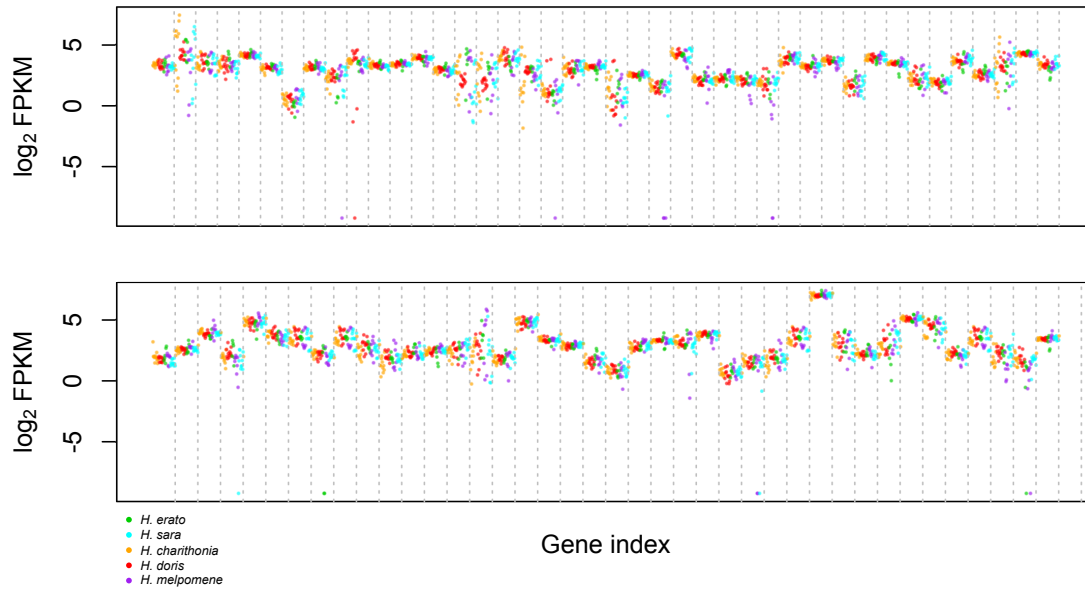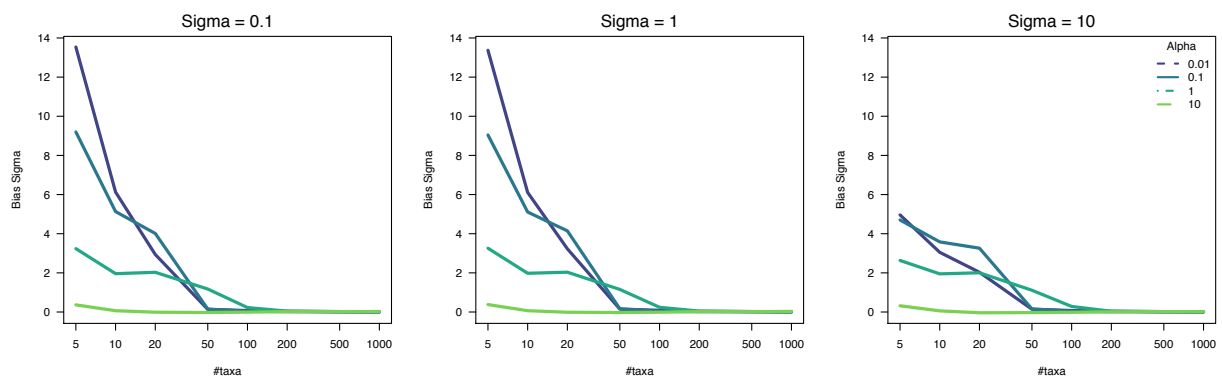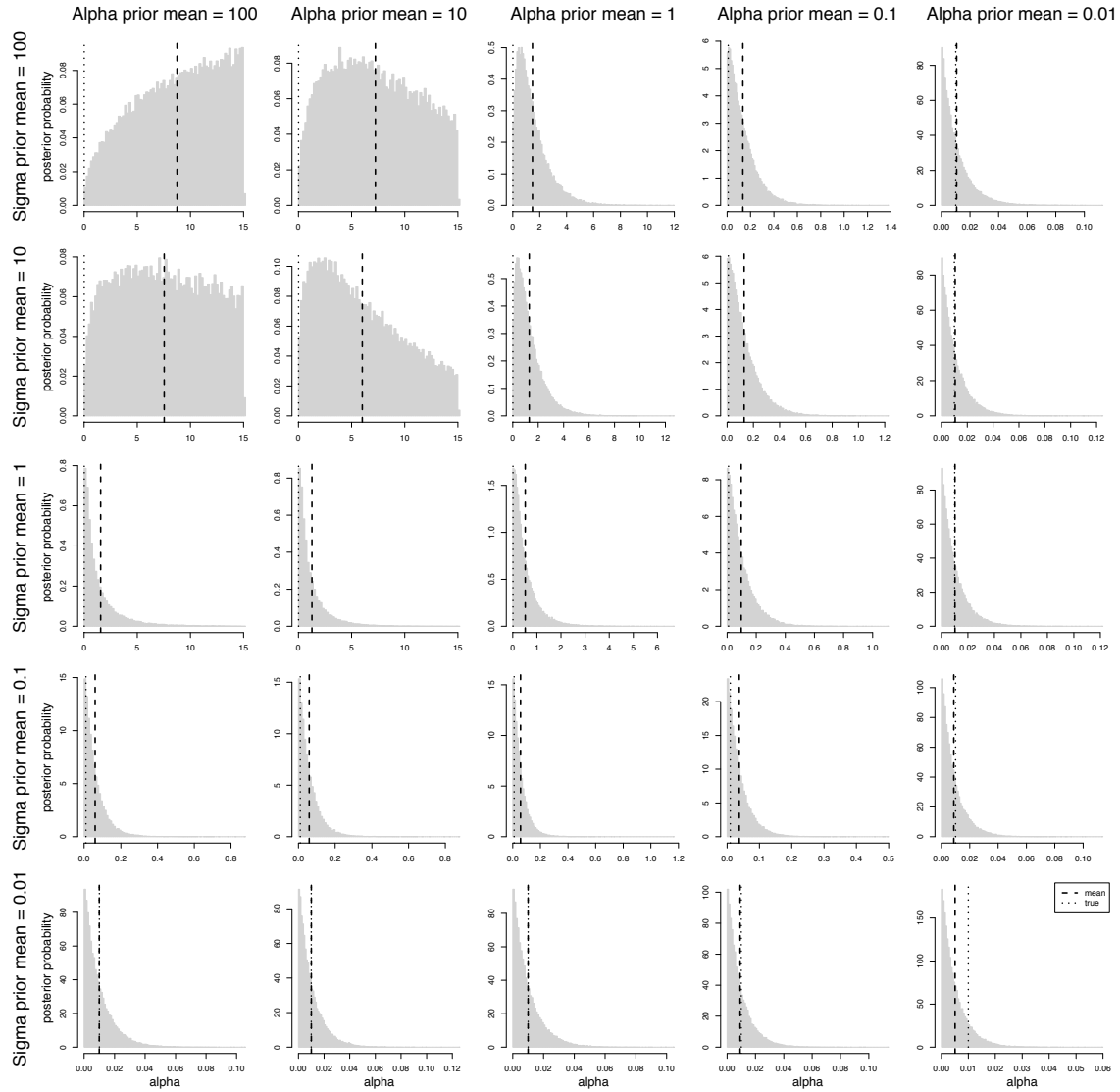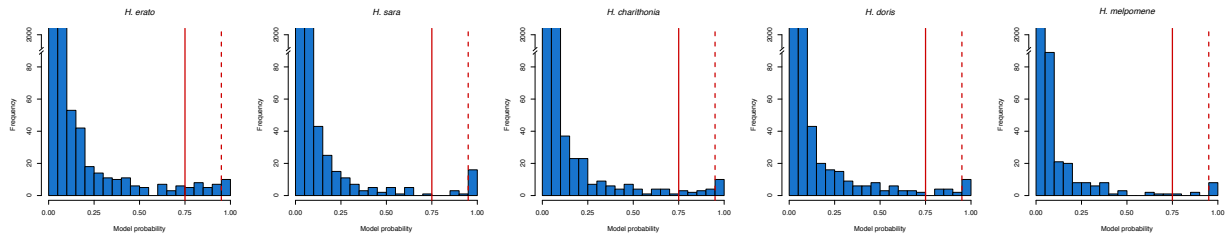# 1   Supplementary figures

2

Figure S1. Orthoclusters with conserved gene expression values across five *Heliconius*

species. The orthoclusters with σ^2 not significantly larger than zero from Figure 4 were used
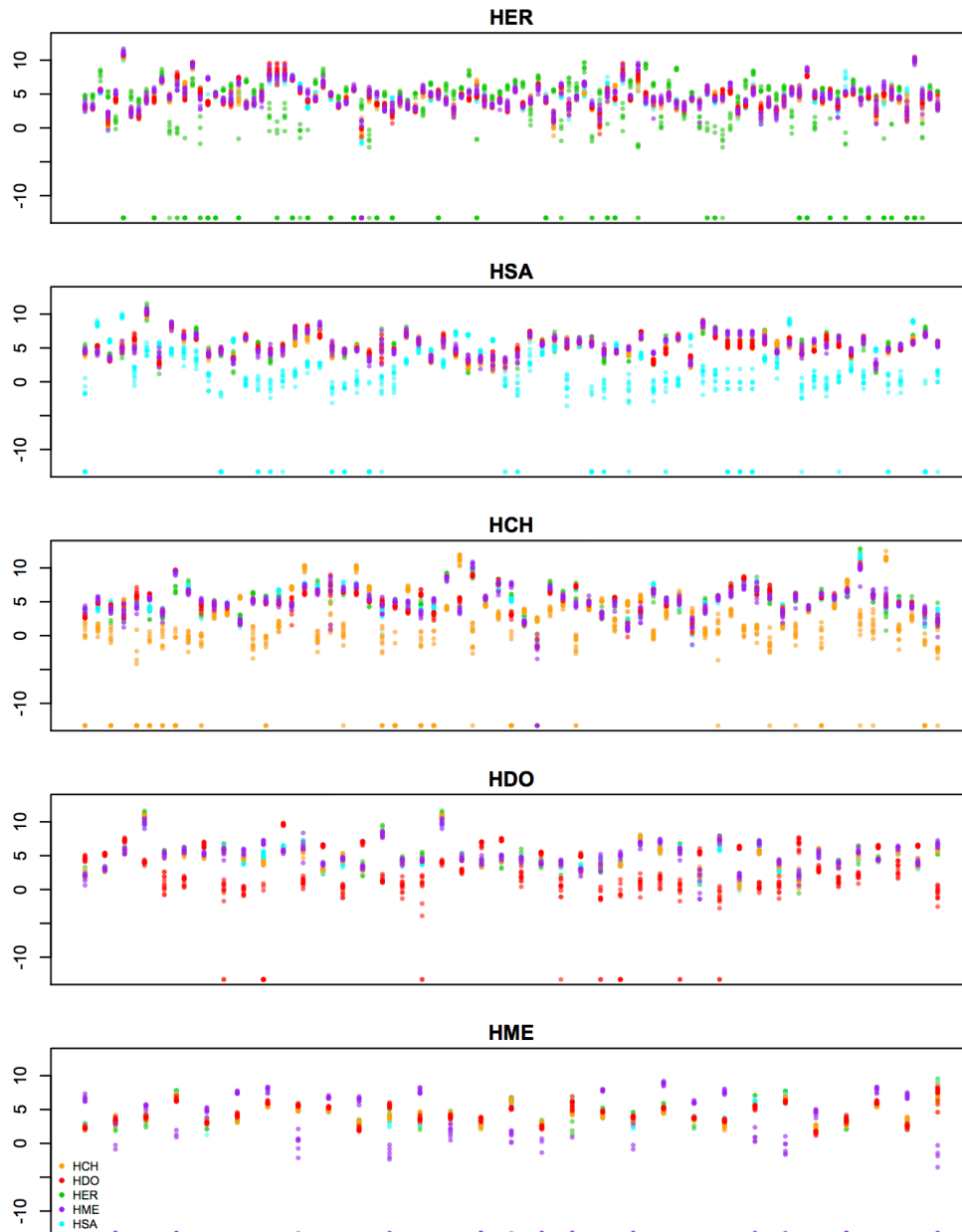
here.

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Figure S2. Simulation study for the assessment of parameter estimation bias under an OU-

model. Parameter (σ^2) bias estimation through 1000 simulations under sigma values ranging

from 0.1 – 10. Simulations were performed for phylogenies with sizes ranging from 5 to 1000
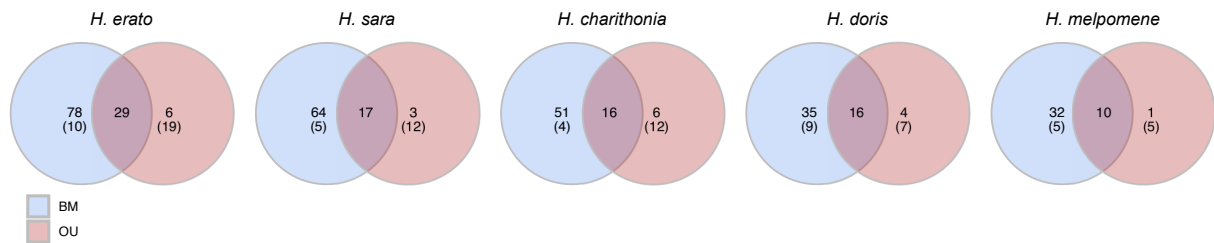
taxa.

Figure S3. Posterior distribution for a single simulated dataset (see Figure S2). Here we used

a phylogeny with 5 taxa, an alpha parameter of 0.01, and a sigma parameter of 0.1. The plot

shows the bias of the mean posterior estimate (dashed line) compared with the true parameter

(dotted line). This example shows why, contrary to intuition, estimates of alpha are biased

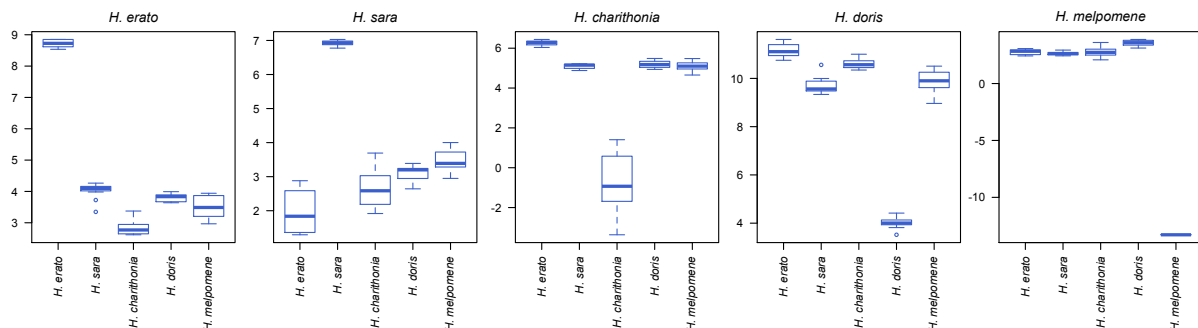towards larger values because the posterior distribution is skewed.

Figure S4. Model probability when testing for directional selection. Significance is shown at model probability > 0.75 (solid red, Bayes factor > 3, positive support) and model probability > 0.95 (solid red, Bayes factor > 20, strong support).
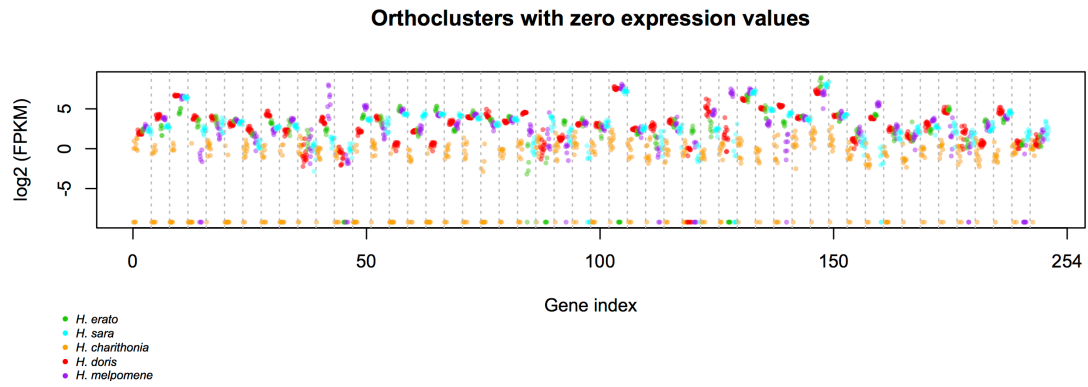
27 **Figure S5.** Orthoclusters where a shift in gene expression optima was detected along a

28 particular terminal *Heliconius* species branch. Panels show shifts in the HER branch (A), in

29 HSA (B), in HCH (C), in HDO (D) and in the HME branch (E).

30

31

32 **Figure S6.** Venn Diagram depicting the number of genes showing a branch-specific shift in

33 σ^2 tested by an BM (blue) model and those genes showing a shift in gene expression optimum

34 assessed with an OU model (red). In parenthesis are shown the number of genes contributing

35 to the overlapping region identified by each of the method.

36

37

38 **Figure S7.** Examples of branch specific shifts in evolutionary rates for each of the five

39 *Heliconius* species. Boxplots depict the distribution of $\log_2$ transformed FPKM expression

40 values. Horizontal solid line indicates median expression level. Shift in: *H. erato*:

41 OrthoGroup_1567_clean, annotated as protein domain with unknown function (Appendix 1).

42 *H. sara*: OrthoGroup_530_clean, annotated as vinculin family involved in cell adhesion and

43 cytoskeleton. *H. charithonia*: OrthoGroup_1276_clean, annotated as photoreceptor

44 hydrogenase. *H. doris*: OrthoGroup_449_clean, annotated as Tropomyosin 1, involved in

45 dendrite morphogenesis.

Orthoclusters with zero expression values

- H. erato
- H. sara
- H. charithonia
- H. doris
- H. melpomene

46

47 **Figure S8.** *Heliconius* orthoclusters where an orthology was identified with zero expression

48 values. A total of 254 orthoclusters were found.