# Demographically explicit scans for barriers to gene flow using gIMble

Dominik R Laetsch[a,†] , Gertjan Bisschop[a,†] , Simon H Martin[a], Simon Aeschbacher[b], Derek Setter[a] and Konrad Lohse[a,*]

**a Institute of Evolutionary Biology, University of Edinburgh, EH9 3FL Edinburgh, Scotland**
**b Department of Evolutionary Biology and Environmental Studies, University of Zurich, Winterthurerstrasse 190, CH-8057 Zurich, Switzerland**

∗ **Corresponding author: konrad.lohse@ed.ac.uk**
† **these authors contributed equally**

## Abstract

Identifying regions of the genome that act as barriers to gene flow between recently diverged taxa has remained challenging given the many evolutionary forces that generate variation in genetic diversity and divergence along the genome, and the stochastic nature of this variation. Here we implement a composite likelihood approach for the quantification of barriers to gene flow. This analytic framework captures background selection and selection against locally maladaptive alleles (i.e. genomic barriers) in a model of isolation with migration (IM) as heterogeneity in effective population size ($N_e$) and effective migration rate ($m_e$), respectively. Variation in both effective demographic parameters is estimated in sliding windows via pre-computed likelihood grids. We have implemented genomewide IM blockwise likelihood estimation (`gIMble`) as a modular tool, which includes modules for pre-processing/filtering of genomic data and performing parametric bootstraps using coalescent simulations. To demonstrate the new approach, we analyse data from a well-studied sister species pair of tropical butterflies with a known history of post-divergence gene flow: *Heliconius melpomene* and *H. cydno*. Our analysis uncovers both large effect barrier loci (including well-known wing-pattern genes) and a genome-wide signal of polygenic barrier architecture.

## Author summary

As a fundamental process generating biological diversity, speciation involves the evolution of reproductive isolation and thus the build-up of barriers to genetic exchange among organismal groups. While population genomic data are arguable the only source of information we have about most recent speciation events, the way such data are analysed remains depressingly superficial: population genomic studies of speciation are phrased either as scans for outliers of genetic differentiation, or are based on models of neutral evolution under the constraint of a single genome-wide demography. Here we introduce a new statistical framework called gIMble to estimate the effective rate of gene flow and the effective population sizes along the genome from population genomic data. By capturing genome-wide variation in these two effective demographic parameters, gIMble disentangles the genomic footprints of different modes of selection and provides a direct quantification of the species barrier. To illustrate the application of this framework, we analyse a classic speciation genomic dataset from Heliconius butterflies and show that barriers to gene flow in this system include both large effect loci – most, but not all, of which were known from functional work – as well as a genome-wide signature of weak-effect polygenic barriers.

## Introduction

How reproductive isolation between species builds up at the genomic level remains a major question in evolutionary biology [1]. Population genomic studies inferring explicit demographic models of speciation for an increasing number of taxa suggest that species divergence in the face of gene flow is common. This poses a conundrum: gene flow homogenizes populations and — in the presence of recombination — leads to the breakdown of co-adapted combinations of alleles, which directly opposes divergent selection [2]. However, divergent selection for locally beneficial alleles, if sufficiently strong, may overcome the influx of maladapted migrant alleles [3, 4] at specific loci in the genome. It has been suggested that such barrier loci of large effect may act as nuclei around which further barrier loci can accumulate [5, 6]. The clustered genetic differentiation that may result from this feedback between migration, local selection and linkage has been referred to using metaphors of "speciation islands" [7] or "genomic islands of divergence" [6, 8]. Despite the fact that we now have access to vast volumes of whole genome sequence (WGS) data, it remains an open question whether the clustered genomic barriers envisaged by these verbal models are important in generating and maintaining new species in nature and, if so, how exactly they arise. More generally, it remains unclear how often species barriers are due to a few loci of large effect and how often barriers have a highly polygenic architecture. Progress on these questions has been stymied by a lack of methods that can extract the relevant information about the population processes involved in the build up

of barriers from WGS data. Current speciation-genomic inference approaches can be classed into three broad categories [9]: **summary statistics scans**, **demographic inference**, and **analyses of the relationship between recombination rate and divergence**. These have proven to be challenging to connect.

### Summary statistics scans

The search for speciation islands has led to an industry of genomic outlier scans that initially interpreted regions of increased differentiation between species as "speciation islands" [7, 8, 10, 11]. Outlier scans are generally based on summary statistics, in particular differentiation as measured by $F_{\mathrm{ST}}$ [1, 12]. However, despite their conceptual appeal, genome scans in general and $F_{\mathrm{ST}}$ scans in particular have been criticized on several grounds. Since $F_{\mathrm{ST}}$ is a relative measure of divergence, it is sensitive to any force that locally reduces diversity. Thus, even in the absence of gene flow, $F_{\mathrm{ST}}$ may be increased simply as a result of past positive selection [13, 14] and/or background selection (BGS) [13, 15] on linked sites [16, 17]. In fact, outlier scans trace back to Lewontin and Krakauer [18] who proposed a test for divergent selection based on the variance in $F_{\mathrm{ST}}$. While a number of other summary statistics have been developed to overcome the limitations of $F_{\mathrm{ST}}$ [19, 20], the fundamental problem of any scan based on a one-dimensional summary statistic is that it cannot distinguish between the alternative population genetic processes we wish to learn about [14, 21, 22]. In other words, it is unclear how often one expects outliers in a summary statistic to arise simply by chance due to genetic drift or under scenarios that have nothing to do with the establishment of barriers to gene flow. As a result, distinguishing interesting outliers from noise has remained a major challenge [22].

### Model-based demographic inference

An arguably more successful line of research has been to use genome data to reconstruct the demographic history of speciation. A range of inference methods exist that fit more or less complex models of demographic history to whole-genome data (see [23–26] for reviews). An important starting point for quantifying gene flow during speciation has been the Isolation with Migration (IM) model [27] which assumes a constant rate of gene flow from the time of divergence until the present. Estimates of gene flow under the IM and related models have been obtained for many recently diverged species and have led to a reappraisal of gene flow as a ubiquitous ingredient of the speciation process [28, 29].

### Modelling the relationship between recombination and divergence

A third type of speciation genomic analysis, which has so far only been applied to a small number of taxa, exploits the genome-wide relationship between genetic divergence and recombination to extract information about the selection on and density of barrier loci [9]. In contrast to the positive correlation between genetic diversity and recombination expected under background selection [15] (and globally positive selection), divergent selection against locally maladapted alleles results in a negative correlation between genetic divergence and recombination [30]. This prediction is based on theory showing that selection against deleterious immigrant alleles reduces the effective rate of gene flow at linked neutral sites [2] below the rate $m$ at neutral sites that are not affected by barriers. The extent of this reduction can be captured by the effective migration rate $m_e$, which decreases with the strength of selection and increases with the map distance from selective targets [2, 31]. Integrating over the unknown genomic positions of barrier loci and conditioning on a recombination map, Aeschbacher et al. [9] developed an approach for jointly estimating the aggregate strength of selection against immigrant alleles in the genome, the divergence time, and the baseline migration rate. However, although this method uses the expected correlation between recombination and genetic divergence under a demographically explicit model, it infers genome-wide parameters and does not allow scanning for barriers to gene flow.

## Demographically explicit genome scans

Given the current state of inference approaches, speciation genomic analyses involve an awkward choice: one either infers a demographic history, which — however detailed — is uninformative about which genomic regions may act as barriers. Alternatively, one may visualize the heterogeneity in differentiation along the genome via summary statistics, which partially sacrifices the ability to learn about the population processes that have given rise to this heterogeneity.

Ideally, we would like to reconstruct a fully specified model of the speciation process, including both the demographic history — parameterized in terms of genetic drift, divergence, and gene flow — as well as all relevant selective forces, i.e. a plausible null model *sensu* [32]. Indeed, since most selection is not likely related to the emergence of reproductive barriers [33], even a very simple cartoon of the speciation process would have to capture both selection on variation that is globally beneficial or deleterious, and local selection that establishes and maintains barriers to gene flow. Unfortunately, it is currently impossible to infer such a detailed model from genome data.

A simpler approach is to model the selective processes that lead to the establishment of barriers to gene flow via heterogeneity in effective migration rate $m_e$. This approach has been pioneered by Roux et al. [34] and has been implemented as an approximate Bayesian computation framework [35] that allows fitting models of heterogeneous gene flow to genome-wide data. However, while this

method estimates variation in $m_e$ (and $N_e$) via aggregate genome-wide distributions, it does not allow inference of local $m_e$ along the genome.

The motivation of the present study is to fill this gap and develop an alternative framework based on likelihood calculation that allows interpreting the heterogeneity of divergence and diversity along the genome through the lens of an explicit, albeit necessarily simple, demographic history. We approximate the effects of background selection (BGS) and selection against migrants as variation in effective population size ($N_e$) and effective migration rate ($m_e$), respectively. Our method infers genome-wide histories of isolation with migration (IM) using a block-wise likelihood calculation. The composite likelihood (CL) calculation at the heart of our approach is based on previous analytic work [36–38] and is implemented as part of a modular, open source bioinformatic toolset: genome-wide IM blockwise likelihood estimation `gIMble` v.1.0.3 (https://github.com/LohseLab/gIMble). `gIMble` allows for an efficient inference of $m_e$ and $N_e$ in windows along the genome as a scan for barriers to gene flow, includes algorithms for the required partitioning, filtering and summarizing of genomic variation, and provides a simulation module for generating parametric bootstraps using the coalescent simulator `msprime` [39].

## *Heliconius melpomene* and *H. cydno* as a test case

Throughout the paper, we describe our approach for demographically explicit scans for barriers to gene flow by way of a re-analysis of data from two sister species of *Heliconius* butterflies: *H. melpomene* and *H. cydno*. This classic model of speciation research provides an excellent test case for our approach given that the genome-wide heterogeneity in divergence and diversity has been studied extensively in this species pair [40–46] and the genetic basis of key barrier traits (wing patterns and preference for them) is known [41–43, 47]. Despite strong behavioural, ecological, and postzygotic isolation (female hybrid sterility), there is overwhelming evidence for ongoing hybridisation and gene flow between these species that affects a large proportion of the genome [37, 42]. Martin et al. [42] found that $F_{\mathrm{ST}}$ between *H. melpomene* and *H. cydno* is highly heterogeneous along the genome, but concluded that this heterogeneity does not provide direct evidence of barriers to introgression. Indeed, $F_{\mathrm{ST}}$ between *H. melpomene* and *H. cydno* is similarly heterogeneous and strongly correlated in both sympatric and allopatic comparisons, despite the fact that allopatric populations are thought to experience no contemporary gene flow [42]. Many $F_{\mathrm{ST}}$ outliers between *H. melpomene* and *H. cydno* may therefore simply reflect increased rates of within-species coalescence [14]. Using this well studied *Heliconius* system set as a test case, we showcase `gIMble` and address the following questions:

1. What fraction of the genome likely acts as a complete barrier ($m_e = 0$) between these taxa?

2. What is the overlap/correlation between barriers defined via $m_e$ and $F_{\mathrm{ST}}$ outliers?
3. Do $m_e$ barriers include known true positives, i.e. major-effect loci controlling phenotypes involved in reproductive isolation?
4. Is there a genome wide relationship between $m_e$ and the rate of recombination, as predicted by models that assume polygenic barriers?

# Results

## The inference problem

The framework implemented in `gIMble` models the genomic landscape of speciation by constructing scans of two kinds of effective demographic parameters: background selection (BGS) is captured via heterogeneity in $N_e$, and barriers to gene flow are modeled via heterogeneity in $m_e$. Unlike previous approaches [9] which rely on the relationship between genetic variation and recombination, our inference is based on sequence variation alone and does not require knowledge of the recombination map.

We focus on an IM history between two species A and B. We assume divergence at a time $T$ (measured in generations) and a constant probability of unidirectional migration, i.e. lineages sampled in species A may trace back to B (backward in time) via migration at rate $m_e$. Effective population sizes are allowed to differ between species, i.e. the most complex model assumes three different $N_e$ parameters: $N_A$, $N_B$ and $N_{\mathrm{anc}}$.

One could in principle co-estimate all five parameters of the IM model ($N_A$, $N_B$, $N_{anc}$, $m_e$, $T$) in sliding windows along the genome. However, in practice, this maximally complex model in which all parameters vary freely along the genome is both hard to infer — given that IM parameters are known to have correlated errors [27] – and difficult to interpret. Thus, we conduct a two-step inference (Fig 1): first, we identify the best-fitting *global* IM history which assumes no heterogeneity in demographic parameters along the genome. In a second step, *local* variation in the $N_e$ and $m_e$ parameters is estimated in sliding windows along the genome. This inference is conditioned on the $T$ estimate under the global model, i.e. we assume that the onset of species divergence is a global event that is shared by the whole genome.

## Implementation

The basic unit of data in the inference implemented in `gIMble` is a block of sequence of a fixed length in which we record sequence variation in a heterospecific pair of individuals. We will refer to this as a "pair-block". An important property of our blocking algorithm is that pair-blocks

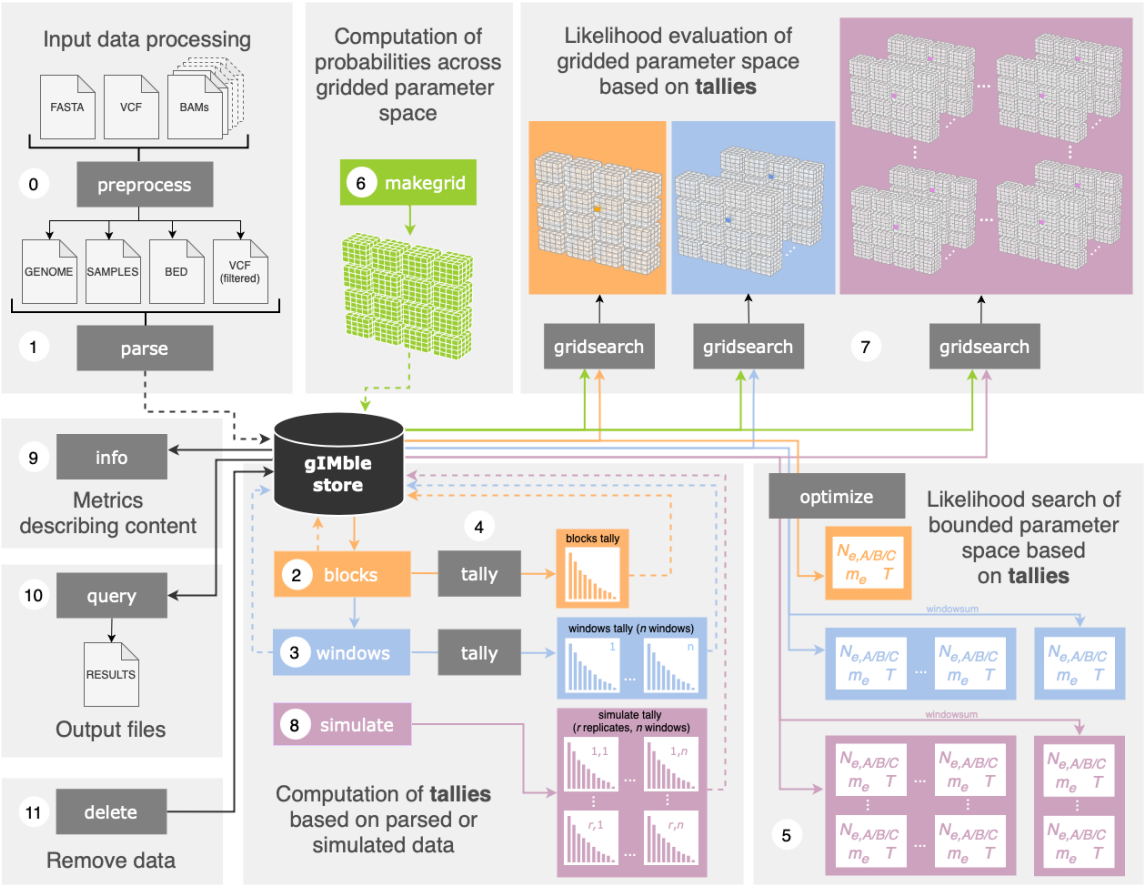



**Fig 1.** `gIMble` workflow. Numbers correspond to the order of steps in the *Heliconius* analysis: `preprocess` (0) assures input data meet standard filters; `parse` (1) reads data into a `gIMble` store, the central data structure that holds all subsequent analysis. The modules `blocks` (2) and `windows` (3) partition the data. The data are then summarised as a `tally` (4) of blockwise mutation configurations (bSFSs) either across all pair-blocks (**blocks tally**) or for pair-blocks in windows (**windows tally**). Tallies may be used either in a bounded search of parameter space via the module `optimize` (5) or to evaluate likelihoods over a parameter grid (which is precomputed using `makegrid`, 6) via the `gridsearch` (7) module. The `simulate` (8) module allows coalescent simulation of tallies (**simulate tally**) based on inferred parameter estimates (either global estimates or gridsearch results of `window`-wise data). Simulated data can be analysed to quantify the uncertainty and/or potential bias of parameter estimates. The results held within a `gIMble` store can be described, written to column-based output files or removed using the modules `info` (9), `query` (10), and `delete` (11).

are defined independently for each pair of individuals. Crucially, we assume that blocks are only indirectly affected by selection at nearby linked sites and otherwise evolve neutrally and under a constant mutation rate. While these assumptions allow us to treat blocks within the same window as statistically exchangeable, they necessitate a careful and consistent filtering strategy for variant and invariant sites. In particular, we avoid sample-size dependent filters and do not restrict inference to data that meet filtering criteria in a fixed fraction of individuals. To facilitate comparability across datasets, we have implemented a minimal set of standard filters within the `preprocess` module.

Inference is based on the block-wise site frequency spectrum (the bSFS *sensu* [37]), i.e. the tallies of SFS mutation types in blocks (formally the counts of joint, folded site frequency spectra). Lohse et al. [36] show how to analytically compute the probability of bSFS counts under the IM and related demographic models in the absence of recombination using the generating function (GF) of genealogies. `gIMble` implements this calculation using `agemo` [38] and supports both global and local inference through a number of modules. The `optimize` module uses the bSFS data to find maximum composite likelihood estimates (MCLE) under the IM model using standard numeric optimization (Fig 1, (3)). In contrast, `gridsearch` infers IM parameters in sliding windows over a grid of bSFS probabilities pre-computed using `makegrid` (Fig 1, (4) & (5)). Importantly, to quantify the uncertainty in parameter estimates `gIMble` includes a module to `simulate` parametric bootstrap replicates under the inferred demographic history. See Methods for a more detailed overview of the `gIMble` modules.

## A butterfly test case

To illustrate `gIMble`, we re-analysed WGS data of 10 wild-caught individuals each of *H. melpomene* and *H. cydno*, sampled from sympatric populations in Panama (of the subspecies *H. melpomene rosina* and *H. cydno chioneous* respectively, Table S1). To maximise the density of variable sites, we focused all analyses on intergenic sequence defined as any sequence without gene or repeat annotation. For the sake of simplicity, we ignore the Z chromosome, which is known to have greatly reduced genetic diversity and gene flow in *Heliconius* taxa [42, 46].

After filtering for coverage and quality, and partitioning the data into pair-blocks of 64 bases, the total block-wise dataset comprised a mean length of 72 Mb of sequence per heterospecific sample pair. This corresponds to 69 % of intergenic sequence and 24 % of the *H. melpomene* genome. We find that average heterozygosity within each species ($H$) in intergenic blocks (Table 1) is comparable to the mean pairwise genetic diversity [48] across all sample pairs ($\pi = 0.0163$ and 0.0156 in *H. cydno* and *H. melpomene*, respectively), which suggests that population structure is weak.

We summarised blockwise genetic variation in windows of 50,000 pair-blocks with a 20 % offset between adjacent windows. This window size corresponds to a minimum window span of $500 \times 64$

**Table 1.** Average heterozygosity ($H$) across all individuals within each species and divergence ($d_{\mathrm{xy}}$) between *H. melpomene* and *H. cydno*. Summaries were generated using `gIMble info` based on intergenic blocks of 64 bases sampled in heterospecific pairs of individuals (top row). Estimates from a similar previous analysis for a single pair of individual genomes and longer blocks of 150 bases [37] (second row) are shown for comparison.

| Study | Data | $H_{\mathrm{Hcyd}}$ | $H_{\mathrm{Hmel}}$ | $d_{\mathrm{xy}}$ | $F_{\mathrm{ST}}$ |
|---|---|---|---|---|---|
| This study | 64 b | 0.01583 | 0.01553 | 0.0220 | 0.169 |
| Ref. [37] | 150 b | 0.0169 | 0.0150 | 0.0218 | 0.155 |

bases, or 32 kb, of "blocked" sequence assuming complete coverage across all 100 hetero-specific sample pairs. However, since we only consider intergenic sequence that meet coverage and quality filters, the span (distance between first and last base) of windows is considerably greater (median 103 kb, Fig S1). Exploring window-wise variation, we recover two known properties of this dataset: first, outliers of $F_{\mathrm{ST}}$ are mostly due to reduced diversity rather than an increase in $d_{\mathrm{xy}}$ (Fig S2). Second, differentiation along the genome is highly variable and includes many peaks of $F_{\mathrm{ST}}$, but no large genomic regions of elevated $F_{\mathrm{ST}}$ (Fig S3).

### Fitting a global background model of divergence and gene flow

Our initial aim was to identify a plausible, yet simple, global background demography for this *Heliconius* species pair. We compared support for IM models with gene flow in either direction, as well as simpler nested histories. Specifically, we fitted a history of strict divergence ($DIV$, $m_e = 0$) and a model of migration only ($MIG$, $T = \infty$) (see Table S2).

The scenario that best fit the global bSFS is an IM model involving gene flow at rate $m_e = 7.41 \times 10^{-7}$ ($M = 4N_{\mathrm{mel}}m_e = 1.62$ individuals) per generation from *H. cydno* into *H. melpomene* (forwards in time) ($IM_{\rightarrow mel}$ Table, 2, Fig S4). We will refer to this model as "the global model" from here on. Our estimates under the global model are consistent with both previous demographic analyses for this species pair [37, 41] (Table 2) and the observation that backcrosses of F1 hybrids are only viable in the *H. melpomene* direction in the lab. Assuming four generations per year and a spontaneous mutation rate estimated for *Heliconius* of $2.9 \times 10^{-9}$ per bp and generation [49], the maximum composite likelihood estimates (MCLE) under the best fitting IM model correspond to effective population sizes ($N_e$) of roughly 0.5, 1.5 and 1 $\times 10^6$ for *H. melpomene*, *H. cydno* and their shared ancestral population, respectively, and a divergence time of approximately 1 MYA (Fig S4, Table 2).

**Table 2.** Divergence and gene flow estimates for *H. melpomene* and *H. cydno* inferred using `gIMble optimise`. By default `gIMble` parameter estimates are scaled in absolute units, i.e. number of individuals and generations given a user-specified $\mu$. We have converted $T$ to millions of years (MYA) assuming four generations per year. 95% CI (based on parametric bootstrap) for parameter estimates are: $N_{\text{mel}} = \left[5.36 \times 10^5, 5.63 \times 10^5\right]$, $N_{\text{cyd}} = \left[1.40 \times 10^6, 1.43 \times 10^6\right]$, $N_{\text{anc}} = \left[9.25 \times 10^5, 9.31 \times 10^5\right]$, $T = [1.046, 1.061]$, and $m = \left[7.30 \times 10^{-7}, 7.53 \times 10^{-7}\right]$. The bottom row gives estimates from a previous analysis [37], which was based on a single pair of individual genomes for comparison.

| model/dataset | $N_{\text{mel}}$ | $N_{\text{cyd}}$ | $N_{\text{anc}}$ | $T$ (MYA) | $m$ ($M = 4N_e m$) |
|---|---|---|---|---|---|
| $IM_{\rightarrow\text{mel}}$ | $5.49 \times 10^5$ | $1.415 \times 10^6$ | $9.279 \times 10^5$ | 1.054 | $7.41 \times 10^{-7}$ (1.62) |
| $IM_{\rightarrow\text{mel}}$ [37] | $1.10 \times 10^6$ | $2.85 \times 10^6$ | $N_{\text{cyd}}$ | 1.04 | $3.41 \times 10^{-7}$ (1.50) |

### Strong support for heterogeneity in $N_e$ and $m_e$

We explored variation in $N_{\text{mel}}$, $N_{\text{cyd}}$, $N_{\text{anc}}$ and $m_e$ along the *Heliconius* genome by exhaustively searching parameter combinations in a pre-computed $12 \times 12 \times 12 \times 16$ grid. The grid was roughly centred on the MCLEs inferred under the global model (Table 2) and $T$, the species divergence time, was fixed to the global estimate (Table 2).

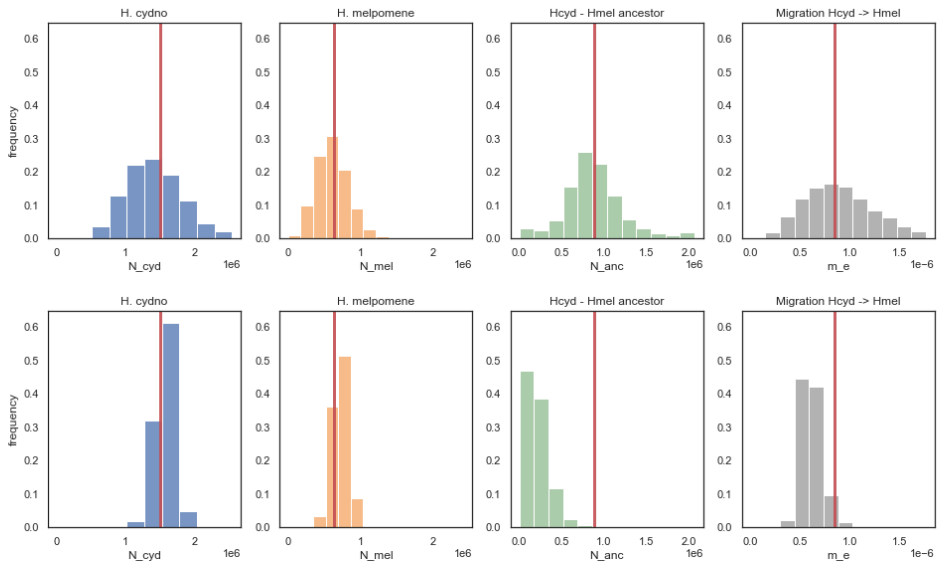
We found that window-wise, i.e. local, MCLEs of all three $N_e$ parameters varied by a factor of three (Fig 2). Similarly, local estimates of $m_e$ have a wide distribution. Importantly, applying the same inference to window-wise data simulated under an IM model without heterogeneity (i.e. the MCLEs under the global model, Table 2) gives much narrower distributions of local estimates (Fig 2, bottom). Thus, the heterogeneity in local $N_e$ and $m_e$ cannot be explained by the randomness of the coalescent and the mutational process, but likely captures true variation in effective demographic history along the genome.

We can quantify the overall support for heterogeneity in $N_e$ and $m_e$ by summing the support ($\ln CL$) across windows: e.g. the support for a maximally heterogeneous model that allows for variation in $N_{mel}$, $N_{cyd}$, $N_{anc}$ and $m_e$ is the sum of $\ln CL$ associated with all local MCLEs. Allowing for heterogeneity in all three effective parameters greatly improved the overall fit of the $IM_{\rightarrow mel}$ model (Table 3).

## Detecting barriers to gene flow

Estimating $m_{e,i}$ for each window $i$ (shorthand $m_i$) along the genome immediately allows us to detect genomic barriers as regions with reduced gene flow. One possibility is to interrogate the point estimates of $m_i$ along the genome. However, in quantifying the support for reduced $m_e$, we want

**Fig 2.** The distribution of $N_{mel}$, $N_{cyd}$, $N_{anc}$ and $m_e$ from *H. cydno* into *H. melpomene* estimated in sliding windows of 32 kb of blocked sequence, for the real data (top row) and bootstrap simulations without $m_e$ and $N_e$ heterogeneity (bottom row). Simulations were conditioned on the *Heliconius* recombination map [50] (see Methods). The red lines indicate estimates under the global model. Note that histogram bins correspond to points in the $12 \times 12 \times 12 \times 16$ parameter grid.




to account for the confounding effects of locally varying effective population sizes $N_i$. To this end, we define $\Delta_B$ as the relative support for a model in which $m_i$ is reduced below some threshold $m_e^*$ relative to a model in which $m_i$ is fixed to $\hat{m}_e$, the value that best fits the data under the global model assuming no heterogeneity in $m_e$.

$$\Delta_B = \max_{\substack{m_i \leq m_e^* \in \mathbf{M} \\ N_i \in \mathbf{N}}} \left\{ \ln CL(m_i, \hat{T}, N_i) \right\} - \max_{N_i \in \mathbf{N}} \left\{ \ln CL(\hat{m}_e, \hat{T}, N_i) \right\} \quad (1)$$

Here, $\hat{m}_e$ and $\hat{T}$ are the best supported parameter estimates globally and $\mathbf{M}$ and $\mathbf{N}$ are the grids

**Table 3.** Model fit measured as $\Delta \ln CL$ relative to the best fitting global model, $IM_{\rightarrow mel,fix}$ (Table 2) which assumes that all effective parameters are fixed.

| Model | $DIV_{fix}$ | $MIG_{\rightarrow mel,fix}$ | $IM_{\rightarrow mel,fix}$ | $IM_{\rightarrow mel,hetN_e}$ | $IM_{\rightarrow mel,hetN_e,m_e}$ |
|---|---|---|---|---|---|
| $\Delta \ln CL$ | -1,057,371 | -194,281 | 0 | 3,933,396 | 5,103,312 |

of $m_e$ and $N_e$ values used for the window-wise optimization. The first term represents the highest support ($\ln CL$) obtained for window $i$ across all possible population sizes $N_i$ and migration rates $m_i$ below the threshold value $m_e^*$. The second term gives the support of the best fitting model when the migration rate is fixed to the global estimate $m_i = \hat{m}_e$ and only $N_i$ may vary. We can think of $\Delta_{B,0} = 0$ as defining a sea level for barriers to gene flow, i.e. windows with a positive value have support for reduced gene flow.

Given that any `gIMble` window is largely composed of neutral sites linked to potential barriers rather than barrier loci itself, we expect $m_i > 0$ even for a window that is tightly associated with a complete barrier to gene flow. We therefore explored the *Heliconius* dataset using two $m_e$ thresholds to define barriers, $m_e^* = 0$ or $m_e^* = 0.2 * \hat{m}_e$ (we refer to these analyses as $\Delta_{B,0}$ and $\Delta_{B,0.2}$ scans respectively). Only 0.78 % of windows (87 out of 11,217) met the strictest barrier definition $\Delta_{B,0} > 0$. We merged all overlapping barrier windows above the "sea-level" of $\Delta_{B,0} = 0$ which defined 25 barrier regions of a total length of 4.96 Mb (1.9 % of autosomal sequence). Individual barrier regions ranged in length from 90 to 408 kb (Fig 3, top). A less stringent barrier definition, $\Delta_{B,0.2} > 0$, identified 7.8 % of windows, spanning 170 barrier regions of a total length of 47.4 Mb (18.2 % of autosomal sequence) (Fig 3, bottom).

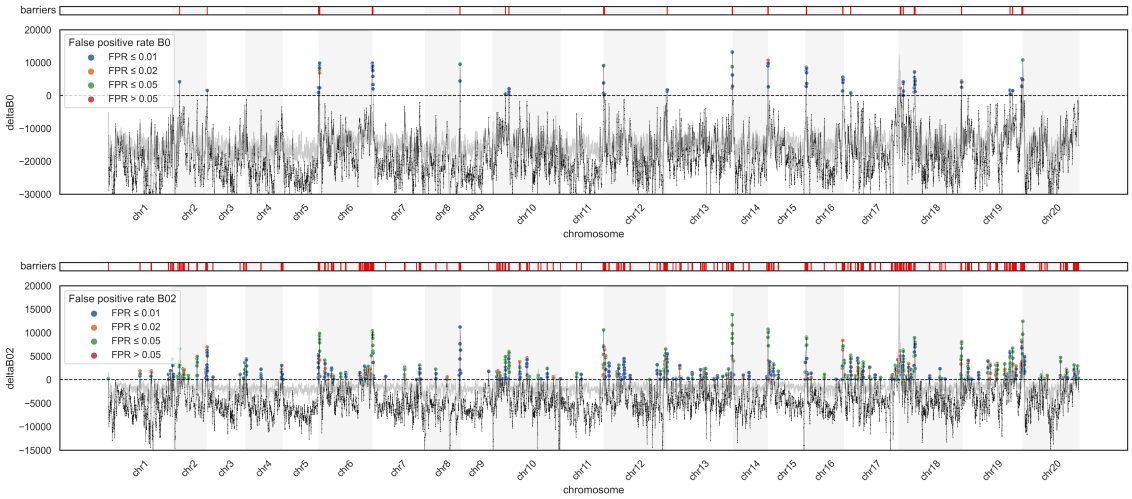
Since the composite-likelihood measure of barrier support $\Delta_B$ depends both on the locally best fitting set of $\mathbf{N}$ and the local recombination rate, we used a parametric bootstrap (based on window-wise simulation replicates generated with `gIMble simulate`, see Methods) to estimate window-wise false positive rates (FPR). We find that the average genome-wide FPR are far lower than the observed fraction of barrier windows: 0.017 % and 0.44 % for $\Delta_{B,0}$ and $\Delta_{B,0.2}$ scans, respectively (Fig 3).

## Barriers only partially overlap $F_{\mathrm{ST}}$ outliers but coincide with known wing-pattern genes

Although $F_{\mathrm{ST}}$ and $\Delta_B$ are correlated (e.g. for $\Delta_{B,0}$ $\rho = 0.783$, $p < 0.000001$, Fig 4), we find that $\Delta_B$ barriers only partially overlap $F_{\mathrm{ST}}$ outliers. This is true irrespective of how $\Delta_B$ barriers are defined. For example, only 42 of the 87 $\Delta_{B,0}$ barrier windows are contained in the analogous $F_{\mathrm{ST}}$ tail (Fig 4). In other words, the demographically explicit scan identified novel and different genomic regions which are not necessarily outliers in $F_{\mathrm{ST}}$.

To investigate if the $\Delta_B$ scan recovers previously identified barrier loci, we focused on the three large-effect genes controlling wing-pattern differences between *H. melpomene* and *H. cydno* that have been studied in detail [43, 51–53]: *wnt-A* (chromosome 10), *cortex* (chromosome 15), and *optix* (chromosome 18). We find that all three of these known positives are barriers as defined by $\Delta_{B,0.2}$ and two (*wnt-A* and *optix*) are also barriers under the most stringent criterion of $\Delta_{B,0} > 0$ (Fig 5).

**Fig 3.** Barriers to gene flow between *H. melpomene* and *H. cydno* inferred using `gIMble`. The $m_e$ threshold used to diagnose barriers is relaxed from top ($\Delta_{B,0}$) to bottom ($\Delta_{B,0.2}$). Regions above the "sea-level" of $\Delta_B = 0$ fit a history of reduced $m_e$ better than a model assuming the global estimate $\hat{m}_e$. Windows with $\Delta_B > 0$ have been coloured to reflect their expected FPR. The $\Delta_B$ threshold corresponding to a FPR of 0.05 is shown in grey.

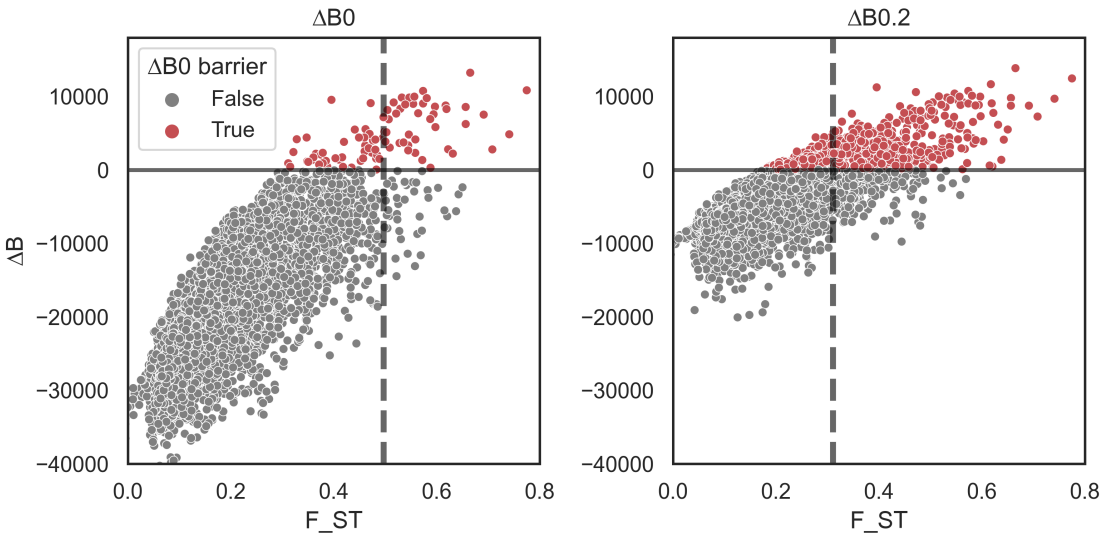



While *optix* is also contained in the analogous tail of $F_{\mathrm{ST}}$ outliers (i.e. the 0.78 % of windows with highest $F_{\mathrm{ST}}$), both *wnt-A* and *cortex* are not. Intriguingly, the two $\Delta_{B,0}$ barrier regions downstream of *optix* on chromosome 18 coincide with a recently identified QTL peak of species-specific visual preference behaviours for the red wing-pattern controlled by *optix* [40]. This region includes five candidate genes [54], specifically cis regulatory differences in *Grik2* and *regucalcin2* (highlighted in orange in Fig 5).

## Barriers are concentrated in regions of low recombination and high coding density

Population-genetic models of species barriers predict a reduction of $m_e$ in the genomic vicinity of barrier loci. This reduction scales with the the map distance from the barrier locus and the strength of divergent selection acting on it [9,57,58]. In regions of low recombination there is thus an increase both in the physical scale at which barrier loci reduce gene flow in flanking neutral regions and in the chance that new barrier loci arise [59,60]. However, since the effect of any form of selection on nearby neutral sequence depends on recombination, quantifying the association between barriers and $r_{\mathrm{bp}}$ has proven hard using summary statistics (but see [61]). Thus, $F_{\mathrm{ST}}$ outliers are expected to be

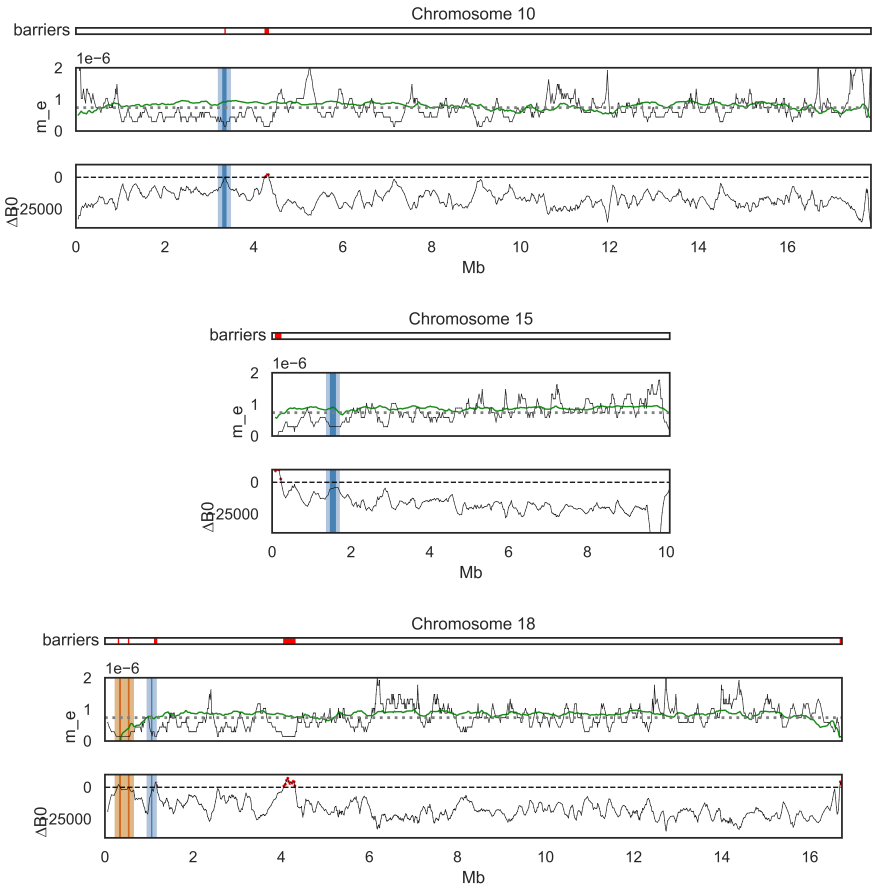
**Fig 4.** $F_{\mathrm{ST}}$ and support for barriers to gene flow between *H. melpomene* and *H. cydno* as measured by $\Delta_B$ (eqn. 1) are partially correlated. Each dot represents an autosomal window of effective length 32 kb. Two $m_e$ thresholds used to distinguish barriers are shown: $\Delta_{B,0}$ (left) and $\Delta_{B,0.2}$ (right) which identify 0.78 % and 7.8 % of windows as barriers. The dashed vertical lines delineate the corresponding percentiles in the $F_{\mathrm{ST}}$ distribution.




concentrated in regions of low recombination simply as a result of the ubiquitous positive correlation between genetic diversity and recombination rate caused by BGS and positive selection [13, 62].

In contrast, our framework of estimating effective parameters and defining barriers of $m_e$ via $\Delta_B$ allows to disentangle selection acting on barriers from BGS and positive selection not contributing to species differentiation. After controlling for heterogeneity in $N_e$, we found a clear association between local $m_e$ and direct estimates of recombination rate ($r_{\mathbf{bp}}$) over two physical scales. At an inter-chromosomal scale, we found a strong negative correlation between $m_e$ and physical chromosome length (Fig 6, Pearson's $\rho = -0.63$, $p = 0.0073$). This pattern is expected under polygenic selection against interspecific gene flow because longer chromosomes tend to have lower $r_{\mathrm{bp}}$ in *Heliconius* [63]. A negative correlation between chromosome length and introgression – measured in terms of $f_d$, a summary statistic that draws information from taxon trios – has also been found in a previous study of the same *Heliconius* species pair [61]. Reassuringly, we found estimates of local $N_e$ (both $N_{cyd}$ and $N_{mel}$) to be even more strongly correlated with chromosome length (Fig S6) than $m_e$, as is expected given the general effect of selection on linked neutral sites [64]. The fact that our estimates of both effective gene flow and effective population sizes correlated with recombination in

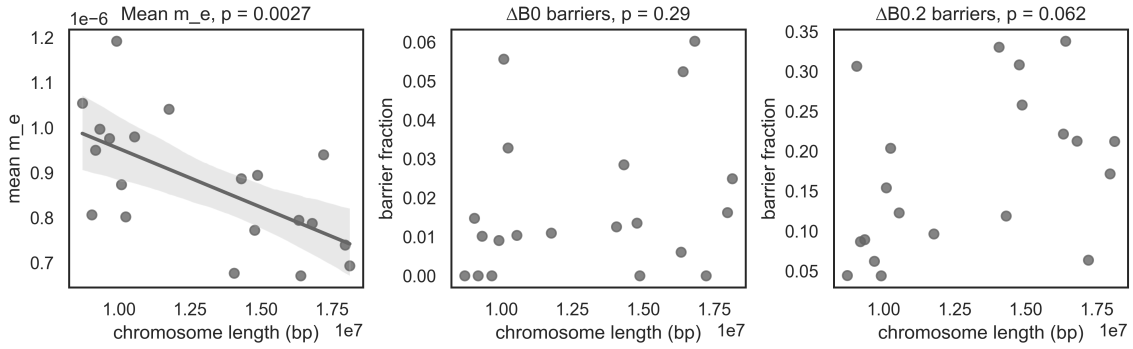
**Fig 5.** Local point estimates of $m_e$ (top of each subplot) and $\Delta_{B,0}$ (bottom of each subplot) for the three chromosomes containing large-effect wing patterning genes (shown in blue). $\Delta_{B,0}$ identifies *wnt-A* [55] on chromosomes 10 (top) and *optix* on chromosome 18 (bottom) as barriers. Given that causal sites may be situated in regulatory regions, which in the case of *optix* extend to $\approx$ 100 kb away from the gene [56], we have highlighted the 100 kb up and downstream of each gene (lighter blue). $\Delta_{B,0} > 0$ barriers for each chromosomes are shown in red on top. Another $\Delta_{B,0} > 0$ barrier on chromosome 18 coincides with *Grik2* and *regucalcin2* (orange), genes that are associated with male wing-pattern preference. The wing-pattern gene *cortex* on chromosome 15 (centre) is not a barrier according to $\Delta_{B,0}$. Point estimates of local $m_e$ are shown in the centre of each subplot, the prediction under a polygenic model (modified from [9]) is shown in green.




the expected direction suggests that we have successfully decomposed the effects of selection acting at barrier loci from BGS and positive selection.

The fraction of chromosomes inferred to be covered by strong barrier regions (i.e. $\Delta_{B,0} > 0$) varies widely between chromosomes. For instance, chromosomes 1, 4, 9, 14 and 20 harbour no $\Delta_{B,0}$ barrier windows at all, while chromosome 18 appears to have 6 % of its physical length covered by strong barriers. However, the fraction of chromosomes covered by barrier regions did not correlate with physical chromosome length (Fig 6, centre and bottom). This is interesting for two reasons. First, under the simplifying assumptions that each barrier region is caused by a single barrier locus and that selection acting on individual barrier loci does not differ among chromosomes, the width of barriers is predicted to scale with $1/r_{\mathrm{bp}}$ [4]. If the rate at which mutations give rise to potential barrier alleles is uniform across chromosomes, we would expect longer chromosomes to have a greater fraction of barrier windows. Second, theory also predicts that existing barrier loci facilitate the establishment of further barrier loci in close linkage [59, 60]. This clustering effect would again be stronger for long chromosomes (lower $r_{\mathrm{bp}}$) compared to short chromosomes (higher $r_{\mathrm{bp}}$), and should thus further contribute to a positive correlation between chromosome length and the fraction of chromosomes covered by barrier regions. The most likely explanation for the absence of this predicted correlation for $\Delta_{B,0} > 0$ barrier regions is a lack of statistical power (i.e. there are only 25 $\Delta_{B,0}$ barrier regions in total). Indeed, when we consider the less-stringent barrier definition (i.e. the 172 barrier regions identified by $\Delta_{B,0.2} > 0$), a weakly positive, albeit non-significant, positive relation between chromosome length and the barrier fraction emerges. (Fig 6, right).

**Fig 6.** Relationship between chromosome length and the strength and abundance of barriers. Barrier strength, measured as the average effective migration rate across windows, mean $m_e$, is negatively correlated with chromosome length (left). In contrast, there is no significant relationship between the relative length of chromosomes defined as barrier regions and chromosome length irrespective of whether barriers are defined via $\Delta_{B,0}$ (center) or $\Delta_{B,0.2}$ (right).




At the second, intra-chromosomal scale, we found that window-wise estimates of $m_e$ are strongly and positively correlated with $r_{\mathrm{bp}}$ [63] (estimated directly from lab crosses, see Methods), again as

predicted under a polygenic architecture of barriers [65]. Similarly, barriers to gene flow defined either by $\Delta_{B,0}$ or $\Delta_{B,0.2}$ are concentrated in regions of reduced recombination: as per a simple resampling procedure, $r_{\mathrm{bp}}$ is significantly lower in barrier windows compared to the average (0.75 vs 1.94 cM/Mb, respectively) (Fig 7 and Table 4). Assuming that selective targets (both of BGS and selection on barrier loci) reside in or near coding DNA sequence (CDS), we also expected a negative relationship between the density of CDS and local $N_e$ and $m_e$ estimates. We found clear support for both relationships (Fig S7, e.g. for $m_e$ and CDS measured at a 250 kb scale $\rho = -0.164$, $p < 0.000001$). Thus, overall, and in agreement with a model of polygenic selection against inter-specific gene flow, we found that barriers to gene flow are concentrated in regions of low recombination and high CDS density.

**Fig 7.** Top: genomic windows diagnosed as barriers to gene flow by $\Delta_{B,0}$ (centre) and $\Delta_{B,0.2}$ (right) have lower than average recombination rates per base pair ($r_{\mathrm{bp}}$; left). Median $r_{\mathrm{bp}}$ for each type of window is shown in red. Bottom: $\Delta_{B,0}$ and $\Delta_{B,0.2}$ barriers are enriched for coding sequence (CDS).

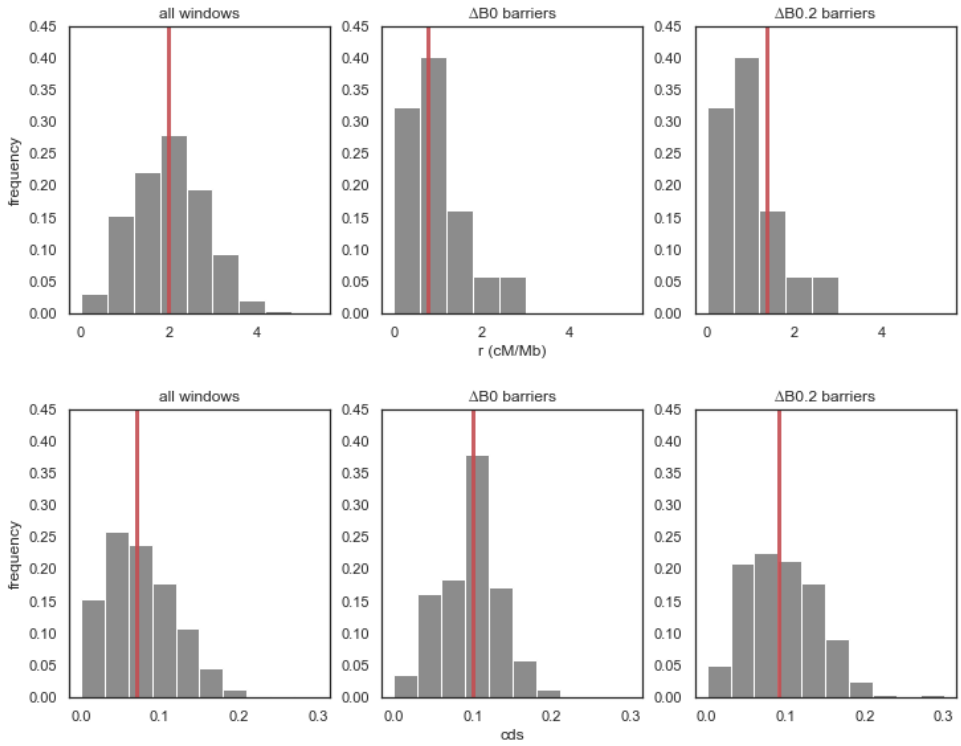


**Table 4.** Average properties and $N_e$ estimates for genomic windows defined via $\Delta_B$ for *H. melpomene* and *H. cydno*.

| Partition | # of windows | # of regions | Total (Mb) | %CDS | $r(cM/Mb)$ | $N_{cyd}$ | $N_{mel}$ |
|---|---|---|---|---|---|---|---|
| $\Delta_{B,0} > 0$ | 87 | 25 | 4.9 | 0.0958 | 0.956 | 1,022,000 | 430,000 |
| $\Delta_{B,0.2} > 0$ | 871 | 170 | 47.4 | 0.0956 | 1.451 | 1,283,000 | 513,000 |
| autosomes | 11, 217 | n/a | 259 | 0.0765 | 2.004 | 1,506,000 | 630,000 |

## How many barrier loci are there?

Aeschbacher et al. [66] proposed a framework to quantify the strength of selection against introgression under a simple model in which barrier loci are uniformly distributed across the genome with density $\nu$ per base pair. Under this model, the expected local reduction in $m_e$ at a genomic window $i$ relative to the background rate (i.e. $m_{e,i}/m$) is a function solely of the per-base pair recombination rate ($r_{\text{bp}}$) and the "selection density" $\sigma = \nu s$, where $s$ is the selection coefficient at each barrier locus [66, eqn. 1.9 in Appendix 1]. We fitted a version of this model in which the reduction in gene flow at a focal genomic window depends only on the closest flanking barrier locus on either side of it. To account for variation in CDS as a proxy for the density of barrier loci, we modified this model by scaling $\nu$ by the inverse of the CDS density. We inferred $\sigma$ and the background $m$ by minimising the sum of squared differences between the expected local $m_e$ (given $r_{\text{bp}}$ and CDS) and the $m_e$ estimates inferred by `gIMble` across all genomic windows. Exploring CDS and $r_{\text{bp}}$ measured at a range of physical scales around each window (see Methods) revealed that $r_{\text{bp}}$ estimated at a scale of 2 Mb and CDS measured at a 250 kb scale best explained the variation in inferred $m_e$ (Fig S8). However, given the edge effects accumulating at chromosome ends when measuring $r_{\text{bp}}$ over 2 Mb, especially for the shorter chromosomes with a length on the order of 10 Mb (Fig S6), we chose to use $r_{\text{bp}}$ values estimated over 1 instead of 2 Mb. With this combination of ranges (1 Mb for $r_{\text{bp}}$, 250 kb for CDS), we obtained estimates of $\hat{\sigma} \approx 7.77 \times 10^{-9}$ (95% CI: $\left[6.95 \times 10^{-9},\ 8.59 \times 10^{-9}\right]$) and of the background migration $\hat{m} \approx 9.87 \times 10^{-7}$.

The compound nature of $\sigma$ means that similar values can be produced by a small number of barrier loci under strong selection and a large number of barrier loci under weaker selection. One question we can ask is what strength of selection $s$ per barrier locus would be required to produce our estimate of $\sigma$, assuming the signal was caused only by the 25 strong (i.e. $\Delta_{B,0}$) barrier regions we inferred. For the sake of simplicity, we assume that each barrier locus experienced the same selection, and that each barrier region contains exactly one barrier locus. Given a total length of 259 Mb for *H. melpomene* autosomes [50], we find $\hat{s} = \hat{\sigma}/(25/[259 \times 10^6]) = 0.0805$, which would imply extremely strong selection at each individual barrier locus. However, the estimate of $\sigma$ is clearly

driven by the genome-wide relationship between $m_e$ and $r_{\mathrm{bp}}$ (and CDS). Indeed, exclusion of the 25 $\Delta_{B,0}$ outlier regions had almost no effect on our estimate of $\sigma$ and $m$: we estimated $\hat{\sigma} \approx 7.07 \times 10^{-9}$ (compared to $\hat{\sigma} \approx 7.77 \times 10^{-9}$) and $\hat{m} \approx 9.82 \times 10^{-7}$ (compared to $\hat{m} \approx 9.87 \times 10^{-7}$). We therefore conclude that there must be many more barrier loci than $\Delta_{B,0}$ regions. We can obtain an upper bound for the number of barrier loci if we consider that $2N_e s > 1$ for each barrier locus for selection to be stronger than genetic drift. At the same time, selection at each barrier locus has to be stronger than the background migration rate if the barrier is to be maintained (i.e. $s > m$) [3]. Given our estimates of $\hat{N}_{mel} \approx 549,000$ (Table 2) and $\hat{m} \approx 9.87 \times 10^{-7}$, the former threshold ($2N_e s > 1$) is the limiting one, and the maximum number of barrier loci implied by $\hat{\sigma} = 7.77 \times 10^{-9}$ is $\approx 1,100,000$. Thus, given this upper limit for the number of barrier loci, we cannot rule out that the overall species barrier between *H. melpomene* and *H. cydno* has an omnigenic architecture. In other words, while a moderate number of strong barriers – including those associated with the candidate genes *optix*, *regucalcin*, and *wnt-A* – appear to locally reduce $m_e$ to a large extent, a much larger number of weaker barrier loci likely contribute to reproductive isolation between these taxa.

### Robustness to model choice

Our reconstruction of $m_e$ assumes an $IM$ model, while our analysis of how polygenic selection generates this $m_e$ variation assumes a model of migration–selection balance (i.e. $T \to \infty$, and genetic variation at barrier loci maintained migration–selection equilibrium). Interestingly, the second best global model to the *Heliconius* data is a history of constant migration ($MIG$). This and the fact that global parameter estimates of $m$, $N_{cyd}$ and $N_{mel}$ under the $MIG$ model are very similar to those under the $IM$ model (Table S2) implies that the information about both $T$ and the ancestral $N_e$ are limited. This raises the question whether these two parameters matter at all for our inference of $m_e$ heterogeneity and barriers.

To assess the extent to which the *Heliconius* barrier analysis is model dependent, we set up a simpler `gIMble` scan under the $MIG$ model (varying $N_{cyd}$, $N_{mel}$ and $m_e$). While $\Delta_{B,0}$ barriers are undefined under the $MIG$ model, we found that estimates of both $m_e$ variation and $\Delta_{B,0.2}$ barriers are extremely similar to the analogous results under the $IM$ model (Fig S9). This demonstrates that the inference of barriers between *H. melpomene* and *H. cydno* is robust to the choice of model.

## Discussion

Progress in speciation genomics has been impeded by a conceptual and methodological divide between approaches that model speciation history under neutral scenarios and analyses that aim to identify loci under divergent selection that act as barriers to gene flow. Here, we bridge this

divide by introducing `gIMble`: a single framework to model both the overall species history and the genomic landscape of speciation. We used `gIMble` to demonstrate a two-step inference procedure that fits a genome-wide (global) demography under the IM model, and then relaxes constraints on $N_e$ and $m_e$ to estimate these two parameters locally in sliding windows along the genome. Unlike previous approaches that attempt to describe species barriers via summary statistics such as $F_{\mathrm{ST}}$ that are affected by all forms of selection, the effective demographic parameters inferred by `gIMble` have straightforward interpretations in terms of evolutionary processes: while heterogeneity in $N_e$ largely reflects BGS, $m_{e,i}$ measures the long-term combined effects of barriers to gene flow at a local genomic window [57]. The ability of our framework to identify both the position and strength of barriers to gene flow based on estimates of $m_e$ holds promise for comparative studies of species barriers [31].

Applying our inference procedure to the neotropical butterfly species *H. cydno* and *H. melpomene*, we found strong support for genome-wide variation in $N_e$ and $m_e$. Genomic windows of very low $m_e$ partially overlapped with well-known large-effect loci controlling wing patterning (*optix*, *cortex* and *wnt-A*) and female preference for wing pattern (*regucalcin*). We also confirmed previous studies that estimated higher (effective) gene flow from *H. cydno* into *H. melpomene* than in the opposite direction [61, 67]. Finally, we illustrated how the inferred genome-wide variation in $m_e$ can be leveraged to infer the aggregate strength of selection against gene flow given maps of the CDS density and recombination rate. Exclusion of the strongest barrier regions from this inference had little effect on our estimate of aggregate selection strength, which suggests that the divergent selection that keeps *H. cydno* and *H. melpomene* separate species is highly polygenic.

The inference framework we proposed is a first step towards model-based characterisation of species barriers. However, there is extensive room for further development of this framework, both in terms of model complexity and optimal exploitation of genomic data. It is important to reiterate that modelling the effect of barriers via demographic parameters is a simplification. While the approximation implemented in `gIMble` does capture the effect of selection *maintaining* barriers once they have been established, it does not consider the processes by which alleles at barrier loci (and locally beneficial alleles more generally) become established. While Bateson–Dobzhansky–Muller incompatibilities can arise through drift alone only in the absence of migration [68], their establishment in the face of gene flow involves selection favouring locally adaptive alleles. Thus, developing methods that capture the joint effect of selection establishing barriers and the resulting reduction in $m_e$ remains an important goal of future work.

While `gIMble` is focused on subsamples of pairs of diploid genomes, its modular design facilitates the implementation of generalisations to larger samples and more complex demographic models. There are several obvious and useful avenues for future extensions. First, `gIMble` assumes a constant rate of unidirectional gene flow until the present. Since many taxon pairs that are of interest in

speciation research are likely to have completed speciation (i.e. there is no contemporary gene flow), it would be useful to generalise the method to allow for a time at which gene flow ceases – the Isolation with initial migration (IIM) model [69] – as well as bi-directional gene flow. While an approximate solution to the problem of bi-directional gene flow has been described [67], we currently do not know of a closed-form analytic solution to account for the multiple discrete demographic events in the IIM model as required by `agemo` [38] (see Methods). This limitation applies equally to other extensions of model space involving multiple discrete events, such as inference under models of instantaneous admixture or the inclusion of explicit selective sweeps [70].

Second, `gIMble` currently does not make use of phased sequence data. While this makes it useful for studies where phasing is not achievable (e.g. short-read sequencing data and small sample sizes as considered here), extension of `gIMble` to phased data would increase its statistical power.

A further and more profound extension would be to incorporate haplotype information in the "blocking" algorithm. Currently, blocks of fixed length are defined by blind discretisation of the genome. Given that blocks are assumed not to have experienced recombination, they should ideally be defined via topologically informative sites (e.g. using the four-gamete-test and other topology information) to ensure that each block meets the assumption of a single genealogy [71]. Recently, progress has been made on approaches that infer ancestral recombination graphs (ARGs) from phased data. In particular, several minimal-assumption heuristics exist to infer topology-only ARGs [72–74]. These methods use information from neighbouring haplotypes to reconstruct marginal tree topologies and would allow to define blocks by the spans of marginal trees, rather than disceretizing the genome at random and assuming no recombination within blocks, as we have done here. Thus, developing sampling strategies and likelihood calculations that incorporate topology and haplotype information will be a priority of future work.

Our analysis of *Heliconius* data illustrates the potential of our framework for speciation research. Specifically, we show that the $m_e$ variation inferred by `gIMble` can be used to answer two different, yet complementary, sets of questions about the speciation process. On the one hand, one may assume that reproductive isolation is conferred at least in part by a small to moderate number of large-effect barrier loci [1] that can be identified individually from their strong effect on $m_e$, a scan based on $\Delta_{B,0}$ yields a meaningful estimate of the fraction of genome in this category. Importantly, our approach – unlike outlier scans based on summary statistics such as $F_{\mathrm{ST}}$ – avoids arbitrary significance thresholds. Instead, the power to estimate barriers to gene flow (which can be quantified readily using `gIMble simulate`) is a function of the background demography (e.g. the higher the genome-wide background $m_e$, the greater the power to identify $\Delta_{B,0}$ barriers) and the recombination rate. On the other hand, reproductive isolation may be largely if not entirely polygenic, with a large number of weak barrier loci reducing gene flow genome-wide [57]. In this case, it becomes impossible to identify individual barriers [75]. Instead, the main objective will be to understand the

genomic predictors/correlates of $m_e$. One advantage of `gIMble` is that its inference of $m_e$ and $N_e$ is purely based on genomic variation and can be intersected with many other data types that are relevant for speciation, e.g. gene expression data or information about barriers to gene flow from crossing experiments or hybrid zone studies.

Importantly, `gIMble` also does not rely on recombination information (we assume blocks are non-recombining and statistically exchangeable), which means that the interaction between recombination rate and $m_e$ can be modelled in a second step to make an aggregate inference on the nature of the polygenic species barrier. We have provided an example of such an inference using the framework of Aeschbacher et al [9], extended here to account for variation in CDS density as a proxy for the density of barrier loci.

Combining these two approaches, we found that *H. melpomene* and *H. cyndo* are separated by a mixture of large-effect and polygenic barriers. Three out of four known large effect loci controlling barrier phenotypes in this *Heliconius* species pair are contained in the 2 % of the genome identified as a barrier by the strictest definition of no effective migration, $\Delta_{B,0}$. Yet at the same time, it is clear that $\Delta_{B,0}$ barriers do not drive the genome-wide correlation we find between $m_e$ and $r_{\mathrm{bp}}$ which is little affected by the exclusion of $\Delta_{B,0}$ barriers. Simple scaling arguments lead us to conclude that, apart from the 25 major barrier loci we identified, up to hundreds of thousands of weak barrier loci scattered across the genome might contribute to reproductive isolation between *H. melpomene* and *H. cyndo*. In fact, the large-effect wing pattern loci may be a rather unique feature of the strong selection pressures generated by Müllerian mimicry in *Heliconius* and it is unlikely that they function as the sole, or even the truly significant, species barriers between *H. melpomene* and *H. cydno* for several reasons. First, these species are known to differ in numerous other ecological traits, including habitat preference [76]. Second, *H. melpomene* alleles at both *optix* and *cortex* are shared between taxa [77, 78] and are known to have adaptively introgressed into *H. timareta* (a relative of *H. cydno*), yet the species barrier between *H. melpomene* and *H. timareta* remains intact [79, 80]. Finally, only three of the 25 strong barrier regions we identified map to genes that are known to control wing patterns or preference for wing patterns.

Given that much of the past progress in speciation research has come from comparative analyses [28, 81], the fact that even the relatively simple model-based scans we have implemented here open the door to comparative studies of species barriers is cause for optimism. In particular, we argue that systematic comparisons of demographically explicit genome scans across many taxon pairs should reveal whether the combination of a few strong barrier regions embedded in a genome-wide, highly polygenic barrier signal seen in *Heliconius* is the rule or an exception.

# Materials and Methods

## Implementation

The `gIMble` analysis workflow is designed for WGS data in which all samples have been mapped to a single reference and requires minimal deviation from established bioinformatic pipelines and file formats. Figure 1 shows the basic workflow we have used to analyse the *Heliconius* data. Currently, `gIMble` only supports analyses of data from population/species pairs.

The different steps of the workflow are implemented as distinct modules within `gIMble` allowing users to string together analysis steps in a way and order they deem fit for a particular dataset and research question:

- `preprocess` applies simple coverage and quality filters to the input files to ensure consistency in how variation along the genome is sampled and summarised.
- `parse` reads the pre-processed input files into a compressed (`zarr`) datastore.
- `blocks` partitions genomic variation data into pair-blocks based on population membership of samples.
- `windows` creates (sliding) windows of a fixed number of pair-blocks along the genome.
- `info` displays information for the output of `blocks` and `windows` data.
- `tally` prepares `blocks`/`windows` data for modules `optimize` and `gridsearch`
- `optimize` performs parameter optimization given a tally and a bounded parameter space under a specified model.
- `makegrid` pre-computes and stores probabilities for a grid in parameter space.
- `gridsearch` computes and stores likelihoods of `tally` data given a pre-computed `makegrid`.
- `query` writes output files describing `windows`, `tally`, `optimize`, and `gridsearch` results.
- `simulate` uses `msprime` [39] to simulate data tallies which can be used for parametric bootstrapping and/or power analyses.

Below, we describe the pre-processing of data, the organisation of `gIMble` analyses into a `zarr` datastore, the modules that allow the fitting of global and local models, and the parametric bootstrapping of `gIMble` estimates.

## Data processing

### `gIMble preprocess`

The `gIMble preprocess` module implements a simple and consistent filtering of input files for subsequent analyses. The purpose of this module is to standardise the quality of data analysed by `gIMble` using a minimal set of filters that apply to both variant and invariant sites while maximizing the proportion of the data retained for analysis.

The required input for `gIMble preprocess` consists of the reference genome (FASTA format), read sets of each sample aligned to the reference (BAM format), and the called variants (VCF format).

The output is composed of the following four files:

- **genome file** recording the name and length of each sequence in the FASTA file.
- **sample file** specifying the name of each sample in the VCF file. This file needs to be manually edited by the user in order to partition samples into two populations/species.
- **VCF file** containing those variants that passed the filter criteria: only SNPs with a minimum distance from non-SNP variants, adequate read depth (based on min/max thresholds inferred from BAM files), minimum genotype quality, and minimum read mapping (SAF, SAR, RPR, RPL) parameters are retained.
- **BED file** describing the "callable" genomic regions for which each sample BAM has adequate coverage (based on min/max thresholds). All variants in the VCF which failed filtering are subtracted from these regions.

The genome and sample files give the user control over what data are processed in subsequent analysis steps of `gIMble`: the genome file delimits the parts of the reference to analyse and the sample file specifies the samples (and their population membership) from which block-wise data will be tabulated.

The VCF and BED files generated by `gIMble preprocess` describe the genomic variation across samples satisfying the defined quality thresholds and can therefore serve as a starting point for any complementary or alternative analyses the user may wish to run on the same data.

### `gIMble parse`

This module parses the four input files generated by `gIMble preprocess`. Based on the parsed data, a compressed `zarr` datastore is created which is central to all subsequent analyses.

### `gIMble blocks`

The output of this step determines which parts of the sampled sequences are available for analysis. The module `blocks` uses the "callable" regions specified in the BED file and the variants contained in the VCF file to define sequence blocks of a fixed number of callable sites. Defined this way, blocks can also span un-callable regions and therefore have variable physical span in the genome. To maximise the genomic regions sampled and to avoid biases due to missing data, blocks are constructed independently for each sample pair (hence our term pair-blocks). This ameliorates the asymmetry in coverage profiles among the samples due to stochastic variation in sequencing depth and/or reference bias. If, instead, blocks were conditioned on overall coverage (and other filtering criteria) across all samples, blocking success would be dominated by poorly covered samples and the "block-able" fraction of genome would be increasingly biased/reduced with increasing sample size.

Blocks are constructed for all pairwise sample combinations between the two populations (X) and within each population (A and B). However, only heterospecific pair-blocks (X) are used in the inference steps. The blocking of genomic data is controlled by the parameters `--block_length` — the number of callable sites in each block — and `--block_span` — the maximum distance between the first and last site in a block. While `gIMble` only considers biallelic genotypes in subsequent steps, the block-cutting algorithm allows for multiallelic and missing genotypes, which are controlled with the parameters `--max_multiallelic`, and `--max_missing`. It should be noted that only one set of data blocks can be stored in a `gIMble` datastore.

### `gIMble` windows

Windows are constructed by traversing each sequence of the reference from start to end, incorporating the heterospecific pair-blocks (X) as they appear based on their start positions. The parameter `--blocks` controls how many blocks are incorporated into each window and the parameter `--steps` by how many blocks the next window is shifted. Since coverage differences or mapping biases between samples can cause uneven distributions of blocks along the genome, there is no guarantee that blocks from all heterospecific pairs are equally represented within a window. However, by choosing a large enough value for `--blocks` this problem can be mitigated. Analogous to blocks, only one set of data windows can be stored in a `gIMble` datastore at a given time.

### `gIMble info`

Information about the data contained in a `gIMble` datastore can be obtained using `gIMble info` which computes standard population genetic summary statistics ($\pi$, $d_{xy}$ and $H$ mean heterozygosity) based on blocks sampled between (X) and within species/populations (A and B).

### `gIMble` query

Data contained in the `gIMble` datastore can be queried using this module and written into BED and TSV files. Currently, this functionality is supported by the data generated from the modules `windows`, `tally`, `optimize`, and `gridsearch`.

### `gIMble` tally

Having created blocks (with `gIMble blocks`) and windows (with `gIMble windows`), the module `gIMble tally` generates the bSFSs for heterospecific sample pairs, each consisting of a single diploid sample from each population/species A and B. The bSFS [67] is a tally of the mutation configurations of blocks which are themselves described by vectors of the form $\underline{k}_i$, which count the four possible mutation types found within a pair-block $i$.

- $m_1$ sites with homozygous genotype in A, heterozygous genotype in B
- $m_2$ sites with heterozygous genotype in A, homozygous genotype in B
- $m_3$ sites with shared heterozygous genotype in both populations
- $m_4$ sites with distinct homozygous genotype in both populations

The argument `--kmax` limits the counts of mutations of each type in the bSFS tally. Counts of mutations inside blocks $(m_1, m_2, m_3, m_4)$ exceeding the values in `--kmax` (default: $2, 2, 2, 2$) are combined. Thus the choice of `--kmax` involves a trade-off between capturing the probabilities of rare mutation configurations fully and computational complexity. The bSFS tallies of heterospecific data in blocks can be generated for all blocks (`-d blocks`), per window (`-d windows`), or for all blocks that are included in windows (`-d windowsum`).

## Calculating likelihoods

Probabilities of block-wise data under the coalescent with arbitrary demographic history can be calculated using the method described in [36, 37]. In this framework, the generating function (GF) for the distribution of genealogical branch lengths is described recursively as the convolution of independent Laplace-transformed exponential random variables. The GF for a particular model and sampling configuration can be represented most simply as a directed graph involving all possible ancestral states of the sample, and the probability of any bSFS configuration $\underline{k}_i$ can be calculated as a partial derivative of the GF [37].

Previous automation of this calculation relied on rather inefficient use of *Mathematica*'s [82] symbolic algebra functionality. `gIMble` now uses `agemo` [38], a user-friendly re-implementation of

the GF approach in python which extracts the probabilities of bSFS configurations efficiently by means of a graph traversal algorithm.

Since both the number of possible bSFS configurations and the coalescent state space graph grow exponentially with sample size $n$, computing probabilities becomes prohibitive for $n = n_A + n_B > 6$. `gIMble` accommodates large samples by considering the composite likelihood (CL) across all $n_A \times n_B$ heterospecific sample pairs (each pair $j$ consists of single diploid individual each from $A$ and $B$). Using $n_{i,j}$ as shorthand notation for the number of blocks in sample pair $j$ with bSFS configuration $\underline{k}_i$, the joint log composite likelihood ($\ln CL$) is given by:

$$\ln CL(\Theta) = \sum_{i} \sum_{j=1}^{n_A \times n_B} n_{i,j} \ln p(\underline{k}_i|\Theta) \tag{2}$$

where $\Theta$ is the vector of model parameters. The composite likelihood can be calculated dynamically during an optimization run for a given data set (see `optimize`). However, when scanning WGS data, it is more efficient to cast a grid over parameter space and pre-compute $\ln p(\underline{k}_i|\Theta_h)$ for all $\underline{k}_i$ and each possible possible parameter combination $h$ in the grid (see `makegrid`). Composite likelihoods can then be computed efficiently for tallies (based on `blocks`, `windows`, or `simulate`) using `gridsearch`.

### `gIMble optimize`

This module optimizes model parameters under a given model for a given data tally (based on parsed or simulated tallies) using an optimization algorithm with bounded constraints (CSR2, sbplx, or neldermead), as implemented in `nlopt` [83]. Given a set of bounds, optimization can be initiated either at the midpoint of the bounded parameter space or at a random start point. Optimizations finalize after user-defined stopping criteria are met and the user can assess convergence of optimizations by consulting the log-file.

### `gIMble makegrid` and `gridsearch`

Pre-computing the probabilities of bSFS configurations in a grid across parameter space is computationally efficient (relative to using an optimization algorithm) and therefore useful when we wish to interrogate data in replicate, i.e. across windows or simulation replicates. Grids are pre-computed using `makegrid`, saved in the `gIMble` store, and can be used to analyse any dataset contained within the same datastore using `gridsearch`. Grid searches may be used either for an initial exploration of the likelihood surface (i.e. prior to defining the parameter space across which to run *optimize*), or to fit models to window-wise data.

## Parametric bootstrap

Maximizing $\ln CL$ across blocks and sample pairs ignores both linkage between blocks and the pseudo-replication inherent in subsampling heterospecific pairs. To facilitate quantifying uncertainty in parameter estimates, critical values for obtaining $\Delta \ln CL$ (when comparing models) and measuring the power of $\Delta_B$ scans using parametric bootstrap, we implemented a wrapper for `msprime` [39].

### `gIMble simulate`

This module simulates replicate datasets under either a user specified IM type model and set of parameter values, or based on a previous `gridsearch` result of windows. Each simulation replicate consists of windows and the associated bSFS tallies saved to the `gIMble` store. Simulated windows are assumed to be independent and can be analysed in the same way as real data parsed via `parse`. This allows users to perform parametric bootstraps on both global (genome-wide) and local (window-wise) estimates and to quantify the power and potential biases (due to model misspecification) of `gIMble` analyses, in particular the assumption of no recombination within blocks. Parametric bootstraps on local estimates are set up by providing the key of the corresponding `gridsearch` analysis.

The module *simulate* uses `demes` [84] internally for unequivocal model specification. `gIMble` can also output the results of `optimize` as a `demes`-readable yaml-file, allowing users to connect `gIMble` to the growing list of other population genetics tools that support the `demes` format [39, 85–89].

## Heliconius analyses

We provide a list of all `gIMble` commands used to analyse the *Heliconius* dataset in the Supplementary Information (`gIMble` commands).

### Sequence data

Raw reads (Illumina 150 base paired-end) (Table S1) were downloaded from ENA and quality trimmed using `fastp 0.20.1` [90] (`--length_required 50`). Trimmed reads of each read set were aligned against the chromosome-level assembly of *H. melpomene* Hmel2.5 [63] using `bwa mem 0.7.17-r1188` [91]. Alignment files were sorted with `samtools 1.12` [92] and duplicated read pairs were marked with `sambamba markdup 0.8.1` [93]. Variants were called using `freebayes v1.3.3` [94] (`-k -w -j -T 0.02 -E -1 --report-genotype-likelihood-max`).

### Data processing

We used the following `gIMble preprocess` parameters for the *Heliconius* analysis: `--min_qual 1`, `--snpgap 2`, `--min_depth 8` (i.e. 8 reads), and `--max_depth 3` (i.e. 3 × mean coverage of each BAM

file). Blocks were cut using the following parameters: `--block_length 64`, `--block_span 128`, `--max_multiallelic 3`, and `--max_missing 3`. Blocks were grouped into windows using `gIMble windows` with these parameters: `--blocks 50000` (on average, each of the 100 heterospecific sample pairs should occur in $50,000/100 = 500$ blocks within a window) and `--steps 10000`. Tallies were made using `gIMble tally` and `--kmax 2,2,2,2` for 'blocks' and 'windows', and 'windowsum' (all blocks in windows).

### Recombination map and parametric bootstrap

For each data window in the *Heliconius* analysis, we obtained direct recombination rate estimate $r_{\mathrm{bp}}$ over a range of scales as the total number of crossover events recorded by Davey et al [63]: we averaged the recombination rate estimates in *H. melpomene*, *H. cydno* and hybrid crosses in a fixed physical region (125 kb, 250 kb, 500 kb, 1 Mb, 2 Mb) centred on the window mid-point using a custom script `get_recombination_rate_KL.py`.

We performed a parametric bootstrap on the global $IM$ estimates obtained for *Heliconius*. To obtain 95 % confidence intervals (CI) of MCLEs of parameters under the best fitting global $IM$ model (Table 2), we simulated 100 replicate datasets under the inferred $IM_{\rightarrow\mathrm{mel}}$ model. Each replicate consisted of a dataset partitioned into windows analogous to the real data (i.e. 500 blocks of 64 bases sampled across $10 \times 10$ heterospecific pairs). Importantly, simulations were conditioned on the *H. melpomene* recombination rates ($r_{\mathrm{bp}}$ measured at a 1 Mb scale). Given that real data windows partially overlap and are in LD, whereas simulated windows are assumed to be statistically independent, we simulated datasets containing 50 times fewer windows than the real data (this ensures a distance of around 500 kb between windows). We estimated the 95 % CIs of data MCLEs as +/- 2SD of the MCLEs across replicates.

To estimate the uncertainty in the `gIMble` scan, we used `gIMble simulate` as follows: For each window and its associated $r_{\mathrm{bp}}$ measured at a 1 Mb scale, we simulated 100 replicates under the locally best fitting set of $\mathbf{N}$ estimates but fixing $m_e$ to $\hat{m}_e$ and $\hat{T}$ to the MCLE under the global model (Table 2). We used `gIMble gridsearch` to estimate the false positive rate as the fraction of simulation replicates for which $\Delta_B > 0$.

## Power analysis

While the choice of block length involves a trade-off between power and bias, the definition of windows involves a trade-off between power and resolution. Our analysis scanned the *Heliconius* genomes for barriers using windows on the scale of 100 kb (i.e. $50,000 \times 64$ base blocks),but we find that given the *Heliconius* background demography, there is high power to detect genomic barriers for a wide range of window sizes (Fig S10).

We quantified the impact of window size, recombination rate and sample size on the power to detect barriers using `gIMble simulate` and two extreme barrier definitions: the complete absence of gene flow ($m_e^* = 0$, $\Delta_{B,0}$), or a very relaxed definition of a reduction in $m_e$ by half ($m_e^* < \hat{m}_e/2$, $\Delta_{B,0.5}$). Note that the latter is more permissive than the weaker barrier definition we used in the *Heliconius* scan ($\Delta_{B,0.2}$). When fitting window-wise models, the split time $T$ was fixed to the background value while $N_e$ parameters were allowed to vary freely. We assumed an average per base and generation recombination rate $r_{\text{bp}} = 1.89 \times 10^{-8}$ (given a male map length of 1083 cM and a genome size of 272.6 Mb [63]). ROC curves were constructed using 1,000 replicate simulations under the background IM model (Table 2) and background model with a reduced local $m_e$ ($m_i = 0$ or $\hat{m}_e/2$).

Figure S11 shows the relationship between the recombination rate and the power to detect barriers. The impact of recombination is two-fold: on the one hand, with an increase in recombination rate we expect to see more topologies within a single window. On the other hand, an increase in recombination for the same block size increases the probability of observing four-gamete-test violations. Blocks containing mutation configurations that cannot be placed on a single topology are non-informative in this framework. Consistently leaving out a particular subset of topologies might however bias the inference. Increasing the recombination rate therefore increases power but also leads to bias.

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# 1 Supplementary section

# Supplementary Information

## gIMble commands

```
# gimble parse (elapsed: 00h:22m:30.888s)
~/gIMble/gimble parse \
    -g input/heliconius_20220202.genomefile \
    -v input/heliconius_20220202.vcf.gz \
    -b input/heliconius_20220202.non_genic_non_repeats.bed \
    -s input/heliconius_20220202.samples.csv \
    -z heliconius.v1

# gimble blocks (elapsed: 01h:25m:29.398s)
~/gIMble/gimble blocks -z heliconius.v1.z -l 64

# gimble windows (elapsed: 00h:34m:07.976s)
~/gIMble/gimble windows -z heliconius.v1.z -w 50000 -s 10000

# gimble tally
## tally of blocks (elapsed: 00h:07m:43.094s)
~/gIMble/gimble tally -z heliconius.v1.z -d blocks -l blocks
## tally of windows (elapsed: 00h:32m:06.685s)
~/gIMble/gimble tally -z heliconius.v1.z -d windows -l windows

# gimble optimize

## optimize windows-windowsum tally under model DIV (elapsed: 00h:03m:48.604s)
~/gIMble/gimble optimize -z heliconius.v1.z -d tally/windows -w \
    -r A -u 2.9e-9 -g CRS2 -p 1 -s midpoint -i 10000 \
    -A=100_000,3_000_000 -B=100_000,3_000_000 \
    -C=100_000,3_000_000 -T=0,5_000_000 -m DIV -l DIV

## optimize windows-windowsum tally under model MIG_AB (elapsed: 00h:03m:15.508s)
~/gIMble/gimble optimize -z heliconius.v1.z -d tally/windows -w \
    -r A -u 2.9e-9 -g CRS2 -p 1 -s midpoint -i 10000 \
    -A=100_000,3_000_000 -B=100_000,3_000_000 \
    -M=1e-10,2.21E-06 -m MIG_AB -l MIG_AB

## optimize windows-windowsum tally under model MIG_BA (elapsed: 00h:03m:14.747s)
~/gIMble/gimble optimize -z heliconius.v1.z -d tally/windows -w \
    -r A -u 2.9e-9 -g CRS2 -p 1 -s midpoint -i 10000 \
    -A=100_000,3_000_000 -B=100_000,3_000_000 \
```

```
    -M=1e-10,2.21E-06 -m MIG_BA -l MIG_BA

## optimize windows-windowsum tally under model IM_AB (elapsed: 01h:38m:10.970s)
~/gIMble/gimble optimize -z heliconius.v1.z -d tally/windows -w \
    -r A -u 2.9e-9 -g CRS2 -p 1 -s midpoint -i 10000 \
    -A=100_000,3_000_000 -B=100_000,3_000_000 \
    -C=100_000,3_000_000 -T=0,5_000_000 \
    -M=0,2.21E-06 -m IM_AB -l IM_AB

## optimize windows-windowsum tally under model IM_BA (elapsed: 00h:47m:37.386s)
~/gIMble/gimble optimize -z heliconius.v1.z -d tally/windows -w \
    -r A -u 2.9e-9 -g CRS2 -p 1 -s midpoint -i 10000 \
    -A=100_000,3_000_000 -B=100_000,3_000_000 \
    -C=100_000,3_000_000 -T=0,5_000_000 \
    -M=0,2.21E-06 -m IM_BA -l IM_BA

# gimble makegrid (elapsed: 00h:06m:47.222s)
~/gIMble/gimble makegrid -z heliconius.v1.z -m IM_BA \
    -b 64 -r A -u 2.9e-9 -k 2,2,2,2 \
    -A=200_000,3_000_000,12,lin \
    -B=100_000,2_000_000,12,lin \
    -C 100_000,2_000_000,12,lin \
    -T 4_256_034 -M 0,2.21E-06,16,lin \
    -p 48 -e 19 -l IM_BA_final

# gimble gridsearch of windows (elapsed: 00h:01m:35.828s)
~/gIMble/gimble gridsearch -z heliconius.v1.z \
    -g makegrid/IM_BA_final -d tally/windows \
    -p 50 -c 1000

# gimble simulate

## gimble simulate of best model

## gimble simulate of unconstrained gridsearch result (elapsed: 16h:24m:34.610s)
~/gIMble/gimble simulate -z heliconius.v1.z \
    --seed 19 --replicates 100 --windows 11217 --blocks 500 \
    --block_length 64 -a 10 -b 10 \
    --gridsearch_key gridsearch/windows/IM_BA_final \
    --rec_map heliconius.v1.rec_final_Hmel_1000kb_2023.txt \
    -k 2,2,2,2 -s IM_BA_grid_unconstrained -p 55 \
    -u 2.9e-9

## gridsearch of simulation (elapsed: 03h:00m:43.823s)
~/gIMble/gimble gridsearch -z heliconius.v1.z \
```

```
    -g makegrid/IM_BA_final \
    -d simulate/IM_BA_grid_unconstrained \
    -p 10 -c 500

## gimble simulate of  constrained gridsearch result (elapsed: 21h:27m:05.082s)
~/gIMble/gimble simulate -z heliconius.v1.z \
    --seed 19 --replicates 100 --windows 11217 --blocks 500 \
    --block_length 64 -a 10 -b 10 \
    --gridsearch_key gridsearch/windows/IM_BA_final \
    -t me=7.366666666666667e-07 \
    --rec_map heliconius.v1.rec_final_Hmel_1000kb_2023.txt \
    -k 2,2,2,2 -s IM_BA_grid_constrained \
    -p 30 -u 2.9e-9

## gridsearch of simulation (elapsed: 07h:41m:01.082s)
~/gIMble/gimble gridsearch -z heliconius.v1.z \
    -g makegrid/IM_BA_final \
    -d simulate/IM_BA_grid_constrained \
    -p 10 -c 500
```

**Listing 1.** gIMble commands

**Table S1.** *Heliconius* Illumina read sets downloaded from ENA. COV-mean and COV-sd represent mean and standard deviation of read coverage when mapping quality-trimmed reads against the Hmel2.5 assembly.

| Read group ID | ENA run accession | sex | taxon | COV-mean | COV-sd |
|---|---|---|---|---|---|
| chi.CAM25091.f | ERR2298220 | F | *H. cydno chioneus* | 27.71 | 78.67 |
| chi.CAM25137.f | ERR2298222 | F | *H. cydno chioneus* | 29.6 | 77.41 |
| chi.CAM580.m | ERR2298196 | M | *H. cydno chioneus* | 23.41 | 44.5 |
| chi.CAM582.m | ERR2298197 | M | *H. cydno chioneus* | 25.7 | 48.83 |
| chi.CAM585.m | ERR2298199 | M | *H. cydno chioneus* | 24.96 | 52.37 |
| chi.CAM586.m | ERR2298200 | M | *H. cydno chioneus* | 24.3 | 49.73 |
| chi.CJ553.m | ERR260295 | M | *H. cydno chioneus* | 37.62 | 68.71 |
| chi.CJ560.m | ERR260296 | M | *H. cydno chioneus* | 37.11 | 67.13 |
| chi.CJ564.m | ERR260297 | M | *H. cydno chioneus* | 41.27 | 78.71 |
| chi.CJ565.m | ERR260298 | M | *H. cydno chioneus* | 48.36 | 76.16 |
| ros.CAM1841.m | ERR2298205 | M | *H. melpomene rosina* | 28.7 | 47.35 |
| ros.CAM1880.m | ERR2298206 | M | *H. melpomene rosina* | 31.94 | 52.82 |
| ros.CAM2045.m | ERR2298208 | M | *H. melpomene rosina* | 23.19 | 30.51 |
| ros.CAM2059.m | ERR2298209 | M | *H. melpomene rosina* | 26.35 | 34.71 |
| ros.CAM2519.m | ERR2298210 | M | *H. melpomene rosina* | 28.48 | 31.07 |
| ros.CAM2552.m | ERR2298211 | M | *H. melpomene rosina* | 25.16 | 56.86 |
| ros.CJ2071.m | ERR260280 | M | *H. melpomene rosina* | 38.2 | 57.43 |
| ros.CJ531.m | ERR260277 | M | *H. melpomene rosina* | 28.26 | 44.68 |
| ros.CJ533.m | ERR260278 | M | *H. melpomene rosina* | 28.27 | 43.12 |
| ros.CJ546.m | ERR260279 | M | *H. melpomene rosina* | 27.97 | 45.36 |

**Table S2.** Estimates of demographic history of *H. melpomene* and *H. cydno* inferred using `gIMble` *optimise* under models allowing either migration only ($MIG$), strict isolation ($DIV$) or isolation with migration ($IM$). Inference based on the total count of bSFS configurations of blocks that contribute to overlapping windows (windowsum) is near identical to the total bSFS (blocks). By default `gIMble` parameter estimates are scaled in absolute units, i.e. number of individuals and generations given a user specified $\mu$. We have converted $T$ to millions of years (MYA) assuming four generations per year.

| model | $\ln CL$ | $N_{\text{mel}}$ | $N_{\text{cyd}}$ | $N_{\text{anc}}$ | $T$ (MY) | $m$ |
|---|---|---|---|---|---|---|
| blocks | | | | | | |
| $DIV$ | $-394,846,023$ | $1.12 \times 10^6$ | $1.49 \times 10^6$ | $1.42 \times 10^6$ | 0.243 | |
| $MIG_{\rightarrow cyd}$ | $-395,756,127$ | $1.255 \times 10^6$ | $6.96 \times 10^5$ | | | $7.86 \times 10^{-7}$ |
| $MIG_{\rightarrow mel}$ | $-393,957,100$ | $0.529 \times 10^5$ | $1.29 \times 10^6$ | | | $7.80 \times 10^{-7}$ |
| $IM_{\rightarrow cyd}$ | $-394,051,624$ | $1.14 \times 10^6$ | $1.40 \times 10^6$ | $1.40 \times 10^6$ | 0.261 | $7.50 \times 10^{-8}$ |
| $IM_{\rightarrow mel}$ | $-393,767,380$ | $5.45 \times 10^5$ | $1.41 \times 10^6$ | $9.23 \times 10^5$ | 1.064 | $7.38 \times 10^{-7}$ |
| windowsum | | | | | | |
| $DIV$ | $-391,893,600$ | $1.13 \times 10^6$ | $1.50 \times 10^6$ | $1.42 \times 10^6$ | 0.243 | |
| $MIG_{\rightarrow cyd}$ | $-392,780,725$ | $1.26 \times 10^6$ | $7.00 \times 10^5$ | | | $7.91 \times 10^{-7}$ |
| $MIG_{\rightarrow mel}$ | $-391,030,511$ | $5.33 \times 10^5$ | $1.30 \times 10^6$ | | | $7.85 \times 10^{-7}$ |
| $IM_{\rightarrow cyd}$ | $-391,889,506$ | $1.16 \times 10^6$ | $1.41 \times 10^6$ | $1.40 \times 10^6$ | 0.263 | $8.30 \times 10^{-8}$ |
| $IM_{\rightarrow mel}$ | $-390,836,229$ | $5.49 \times 10^5$ | $1.415 \times 10^6$ | $9.28 \times 10^5$ | 1.054 | $7.41 \times 10^{-7}$ |

**Fig S1.** The *Heliconius* genome was analysed in sliding windows comprised of $L = 50,000$ (64 b) pair-blocks of intergenic sequence. Since each block is sampled in a single heterospecific pair, this corresponds to a minimum window span of $(L * 64)/(n_A * n_B) = 32kb$ (black dashed line). Windows show a wide distribution of spans with a median (103 kb, red line) substantially greater than this minimum.

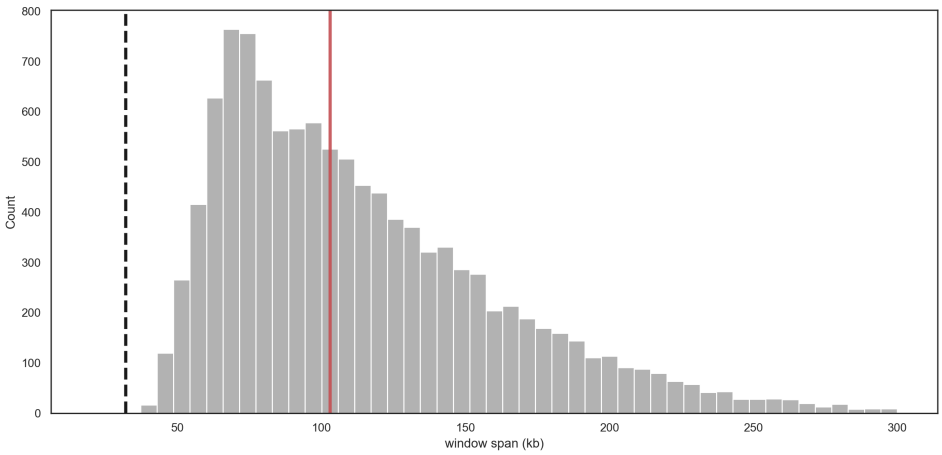

**Fig S2.** Mean heterozygosity $H$ in *H. cydno* (left) and *H. melpomene* (right) against pairwise divergence ($d_{xy}$) in intergenic windows (see Fig. S1). The upper 5 % tail of window-wise $F_{\mathrm{ST}}$ is shown in blue.

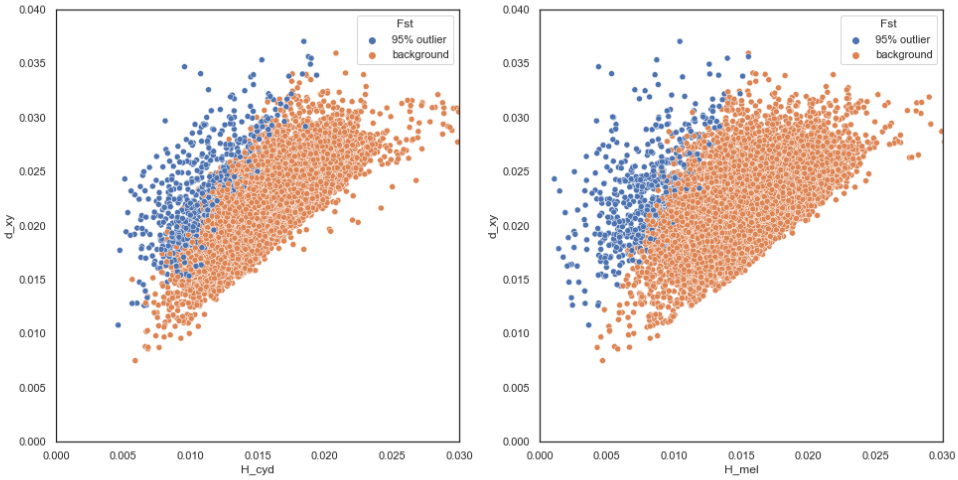

**Fig S3. Top**: Divergence ($d_{xy}$, black), heterozygosity in *H. cydno* ($H_{cyd}$, green) and *H. melpomene* ($H_{mel}$, blue) along the genome in windows of intergenic sequence (50,000 × 64 b blocks). Mean values are shown as horizontal lines. **Bottom**: $F_{\mathrm{ST}}$ computed from the summaries above. The upper 5 % tail of window-wise $F_{\mathrm{ST}}$ is delineated by the black dashed line, the median is shown in gray.

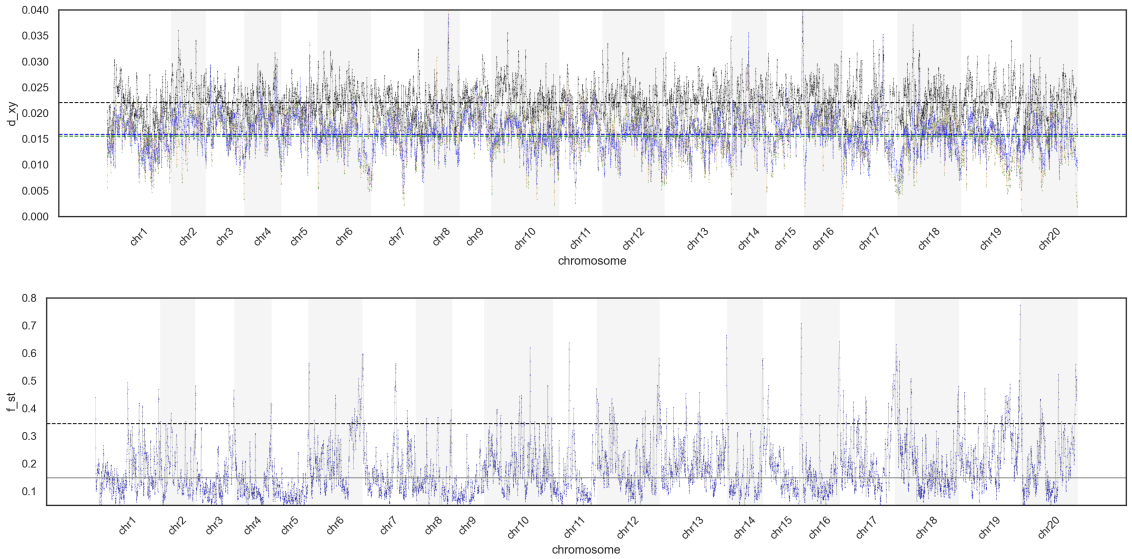

**Fig S4.** The best supported global history of divergence and gene flow between *H. melpomene* and *H. cydno* inferred using `gIMble optimize`.

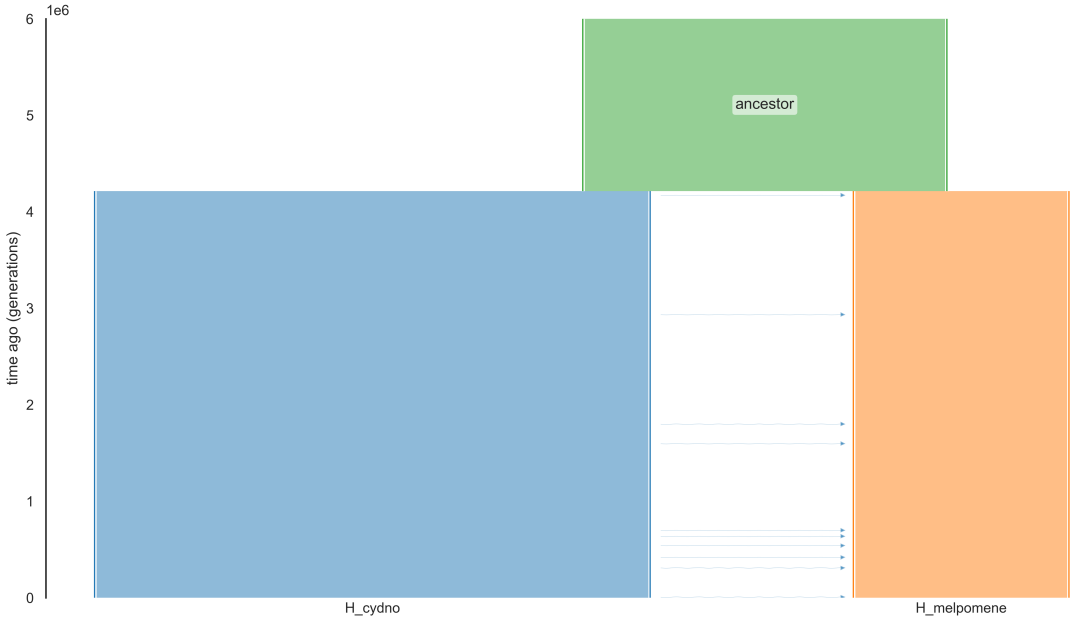

**Fig S5.** $F_{\mathrm{ST}}$ between *H. melpomene* and *H. cydno* is negatively correlated with recombination rate.

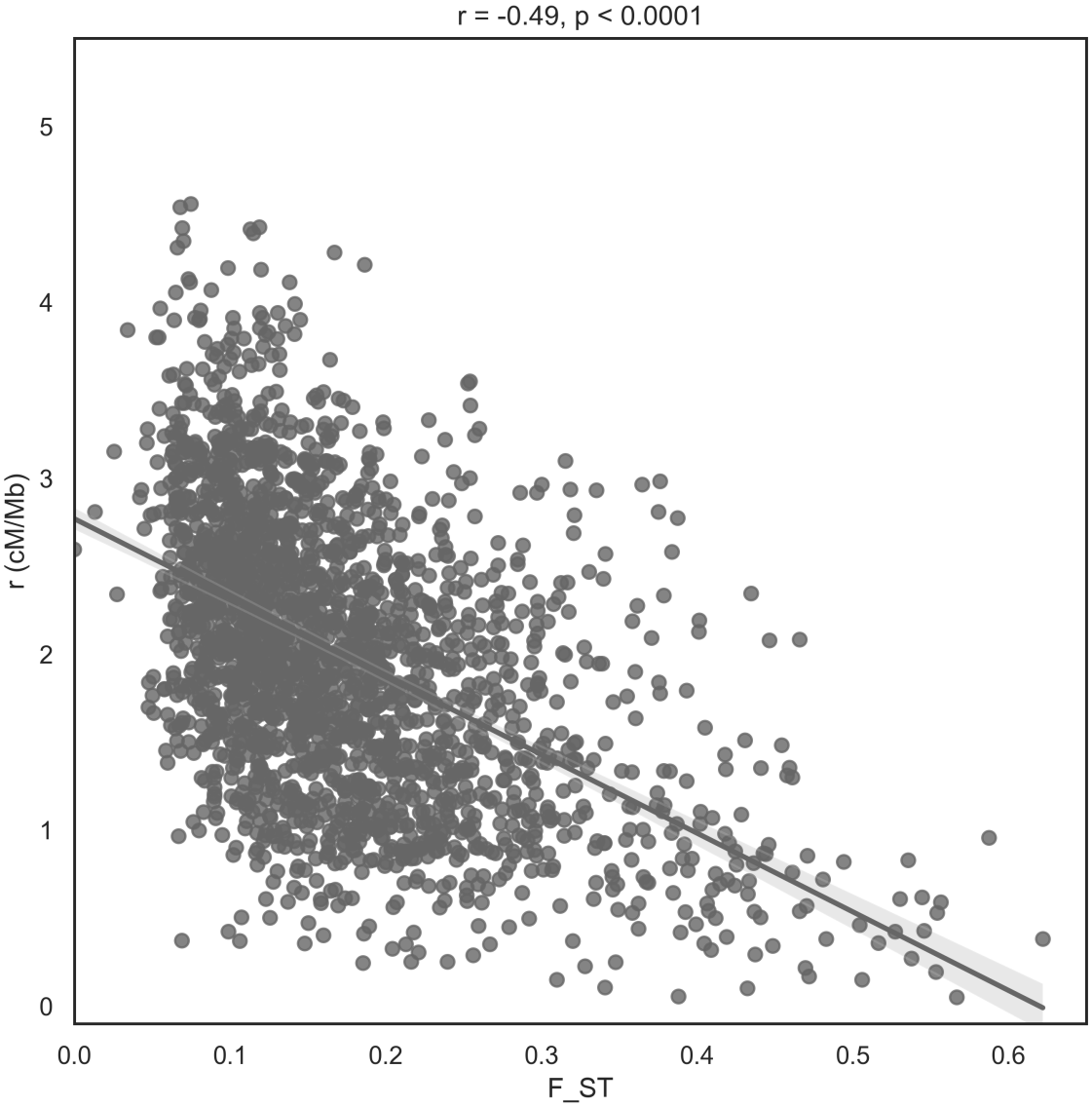

**Fig S6.** Local estimates of $N_e$ in *H. melpomene* (left) and *H. cydno* (right) are negatively correlated with chromosome length.

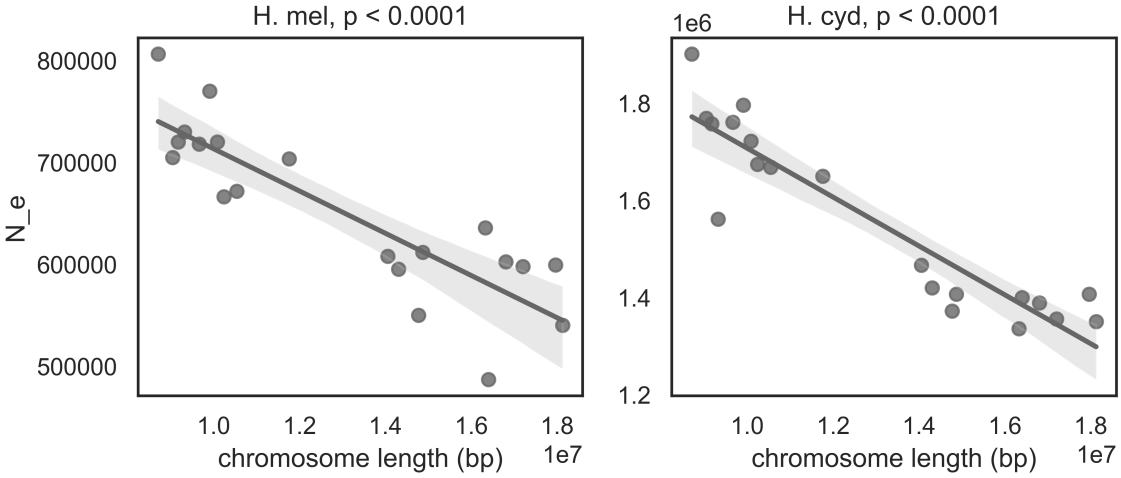

**Fig S7.** Estimates of local $N_e$ in *H. melpomene* and *H. cydno* and $m_e$ are correlated positively with the recombination rate (top) and negatively with the density of CDS (bottom).

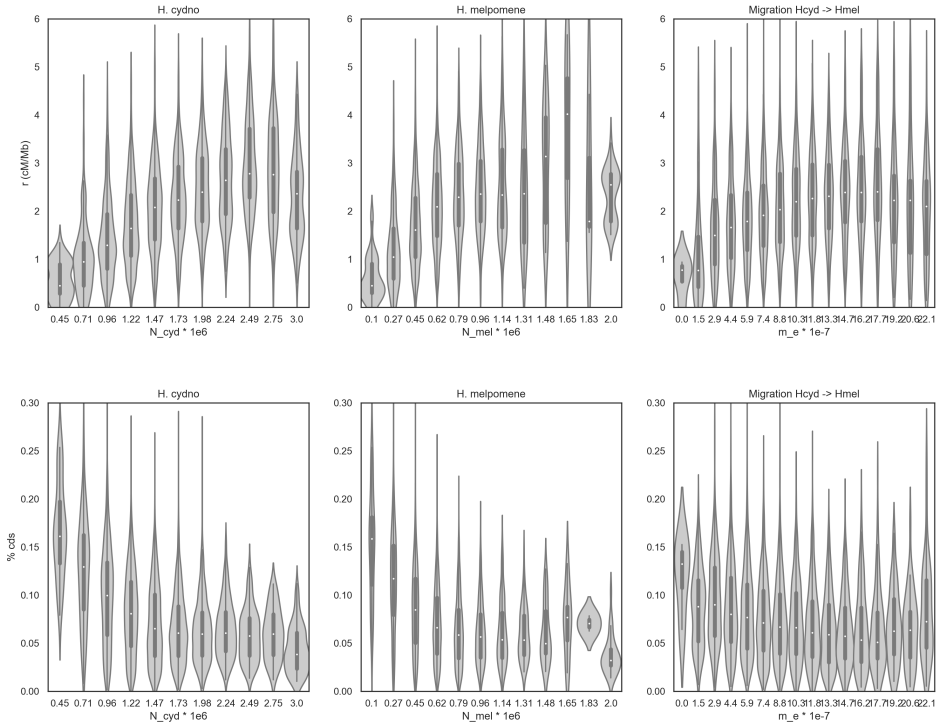

**Fig S8.** Goodness-of-fit of the aggregate migration–selection model by Aeschbacher et al. [9] fit to the $m_e$ estimates inferred with `gIMble` from *H. melpomene* and *H. cydno*. The goodness-of-fit is expressed in terms of the residual standard error (left) and the natural logarithm of the difference in AIC from the best-fitting model (shifted by 1 to avoid indefinite values; right), and for combinations of estimation ranges for the percentage of CDS and the recombination rate $r_{\mathrm{bp}}$. Darker shading indicates a better fit.

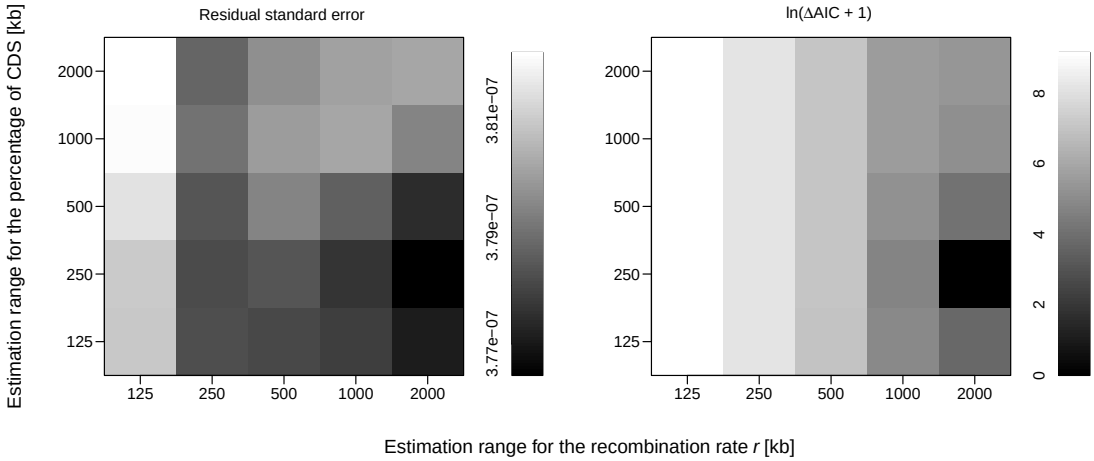

**Fig S9.** Local point estimates of $m_e$ (middle of each subplot) and $\Delta_{B,02}$ (bottom of each subplot) for the three chromosomes containing large effect wing patterning genes. Results from the (best-fitting) $IM_{\rightarrow mel}$ model are in black, results from the $MIG_{\rightarrow mel}$ model are shown in blue, and the predicted $m_e$ under a polygenic model is shown in green. For each chromsome, the $\Delta_{B,02} > 0$ barriers under the $IM_{\rightarrow mel}$ model are shown in red (top of each subplot). $\Delta_{B,02}$ identifies all three known wing pattern genes as barriers (dark blue):*wnt-A*, *cortex* and *optix*) on chromosomes 10 (top), 15 (centre) and 18 (bottom), respectively. Given that causal sites may be situated in regulatory regions, which in the case of *optix* extends to ≈ 100 kb away from the gene [56], we have highlighted the 100 kb up and downstream in lighter blue. In orange, we highlight an additional $\Delta_{B,02} > 0$ barrier on chromosome 18 that coincides with the *Grik2* and *regucalcin2* that are associated with male wing pattern preference.

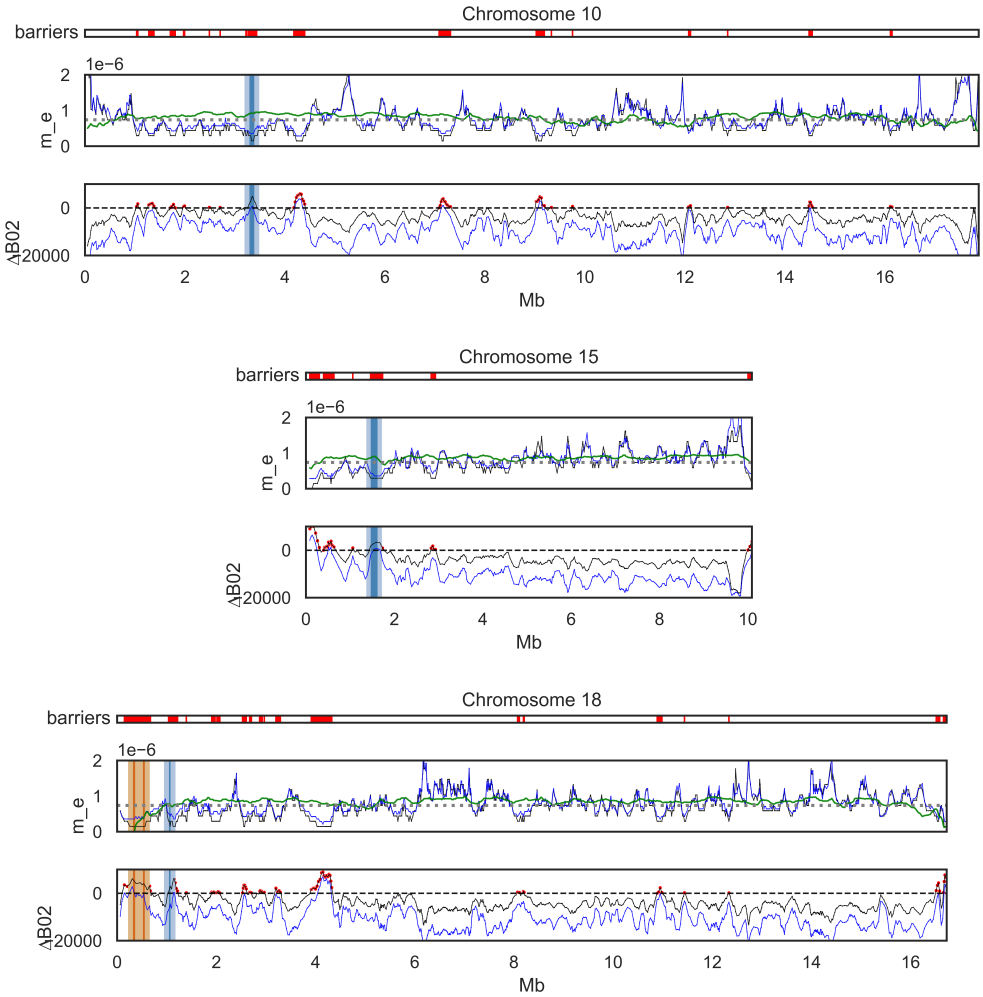

**Fig S10.** The impact of window size on the power to detect a local reduction in $m_e$ relative to the estimated background migration rate. The black line corresponds to the window size used in the *Heliconius* analysis.

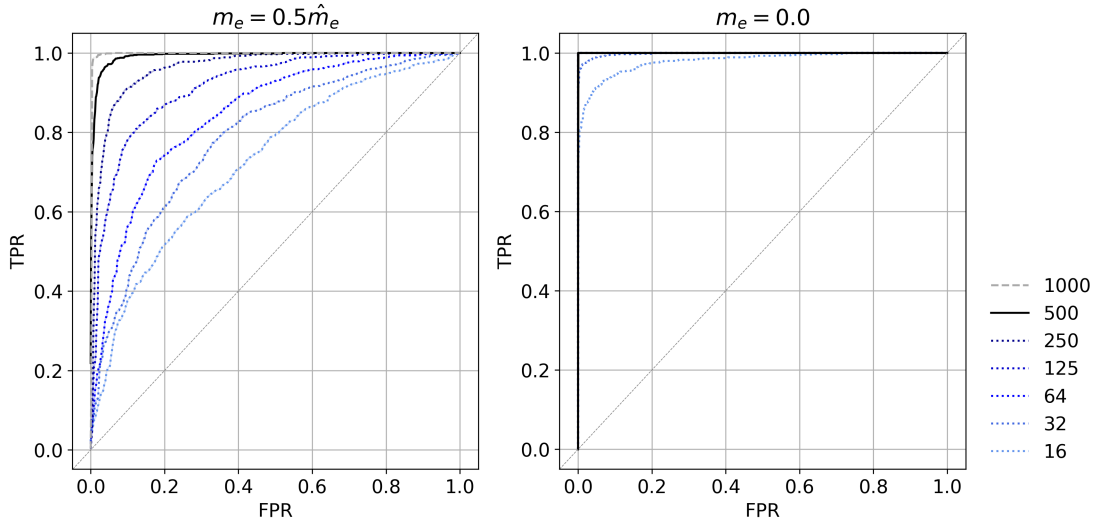
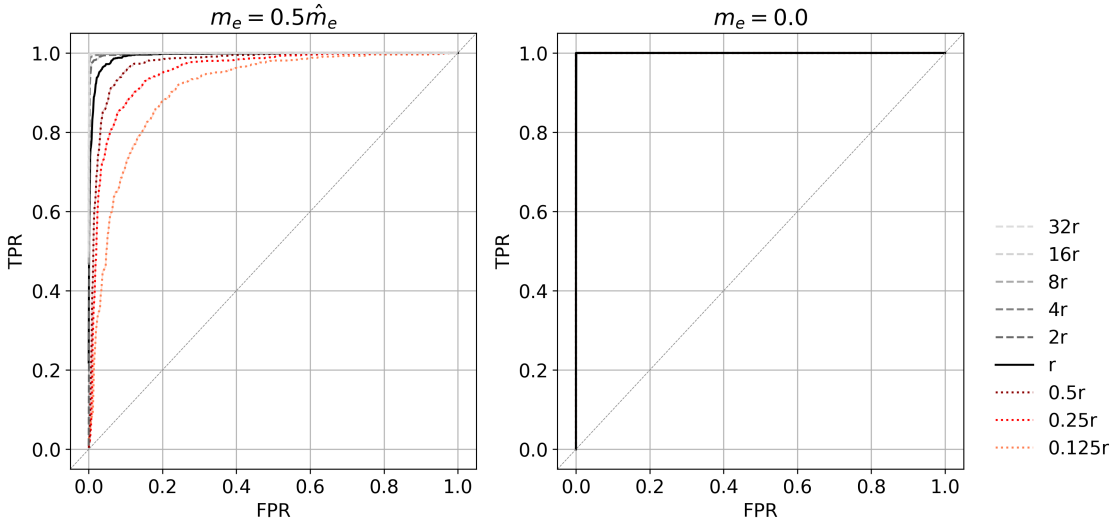
**Fig S11.** The impact of $r_{\mathrm{bp}}$ on the power to detect a local reduction in $m_e$ relative to the estimated background migration rate. The black line indicates the average recombination rate in *Heliconius*.