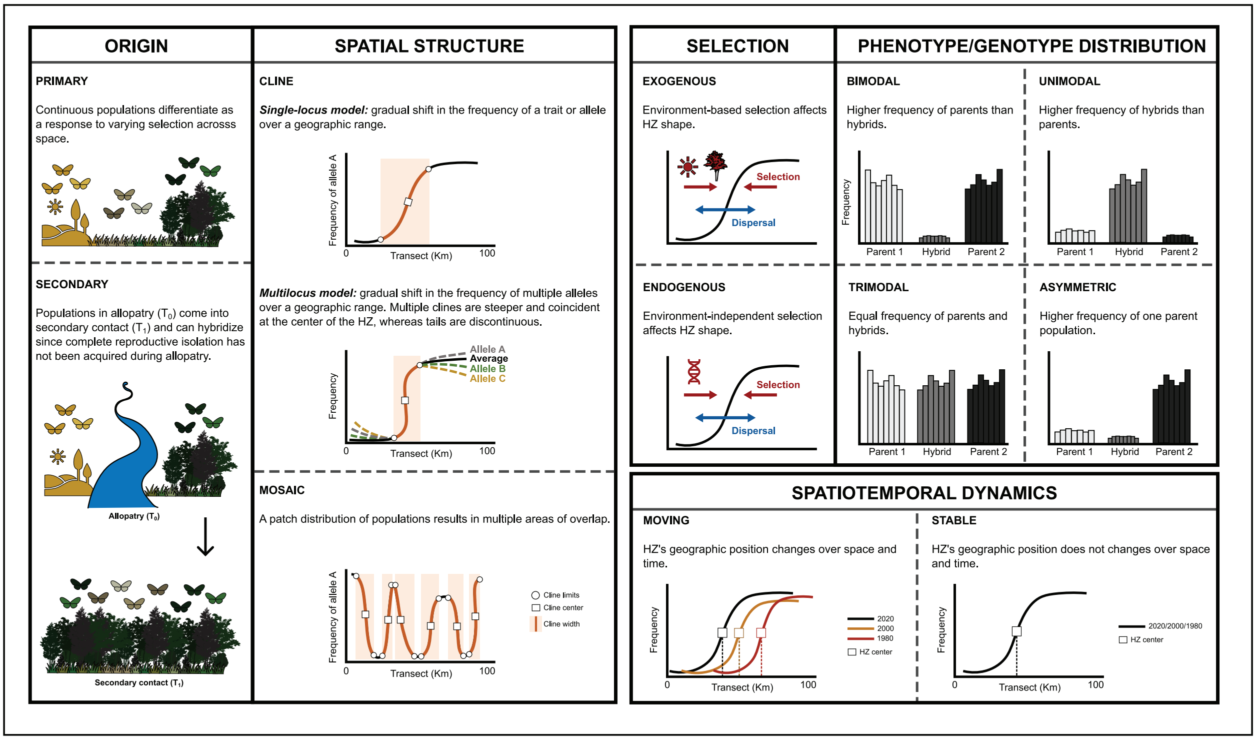


Fig. 2 Hybrid zone classification according to different criteria (Endler, 1977; Barton, 1983; Barton & Hewitt, 1985, 1989; Harrison & Bogdanowicz, 1997; Jiggins & Mallet, 2000; Harrison & Larson, 2016; Abdelaziz *et al.*, 2021).

on HZ spatiotemporal dynamics under the context of global environmental changes.

Literature search

We searched articles on Google Scholar, Web of Science, Scopus, and the WorldCat platforms using the following terms: “Hybrid zone” OR “cline” OR “tension zone” AND “movement” OR “moving” OR “shift” OR “stasis” OR “stability” OR “static” OR “stable” OR “maintain” OR “maintained” OR “maintenance” OR “trapped” OR “dynamics” OR “spatiotemporal dynamics” OR “position” OR “location” AND “butterfly” OR “butterflies” OR “Lepidoptera.” We selected publications that explicitly tested for hybrid zone spatiotemporal dynamics, as well as papers from which we could hypothesize a spatiotemporal classification based on indirect evidence or authors’ untested suggestions. We excluded review articles, book chapters, and studies in which the hybridizing taxa were unclear. Our final database contains 36 articles, encompassing 13 butterfly genera (Table 1). Among these, two articles focused on hybrid zones of multiple heliconiine and ithomiine species. We included them in our database as they contain important generalizations regarding suture zones (i.e., geographic areas in which multiple hybrid zones overlap; Remington, 1968).

Mechanisms driving hybrid zones spatiotemporal dynamics

Hybrid zones can be classified according to different criteria (Fig. 2). Most HZs are suggested to be clines subject to environment-independent selection (Barton & Hewitt, 1985) (Figs. 1 and 2). These are known as tension zones and can move across the geographic landscape when the balance between endogenous selection against hybrids and dispersal is disturbed (Barton, 1979; Barton & Hewitt, 1985, 1989). Various forces can disrupt the tension zone equilibrium, including dominance drive (i.e., a mechanism of allelic asymmetric selection in which movement is determined by the spatial expansion and fixation of a dominant allele [Mallet, 1986; Mallet & Barton, 1989]), gradients in population density, differential parental migration, and asymmetries in selection (Barton, 1979; Barton & Hewitt, 1985; Mallet, 1986). In

Table 1 Studies on butterflies' hybrid zones.

Hybridizing taxa[†]	Location	Type of data	Moving or stable	Evidence	Main mechanism(s)	References
Anartia fatima *A. amathea*	Panama	Genetic and phenotypic clines	Moving	Temporal comparison	Density differences, asymmetric migration, and/or hybridization	Dasmahapatra *et al.* (2002)
Coenonympha gardetta *C. arcania* *C. darwiniana* (hybrid sp.)	France, Switzerland and Italy	Simulation of divergent demographic scenarios, population genetic, and phenotypic structures, and climatic niche analysis	Stable Past movements?	Model results, genetic and phenotypic clustering, climatic niche separation	Ecological adaptation (altitude), differential backcrossing rates of two *C. darwiniana* lineages, and parental species	Capblancq *et al.* (2015)[‡]
Coenonympha gardetta *C. arcania* *C. macromma* (hybrid sp.) *C. darwiniana* (hybrid sp.)	France, Switzerland, Italy, and Austria	Simulation of divergent demographic scenarios, population genetic structure and correlations between ecological divergence and genetic isolation	Stable Past movements?	Model results, ancestry patterns, genotypic distribution, genetic, and phenotypic clustering	Ecological adaptation (altitude)	Capblancq *et al.* (2019)[‡]
Coenonympha gardetta *C. macromma*	France	Genetic, phenotypic, and spatial clines; population genetic and phenotypic structures	Stable Movement prediction	Low heterozygosity, clines with steep and narrow widths, and association with environment	Adaptation to climatic gradient (stability); asymmetric selection due to climate change (movement prediction: rise of temperature and change of vegetation cover)	Capblancq *et al.* (2020)[‡]
Erebia cassioides *E. tyndarus* *E. nivalis*	Switzerland	Genetic, phenotypic, ecological, and parasitic clines; population genetic and phenotypic structures, patterns of introgression (D-statistics); and niche differentiation	Stable	Few F1 hybrids, concordant narrow genotypic, phenotypic, and parasitic clines; population clustering, bacterial infection differences, D-statistics results	Postzygotic incompatibilities, strong selection against hybrids, chromosome number difference as a strong but incomplete barrier to gene flow	Lucek *et al.* (2020)[‡]

(to be continued)

Table 1 (Continued).

Hybridizing taxa[†]	Location	Type of data	Moving or stable	Evidence	Main mechanism(s)	References
Erebia tyndarus *E. cassioides*	Switzerland	Population genetic and phenotypic structures; genetic, phenotypic, and environmental clines	Stable	Few F1 hybrids, population clustering, concordance of genetic and phenotypic narrow clines, and wide environment clines	Strong (endogenous) selection against hybrids, differences in bacterial infection as an intrinsic postzygotic barrier, reinforcement could have occurred in the past	Augustijnen *et al.* (2022)[‡]
Heliconius erato hydara *H. e. petiverana/ H. e. demophoo*[§]	Panama	Simulation, genetic Cline, and associations with land cover	Moving	Model results and temporal comparison	Dominance drive, asymmetric selection, and/or migration	Mallet (1986) Blum (2002) Thurman *et al.* (2019)
Heliconius erato favorinus *H. e. emma*	Peru	Simulation, genetic Cline, and associations with climate	Stable Movement prediction	Model results and temporal comparison	Local barrier trapping hybrid zone, constant selection, low population density	Mallet *et al.* (1990) Rosser *et al.* (2014)
Heliconius melpomene amaryllis *H. m. aglaope*	Peru	Simulation, genetic cline, and associations with climate	Stable Movement prediction	Model results and temporal comparison	Local barrier trapping hybrid zone, constant selection, low population density	Mallet *et al.* (1990) Rosser *et al.* (2014)
Heliconius erato *H. himera*	Ecuador and Peru	Genetic cline	Stable	Narrow cline widths and associations with habitat gradient	Predator FDS,[¶] ecological adaptation and assortative mating	Jiggins *et al.* (1996)[‡]
Heliconius erato venus *H. e. chestertonii*	Colombia	Population genetic structure, LD,[††] Hardy-Weinberg estimates, and phylogeny	Stable	Patterns of ancestry, deficit of heterozygotes, and monophyletic subspecies	Ecological adaptation, assortative mating, strong selection against hybrids	Arias *et al.* (2008)[‡]
Heliconius erato hydara *H. e. erato*	French Guiana	Genetic, phenotypic, and spatial clines	Stable	Temporal comparison	(*balancing*) Dominance drive and asymmetric selection	Blum (2008)

(to be continued)

Table 1 (Continued).

Hybridizing taxa[†]	Location	Type of data	Moving or stable	Evidence	Main mechanism(s)	References
Heliconius erato lativitta *H. e. notabilis*	Ecuador	Genetic and phenotypic clines, and deterministic two-locus FDS[¶] simulation	Stable Movement prediction	Asymmetric introgression and model results	(*balancing*) Dominance drive, predator FDS, [¶]or hybrid advantage	Meier *et al.* (2021)
Heliconius melpomene malleti *H. m. plesseni*	Ecuador	Genetic and phenotypic clines, and deterministic two-locus FDS[¶] simulation	Stable Movement prediction	Asymmetric introgression and model results	(*balancing*) Dominance drive, predator FDS,[¶] or hybrid advantage	Meier *et al.* (2021)
Heliconius erato Postman X dennis-ray phenotypes	Brazil	Phenotypic clines	Stable	Location of hybrid zones of comimetic subspecies pairs	Amazon River as a dispersal barrier	Pereira Martins *et al.* (2022)[‡]
Heliconius melpomene Postman X dennis-ray phenotypes	Brazil	Phenotypic clines	Moving/past movement	Location of hybrid zones of comimetic subspecies pairs	Historical dispersal	Pereira Martins *et al.* (2022)[‡]
Ithomia salapia derasa *I. s. aquinia*	Peru	Genetic cline, population genetic and phenotypic structures	(Past) Moving (Current) Stable	(Past) Asymmetric introgression and patterns of ancestry; (Current) Low heterozygosity	(Past) Asymmetric hybridization; (Current) selection against hybrids and adaptation to environmental gradient	Gauthier *et al.* (2020)[‡]
Limenitis weidemeyerii *L. lorquini*	USA[‡‡]	Phenotypic population structure across space	1. Moving or stable (context dependent) 2. Stable	Hybrids compared to parental scores	1. Drift and asymmetric selection (moving regions); selection and dispersal balance (stable regions) 2. Hybrid superiority and weak parental dispersal	Boyd *et al.* (1999)[‡‡]

(to be continued)

Table 1 (Continued).

Hybridizing taxa[†]	Location	Type of data	Moving or stable	Evidence	Main mechanism(s)	References
Lycaeides melissa *L. idas*	USA and Canada	Population genetic and phenotypic structures, genetic and phenotypic clines, and LD[††]	Moving	Ancestry patterns, discordant and wide clines, asymmetric genomic composition cline, no association between LD,[††] and geographic location	Patchy distribution, local extinctions and recolonizations, environmental variation, weak selection against hybrids, differential selection among hybrid genotypes, and high parental dispersal	Gompert *et al.* (2010)[‡]
Jackson Hole *Lycaeides* *Lycaeides melissa*	USA	Population genetic structure and genetic clines	Moving?	Asymmetric introgression and asymmetric patterns of ancestry	Asymmetric selection or an excess contribution of DMIs[§§]	Zhang *et al.* (2022)[‡]
Lycaena bleusei *L. tityrus*	Iberian Peninsula	Population genetic and phenotypic structures, phylogeny, divergence times estimates, and species distribution modeling	Stable Past movements	Modeling results, monophyletic species, infrequent hybrids, and genetic and phenotypic population clustering	Different environmental variables drive distinct species distributions, selection against hybrids; range expansions and retractions (Past movements)	Marabuto *et al.* (2023)[‡]
Oleria onega janarilla *O. o. ssp nov2*	Peru	Genetic cline, population genetic and phenotypic structures	(Past) Moving (Current) Stable	(Past) Asymmetric introgression and ancestry patterns; (Current) low heterozygosity	(Past) Asymmetric hybridization; (Current) selection against hybrids and adaptation to environmental gradient	Gauthier *et al.* (2020)[‡]

(to be continued)

Table 1 (Continued).

Hybridizing taxa[†]	Location	Type of data	Moving or stable	Evidence	Main mechanism(s)	References
Papilio canadensis *Papilio glaucus*	USA	Population phenotypic structure, fitness estimates, divergence times and phylogeny, genetic and spatial clines, LD[††] and search for candidate genes	Stable Movement prediction	Hybrids compared to parental scores, survival differences, patterns of ancestry, rates of introgression, LD[††] patterns, species-specific polymorphisms and haplotypes proportions, developmental threshold distribution, no association between candidate genes, and ecological adaptations	(*balancing*) Unknown genetic factors, ecological adaptation, and mate choice; asymmetric selection due to climate change (movement prediction); hybrid occupation of a novel adaptive peak and local adaptation (stability of hybrid species: *P. appalachiensis*)	Scriber (1990)[‡,¶¶] Scriber *et al.* (2002)[‡] Kunte *et al.* (2011)[‡] Ryan *et al.* (2017)
Papilio glaucus *Papilio canadensis*	USA 1. Large scale 2. Fine scale	Phenotypic and climate change associations	Stable	Temporal and scale comparisons	1. Regions with no association with climate warming or no increasing of temperature detected 2. Phenotypic plasticity	Scriber *et al.* (2014)[‡]
Papilio glaucus *Papilio canadensis*	USA	Phenotypic and climate associations using latitudinal and local scales, genetic and phenotypic frequencies across space, and developmental threshold distribution simulation	Moving	Differences in preferences and performance associated with varying thermal constrains, asymmetric introgression	Asymmetric selection due to climate change (rise of temperature) and asymmetric mating	Scriber (2002)[‡,†††] Stump *et al.* (2003)[‡] Ryan *et al.* (2018)

(to be continued)

Table 1 (Continued).

Hybridizing taxa[†]	Location	Type of data	Moving or stable	Evidence	Main mechanism(s)	References
Papilio machaon *P. zelicaon*	Canada	Population genetic structure	Stable	Temporal comparison	?	Dupuis and Sperling (2016)[‡]
Pieris napi *P. bryoniae*	Switzerland	Fitness estimates	Stable Movement prediction	Survival and selection differences	(*balancing*) Asymmetric selection, unknown genetic factors, and ecological adaptation	Porter (1997)[‡]
Polyommatus agestis *P. artaxerxes*	Scotland, England, and North Wales	Phylogeny and population genetic structure across space	Moving	Genetic proportions and mtDNA haplotypes across the landscape	Asymmetric selection due to climate change (rise of temperature)	Mallet *et al.* (2011)[‡]
Polyommatus celina *P. icarus*	Europe Hybrid zone: Spain and France	Phylogeographical patterns: population genetic and phenotypic structures	Stable Past movements	Low number of hybrids, phylogenetic distance between species, phenotypic differences	Competition, partial Reproductive barrier, strong selection against hybrids	Dincă *et al.* (2011)[‡]
Pontia daplidice *P. edusa*	Italy	Genetic clines, estimates of selection, dispersal, and barrier strength (calculated based on geographic distance)	Stable Past movements	Strong selection and high parental dispersal forming steep clines with similar long tails of introgression in both sides of the cline	Selection and dispersal balance	Porter *et al.* (1997)[‡]
22 species of ithomiine and heliconiine butterflies (each species presenting a pair of hybridizing subspecies)[‡‡‡]	Peru	Divergence times modeling using mutilocus sequence data	Stable Past movements	Model results (variable split times)	Cordillera Escalera (Andes) represents a barrier trapping multiple independently moving clines (region of low population density)	Dasmahapatra *et al.* (2010)[‡,‡‡‡]

(to be continued)

Table 1 (Continued).

Hybridizing taxa[†]	Location	Type of data	Moving or stable	Evidence	Main mechanism(s)	References
25 species of heliconiine butterflies representing four mimicry rings [‡‡‡]	Brazil	Models to test associations between color diversity and geographic location, and phenotypic clines	Stable Past movements	Model results, concordance of clines (Red mimicry ring)	Amazon River is a semipermeable barrier that traps multiple independently moving clines (region of low population density)	Rosser *et al.* (2021)[‡‡‡]

[†] If it is a moving hybrid zone, the advancing taxon comes first.

[‡] The study does not directly focus on hybrid zone spatiotemporal dynamics. Potential classification is based on indirect evidence or authors' untested suggestions.

[§] *H. e. petiverana* and *H. e. demophoon* are two *H. erato* subspecies presenting very similar phenotypes, and as both occur in Panama, it is difficult to classify a *H. erato* "yellow bar" individual based solely on color pattern.

[¶] FDS: Frequency-dependent selection.

[††] LD: linkage disequilibrium.

[‡‡] Study evaluated two hybrid zones. (1) A hybrid zone termed "Mono" extending from the Wassuk Range in western Nevada to the canyons on the east slope of the Sierra Nevada in eastern California. (2) A hybrid zone termed "Humboldt" located in northern Nevada in the Santa Rosa and Pine Forest ranges.

[§§] DMIs: Dobzhansky-Müller incompatibilities.

[¶¶] In Scriber (1990), the subspecies names *Papilio glaucus canadensis* and *Papilio glaucus glaucus* are used instead of the current accepted *Papilio canadensis* and *Papilio glaucus*.

[†††] Scriber (2002) compared populations with and without phenological thermal constrains within each species using large (latitudinal) and local ("cold pockets") spatial scales.

[‡‡‡] The study does not focus on a hybrid zone between two delimited taxa, but we decided to include in our database as there are important generalizations regarding multiple concordant clines.

addition, stochastic processes, such as random changes in population sizes and random dispersal can also affect tension zone dynamics (Barton, 1979; Barton & Hewitt, 1989). In particular, genetic drift may affect population structures, leading to misleading cline estimates (Polechová & Barton, 2011).

There are many examples of moving and stable tension zones in butterflies (Table 1). However, despite many studies assuming an environment-independent cline model, it is unrealistic to completely exclude environmental factors when empirically testing HZ spatiotemporal dynamics. Since HZs occupy a geographic space, they are intrinsically subjected to exogenous influences (Barton & Hewitt, 1985; Mallet, 1993; Curry, 2015), even if these influences are relatively weak compared to endogenous selection. For this reason, studies proposing the stability or movement of a tension zone generally do not discard minor and indirect effects of exogenous selection. Thurman *et al.* (2019), for example, showed the westward movement of a tension zone in Panama following the advance of the genetically dominant *Heliconius erato hydara* and the retraction of *H. e. demophoon*/*petiverana* (Fig. 1A). This result corroborated the prediction made more than 30 years earlier (Mallet, 1986) and tested 17 years later (Blum, 2002). These studies suggested that westward movement was mainly caused by dominance drive (Mallet, 1986; Mallet & Barton, 1989). Nevertheless, Blum (2002) hypothesized that differences in habitat preferences, particularly forest type, also contributed to the cline's movement as a consequence of the increasing deforestation in Panama. As such, environmental factors and dominance drive would be acting in concert, leading to asymmetric selection and migration favoring *H. e. hydara* expansion. Thurman *et al.* (2019) tested the habitat-preference hypothesis and did not find strong evidence for associations between HZ movement and forest change. Yet, they did not discard indirect and/or weak environmental effects on the HZ, since results pointed to a decrease in the movement velocity and an increase in the cline width over the years. These findings suggested that selection has become weaker, possibly because intense deforestation has impacted bird populations and consequently reduced *Heliconius* predation.

In a different tension zone in Panama, Dasmahapatra *et al.* (2002) documented an eastward movement favoring the advance of *Anartia fatima* and the spatial replacement of *A. amathea* (Table 1). The authors suggested that differences in population density have caused asymmetric migration and/or asymmetric hybridization, initiating movements in the direction of the lower density region. As in Thurman *et al.* (2019) and Blum (2002), this study also discussed the potential influences of deforestation across Panama in the HZ. However, like *Heliconius erato*, *Anartia* butterflies are adapted to open areas. As such, if deforestation were a major force influencing movement, it would be expected that *Heliconius* and *Anartia* HZs would shift in the same direction, which was not the case. The authors suggested that rather than an abiotic environmental effect, unknown ecological and genetic factors are creating population density differences and driving these distinct movement patterns (Dasmahapatra *et al.*, 2002).

Our compiled dataset also contains studies indicating the stability of butterfly tension zones (Table 1). For example, Mallet *et al.* (1990) predicted a westward movement of two Müllerian comimic tension zones in Peru, one between subspecies of *Heliconius erato* and another between subspecies of *H. melpomene*. However, in this study, tension zones appeared to be stable on the slopes of the Andes. This pattern is probably due to the presence of a physical barrier and/or high rainfall rates, which impede dispersal. Indeed, high rainfall can suppress butterfly activity (Mallet *et al.*, 1990; Mallet, 1993), resulting in low population density and preventing cline movements (Barton, 1979). Rosser *et al.* (2014) revisited these tension zones and found stability over a period of 25 years. The authors found a strong positive correlation between rainfall and the proportion of heterozygotes and argued that the low population density on the slopes of the Andes is probably due to the intense pluviosity, and thus that low population density in the transition zone was the main cause of the stability.

Hybrid zones that are moving or stable as a primary response to exogenous selection do not fit the tension zone model. Studies focusing on HZs between *Papilio glaucus* and *P. canadensis*, for example, have showed varying patterns, including movement due to asymmetric selection associated with climate warming (Scriber, 2002; Ryan *et al.*, 2018), stability despite temperature increasing due to phenotypic plasticity (Scriber *et al.*, 2014), and stability as a response to the coupling effects of endogenous barriers and local adaptation to climatic gradients (Ryan *et al.*, 2017). Importantly, HZs that are mainly subject to exogeneous selection can still be characterized as clines when the frequency of traits or alleles follow environmental gradients (e.g., Ryan *et al.*, 2017; Capblancq *et al.*, 2020). However, empirical examples have documented significant variation in allele/trait frequency between populations across distinct regions, potentially due to habitat preference and abiotic local selection (e.g., Ross & Harrison, 2002; Vines *et al.*, 2003; Curry, 2015). Therefore, HZs characterized by high levels of spatial structure are better represented under a two-dimensional model, termed as mosaic hybrid zones (Fig. 2) (Harrison & Rand, 1989). Accordingly, spatiotemporal dynamics characteristics will depend on the location of the zone. In HZs between *Papilio canadensis* and *P. glaucus*, for instance, it has been shown that specific geographical locations on the thermal landscape created distinct selection regimes, leading to different voltinism patterns (Scriber, 2002).

We emphasize that caution is warranted while assessing HZ spatiotemporal dynamics, as well as with its potential causes, since multiple mechanisms could create genetic patterns, fitness estimates, and population structures similar to those produced by movement or stability (e.g., Zhang *et al.*, 2022). In this review, we have included studies that did not directly test spatiotemporal dynamics as these can also offer important insights on HZ characteristics, along with inferences based on indirect evidence or authors' untested suggestions. In the following sections, we discuss how different lines of evidence can be used to make hypotheses about the spatiotemporal dynamics of hybrid zones, highlighting the strengths and limitations of each approach.

Interpreting distinct lines of evidence

Repeated observations of clines over time are the most reliable evidence of a HZ movement (Buggs, 2007; Wielstra, 2019). Using this approach, Dasmahapatra *et al.* (2002) reported a spatial movement of 50 km in the HZ between *Anartia fatima* and *A. amathea* over a period of 23 years. In addition, repeated sampling over multiple years showed an acceleration in the rate of HZ movement during the study period. Blum (2002) and Thurman *et al.* (2019) also inferred movements of 47 and 64 km after 17 and 33 years, respectively, when revisiting a *Heliconius* HZ that had previously been assessed by Mallet (1986). Ryan *et al.* (2018) projected a movement of 68 km using simulation modeling and documented a 40 km shift using empirical evidence over the period of 32 years in a HZ between *Papilio glaucus* and *P. canadensis* (Table 1). Besides movement, our literature review also revealed studies suggesting stability based on temporal monitoring. For instance, the overall stability of *Heliconius* HZs in French Guiana (Blum, 2008) and Peru (Rosser *et al.*, 2014) was proposed based on temporal comparisons of 31 and 27 years, respectively.

Even when cline parameters (i.e., position, width, and slope) are not temporally compared, indirect evidence can be useful when studying HZ dynamics. For example, two studies using 30-year temporal comparisons suggested stability in the HZs between *Papilio glaucus* and *P. canadensis* based on results of phenotypic and climate change associations (Scriber *et al.*, 2014), and between *P. machaon* and *P. zelicaon* based on genetic clustering of microsatellite and single nucleotide polymorphism data (Dupuis & Sperling, 2016). However, when direct evidence is lacking, a spatiotemporal hypothesis should be proposed by combining varying lines of indirect evidence and assessing HZ classification using different criteria (Fig. 2). This is justified because distinct HZ characteristics have specific assumptions. For instance, the cline model predicts that HZ movement will occur in the direction of the lower fitness phenotype and/or lower population density. The best-adapted phenotype tends to increase in frequency and is more likely to send out emigrants, reducing the distribution of the lower fitness phenotype (Barton, 1979; Barton & Hewitt, 1985, 1989). On the other hand, in a mosaic structure, hybridization occurs in several areas because discontinuous parental distributions create distinct contact zones. For this reason, the spatial scale of sampling is crucial, as at a large scale, this structure can be represented by several clines with geographically varying parameters and dynamics (Bridle *et al.*, 2001; Ross & Harrison, 2002; Curry, 2015) (Fig. 2). In the next section, we explore in more detail how distinct lines of evidence may contribute to our understanding of HZs spatiotemporal dynamics.

Simulation models

Simulation models are simplified representations of the real world in which, using computer programs, it is possible to manipulate distinct parameters of complex systems (Peck, 2004; Jørgensen & Fath, 2011). As such, in biology, models represent important tools to predict species distributions and population dynamics while also including information about past, current, and/or future climatic scenarios (Getz *et al.*, 2018). Our compiled data presents nine studies in which simulation models were used (Table 1). Of these, we highlight a few studies simulating changes in *Heliconius* genotype frequencies to predict whether a HZ is moving or stable.

Mallet (1986) showed a HZ tendency to move due to dominance drive, using a single-locus, two-allele, and one-dimension cline model in *Heliconius erato* (Fig. 1), assuming environment-independent selection. The author discussed movement under distinct selection and dispersal scenarios and their consequences for the divergence process. If selection and dispersal were assumed to be low, the model predicted slow HZ movement and that the current parapatric distribution is most likely the result of secondary contact. If selection and dispersal were considered high, rapid HZ movement could hide patterns of past distributions or movement could be hampered by counter-effect factors, such as population structure (Barton, 1979). Importantly, Mallet (1986) recognized the necessity of better estimates of dispersal and selection for more robust inferences regarding the velocity of movement.

Using a three-locus model, Mallet *et al.* (1990) also predicted movement in *H. erato* and *H. melpomene* HZs in Peru as a result of dominance drive. However, the authors suggest that the presence of the Andes might hamper cline movement, possibly because it represents a barrier to butterflies' dispersal and/or harsh ecological conditions that could reduce population density. Thus, although modeling results pointed to the movement of comimics' hybrid zones, knowledge about butterfly habitat suitability and the presence of physical barriers support a hypothesis contrary to the one suggested by the model.

Combining empirical phenotypic and haplotype cline analyses, Meier *et al.* (2021) proposed the coemergence of a hybrid morph in two parallel *Heliconius* HZs across an elevation gradient. The authors simulated two loci with complete dominance to test whether positive frequency-dependent selection could maintain the hybrid morph. Model results suggested that comimetic clines are expected to move together, although field collections and literature indicate stability. The authors discussed whether additional balancing factors that were not included in the model could be acting in the system or if this could be a case of hybrid fitness superiority (Table 1).

As shown by our compiled data (Table 1), modeling studies can be performed using different methods, such as estimating cline parameters (Mallet, 1986; Mallet *et al.*, 1990; Meier *et al.*, 2021), using species distribution modeling (Ryan *et al.*, 2018; Marabuto *et al.*, 2023), simulating demographic scenarios and times of divergence (Dasmahapatra *et al.*, 2010; Capblancq *et al.*, 2015, 2019), or testing associations between phenotypic diversity and spatial landscape (Rosser *et al.*, 2021). While simulations have the advantage of not requiring sampling across multiple time frames and/or locations, which is often financially and logistically consuming (Buggs, 2007; Wielstra, 2019), it should be noted that models can miss important variables and sometimes depend on scarce and/or dubious occurrence data, or can include erroneous or biased prior assumptions (Merow *et al.*, 2013; Wisz *et al.*, 2013; Duputié *et al.*, 2014; Guillera-Arroita *et al.*, 2015; Marabuto *et al.*, 2023).

Phenotypic and fitness estimates

We identified two studies using fitness estimates and 18 articles reporting population phenotypic structures, phenotypic clines, and/or testing for phenotypic and climatic associations (Table 1).

Studies of fitness estimates can offer valuable insights on the dynamics of HZs even if not reporting a spatial context. For instance, testing the effects of host plants on larval survival of pure forms of *Pieris napi* and *P. bryoniae*, Porter (1997) showed a slightly higher survival of *P. napi*, even on plants that are restricted to *P. bryoniae* habitat. As such, a potential HZ movement could occur due to the expansion of *P. napi* distribution range, although other factors besides host plant availability could also determine range expansions and contractions (Martelli *et al.*, 2022; Hällfors *et al.*, 2023).

Of the 18 articles using phenotypic data, we highlight Boyd *et al.* (1999) and Pereira Martins *et al.* (2022). Both studies investigated the distribution of phenotypes across space using transects crossing distinct hybrid zones. Boyd *et al.* (1999) used wing traits and male genitalia to score samples across two HZs between *Limenitis weidemeyerii* and *L. lorquini* in different regions of the United States. Importantly, although both hybrid zones could be classified as stable, they presented very distinct characteristics. The first showed a bimodal distribution and presented overall stability due to a balance between selection and dispersal, although some sites showed a moving pattern favoring *L. weidemeyerii;* while the second HZ showed a unimodal phenotypic distribution, and its stability was suggested to be due to hybrid superiority and weak parental dispersal.

In Pereira Martins *et al.* (2022), phenotypic frequency changes between forms of the comimics *Heliconius erato* and *H. melpomene* were analyzed in pairs of HZs located in distinct regions of the Brazilian Amazon Forest. Interestingly, the expectation for similar comimic cline patterns was not observed in the first HZ. The authors suggested that differences in historical or contemporary dispersal patterns could be driving the distinct patterns between mimetic species. On the other hand, results from the second HZ showed that frequency change of comimics were highly concordant. Overall, results from studies such as Boyd *et al.* (1999) and Pereira Martins *et al.* (2022) highlight the importance of context and scale dependence, as HZs can show distinct patterns in different regions and even within the same HZ.

Genetic patterns

The majority of our compiled studies combined genetic evidence with other types of data, such as phenotypic patterns, interspecific crosses, mating experiments, and fitness estimates (Table 1). While temporal comparisons using genetic clines were discussed in previous sections, here we highlight studies using patterns of introgression, measures of linkage disequilibrium, Hardy-Weinberg estimates, and population genetic structure as indirect evidence to hypothesize HZ dynamics.

According to Barton and Hewitt (1985), HZ movement can be inferred when consistent asymmetric introgression is observed in multiple unlinked neutral molecular markers. One expects to observe a set of long cline tails in the advancing population, representing the genetic traces of the displaced genotype (Riemsdijk *et al.*, 2019) (Fig. 3A). It is important to mention, however, that despite being widely used, asymmetric introgression has been considered dubious evidence for HZ movement because differential introgression can also be the outcome of other processes (Sequeira *et al.*, 2020). Zhang *et al.* (2022), for example, investigated patterns of population differentiation and introgression between *Lycaeides melissa* and the Jackson Hole *Lycaeides* population. Overall, asymmetric introgression was observed, with an excess of Jackson Hole population ancestry. These patterns suggest HZ movement attributed to environment-driven asymmetric selection. However, the same asymmetry could be the result of mechanisms that do not require a HZ movement. In this case, the authors suggested that the observed asymmetry could be the result of Dobzhansky-Müller incompatibilities (DMIs) accumulation. Since DMIs are the outcome of negative epistatic interactions between alleles from distinct parental populations (Matute *et al.*, 2010; Moyle & Nakazato, 2010; Xiong & Mallet, 2022), there would be stronger selection against *L. melissa* alleles in the formation of hybrids (Fig. 4). Notably, as the Jackson Hole *Lycaeides* population is an ancient hybrid taxon in which *L. melissa* is one of the ancestors, it is expected that contemporary hybrids between *L. melissa* and Jackson Hole *Lycaeides* present a skewed contribution of ancestry.

Asymmetric introgression can also show patterns that contradict other lines of evidence (Devitt *et al.*, 2011; Riemsdijk *et al.*, 2019) as it can be context and scale dependent (Bierne *et al.*, 2013; Zieliński *et al.*, 2019). It is also possible that markers that are assumed to be neutral are actually under selection, resulting in erroneous inferences about HZ dynamics (Toews & Brelsford, 2012; Wang *et al.*, 2019). For instance, mitochondrial (mtDNA) markers are often assumed to be selectively neutral (Harrison, 1989). As a result, mtDNA asymmetries are often presented as evidence of HZ movement. However, the neutrality assumption does not always hold as true (Mishmar *et al.*, 2003; Kivisild *et al.*, 2006; Ruiz-Pesini & Wallace, 2006). In particular, mtDNA can exhibit pervasive interactions with the nuclear genome and be subject to strong selection pressure (Burton & Barreto, 2012). In the *Anartia* HZ studied by Dasmahapatra *et al.* (2002), for instance, eastward movement was shown using temporal comparisons, but mtDNA clines did not show asymmetric patterns and instead coincided very closely with nuclear clines, suggesting strong epistasis. Without the use of temporal tracking, these patterns could be falsely interpreted as a stable zone. The authors suggested that asymmetric introgression is not a strong line of evidence for describing HZ dynamics and that measures of linkage disequilibrium should be more reliable.

Patterns of linkage disequilibrium (LD) and Hardy-Weinberg Equilibrium can be informative when hypothesizing hybrid zone dynamics. For instance, Ryan *et al.* (2017) suggested HZ stability between *Papilio glaucus* and *P. canadensis* based on the coincident geographic positions of the LD peak and cline centers. In contrast, elevated LD is expected to be located at the leading edge of a moving hybrid zone due to a higher concentration of early-generation hybrids and lower frequency of recombined haplotypes (Fig. 3B). Arias *et al.* (2008) combined population genetic structure, patterns of LD, phylogenetic data, and Hardy-Weinberg estimates to suggest the bimodality of a hybrid zone between *Heliconius erato venus* and *H. e. chestertonii*. The deficit of heterozygotes, the monophyletic grouping of subspecies, and the high LD estimate between color loci under strong selection and neutral markers imply strong reproductive isolation. In sum, these results were suggestive of a stable hybrid zone.

Another challenge in using genetic data to make inferences about current spatiotemporal status is inferring whether the HZ has a primary or secondary origin (Endler, 1977; Barton & Hewitt, 1985) (Fig. 2). Secondary HZs occur from historical demographic expansion after the disappearance of a geographical barrier, whereas a primary contact scenario occurs when differentiation has occurred along an environmental gradient (Barton & Hewitt, 1985). If suitable habitats have been stable over time, the HZ position will also be stable (Vines *et al.*, 2003; Morales-Rozo *et al.*, 2017). Here, genetic data can be misleading when evaluating the origins of a HZ because genetic signatures can erode by recombination over time and hinder the detection of past movements (Endler, 1977; Durrett *et al.*, 2000). Thus, the

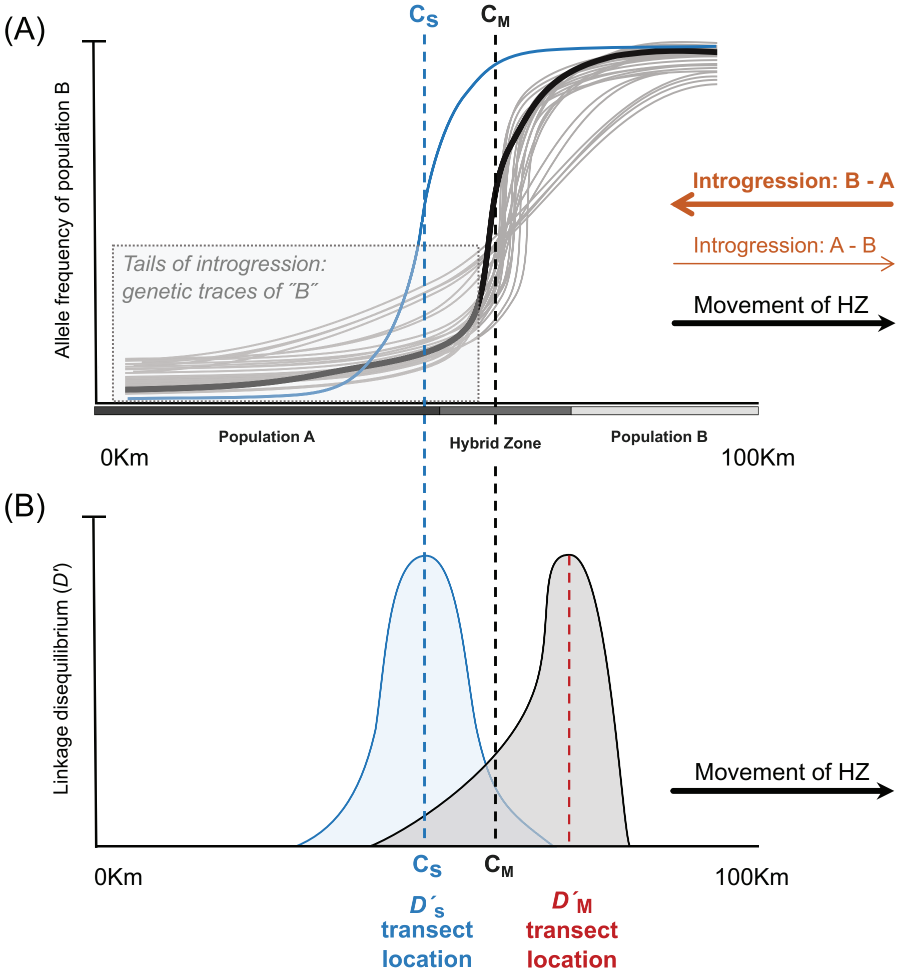
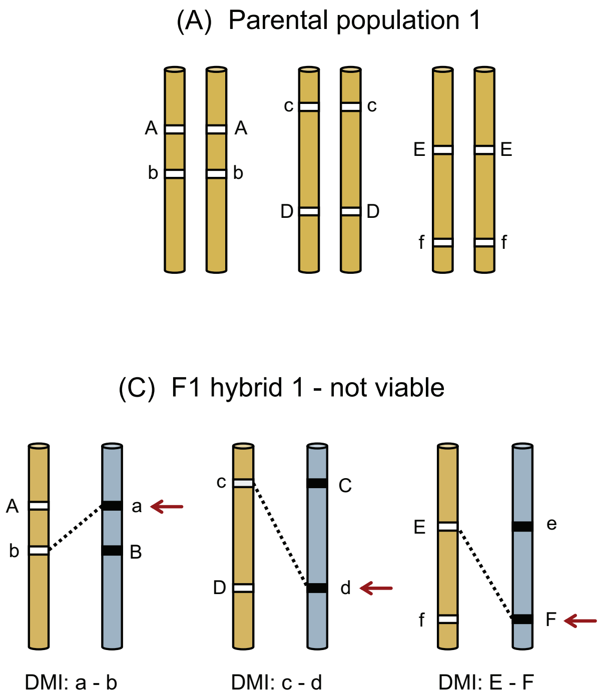


Fig. 3 Genetic patterns used as indirect evidence of stable and moving hybrid zones. (A) Cline patterns. Gray lines represent multiple asymmetric clines resulting from a greater introgression of neutral alleles from population B to population A (direction represented by a bold orange arrow) than from population A to population B (direction represented by a thin orange arrow). Asymmetric introgression produces tails of introgression, which has been used as indirect evidence of hybrid zone movement toward the introgressive population (movement direction represented by a bold black arrow). The black cline represents the average pattern of asymmetric clines, and the vertically traced black line represents the dislocated center of the moving hybrid zone (C_M). The blue cline represents a symmetric cline pattern, and the vertically traced blue line shows the center of a stable hybrid zone (C_S). The *Y* axis is the allele frequency of population B, and the *X* axis shows a transect of 100 Km, crossing the pure population A (dark gray area in the *X* axis), a hybrid zone (gray area in the *X* axis), and the pure population B (light gray area in the *X* axis). (B) Admixture linkage disequilibrium (*D'*) across stable and moving hybrid zones. Blue curve represents a stable hybrid zone in which the peak of *D'* and the center of the hybrid zone (C_S) are coincident. Black curve represents a moving hybrid zone with the peak of *D'* (red traced line—D'_M) dislocated from the hybrid zone center (black traced line—C_M), following the direction of movement. According to theory, the peak of *D'* is predicted to be in the lead edge of a moving hybrid zone, where early-generation hybrids are found (i.e., *D'* is highest when two parental populations first hybridize, and it decays over time due to recombination). Figure based on the model presented in Currat *et al.* (2008), results from Gay *et al.* (2008), Wang *et al.* (2011), and Riemsdijk *et al.* (2019).

genomic signatures between primary and secondary contact HZ will be very similar despite very different histories.

Nevertheless, the advantage of using genetic patterns in HZ spatiotemporal studies are significant, allowing a better understanding of historical processes that can be challenging with phenotypic data alone (Buggs, 2007; Savolainen *et al.*, 2013). However, the use of a single type of analysis can lead to erroneous conclusions as discussed in this section. As shown by the selected



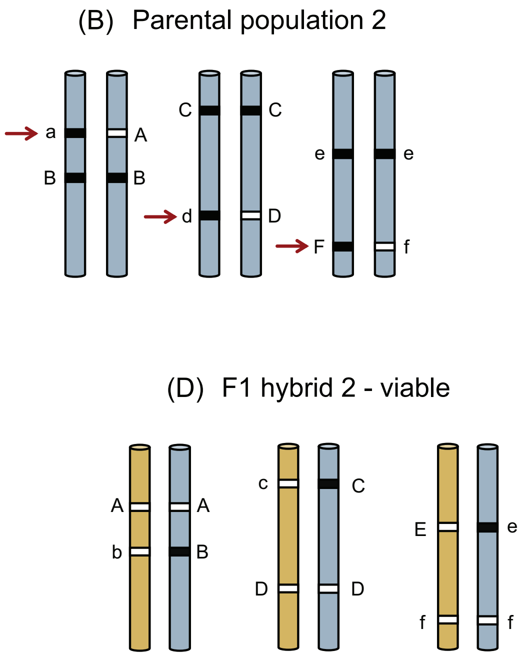


Fig. 4 Simplified view of the Dobzhansky-Müller incompatibility model. The model predicts that hybrid breakdown is a response to negative epistatic interactions between alleles from different parental genetic backgrounds. (A) Hypothesized parental population 1. (B) Hypothesized parental population 2. (C) F1 hybrid is not viable as it accumulates three Dobzhansky-Müller incompatibilities (DMIs): a–b, c–d, and E–F (represented by black dotted lines). (D) F1 viable hybrid as it does not present any DMIs. Red arrows in (B) and (C) indicate alleles in which selection is acting negatively due to DMI.

literature (Table 1), stronger hypotheses are built with the combination of multiple analysis using genetic data, and preferably including different lines of evidence.

Effects of hybrid zone spatiotemporal dynamics

Evolution The HZ concept has important implications for our view of speciation as a dynamic and continuous process (Fig. 5A) (Mallet *et al.*, 2007; Kronforst *et al.*, 2013; Wolf & Ellegren, 2017). Hybridization can either facilitate or hamper speciation depending on the stage of the process, the spatial scale evaluated, the regional and temporal contexts, and the specific taxa involved (Abbott, 2017). Considering that HZs can alternate between stability and movement, a pair of hybridizing taxa can move back and forth across the speciation continuum depending on the varying factors acting on the HZ during different periods of time (Barton, 1979). Thus, studying spatiotemporal dynamics will typically reveal a snapshot of the entire history of interactions among populations, and inevitably do not capture the impacts of earlier changes in population sizes, connectivity, and interactions with other taxa and abiotic factors (Hewitt, 1996, 2011; Abbott *et al.*, 2013).

In our review, we examine butterfly HZs belonging to different stages along the speciation continuum. At one end of the continuum are HZs between taxa where hybridization is rare and parental forms maintain their distinctiveness in sympatry. For instance, in the HZ between *Erebia tyndarus* and *E. cassioides* (Augustijnen *et al.*, 2022), genomic and phenotypic data showed clear species separation, few F1 hybrids, and absence of backcrossing. We hypothesize this HZ is stable, represents a well-advanced stage along the continuum, and is nearly completely reproductively isolated (Fig. 5A). Other HZs are more intermediate; for example, in HZs between *Heliconius erato* and *H. himera*, hybrids and backcross individuals are found in areas where the two species cooccur, but they are rare relative to the parental types and gene flow remains low. Thus, genetic differences between species are maintained resulting in a bimodal distribution of genotypes (Fig. 2). In the case of *H. erato* and *H. himera*, divergence is driven by the coupling of strong premating isolation with ecological adaptation to distinct habitats (i.e., *H. erato* is common

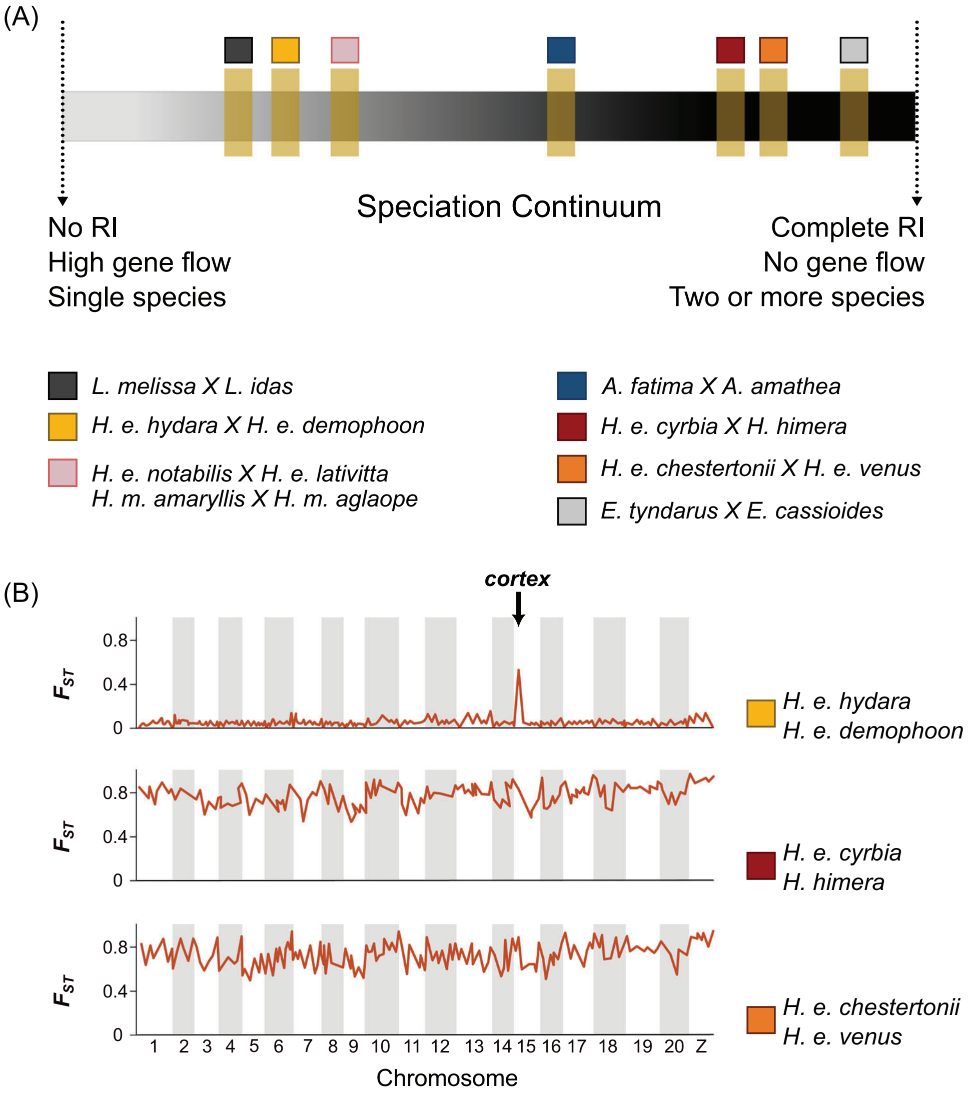


Fig. 5 Speciation continuum and heterogeneous divergence across the genome. (A) Speciation continuum ranging from a stage in which there is no reproductive isolation (RI) between populations and high levels of gene flow, forming a single species, to the opposite end characterized by complete reproductive isolation, absence of gene flow, and the formation of multiple species. Positions of butterfly hybrid zone examples (yellow rectangles) along the speciation continuum are proposed based on distinct hybrid zone classification criteria (Fig. 2) and genomic patterns of divergence. *Lycaeides melissa* X *L. idas* (Gompert *et al.*, 2010); *Heliconius erato hydara* X *H. e. demophoon* (Mallet, 1986; Blum, 2002; Thurman *et al.*, 2019); *H. e. notabilis* X *H. e. lativitta* (Meier *et al.*, 2021); *H. melpomene amaryllis* X *H. m. aglaope* (Mallet *et al.*, 1990; Rosser *et al.*, 2014; Van Belleghem *et al.*, 2021); *Anartia fatima* X *A. amathea* (Dasmahapatra *et al.*, 2002); *H. e. cyrbia* X *H. himera* (Jiggins *et al.*, 1996; McMillan *et al.*, 1997; Supple *et al.*, 2015; Van Belleghem *et al.*, 2021); *H. e. chestertonii* X *H. e. venus* (Arias *et al.*, 2008; Van Belleghem *et al.*, 2021); and *Erebia tyndarus* X *E. cassioides* (Lucek *et al.*, 2020; Augustijnen *et al.*, 2022). (B) Heterogeneous pattern of genomic divergence between *Heliconius* butterflies along 21 chromosomes (calculated as the fixation index, F_{ST}). Orange curves represent the genomic divergence pattern between hybridizing taxa (colored squares represent the same taxa from Fig. 5A). In the first panel, divergence between *Heliconius erato hydara* and *H. e. demophoon* has only one peak of differentiation associated with the gene *cortex*, which affects the yellow hindwing bar trait. The other two panels show high levels of divergence across the entire genome, including regions known to underlie wing color pattern differences. Fig. 5B is adapted from Van Belleghem *et al.* (2021).

in wet forests, while *H. himera* is typically found in dry forests) and selection by predators (McMillan *et al.*, 1997). Interestingly, Van Belleghem *et al.* (2021) showed that different subspecies of *H. erato* show varying patterns of genomic differentiation with *H. himera*. Within the continuum framework, *H. erato* and *H. himera* HZs are closer to complete reproductive isolation but not as close to the end as found in *E. tyndarus* and *E. cassioides*, given the presence of backcrossing individuals in the *Heliconius* HZs (Fig. 5A). Somewhat intermediate between *E. tyndarus*/*E. cassioides* and *H. erato*/*H. himera* are the hybridizing populations of *H. erato chestertonii* and *H. e. venus* (Fig. 5A). This HZ is characterized by a deficit of heterozygotes and strong pre- and postmating reproductive isolation (Arias *et al.*, 2008; Muñoz *et al.*, 2010). In addition, genomic data reveal large differentiation between these subspecies, with several peaks of divergence across the genome, which could be comparable to patterns between species (Fig. 5B). Indeed, results from Van Belleghem *et al.* (2021) suggest stronger reproductive isolation between these subspecies of *H. erato* than between the distinctive species *H. e. cyrbia* and *H. himera*.

In the previous examples, speciation has arguably occurred, and hybridizing taxa can maintain species defining differences in ecology and behavior. In these cases, differentiation can build up rapidly across the genome (Van Belleghem *et al.*, 2021). However, many butterfly HZs fall close to the panmictic end of the speciation continuum. This area is often referred to as the "gray zone" of speciation and represents the states in which species definition is controversial and pairs of populations can change their positions relatively rapidly (De Queiroz, 2007; Supple *et al.*, 2015; Roux *et al.*, 2016; Zhang *et al.*, 2019; Xiong & Mallet, 2022). Dasmahapatra *et al.* (2002), for example, suggested that the Anartia HZ represented an intermediate case between a panmictic and a bimodal distribution, in which there are moderate barriers to reproduction and frequent hybridization (Fig. 5A). According to authors, this case was "*at or just beyond the threshold of speciation*." Notably, HZs located at the "gray zone" and closer to the end of the continuum representing a single species are very often characterized as the distributional overlap of subspecies. Accordingly, the term "subspecies" was proposed to indicate varieties within species that replaced one another across the geographic landscape (Mayr, 1966, 1970, 1982; Mallet, 2007; Braby *et al.*, 2012), and its use is very common in the butterfly literature (Braby *et al.*, 2012) (Table 1).

Several HZs between distinctive wing patterns of *Heliconius erato* and *H. melpomene* fall closer to the end where reproductive isolation is weak and gene flow is high. Accordingly, HZs between *H. e. notabilis* and *H. e. lativitta* (Meier *et al.*, 2021), *H. m. amaryllis* and *H. m. aglaope* (Mallet et al., 1990; Rosser *et al.*, 2014), and *H. e. hydara* and *H. e. demophoon* (Mallet, 1986; Blum, 2002; Thurman *et al.*, 2019) are placed in proximity to the single species end, although we suggest the last one is nearest to the end due to its wider HZ width and minor color pattern differences (Fig. 5A). Similarly, despite being classified as distinct species, we also putatively include the *Lycaeides melissa* and *L. idas* HZ (Gompert *et al.*, 2010) as closer to this extreme based on the overall weak selection against hybrids, the observed extensive hybridization, and the high parental dispersal. The authors reported a unimodal genomic distribution (Fig. 2), which could be used as an indicator of HZ stability since hybrids would have higher local fitness compared to parental genotypes and movements would only be expected with an environmental change (Stankowski *et al.*, 2021). However, we suggest there is still potential for movement due to the reported absence of elevated LD in the center of the hybrid zone (Fig. 3B), presence of asymmetric clines, and the absence of an isolated hybrid lineage.

According to the genic view of speciation, divergence is heterogeneous across the genome, with some regions exhibiting high levels of differentiation between populations as they are subjected to stronger selection and/or low gene flow, in contrast to the rest of the genome, which is predictably neutral and homogenized by gene flow (Fig. 5B) (Wu, 2001). Thus, in *Heliconius* HZs, for instance, it is possible to observe varying patterns of genomic divergence, such that HZs between subspecies will often show high levels of gene flow at most of the genome, except for genomic regions involved in wing color patterns, which are under strong selection (Fig. 5B). Following this view, speciation is a process of gradual accumulation of loci under selection (and loci linked to those under selection) (Turner *et al.*, 2005; Harr, 2006; Nosil *et al.*, 2009).

Studies with HZs showing high levels of gene flow can also take advantage of admixture mapping to identify variation potentially under selection and, consequently, involved in the buildup of reproductive isolation or the maintenance of adaptive differences (reviewed in Buerkle & Lexer, 2008; Gompert *et al.*, 2017). This approach tests for associations between genetic variants and phenotypes based on ancestry patterns and linkage disequilibrium (Fig. 6). As an example, Meier *et al.* (2021) recently used admixture mapping to identify functionally important genetic variation contributing to wing pattern mimicry and divergence between high- and lowland subspecies of distantly related, yet phenotypically identical, *Heliconius* species. The authors revealed strong association between

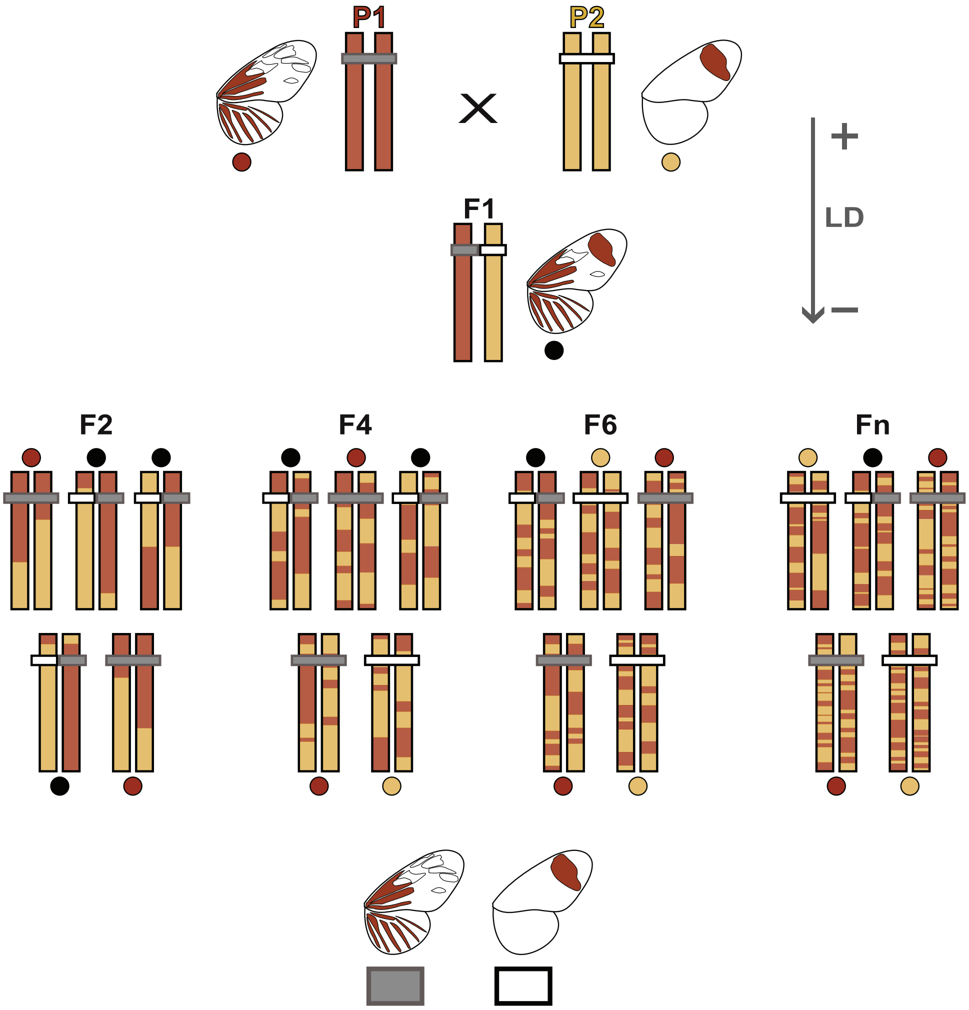


Fig. 6 Admixture mapping in *Heliconius erato*. P1 parental individual has red hindwing rays and a red patch at the forewing base, known as the "dennis patch" (represented by a red circle), P2 parental individual has a red forewing bar (represented by a yellow circle), and the F1 generation has a distinct intermediate phenotype (represented by a black circle). F1 crosses and backcrosses will result in recombined chromosomes, such that later generations (e.g., F2, F4, F6, and Fn) will have a higher frequency of recombined haplotypes and lower linkage disequilibrium (LD), revealing more precise associations between phenotypes and genetic variants. In the example, the phenotype characterized by red hindwing rays and a dennis patch is strongly associated with the genetic variant represented by the gray rectangle, while the red forewing bar is strongly associated with the genetic variant represented by the white rectangle. Fig. 6 is based on Van Belleghem *et al.* (2017).

highly differentiated genomic regions and wing color pattern differences in two-comimetic pairs, suggesting that color pattern mimicry was achieved using the same genomic intervals. These intervals were in noncoding regions of known mimicry genes, suggesting parallel regulatory changes. In *Heliconius*, admixture mapping has been used to better reveal genotype by phenotype associations. For example, Bainbridge *et al.* (2020) suggested that although the same major effect color loci are involved in mimetic patterns, minor effect loci control subtle changes in the shape and size of *Heliconius erato* and *H. melpomene* mimic red forewing bands. Similarly, recent studies using other methods, such as genome editing, transcriptome analysis, and epigenomic profiling, are highlighting the molecular nature of convergent evolution. These studies demonstrate that, although a small number of major effect genes are generating pattern variation across the genus, how these loci are deployed to output matching phenotypes differs between comimetic species (Concha *et al.*, 2019; Hanly *et al.*, 2019;

Lewis & Van Belleghem, 2020; Van Belleghem *et al.*, 2020, 2023).

HZs located nearer the "no reproductive isolation" end of the continuum will have fewer divergent genomic regions and consequently are more likely to move across space and along the speciation continuum (Fig. 5). We argue that while defining populations as subspecies and species has been useful for the systematic study of evolutionary, ecological, and biogeographical patterns (Descimon & Mallet, 2009), the use of a single and universal criterion to draw boundaries in nature may not be realistic. Ultimately, the decision of how to classify hybridizing populations should depend on the evaluation of how populations are structured, which species definition is adopted, and the purposes of the study (Wu, 2001; Descimon & Mallet, 2009).

Conservation The alteration of abiotic factors can have major effects on HZ characteristics, and consequently have important implications for conservation management. HZ movements due to climatic warming have been predicted in *Papilio glaucus* and *P. canadensis* (Scriber, 2002; Scriber *et al.*, 2002; Ryan *et al.*, 2018), *Polyommatus agestis* and *P. artaxerxes* (Mallet *et al.*, 2011) and *Coenonympha macromma* and *C. gardetta* (Capblancq *et al.*, 2020) (Table 1), indicating potential threats to the dislocated species. The threat to butterfly populations is heightened because even rare weather extreme events are enough to disrupt the stability of butterfly geographic distributions (Scriber *et al.*, 2002). In addition to rising temperatures, other anthropogenic activities can directly and/or indirectly affect the equilibrium of HZ distributions. For instance, deforestation can have large effects on butterfly population densities (Dasmahapatra *et al.*, 2002), on the intensity of predation (Thurman *et al.*, 2019), and on the availability of host plants (Benson, 1978; Merrill *et al.*, 2013). The high sensitivity of butterfly HZs to habitat alterations, together with the multiple methods for tracking their population changes, can potentially provide a useful tool for assessing human environmental impacts.

Conclusion

Our findings show that butterflies, especially *Heliconius* and *Papilio*, represent an important model organism in which foundational knowledge regarding HZs and their evolutionary consequences has been developed. However, we highlight the need for research concentrating on other butterfly genera, and in a range of spatial contexts, because regional, scale, and taxon-specific characteristics can have important effects on HZ patterns. We also highlight the importance of including the investigation of spatiotemporal dynamics as a primary research goal. These needs are justified by the importance of butterflies and HZ dynamics as indicators of environmental change, which may contribute to the development of useful conservation strategies. We argue that the temporal comparison of phenotypic and/or genotypic data across space is still the most reliable evidence of HZs dynamics. However, as an alternative to the high demands of this type of study, a good alternative is the combination of multiple lines of evidence using different types of data, distinct transects crossing the same HZ, HZs in different regions, and multiple spatial scales. Accordingly, we suggest three key research areas for future investigation that do not necessarily require temporal comparisons: (1) predictions of HZs distributions using future climatic scenarios, different butterfly species, and multiple spatial scales; (2) HZ investigations within areas of current intense environmental change; and (3) comparisons of HZs located in distinct regions, as well as varying locations within a single HZ to evaluate the effects of context-dependent factors.

Acknowledgments

We thank the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior—Brasil (CAPES)—Finance Code 001 (grant 99999.002113/2015-05), the National Sciences and Engineering Research Council of Canada (NSERC CREATE in Biodiversity, Ecosystem, Services and Sustainability—466 and a Canada Research Chair and NSERC Discovery Grant to RDHB), and the Smithsonian Tropical Research Institute for funding this research. We are grateful to the members of the Barrett and Hendry Lab (Redpath Museum, McGill University) for all the discussions and support during the conceptualization and development of this study. To Dr. James Mallet for providing helpful comments, and to Dr. Lucas Pereira Martins and Dr. Silma Regina F. Pereira for insights during the writing process of this review.

Disclosure

The authors declare no conflict of interest. All authors have read and agreed to the submitted version of the manuscript.

References

Abbott, R., Albach, D., Ansell, S., Arntzen, J.W., Baird, S.J.E., Bierne, N. *et al.* (2013) Hybridization and speciation. *Journal of Evolutionary Biology*, 26, 229–246.

Abbott, R.J. (2017) Plant speciation across environmental gradients and the occurrence and nature of hybrid zones: hybrid zones and plant speciation. *Journal of Systematics and Evolution*, 55, 238–258.

Abdelaziz, M., Muñoz-Pajares, A.J., Berbel, M., García-Muñoz, A., Gómez, J.M. and Perfectti, F. (2021) Asymmetric reproductive barriers and gene flow promote the rise of a stable hybrid zone in the Mediterranean high mountain. *Frontiers in Plant Science*, 12, 687094.

Arias, C.F., Muñoz, A.G., Jiggins, C.D., Mavárez, J., Bermingham, E. and Linares, M. (2008) A hybrid zone provides evidence for incipient ecological speciation in *Heliconius* butterflies. *Molecular Ecology*, 17, 4699–4712.

Arnold, M.L. and Martin, N.H. (2009) Adaptation by introgression. *Journal of Biology*, 8, 82.

Augustijnen, H., Patsiou, T. and Lucek, K. (2022) Secondary contact rather than coexistence-*Erebia* butterflies in the Alps. *Evolution; Internation Journal of Organic Evolution*, 76, 2669–2686.

Bainbridge, H.E., Brien, M.N., Morochz, C., Salazar, P.A., Rastas, P. and Nadeau, N.J. (2020) Limited genetic parallels underlie convergent evolution of quantitative pattern variation in mimetic butterflies. *Journal of Evolutionary Biology*, 33, 1516–1529.

Barton, N.H. (1983) Multilocus Clines. *Evolution; Internation Journal of Organic Evolution*, 37, 454–471.

Barton, N.H. (1979) The dynamics of hybrid zones. *Heredity*, 43, 341–359.

Barton, N.H. and Gale, K.S. (1993) Genetic analysis of hybrid zones. In *Hybrid Zones and the Evolutionary Process* (ed. R.G. Harrison), pp. 13–45. Oxford University Press, New York.

Barton, N.H. and Hewitt, G.M. (1989) Adaptation, speciation and hybrid zones. *Nature*, 341, 497–503.

Barton, N.H. and Hewitt, G.M. (1985) Analysis of hybrid zones. *Annual Review of Ecology and Systematics*, 16, 113–148.

Barton, N.H. and Shpak, M. (2000) The effect of epistasis on the structure of hybrid zones. *Genetical Research*, 75, 179–198.

Bates, H.W. (1862) XXXII. Contributions to an insect fauna of the Amazon Valley. Lepidoptera: Heliconidae. *Transactions of the Linnean Society of London*, 23, 495–566.

Benson, W.W. (1978) Resource partitioning in passion vine butterflies. *Evolution; Internation Journal of Organic Evolution*, 32, 493–518.

Bierne, N., Gagnaire, P.A. and David, P. (2013) The geography of introgression in a patchy environment and the thorn in the side of ecological speciation. *Current Zoology*, 59, 72–86.

Blum, M.J. (2008) Ecological and genetic associations across a *Heliconius* hybrid zone. *Journal of Evolutionary Biology*, 21, 330–341.

Blum, M.J. (2002) Rapid movement of a *Heliconius* hybrid zone: evidence for phase III of Wright's shifting balance theory? *Evolution; Internation Journal of Organic Evolution*, 56, 1992–1998.

Boggs, C.L., Watt, W.B. and Ehrlich, P.R. (2003) *Butterflies: Ecology and Evolution Taking Flight*. University of Chicago Press, Chicago.

Bonebrake, T.C., Ponisio, L.C., Boggs, C.L. and Ehrlich, P.R. (2010) More than just indicators: a review of tropical butterfly ecology and conservation. *Biological Conservation*, 143, 1831–1841.

Boyd, B.M., Boyd, B.M., Austin, G.T. and Murphy, D.D. (1999) Hybridization of *Limenitis* in the Western Great Basin (Lepidoptera: Nymphalidae). *Holarctic Lepidoptera*, 6, 37–74.

Braby, M.F., Eastwood, R. and Murray, N. (2012) The subspecies concept in butterflies: has its application in taxonomy and conservation biology outlived its usefulness? *Biological Journal of the Linnean Society*, 106, 699–716.

Bridle, J.R., Baird, S.J.E. and Butlin, R.K. (2001) Spatial structure and habitat variation in a grasshopper hybrid zone. *Evolution; Internation Journal of Organic Evolution*, 55, 1832–1843.

Brooks, S.J., Self, A., Toloni, F. and Sparks, T. (2014) Natural history museum collections provide information on phenological change in British butterflies since the late-nineteenth century. *International Journal of Biometeorology*, 58, 1749–1758.

Brown, K.S. (1991) Conservation of neotropical environments: insects as indicators. In *The Conservation of Insects and their Habitats* (eds. N.M. Collins & J.A. Thomas), pp. 349–404. Royal Entomological Society of London Symposium 1989, London.

Buerkle, C.A. and Lexer, C. (2008) Admixture as the basis for genetic mapping. *Trends in Ecology & Evolution*, 23, 686–694.

Buggs, R.J.A. (2007) Empirical study of hybrid zone movement. *Heredity*, 99, 301–312.

Burton, R.S. and Barreto, F.S. (2012) A disproportionate role for mtDNA in Dobzhansky-Muller incompatibilities? *Molecular Ecology*, 21, 4942–4957.

Capblancq, T., Després, L. and Mavárez, J. (2020) Genetic, morphological and ecological variation across a sharp hybrid zone between two alpine butterfly species. *Evolutionary Applications*, 13, 1435–1450.

Capblancq, T., Després, L., Rioux, D. and Mavárez, J. (2015) Hybridization promotes speciation in *Coenonympha* butterflies. *Molecular Ecology*, 24, 6209–6222.

Capblancq, T., Mavárez, J., Rioux, D. and Després, L. (2019) Speciation with gene flow: Evidence from a complex of alpine butterflies (*Coenonympha*, Satyridae). *Ecology and Evolution*, 9, 6444–6457.

Concha, C., Wallbank, R.W.R., Hanly, J.J., Fenner, J., Livraghi, L., Rivera, E.S. *et al*. (2019) Interplay between developmental flexibility and determinism in the evolution of mimetic *Heliconius* wing patterns. *Current Biology*, 29, 3996–4009.

Cordell, H.J. (2002) Epistasis: what it means, what it doesn't mean, and statistical methods to detect it in humans. *Human Molecular Genetics*, 11, 2463–2468.

Currat, M., Ruedi, M., Petit, R.J. and Excoffier, L. (2008) The hidden side of invasions: massive introgression by local genes. *Evolution; Internation Journal of Organic Evolution*, 62, 1908–1920.

Curry, C.M. (2015) An integrated framework for hybrid zone models. *Evolutionary Biology*, 42, 359–365.

Dasmahapatra, K.K., Blum, M.J., Aiello, A., Hackwell, S., Bermingham, E.P. and Mallet, J. (2002) Inferences from a rapidly moving hybrid zone. *Evolution; Internation Journal of Organic Evolution*, 56, 741–753.

Dasmahapatra, K.K., Lamas, G., Simpson, F. and Mallet, J. (2010) The anatomy of a 'suture zone' in Amazonian butterflies: a coalescent-based test for vicariant geographic divergence and speciation. *Molecular Ecology*, 19, 4283–4301.

De Queiroz, K. (2007) Species concepts and species delimitation. *Systematic Biology*, 56, 879–886.

Descimon, H. and Mallet, J. (2009) Bad species. In *Ecology of Butterflies in Europe* (eds. J. Settele, T.G. Shreeve, M. Konvicka & V.H. Dyck), pp. 219–249. Cambridge University Press, Cambridge.

Devitt, T.J., Baird, S.J. and Moritz, C. (2011) Asymmetric reproductive isolation between terminal forms of the salamander ring species *Ensatina eschscholtzii* revealed by fine-scale genetic analysis of a hybrid zone. *BMC Evolutionary Biology*, 11, 245.

Dincă, V., Dapporto, L. and Vila, R. (2011) A combined genetic-morphometric analysis unravels the complex biogeographical history of *Polyommatus icarus* and *Polyommatus celina* Common Blue butterflies. *Molecular Ecology*, 20, 3921–3935.

Dupuis, J.R. and Sperling, F.A.H. (2016) Hybrid dynamics in a species group of swallowtail butterflies. *Journal of Evolutionary Biology*, 29, 1932–1951.

Duputié, A., Zimmermann, N.E. and Chuine, I. (2014) Where are the wild things? Why we need better data on species distribution. *Global Ecology and Biogeography*, 23, 457–467.

Durrett, R., Buttel, L. and Harrison, R. (2000) Spatial models for hybrid zones. *Heredity*, 84, 9–19.

Edelman, N.B. and Mallet, J. (2021) Prevalence and adaptive impact of introgression. *Annual Review of Genetics*, 55, 265–283.

Endler, J.A. (1977) *Geographic Variation, Speciation, and Clines*. Princeton University Press, New Jersey.

Freitas, A.V.L., Santos, J.P., Rosa, A.H.B., Iserhard, C.A., Richter, A., Siewert, R.R. *et al*. (2021) Sampling methods for butterflies (Lepidoptera). In *Measuring Arthropod Biodiversity* (eds. J.C. Santos & G.W. Fernandes), pp. 101–123. Springer Cham, Switzerland.

Gauthier, J., Silva, D.L., Gompert, Z., Whibley, A., Houssin, C., Le Poul, Y. *et al*. (2020) Contrasting genomic and phenotypic outcomes of hybridization between pairs of mimetic butterfly taxa across a suture zone. *Molecular Ecology*, 29, 1328–1343.

Gay, L., Crochet, P.A., Bell, D.A. and Lenormand, T. (2008) Comparing clines on molecular and phenotypic traits in hybrid zones: a window on tension zone models. *Evolution; Internation Journal of Organic Evolution*, 62, 2789–2806.

Getz, W.M., Marshall, C.R., Carlson, C.J., Giuggioli, L., Ryan, S.J., Romañach, S.S. *et al.* (2018) Making ecological models adequate. *Ecology Letters*, 21, 153–166.

Gilbert, L.E. and Singer, M.C. (1975) Butterfly ecology. *Annual Review of Ecology and Systematics*, 6, 365–397.

Gompert, Z. and Buerkle, C.A. (2009) A powerful regression-based method for admixture mapping of isolation across the genome of hybrids. *Molecular Ecology*, 18, 1207–1224.

Gompert, Z., Lucas, L.K., Fordyce, J.A., Forister, M.L. and Nice, C.C. (2010) Secondary contact between *Lycaeides idas* and *L. melissa* in the Rocky Mountains: extensive admixture and a patchy hybrid zone. *Molecular Ecology*, 19, 3171–3192.

Gompert, Z., Mandeville, E.G. and Buerkle, C.A. (2017) Analysis of population genomic data from hybrid zones. *Annual Review of Ecology, Evolution, and Systematics*, 48, 207–229.

Guillera-Arroita, G., Lahoz-Monfort, J.J., Elith, J., Gordon, A., Kujala, H., Lentini, P.E. *et al.* (2015) Is my species distribution model fit for purpose? Matching data and models to applications. *Global Ecology and Biogeography*, 24, 276–292.

Hällfors, M.H., Heikkinen, R.K., Kuussaari, M., Lehikoinen, A., Luoto, M., Pöyry, J. *et al.* (2023) Recent range shifts of moths, butterflies, and birds are driven by the breadth of their climatic niche. *Evolution Letters*, qrad004. https://doi.org/10.1093/evlett/qrad004

Hanly, J.J., Wallbank, R.W.R., McMillan, W.O. and Jiggins, C.D. (2019) Conservation and flexibility in the gene regulatory landscape of heliconiine butterfly wings. *EvoDevo*, 10, 15.

Harr, B. (2006) Genomic islands of differentiation between house mouse subspecies. *Genome Research*, 16, 730–737.

Harrison, R.G. (1989) Animal mitochondrial DNA as a genetic marker in population and evolutionary biology. *Trends in Ecology & Evolution*, 4, 6–11.

Harrison, R.G. and Bogdanowicz, S.M. (1997) Patterns of variation and linkage disequilibrium in a field cricket hybrid zone. *Evolution; Internation Journal of Organic Evolution*, 51, 493–505.

Harrison, R.G. and Larson, E.L. (2016) Heterogeneous genome divergence, differential introgression, and the origin and structure of hybrid zones. *Molecular Ecology*, 25, 2454–2466.

Harrison, R.G. and Larson, E.L. (2014) Hybridization, Introgression, and the Nature of Species Boundaries. *Journal of Heredity*, 105, 795–809.

Harrison, R.G. and Rand, D.M. (1989) Mosaic hybrid zones and the nature of species boundaries. In *Speciation and Its Consequences* (eds. D. Otte & J.A. Endler), pp. 111–134. Sinauer Association, Sunderland.

Hewitt, G.M. (1988) Hybrid zones-natural laboratories for evolutionary studies. *Trends in Ecology & Evolution*, 3, 158–167.

Hewitt, G.M. (2011) Quaternary phylogeography: the roots of hybrid zones. *Genetica*, 139, 617–638.

Hewitt, G.M. (1996) Some genetic consequences of ice ages, and their role in divergence and speciation. *Biological Journal of the Linnean Society*, 58, 247–276.

Hill, G.M., Kawahara, A.Y., Daniels, J.C., Bateman, C.C. and Scheffers, B.R. (2021) Climate change effects on animal ecology: butterflies and moths as a case study. *Biological Reviews*, 96, 2113–2126.

Jay, P., Leroy, M., Le Poul, Y., Whibley, A., Arias, M., Chouteau, M. *et al*. (2022) Association mapping of colour variation in a butterfly provides evidence that a supergene locks together a cluster of adaptive loci. *Philosophical Transactions of the Royal Society B*, 377, 20210193.

Jiggins, C.D., Estrada, C. and Rodrigues, A. (2004) Mimicry and the evolution of premating isolation in *Heliconius melpomene* Linnaeus. *Journal of Evolutionary Biology*, 17, 680–691.

Jiggins, C.D. and Mallet, J. (2000) Bimodal hybrid zones and speciation. *Trends in Ecology & Evolution*, 15, 250–255.

Jiggins, C.D., McMillan, W.O., Neukirchen, W. and Mallet, J. (1996) What can hybrid zones tell us about speciation? The case of *Heliconius erato* and *H. himera* (Lepidoptera: Nymphalidae). *Biological Journal of the Linnean Society*, 59, 221–242.

Jørgensen, S.E. and Fath, B.D. (2011) Introduction. In *Developments in Environmental Modelling* (eds. S.E. Jørgensen & B.D. Fath), pp. 1–18. Elsevier, Amsterdam.

Kivisild, T., Shen, P., Wall, D.P., Do, B., Sung, R., Davis, K. *et al*. (2006) The role of selection in the evolution of human mitochondrial genomes. *Genetics*, 172, 373–387.

Kral, K., Harmon, J., Limb, R. and Hovick, T. (2018) Improving our science: the evolution of butterfly sampling and surveying methods over time. *Journal of Insect Conservation*, 22, 1–14.

Kronforst, M.R., Hansen, M.E.B., Crawford, N.G., Gallant, J.R., Zhang, W., Kulathinal, R.J. *et al.* (2013) Hybridization reveals the evolving genomic architecture of speciation. *Cell Reports*, 5, 666–677.

Kunte, K., Shea, C., Aardema, M.L., Scriber, J.M., Juenger, T.E., Gilbert, L.E. *et al*. (2011) Sex chromosome mosaicism and hybrid speciation among Tiger Swallowtail Butterflies. *PLoS Genetics*, 7, e1002274.

Larson, E.L., White, T.A., Ross, C.L. and Harrison, R.G. (2014) Gene flow and the maintenance of species boundaries. *Molecular Ecology*, 23, 1668–1678.

Lewis, J.J. and Van Belleghem, S.M. (2020) Mechanisms of change: a population-based perspective on the roles of modularity and pleiotropy in diversification. *Frontiers in Ecology and Evolution*, 8, 261.

Livraghi, L., Hanly, J.J., Van Bellghem, S.M., Montejo-Kovacevich, G., van der Heijden, E.S., Loh, L.S. *et al*. (2021) Cortex cis-regulatory switches establish scale colour identity and pattern diversity in *Heliconius*. *eLife*, 10, e68549.

Lucek, K., Butlin, R.K. and Patsiou, T. (2020) Secondary contact zones of closely-related *Erebia* butterflies overlap with narrow phenotypic and parasitic clines. *Journal of Evolutionary Biology*, 33, 1152–1163.

Mallet, J. (1986) Hybrid zones of *Heliconius* butterflies in Panama and the stability and movement of warning colour clines. *Heredity*, 56, 191–202.

Mallet, J. (2005) Hybridization as an invasion of the genome. *Trends in Ecology & Evolution*, 20, 229–237.

Mallet, J. (1993) Speciation, raciation, and color pattern evolution in *Heliconius* butterflies: evidence from hybrid zones. In *Hybrid Zones and the Evolutionary Process* (ed. R.G. Harrison), pp. 226–260. Oxford University Press, New York.

Mallet, J. (2007) Subspecies, semispecies, superspecies. *Encyclopedia of Biodiversity*, 5, 523–526.

Mallet, J. and Barton, N. (1989) Inference from clines stabilized by frequency-dependent selection. *Genetics*, 122, 967–976.

Mallet, J., Barton, N., Lamas, G.M., Santisteban, J.C., Muedas, M.M. and Eeley, H. (1990) Estimates of selection and gene flow from measures of cline width and linkage disequilibrium in *Heliconius* hybrid zones. *Genetics*, 124, 921–936.

Mallet, J., Beltrán, M., Neukirchen, W. and Linares, M. (2007) Natural hybridization in heliconiine butterflies: the species boundary as a continuum. *BMC Evolutionary Biology*, 7, 28.

Mallet, J., Wynne, I.R. and Thomas, C.D. (2011) Hybridisation and climate change: brown argus butterflies in Britain (*Polyommatus* subgenus *Aricia*). *Insect Conservation and Diversity*, 4, 192–199.

Marabuto, E., Nunes, M.S., Martins, R., Mendes, R., Moreira-Pinhal, T.C., Raimundo, J. *et al.* (2023) Integrative analysis reveals the divergence and speciation between sister Sooty Copper butterflies *Lycaena bleusei* and *L. tityrus*. *Molecular Phylogenetics and Evolution*, 180, 107699.

Martelli, F., Paradiso, F., Ghidotti, S., Viterbi, R., Cerrato, C. and Bonelli, S. (2022) Invasion patterns and niche comparison of the butterfly *Cacyreus marshalli* among native and non-native range. *Biological Invasions*, 24, 3935–3952.

Matute, D.R., Butler, I.A., Turissini, D.A. and Coyne, J.A. (2010) A test of the snowball theory for the rate of evolution of hybrid incompatibilities. *Science*, 329, 1518–1521.

Mayr, E. (1966) *Animal Species and Evolution*. Belknap Press of Harvard University Press, Cambridge.

Mayr, E. (1970) *Populations, Species, and Evolution: An Abridgment of Animal Species and Evolution*. Belknap Press of Harvard University Press, Cambridge.

Mayr, E. (1982) *The Growth of Biological Thought: Diversity, Evolution, and Inheritance*. Belknap Press of Harvard University Press, Cambridge.

McMillan, W.O., Jiggins, C.D. and Mallet, J. (1997) What initiates speciation in passion-vine butterflies? *Proceedings of the National Academy of Sciences USA*, 94, 8628–8633.

Meier, J.I., Salazar, P.A., Kučka, M., Davies, R.W., Dréau, A., Aldás, I. *et al.* (2021) Haplotype tagging reveals parallel formation of hybrid races in two butterfly species. *Proceedings of the National Academy of Sciences USA*, 118, e2015005118.

Merow, C., Smith, M.J. and Silander, J.A. (2013) A practical guide to MaxEnt for modeling species' distributions: what it does, and why inputs and settings matter. *Ecography*, 36, 1058–1069.

Merrill, R.M., Dasmahapatra, K.K., Davey, J.W., Dell'Aglio, D.D., Hanly, J.J., Huber, B. *et al*. (2015) The diversification of *Heliconius* butterflies: what have we learned in 150 years? *Journal of Evolutionary Biology*, 28, 1417–1438.

Merrill, R.M., Naisbit, R.E., Mallet, J. and Jiggins, C.D. (2013) Ecological and genetic factors influencing the transition between host-use strategies in sympatric *Heliconius* butterflies. *Journal of Evolutionary Biology*, 26, 1959–1967.

Mishmar, D., Ruiz-Pesini, E., Golik, P., Macaulay, V., Clark, A.G., Hosseini, S. *et al.* (2003) Natural selection shaped regional mtDNA variation in humans. *Proceedings of the National Academy of Sciences USA*, 100, 171–176.

Monteiro, A. (2015) Origin, development, and evolution of butterfly eyespots. *Annual Review of Entomology*, 60, 253–271.

Morales-Rozo, A., Tenorio, E.A., Carling, M.D. and Cadena, C.D. (2017) Origin and cross-century dynamics of an avian hybrid zone. *BMC Evolutionary Biology*, 17, 257.

Morris, J., Navarro, N., Rastas, P., Rawlins, L.D., Sammy, J., Mallet, J. *et al*. (2019) The genetic architecture of adaptation: convergence and pleiotropy in *Heliconius* wing pattern evolution. *Heredity*, 123, 138–152.

Moyle, L.C. and Nakazato, T. (2010) Hybrid incompatibility "Snowballs" between *Solanum* species. *Science*, 329, 1521–1523.

Muñoz, A.G., Salazar, C., Castaño, J., Jiggins, C.D. and Linares, M. (2010) Multiple sources of reproductive isolation in a bimodal butterfly hybrid zone. *Journal of Evolutionary Biology*, 23, 1312–1320.

Nordborg, M. and Tavaré, S. (2002) Linkage disequilibrium: what history has to tell us. *Trends in Genetics*, 18, 83–90.

Nosil, P., Funk, D.J. and Ortiz-Barrientos, D. (2009) Divergent selection and heterogeneous genomic divergence. *Molecular Ecology*, 18, 375–402.

Orteu, A. and Jiggins, C.D. (2020) The genomics of coloration provides insights into adaptive evolution. *Nature Reviews Genetics*, 21, 461–475.

Pardo-Diaz, C., Salazar, C., Baxter, S.W., Merot, C., Figueiredo-Ready, W., Joron, M. *et al.* (2012) Adaptive introgression across species boundaries in *Heliconius* butterflies. *PLoS Genetics*, 8, e1002752.

Parmesan, C. (2003) Butterflies as bioindicators for climate change effects. In *Butterflies: Ecology and Evolution Taking Flight* (eds. C.L. Boggs, W.B. Watt & P.R. Ehrlich), pp. 541–560. University of Chicago Press, Chicago.

Peck, S.L. (2004) Simulation as experiment: a philosophical reassessment for biological modeling. *Trends in Ecology & Evolution*, 19, 530–534.

Pereira Martins, A.R., Martins, L.P., Ho, W., McMillan, W.O., Ready, J.S. and Barrett, R.D.H. (2022) Scale-dependent environmental effects on phenotypic distributions in Heliconius butterflies. *Ecology and Evolution*, 12, e9286.

Phillips, P.C. (2008) Epistasis—the essential role of gene interactions in the structure and evolution of genetic systems. *Nature Reviews Genetics*, 9, 855–867.

Polechová, J. and Barton, N. (2011) Genetic drift widens the expected cline but narrows the expected cline width. *Genetics*, 189, 227–235.

Porter, A. (1997) The *Pieris napi/bryoniae* hybrid zone at Pont de Nant, Switzerland: broad overlap in the range of suitable host plants. *Ecological Entomology*, 22, 189–196.

Porter, A.H., Wenger, R., Geiger, H., Scholl, A. and Shapiro, A.M. (1997) The *Pontia daplidice-edusa* hybrid zone in Northwestern Italy. *Evolution; Internation Journal of Organic Evolution*, 51, 1561–1573.

Remington, C.L. (1968) Suture-zones of hybrid interaction between recently joined biotas. In *Evolutionary Biology* (eds. T. Dobzhansky, M.K. Hecht & W.C. Steere), pp. 321–428. Springer International Publishing, Boston.

Riemsdijk, I., Butlin, R.K., Wielstra, B. and Arntzen, J.W. (2019) Testing an hypothesis of hybrid zone movement for toads in France. *Molecular Ecology*, 28, 1070–1083.

Ross, C.L. and Harrison, R.G. (2002) A fine-scale spatial analysis of the mosaic hybrid zone between *Gryllus firmus* and *Gryllus pennsylvanicus*. *Evolution; Internation Journal of Organic Evolution*, 56, 2296–2312.

Rosser, N., Dasmahapatra, K.K. and Mallet, J. (2014) Stable *Heliconius* butterfly hybrid zones are correlated with a local rainfall peak at the edge of the Amazon basin. *Evolution; Internation Journal of Organic Evolution*, 68, 3470–3484.

Rosser, N., Shirai, L.T., Dasmahapatra, K.K., Mallet, J. and Freitas, A.V.L. (2021) The Amazon river is a suture zone for a polyphyletic group of co-mimetic heliconiine butterflies. *Ecography*, 44, 177–187.

Rossi, M., Hausmann, A.E., Thurman, T.J., Montgomery, S.H., Papa, R., Jiggins, C.D. *et al*. (2020) Visual mate preference evolution during butterfly speciation is linked to neural processing genes. *Nature Communications*, 11, 4763.

Roux, C., Fraïsse, C., Romiguier, J., Anciaux, Y., Galtier, N. and Bierne, N. (2016) Shedding light on the grey zone of speciation along a continuum of genomic divergence. *PLoS Biology*, 14, e2000234.

Ruiz-Pesini, E. and Wallace, D.C. (2006) Evidence for adaptive selection acting on the tRNA and rRNA genes of human mitochondrial DNA. *Human Mutation*, 27, 1072–1081.

Ryan, S.F., Deines, J.M., Scriber, J.M., Pfrender, M.E., Jones, S.E., Emrich, S.J. *et al*. (2018) Climate-mediated hybrid zone movement revealed with genomics, museum collection, and simulation modeling. *Proceedings of the National Academy of Sciences USA*, 115, E2284–E2291.

Ryan, S.F., Fontaine, M.C., Scriber, J.M., Pfrender, M.E., O'Neil, S.T. and Hellmann, J.J. (2017) Patterns of divergence across the geographic and genomic landscape of a butterfly hybrid zone associated with a climatic gradient. *Molecular Ecology*, 26, 4725–4742.

Savolainen, O., Lascoux, M. and Merilä, J. (2013) Ecological genomics of local adaptation. *Nature Reviews Genetics*, 14, 807–820.

Scriber, J. (2002) Latitudinal and local geographic mosaics in host plant preferences as shaped by thermal units and voltinism in *Papilio* spp. (Lepidoptera). *European Journal of Entomology*, 99, 225–239.

Scriber, J., Elliot, B., Maher, E., McGuire, M. and Niblack, M. (2014) Adaptations to "Thermal Time" Ccnstraints in *Papilio*: latitudinal and local size clines differ in response to regional climate change. *Insects*, 5, 199–226.

Scriber, J.M. (1990) Interaction of introgression from *Papilio Glaucus Canadensis* and diapause in producing "Spring Form" Eastern Tiger Swallowtail Butterflies, *P. Glaucus* (Lepidoptera: Palilionidae). *The Great Lakes Entomologist*, 23, 127–138.

Scriber, J.M., Keefover, K. and Nelson, S. (2002) Hot summer temperatures may stop movement o*f Papilio canadensis* butterflies and genetic introgression south of the hybrid zone in the North American Great Lakes Region. *Ecography*, 25, 184–192.

Sequeira, F., Bessa-Silva, A., Tarroso, P., Sousa-Neves, T., Vallinoto, M., Gonçalves, H. *et al*. (2020) Discordant patterns of introgression across a narrow hybrid zone between two cryptic lineages of an Iberian endemic newt. *Journal of Evolutionary Biology*, 33, 202–216.

Slatkin, M. (2008) Linkage disequilibrium—understanding the evolutionary past and mapping the medical future. *Nature Reviews Genetics*, 9, 477–485.

Smadja, C.M. and Butlin, R.K. (2011) A framework for comparing processes of speciation in the presence of gene flow. *Molecular Ecology*, 20, 5123–5140.

Stankowski, S., Shipilina, D. and Westram, A.M. (2021) Hybrid zones. *eLS*, 2, 1–16.

Stump, A.D., Sperling, F.A.H., Crim, A. and Scriber, J.M. (2003) Gene flow between Great Lakes region populations of the Canadian Tiger Swallowtail Butterfly, *Papilio canadensis*, near the hybrid zone with *P. glaucus* (Lepidoptera: Papilionidae). *The Great Lakes Entomologist*, 36, 41–53.

Supple, M.A., Papa, R., Hines, H.M., McMillan, W.O. and Counterman, B.A. (2015) Divergence with gene flow across a speciation continuum of *Heliconius* butterflies. *BMC Evolutionary Biology*, 15, 204.

Szymura, J.M. and Barton, N.H. (1986) Genetic analysis of a hybrid zone between the Fire-Bellied Toads, *Bombina bombina* and *B. variegata*, near Cracow in Southern Poland. *Evolution; Internation Journal of Organic Evolution*, 40, 1141–1159.

Szymura, J.M. and Barton, N.H. (1991) The genetic structure of the hybrid zone between the Fire-Bellied Toads Bombina bombina and B. variegata: comparisons between transects and between loci. *Evolution; Internation Journal of Organic Evolution*, 45, 237–261.

Taylor, S.A., Larson, E.L. and Harrison, R.G. (2015) Hybrid zones: windows on climate change. *Trends in Ecology & Evolution*, 30, 398–406.

Thomas, J.A. (2016) Butterfly communities under threat. *Science*, 353, 216–218.

Thomas, J.A. (2005) Monitoring change in the abundance and distribution of insects using butterflies and other indicator groups. *Philosophical Transactions of the Royal Society B*, 360, 339–357.

Thurman, T.J., Szejner-Sigal, A. and McMillan, W.O. (2019) Movement of a *Heliconius* hybrid zone over 30 years: a Bayesian approach. *Journal of Evolutionary Biology*, 32, 974–983.

Toews, D.P.L. and Brelsford, A. (2012) The biogeography of mitochondrial and nuclear discordance in animals. *Molecular Ecology*, 21, 3907–3930.

Turner, T.L., Hahn, M.W. and Nuzhdin, S.V. (2005) Genomic islands of speciation in *Anopheles gambiae*. *PLoS Biology*, 3, e285.

Van Belleghem, S.M., Cole, J.M., Montejo-Kovacevich, G., Bacquet, C.N., McMillan, W.O., Papa, R. *et al.* (2021) Selection and isolation define a heterogeneous divergence landscape between hybridizing *Heliconius* butterflies. *Evolution; International Journal of Organic Evolution*, 75, 2251–2268.

Van Belleghem, S.M., Rastas, P., Papanicolaou, A., Martin, S.H., Arias, C.F., Supple, M.A. *et al.* (2017) Complex modular architecture around a simple toolkit of wing pattern genes. *Nature Ecology & Evolution*, https://doi.org/10.17863/CAM.8233.

Van Belleghem, S.M., Ruggieri, A.A., Concha, C., Livraghi, L., Hebberecht, L., Rivera, E.S. *et al*. (2023) High level of novelty under the hood of convergent evolution. *Science*, 379, 1043–1049.

Van Belleghem, S.M.V., Roman, P.A.A., Gutierrez, H.C., Counterman, B.A. and Papa, R. (2020) Perfect mimicry between *Heliconius* butterflies is constrained by genetics and development. *Proceedings of the Royal Society B*, 287, 20201267.

Vines, T.H., Kohler, S.C., Thiel, M., Ghira, I., Sands, T.R., MacCallum, C.J. *et al.* (2003) The maintenance of reproductive isolation in a mosaic hybrid zone between the fire-bellied toads *Bombina bombina* and *B. variegata*. *Evolution; Internation Journal of Organic Evolution*, 57, 1876–1888.

Wang, L., Luzynski, K., Pool, J.E., Janoušek, V., Dufková, P., Vyskočilová, M.M. *et al*. (2011) Measures of linkage disequilibrium among neighbouring SNPs indicate asymmetries across the house mouse hybrid zone. *Molecular Ecology*, 20, 2985–3000.

Wang, S., Rohwer, S., Delmore, K. and Irwin, D.E. (2019) Cross-decades stability of an avian hybrid zone. *Journal of Evolutionary Biology*, 32, 1242–1251.

Watt W.B. and Boggs C.L. (2003) Synthesis: Butterflies as model systems in ecology and evolution—present and future. In *Butterflies: Ecology and Evolution Taking Flight* (eds. Boggs, C.L., Watt, W.B. & P.R. Ehrlich), pp. 603–613. University of Chicago Press, Chicago.

Wielstra, B. (2019) Historical hybrid zone movement: More pervasive than appreciated. *Journal of Biogeography*, 46, 1300–1305.

Wisz, M.S., Pottier, J., Kissling, W.D., Pellissier, L., Lenoir, J., Damgaard, C.F. *et al*. (2013) The role of biotic interactions in shaping distributions and realised assemblages of species: implications for species distribution modelling. *Biological Reviews*, 88, 15–30.

Wolf, J.B.W. and Ellegren, H. (2017) Making sense of genomic islands of differentiation in light of speciation. *Nature Reviews Genetics*, 18, 87–100.

Wu, C.I. (2001) The genic view of the process of speciation. *Journal of Evolutionary Biology*, 14, 851–865.

Xiong, T. and Mallet, J. (2022) On the impermanence of species: the collapse of genetic incompatibilities in hybridizing populations. *Evolution; Internation Journal of Organic Evolution*, 76, 2498–2512.

Zhang, L., Chaturvedi, S., Nice, C.C., Lucas, L.K. and Gompert, Z. (2022) Population genomic evidence of selection on structural variants in a natural hybrid zone. *Molecular Ecology*, 32, 1497–1514.

Zhang, L., Thibert-Plante, X., Ripa, J., Svanbäck, R. and Brännström, Å. (2019) Biodiversity loss through speciation collapse: mechanisms, warning signals, and possible rescue. *Evolution; International Journal of Organic Evolution*, 73, 1504–1516.

Zhang, Y., Teng, D., Lu, W., Liu, M., Cao, L., Southcott, L. *et al*. (2021) A widely diverged locus involved in locomotor adaptation in *Heliconius* butterflies. *Science Advances*, 7, eabh2340.

Zieliński, P., Dudek, K., Arntzen, J.W., Palomar, G., Niedzicka, M., Fijarczyk, A. *et al.* (2019) Differential introgression across new hybrid zones: evidence from replicated transects. *Molecular Ecology*, 28, 4811–4824.

Manuscript received May 2, 2023
Final version received July 13, 2023
Accepted July 21, 2023