Complex multi-trait responses to multivariate environmental cues in a seasonal butterfly

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Abstract

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

Keywords

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

Introduction

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

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

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

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

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

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

The transitory period from the wet to dry season (March and April) is characterised by a decline in temperature (from >25°C to <21°C) and a gradual drying out of the environment which likely affects host plant quality (Windig et al. 1994; van Bergen et al. 2016; Nokelainen et al. 2018). The larvae that develop during the early dry season (April to July) experience relatively low levels of precipitation and cooler temperatures (<21°C). Dry season individuals accumulate higher mass and fat reserves during development; have small or absent eyespots, a higher resting metabolic rate, delayed reproduction (with larger eggs) until the following wet season, and a longer lifespan (Brakefield and Reitsma 1991; Pijpe et al. 2007; Geister et al. 2008; Oostra et al. 2011; Halali et al. 2020b). No recruitment occurs during the final part of the dry season (August to October) since larval host plants dry out and disappear completely (Brakefield and Reitsma 1991; van Bergen et al. 2016). In addition to 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_2 respiration rate
169 varies in response to temperature (Brakefield et al. 2007; Pijpe et al. 2007), but O_2
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.

Materials and Methods

Study organism

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

Experimental design and rearing

An outbred laboratory stock of the butterfly *B. anynana* was used for the experiment. The stock was established in 1988 from numerous gravid females collected in Malawi. Adults are fed on banana, and the larvae are reared on maize (*Zea mays*) (Brakefield et al. 2009). The larvae are oligophagous and are known to utilize a variety of Poaceae (grass) species (Kooi 1992; Kooi et al. 1996). Although maize is widely cultivated in Malawi, it is a native plant of Central America and is not a natural host plant. Maize is a grass species that uses the C_4 photosynthetic pathway and the associated high growth rates are beneficial for rearing large laboratory stock populations. Previous experiments have shown that larval performance is high when individuals utilize this host plant (Kooi et al. 1996; Brakefield et al. 2009), and

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

We used a full-factorial design to investigate the effects of larval host plant quality, pre-adult (i.e. larval and pupal) temperature, sex, and their interactions, on a suite of life-history traits. Three temperature treatments (19, 23 and 27°C, representing dry, intermediate, and wet season conditions, respectively) and two plant quality treatments (old maize, young maize) were used. Eggs were collected from the stock population and one day after hatching, larvae were randomly allocated to cages (35cm x 44cm x 65cm) with young maize plants set up in climate rooms (2.6 x 2 x 2.5 m^3) at 19°C and 27°C, and in smaller climate-cabinets (Sanyo/Panasonic MLR-350H, 0.76 x 0.7 x 1.835 m^3) at 23°C (all at 75% relative humidity and a 12h:12h day:night light cycle), similar to previous experiments (de Jong *et al.*, 2010; Oostra *et al.*, 2011). Initially, each temperature had 280 larvae in two cages (140 larvae per cage), except at 19°C that had 390 larvae in 3 cages (110-140 larvae per cage), such that we had 950 larvae in total. Each cage had multiple (~16) plants (with <9 larvae per plant). One day after they moulted to the 4^{th} instar, larvae were randomly distributed to new cages containing either old or fresh young plants (host plant treatment) at that temperature, while controlling for density (Supplementary Table 1). To keep the density of larvae per plant low and accommodate the large size of old plants, we used multiple cages for the old host plant treatment and for 19°C young host plant treatment (which had 250 larvae), while we only used one cage per experimental treatment for young host plants at 23°C and 27°C. The larvae were only exposed to the host plant treatment during the final two larval instars, which is the period when most growth occurs and the effect of food quality should be most prominent. Importantly, the temperature experienced during the end of the 5^{th} instar (and early pupal stage) are known to be crucial cues for plasticity in this species and is the period when the

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

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

Host plant quality treatments

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

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

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

Life-history traits

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

movement and keep them immobile, since activity during the measurement can lead to changes in respiration rate. Each RMR cycle consisted of three runs of 20 minutes during which RMR was measured as the individual rate of CO_2 and O_2 respiration (millilitre per minute), using stop-flow respirometry (Pijpe *et al.*, 2007). CO_2 and O_2 production were measured using a LI-7000 CO_2 gas analyser (Li-Cor) and an Oxzilla FC-2 Differential Oxygen Analyzer (Sable Systems), respectively, and acquired data were handled in Expedata (Sable Systems). The CO_2 and O_2 respiration rates were scaled to mass by dividing respiration rate by adult mass. Measurements were taken around the same time of the day (taken between 0900 hrs and 1500 hrs) for all individuals, and the data from the second and third runs were averaged. The first run was excluded for each individual as this occurred during the butterfly's acclimation phase. The respiratory quotient was calculated as the CO_2 respiration rate divided by the O_2 respiration rate (Richardson 1929).

Statistical analyses

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

In addition, for each dependent variable (larval development time, pupal development time, pupal mass, adult mass, CO_2 and O_2 respiration rates (scaled by mass), and the respiratory

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

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

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

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

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

Results

Limited effect of host plant quality on pre-adult survivorship

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

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

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

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_2 respiration rates in this species (Brakefield et al. 2007; Pijpe
380 et al. 2007), both the CO_2 and O_2 respiration rate increased with temperature (temperature; P
381 < 0.0001 for both variables, with 27°C > 23°C > 19°C for CO_2, 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_2 and O_2 respiration rates (but

note that the 3-way interaction term was significant for CO_2 respiration rate, Table 3). The respiratory quotient was not affected by the sex of the individual, the thermal environment nor the food quality ($P > 0.05$ for all factors, see Table 3 and Supplementary Figure 1).

Poor host plant quality affects phenotypic integration

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

Discussion

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

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

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

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

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

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

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

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_2 and O_2 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

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

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

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

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Tables

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

Dependent variable	Fixed effects	df	χ^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

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

Dependent variable	Fixed effects	df	partial η^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 η^2). All dependent variables were log-transformed.

Dependent variable	Fixed effects	df	partial η^2	F	P
CO_2 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_2 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.

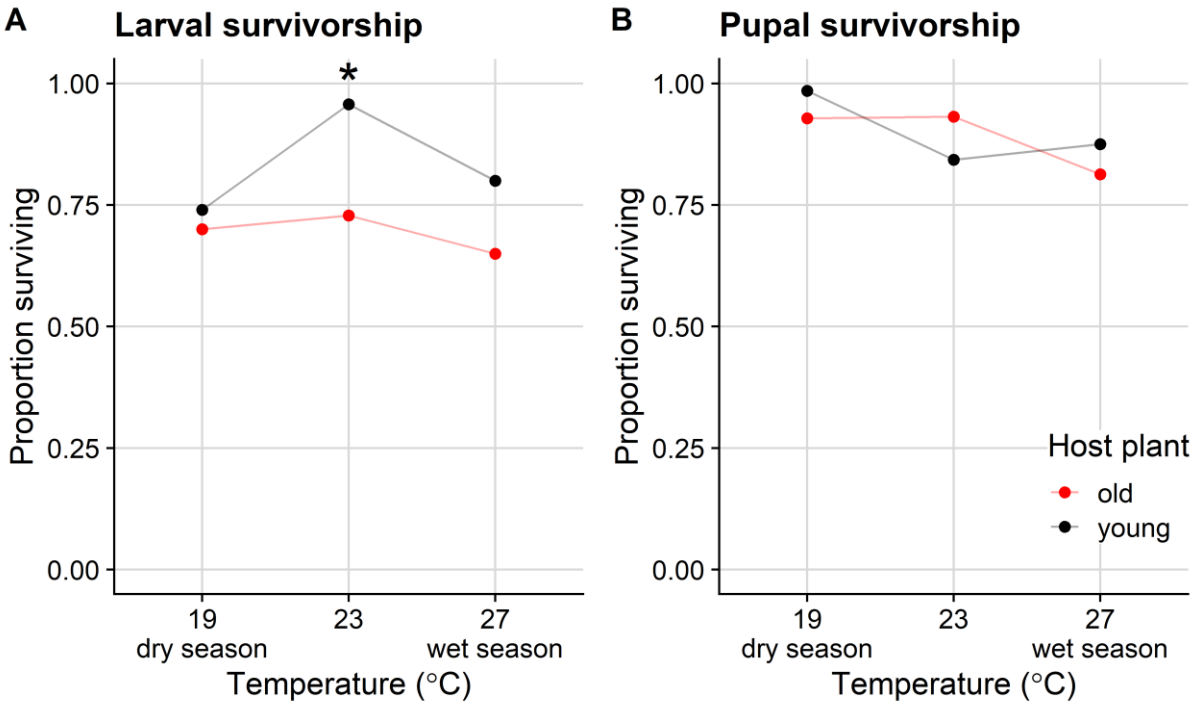
916 Figure 2. Slower development due to poor host plant quality at 23°C: Effect of host plant
917 quality and temperature on larval development time (top row) and pupal development time
918 (bottom row) is shown for females (left) and males (right), with data for young and old maize
919 indicated by 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_2 (top row) and O_2 (bottom row)
928 respiration rates (ml hr^{-1} mg^{-1}). 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



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938 Figure 1.

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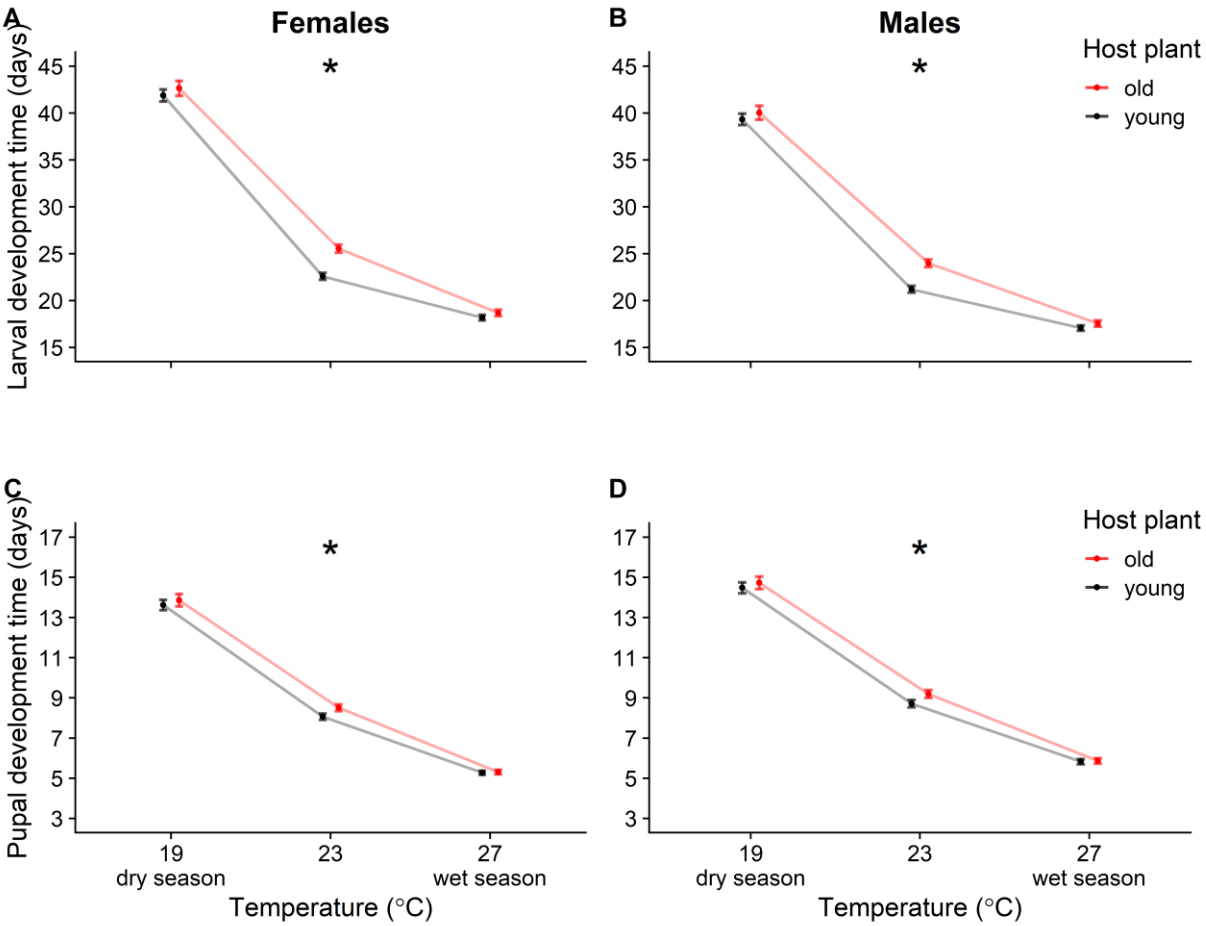

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942 Figure 2.

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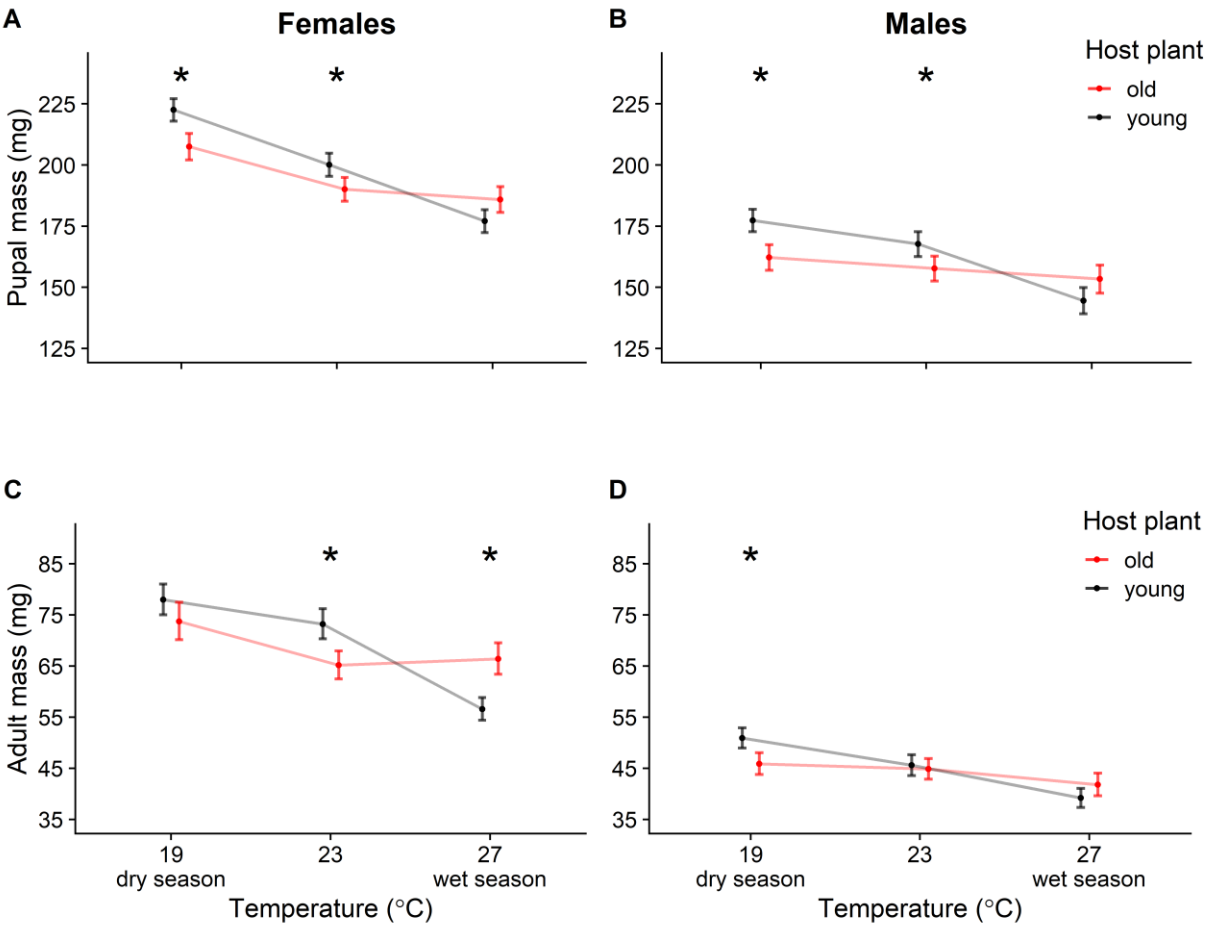

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945 Figure 3.

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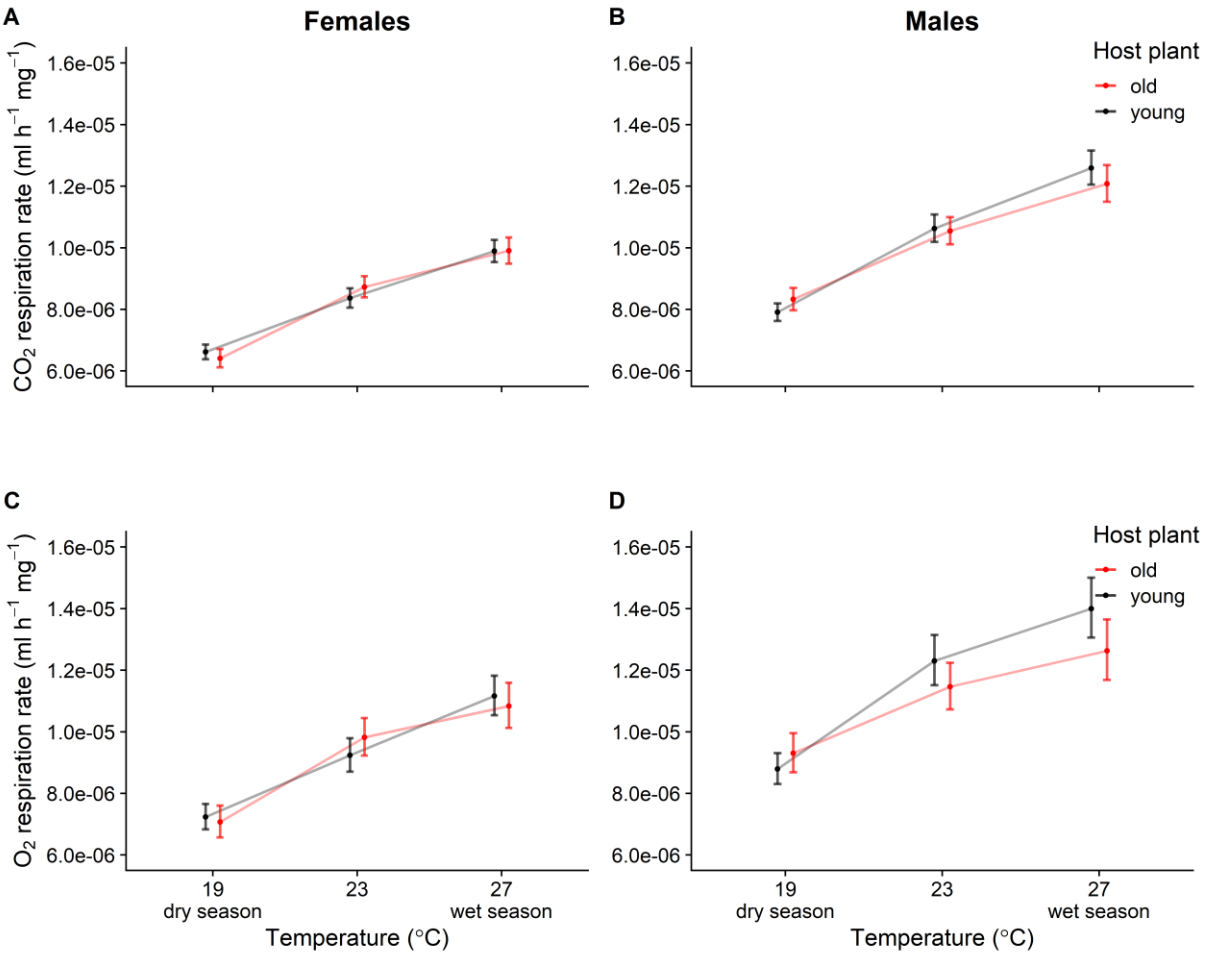

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948 Figure 4.

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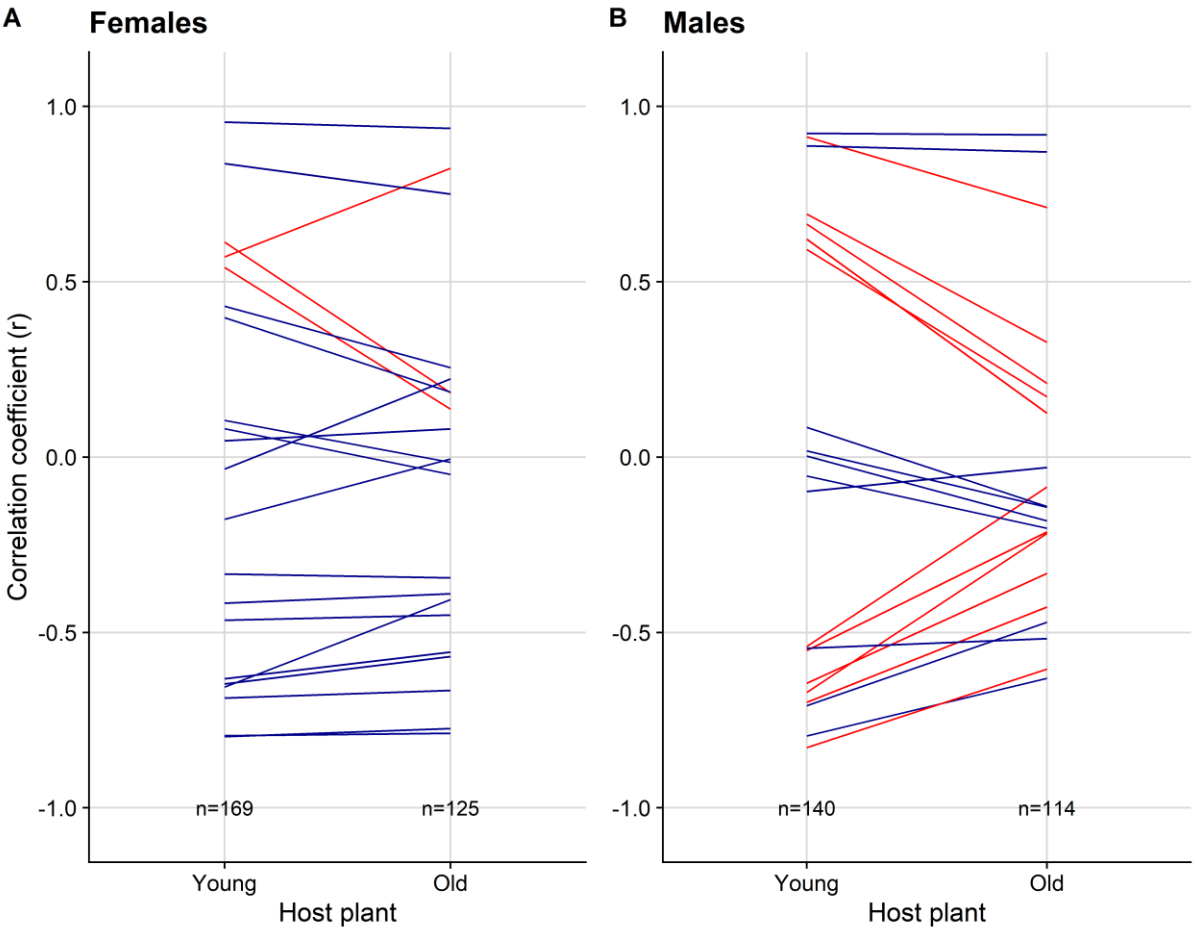

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952 Figure 5.