

Locomotion leads to speciation in *Heliconius* butterflies

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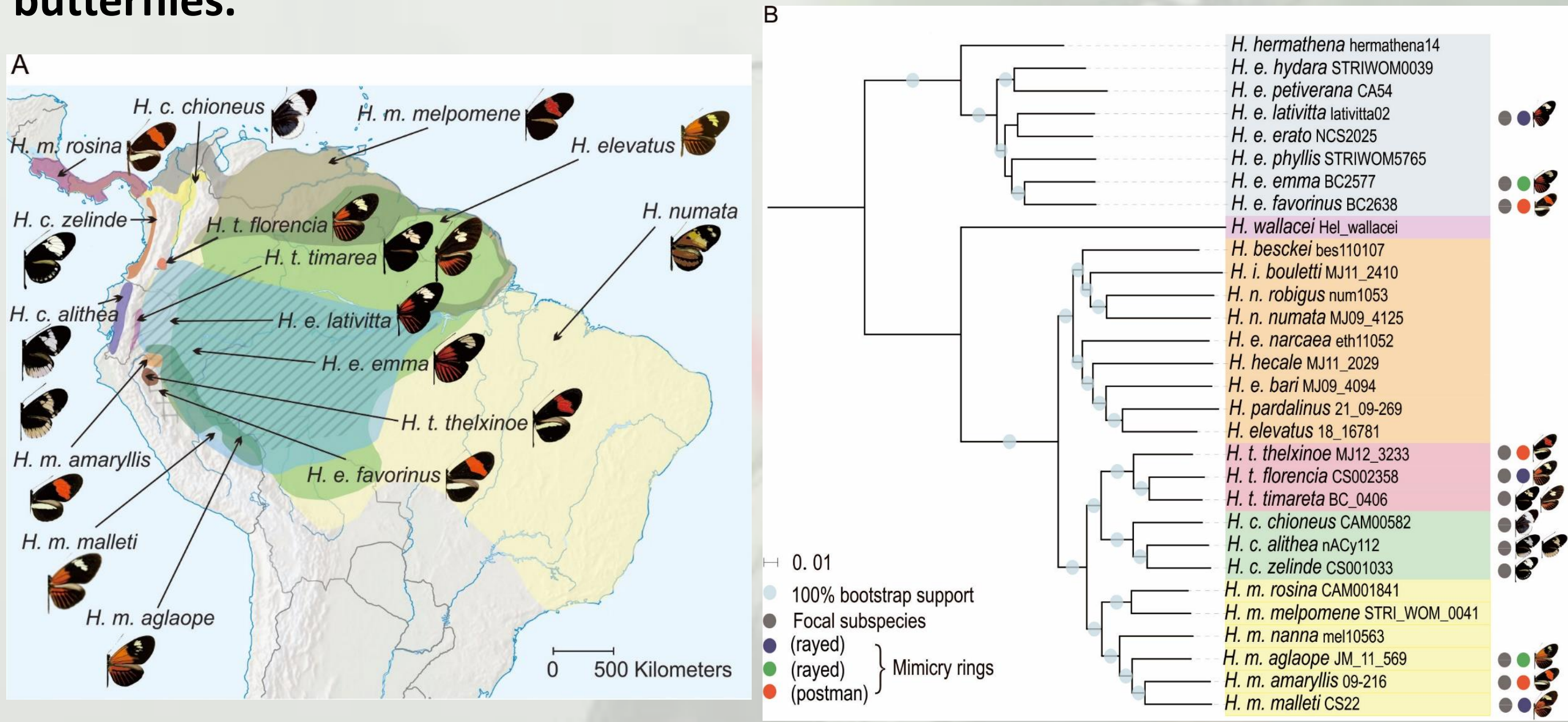
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Introduction

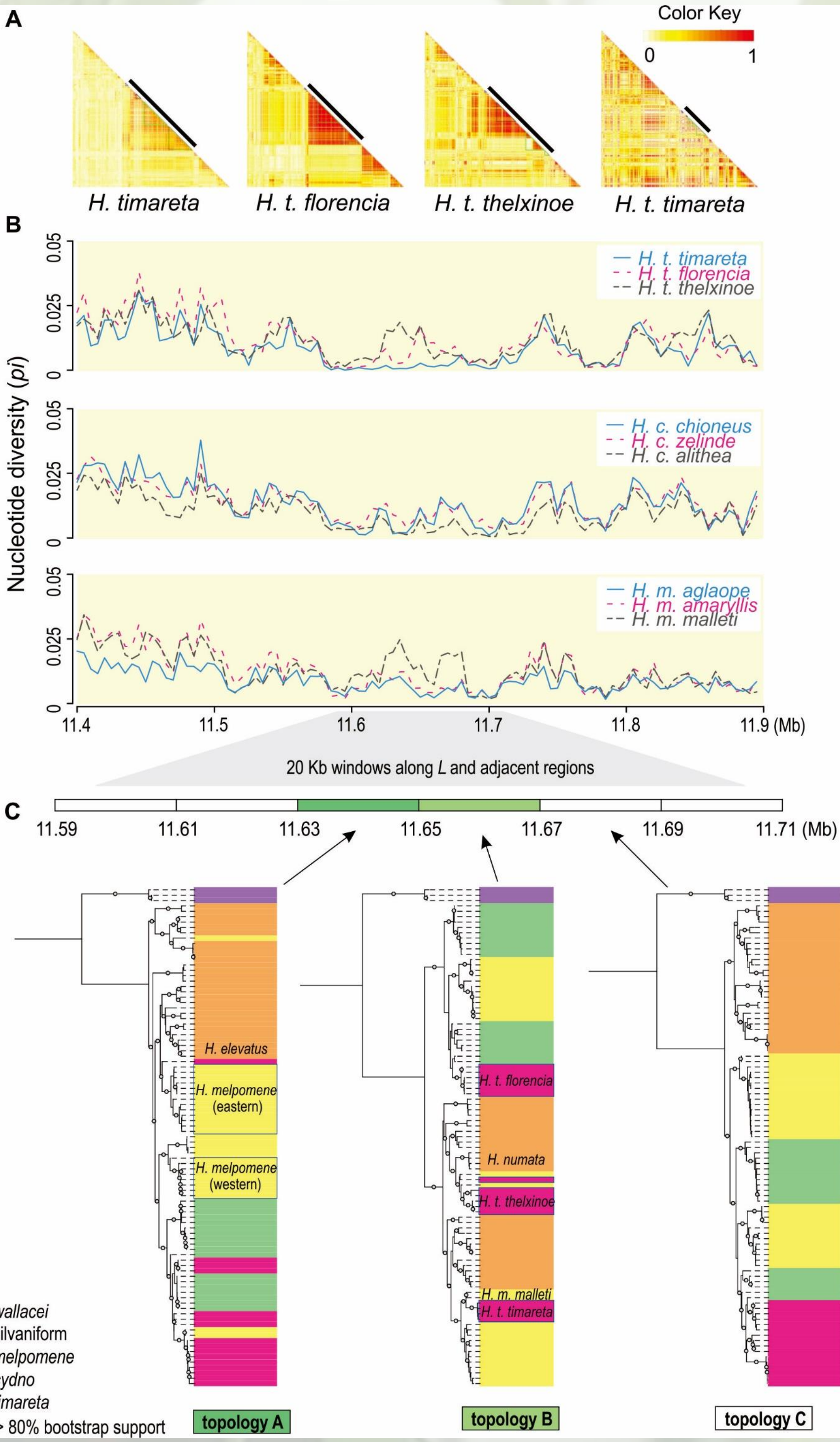
Speciation is a comprehensive process, yet genetic evidence of the biomechanical traits involved in this process and their interplay with other traits is still lacking. *Heliconius* butterflies have undergone adaptive radiation and therefore serve as an excellent system for the study of speciation. However, there is a long-last paradox between their convergence of mimetic wing patterns and rapid divergence in speciation. Here, with the aim of addressing the role of wing locomotion, we examine the genome-wide patterns of differentiation in *Heliconius* butterflies and identify a locomotor locus, *L*, that displays strong linkage disequilibrium and high divergence. We elucidate the complex evolutionary history of *L* and reveal that it is a hotspot region for introgression. The *L* locus contains a group of locomotion-related genes clustered only in Lepidoptera as a single locus. Taken together, we illustrate a dynamic relationship between wing patterns and locomotion and summarize multiple roles of locomotion.

Taxon sampling according to whole-genome phylogenetic analysis

The geographical distribution and genome-wide phylogeny of *Heliconius* butterflies.



Evidence of selection and introgression at the *L* locus



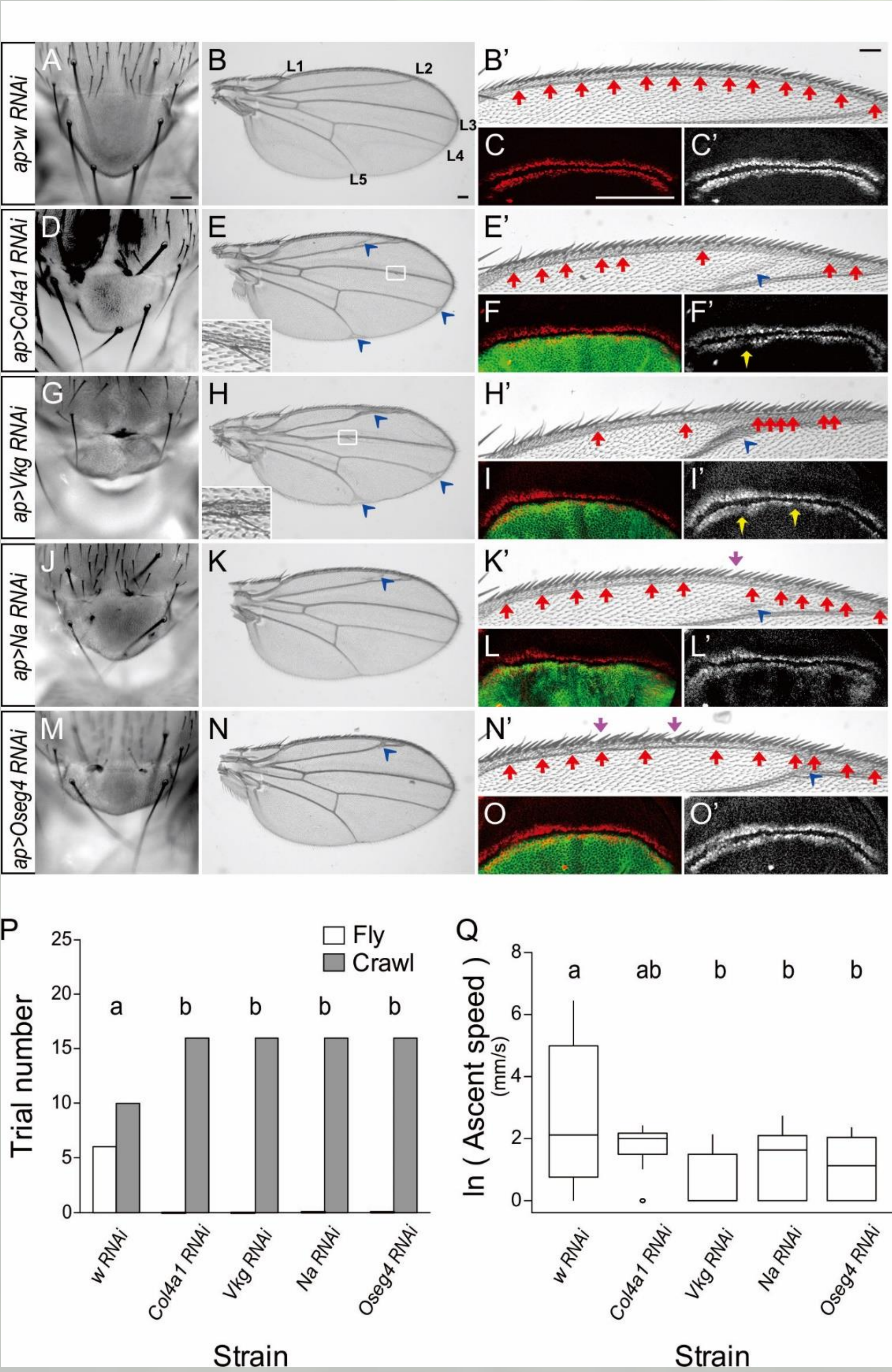
Local patterns of linkage disequilibrium, genetic diversity and phylogenetic trees along the *L* locus.

Pairwise LD (r^2), is estimated along *L* and its adjacent regions (A), with *L* indicated by bold black bars. Values of nucleotide diversity (π) are calculated along *L* (B). Maximum likelihood phylogenetic trees are constructed for every 20 kb window along *L* and adjacent regions (C). The *H. timareta* subspecies are grouped polyphyletically. The eastern and western *melpomene* populations join the silvaniform and *cydno-timareta* clades, respectively, in the window of topology A, with a signature of introgression between eastern *melpomene* and *H. elevatus*.

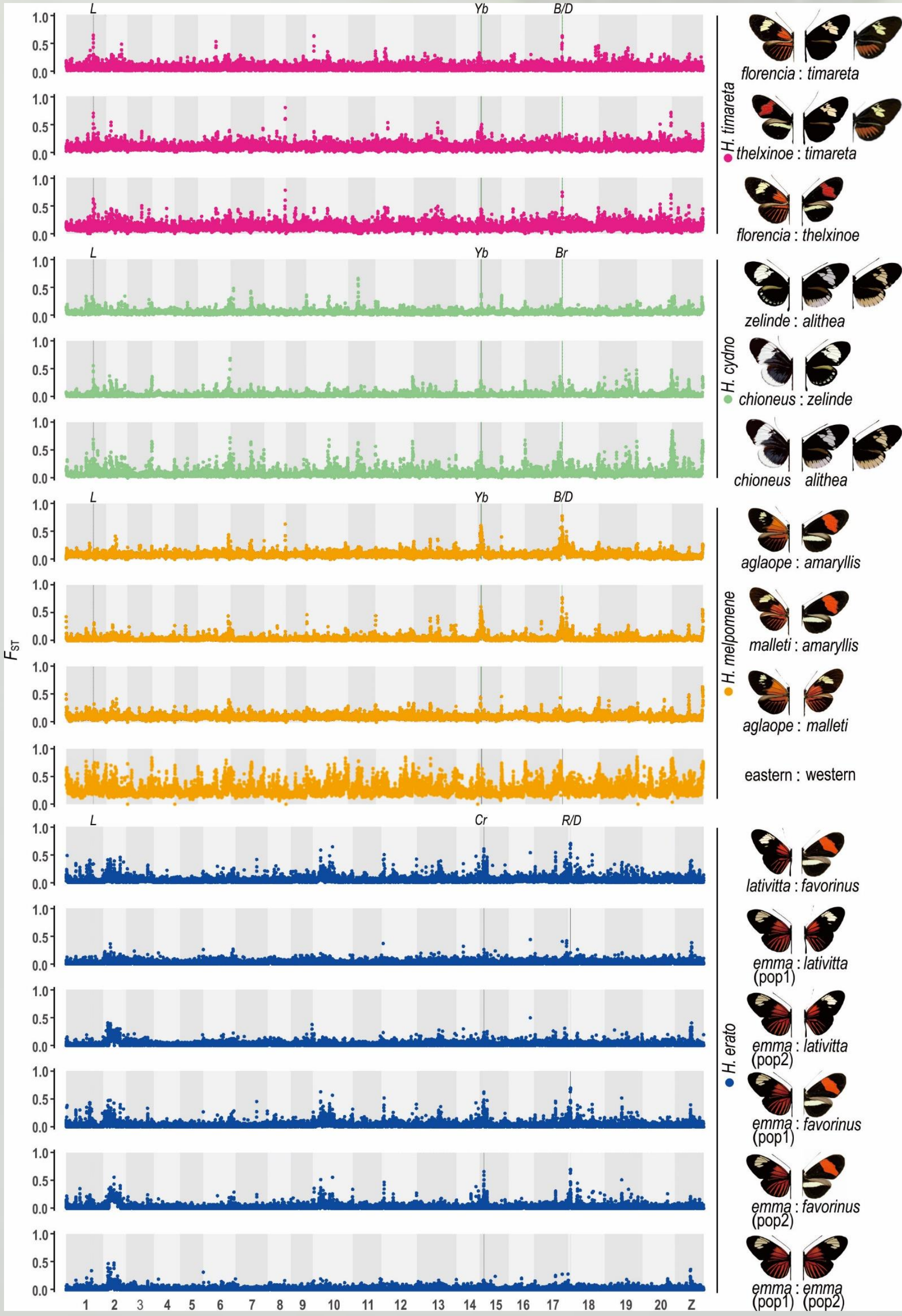
Functional implications of the *L* locus

The *Drosophila* orthologous genes at the *L* locus are functionally related to locomotion.

The expression of *w* RNAi alone by *ap*-Gal4 does not produce any obvious defect in the adult *Drosophila* scutellum (A) or wing blade (B, B'). Accordingly, the expression of *Senseless* in the third-instar imaginal discs is not altered (C, C'). Knocking down each of the genes in the *L* locus (*Col4a1*, *Vkg*, *Na* or *Oseg4*) produces similar defects associated with locomotion (D-N'). The expression pattern of *Senseless* is also altered in the third-instar imaginal discs of KD flies (F', I', L' and O'). The KD flies fail to fly in response to the dropping stimulus (P) and show a slower ascent speed (Q). Different letters (a and b) indicate statistically significant differences across groups ($p < 0.05$) (P, Q). Scale bars, 100 μm .



Genetic divergence across the *L* locus and wing pattern loci



Genomic divergence during early speciation.

Pairwise F_{ST} values are calculated across the genome in 50 kb sliding windows with a step size of 20 kb, between subspecies of *H. timareta*, *H. cydno*, *H. melpomene* and *H. erato* and between the eastern and western populations of *H. melpomene*. Twenty kb sliding windows are used for the calculation of F_{ST} values between subspecies of *H. erato*. The locomotor locus, *L*, and other wing patterning loci are labeled with gray bars. In *H. erato*, the orthologous loci of *Yb* and *B/D* are known as *Cr* and *R/D*, respectively.

Conclusions

We characterized a locomotor locus that acted as an introgression hotspot and played a consistent role in early *Heliconius* speciation. We further showed that it is likely a gene cluster in Lepidoptera. These findings involve a handful of fundamental issues in evolution, including speciation, adaptation and hybridization. Understanding the role of locomotion appears to be the key to disentangling this complexity. Butterfly wings exhibit a variety of functions and have been favored by natural selection and sexual selection, and wing patterns have been recognized as an important morphological trait involved in speciation and adaptation. In this study, we showed that a biomechanical trait, wing locomotion, has been favored by selection in multiple *Heliconius* species and plays multiple roles in speciation and adaptation: it might serve as either a classic magic trait or an ecological trait, and during this process, hybridization directly fueled its divergence in speciation. Although locomotion is a less intuitive biomechanical feature, our results, for the first time, decode combinations of wing patterns and locomotion that cannot be overlooked: they might either increase divergence in speciation or promote mimicry in adaptation.

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