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Supplementary Information for

A release from developmental bias accelerates morphological diversification in butterfly eyespots

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Other supplementary materials for this manuscript include the following:
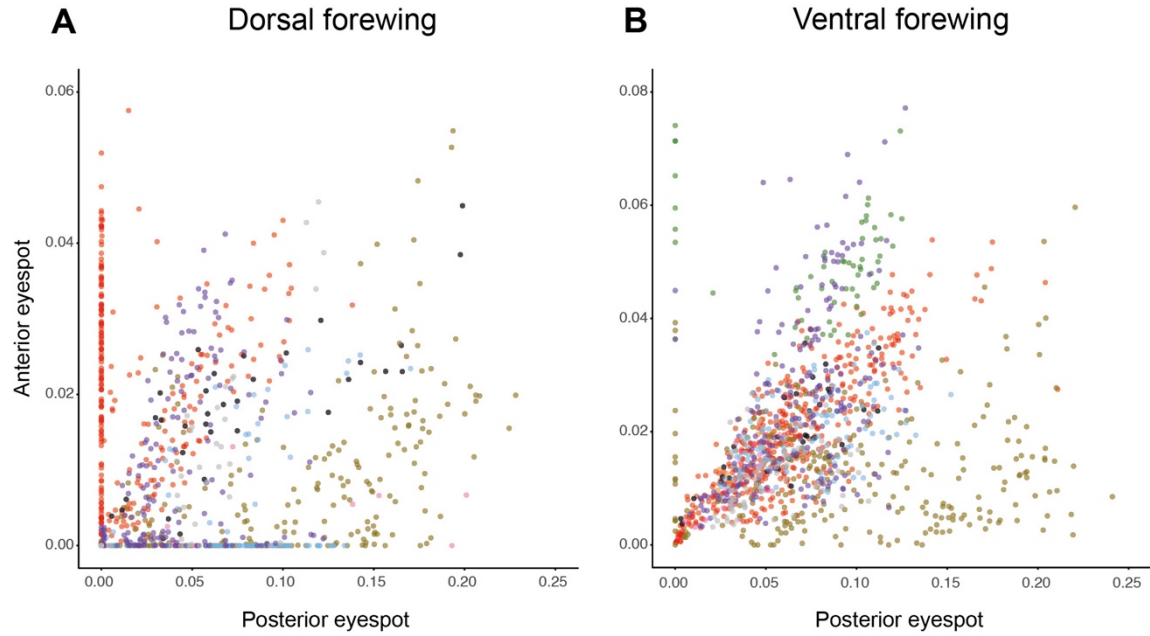
> Datasets S1 to S2

Fig. S1. Relative size of forewing eyespots. Plots show the distribution of the total area (including all color elements from center to the outer edge of the yellow ring if present) of forewing eyespots for 1249 measured specimens of Mycalesina butterflies representing 288 taxa. The data shown in the plots is the relative area of eyespots calculated by dividing the absolute eyespot area by an index of wing size squared (see Material and Methods). (*A*) Dorsal wing surface (16 outliers not shown) and (*B*) Ventral wing surface (5 outliers not shown). The different genera are color coded following the color scheme of Figure 2 in the main text.
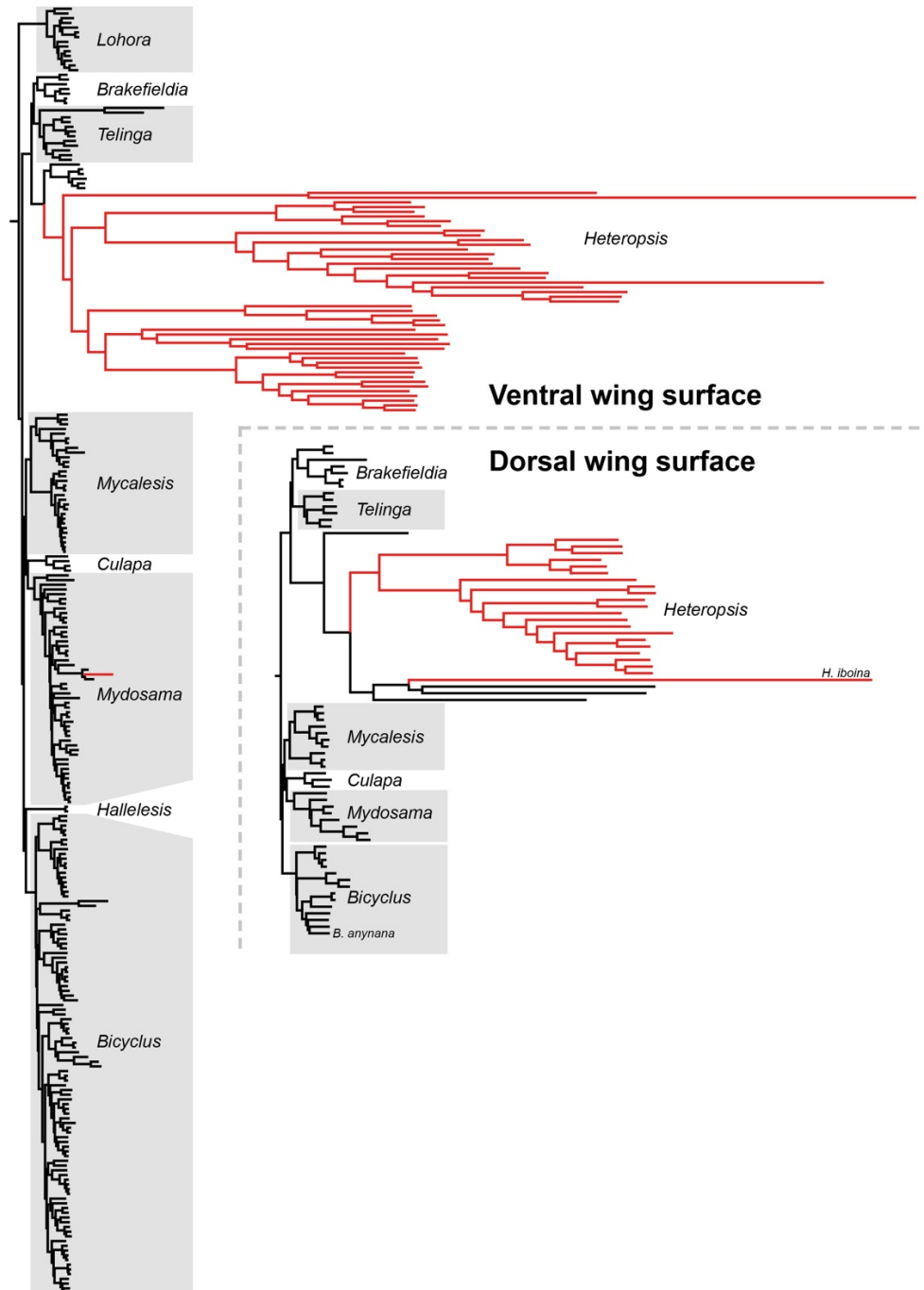
Fig. S2. Comparison of evolutionary dynamics on dorsal and ventral wing surfaces. Estimated historical rate of morphological evolution in forewing eyespot similarity on the dorsal and ventral wing surface. Branches in red show evidence of significant positive phenotypic selection following the method suggested by Baker *et al.* (1). The data shown for the ventral surface is the same as displayed in the circular tree in Figure 2 in the main text. Sample size for the dorsal surface was reduced owing to taxa missing either one of the eyespots on this surface or because the eyespots did not contain yellow outer rings. In the dorsal eyespot phylogeny the position of the two species for which grafting experiments have been performed (*Bicyclus anynana* and *Heteropsis iboina*) are indicated.
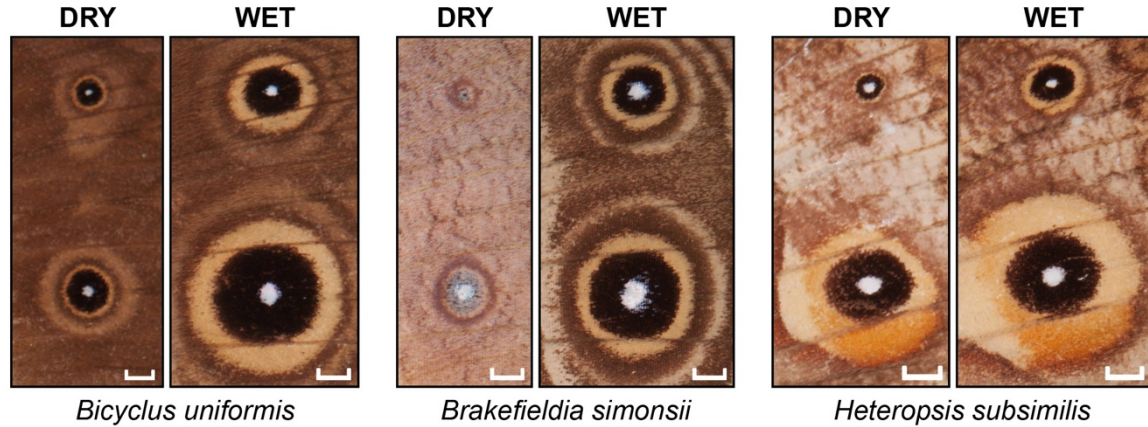
| DRY | WET | DRY | WET | DRY | WET |

Bicyclus uniformis   *Brakefieldia simonsii*   *Heteropsis subsimilis*

Fig. S3. Examples of dry (left) and wet (right) season morphologies in Mycalesina eyespots. The anterior (top) and posterior (bottom) eyespots of the ventral forewing are shown from three representative species. In this study we focused on specimens that showed full expression of the wet season form phenotype. Dry season specimens have smaller eyespots, and the color-compositions can differ from those of the wet season specimens (e.g. *B. simonsii*), but there is no indication that they do not follow the same bias in color-composition in the majority of the species (e.g. *B. uniformis* and *B. simonsii*). The release from this developmental bias in the genus *Heteropsis* involves displaying a larger relative proportion of yellow in the posterior spot regardless of seasonal phenotype, and is represented by *H. subsilimis* in this figure. The small eyespots of the dry season form are harder to measure with high repeatability, not only because they represent smaller areas on the images, but also the nature of the wing scale cells makes them less reliable. Since each individual scale can take on only a single color, the smaller eyespots will have less regular outlines, and changes in single, or a few, scales can have comparatively large effects on the proportional areas. Scale bars correspond to 1 mm.

Table S1. Sources of photographed specimens.

Museum/Collection (Acronym), City, Country	Specimens
African Butterfly Research Institute (ABRI), Nairobi, Kenya	513
Natural History Museum (NHMUK), London, UK	429
Museum für Naturkunde (MNHB), Berlin, Germany	151
Oxford University Museum of Natural History (OUMNH), Oxford, UK	44
Naturalis Biodiversity Center (NBNC), Leiden, The Netherlands	34
Natutralhistorische Museum (NHMW), Vienna, Austria	18
Musée royal de l'Afrique central (MRAC), Tervuren, Belgium	23
Personal research collection of Oskar Brattström	18
McGuire Center for Lepidoptera & Biodiversity (MGCL), Gainesville, USA	11
Stuttgart State Museum of Natural History (SMNS), Stuttgart, Germany	3
Swedish Museum of Natural History (NHRS), Stockholm, Sweden	3
Personal research collection of Peter Roos	1
Personal research collection of Robert Tropek	1
Total	**1249**

Table S2. Taxonomic distribution of taxa analyzed shown for all currently recognized genera of Mycalesina. Mean number of measured samples per taxa, grouped by genera, is also shown.

Genus	Total taxa	Species	Additional subspecies	Undescribed taxa	Total samples (mean/taxon)
Bicyclus	102	93	7	2	485 (4.75)
Brakefieldia	7	7	-	-	28 (4.71)
Culapa	4	4	-	-	19 (4.75)
Devyatkinia	0	0	-	-	0 (0)
Hallelesis	2	2	-	-	10 (5.00)
Heteropsis	64	62	2	2	241 (3.77)
Lohora	16	16	-	-	62 (3.88)
Mycalesis	30	19	11	-	127 (4.23)
Mydosama	51	41	9	1	218 (4.27)
Telinga	12	12	-	-	54 (4.50)
Total	**288**	**254**	**29**	**5**	**1249 (4.34)**

Table S3. Summary of repeatability tests performed by remeasuring the ventral spots from 50 specimens picked at random from the full dataset. The correlation coefficient and r2 values for the correlations between original and remeasured values are given for all raw data traits (bold) and variables calculated from the raw data (normal text).

Measurement	coeff.	r^2
Total Area M_1	**0.983**	**1.00**
Black + Focus Area M_1	**0.994**	**1.00**
Yellow Area M_1	0.949	0.98
Yellow Proportion M_1	1.004	0.93
Total Area CuA_1	**0.994**	**1.00**
Black + Focus Area CuA_1	**0.967**	**1.00**
Yellow Area CuA_1	1.021	0.99
Yellow Proportion CuA_1	1.011	0.94
Size Index	**1.011**	**1.00**
Eyespot Similarity Index	0.853	0.81

Table S4. Mean Log Marginal Likelihood for the complex Variable rates model, the simple Fixed rate model, and the resulting Mean Log Bayes Factor. Values were estimated using the Stepping Stone Sampler implemented in Bayes Traits 3.1. set to sample 1000 stones with 100000 iterations. We ran five separate analyses of each model and resulting minimum and maximum values are shown in brackets.

Wing surface	Log Marg. Lh. Variable Rates	Log Marg. Lh. Fixed Rate	Log Bayes Factor
Dorsal	-12.75 [-12.84, -12.60]	-24.04 [-24.08, -24.01]	22.6 [22.4, 23.0]
Ventral	153.98 [153.84, 154.27]	73.77 [73.71, 73.80]	160.4 [160.2, 160.9]

Table S5. Summary of grafting experiment showing number of grafts per position (one per specimen) and the number of successful eclosions with measurable effects of the transplanted tissue.

Donor	Host	Grafts	Eclosions	Ecl. rate
CuA_1	Distal M_1	112	97	86.6%
CuA_1	Distal M_2	112	95	84.8%
CuA_1	Distal M_3	113	94	83.2%
CuA_1	Proximal M_1	107	94	87.9%
CuA_1	Proximal M_2	112	93	83.0%
Total		**556**	**473**	**85.1%**

Dataset S1 (separate file). Raw data from the image analyses, input data for the evolutionary analyses, and information on all voucher specimens used in the phylogeny.

Dataset S2 (separate file). The phylogenetic consensus tree used for the evolutionary analyses.

SI References

1. J. Baker, A. Meade, M. Pagel, C. Venditti, Positive phenotypic selection inferred from phylogenies. *Biol. J. Linn.Soc.* **118,** 95–115 (2016).