

1 ***Complex multi-trait responses to multivariate environmental cues in a***

2 ***seasonal butterfly***

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24

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26 ***Abstract***

27 Many organisms inhabiting seasonal environments exhibit adaptive developmental plasticity,  
28 allowing them to optimally match life-history traits with fluctuating conditions. This critically  
29 relies on environmental cues, such as temperature, as predictors for seasonal transitions. In  
30 most seasonal environments, multiple factors vary together, but might not be equally relevant  
31 as cue, making it crucial to understand their combined effects on an organism's phenotype.  
32 Here, we study plasticity in a multivariate environment in the seasonally polyphenic butterfly  
33 *Bicyclus anynana*. Using a full-factorial design, we test how developmental temperature and  
34 host plant quality interact to affect life-history traits. Our results show that the cues interact:  
35 reduced food quality can act as a predictive cue at temperatures normally associated with the  
36 food-rich wet season, inducing a partial dry season phenotype. At low temperatures, normally  
37 associated with the food-poor dry season, reduced food quality had an adverse effect on life  
38 history, with decreased body mass and prolonged development time. However, metabolic  
39 rates in adults were not affected, indicating that individuals could partly compensate for  
40 stressful juvenile conditions. Thus, under certain environmental conditions, a single cue (e.g.  
41 temperature) might suffice to shape an organisms' phenotype, while under other conditions  
42 additional cues (like plant quality) might be needed in shaping the organism's phenotype to  
43 optimally match seasonal conditions. Our study reveals complex interactive effects of two  
44 environmental variables on seasonal plasticity, highlighting the importance of studying  
45 multivariate environmental factors to better understand the regulation of phenotypic plasticity  
46 in the wild.

47 ***Keywords***

48 developmental plasticity, plant quality, seasonal polyphenism, *Bicyclus anynana*, reaction  
49 norm

## 50 *Introduction*

51 Environmental seasonality is frequent in nature and can lead to the evolution of phenotypic  
52 plasticity (Tauber et al. 1986; Gotthard and Nylin 1995; Lafuente and Beldade 2019), which  
53 can help to ensure that the phenotype expressed by an organism is in sync with its  
54 environment (Nylin 1992; Flatt and Heyland 2011; Torres-Dowdall et al. 2012). Examples of  
55 such adaptive seasonal plasticity are reproductive diapause and seasonal polyphenism, both of  
56 which are widespread in insects, where they constitute an important strategy for coping with  
57 unfavourable environmental conditions (Tauber et al. 1986; Halali et al. 2020b). While  
58 phenotypic plasticity has often been examined in single traits, organismal responses to  
59 environmental variation are usually manifested via changes in multiple traits, leading to  
60 multivariate plasticity (Boggs 2009; Robinson and Beckerman 2013; Plaistow and Collin  
61 2014). Rather than independently responding to the environment, plastic responses in multiple  
62 traits are often regulated via shared genetic, developmental or/and physiological mechanisms.  
63 The resulting integrated phenotypic response manifests as trait correlations and life-history  
64 trade-offs, and is often adaptive in predictable environments (Zelditch 1988; Murren 2012;  
65 Plaistow and Collin 2014; van Bergen et al. 2017). Environmental stress can alter these  
66 underlying associations, and hence the correlation between life-history traits, leading to a  
67 potentially maladaptive reduction in plastic trait integration (Antonovics 1976; Schlichting  
68 1989; Pigliucci and Preston 2004).

69

70 A common mechanism of seasonal plasticity is developmental plasticity, where phenotypic  
71 changes are induced by the environment experienced during development (Beldade et al.  
72 2011). Developmental plasticity can be adaptive in seasonal environments as it can allow  
73 organisms to adjust their life history strategy for future conditions well before the new season  
74 starts, using predictive environmental cues present during the course of development. For

75 example, diapause is known to be regulated by multiple factors, including abiotic factors such  
76 as photoperiod and temperature (de Wilde 1962; Tauber et al. 1986; Brodeur and McNeil  
77 1989), and biotic factors such as food quality and predation risk (Tauber et al. 1986; Hunter  
78 and Mcneil 1997; Wedell et al. 1997; Kroon et al. 2004; Liu et al. 2010). In a seasonal  
79 environment multiple environmental factors often vary together (Jackson et al. 2009; Chevin  
80 and Lande 2015), leading to key open questions about whether organisms sense their  
81 environment through one or multiple cues, whether these cues interact or act as independent  
82 predictors, and whether they induce similar phenotypic responses.

83

84 In a case where multiple cues are used by an organism to respond to the environment, the  
85 responses to a single cue might be nonintuitive and misleading (Chevin and Lande 2015). For  
86 example, studies have shown that responses to temperature can be modulated by the presence  
87 of other factors, such as precipitation, predation, photoperiod or food, and these interactions  
88 not only influence an organisms physiology, e.g. diapause or melanisation, but can also affect  
89 the population dynamics and stability of ecological communities (Tauber et al. 1986; Alto and  
90 Juliano 2001; Stoehr and Wojan 2016; Sentis et al. 2017). Use of multiple cues is especially  
91 favoured in situations where one of the environmental cues has only limited predictive  
92 reliability on the pertinent timescale, such that the cues together are more dependable  
93 indicators of future conditions (Hoffman 1978; Shapiro 1978; Kingsolver and Huey 1998).  
94 On the other hand, theoretical work has shown that under certain conditions, such as when  
95 there is imperfect correlation between two cues leading to contradictory information,  
96 organisms may be favoured to ignore one of the cues, even if this cue is also predictive of  
97 future conditions (van Baalen 2014). Additionally, theoretical work suggests that when the  
98 relationship between an environmental cue and future conditions is weak, plasticity may not  
99 evolve in response to the environmental predictor (Tufto 2000; Leimar et al. 2006; Rickard

100 and Lummaa 2007; Reed et al. 2010; Chevin and Hoffmann 2017). Thus, there can be  
101 different predictions for how environmental factors interact to affect organismal phenotypes.

102

103 Here, we investigate the effect of a multivariate environment on developmental plasticity of  
104 life history traits, using the seasonally polyphenic butterfly, *Bicyclus anynana*. This species  
105 exhibits two alternative seasonal forms (wet and dry) which correspond to a warm and a cool  
106 season, respectively. The wet season butterflies experience high temperatures and  
107 precipitation during development ( $>25^{\circ}\text{C}$ ; November to March), and adults have larger, more  
108 conspicuous eyespots on their ventral wing surfaces, shorter larval and pupal developmental  
109 periods, lower pupal and adult mass, shorter lifespan and reproduce relatively early than dry  
110 season adults (Brakefield and Reitsma 1991; Brakefield et al. 2009; Oostra et al. 2011).

111

112 The transitory period from the wet to dry season (March and April) is characterised by a  
113 decline in temperature (from  $>25^{\circ}\text{C}$  to  $<21^{\circ}\text{C}$ ) and a gradual drying out of the environment  
114 which likely affects host plant quality (Windig et al. 1994; van Bergen et al. 2016;  
115 Nokelainen et al. 2018). The larvae that develop during the early dry season (April to July)  
116 experience relatively low levels of precipitation and cooler temperatures ( $<21^{\circ}\text{C}$ ). Dry season  
117 individuals accumulate higher mass and fat reserves during development; have small or  
118 absent eyespots, a higher resting metabolic rate, delayed reproduction (with larger eggs) until  
119 the following wet season, and a longer lifespan (Brakefield and Reitsma 1991; Pijpe et al.  
120 2007; Geister et al. 2008; Oostra et al. 2011; Halali et al. 2020b). No recruitment occurs  
121 during the final part of the dry season (August to October) since larval host plants dry out and  
122 disappear completely (Brakefield and Reitsma 1991; van Bergen et al. 2016). In addition to  
123 above, the seasonal forms also differ in their behaviour (e.g. Bear and Monteiro 2013; van

124 Bergen and Beldade 2019) and investment in secondary sexual traits (e.g. Balmer et al. 2018;  
125 Huq et al. 2019). Results from field, laboratory and computational experiments have provided  
126 ample support for the adaptive advantage of these seasonal forms in their respective  
127 environments (Brakefield and Frankino 2009; van den Heuvel et al. 2013; Prudic et al. 2015).

128

129 Previous studies have shown that the temperature experienced during the (late) larval and  
130 (early) pupal stages are crucial cues for plasticity in this species (Brakefield and Reitsma  
131 1991; Brakefield et al. 2007, 2009; Bear and Monteiro 2013). Interestingly, variation in  
132 temperature alone does not produce the full extent of plasticity in life-history traits as  
133 observed in the wild (Roskam and Brakefield 1999), suggesting that other predictive  
134 environmental factors may act in conjunction with temperature (Brakefield 1987; Brakefield  
135 and Reitsma 1991). Here, we hypothesise that larval host plant quality could be an important  
136 environmental cue, in addition to temperature, for developing individuals in the field as  
137 during the transition from wet to dry season in the field, the host plants on which the larvae  
138 feed tend to be older, drier and of poor quality (Brakefield and Reitsma 1991; Kooi et al.  
139 1996). A proxy for the availability and quality of the host plants is rainfall, and the latter is  
140 highly correlated with temperature in parts of range where *B. anynana* occurs, such as Malawi  
141 (de Jong et al. 2010; Oostra et al. 2018). Food quality has been shown to be an important  
142 environmental cue for plasticity in many species, with poor food quality leading to longer  
143 development time, higher mortality (Nylin and Gotthard 1998), decreased fecundity (Awmack  
144 and Leather 2002), reduced growth rates (Atkinson and Sibly 1997), and smaller body size  
145 (Berrigan and Charnov 1994). Moreover, earlier work in *B. anynana* has shown that under  
146 conditions of larval food limitation, this species is better adapted to cope with stressful  
147 conditions as an adult (Saastamoinen et al. 2010; van den Heuvel et al. 2013). Here, we  
148 hypothesise that temperature and plant quality could act together as cues to predict future

149 environmental conditions, in which case we would expect that variation in food quality alters  
150 phenotypic traits in the same direction as temperature, i.e. making each cohort more dry or  
151 wet season-like. Alternatively, if temperature acts as the sole cue, with the plant quality not  
152 being perceived or processed at all, or even acting as a stressor, we would predict general  
153 detrimental effects of life history traits, irrespective of seasonal conditions, and a reduction in  
154 integration of plastic responses. We also expect the sexes to differ in their response as key  
155 life-history traits such as development time, growth rate and body size can have sex-specific  
156 effects on fitness. Moreover, we can expect secondary cues like host plant quality to have a  
157 larger effect (i.e. increased sensitivity) at intermediate temperatures that are typical of the  
158 transition between the seasons in the wild.

159

160 In our study, we test how larval host plant quality—in conjunction with temperature—affects a  
161 suite of life history traits: larval and pupal development time, pupal and adult mass, resting  
162 metabolic rate (RMR) and the respiratory quotient (RQ) of adults. Using old host plants that  
163 mimic the deteriorating conditions in dry season, we feed cohorts of individuals during a  
164 critical window of larval development on old (poor quality) plants, whereas control cohorts  
165 are reared on young (high quality) plants. We tested the effect of host plant quality at three  
166 different temperatures that correspond to wet, intermediate, and dry season temperatures in  
167 the field. This design allows testing of how larval host plant quality and temperature interact  
168 to affect life history traits. Earlier studies in *B. anynana* have shown that CO<sub>2</sub> respiration rate  
169 varies in response to temperature (Brakefield et al. 2007; Pijpe et al. 2007), but O<sub>2</sub>  
170 consumption or RQ have so far not been examined. Analysing RQ allows us to evaluate  
171 whether adults differ in their macronutrient metabolism in response to environmental  
172 conditions (i.e. whether they burn different fuels, in particular fat, protein and carbohydrates).  
173 Finally, we tested whether the host plant quality affects the organismal integration of



174 phenotypic traits by examining the correlations between life-history traits across all  
175 temperatures. This allows us to analyse how the thermally induced plastic responses are  
176 integrated across traits, and if this integration is altered due to poor food quality. From earlier  
177 studies we know that the responses of different phenotypic traits to temperature are correlated  
178 (van Bergen et al. 2017), partly due to shared underlying hormone physiology (Mateus et al.  
179 2014; Oostra et al. 2014; Bear et al. 2017). However, under different environmental  
180 conditions, such as poor host plant quality, we might expect different traits to respond  
181 differently and phenotypic integration to decrease.

## 182 ***Materials and Methods***

### 183 ***Study organism***

184 *Bicyclus anynana* is a Nymphalid butterfly from East Africa and a model organism for  
185 studying seasonal and developmental plasticity (Brakefield et al. 2009). It is found in  
186 savannah grasslands and open woodlands (both seasonal ecosystems) and has probably  
187 evolved developmental plasticity as an adaptation to seasonality in the environment. The two  
188 seasons that *B. anynana* experiences are the warm wet season and the cool dry season, and the  
189 species expresses alternative morphs in these two alternative seasons (see Introduction).  
190 Along with differing in temperature and precipitation, the seasons also differ drastically in the  
191 availability of resources, with the cool dry season having a reduced host plant quantity and  
192 quality (Roskam and Brakefield 1999; van Bergen et al. 2016). The adults of this butterfly  
193 species feed on rotting and fermenting fruit and the larvae utilize grasses.

194

### 195 ***Experimental design and rearing***

196 An outbred laboratory stock of the butterfly *B. anynana* was used for the experiment. The  
197 stock was established in 1988 from numerous gravid females collected in Malawi. Adults are  
198 fed on banana, and the larvae are reared on maize (*Zea mays*) (Brakefield et al. 2009). The  
199 larvae are oligophagous and are known to utilize a variety of Poaceae (grass) species (Kooi  
200 1992; Kooi et al. 1996). Although maize is widely cultivated in Malawi, it is a native plant of  
201 Central America and is not a natural host plant. Maize is a grass species that uses the C<sub>4</sub>  
202 photosynthetic pathway and the associated high growth rates are beneficial for rearing large  
203 laboratory stock populations. Previous experiments have shown that larval performance is  
204 high when individuals utilize this host plant (Kooi et al. 1996; Brakefield et al. 2009), and

205 similar estimates of developmental time and body mass are obtained when larvae are fed more  
206 natural larval host plants, such as *Oplismenus compositus* (Halali et al. 2020a).

207

208 We used a full-factorial design to investigate the effects of larval host plant quality, pre-adult  
209 (i.e. larval and pupal) temperature, sex, and their interactions, on a suite of life-history traits.

210 Three temperature treatments (19, 23 and 27°C, representing dry, intermediate, and wet

211 season conditions, respectively) and two plant quality treatments (old maize, young maize)

212 were used. Eggs were collected from the stock population and one day after hatching, larvae

213 were randomly allocated to cages (35cm x 44cm x 65cm) with young maize plants set up in

214 climate rooms (2.6 x 2 x 2.5 m<sup>3</sup>) at 19°C and 27°C, and in smaller climate-cabinets

215 (Sanyo/Panasonic MLR-350H, 0.76 x 0.7 x 1.835 m<sup>3</sup>) at 23°C (all at 75% relative humidity

216 and a 12h:12h day:night light cycle), similar to previous experiments (de Jong *et al.*, 2010;

217 Oostra *et al.*, 2011). Initially, each temperature had 280 larvae in two cages (140 larvae per

218 cage), except at 19°C that had 390 larvae in 3 cages (110-140 larvae per cage), such that we

219 had 950 larvae in total. Each cage had multiple (~16) plants (with <9 larvae per plant). One

220 day after they moulted to the 4<sup>th</sup> instar, larvae were randomly distributed to new cages

221 containing either old or fresh young plants (host plant treatment) at that temperature, while

222 controlling for density (Supplementary Table 1). To keep the density of larvae per plant low

223 and accommodate the large size of old plants, we used multiple cages for the old host plant

224 treatment and for 19°C young host plant treatment (which had 250 larvae), while we only

225 used one cage per experimental treatment for young host plants at 23°C and 27°C. The larvae

226 were only exposed to the host plant treatment during the final two larval instars, which is the

227 period when most growth occurs and the effect of food quality should be most prominent.

228 Importantly, the temperature experienced during the end of the 5<sup>th</sup> instar (and early pupal

229 stage) are known to be crucial cues for plasticity in this species and is the period when the

230 adult phenotype is differentiated (Kooi and Brakefield 1999; Monteiro et al. 2015). The  
231 resulting pupae were then individually placed in transparent pots, assigned an ID and kept at  
232 their temperature treatment, until they eclosed.

233

234 After randomly discarding excess pupae raised at 19°C and excluding 51 adult individuals  
235 due to missing information about one or multiple life-history traits, the final sample size for  
236 examining the life-history traits was 191 individuals (35 females and 40 males on old maize,  
237 58 females and 58 males on young maize) at 19°C; 189 individuals (49 females and 43 males  
238 on old maize, 54 females and 43 males on young maize) at 23°C, and 168 individuals (41  
239 females and 31 males on old maize, 57 females and 39 males on young maize) at 27°C.

240

#### 241 *Host plant quality treatments*

242 All maize plants were grown from seed and reared in a climate-controlled greenhouse in  
243 Madingley (United Kingdom), with regular watering to keep the soil moist at all times. Young  
244 maize plants were 2-3 weeks old whereas old maize plants were at least 5-7 weeks old,  
245 mimicking the deteriorating conditions in dry season. Earlier studies across a wide range of  
246 plant taxa have shown that plant quality varies with age. Older plants typically have tougher  
247 leaves (Choong 1996; Loney et al. 2006), lower nutritional values (Hikosaka et al. 1994) and  
248 different chemical/physical defences against herbivory (Barton and Koricheva 2010) than  
249 younger plants. For example, there can be differences in the composition and concentration of  
250 defensive chemical compounds depending on the age of maize plants (Cambier et al. 2000;  
251 Makleit et al. 2018). These differences in toughness, nutrition and defences can have  
252 pronounced effects on herbivory (Price et al. 1987; Loney et al. 2006), with the incidence of  
253 herbivorous invertebrates on old host plants typically being lower than on young plants

254 (Choong 1996; Fenner et al. 1999; Boege and Marquis 2005). Thus, older host plants are  
255 inferred to be of poor quality relative to younger host plants, and 'herbivore performance'  
256 (quantified as preference, performance, and density) is reduced on older herbs and grasses  
257 compared to younger plants of the same species (reviewed in Barton and Koricheva 2010  
258 using data from 116 studies). Moreover, host plant quality can also directly regulate  
259 phenotypic plasticity in herbivorous insects (Lin et al. 2018).

260

261 In our experiment, we measured the maximum leaf width and height of each maize plant  
262 before feeding it to the larvae. For old maize plants, plant height was  $92.2 \pm 33.2$  (mean $\pm$ sd)  
263 cm and maximum leaf-width was  $4.2 \pm 0.6$  cm. For young maize plants, plant height was  
264  $69.6 \pm 4.5$  cm and maximum leaf-width was  $1.4 \pm 0.2$  cm. The larvae were reared on whole  
265 plants, and *ad libitum* feeding was ensured by providing new plants whenever needed. When  
266 the old plants were too large to be completely accommodated inside the cage, only a part of  
267 the (whole) plant was put in, while ensuring that the larvae could not escape from the cage.

268

### 269 *Life-history traits*

270 For each individual, larval development time was recorded as the number of days between  
271 hatching of the egg and pupation of the larvae, and pupal development time was recorded as  
272 the number of days between pupation and eclosion of the butterfly. Pupae were weighed  
273 approximately 24 h after pupation. Adults were weighed and resting metabolic rate (RMR)  
274 measurements made one day after eclosion following established procedures (Pijpe et al.  
275 2007; Brakefield et al. 2009; Oostra et al. 2011). For the RMR, individual butterflies were  
276 measured in the dark –at their rearing temperature–in small cylindrical glass containers (4 cm  
277 in diameter  $\times$  9 cm in height). The RMR was measured in the dark to avoid butterfly

278 movement and keep them immobile, since activity during the measurement can lead to  
279 changes in respiration rate. Each RMR cycle consisted of three runs of 20 minutes during  
280 which RMR was measured as the individual rate of CO<sub>2</sub> and O<sub>2</sub> respiration (millilitre per  
281 minute), using stop-flow respirometry (Pijpe *et al.*, 2007). CO<sub>2</sub> and O<sub>2</sub> production were  
282 measured using a LI-7000 CO<sub>2</sub> gas analyser (Li-Cor) and an Oxzilla FC-2 Differential  
283 Oxygen Analyzer (Sable Systems), respectively, and acquired data were handled in Expedata  
284 (Sable Systems). The CO<sub>2</sub> and O<sub>2</sub> respiration rates were scaled to mass by dividing respiration  
285 rate by adult mass. Measurements were taken around the same time of the day (taken between  
286 0900 hrs and 1500 hrs) for all individuals, and the data from the second and third runs were  
287 averaged. The first run was excluded for each individual as this occurred during the  
288 butterfly's acclimation phase. The respiratory quotient was calculated as the CO<sub>2</sub> respiration  
289 rate divided by the O<sub>2</sub> respiration rate (Richardson 1929).

290

### 291 *Statistical analyses*

292 For larval survivorship, we counted the number of larvae that survived the larval stage and  
293 pupated, which did not allow testing sex-specificity as we did not sex pupae. For pupal  
294 survivorship, we counted the number of pupae that survived the pupal stage and eclosed  
295 (Supplementary Table 1). We assessed the effects of temperature, host plant quality and their  
296 interaction on larval or pupal survivorship using a Generalized Linear Model with binomial  
297 response, followed by post hoc pairwise comparisons (Tukey's HSD;  $\alpha = 0.05$ ) using the  
298 *emmeans* package (Lenth et al. 2020).

299

300 In addition, for each dependent variable (larval development time, pupal development time,  
301 pupal mass, adult mass, CO<sub>2</sub> and O<sub>2</sub> respiration rates (scaled by mass), and the respiratory

302 quotient), we constructed a linear model with temperature, host plant quality, sex, and all their  
303 interactions, as independent fixed effects. For all models, step-wise model selection based on  
304 AIC values was performed using the *step()* function in R. Post hoc pairwise comparisons  
305 (Tukey's HSD;  $\alpha = 0.05$ ) were performed using the *emmeans* package (Lenth et al. 2020).  
306 Prior to statistical analyses, the data was graphically checked for the assumptions of  
307 parametric tests, and all traits (except pupal mass) were log-transformed as this improved the  
308 normality.

309

310 To assess whether host plant quality had an effect on phenotypic integration, we calculated  
311 Pearson's correlation coefficients among the log-transformed life-history traits for individuals  
312 reared on both young and old host plants for both sexes across all temperatures. Thus, we  
313 obtained two correlation matrices per sex. We tested whether poor host plant quality disrupted  
314 the seasonal morphs by comparing the correlation matrices for each sex using matrix  
315 correlation, which measures the strength of association, with values ranging from  $-1$  to  $+1$ ,  
316 such that zero indicates no similarity between the matrix on old maize and young maize. We  
317 evaluated the statistical significance of the association between the matrices using the Mantel  
318 test (Mantel 1967) at each temperature, using the *MantelCor()* in *evolqg* function in R (Melo  
319 et al. 2015). After getting the overall association between the correlation matrices on old and  
320 young maize, we examined the specific changes by comparing the correlation coefficients  
321 between old and young host plants for each trait combination for both sexes. For this, we  
322 converted the correlation coefficient into a  $z$ -score using Fisher's  $r$ -to- $z$  transformation (Fisher  
323 1915, 1921) and compared these  $z$ -scores using the sample size for each coefficient, using the  
324 following formula (Cohen et al. 2003):

325

$$z_{observed} = \frac{(z_{young} - z_{old})}{\sqrt{\frac{1}{n_{young} - 3} + \frac{1}{n_{old} - 3}}}$$

326 where  $Z_{\text{young}}$  and  $Z_{\text{old}}$  are correlation coefficients and  $n_{\text{young}}$  and  $n_{\text{old}}$  are the sample sizes for  
327 individuals on young and old host plants, respectively. We performed 21 comparisons for  
328 each sex. We checked if the absolute value of  $Z_{\text{observed}}$  was greater than 3.03, which is the  
329 critical value for the two-tailed  $\alpha = 0.0024$  significance criterion for normal distribution ( $\alpha$  for  
330 each comparison corrected to account for multiple testing), which would imply that the  
331 difference between the correlation coefficients was statistically significant. We also  
332 performed a Chi-Square test for Independence to assess if host plant quality had a sex-specific  
333 effect on phenotypic disintegration, by examining the number of trait combinations that were  
334 disrupted for males and females.

335 All the analyses were done in R version 3.6.1 (R Core Team 2019).

336



337 **Results**

338 ***Limited effect of host plant quality on pre-adult survivorship***

339 Development on old maize had a temperature specific effect on larval survivorship (Figure 1,  
340 Table 1, Supplementary Table 1), with fewer larvae surviving on old host plants at 23°C  
341 ( $P=0.0001$ ), while there was no significant effect at 19°C ( $P=0.96$ ) and only a marginal effect  
342 at 27°C ( $P=0.06$ ). For pupal survivorship, there was a significant interaction effect of  
343 temperature and host plant quality (Figure 1, Table 1, Supplementary Table 1), which  
344 signifies that the response to temperature is dependent on the host plant used by the larvae  
345 (and vice versa), although the difference in pupal survival on old and young maize was not  
346 significant at any of the temperatures (pairwise comparisons at 19°C:  $P=0.36$ , 23°C:  $P=0.33$ ,  
347 and 27°C:  $P=0.83$ ).

348

349 ***Prolonged development at 23°C due to poor host plant quality***

350 Host plant quality interacted with temperature (Table 2) such that, in contrast to the  
351 treatments at both ends of the thermal gradient (19°C and 27°C), host plant quality had a  
352 significant effect on larval (pairwise comparisons at 23°C,  $P<0.0001$ , Figure 2A,B) and pupal  
353 development time (pairwise comparisons at 23°C,  $P=0.0004$ , Figure 2C,D), the intermediate  
354 temperature. At this thermal environment, the larvae took nearly 13% more time to complete  
355 development on old plants, while pupal development time was about 6% longer. Consistent  
356 with earlier studies (Pijpe et al. 2007; de Jong et al. 2010; Oostra et al. 2011; Mateus et al.  
357 2014), development time decreased with increasing temperature, and males had a shorter  
358 larval but longer pupal development time than females (Figure 2, Table 2).

359

360 ***Temperature-dependent effects of host plant quality on body mass***

361 Similar to development time, host plant quality had a temperature-specific effect on body  
362 mass (Table 2), with the effect of temperature on body mass being less pronounced in  
363 individuals utilizing old host plants, i.e. thermal reaction norms are flatter (Figure 3).  
364 Utilizing old maize during the final instars of development led to a greater than 5% reduction  
365 in pupal mass (in both sexes) compared to being reared on young maize at the two lower  
366 temperatures (pairwise comparisons at 19°C:  $P < 0.0001$ , and 23°C:  $P = 0.03$ , see Figure 3A,B).  
367 In contrast, at the higher temperature (27°C) the pupal mass of both sexes was enlarged when  
368 reared on old host plants, though the differences at this temperature were not statistically  
369 significant (pairwise comparisons at 27°C:  $P = 0.1641$ ). For adult mass, host plant quality had  
370 a temperature and sex-specific effect (Figure 3C,D). Adult mass of females was about 17%  
371 higher at 27°C (pairwise comparison:  $P = 0.0001$ ) and 11% lower at 23°C (pairwise  
372 comparisons,  $P = 0.005$ ), when they fed on old plants instead of younger ones. For males the  
373 effect of poor host quality led to a 10% reduction in adult mass at 19°C (pairwise comparison  
374 at 19°C:  $P = 0.04$ ). In general, both pupal and adult mass decreased with increasing  
375 temperature, and both size estimates were higher in females across all experimental treatments  
376 (Table 2, Figure 3).

377

378 ***No effect of host plant quality on mass-scaled respiration rates and respiratory quotient***

379 Similar to earlier studies on CO<sub>2</sub> respiration rates in this species (Brakefield et al. 2007; Pijpe  
380 et al. 2007), both the CO<sub>2</sub> and O<sub>2</sub> respiration rate increased with temperature (temperature;  $P$   
381  $< 0.0001$  for both variables, with 27°C > 23°C > 19°C for CO<sub>2</sub>, see Table 3 and Figure 4) and  
382 males having higher mass-scaled respiration rates than females (sex;  $P < 0.0001$  for both  
383 variables). Host plant quality did not significantly affect the CO<sub>2</sub> and O<sub>2</sub> respiration rates (but

384 note that the 3-way interaction term was significant for CO<sub>2</sub> respiration rate, Table 3). The  
385 respiratory quotient was not affected by the sex of the individual, the thermal environment nor  
386 the food quality ( $P > 0.05$  for all factors, see Table 3 and Supplementary Figure 1).

387

### 388 *Poor host plant quality affects phenotypic integration*

389 The mantel test showed that the host plant quality caused little overall change in the  
390 correlation matrix for life-history traits for both sexes (correlation between matrix for young  
391 maize vs old maize, females:  $r=0.94$ ,  $P=0.0009$ , and for males:  $r=0.90$ ,  $P=0.0009$ ), indicating  
392 similar matrix structures. Examining pairwise combinations, we found that males were more  
393 severely affected ( $\chi^2=6.85$ ,  $df=1$ ,  $P=0.008$ ), with 11 out of 21 correlation coefficients being  
394 significantly different between young and old host plants, while for females only 3 out of 21  
395 correlation coefficients were significantly affected (Figure 5, for details see Supplementary  
396 Table 4). In general, except for 3 cases each for males and females, the sign of the correlation  
397 remained the same, but the absolute correlation became weaker (closer to 0) or stronger  
398 (closer to 1). Amongst the significant changes, for males, all 11 correlation coefficients  
399 decreased (mean decrease ~56%) on old host plants while for females 2 correlation  
400 coefficients decreased (mean decrease ~72%) and 1 correlation coefficients increased (~44%  
401 increase) on old host plants.

402

403 *Discussion*

404 In order to optimally time life cycle events with the seasons, organisms in seasonal  
405 environments exploit environmental cues that predict seasonal transitions. As environments  
406 are complex, there is often more than one cue that is relevant, and relevance of these cues may  
407 depend on other cues. Temperature and food quality are known to be some of the most  
408 important environmental factors affecting the growth and development of insects. Here, we  
409 tested whether food quality acts as a cue in an Afrotropical butterfly, which is known to rely  
410 on temperature as predictor of transitions between wet and dry seasons. We found that the  
411 cues interact: reduced food quality can act as a predictive cue at temperatures normally  
412 associated with the food-rich wet season, inducing a more dry season-like phenotype. At low  
413 temperatures, normally associated with the food-poor dry season, rather than inducing a more  
414 extreme dry season phenotype, reduced food quality had an adverse effect on life history.  
415 Thus, reduced food quality may only be a relevant cue under some conditions, as we discuss  
416 in detail below.

417

418 Food quality or nutrition is known to play a vital role in shaping animal behaviour and  
419 physiology, with studies showing that alteration in nutrient availability can influence diapause  
420 propensity, foraging behaviour, fecundity, life-history strategy, oviposition behaviour, and  
421 sexual selection dynamics in butterflies (Wedell et al. 1997; McKay et al. 2016; Espeset et al.  
422 2019; Jaumann and Snell-Rood 2019; Mitchell et al. 2019). Specifically, food limitation  
423 experienced during development can have enduring effects on adult physiology and life-  
424 history, particularly in holometabolous insects where the resources assimilated during larval  
425 stage are reallocated during metamorphosis to form the adult (Monaghan 2008; Boggs 2009).  
426 While food limitation usually has a negative effect on an organisms physiology and  
427 survivorship, it sometimes leads to compensatory growth during periods of increased food

428 availability, which in turn can shape adult life history, for instance via altered metabolic rate  
429 (Wilson and Osbourn 1960; Metcalfe and Monaghan 2001). Earlier studies testing the effect  
430 of developmental food deprivation in *B. anynana* , showed that food-stressed individuals have  
431 a reduced body mass and prolonged developmental time, but can under some conditions  
432 reallocate resources adaptively (Bauerfeind and Fischer 2005; Saastamoinen et al. 2010,  
433 2013). ~~In our study, the effect of host plant quality on different life history traits was~~  
434 ~~temperature-dependent, indicating that the effect depended on the physiological state of the~~  
435 ~~organism.~~

436

437 When exposed to the thermal conditions of the wet-season (27°C), poor host plant quality  
438 induced an increase in body mass, which was significant for female adult mass. This partial  
439 dry-season-like phenotype could indicate an adaptive response to within-season fluctuations  
440 in food quality, allowing them to better compensate as adults for reduced food (Monaghan  
441 2008). In insects, body size is a key determinant of female fecundity (egg  
442 provisioning)(Honěk 1993; Boggs and Freeman 2005), whereas for males fecundity is more  
443 related to flight capability (as they need to find and court females). Therefore, the increased  
444 adult mass we observed in females may be suggestive of a terminal reproductive investment  
445 (Clutton-Brock 1984; cf. Oostra et al. 2018). Moreover, food quality can vary independently  
446 of temperature (van den Heuvel et al. 2013), making it a potentially important cue under  
447 conditions when the thermal information is inconclusive, and in such situations the use of  
448 multiple cues might be favoured (Hoffman 1978; Shapiro 1978; Kingsolver and Huey 1998).

449

450 In contrast to the pattern observed at high, wet season-like temperatures, at temperatures that  
451 mimic the dry season (19°C) and the transition temperature (23°C), poor host plant quality did

452 not act as a seasonal cue inducing a more dry-season like form. Instead, the treatment resulted  
453 in lower body mass and longer development times (significant only at 23°C), indicating a  
454 stress response. However, there was no change in RMR, suggesting that in some aspects they  
455 could compensate for the adverse earlier conditions. A possible explanation for the lack of a  
456 role of host plant quality as a cue for seasonal progression, at least at 19°C, is that they cannot  
457 become more dry season-like, as they are already maximally in dry season mode.  
458 Alternatively, in thermal conditions of the dry season (19°C), temperature may suffice as a  
459 cue.

460

461 Interestingly, for larval survivorship and development time, we observed a significant effect  
462 of host plant quality only at 23°C, which is the average temperature during the transition from  
463 the wet (27°C) to the dry (19°C) season (Windig et al. 1994; van Bergen et al. 2016). This  
464 may suggest that there is increased sensitivity at this temperature, potentially because  
465 distinguishing the transition between the seasons may require additional environmental  
466 information in order to induce the expression of the appropriate phenotype. The prolonged  
467 development time at this transitional temperature is likely due to the old maize being of a  
468 poorer quality, prolonging the period necessary to reach the critical mass needed for  
469 undergoing hormonal changes and pupation (Coley et al. 2006). In addition, the effect of host  
470 plant quality on body mass was more evident than on survivorship and development time.  
471 This may be related to the fact that the larvae were only exposed to the poor host plant quality  
472 during the final two larval instars. The latter represents the period when most growth occurs,  
473 but it is only a short period of the total development time.

474

475 Our results are consistent with findings in other organisms, where it has been shown that  
476 temperature and food quality generally have interactive effects on the phenotype of an  
477 organism, leading to complex reaction norms (Stamp and Bowers 1990; Gresens 1997; Sultan  
478 et al. 1998; Petersen et al. 2000; Sultan 2001; Ris et al. 2004; Relyea and Auld 2005; Stillwell  
479 et al. 2007). For example, temperature can influence an organisms foraging and performance  
480 (Lindroth et al. 1997; Petersen et al. 2000; Kingsolver et al. 2006; Stillwell et al. 2007; Lee  
481 and Roh 2010; Jang et al. 2015), alter nutritional requirements of an organism and its  
482 sensitivity to plant secondary compounds and hence, host plant usage patterns (Stamp 1993;  
483 Stamp and Yang 1996; Lemoine et al. 2013). Similarly, while decrease in body size with  
484 increase in temperature is a widely observed phenomenon in ectotherms, this effect can be  
485 modulated or even reversed by host plant quality (Diamond and Kingsolver 2010). The  
486 temperature-specific effect of food quality is similar to what is observed for diapause, where  
487 there are thermal limits within which insects respond to photoperiod, such that the  
488 temperature influences whether photoperiod acts to induce diapause or to prevent diapause  
489 (Tauber et al. 1986).

490

491 We also examined, for the first time in this species, the respiratory quotient (RQ) in resting  
492 metabolic rate. This is the ratio between CO<sub>2</sub> and O<sub>2</sub> respiration rate at rest, which reflects  
493 which macronutrients are metabolized for energy, with values of 0.7, 0.8 or 1.0 indicating fat,  
494 protein or carbohydrate metabolism, respectively (Nunes et al. 1997). We found that the RQ  
495 was not influenced by either temperature, sex, host plant quality, or their interactions. Across  
496 all experimental treatments, RQ stayed constant around 0.9, intermediate between protein and  
497 carbohydrate metabolism, indicating that adult macronutrient metabolism was unaffected by  
498 thermal environment or larval food quality. This is surprising, as earlier studies in both field  
499 and laboratory showed that dry season form butterflies have a higher fat content (Brakefield

500 and Reitsma 1991; de Jong et al. 2010; Oostra et al. 2011). However, we measured the  
501 metabolic rates of newly eclosed adults under benign conditions in the laboratory where fat  
502 reserves are likely under-used compared to the wild, where adults often face prolonged  
503 periods of desiccation and/or starvation. Restricted food intake is often associated with  
504 reduced metabolic rates (DeLany et al. 1999; Ramsey et al. 2000; Even et al. 2001; Blanc et  
505 al. 2003; Roark and Bjorndal 2009), and studies have shown that under starvation, animals  
506 usually have a lower respiratory rate (Porter et al. 1982). ~~For example, *Daphnia magna*~~  
507 ~~metabolizes fat under reduced food conditions, while during favourable food conditions it~~  
508 ~~synthesises lipids (Lampert and Bohrer 1984).~~

509

510 Overall, phenotypic integration of traits was structurally similar between individuals reared on  
511 control and old maize. However, pairwise comparisons showed a change in multiple  
512 correlations between life history traits, with most correlation coefficients decreasing on poor  
513 quality host plants, suggesting reduced phenotypic integration, especially in males. This is  
514 consistent with several studies in other organisms, which have reported that stressful  
515 conditions can modify phenotypic variance (usually increase) and phenotypic integration  
516 (usually decrease) (Pigliucci 2002; Pigliucci and Kolodynska 2002, 2006; Badyaev 2005). We  
517 observed the reduction in phenotypic integration mainly in males, not females, likely as a  
518 result of sex-specific regulation and selective pressures. The hormone signalling pathway  
519 responsible for phenotypic integration (Oostra et al. 2011), often plays a sex-specific-  
520 regulatory role (Stillwell et al. 2010; Bhardwaj et al. 2018), thus permitting sex-specific  
521 differences in plastic responses. These are common in insects, for instance, responses to larval  
522 food stress in the Glanville fritillary butterfly, *Melitaea cinxia* (Rosa and Saastamoinen 2017)  
523 are the result of sex-specific selection on different life-history traits (Tarka et al. 2018).

524



525 Our lab population of *B. anynana* originates from a location in Malawi where temperature is a  
526 highly reliable predictor of seasonal transitions (Oostra et al. 2018), which in this population  
527 may override the necessity for additional cues under most conditions. An open question then  
528 is whether food quality may be a more important cue in other parts of the species' range,  
529 where the relevance and reliability of temperature as a cue is lower (Roskam and Brakefield  
530 1996; van Bergen et al. 2017), ~~as seen for different populations of *Colias* butterflies which~~  
531 ~~vary in their dependence on photoperiod or temperature for wing melanisation depending on~~  
532 ~~the ecological conditions in their local environment (Hoffman 1978). Moreover, *B. anynana*~~  
533 ~~larvae might utilize a variety of different grass species in the wild (Kooi 1992; Kooi et al.~~  
534 ~~1996) and for longer periods than exposed in our study (Brakefield et al. 2009; van Bergen et~~  
535 ~~al. 2016), which may trigger more pronounced phenotypic effects (Braby and Jones 1994;~~  
536 ~~Kooi et al. 1996; Jang et al. 2015).~~ Taken together, our study shows that plant quality affects  
537 life history traits in a temperature- and sex-specific manner, indicating that under certain  
538 environmental condition a single cue (e.g. temperature) might suffice to shape an organisms'  
539 phenotype, while under other conditions additional cues (like plant quality) might be needed  
540 in shaping the organism's phenotype to optimally match seasonal conditions. Lastly, being  
541 able to exploit multiple cues and knowing when to use which cue is likely an important  
542 adaptation for organisms living in complex, seasonal environments.

543

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551

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899 Table 1. Generalized Linear Model with binomial response for the effect of developmental temperature, sex (used only for pupal survivorship),  
900 host plant quality and all interaction terms on larval and pupal survivorship.

Dependent variable	Fixed effects	df	$\chi^2$	P
Larval survivorship	Temperature	2	15.85	0.0003
	Host plant quality	1	21.95	<0.0001
	Temperature x Host plant quality	2	17.04	0.0002
Pupal survivorship	Temperature	2	18.01	0.0002
	Host plant quality	1	0.09	0.77
	Temperature x Host plant quality	2	10.63	0.005

901

902

903 Table 2. Minimum adequate models for the effect of developmental temperature, sex and host plant quality on developmental time and body  
 904 mass, related to Figures 1-2. See Supplementary Table 1 for minimum adequate model derivation and Supplementary Table 2 for full models of  
 905 all traits. The standardised effect size of the fixed effects is measured by the partial eta-squared (partial  $\eta^2$ ). All dependent variables (except pupal  
 906 mass) were log-transformed (natural logarithms).

Dependent variable	Fixed effects	df	partial $\eta^2$	F	P
Larval development time	Temperature	2	0.95	5458.1	< 0.0001
	Sex	1	0.14	86.9	< 0.0001
	Host plant quality	1	0.12	75.7	< 0.0001
	Temperature x Host plant quality	2	0.09	26.5	< 0.0001
	Residuals	541			
Pupal development time	Temperature	2	0.96	6030	< 0.0001
	Sex	1	0.19	127.8	< 0.0001
	Host plant quality	1	0.02	14.2	0.0002
	Temperature x Sex	2	0.01	2.5	0.08
	Temperature x Host plant quality	2	0.02	4.3	0.01
	Residuals	539			
Pupal mass	Temperature	2	0.22	77.3	< 0.0001
	Sex	1	0.47	473.6	< 0.0001
	Host plant quality	1	0.02	12.1	0.0006
	Temperature x Sex	2	0.02	6.8	0.001
	Temperature x Host plant quality	2	0.06	17.1	< 0.0001
	Residuals	539			
Adult mass	Temperature	2	0.18	60	< 0.0001
	Sex	1	0.67	1102.9	< 0.0001
	Host plant quality	1	0.003	1.5	0.23
	Temperature x Sex	2	0.003	0.8	0.43
	Temperature x Host plant quality	2	0.08	24.4	< 0.0001
	Sex x Host plant quality	1	0.0002	0.1	0.69
	Temperature x Sex x Host plant quality	2	0.02	5.1	0.006
	Residuals	536			

907 Table 3. Minimum adequate models of the effect of developmental temperature and sex on mass-scaled metabolic rates, related to Figures 3-4.  
 908 See Supplementary Table 1 for minimum adequate model derivation and Supplementary Table 2 for full models of all traits. The standardised  
 909 effect size of the fixed effects is measured by the partial eta-squared (partial  $\eta^2$ ). All dependent variables were log-transformed.

Dependent variable	Fixed effects	df	partial $\eta^2$	F	P
CO <sub>2</sub> respiration rate (scaled for mass)	Temperature	2	0.59	378.1	< 0.0001
	Sex	1	0.38	322.5	< 0.0001
	Host plant quality	1	0.0004	0.23	0.63
	Temperature x Sex	2	0.0003	0.07	0.93
	Temperature x Host plant quality	2	0.003	0.8	0.45
	Sex x Host plant quality	1	0.00001	0.007	0.93
	Temperature x Sex x Host plant quality	2	0.01	3.2	0.04
	Residuals	536			
O <sub>2</sub> respiration rate (scaled for mass)	Temperature	2	0.36	151.6	< 0.0001
	Sex	1	0.19	128.9	< 0.0001
	Host plant quality	1	0.0007	0.4	0.53
	Temperature x Sex	2	0.001	0.3	0.75
	Temperature x Host plant quality	2	0.006	1.5	0.22
	Sex x Host plant quality	1	0.002	1.2	0.28
	Temperature x Sex x Host plant quality	2	0.01	2.8	0.06
	Residuals	536			
Respiratory Quotient	Temperature	2	0.002	0.5	0.61
	Sex	1	0.00001	0.003	0.95
	Host plant quality	1	0.003	1.8	0.18
	Residuals	543			

910

911

912 ***Figure legends***

913 Figure 1. Effect of host plant quality on proportion of larval and sex-specific pupal  
914 survivorship at all temperatures. Statistically significant effects of host plant quality (Tukey's  
915 HSD,  $\alpha = 0.05$ ) are indicated for each temperature with an asterisk.

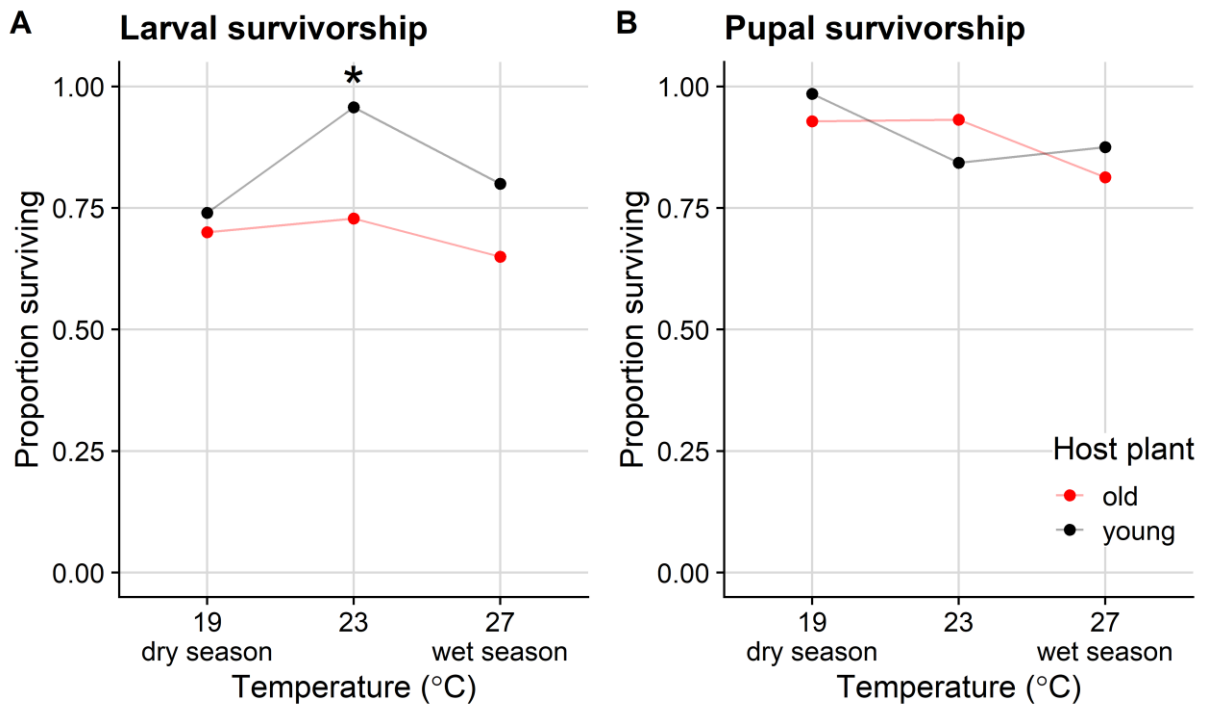
916 Figure 2. Slower development due to poor host plant quality at 23°C: Effect of host plant  
917 quality and temperature on larval development time (top row) and pupal development time  
918 (bottom row) is shown for females (left) and males (right), with data for young and old maize  
919 indicated by black and red, respectively. Typical wet season morphs develop faster compared  
920 to dry season morphs. Plots show estimated marginal means and upper and lower confidence  
921 limits of data. Statistically significant effects of host plant quality (Tukey's HSD,  $\alpha = 0.05$ ) are  
922 indicated for each temperature with an asterisk.

923 Figure 3. Temperature and sex-dependent effects of host plant quality on body mass: Effect of  
924 host plant quality and temperature on pupal mass (top row) and adult mass (bottom row).  
925 Typical wet season morphs have lower body mass compared to dry season morphs. See  
926 legend to Figure 1.

927 Figure 4. No effect of host plant quality on mass-scaled CO<sub>2</sub> (top row) and O<sub>2</sub> (bottom row)  
928 respiration rates (ml hr<sup>-1</sup> mg<sup>-1</sup>). Typical wet season morphs have higher respiration rates  
929 compared to dry season morphs. See legend to Figure 1.

930 Figure 5. Poor host plant quality has an effect on some trait correlations, particularly in males:  
931 Pearson correlation coefficients ( $r$ ) between trait values for a) females and, b) males on young  
932 (high quality) or old (poor quality) host plants. Each line represents the correlation coefficient  
933 between one pair of traits. Correlation coefficients that changed significantly (21 tests for  
934 each sex) due to poor host plant quality are highlighted in red. Sample sizes for calculating  
935 each correlation coefficient are given at the bottom.

936

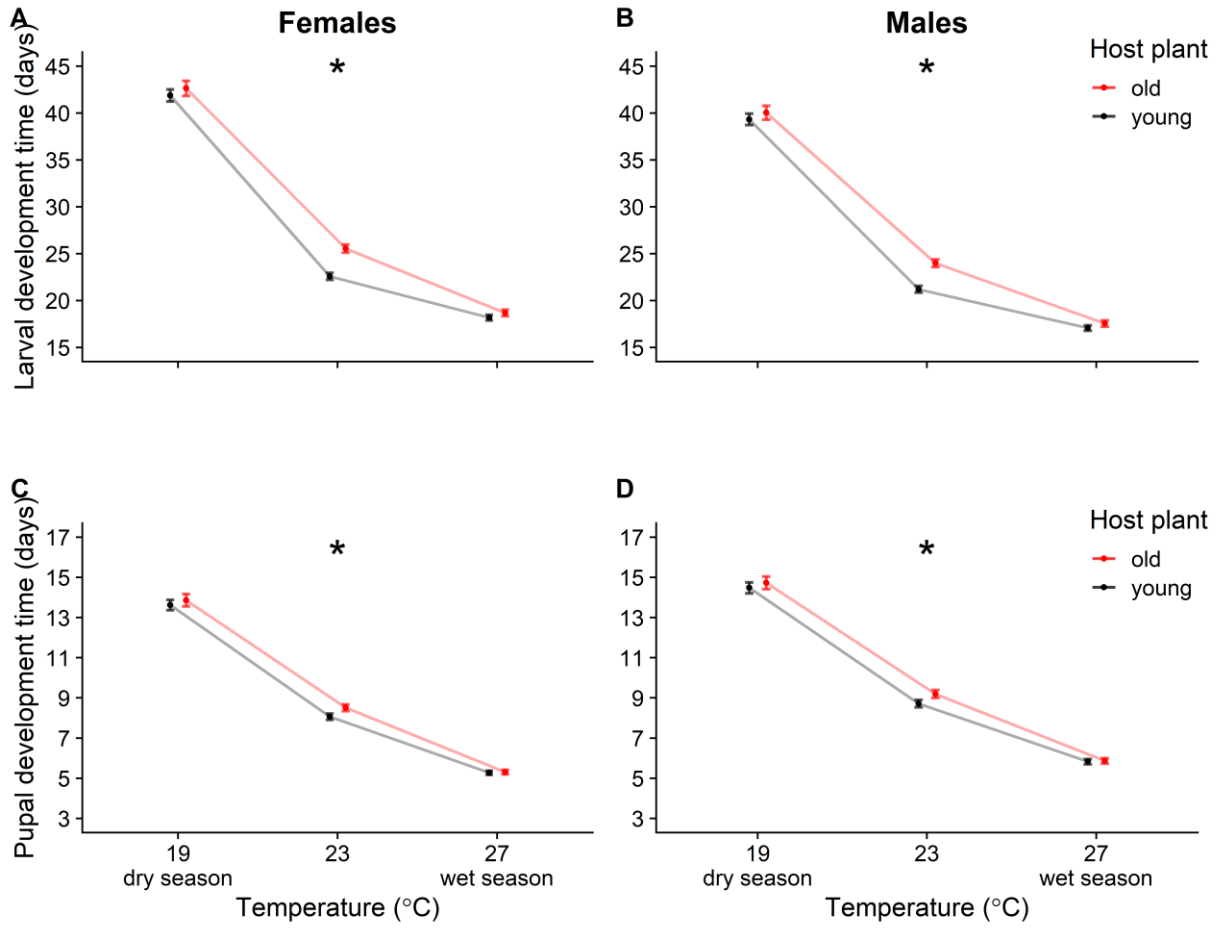


937

938 Figure 1.

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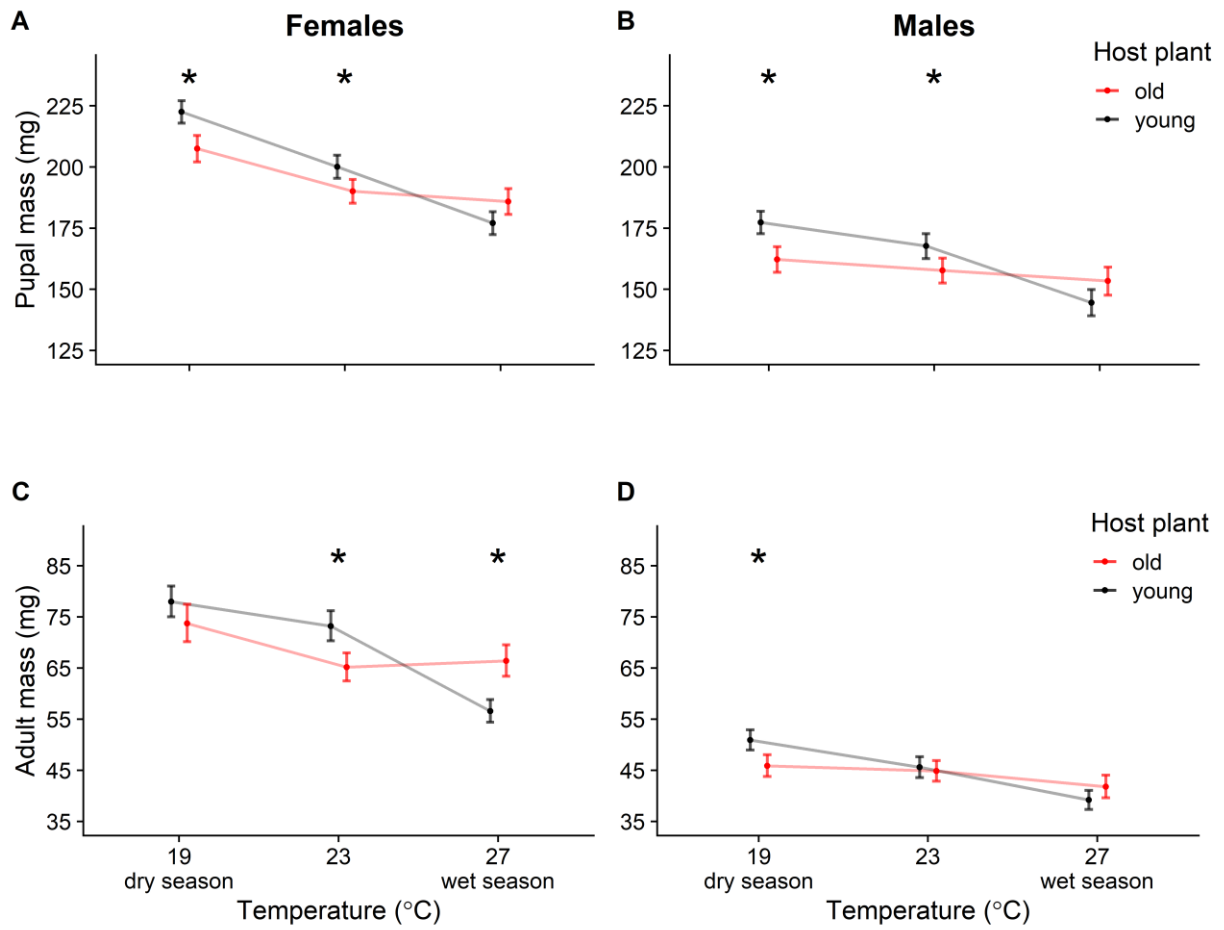
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942 Figure 2.

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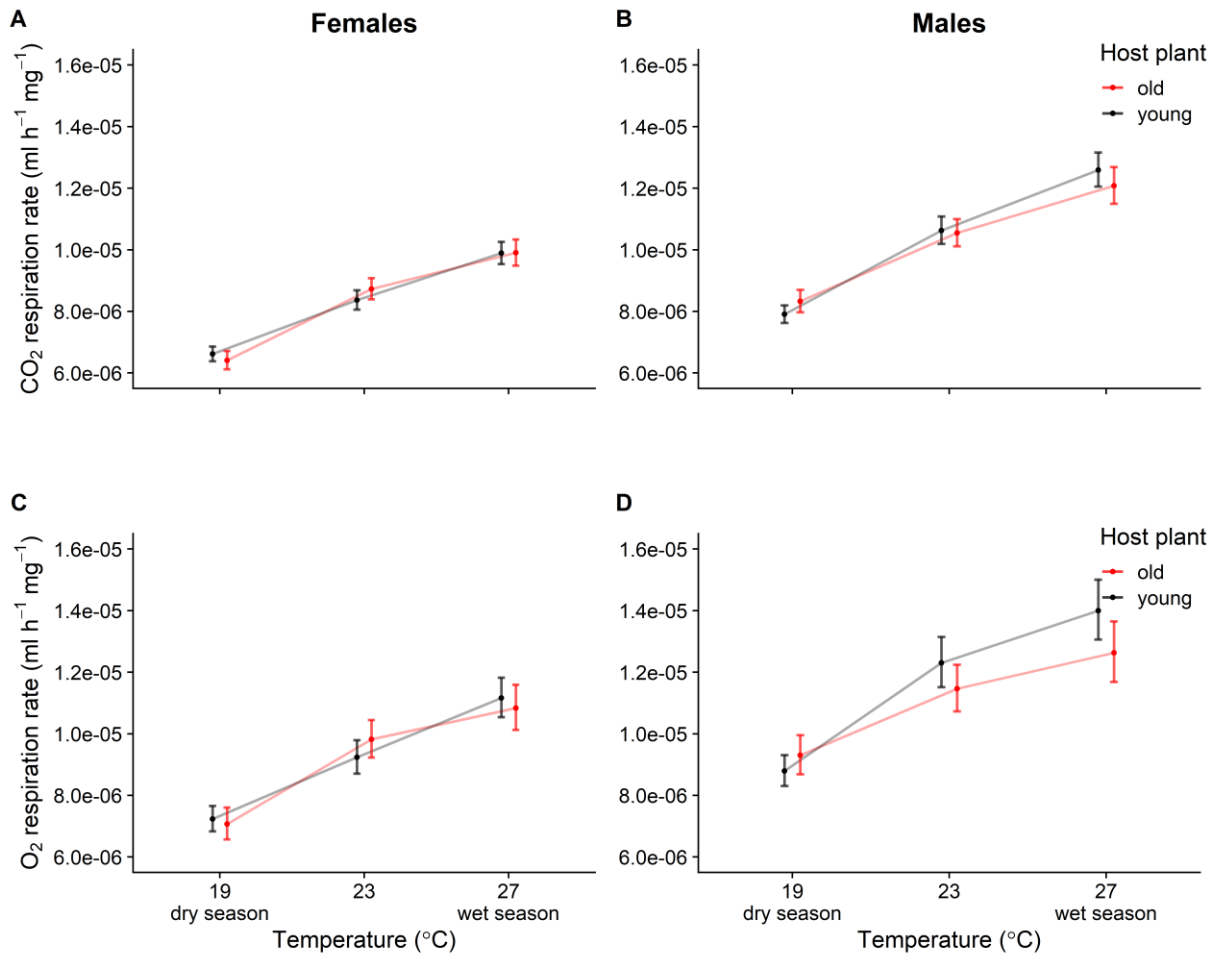


944

945 Figure 3.

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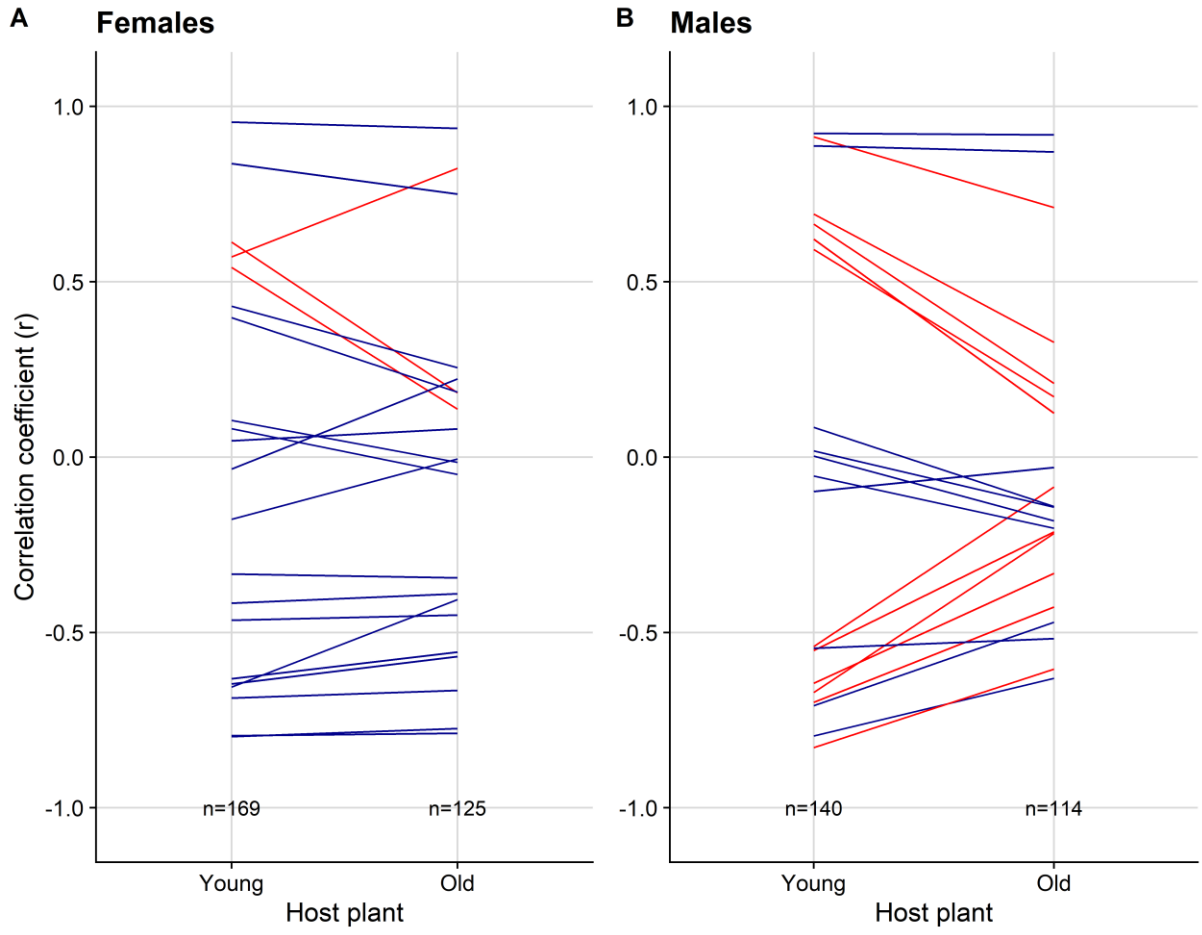




947

948 Figure 4.

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952 Figure 5.