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

Pragya Singh^{1,2,3†}, Erik van Bergen^{3,4}, Oskar Brattström^{3,5}, Dave Osbaldeston³, Paul M. Brakefield³ and Vicencio Oostra^{3,4}

¹ Evolutionary Biology, Zoological Institute, University of Basel, Basel 4055, Switzerland

² Indian Institute of Science Education and Research, Kolkata

³ Department of Zoology, University Museum of Zoology Cambridge, University of Cambridge, Cambridge, CB2 3EJ, United Kingdom

⁴ Research Centre for Ecological Change, University of Helsinki, PO Box 65 (Viikinkaari 1), FI-00014 University of Helsinki, Finland

⁵ School of Life Sciences, University of Glasgow, Glasgow, G12 8QQ, United Kingdom

[†]Corresponding Author:

University of Basel, Zoological Institute, Evolutionary Biology, Vesalgasse 1, 4051 Basel, Switzerland

Email: pragya.singh42019@gmail.com

ORCID: <https://orcid.org/0000-0002-7411-3206>

Acknowledgements: We would like to thank Andrew Balmer for help with the plant measurements, and the Radiating Butterflies Group for helpful discussions. This study was supported by funding for summer studentship from Trinity College, Cambridge, an INSPIRE

22 scholarship travel fund to PS, and an Advanced Grant from the European Research Council
 23 (EMARES - 250325) to PMB.

24 **Author contributions:** All authors designed the experiments. PS and VO performed the
 25 experiments, statistically analysed the data and wrote the manuscript, with input from EvB,
 26 OB and PMB. All authors have read and approved the final version.

27

28 **Data accessibility:** All data in this manuscript will be deposited online on Zenodo (xx).

29 **Abstract**

30 Developmental plasticity in a seasonal environment allows an organism to optimally match its
 31 life-history traits with the fluctuating conditions. This critically relies on abiotic and biotic
 32 factors, such as temperature or food quality, that act as environmental cues and predict
 33 seasonal transitions. In most seasonal environments, multiple factors vary together, making it
 34 crucial to understand their combined effects on an organism's phenotype. Here, we study
 35 plasticity in a multivariate environment in the butterfly *Bicyclus anynana* that exhibits two
 36 distinct seasonal phenotypes. Temperature is an important cue mediating plasticity in this
 37 species, but other environmental cues such as larval host plant quality could also be
 38 informative since plant quality deteriorates during the transition from wet to dry season in the
 39 field. We examine how temperature and host plant quality interact to affect life-history traits.
 40 Using a full-factorial design, we expose cohorts of larvae to either poor (old plants) or high
 41 (young plants) quality plants at different temperatures. Our results show that plant quality had
 42 a temperature and sex-dependent effect on life-history traits. At lower and intermediate
 43 temperatures, it decreased body mass and prolonged development time, indicating that poor
 44 plant quality acted as a stressor. However, metabolic rates in adults were not affected,
 45 indicating that individuals could, at least in part, compensate for stressful juvenile conditions.
 46 In contrast, at higher temperatures poor plant quality induced a partial dry-season phenotype,
 47 indicating that it may have acted as an environmental cue. Moreover, poor plant quality,
 48 particularly in males, also decreased the correlation between life history traits, signifying
 49 disrupted phenotypic integration. Our study reveals complex interactive effects of two
 50 environmental variables on seasonal plasticity, reflecting differences in their reliability as
 51 seasonal cues. This highlights the importance of studying the combined effects of multiple
 52 environmental factors to better understand the regulation of phenotypic plasticity in wild.

53 ***Keywords***

54 developmental plasticity, food stress, plant quality, seasonal polyphenism, *Bicyclus anynana*,

55 reaction norm

56

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). Plasticity can help to ensure that the phenotype expressed by an organism matches the requirements of the organism's environment, and often involves trade-offs between life-history traits such as reproduction and lifespan, or growth rate and body size (Nylin 1992, Flatt and Heyland 2011, Torres-Dowdall et al. 2012). A particular case of phenotypic 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. Environmental cues can range from abiotic factors, like temperature and day-length, to biotic factors, such as food quality and predator presence. A special type of developmental plasticity is the Predictive Adaptive Response (PAR), in which an organism adjusts its phenotype to maximize fitness in future environmental conditions, even though the phenotype might not be immediately advantageous in the current environment. In fact, the phenotype could be maladaptive if the expected environmental change does not occur (Ghalambor et al. 2007, Monaghan 2008, Saastamoinen et al. 2010).

In a seasonal environment multiple environmental factors often vary together (Jackson et al. 2009, Chevin and Lande 2015). For example, low precipitation is usually accompanied by a decline in quantity or quality of resources. This leads to key open questions about i) whether organisms sense the environment through one or multiple cues, ii) whether these cues interact

(E x E) or act as independent predictors, and iii) whether they are processed in similar manners leading to similar phenotypic responses. One hypothesis would be that the factors act together as cues to predict future environmental conditions and potentially lead to an additive adaptive response. Alternatively, one factor could act as a cue, while the other environmental factor may not be perceived or processed at all, or could even be a stressor that disrupts the phenotype or phenotypic integration. Theoretical work has shown 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, Reed et al. 2010). Moreover, 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). While many earlier studies on plasticity have manipulated one environmental cue at a time, it is more likely that multiple environmental cues vary simultaneously in natural seasonal environments, making it important to study plasticity in a multivariate environment.

To investigate the effect of multivariate environment on developmental plasticity, we use the seasonally polyphenic butterfly *Bicyclus anynana*. This species exhibits two alternative seasonal forms (dry and wet) which correspond to a cool and a warm season, respectively. In addition to differences in morphology (Brakefield and Reitsma 1991), physiology (Oostra et al. 2011), and behaviour (van Bergen and Beldade 2019), the forms also differ markedly in their life-history traits (e.g. Oostra et al. 2011). Previous studies have shown that the temperature experienced during the (late) larval stage is a crucial cue for plasticity in this species (Brakefield and Reitsma 1991, Brakefield et al. 2007, 2009). Interestingly, variation in temperature alone does not produce the full extent of plasticity in life-history traits as observed in the wild (Roskam and Brakefield 1999), suggesting that other predictive environmental factors may act in conjunction with temperature (Brakefield 1987, Brakefield

and Reitsma 1991). Here, we hypothesise that larval host plant quality could be an important environmental cue, in addition to temperature, for developing individuals in the field. During the transition from wet to dry season in the field, the plants on which the larvae feed tend to be old and of poor quality (Brakefield and Reitsma 1991, Kooi et al. 1996). Moreover, earlier work in *B. anynana* has shown that this species, under conditions of food limitation, is able to produce a PAR phenotype (i.e. better adapted to cope with stressful conditions as an adult) (Saastamoinen et al. 2010, van den Heuvel et al. 2013).

In our study, we test how larval host plant quality—in conjunction with temperature—affects a suite of life history traits. In particular, using old host plants that mimic the deteriorating conditions in dry season, we feed cohorts of individuals for a part of larval development on old (poor quality) plants. Data collected from these individuals is compared to those of cohorts that had been reared on young (high quality) plants throughout development. These treatments enable an examination of whether larval host plant quality affects the phenotype. Furthermore, we test the effect of host plant quality at three different temperatures that correspond to wet, intermediate and dry season temperatures in the field. This design allows testing of how larval host plant quality and temperature interact to affect larval and pupal development time, pupal and adult mass, resting metabolic rate (RMR) and the respiratory quotient of adults. Earlier studies using this species examined RMR by measuring CO₂ respiration rate (Brakefield et al. 2007, Pijpe et al. 2007), and have showed that it varies in response to temperature. However, as of yet, no study has examined the O₂ consumption or respiratory quotient in *B. anynana*. Examining the respiratory quotient allows us to evaluate whether adults differ in their macronutrient metabolism in response to environmental conditions (i.e. whether they burn different fuels, in particular fat, protein and carbohydrates). Finally, we tested whether the host plant quality affects the organismal integration of

131 phenotypic traits by examining the correlation between life-history traits. The response of
 132 different phenotypic traits to environmental cues might be correlated (van Bergen et al. 2017),
 133 e.g. due to similar underlying physiological processes, but under stress we might expect the
 134 synchronised response of the traits to become uncoupled and phenotypic integration to
 135 decrease.
 136

Materials and Methods

Study organism

Bicyclus anynana is a Nymphalid butterfly from East Africa and is 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. The wet season butterflies experience high temperatures and precipitation during development, 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 (Brakefield et al. 2009, Oostra et al. 2011). In contrast, the dry season larvae that develop during the late wet season experience relatively cooler temperatures and lower precipitation which is associated with the transition from wet to dry season (Windig et al. 1994, van Bergen et al. 2016). Moreover, dry season adults accumulate higher mass and fat reserves during development; have small or absent eyespots, a higher metabolic rate, delayed reproduction until the following wet season, and a longer lifespan (Brakefield and Reitsma 1991, Pijpe et al. 2007, Oostra et al. 2011, Halali et al. 2019). The seasons, along with differing in temperature and precipitation, 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).

We used a full-factorial design to investigate the effects of larval host plant quality, pre-adult (i.e. larval and pupal) temperature, and their interaction 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 daily from the stock population. One day after hatching, larvae were randomly allocated to cages (35cm x 44cm x 65cm) with young maize plants set up in climate rooms at 19°C and 27°C, and in smaller climate-cabinets (Sanyo/Panasonic MLR-350H) at 23°C (all at 75% relative humidity and a 12h:12h day: night light cycle). Each cage had at least 140 larvae (950 larvae in total, Table 1). One day after they moulted to the 4th instar, larvae were switched to the host plant treatment: old maize or fresh young plants. 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. In addition, this period is close to the pupal stage when the adult phenotype is differentiated. The resulting pupae were then individually placed in transparent pots, assigned an ID and kept at their temperature treatment, until they eclosed.

After randomly culling excess pupae raised at 19°C and excluding 51 individuals due to missing information about one or multiple life-history traits, the final sample size across all treatments was 548 (see Table 1).

184

185 *Host plant quality treatments*

186 All maize plants were grown from seed and reared in a climate-controlled greenhouse in
 187 Madingley (United Kingdom), with regular watering to keep the soil moist at all times. Young
 188 maize plants were 2-3 weeks old whereas old maize plants were at least 5-7 weeks old. Earlier
 189 studies have shown that plant quality varies with age. For example, older plants typically have
 190 tougher leaves (Choong 1996, Loney et al. 2006), lower nutritional values (Hikosaka et al.
 191 1994) and different chemical/physical defences against herbivory (Barton and Koricheva
 192 2010) than younger plants. These differences can have pronounced effects on herbivory (Price
 193 et al. 1987, Loney et al. 2006), with the incidence of herbivorous invertebrates on old host
 194 plants typically being lower than on young plants (Choong 1996, Fenner et al. 1999, Boege
 195 and Marquis 2005). Thus, older host plants can be inferred to be of poor quality relative to
 196 younger host plants. Moreover, host plant quality can also directly regulate phenotypic
 197 plasticity in herbivorous insects (Lin et al. 2018).

198

199 In our experiment, we measured the maximum leaf width and height of each maize plant
 200 before feeding it to the larvae. For old maize plants, plant height was 92.2 ± 33.2 (mean $\pm$ sd)
 201 cm and maximum leaf-width was 4.2 ± 0.6 cm. For young maize plants, plant height was
 202 69.6 ± 4.5 cm and maximum leaf-width was 1.4 ± 0.2 cm. The larvae were reared on whole
 203 plants (multiple larvae per plant), and *ad libitum* feeding was ensured by providing new plants
 204 whenever needed. When the old plants were too large to be completely accommodated inside
 205 the cage, only a part of the (whole) plant was put inside, while ensuring that the larvae could
 206 not escape from the cage.

207

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. For the RMR measurements, individual butterflies were measured in the dark—at their rearing temperature—in small cylindrical glass containers (4 cm in diameter × 9 cm in height). Each RMR cycle consisted of three runs of 20 minutes during which RMR was measured as the individual rate of CO₂ and O₂ respiration (millilitre per minute), using stop-flow respirometry (Pijpe et al. 2007). CO₂ and O₂ production were measured using a LI-7000 CO₂ 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 CO₂ and O₂ respiration rates were scaled to mass by dividing respiration rate by adult mass. Measurements were taken at the same time of the day 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 CO₂ respiration rate divided by the O₂ respiration rate (Richardson 1929).

Statistical analyses

We performed a Chi-square Goodness-of-Fit test to test if host plant quality had an effect on the larval and pupal survivorship. We also performed a Chi-Square Test for Independence to assess if the host plant quality had a sex-specific effect on survivorship for each temperature.

In addition, for each dependent variable (larval development time, pupal development time, pupal mass, adult mass, CO₂ and O₂ respiration rates (unscaled and scaled by mass), and respiratory quotient), we constructed a three-way ANOVA with temperature, host plant quality, sex, and all interactions, as independent fixed factors. We evaluated each model and removed the least significant term from this model at 0.05 significance level ($p < 0.05$) in a step-wise fashion to obtain the minimal adequate model. Here we report the results of the minimal adequate model for all the dependent variables (Supplementary S1). Prior to statistical analyses, all traits, except pupal mass, were log-transformed when they did not fulfil the assumptions of a parametric test. We did post-hoc comparisons using t-tests and corrected for multiple testing with Bonferroni correction.

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 (all traits except unscaled CO₂ and unscaled O₂ respiration rates) for individuals reared on both young and old host plants, and for each sex (i.e. four correlation coefficients per pair of traits). We tested whether poor host plant quality disrupted the seasonal morphs by comparing the correlation coefficient of each life-history trait pair between old and young host plants. We converted the correlation coefficient into a z -score using Fisher's r -to- z transformation 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 assessed whether the difference

between the correlation coefficients was statistically significant ($P \leq 0.05$) by checking if the Z_{observed} was greater than the critical value of ± 1.96 .

All the analyses were done in R version 3.1.0 (R Development Core Team 2016).

Results

In general, temperature and sex had a significant effect on all traits except respiratory quotient, as had been found in previous studies (Pijpe et al. 2007, de Jong et al. 2010, Oostra et al. 2011, 2014, Mateus et al. 2014). The effect of host plant quality varied among traits, typically with a temperature and/or sex dependent effect (Table 1, Supplementary S1).

Limited effect of host plant quality on pre-adult survivorship

Development on old maize had a limited effect on larval and pupal survivorship, with a significant effect only on larval survivorship at 23°C, where survival was higher on young maize plants (Table 1). Moreover, host plant quality did not have a sex-specific effect on survivorship (Table 1).

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

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 increase in temperature, and males had a shorter larval but longer pupal development time than females (Supplementary S1). 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 and pupal development time at 23°C (Figure 1). At this intermediate temperature the larvae/pupae took a longer time to develop on old maize.

Temperature-dependent effects of host plant quality on body mass

Host plant quality led to a temperature-specific reduction in pupal mass, and to a temperature and sex-specific effect on adult mass (Supplementary S1, Figure 2). On old maize, both sexes had lower pupal mass at 19°C and 23°C, while both sexes had higher pupal mass on old maize at 27°C, though the latter was not statistically significant. Furthermore, pupal mass significantly decreased with increasing temperature, and was higher in females. Both sexes had lower adult mass on old maize at 19°C, while only females had a lower adult mass on old maize at 23°C. Similar to pupal mass, both sexes had higher adult mass on old maize at 27°C, though this effect was only significant for females. Females had a higher adult mass compared to males for all three temperatures.

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

Similar to pupal mass, unscaled CO₂ and O₂ respiration rate were significantly affected by the interaction between temperature and host plant quality (Supplementary S1, Figure 3, 4). Adults of both sexes had a higher unscaled CO₂ respiration rate when reared on old compared to young maize at 27°C (Figure 3). In contrast, there was no significant effect of plant quality on unscaled O₂ respiration rate at any temperature (Figure 4). Females had significantly higher unscaled CO₂ and O₂ respiration rates than males. Moreover, on both old and young plants, unscaled CO₂ and O₂ respiration rates were significantly lower at 19°C compared to the other temperatures, but did not differ significantly between 23°C and 27°C. However, after scaling (correcting) for body mass, only temperature and sex had a significant effect on

CO₂ and O₂ respiration rates (Supplementary S1, Figure 3, 4), indicating that the effect of food stress on CO₂ and O₂ respiration was modulated by a difference in body mass. Similar to earlier studies on CO₂ respiration rates in this species (Brakefield et al. 2007, Pijpe et al. 2007), both the CO₂ and O₂ respiration rate increased with temperature (with all temperatures being significantly different from each other), and males had higher respiration rates than females. The respiratory quotient was not affected by sex, temperature, food stress, or any interactions between these factors (Supplementary S1, Figure 5).

Poor host plant quality disrupts phenotypic integration

Host plant quality significantly changed the correlation matrix for life-history traits in both sexes (Figure 6, for details see Supplementary S2). Males were more severely affected, with 13 out of 21 correlation coefficients being significantly different between young and old host plants, while for females only 7 out of 21 correlation coefficients were significantly affected. Amongst the significant changes, for males, all 13 correlation coefficients decreased on old host plants while for females 5 correlation coefficients decreased and 2 correlation coefficients increased on old host plants. Thus, in general, poor host plant quality resulted in a less integrated phenotype with a decrease in correlations among life-history traits, especially for males.

Discussion

While several earlier studies have examined the effect of food stress on life-history traits in different organisms, relatively few studies have analysed the role of food quality as a

predictive cue for future conditions, especially in combination with other cues such as temperature (Wilson 1994, Rotem et al. 2003, Bauerfeind and Fischer 2005, Coley et al. 2006, Rosa and Saastamoinen 2017). Examining the effect of food quality in combination with other environmental cues is important, as most seasonal environments in nature are usually characterized by multiple environmental factors varying together. In our experiment, we examined the effect of host plant quality in conjunction with temperature, and found that host plant quality had a temperature- and sex-specific effect on life-history traits.

Earlier studies using *B. anynana* and food deprivation as nutritional stress, had shown that stressed individuals had a reduced body mass and prolonged developmental time (Bauerfeind and Fischer 2005, Saastamoinen et al. 2010, 2013). These results contrast our findings and the difference in effect is likely due to the different approaches used; in the earlier studies individuals were starved (food deprivation) while in our study individuals were fed *ad libitum* on old host plants. These older host plants are expected to be less palatable (i.e. tougher), have lower nutritional values and differ in their composition of secondary metabolites relative to younger plants. Compared to food deprivation, larvae developing on old host plants were likely able to acquire sufficient resources and thus, demonstrated a less dramatic change in the life-history traits studied. Moreover, food deprivation and old host plants may activate different physiological responses.

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 (which can also depend on temperature experienced). If reduced host plant quality can be perceived as an environmental cue indicative of harsh (future) environmental conditions, we

would have expected development on old host plants to lead to a more dry-season phenotype (e.g. an increase in body mass). Indeed, when exposed to the thermal conditions of the wet-season (27°C), poor host plant quality induced a partial dry-season phenotype with an increase in adult mass (significant for females). In contrast to the pattern observed at high temperatures, at temperatures that mimic the dry season (19°C), poor host plant quality did not act as a seasonal cue. Instead, the treatment resulted in lower body mass and longer development times, indicating a stress response. The increase in adult mass in wet-season form butterflies (27°C) could potentially indicate an adaptive response (e.g. PAR-like) for within-season fluctuations in food quality. Food quality can potentially vary independently of temperature, making it an important cue under conditions when the thermal information may be inconclusive (see below).

Interestingly, for several traits, including 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). One possible explanation for observing the above effects only at 23°C could be that increased sensitivity for host plant quality could be adaptive at this temperature. Larvae developing during the transition between the seasons, or during a cold spell in the wet season, may require additional environmental information to induce the expression of the appropriate phenotype (see above). Moreover, the prolonged development time at 23°C is likely due to the old maize being of a poorer quality (e.g. tougher leaves and/or more secondary metabolites), prolonging the period necessary to reach the critical mass needed for undergoing hormonal changes and pupation (Coley et al. 2006). Remarkably, despite the prolonged time to acquire resources, the individuals reared on old plants had a lower pupal mass (both sexes) and adult mass (only significant for females).

372

373 In addition, the effect of host plant quality on body mass was more evident compared to the
374 effect on development time and survivorship at all thermal environments. This may be related
375 to the fact that the larvae were only exposed to the poor host plant quality during the final two
376 larval instars. The latter represents the period when most growth occurs, but it is only a short
377 period of the total development time, potentially resulting in more pronounced effects on
378 body mass compared to development time.

379

380 We also examined for the first time the respiratory quotient, the ratio between CO₂ and O₂
381 respiration rate at rest. We found that it was not influenced by either temperature, sex, host
382 plant quality, or their interactions. The respiratory quotient reflects which macronutrients are
383 metabolized for energy, with values of 0.7, 0.8 or 1.0 indicating fat, protein or carbohydrate
384 metabolism, respectively (Nunes et al. 1997). In our study, the respiratory quotient was
385 around 0.9, which is intermediate between protein and carbohydrate metabolism, across all
386 temperatures. Thus, our results point towards adult macronutrient metabolism remaining
387 similar, irrespective of larval food quality. This is surprising, as earlier studies in both field
388 and laboratory showed that dry season form butterflies have a higher fat content (Brakefield
389 and Reitsma 1991, Pijpe et al. 2007, de Jong et al. 2010, Oostra et al. 2011). However, we
390 measured the metabolic rates of newly eclosed adults under benign conditions in the
391 laboratory where fat reserves are likely under-used compared to the wild, where adults often
392 face prolonged periods of desiccation and/or starvation.

393

394 The overall phenotypic integration of traits was affected by plant quality, with a decrease in
395 the correlation between life history traits on poor quality host plants, signifying disrupted

phenotypic integration, particularly in males. Interestingly, a similar phenomenon of sex-specific effects of larval food stress on larval and adult performance has been found in a study using the Glanville fritillary butterfly, *Melitaea cinxia* (Rosa and Saastamoinen 2017). This could indicate that the underlying physiological processes that are responsible for phenotypic integration, e.g. hormone signalling, may have a sex-specific component, and that stressors affecting these processes result in a more extensive phenotypic disintegration in only one of the sexes.

A possible explanation for the lack of a role of host plant quality as a cue of seasonal progression, at least in thermal conditions of the dry season, could be that temperature suffices as a cue, as our lab population of *B. anynana* originated from a location in Malawi where temperature is a highly reliable predictor of seasonal transitions (Oostra et al. 2018). Thus, the reliability of temperature may override the necessity for additional cues in this population. Hence, an interesting open question 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 less (Roskam and Brakefield 1996, van Bergen et al. 2017). Future studies could investigate the effect of host plant quality on life-history traits in populations or species originating from regions where temperature is a relatively unreliable cue. In addition, here we only exposed the larvae to old maize plants during the last two instars of development, while the larvae of this species utilize a variety of grass species in the wild and are likely to feed on poor quality host plants for longer periods (Brakefield et al. 2009, van Bergen et al. 2016). The deteriorating effects of the dry season conditions on natural grasses are likely to be host specific and longer exposure to poor quality host during development may trigger more pronounced phenotypic effects.

Taken together, plant quality affected life history traits in a temperature- and sex-specific manner in our study, 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 also become relevant in shaping the organism's phenotype. Thus, it is important to study phenotypic plasticity in a multivariate environment as multiple environmental factors can interact to produce different phenotypes, and are better representative of conditions occurring in the wild.

References

- Barton, K. E. and Koricheva, J. 2010. The ontogeny of plant defense and herbivory: characterizing general patterns using meta-analysis. - *Am. Nat.* 175: 481–493.
- Bauerfeind, S. S. and Fischer, K. 2005. Effects of food stress and density in different life stages on reproduction in a butterfly. - *Oikos* 111: 514–524.
- Beldade, P. et al. 2011. Evolution and molecular mechanisms of adaptive developmental plasticity. - *Mol. Ecol.* 20: 1347–1363.
- Boege, K. and Marquis, R. J. 2005. Facing herbivory as you grow up: the ontogeny of resistance in plants. - *Trends Ecol. Evol.* 20: 441–448.
- Brakefield, P. M. 1987. Tropical dry and wet season polyphenism in the butterfly *Melanitis leda* (Satyridae): Phenotypic plasticity and climatic correlates. - *Biol. J. Linn. Soc.* 31: 175–191.
- Brakefield, P. M. and Reitsma, N. 1991. Phenotypic plasticity, seasonal climate and the population biology of *Bicyclus* butterflies (Satyridae) in Malawi. - *Ecol. Entomol.* 16: 291–303.
- Brakefield, P. M. et al. 2007. Developmental plasticity and acclimation both contribute to adaptive responses to alternating seasons of plenty and of stress in *Bicyclus* butterflies. - *J. Biosci.* 32: 465–475.
- Brakefield, P. M. et al. 2009. The African Butterfly *Bicyclus anynana*: A Model for Evolutionary Genetics and Evolutionary Developmental Biology. - Cold Spring Harb. Protoc. 2009: pdb.emo122-pdb.emo122.
- Chevin, L. M. and Lande, R. 2015. Evolution of environmental cues for phenotypic plasticity. - *Evolution* (N. Y). 69: 2767–2775.
- Choong, M. F. 1996. What makes a leaf tough and how this affects the pattern of castanopsis fissa leaf consumption by caterpillars. - *Funct. Ecol.* 10: 668.
- Coley, P. D. et al. 2006. The effects of plant quality on caterpillar growth and defense against natural enemies. - *Oikos* 115: 219–228.
- de Jong, M. et al. 2010. Geographic variation in thermal plasticity of life history and wing pattern in *Bicyclus anynana*. - *Clim. Res.* 43: 91–102.
- Fenner, M. et al. 1999. Comparison of seedling and adult palatability in annual and perennial plants. - *Funct. Ecol.* 13: 546–551.

- 460 Flatt, T. and Heyland, A. 2011. Mechanisms of Life History Evolution: The Genetics and
461 Physiology of Life History Traits and Trade-Offs. - Genetics: 540.
- 462 Ghalambor, C. K. et al. 2007. Adaptive versus non-adaptive phenotypic plasticity and the
463 potential for contemporary adaptation in new environments. - Funct. Ecol. 21: 394–407.
- 464 Gotthard, K. and Nylin, S. 1995. Adaptive plasticity and plasticity as an adaptation: a
465 selective review of plasticity in animal morphology and life history. - Oikos 74: 3.
- 466 Halali, S. et al. 2019. To mate, or not to mate: the evolution of reproductive diapause
467 facilitates insect radiation into African savannahs in the Late Miocene. - bioRxiv:
468 693812.
- 469 Hikosaka, K. et al. 1994. Effects of leaf age, nitrogen nutrition and photon flux density on the
470 distribution of nitrogen among leaves of a vine (*Ipomoea tricolor* Cav.) grown
471 horizontally to avoid mutual shading of leaves. - Oecologia 97: 451–457.
- 472 Jackson, S. T. et al. 2009. Ecology and the ratchet of events: climate variability, niche
473 dimensions, and species distributions. - Proc. Natl. Acad. Sci. U. S. A. 106 Suppl:
474 19685–92.
- 475 Kooi, R. E. et al. 1996. Effects of food plant on phenotypic plasticity in the tropical butterfly
476 *Bicyclus anynana*. - Entomol. Exp. Appl. 80: 149–151.
- 477 Lafuente, E. and Beldade, P. 2019. Genomics of Developmental Plasticity in Animals. -
478 Front. Genet. in press.
- 479 Leimar, O. et al. 2006. A new perspective on developmental plasticity and the principles of
480 adaptive morph determination. - Am. Nat. 167: 367–376.
- 481 Lin, X. et al. 2018. Host quality induces phenotypic plasticity in a wing polyphenic insect. -
482 Proc. Natl. Acad. Sci. 115: 7563–7568.
- 483 Loney, P. E. et al. 2006. How does ontogeny in a Eucalyptus species affect patterns of
484 herbivory by Brushtail Possums? - Funct. Ecol. 20: 982–988.
- 485 Mateus, A. R. A. et al. 2014. Adaptive developmental plasticity: Compartmentalized
486 responses to environmental cues and to corresponding internal signals provide
487 phenotypic flexibility. - BMC Biol. 12: 97.
- 488 Monaghan, P. 2008. Early growth conditions, phenotypic development and environmental
489 change. - Philos. Trans. R. Soc. Lond. B. Biol. Sci. 363: 1635–45.
- 490 Nunes, L. et al. 1997. On the respiratory quotient (RQ) of termites (Insecta: Isoptera). - J.
491 Insect Physiol. 43: 749–758.
- 492 Nylin, S. 1992. Seasonal plasticity in life history traits: growth and development in *Polygonia*
493 *c-album* (Lepidoptera: Nymphalidae). - Biol. J. Linn. Soc. 47: 301–323.
- 494 Oostra, V. et al. 2011. Translating environmental gradients into discontinuous reaction norms
495 via hormone signalling in a polyphenic butterfly. - Proc. R. Soc. B Biol. Sci. 278: 789–
496 797.
- 497 Oostra, V. et al. 2014. Ecdysteroid Hormones Link the Juvenile Environment to Alternative
498 Adult Life Histories in a Seasonal Insect. - Am. Nat. 184: E79–E92.
- 499 Oostra, V. et al. 2018. Strong phenotypic plasticity limits potential for evolutionary responses
500 to climate change. - Nat. Commun. 9: 1005.
- 501 Pijpe, J. et al. 2007. Phenotypic plasticity of starvation resistance in the butterfly *Bicyclus*
502 *anynana*. - Evol. Ecol. 21: 589–600.
- 503 Price, P. W. et al. 1987. Plant age and attack by the bud galler, *Euura mucronata*. - Oecologia
504 73: 334–337.
- 505 R Development Core Team 2016. R: A Language and Environment for Statistical Computing.
506 - R Found. Stat. Comput. Vienna Austria 0: {ISBN} 3-900051-07-0.
- 507 Reed, T. E. et al. 2010. Phenotypic plasticity and population viability: The importance of
508 environmental predictability. - Proc. R. Soc. B Biol. Sci. 277: 3391–3400.
- 509 Richardson, H. B. 1929. The respiratory quotient. - Physiol. Rev. 9: 61–125.

- 510 Rosa, E. and Saastamoinen, M. 2017. Sex-dependent effects of larval food stress on adult
511 performance under semi-natural conditions: only a matter of size? - *Oecologia* 184: 633–
512 642.
- 513 Roskam, J. C. and Brakefield, P. M. 1996. A comparison of temperature-induced
514 polyphenism in african bicylus butterflies from a seasonal savannah-rainforest ecotone. -
515 *Evolution* (N. Y). 50: 2360.
- 516 Roskam, J. C. and Brakefield, P. M. 1999. Seasonal polyphenism in *Bicyclus* (Lepidoptera:
517 Satyridae) butterflies: Different climates need different cues. - *Biol. J. Linn. Soc.* 66:
518 345–356.
- 519 Rotem, K. et al. 2003. Parental effects in *Pieris rapae* in response to variation in food quality:
520 adaptive plasticity across generations? - *Ecol. Entomol.* 28: 211–218.
- 521 Saastamoinen, M. et al. 2010. Predictive Adaptive Responses: Condition-Dependent Impact
522 of Adult Nutrition and Flight in the Tropical Butterfly *Bicyclus anynana*. - *Am. Nat.* 176:
523 686–698.
- 524 Saastamoinen, M. et al. 2013. Quantitative genetic analysis of responses to larval food
525 limitation in a polyphenic butterfly indicates environment and trait-specific effects. -
526 *Ecol. Evol.* 3: 3565–3575.
- 527 Tauber, M. J. et al. 1986. Seasonal adaptations of insects. - Oxford University Press on
528 Demand.
- 529 Torres-Dowdall, J. et al. 2012. Local adaptation and the evolution of phenotypic plasticity in
530 trinidadian guppies (*Poecilia reticulata*). - *Evolution* (N. Y). 66: 3432–3443.
- 531 Tufto, J. 2000. The Evolution of Plasticity and Nonplastic Spatial and Temporal Adaptations
532 in the Presence of Imperfect Environmental Cues. - *Am. Nat.* 156: 121–130.
- 533 van Bergen, E. and Beldade, P. 2019. Seasonal plasticity in anti-predatory strategies:
534 Matching of color and color preference for effective crypsis. - *Evol. Lett.* 3: 313–320.
- 535 van Bergen, E. et al. 2016. The stable isotope ecology of mycalesine butterflies: implications
536 for plant-insect co-evolution (KL Purn, Ed.). - *Funct. Ecol.* 30: 1936–1946.
- 537 van Bergen, E. et al. 2017. Conserved patterns of integrated developmental plasticity in a
538 group of polyphenic tropical butterflies. - *BMC Evol. Biol.* 17: 59.
- 539 van den Heuvel, J. et al. 2013. The predictive adaptive response: modeling the life-history
540 evolution of the butterfly *Bicyclus anynana* in seasonal environments. - *Am. Nat.* 181:
541 E28–42.
- 542 Wilson, L. J. 1994. Plant-Quality Effect on Life-History Parameters of the Twospotted Spider
543 Mite (Acari: Tetranychidae) on Cotton. - *J. Econ. Entomol.* 87: 1665–1673.
- 544 Windig, J. J. et al. 1994. Seasonal polyphenism in the wild: survey of wing patterns in five
545 species of *Bicyclus* butterflies in Malawi. - *Ecol. Entomol.* 19: 285–298.

549 **Tables**

550 Table 1. Larval and pupal survival (% in brackets) for different temperatures. The larval survivorship was computed as the number of pupations
 551 and is expressed as a percentage of the initial number of larvae reared. Pupal survivorship was the number of adults which eclosed and is
 552 expressed as a percentage of the initial number of pupae.

553 ** We discarded excess pupae at 19°C and only kept 130 pupae.

Temperature (°C)	Maize	No. of larvae	Larval survivorship	Chi-square test analysis of larval survivorship			Pupal survivorship	Chi-square test analysis of pupal survivorship			No. of female adults	No. of male adults	Chi-square test analysis of survivorship of sexes		
				χ^2	df	p		χ^2	df	p			χ^2	df	p
19	old	140	98 (70%)	0.2	1	0.66	91 (93%)	0.18	1	0.67	46 (100%)	45 (98%)	0.07	1	0.79
	young	250	185 (74%)				128 (98%) **				67 ()	61			
23	old	140	102 (73%)	4.34	1	0.04	95 (93%)	0.51	1	0.48	49	46	0.08	1	0.77
	young	140	134 (96%)				113 (84%)				56	57			

27	old	140	91 (65%)	2.17	1	0.14	74 (81%)	0.23	1	0.63	42	32	0.21	1	0.65
	young	140	112 (80%)				98 (87%)				59	39			

554

555

556

557

Figures

Figure 1. Slower development due to poor host plant quality at certain temperatures: Effect of host plant quality and temperature on larval development time (top row) and pupal development time (bottom row) in each sex. Plots show means and 95% confidence intervals of data. Statistically significant effect of host plant quality on development time is indicated for each temperature with *, indicating $P \leq 0.05$. Red lines represent old host plants and black lines represents young host plants.

Figure 2. Temperature and sex-dependent effects of host plant quality on body mass: Effect of host plant quality and temperature on pupal mass (top row) and adult mass (bottom row) in each sex. Plots show means and 95% confidence intervals of data. Statistically significant effect of host plant quality on development time is indicated for each temperature with *, indicating $P \leq 0.05$. Red lines represent old host plants and black lines represents young host plants.

Figure 3. Limited effect of host plant quality on unscaled and scaled CO₂ respiration rates (production per hour): Effect of host plant quality and temperature on unscaled CO₂ respiration rate (top row) and CO₂ respiration rate scaled by mass (bottom row) in each sex. Plots show means and 95% confidence intervals of data. Statistically significant effect of host plant quality on development time is indicated for each temperature with *, indicating $P \leq 0.05$. Red lines represent old host plants and black lines represents young host plants.

Figure 4. No effect of host plant quality on unscaled and scaled O₂ respiration rates (production per hour): Effect of host plant quality and temperature on unscaled O₂ respiration rate (top row) and O₂ respiration rate scaled by mass (bottom row) in each sex. Plots show means and 95% confidence intervals of data. Red lines represent old host plants and black lines represents young host plants.

Figure 5. No effect of temperature and host plant quality on respiratory quotient in each sex. Plots show means and 95% confidence intervals of data. Red lines represent old host plants and black lines represents young host plants.

Figure 6. Poor host plant quality disrupts phenotypic integration, particularly for males:

Correlation coefficients (r) between log transformed trait values for a) females and, b) males on old (poor quality) or young (high quality) host plants. Each line represents the correlation coefficient between one pair of traits. Correlation coefficients that changed significantly ($P \leq 0.05$), due to poor host plant quality, are highlighted in red. Sample sizes for calculating each correlation coefficient are given at the bottom.