SUPPLEMENTARY MATERIAL

Title: The stable isotope ecology of mycalesine butterflies: implications for plant-insect co-evolution

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Table S1: Results of experiment B. The $\delta^{13}C$ values obtained from adult tissue are significantly affected by the photosynthetic pathway of the host plant used during larval development. The effect during the final instar is greater than the effect during the first four instars; see figure 1 in the main text.

dependent variable	**fixed effects**	**F**	**df**	**P**
$\delta^{13}C$ leg tissue	Host plant first instars	101.7	1,37	<0.001
	Host plant final instar	13095.7	1,37	<0.001
$\delta^{13}C$ antenna tissue	Host plant first instars	150.2	1,37	<0.001
	Host plant final instar	9373.0	1,37	<0.001
$\delta^{13}C$ wing tissue	Host plant first instars	128.1	1,37	<0.001
	Host plant final instar	19681.4	1,37	<0.001

Table S2: Minimum adequate generalized linear model, see table 2 in the main text. A non-significant seasonal trend in host plant use towards an increased relative consumption of C_3 host plants in DSF was found (in bold).

	Estimate	**Std. Error**	**z value**	**P**
(Intercept)	2.25657	0.28849	7.822	<0.001
SpeciesSAF	-0.01912	0.39044	-0.049	0.961
SpeciesVAN	2.00724	0.30396	6.604	<0.001
SeasonWSF	-1.01827	0.29923	-3.403	<0.001
HostC4	2.37280	0.29799	7.963	<0.001
SpeciesSAF:SeasonWSF	0.31350	0.20879	1.501	0.133
SpeciesVAN:SeasonWSF	1.08414	0.28852	3.758	<0.001
SpeciesSAF:HostC4	0.12270	0.40030	0.307	0.759
SpeciesVAN:HostC4	-3.80814	0.34700	-10.974	<0.001
SeasonWSF:HostC4	**0.47572**	**0.27140**	**1.753**	**0.080**

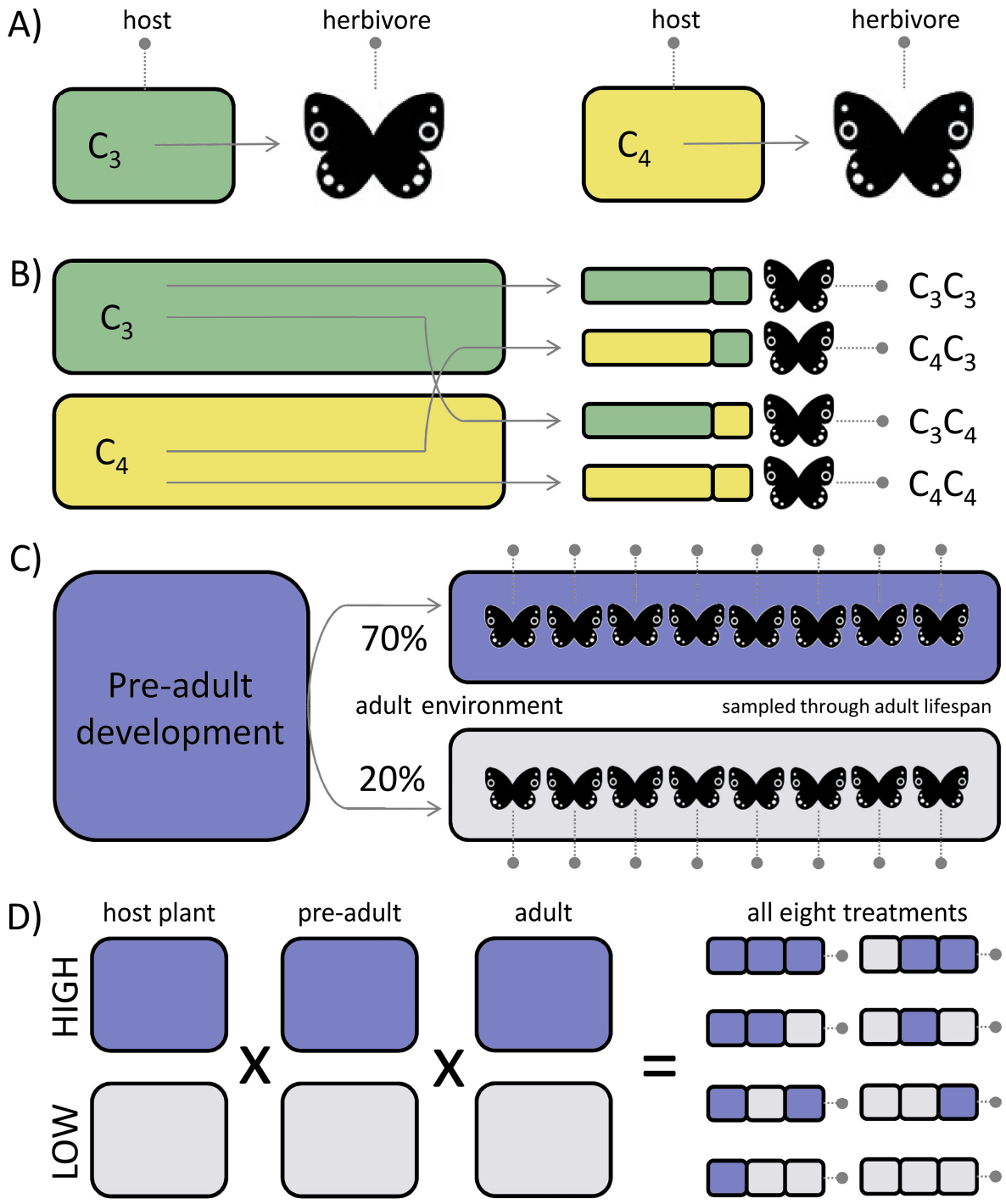

Figure S1: Schematic representations of laboratory experiments A, B, C and D. Experiments A and B investigated whether the feeding history of individual larvae on C_3 and C_4 grasses could be traced by analysing the $\delta^{13}C$ of adult leg tissue. Experiments C and D were designed to examine the extent to which the relative humidity (RH) of the environment affects ^{18}O composition in the organic material of mycalesine butterflies.

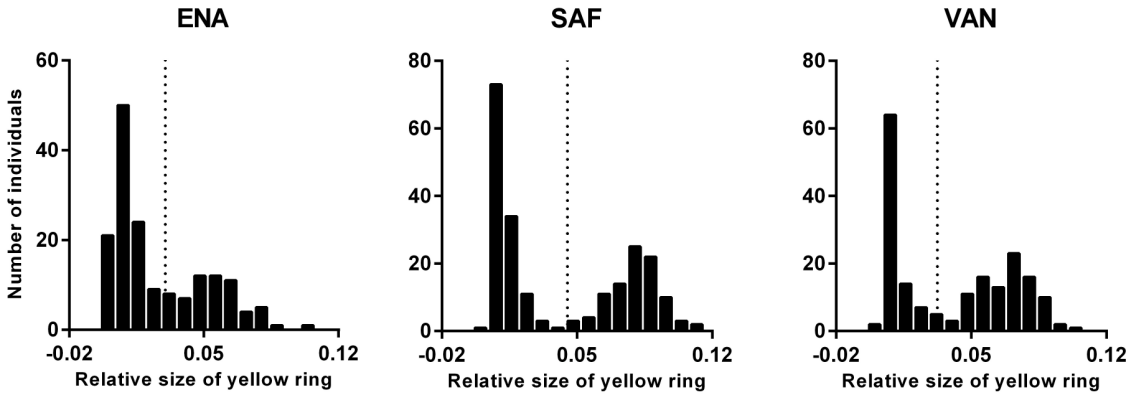

Figure S2: Bimodal distributions of wing pattern elements, relative size of the yellow ring of the large eyespot on the hindwing, used to classify the distinct seasonal forms. Dashed lines represent the chosen threshold; individuals with values smaller than the threshold were classified as a DSF while specimens with greater values were classified as WSF.

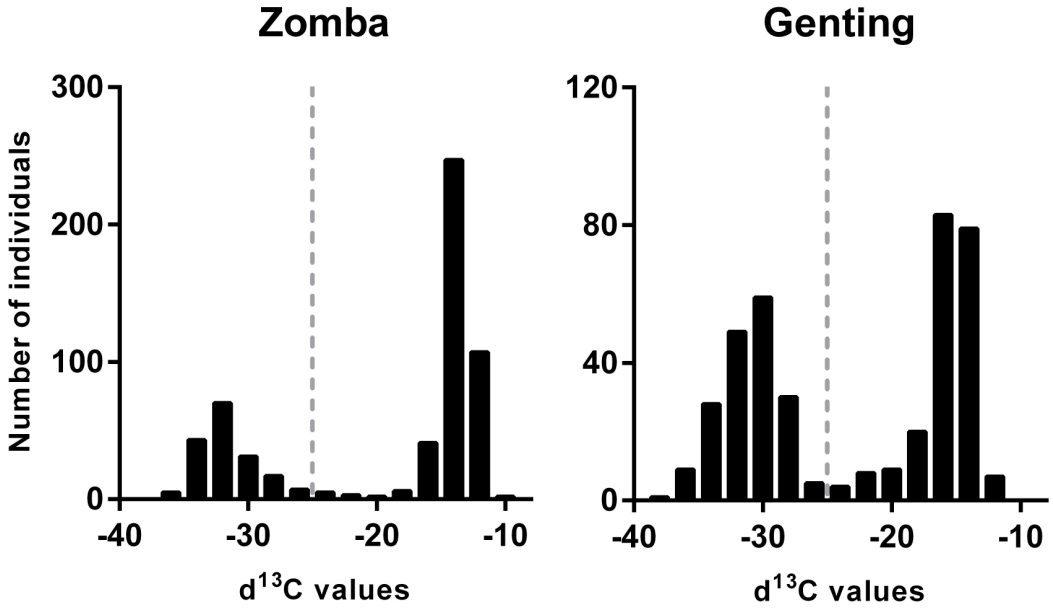

Figure S2: Bimodal distributions of $\delta^{13}C$ values of three *Bicyclus* species collected in Zomba, Malawi (left) and eight Mycalesine species collected in Genting, Malaysia (right). Plants discriminate against $^{13}CO_2$ during photosynthesis. C_4 photosynthesis dramatically lowers this discrimination, leading to distinct non-overlapping differences in $\delta^{13}C$ values between C_3 (left part of the distribution) and C_4 (right part of the distribution) plants. Differences in ^{13}C isotope ratios between these C_3 and C_4 grasses are transmitted to the tissues of the herbivores that feed on the plant material. Individuals with $\delta^{13}C$ values less than -25‰ were classified as C_3 feeders.