

1 Selection and gene flow define polygenic barriers between incipient butterfly species

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17 Abstract

18 Characterizing the genetic architecture of species boundaries remains a difficult task.
19 Hybridizing species provide a powerful system to identify the factors that shape genomic variation and,
20 ultimately, identify the regions of the genome that maintain species boundaries. Unfortunately, complex
21 histories of isolation, admixture and selection can generate heterogenous genomic landscapes of
22 divergence which make inferences about the regions that are responsible for species boundaries
23 problematic. However, as the signal of admixture and selection on genomic loci varies with
24 recombination rate, their relationship can be used to infer their relative importance during speciation.
25 Here, we explore patterns of genomic divergence, admixture and recombination rate among hybridizing
26 lineages across the *Heliconius erato* radiation. We focus on the incipient species, *H. erato* and *H. himera*,
27 and distinguish the processes that drive genomic divergence across three contact zones where they
28 frequently hybridize. Using demographic modeling and simulations, we infer that periods of isolation
29 and selection have been major causes of genome-wide correlation patterns between recombination
30 rate and divergence between these incipient species. Upon secondary contact, we found surprisingly
31 highly asymmetrical introgression between the species pair, with a paucity of *H. erato* alleles
32 introgressing into the *H. himera* genomes. We suggest that this signal may result from a current
33 polygenic species boundary between the hybridizing lineages. These results contribute to a growing
34 appreciation for the importance of polygenic architectures of species boundaries and pervasive genome-
35 wide selection during the early stages of speciation with gene flow.

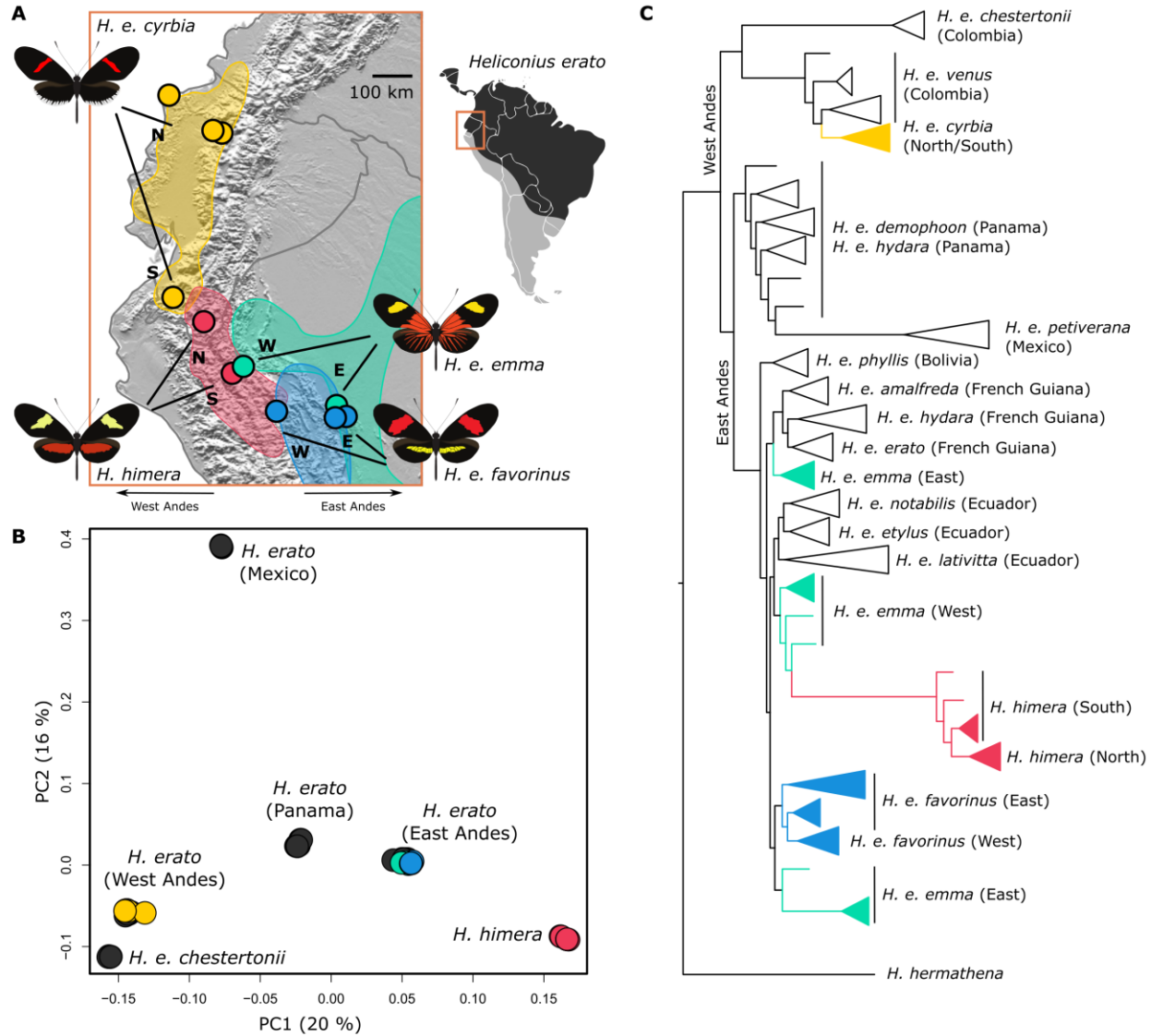
36 Introduction

37 Disentangling the factors that drive genomic divergence is necessary for advancing our
38 understanding of speciation. Targets of selection, for example, may be responsible for adaptive
39 differences between species; and their number, distribution and effect on gene flow along the genome
40 define the architecture of species boundaries. In population genomic studies, targets of selection are
41 expected to show elevated divergence between species and reduced genetic diversity within species [1].
42 These highly divergent loci often reflect local adaptation and/or incompatibilities between species, and
43 can be considered the loci that define the species [2,3]. This is because natural selection acts as a local
44 genomic “barrier” to gene flow between hybridizing species [4–6]. In contrast, the rest of the genome,
45 which is not under such selective pressures, may be expected to exchange more freely between the
46 species (i.e. admixture). However, genetic variation at these latter genomic regions can be greatly
47 impacted by neutral demographic processes (i.e. population size and migration) and the indirect effects
48 of nearby targets of selection (i.e. linked selection). Local recombination rates can further influence how
49 these processes impact genetic variation, which collectively result in highly heterogenous patterns of
50 genome-wide divergence [1,4,7–10]. Thus, the challenge is to distinguish those targets of selection and
51 demographic processes that generate the genomic landscape of divergence.

52 Here, we reconstruct the history of demographic isolation and characterize the extent to which
53 selection has shaped genomic divergence between two closely related, hybridizing *Heliconius* species.
54 More precisely, we first use a demographic modeling approach to reconstruct the history of population
55 sizes and isolation during divergence of these species [11–18]. Next, with knowledge of the most likely
56 demographic history, we use coalescent simulations to test for the importance of selection as the
57 underlying mechanism driving heterogeneous patterns of genomic divergence. The coalescent
58 simulation approach allows us to explore other genomic factors, such as recombination rate, on
59 genomic divergence. Specifically, we can test for genome-wide impacts of linked selection by using the
60 expectation that linked selection is higher in regions of the genome where recombination rate is lower
61 [19]. Hence, we would expect a negative association between recombination rate and divergence across
62 the genome [8,9,20,21]. Similarly, we expect a positive association between recombination rate and
63 admixture, if the species continue to hybridize [4,22], but not necessarily if they diverged in isolation
64 [8,23]. Thus, the relationships of recombination rate with divergence and admixture can be used to infer
65 the relative importance of different evolutionary processes during speciation.

66 To provide a relative perspective of the divergence between our focal hybridizing *Heliconius*
67 species, we first investigate the relationship between reproductive isolation and genomic divergence
68 across 15 pairs of increasingly divergent populations and species in the *H. erato* clade. Next, we use the
69 incipient species *Heliconius erato* and *Heliconius himera* that hybridize across three geographically
70 distinct contact zones to test for the relative contribution of demographic and selective factors in the
71 evolution of the divergence landscape. *Heliconius himera* is found in dry forest areas of southern
72 Ecuador and northern Peru [24]. It comes into contact with *Heliconius erato cyrba* on the western
73 slopes of the Ecuadorian Andes and with *Heliconius erato favorinus* and *Heliconius erato emma* on the
74 eastern slopes of the Andes, both areas with wet forest (Figure 1A). Hybridization is ongoing and hybrids
75 are easily identifiable by their wing color patterns. In Ecuador, hybrids compose approximately 5% of the
76 population in the contact zone [25], and, although poorly characterized, hybridization is known to occur
77 in the other contact zones. The two species show strong premating isolation but little or no postmating
78 reproductive barriers [25]. The eastern and western *H. erato* populations that hybridize with *H. himera*
79 do not come into contact with each other and show deep genetic divergence in the *H. erato* clade [26].

80 Our findings demonstrate the importance of both isolation and selection in establishing the
81 heterogeneous genomic landscape of divergence that characterizes our hybridizing species. The
82 reconstructed demographic history supports a complex dynamics of population fluctuations and varying
83 migration rates, that with selection, resulted in the observed heterogeneous patterns of genomic
84 divergence. Further, our coalescent simulations well fit the observed relationship of genomic divergence
85 and recombination when we consider genome-wide impacts of recent and strong selective events in the
86 absence of gene flow. Finally, we show that the species boundary between *H. himera* and *H. erato* is
87 highly porous and that gene flow is highly asymmetrical and distinct across each of the three contact
88 zones. We suggest that this asymmetrical signal of gene flow may be the result of polygenic species
89 boundaries that restrict introgression in *H. himera*. Overall, our results highlight that the study of
90 heterogeneous landscapes of divergence can help us understand how demographic and selective
91 processes drive speciation.



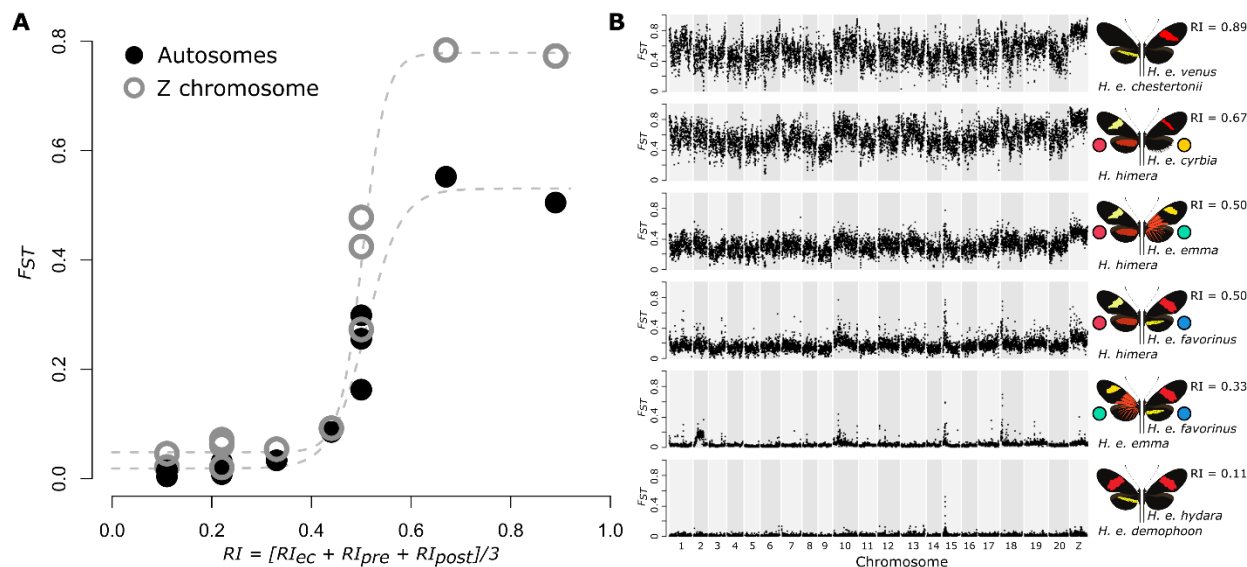
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93 **Figure 1. Geographical distribution, population structure and phylogeny of the focal populations in relation to the *Heliconius***
 94 ***erato* radiation. (A)** We sampled two populations of *H. himera*, *H. e. cyrbia*, *H. e. emma* and *H. e. favorinus*. The distribution of
 95 *H. himera* (red) covers dry valleys in the Andes of South Ecuador and North Peru. In the North, *H. himera* (N) comes into contact
 96 with a *H. e. cyrbia* (S) population. In the South, *H. himera* comes into contact with a *H. e. emma* (W) and *H. e. favorinus* (W)
 97 population. **(B)** Principal Component Analysis (PCA) of the focal samples (colored points) among all the available whole genome
 98 data for the *H. erato* radiation (black points). **(C)** Maximum likelihood tree built using FastTree and using only autosomal sites
 99 from 121 whole genome resequenced individuals (see Figure S1 for the uncollapsed tree). Nodes in the tree that represent the
 100 major clades within *H. erato* (east and west of Andes) obtained high support (= 1) from the Shimodaira-Hasegawa test.

101 **Results & discussion**

102 *Genomic landscape of divergence among hybridizing races and incipient species*

103 We first sought to describe the general patterns of genome-wide divergence and how they
104 varied based on the varying degrees of reproductive isolation. To do this, we compared genome-wide
105 patterns of divergence across 15 contact zones in the *H. erato* clade that have varying degrees of
106 reproductive isolation (*RI*, Figure 2; Table S1). Correlations of *RI* with genome-wide estimates of relative
107 divergence (F_{ST}) show that divergently selected color patterns between hybridizing races of *H. erato* with
108 absence of other pre- or post-mating barriers are not sufficient to drive genome-wide increases in
109 divergence (Figure 2A). In these hybridizing *H. erato* races, there are only narrow peaks of divergence
110 largely centered over the loci known to be responsible for color pattern differences (Figure 2B). In
111 contrast, between the incipient species *H. erato cyrbia* and *H. himera*, which have strong differences in
112 mate preference, divergence is much higher across the entire genome. Divergence between these
113 species has increased to the extent that F_{ST} peaks near the known color pattern loci *WntA* (chr 10),
114 *cortex* (chr 15), and *optix* (chr 18) are not detectable (Figure 2B). The *H. himera* and *H. erato* contact
115 zones on the eastern Andes (*H. e. emma* and *H. e. favorinus*) also show elevated genome-wide
116 divergence, but much lower overall than in the western Andes contact zone. As expected, genomic
117 divergence was highest between *H. e. venus* and *H. e. chestertonii* from Colombia, where both mate
118 preference and hybrid sterility have been reported [27]. We also note a dramatic increase in divergence
119 on the Z chromosome relative to the autosomes (Figure 2). This is in line with previous work on *H. e.*
120 *chestertonii*, which suggested the important role played by the Z chromosome as a barrier to gene flow
121 [28].



122

123 **Figure 2. Reproductive isolation and divergence among *Heliconius erato* populations.** (A) Genome-wide averages of relative
 124 divergence (F_{ST}) show a sharp increase with increasing measures of reproductive isolation between parapatric *H. erato*
 125 populations. The measure of reproductive isolation (RI) was obtained by equally weighting ecological (RI_{ec}), pre-isolation (RI_{pre})
 126 and post-isolation (RI_{post}) components (Table S1). Higher relative divergence on the Z chromosome can be observed for the
 127 more divergent parapatric comparisons, however, incompatibilities that are potentially Z-linked have only been suggested for
 128 *H. e. chesteronii* crosses [27,28]. (B) Plots of relative divergence (average F_{ST} in 50 kb windows) between parapatric *H. erato*
 129 populations along the genome. Plots are ordered according to the measure of reproductive isolation (RI). Colored circles match
 130 color codes used for the focal populations in this study. Divergence peaks on chromosome 10, 15 and 18 correspond to the
 131 divergently selected color pattern genes *WntA* (affecting forewing band shape), *cortex* (affecting yellow hindwing bar), and
 132 *optix* (affecting red color pattern elements), respectively [26].

133

134 *Demographic change and selection jointly drive genomic divergence among incipient species*

135 Collectively, our results provide a view into the genomic landscape among lineages with
 136 increasing degrees of reproductive isolation (Figure 2)[29,30]. However, the increase in divergence does
 137 not seem to be a linear process as there is a marked increase in divergence between the incipient
 138 species that are known to still frequently and continuously hybridize over many generations. To
 139 understand what drives these elevated patterns of divergence, we have to recognize that each of these
 140 contact zones reflect hybridization between evolutionary distinct lineages. In this regard, the genomic
 141 landscapes do not reflect a continuum of genomic divergence throughout speciation, but rather they
 142 each are the evolutionary outcomes of various neutral and adaptive processes that shaped each of the
 143 populations coming into contact. For example, we find increased divergence between *H. himera* and *H.*
 144 *erato* from the western Andes slopes compared to *H. erato* from eastern Andes slopes. As seen in the
 145 PCA and phylogenetic inference, this results from a deeper split between *H. himera* and *H. e. cyrbia* from
 146 the western Andes slope compared to *H. himera* and the *H. erato* races that are found east of the Andes,

147 including *H. e. emma* and *H. e. favorinus* (Figure 1B, C). This is consistent with previous studies that
148 placed *H. himera* nested within the *H. erato* clade and not as a sister species to *H. erato* [26,31].

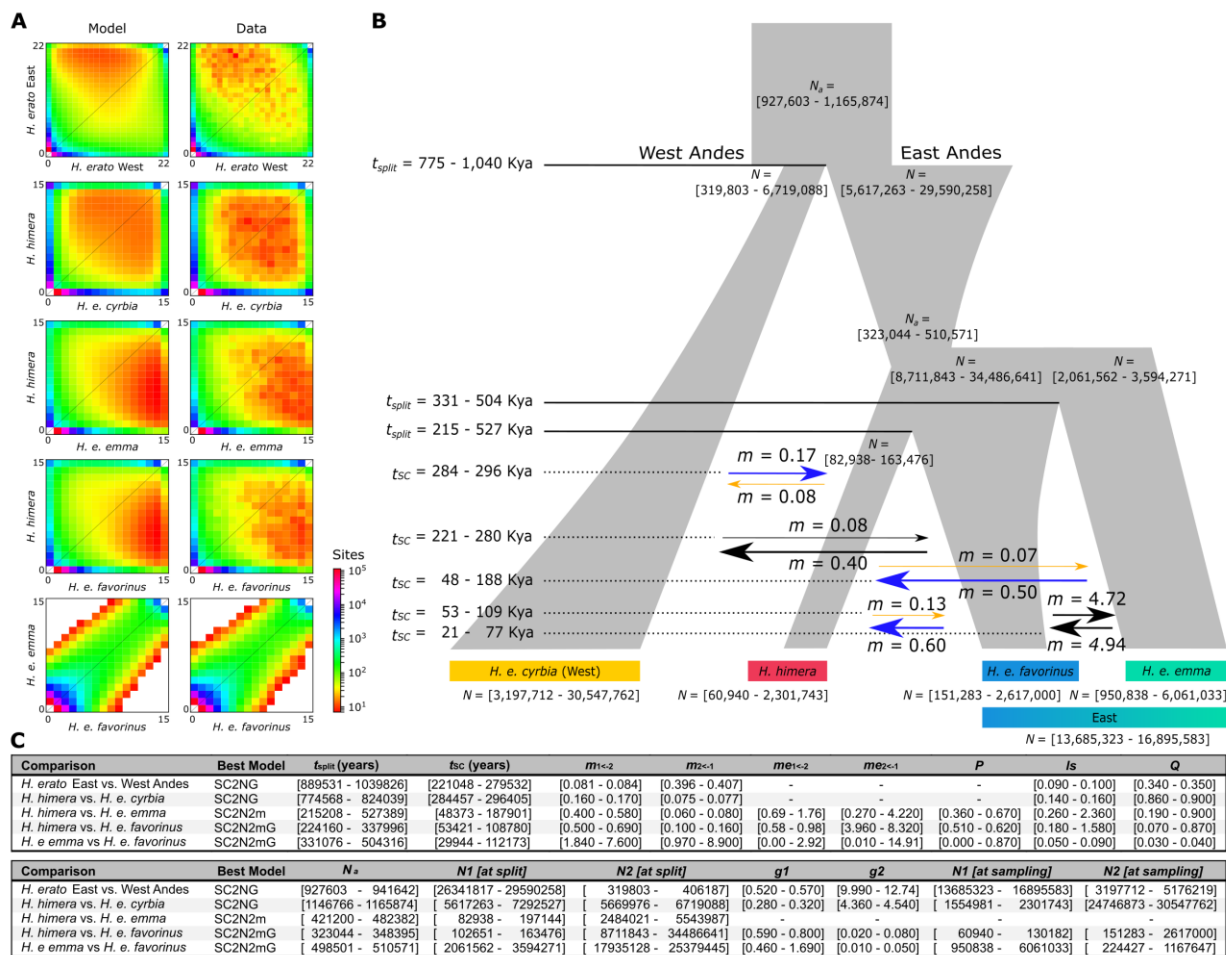
149 To understand what forces are likely driving differences in patterns of genome-wide divergence
150 between *H. erato* and *H. himera*, we fit the estimated joint site-frequency spectrum (JSFS) for three
151 geographically distinct contact zones to 26 alternative demographic scenarios that varied in split times,
152 migration rates and population sizes (Figure 3). All three *H. erato* and *H. himera* contact zones best fit
153 models that included secondary contact (SC) after a period of isolation without gene flow (Figure S2-3;
154 Table S2). For all three zones we found support for asymmetrical migration. In each case, migration
155 rates were predominantly in one direction, with on average 0.5 to 0.6 migrants per generation moving
156 from *H. erato* into *H. himera*, compared to 0.07 to 0.13 moving from *H. himera* into *H. erato*. This result
157 is consistent with the effective migration rates being driven by the marked population size differences
158 between the two species (Figure 3B).

159 Nearly all the models that included exponential population growth (G) best fit the JSFS, with the
160 exception of the *H. himera* and *H. e. emma* comparison. Estimates of ancestral and contemporary
161 population sizes suggest strong expansions in *H. himera* and *H. erato*. These inferred changes in
162 population sizes broadly fit previous results obtained from pairwise sequentially Markovian coalescent
163 (PSMC) analysis (Van Belleghem et al. 2018), which suggested an overall population growth in *H. erato*
164 east and west of the Andes in the past 1 My and size reduction for *H. himera* in the past 200 Ky (Figure
165 S3). However, we found that estimates of contemporary population sizes varied greatly depending on
166 the population comparison (Figure 3B, C), a result possibly explained by unaccounted population
167 structure and difficulties in estimating growth (G). For *H. e. favorinus*, estimates of contemporary
168 population size were generally much smaller than the ancestral population. This result fits the
169 observation that *H. e. favorinus* is a smaller Andean population of the “postman” color pattern (i.e. red
170 forewing band), which has a much larger distribution throughout the Neotropics. Collectively, the
171 models support a history that includes periods of allopatry, followed by lineage specific changes in
172 population size that coincide with more recent gene flow.

173 To investigate if the JSFS contained evidence of selection driving patterns of divergence across
174 the contact zones, we incorporated heterogeneity in population size (2N) and migration rate (2M) into
175 the models, similar to what was done by Rougeux *et al.* 2017 [32] and Tine *et al.* 2014 [16], respectively.
176 The 2N model allows heterogeneity in population size estimates across loci that result from the
177 differences in allelic variation caused by linked selection (l/s = effective population size of locus relative to

178 neutral loci; Q = proportion of the genome affected by I_s). We found that all contact zones between *H.*
179 *erato* and *H. himera* well supported 2N models, suggesting the effect of linked selection in shaping
180 patterns of genomic variation and divergence between the incipient species. The strongest I_s was
181 observed for the population comparisons of *H. erato* East and West ($I_s = 0.10$; $Q = 0.35$) and *H. himera*
182 and *H. erato* West ($I_s = 0.15$; $Q = 0.90$) and the lowest observed between *H. e. emma* and *H. e. favorinus*
183 ($I_s = 0.07$; $Q = 0.04$) (Figure 3C, Table S3).

184 The result of selection on locally adapted alleles is a heterogeneous landscape with regions
185 containing these variants showing much lower rates of admixture compared to the rest of the genome.
186 The 2M models allow for this type of heterogeneity in migration rates across the genome. Both contact
187 zones in the eastern Andes supported these models for *H. himera* and *H. erato*, suggesting that the
188 model fits the eastern Andean populations having genomic regions with much lower rates of
189 introgression than other parts of the genome.



190

191 **Figure 3. Secondary Contact (SC) best demographic model of the *H. himera* and *H. erato* population history.** (A) Joint Site
 192 Frequency Spectra (JSFS) for data and best model (see Table S2 and Figure S3-4 for AIC values (Akaike Information Criterion)).
 193 (B) Reconstruction of historical demography of *H. himera* and *H. erato* populations using models with best AIC scores. All best
 194 models included a period of isolation and secondary contact. Arrows indicate effective migration rates ($2N_e m$). Migration from
 195 *H. himera* into *H. erato* is indicated in orange, migration from *H. erato* into *H. himera* is indicated in blue. (C) Table with
 196 parameter ranges obtained from five best scoring models out of twenty runs. N_a = ancestral population size, N_1 = Size of
 197 population 1, N_2 = size of population 2, g_1 = growth coefficient of population 1, g_2 = growth coefficient of population 2, ls =
 198 linked selection, Q = proportion of the genome with a reduced effective size due to linked selection (ls), $m_{1<2}$ = migration from
 199 population 2 into 1, $m_{2<1}$ = migration from populations 1 into 2, t_{split} = split time, t_{sc} = time of secondary contact, P = proportion
 200 of the genome evolving neutrally.

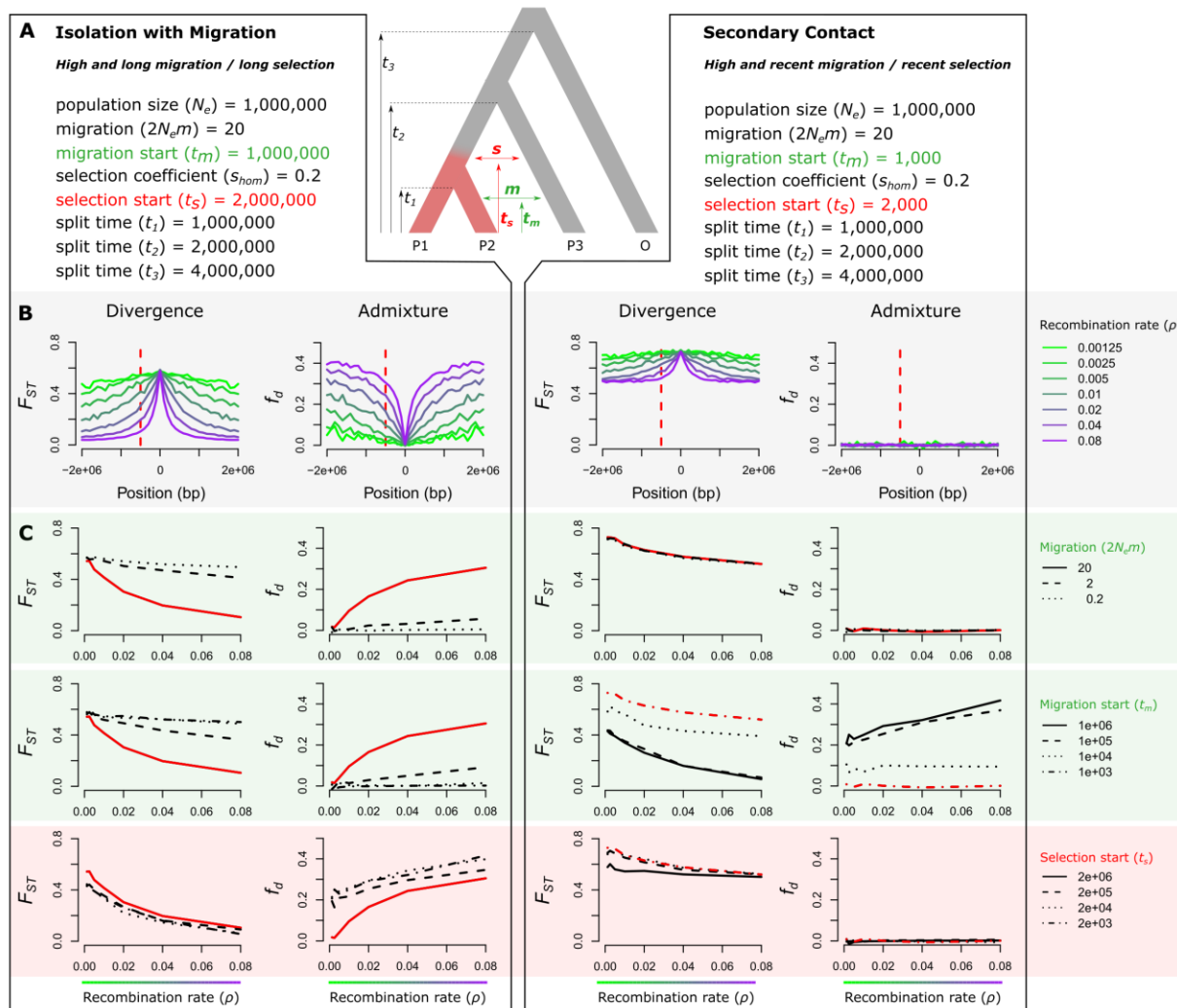
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202 *Simulations of linked selection and secondary contact predict correlation of recombination rate with*
 203 *divergence, not admixture*

204 The demographic models provide a comprehensive reconstruction of the evolutionary history of
 205 divergence between *H. erato* and *H. himera* and allow us to estimate time intervals of lineage splits, size
 206 changes and migration changes that span the past million year of divergence between *H. himera* and
 207 *erato* in the Andes (Figure 3). We next use these estimates to inform simulations and conduct further

208 tests for the impact of selection and demography during genomic divergence of the incipient species. To
209 do this, we simulated the expected relationship of recombination rate to relative divergence (F_{ST}) and
210 admixture (f_d) at a locus linked to a site under selection. We used two distinct scenarios of migration
211 broadly applicable to *H. erato* and *H. himera* and compared the simulation results to real data (Figure
212 4A, B).

213 As demonstrated by other studies, in a scenario of “isolation with migration” (IM) with long periods of
214 migration and divergent selection, our simulations predict a strong negative correlation between
215 recombination rate and F_{ST} [20,21,33] and a strong positive correlation between recombination rate and
216 f_d [4,8] (Figure 4C, left). This pattern reflects the degree of linkage between neutral sites and loci under
217 divergent selection [23,34]. In contrast, our simulations show that the relationship between
218 recombination rate and f_d is reduced when migration is more recent or low (Figure 4C, left). As expected,
219 in a “secondary contact” (SC) scenario that is characterized by a more recent onset of migration, the
220 simulations show that F_{ST} values are generally high and f_d values close to zero (Figure 4C, right). This
221 results in the absence of a relationship between recombination rate and F_{ST} or f_d in most SC scenarios.
222 However, when selection in the genome is recent (~selective sweeps), a negative relationship between
223 recombination rate and divergence, but not admixture, emerges in the SC scenario (Figure 4C). This
224 relationship arises due to linked selection that reduces diversity within populations and increases the
225 relative divergence, F_{ST} , between populations [35]. The relationship holds for lower selection strengths
226 at relatively short distances from the selected site ($s = 0.02$; Figure S5).



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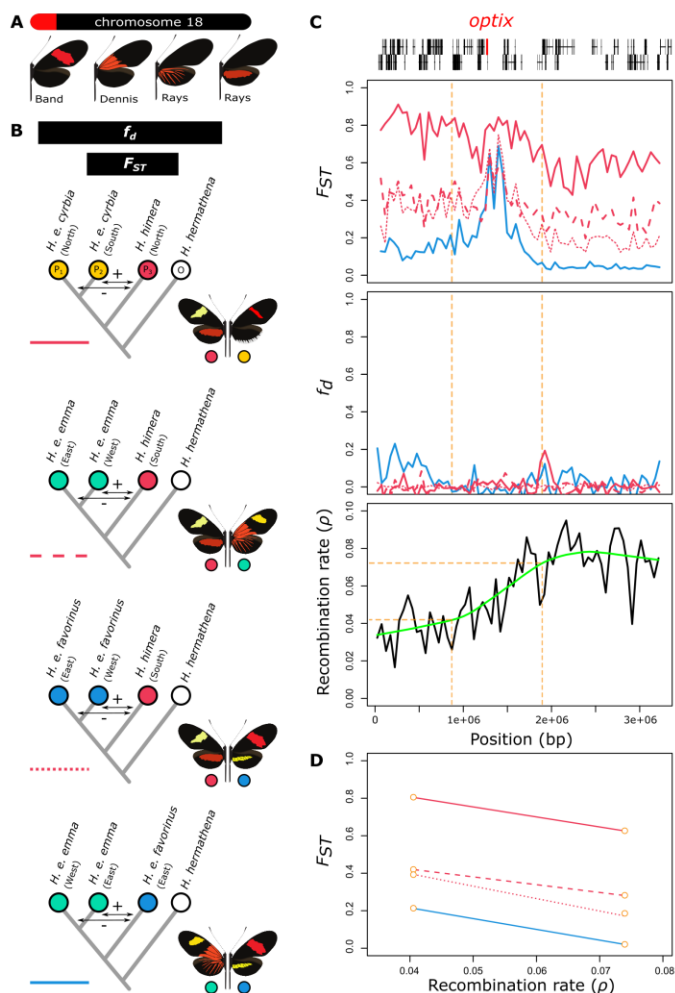
228 **Figure 4. Expected relationship of recombination rate with divergence (F_{ST}) and admixture (f_d) near a divergently selected**
 229 **locus. (A)** The population tree shows the simulated scenario with the onset of divergent selection on a derived allele indicated
 230 in red and in which migration rate (m) and migration time (t_m) between P2 and P3 and selection start time (t_s) are varied. Left
 231 and right of the simulated scenario are parameter combinations for two extreme scenarios that both include linked selection;
 232 on the left a scenario with Isolation with Migration (IM) and on the right a scenario reflecting Secondary Contact (SC). **(B)** Effect
 233 of population recombination rate (ρ) on relative divergence (F_{ST}) and admixture (f_d) near a divergently selected locus for the
 234 two simulated scenarios with parameter combinations as in panel A. The selected allele occurs at position 0. The dashed red
 235 line indicates a locus at 500 kb from the selected locus at which the relationship between ρ , divergence and admixture is
 236 assessed in panel C. **(C)** The effect of migration start time (t_m) and selection start time (t_s) on the relation between ρ , divergence
 237 and admixture. Apart from the respective parameters being evaluated, other parameters were fixed as in panel A, with the red
 238 lines indicating the exact parameter combinations as in panel A and B. For simulations with a lower selection coefficient ($s =$
 239 0.02), see Figure S5.

240

241 *Patterns of divergence are shaped by linked selection*

242 The differences in the expected relationships between recombination rate and F_{ST} and between
 243 recombination rate and f_d under the IM and SC scenarios provide specific predictions that we can use to

244 determine how linked selection may have shaped genomic divergence between *H. erato* and *H. himera*.
245 We first explored this relationship of recombination rate, relative divergence and admixture across the
246 *optix* locus using the expected patterns obtained from our simulations. The *optix* locus controls red wing
247 pattern differences among *H. erato* races, as well as between *H. erato* and *H. himera* and is a target of
248 strong selection (Figure 5A) [26,31,36]. We found recombination rate (ρ) estimates were markedly lower
249 upstream, compared to downstream of the *optix* gene (Figure 5C). Such sharp decreases in
250 recombination rate near chromosome ends have been observed in other *Heliconius* species [4] and likely
251 explain the decrease in recombination rate upstream of the *optix* gene. To test if selection at the *optix*
252 locus has produced the expected relationship of recombination rate with divergence and admixture, as
253 predicted above in our simulations (Figure 4C), we sampled sites 500 kb from the center of the peak of
254 the divergence at *optix* and plotted the recombination rate and divergence estimates for those sites
255 (Figure 5D). As expected from the scenario of SC with linked selection, we found negative relationships
256 between recombination rate and divergence (F_{ST}) and no correlations between recombination rate and
257 admixture (f_d). These findings demonstrate that our simulated patterns of recombination, divergence
258 and admixture, reflect real observed patterns in a region known to be under strong selection.



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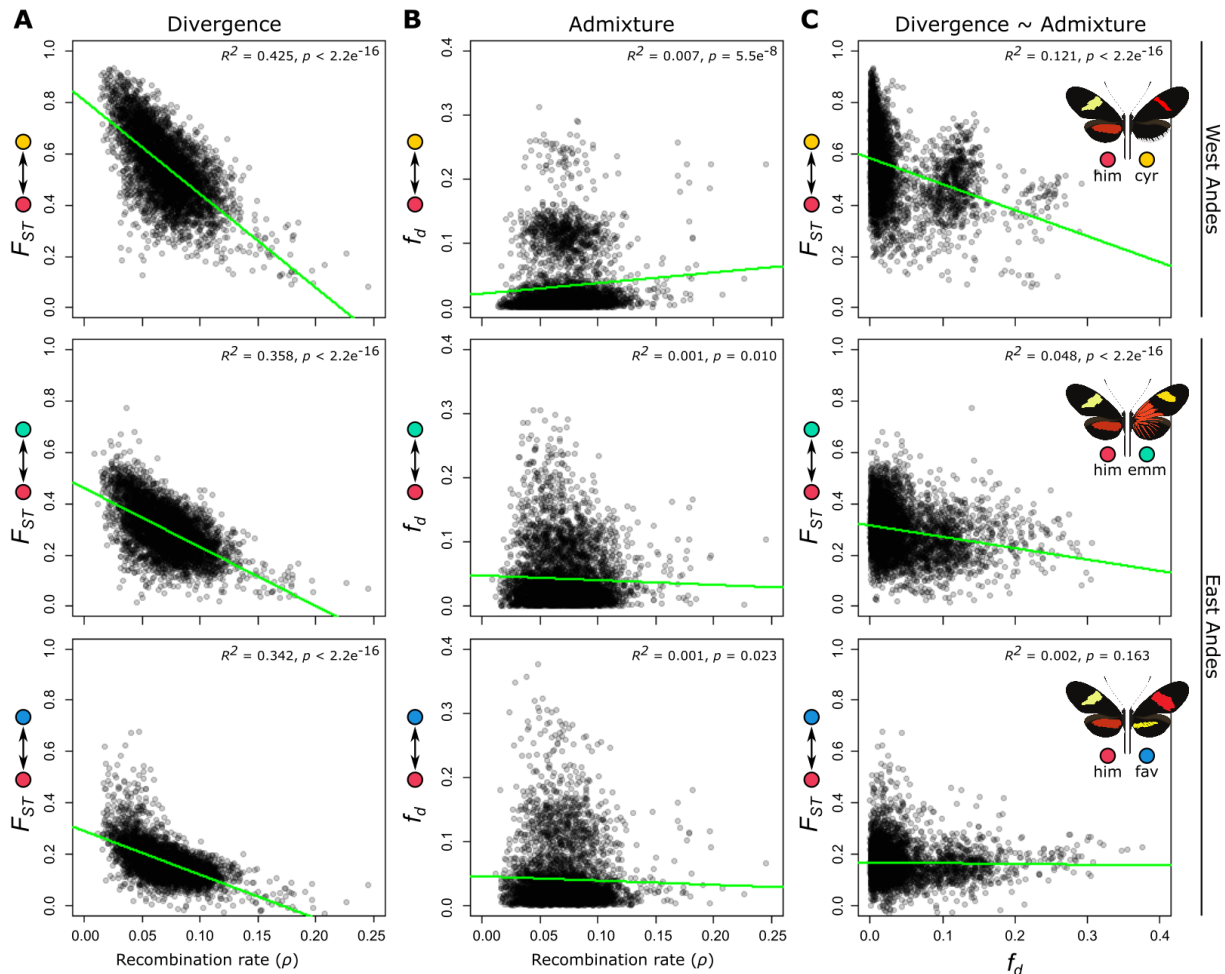
260 **Figure 5. Divergence (F_{ST}), admixture (f_d) and recombination rate (ρ) near the red color pattern gene *optix*.** (A) The *optix* gene
 261 is located near the start of chromosome 18 and has been demonstrated to control the expression of red color pattern elements
 262 in *Heliconius* wings [37]. (B) Relative divergence (F_{ST}) and admixture (f_d) comparisons performed between *H. himera*, *H. e.*
 263 *cyrba*, *H. e. emma* and *H. e. favorinus*. Colored circles match color codes in Figure 2. (C) Lines show F_{ST} , f_d and recombination
 264 rate (ρ) calculated in 50 kb non-overlapping windows. The green line in the bottom plot shows a loess fit of the recombination
 265 rate. Gene models including the location of the *optix* gene are presented at the top. (D) Relationship between F_{ST} and
 266 recombination rate (ρ) 500 kb left and right from the center of the *optix* regulatory sequence divergence peak.

267

268 To test for evidence that linked selection has driven genome-wide patterns of divergence, we
 269 compared recombination rates with divergence and admixture across the whole genome. We found a
 270 significant negative association between recombination rate (ρ) and relative divergence (F_{ST}) between *H.*
 271 *himera* and *H. erato* populations (Figure 6A) but no association with admixture (f_d) (Figure 6B). These
 272 genome-wide patterns are identical to those observed at the *optix* locus (Figure 5D) and the simulations
 273 of SC with linked selection (Figure 4C). Further, we observed a positive association between proportion
 274 of coding sequence and relative divergence, which again suggests the importance of linked selection for

275 genome divergence (Figure S6). Additionally, we found a significant relationship between F_{ST} and f_d in
 276 the *H. himera* – *H. erato* hybrid zones, which suggests that, although admixture can partly explain
 277 reduced F_{ST} (Figure 6C), the rates of migration have been too low or recent to build up a significant
 278 association with recombination rate. Finally, we note that the observed patterns of linked selection may
 279 include the effects of both genetic hitchhiking and background selection. While our genomic dataset
 280 does not allow us to differentiate between them, our simulations suggest that the observed patterns
 281 can be explained by genetic hitchhiking alone and other studies suggest background selection may be
 282 too subtle to cause these patterns [9].

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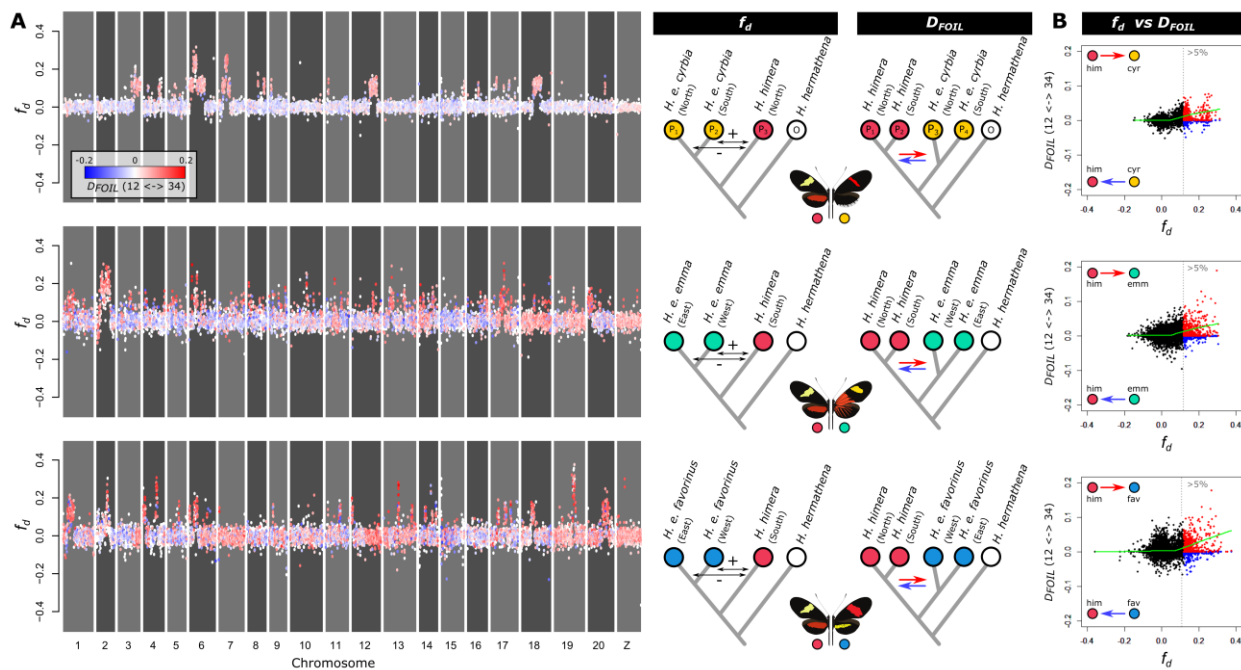
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285 **Figure 6. Correlations of divergence and admixture proportions with recombination rate in the three *H. himera* – *H. erato***
 286 **contact zones. (A)** Relative divergence (F_{ST}) versus recombination rate (ρ). **(B)** Admixture proportion (f_d) versus recombination
 287 rate (cM/Mb). **(C)** Relative divergence (F_{ST}) versus admixture proportion (f_d). Statistics were calculated in 50 kb non-overlapping
 288 windows. Recombination rates were calculated from each *H. erato* population separately and averaged over populations (see
 289 methods) and showed a genome-wide average of ρ equal to 0.071 (SD = 0.026; $\rho = 4N_e r$). Colored circles match geographic
 290 distributions and contact zones in Figure 2.

291 *Asymmetrical admixture suggests a polygenic species boundary*

292 The combination of empirical and simulation data suggests that periods of isolation and linked
293 selection within populations have played a major role in shaping the divergence landscape between *H.*
294 *erato* and *H. himera*. To explore how these factors influence the finer details of genomic divergence we
295 investigated patterns of admixture along chromosomes. We were both interested in the patterns of
296 admixture across our three replicate hybrid zones and inferring the direction of admixture. To
297 determine if patterns of admixture were similar across the three contact zones, we used a modified
298 four-taxon D -statistic called f_d [38]. Across several chromosomes we observed large blocks of increased
299 f_d , particularly in the hybrid zone between *H. himera* and *H. e. cyrbia* west of the Andes (see
300 chromosomes 3, 4, 6, 7, 8, 12 and 18 in Figure 7A). The large size of these admixture tracks indicates
301 that these signals are recent and there has not been sufficient time for recombination to break down
302 the haplotypes. In contrast, high admixture values (f_d) between *H. himera* and *H. e. emma* and *H. himera*
303 and *H. e. favorinus* east of the Andes are distributed more evenly along the chromosomes (Figure 7A),
304 which would agree with older admixture events between these populations. In general, there was a lack
305 of overlap between genomic regions with high f_d in the different hybrid zone comparisons further
306 reinforcing the idea that we are examining independent admixture events.

307 To determine the directionality of introgression across the genome we used a five-taxon D_{FOIL} -
308 statistic [39]. This test considers all available taxa (i.e. two populations of *H. himera* and two populations
309 of *H. erato*) and calculates all possible four-taxon D -statistic comparisons to infer admixture as well as
310 directionality of admixture. While the D_{FOIL} -statistic is calculated using a single genome for each taxon,
311 we performed this test on all possible combinations of available samples and represented the D_{FOIL} signal
312 as the proportion of significant comparisons (Figure 7A). Comparing f_d and D_{FOIL} results revealed that
313 among loci that show strong evidence of admixture, there is a relative paucity of loci in *H. himera*
314 individuals carrying *H. erato* alleles (Figure 7B). This asymmetry suggests *H. erato* is more porous to
315 introgressed alleles from *H. himera* than vice-versa. Consistent with a well characterized “large X(Z)
316 effect” in speciation, on the sex (Z) chromosome there are only a few loci that show signals of admixture
317 (high f_d) and again the D_{FOIL} tests only show evidence of introgression of *H. himera* alleles into *H. erato*
318 (Figure 7A).



319

320 **Figure 7. Admixture (f_d) and admixture directionality (D_{FOIL}) between *H. himera* and *H. erato* in three contact zones. (A)** Points
 321 show admixture (f_d) values, whereas coloring shows directionality (D_{FOIL}) in 50 kb non-overlapping windows for the contact
 322 zones *H. himera* – *H. e. cyrba* (top), *H. himera* – *H. e. emma* (middle) and *H. himera* – *H. e. favorinus* (bottom). Blue indicates
 323 predominant admixture from *H. erato* into *H. himera* (12 <- 34), whereas red indicates predominant admixture from *H. himera*
 324 into *H. erato* (12 -> 34) based on the D_{FOIL} tests. (B) Summary of admixture versus directionality with points above the 95%
 325 quantile indicated in blue (12 <- 34) and red (12 -> 34) demonstrates that the majority of windows with high f_d indicate
 326 admixture from *H. himera* into *H. erato*. The green line indicates a loess fit of the data. Colored circles match color codes in
 327 Figure 2.

328

329 We propose that the multitude of loci with asymmetrical gene flow may represent the genetic signal of
 330 a polygenic species barrier. These barriers could result from co-adapted loci in *H. himera* that cannot be
 331 replaced by *H. erato* alleles without fitness consequences. The inference that this pattern results from a
 332 polygenic species barrier is further strengthened by two observations. First, *H. himera* males have been
 333 shown to mate more frequently with F1 hybrids compared to *H. erato* males [25], which should result in
 334 an opposite asymmetric admixture pattern than we found [40], with more alleles introgressing from *H.*
 335 *erato* into *H. himera*. Second, the smaller effective population size estimates of *H. himera* should also
 336 result in an opposite pattern of asymmetric admixture [13], with more alleles introgressing from *H.*
 337 *erato* into *H. himera*. This latter expectation is observed in the demographic modeling results, which
 338 show greater migration of alleles from *H. erato* into *H. himera* and corresponds to their estimated
 339 differences in population size between the species (Figure 3B), but is not seen in the more recent signals
 340 of admixture as measured by f_d .

341 *Different histories can generate similar heterogeneous divergence patterns*

342 Although the genomic landscape of divergence between hybridizing taxa reflects the history of
343 selection and demographic changes they have experienced, different histories can generate strikingly
344 similar heterogeneous patterns. This can greatly limit our ability to make inferences about the
345 evolutionary processes driving genomic change [2]. For example, in *Heliconius melpomene* there are
346 strikingly similar heterogeneous landscapes of divergence to those we report here for *H. erato* and *H.*
347 *himera*, despite known differences in their evolutionary histories [41,42]. Here, we show that through a
348 comprehensive set of analytical approaches we can disentangle these evolutionary histories and reveal
349 that different evolutionary processes have resulted in similar divergence landscapes among the *H. erato*
350 and *H. melpomene* clades.

351 *Heliconius melpomene* and *H. erato* are co-mimics and experience similar strong selective
352 pressures on wing color patterns throughout their distributions. In the *H. melpomene* clade, *H.*
353 *melpomene* comes into contact and hybridizes with *H. cydno* in Panama and occasionally with *H.*
354 *timareta* in Ecuador and northern Peru. Although at first sight the genomic landscapes appear similar,
355 correlation analyses reveal differences in the relative importance of admixture. The *H. melpomene*
356 comparisons showed strong correlations of recombination rate with divergence as well as admixture,
357 which supports a longer history of divergent selection with gene flow (Martin et al. 2019). In contrast, *H.*
358 *himera* and *H. erato* comparisons showed recombination rate correlated with divergence, but not
359 admixture. We suggest the lack of correlation may be because the secondary contact is recent and the
360 rates of gene flow are low between *H. erato* and *H. himera*. Thus, there has not been enough time for a
361 correlation between recombination rate and admixture to arise. Instead, we argue that differences
362 accumulated in periods of isolation have had more profound effects on the divergence landscape of *H.*
363 *erato* and *H. himera* whereas gene flow has likely been more continuous throughout the divergence
364 history of the young species pair in the *H. melpomene* clade. These differences in the history of
365 divergence among co-mimetic species highlights the power of approaches like those applied here to
366 resolve the roles of different evolutionary processes in generating seemingly similar heterogeneous
367 patterns of genomic divergence.

368

369

370 *Conclusions*

371 We observed genome-wide increase of divergence as reproductive barriers increase between
372 hybridizing populations or species. Nonetheless, it remains difficult to determine if peaks of divergence
373 result from barrier loci that are resistant to ongoing gene flow (heterogeneous gene flow), from recent
374 selective sweeps in isolated populations (heterogeneous selection), or both. Fortunately, using a
375 combination of demographic modeling, simulations, and correlation analyses we can characterize the
376 evolutionary processes responsible for the heterogeneous landscapes of divergence. For the incipient
377 species *H. erato* and *H. himera*, our results suggest a disproportionately large effect of the Z
378 chromosome on the evolution of species barriers in *H. erato*. The data also favor a scenario of periods of
379 isolation accompanied by both selection and gene flow. The overall patterns of asymmetric admixture
380 suggest that during periods of isolation, selection at multiple loci may have resulted in a polygenic
381 species boundary. This finding adds to a number of studies that illustrate how fluctuating gene flow and
382 pervasive selection along the genome lead to the evolution of polygenic architectures of species
383 boundaries [4,8,21,43,44].

384 **Methods**

385 *Sampling*

386 We obtained whole genome resequence data for a total of 122 *Heliconius* butterflies (Tables S4).
387 These include northern (North Ecuador, n = 10) and southern (South Ecuador *H. himera* contact zone, n
388 = 4) *H. e. cyrbia*, western (Peru *H. himera* contact zone, n = 4) and eastern (Peru *H. e. favorinus* contact
389 zone, n = 7) *H. e. emma* and western (Peru *H. himera* contact zone, n = 4) and eastern (Peru *H. e. emma*
390 contact zone, n = 8) *H. e. favorinus* used to study admixture patterns with northern (Ecuador *H. e.*
391 *emma/favorinus* contact zone, n = 5) and southern *H. himera* (Peru *H. e. cyrbia* contact zone, n = 4).
392 Additionally, samples from *H. e. petiverana* (Mexico, n = 5), *H. e. demophoon* (Panama, n = 10), *H. e.*
393 *hy dara* (Panama, n = 6 and French Guiana, n = 5), *H. e. erato* (French Guiana, n = 6), *H. e. amalfreda*
394 (Suriname, n = 5), *H. e. notabilis* (Ecuador *Heliconius erato lativitta* contact zone, n = 5 and Ecuador *H. e.*
395 *etylus* contact zone, n = 5), *H. e. etylus* (Ecuador, n = 5), *H. e. lativitta* (Ecuador, n = 5), *H. e. phyllis*
396 (Bolivia, n = 4), *H. e. venus* (Colombia, n = 5) and *H. e. chestertonii* (Colombia, n = 7) were used for
397 contact zone divergence analysis and population structure visualization as well as samples of *H.*
398 *hermathena* (Brazil, n = 3) as an outgroup to root the phylogenetic inference and polarize site frequency
399 spectra. All data have been previously published [26,28], apart from the ten *H. e. cyrbia* from North
400 Ecuador.

401

402 *Sequencing and genotyping*

403 Genotypes were obtained as in [28]. In short, whole-genome 100-bp paired-end Illumina
404 resequencing data from *H. erato* samples were aligned to the v1 [26] reference genome, using BWA v0.7
405 [45]. PCR duplicated reads were removed using PICARD v1.138 (<http://picard.sourceforge.net>) and
406 sorted using SAMTOOLS [46]. Genotypes were called using the genome analysis tool kit (GATK)
407 Haplotypecaller [47]. Individual genomic VCF records (gVCF) were jointly genotyped using GATK's
408 genotype GVCFs. Genotype calls were only considered in downstream analysis if they had a minimum
409 depth (DP) ≥ 10 , maximum depth (DP) ≤ 100 (to avoid false SNPs due to mapping in repetitive regions),
410 and for variant calls, a minimum genotype quality (GQ) ≥ 30 . Specific data filtering steps for running
411 population structure, phylogenetic and demographic analysis are explained in the respective sections.

412 *Population structure and phylogenetic relationships*

413 We estimated levels of relative divergence (F_{ST}) [48] between populations in nonoverlapping 50
414 kb windows using python scripts and egglib [49]. For this analysis, we only considered windows for
415 which at least 10% of the positions were genotyped for at least 75% of the individuals within each
416 population. On average 96% of windows met this criterium. To discern population structure among the
417 *H. erato* and individuals, we performed principal component analysis (PCA) using EIGENSTRAT SmartPCA
418 [50]. For this analysis, we only considered autosomal biallelic sites that had coverage in all individuals
419 and excluding the Z chromosome (5,058,785 SNPs). Using the same filtering but including the outgroup
420 *H. hermathena* (4,927,152 SNPs), we used FastTree v2.1 [51] to infer an approximate maximum
421 likelihood phylogeny using the default parameters.

422

423 *Demographic modeling*

424 We performed demographic analyses on the joint site-frequency spectra (JSFS) of five
425 population comparisons using a modified version of *dad*i v1.7 [11]. Genotype calls were filtered for
426 biallelic autosomal SNPs with a threshold of at least 50 % minimum genotype calls for each population
427 of interest. In order to ensure demographic analyses were performed on unlinked loci, we subsampled
428 our data so that a single SNP was selected at least every ~1000 bp (based on linkage disequilibrium maps
429 from [52] in Figure S1). Unfolded joint site-frequency spectra (JSFS) were created from the filtered calls
430 data using *H. hermathena* as an outgroup, including on average 316090.3 SNPS (Table S3). The
431 proportion of accurate SNP orientation (O) was consistent across all pairwise comparisons (~97 %),
432 which suggests that ancestral state identification was correct for the majority of SNPs using *H.*
433 *hermathena* as an outgroup.

434 Models tested include four basic scenarios in addition to 22 extensions of the basic models that
435 allowed for independent assessment of additional selective and demographic parameters [32] (Table
436 S5). Basic model scenarios included strict isolation (SI), ancient migration (AM), isolation-with-migration
437 (IM), and secondary contact (SC). The standard four models involved the divergence of an ancestral
438 population (N_{ref}) at t generations into two resulting daughter populations with an effective size of N_1 and
439 N_2 , respectively. Migration occurred in IM, AM, and SC at rate m_{12} from population 2 into population 1
440 and m_{21} in the opposite direction. Model extensions included growth rate parameters (g) which account
441 for changes in the effective population size over time (bottlenecks and expansions) by taking a ratio of
442 the contemporary and ancient population sizes. Note that *dad*i cannot infer both heterogeneous

443 migration and genetic drift when gene flow is not temporally localized (i.e. IM model). To capture the
444 effects of linked selection that are due to sweeps and background selection (Hill and Robertson, 1966;
445 Maynard Smith and Haigh, 1974; Charlesworth et al, 1993), further model extensions incorporated two
446 categories of loci ($2N$) that occur in proportions of Q and $1-Q$ in order to account for heterogeneity in N_e
447 across the genome. A Hill-Robertson scaling factor (*hrf*) was included with these models that relates the
448 effective size of loci experiencing selection to that of neutral loci. Models that infer migration (IM, AM,
449 SC) were extended to include parameters that capture heterogeneous migration rates ($2m$) across the
450 genome resulting from selection on barrier loci during adaptive divergence [16]. These extensions
451 allowed for the estimation of a proportion of loci (P) experiencing standard migration rates (m_{12} and m_{21})
452 and a second category of loci ($1-P$) undergoing rates me_{12} and me_{21} . Given that the JSFS was polarized
453 using *H. hermathena* as the outgroup for all comparisons, a SNP orientation parameter (O) was included
454 in all models to account for ancestral state misidentification. Additionally, the effective mutation rate (ϑ)
455 of N_{ref} was estimated as a free parameter in all comparisons.

456 To check for model convergence, a total of 20 independent optimizations were performed for
457 each model on each population comparison. When running these models, consistency in the likelihood
458 scores generally increased as the best optimized parameters from previous steps were incorporated into
459 subsequent steps. To score the models and account for overparameterization, the Akaike Information
460 Criterion (AIC: Burnham and Anderson 2004) was used and parameters from the top five scoring runs for
461 each model were averaged. The models with the best average AIC score for each comparison were
462 retained for each comparison. The highest and lowest optimized parameter values in the top five
463 replicate runs for each model were used to construct intervals to estimate uncertainty.

464 Model parameter estimates for effective population sizes, migration rates and divergence times
465 were transformed into absolute units using a *Heliconius* mutation rate of 2×10^{-9} per generation (i.e.
466 spontaneous *Heliconius* mutation rate corrected for selective constraint; Keightley et al. 2014; Martin,
467 Eriksson, et al. 2015) and assuming a generation time of 0.25 years. Ancestral effective population size
468 was calculated using the optimized theta value for each model comparison ($N_{ref} = \vartheta/4\mu L$), where L
469 represents the effective sequence length and μ the estimate of the mutation rate. Effective sequence
470 length for each pairwise comparison is estimated as $L = (x/y)z$, where x is the number of SNPs used in the
471 ∂adi demographic analysis and y is the number of segregating sites detected in the original sample out
472 of z total sites. Migration rates are presented in units of $2N_{ref}$ to represent the number of individuals per
473 generation that migrate into each population.

474 *Divergence and admixture simulations*

475 To compare patterns in our data to expectations, we simulated genealogies near a selected
476 locus. Genealogies were simulated using the coalescent simulator *msms* [56] and from these
477 genealogies 10 kb sequences were simulated with a mutation rate of $2e^{-9}$ (i.e. spontaneous *Heliconius*
478 mutation rate corrected for selective constraint; Keightley et al. 2014; Martin, Eriksson, et al. 2015) and
479 a Hasegawa–Kishino–Yano (HKY) substitution model using *seq-gen* v1.3.4 [57]. With *msms*, we simulated
480 four populations with a split history given by ((P1, P2), P3), O where t_1 , t_2 and t_3 denote the split times
481 between P1 and P2, (P1, P2) and P3 and ((P1, P2), P3) and O, respectively (Figure 4A). Population size
482 (N_e) was fixed to 1,000,000 individuals and t_2 and t_3 were fixed to 0.5 (2,000,000 generations) and 1
483 (4,000,000 generations) coalescent units ($4N_e$), respectively. Within the range of selection on color
484 pattern in *Heliconius* [58], we simulated divergent selection at a single locus with selection coefficients
485 (s) of 0.2 and 0.02 for homozygous genotypes and 0.1 and 0.01 for heterozygous genotypes and
486 selection strength specified in units of $2N_e s$. Selection was set to work in opposite direction for P1 and
487 P2 versus P3 and O and was set to start at time t_s . After population splits, migration (m) was restricted
488 between P2 and P3 only, with symmetrical migration rates and a start time equal to t_m . In relevance to
489 our demographic modeling results, we ran simulations by varying the parameters t_s (selection start time;
490 $2e^3$ - $2e^6$ generations), t_m (migration start time; $2.5e^{-5}$ - $5e^{-1}$ generations), m (migration rate; $1e^{-7}$ - $1e^{-5}$
491 generations) and ρ (population recombination rate $4N_e r$; r = probability of recombination per generation
492 per bp; $6.25e^{-4}$ - $8e^{-2}$). A maximum ρ of $8e^{-2}$ was used for computational feasibility. The genealogies were
493 sampled at 100 kb increments from the selected locus. This was achieved by using an infinite
494 recombination sites model and changing the position of the selected locus in increments of 10 neutral
495 locus units (i.e. 10 x 10 kb) away from the sampled locus. Divergence (F_{ST}) was calculated as in Hudson *et*
496 *al.* 1992 and admixture (f_d) was calculated as in Martin *et al.* 2016 using python scripts and *egglib* v3 (De
497 Mita & Siol, 2012). We investigated the correlation between F_{ST} , f_d and recombination rate at a distance
498 of 500 kb from a selected locus but similar expectations are obtained from wide range of distances to
499 the selected locus. Simulations were run with 100 replicates for each parameter combination.
500 Pseudocode to run the *msms* command lines are provided in Tables S6.

501

502 *Recombination rate estimates*

503 We estimated fine-scale variation in population recombination rate ($\rho = 4N_e r$; r = probability of
504 recombination per generation per bp) along the *H. erato* chromosomes from linkage-disequilibrium in

505 population genetic data using *LDhelmet* v1.7 [59]. We phased quality filtered genotypes from thirteen *H.*
506 *erato* populations (i.e. *H. e. cyrba*, *H. e. venus*, *H. e. demophoon*, *H. e. hydara* (Panama), *H. e. emma*, *H.*
507 *e. etylus*, *H. e. lativitta*, *H. e. notabilis*, *H. e. favorinus*, *H. e. phyllis*, *H. e. erato*, *H. e. hydara* (French
508 Guiana) and *H. e. amalfreda*) using Beagle v4.1 [60] with default parameters. From the phased
509 genotypes, fasta sequences were generated for 50 kb windows. These 50 kb windows were transformed
510 to haplotype configuration files with the recommended window size of 50 SNPs used by *LDhelmet* to
511 estimate composite likelihoods of the recombination rate. From the haplotype configuration files,
512 lookup tables for two-locus pairwise recombination likelihoods and Padé coefficients were generated
513 within the recommended value range. Transition matrices were calculated for each chromosome
514 separately by comparing genotypes obtained from *H. erato demophoon* to the outgroup species *H.*
515 *hermathena*. The likelihood lookup tables, Padé coefficients and transition matrices were used in the
516 rjMCMC procedure of *LDhelmet* to estimate the recombination map. In this latter step, 1000,000
517 Markov chain iterations were run with a burn-in of 100,000 iterations, a window size of 50 SNPs and
518 block penalty of 50. To reduce the potential effect of locus-specific changes in effective population size
519 (N_e) on population recombination rate (ρ) estimates (e.g. due to population specific selective sweeps or
520 background selection), we estimated ρ for each *H. erato* population separately and obtained averages
521 for each 50 kb interval.

522

523 *Admixture statistics*

524 We estimated admixture proportions for 50 kb non-overlapping windows using the f_d statistic
525 [38]. This statistic is based on the ABBA-BABA test or Patterson's D statistic which measures an excess of
526 derived allele sharing between sympatric non-sister taxa [61]. This excess is tested by comparing the
527 relative abundance of SNP patterns termed ABBAs and BABAs in a tree of three populations and an
528 outgroup with the relationship (((P1, P2), P3), O). ABBAs are sites where a derived allele is shared
529 between P2 and P3, whereas BABAs are sites where a derived allele is shared between P1 and P3. Under
530 a neutral coalescent model, such sites are only expected to be found due to incomplete lineage sorting
531 or recurrent mutation and a D statistic of 0 is expected. In the presence of admixture, however, an
532 excess of either ABBAs or BABAs can be observed and a D statistic that significantly deviates from 0 may
533 be obtained. The f_d statistic is derived from the D statistic by calculating the difference between ABBA
534 and BABA sites and normalizing this difference by a scenario of complete admixture. The estimator is
535 dynamic in that for the complete admixture scenario used to normalize, a donor population for the

536 admixture is chosen with the highest frequency of the derived site. The resulting normalized measure is
537 approximately proportional to the effective migration rate and has been evaluated not to be
538 confounded by locus-specific changes in effective population size due to background selection or
539 reductions in diversity due to selective sweeps [4,38]. The populations included as P1, P2, P3 and O are
540 indicated in the figures. *Heliconius hermathena* samples were consistently used as the outgroup taxa.

541

542 *Admixture directionality*

543 By expanding the four-taxon D statistic to a five-taxon scenario, it is possible to obtain
544 information on the directionality of admixture (i.e. donor versus recipient population). A set of statistical
545 measures that use a five-taxon symmetric phylogeny to infer both the taxa involved in and the direction
546 of admixture are called the D_{FOIL} statistics [39]. The D_{FOIL} statistics identify taxa involved in admixture by
547 performing four possible D tests with different combinations of three ingroup taxa within a five-taxon
548 phylogeny defined as (((P1, P2), (P3, P4)), O). These four D tests considered collectively can provide
549 information on the directionality of admixture. This is because admixture does not only change the
550 position of the donor sample in the topology but will also change the relationship of the donor's sister
551 taxon to the other taxa in the phylogeny. For instance, if admixture occurs from P2 into P3, the sampled
552 topology becomes (((P2, P3), P1), P4), O) and P1 will group more closely to (P2, P3) because of more
553 recent sharing of variation with P2, whereas if admixture occurs from P3 into P2, the topology becomes
554 (((P2, P3), P4), P1), O). For the latter instance, this will be reflected by a similar sign of the D test
555 statistics that include (((P1, P2), P3), O) or (((P1, P2), P4), O) but a different sign for the D test that
556 includes (((P3, P4), P2), O) and no significant D test for (((P3, P4), P1), O). Hence, by comparing the
557 combinations of different signs (+, -, or 0) of the four D tests within the five-taxon topology, the
558 directionality of admixture can be assessed [39].

559 We assessed directionality of admixture in 50 kb non-overlapping windows in the three *H.*
560 *himera* contact zones with *H. erato* populations using the D_{FOIL} tests explained above using the available
561 *dfoil* software (www.github.com/jbpease/dfoil). Samples from the *H. himera* North and South
562 populations were specified as the P1 and P2 group, whereas samples from the considered *H. erato*
563 populations were specified as P3 and P4. *Heliconius hermathena* was used as the outgroup taxon (ID
564 hermathena_13 in Table S4). The D_{FOIL} statistics were calculated between each possible combination of
565 available ingroup taxa (i.e. one sample for each taxon group); 800 combinations for *H. himera* – *H. e.*
566 *cyrba*, 500 for *H. himera* – *H. e. emma* and 480 for *H. himera* – *H. e. favorinus*. Among these sample

567 combinations, significant D_{FOIL} signatures (χ^2 goodness-of-fit test) were counted and used to obtain
568 heterogeneous patterns of admixture directionality along the genome.

569

570 **Data accessibility**

571 For GenBank accession numbers of whole genome resequence data see Table S4.

572

573 **Author contributions**

574 The study was conceived and designed by SVB and BAC in collaboration with RP and WOM.
575 Genomic analyses were performed by SVB and JC. Demographic modeling with *dadl* was conducted by
576 JC. Samples of *H. e. cyrbia* from northern Ecuador were contributed by GMK, and CB assisted with
577 permits. RP, GMK and WOM provided input on results and manuscript preparation. The manuscript was
578 written and figures were made by SVB, JC, and BAC.

579

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592

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