Arthropod-Plant Interactions
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Red foliage color reliably indicates low host quality and increased metabolic load for development of an herbivorous insect

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Abstract Plant chemical defense and coevolved detoxification mechanisms in specialized herbivorous insects are fundamental in determining many insect–plant interactions. For example, Brassicale plants protect themselves from herbivory by producing glucosinolates, but these secondary metabolites are effectively detoxified by larvae of Pierid butterflies. Nevertheless, not all Brassicales are equally preferred by these specialist herbivores. Female *Pieris* butterflies avoid laying eggs on anthocyanin-rich red foliage, suggesting red color is a visual cue affecting oviposition behavior. In this study, we reared *P. brassicae* larvae on green and red cabbage leaves, to determine whether foliage color reliably indicates host plant quality. We did not find a difference in survival rates or maximal larval body mass in the two food treatments. However, larvae feeding on red cabbage leaves exhibited significantly lower growth rates and longer durations of larval development. Interestingly, this longer development was coupled with a higher consumption rate of dry food matter. The lower ratio of body mass gain to food consumption in larvae feeding on red cabbage leaves was coupled with significantly higher (ca. 10 %) larval metabolic rates. This suggests that development on red foliage may incur an increased metabolic load associated with detoxification of secondary plant metabolites. Energy and oxygen allocation to detoxification could come at the expense of growth and thus compromise larval fitness as a result of extended development. From an evolutionary perspective, red foliage color may serve as an honest defensive cue, as it reliably indicates the plant's low quality as a substrate for larval development.

Keywords Defensive plant coloration · Detoxification · Development · Fitness · Metabolic rate · *Pieris* · Secondary metabolites

Introduction

Plant secondary metabolites are substances that are not universal to all higher plants. Rather, many of them are restricted to certain taxa or appear in some taxa and plant organs in considerably higher concentrations compared with others. Initially thought to be waste products, many secondary metabolites are of no nutritional value to herbivorous insects and are often toxic. However, secondary metabolites are currently recognized as having great ecological and economic importance as they affect the biology of plant–insect interactions, including pollinator attraction and herbivore deterrence (Schoonhoven et al. 1998). According to coevolutionary theory, certain insects have been so successful in overcoming the adverse effect of plant secondary metabolites that they have become specialists on a clade of host plants that share a common chemical defense mechanism (Ehrlich and Raven 1964).

Glucosinolates, a group of sulfur- and nitrogen-containing compounds, form a diverse group of plant secondary metabolites occurring mainly in the Brassicales plant order (Schoonhoven et al. 1998). Their rapid hydrolysis upon plant tissue rupture makes the plant unpalatable or even toxic to most generalist insect herbivores (e.g., Wittstock et al. 2004). However, evolved detoxification mechanisms in Pierinae butterflies (Lepidoptera: family

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Pieridae) allowed the adaptive radiation of Pierinae shortly after the evolution of their Brassicale hosts (Wittstock et al. 2004; Wheat et al. 2007). Observations of the specificity of *Pieris* larvae to Brassicale hosts date back to 1660 (see Mickel 1973). A number of studies have since confirmed that the specificity of these lepidopterans to host plants is based on stimulation of egg laying and larval feeding by glucosinolates (reviewed by Chew and Renwick 1995). Although larvae of the cabbage white butterflies, *Pieris brassicae*, are largely restricted to Brassicaceae plants, they can also be found on other glucosinolate-producing plants (e.g., *Nasturtium*; Schoonhoven et al. 1998). Glucosinolates in the plant leaves do not yield volatiles prior to plant tissue rupture, and therefore, gustation is involved in locating suitable plants for oviposition (Chew and Renwick 1995). Still, visual cues are critical in searching and orientating toward the host plant, earlier in the decision-making behavioral sequence (Renwick and Chew 1994). Visual cues affecting oviposition behavior in *Pieris* include host color (Irwin et al. 2003) and size (e.g., Firake et al. 2012), as well as non-plant factors such as the presence of eggs previously laid by conspecifics (Schoonhoven 1990).

The effect of foliage color or color-related traits on herbivore reproductive behavior has been reported in studies showing that *P. rapae* (Radcliffe and Chapman 1966) and *P. brassicae* (Metspalu et al. 2003) are less likely to lay eggs on red than on green cabbage leaves. These observations are particularly intriguing in light of the considerably higher concentrations of the favored glucosinolates in the former (Rosen et al. 2005; Fritz et al. 2010). Possibly, other secondary metabolites mediate *Pieris*' deterrence from red leaves. Anthocyanin pigments are obvious candidates when the visual aspect of deterrence is considered.

Anthocyanins are a group of flavonoids, a major class of phenolic plant secondary metabolites, comprising most of the red and blue pigments in plants (Schoonhoven et al. 1998). Apart from enhancing plant reproduction by attracting pollinators, anthocyanins also play a role in plant resistance to abiotic (e.g., Gould et al. 1995) and biotic stress (Lev-Yadun and Gould 2009). However, despite their antimicrobial and antifungal properties, anthocyanins appear to be harmless to higher animals (Lee et al. 1987; but see Johnson et al. 2008). Therefore, they have been suggested to serve as coevolved signals for plant defense capacities (Archetti 2000; Hamilton and Brown 2001; Archetti et al. 2009), undermining herbivore crypsis or as aposematic signals (Lev-Yadun and Gould 2009). According to the alternative non-exclusive "defense indication hypothesis" (Schaefer and Rolshausen 2006), the biochemical pathway of anthocyanin synthesis includes precursors for other compounds that (among other functions) inhibit food intake rate and digestion efficiency in insects. Therefore, despite the innocuous nature of anthocyanins to insect herbivores, their color may advertise the presence of other co-occurring metabolites that are more toxic. This may provide potential herbivores with a reliable visual indicator of the low quality of the host plant (see also Fineblum and Rausher 1997).

Anthocyanin levels in leaves of red cabbage are considerably higher compared with those of green cabbage (Gerchman et al. 2011). Nevertheless, despite the consistent preference of female *Pieris* butterflies for laying eggs on green compared with red leaves, experimental evidence for the consequence of choosing the latter is equivocal. In *P. rapae*, larval survival rates (Radcliffe and Chapman 1966) and mass gain (Irwin et al. 2003) were higher when feeding on anthocyanin-producing hosts. In *P. brassicae*, no difference was found in developmental time or pupal mass between larvae feeding on red and green cabbage (Metspalu et al. 2003). However, survival to pupation was higher on green cabbage leaves, in contrast to the finding of Radcliffe and Chapman (1966).

In this study, we measured larval survival rates, developmental time, food intake and growth rates of *P. brassicae* larvae reared on red and green cabbage leaves. We hypothesized that development on red leaves will incur a cost in terms of the butterfly's fitness if anthocyanins are a reliable signal for poor food quality. We complemented the determination of growth indices with measurements of larval gas exchange rates. To the best of our knowledge, this is the first study using larval respirometry in order to establish whether development on anthocyanin-rich leaves is associated with resource investment by their insect herbivores, and with increased metabolic loads resulting from the need for detoxification of allelochemicals (Whittaker and Feeny 1971).

Materials and methods

Larvae

Adult *P. brassicae* were collected in the botanical gardens of the Oranim campus, in Kiryat Tivon, Israel. They were then held in a semi-shaded outdoors enclosure (300 × 280 × 300 cm) where they mated and laid eggs on *Tropaeolum majus* (Brassicaceae) leaves. Additional egg masses were collected from *T. majus* leaves on the Oranim campus and from nearby areas in Kiryat Tivon, Israel. Each egg mass was cut from the host plant and carefully separated and distributed between two food type treatments, namely green and red cabbage (*Brassica oleracea* L. var. *capitata*) leaves. Both cabbage variants were purchased from a grocery store and kept refrigerated for providing larvae with fresh leaves daily. In a preliminary experiment,

no difference was recorded in hatching rate (90 and 89 % for green and red cabbage groups, respectively). Larvae were held in 150-mL plastic containers covered with cloth to allow gas exchange and were kept in a temperature cabinet at 24 °C (12L:12D) throughout the experiment. Larvae were reared in groups until after the second molt, after which they were placed individually in the containers. Growth and development data are based on larvae from four cohorts (egg batches), whereas food consumption and metabolic rate data also include individuals from two additional cohorts.

Survival, development and growth

The experimental subjects were inspected daily to determine hatching date, timing of larval molts, survival and developmental time (egg to adult eclosion). Dividing dense egg masses for distribution between the two food treatments resulted in unavoidable damage to some eggs, and therefore, survival rates were calculated as the proportion of individuals completing development to successful pupation, out of a total of 116 inspected third instar larvae. Growth and development were recorded for these larvae, but data from individuals that did not develop to successful pupation were discarded from development analyses. Thus, growth and development curves are based on healthy individuals only. Overall, a total of 95 larvae from four cohorts were included in the growth and development analyses. The occurrence of larval molts was determined morphologically and by the presence of exuviae. Following their transfer to separate containers after the second molt, body mass of individual larvae was determined (±0.1 mg) daily. Growth rates (g h^{-1}) were calculated for fourth and fifth larval instars as follows:

$$\left(\mathrm{Mb}_{(3)} - \mathrm{Mb}_{(2)}\right)/t$$

where $\mathrm{Mb}_{(2)}$ and $\mathrm{Mb}_{(3)}$ are larval body masses on the second and third day, respectively, after molting from the previous instar, and t is the time (in hours) between mass measurements.

Food intake

Food intake rate was calculated by weighing the cabbage leaves before larval feeding on the second day following molting to the fourth and fifth larval instars and again on the following day. These instars typically lasted ~4 days, and thus, second day feeding was unlikely to be affected by reduced rates before and after a molt. Food intake calculation was corrected for water evaporation from the leaves by placing similar-sized leaves ($N = 15$ each) without larvae in identical containers and recording changes in mass following 24 h of incubation at 24 °C. Similar evaporative water loss rates ($F_{1,27} = 0.03$, $P = 0.85$; ANCOVA with initial leaf mass as a covariate) were recorded for the two cabbage variants. A pooled dataset yielded a 16.5 % loss of initial leaf mass during 24 h at 24 °C. Therefore, we estimated food intake rate (g h^{-1}) using the following equation:

$$\left(\mathrm{M}f_{(\mathrm{b})} - \mathrm{M}f_{(\mathrm{a})}/0.835\right)/t$$

where $\mathrm{Mf}_{(\mathrm{b})}$ and $\mathrm{Mf}_{(\mathrm{a})}$ are the leaf masses before and after larval feeding, respectively, and t is the time (in hours) between mass measurements.

Dry mass/water content of green and red cabbage was determined by weighing leaves before and after drying to constant mass at 60 °C.

Metabolic rate determination

A total of 75 larvae were measured, from at least three different cohorts for each combination of food treatment and larval instar ($N = 12$–14 for each). Larval metabolic rates were measured as CO_2 emission rates using flow-through respirometry at 24 ± 0.2 °C (MIR-554 cooling incubator, Panasonic, Japan), 2 days after the second, third and fourth larval molts. Larvae were placed individually in glass metabolic chambers that were flushed with dry, CO_2-free air by passing outside air through silica gel–ascarite–silica gel columns at 25 mL min^{-1} (factory-calibrated FMA-2617A mass flow controllers; Omega engineering, Stanford, CT, USA). Excurrent air was passed through a CO_2 analyzer (LI-7000, Li-Cor Bioscience, Lincoln, NE, USA). Each animal was measured for 15 min after a 15-min acclimation period to the metabolic chamber environment. Data recorded during the last 5 min were averaged for calculating CO_2 emission rate for each larva. Output from the gas analyzer was collected, stored and analyzed using Expedata acquisition and analysis software (Sable Systems International, Las Vegas, NV, USA). Dry, CO_2-free air was passed directly through the analyzer every 45–60 min for baselining (Lighton 2008).

Statistics

Fisher's exact tests for comparing larval survival rates and chi-square tests for developmental time frequencies were carried out using GraphPad Software online calculator (http://graphpad.com/quickcalcs/). Elsewhere, we used mixed model analysis of variance (ANOVA) with food treatment as a fixed factor and cohort as a random factor. Data were pooled across cohorts for a single factor ANOVA when the random factor was not found significant, with a cutoff value of $P = 0.25$ in order to reduce the risk of type II error (Underwood 1997). Analyses of covariance (ANCOVA, body mass as a covariate) were used for comparing food treatment effect on larval food consumption,

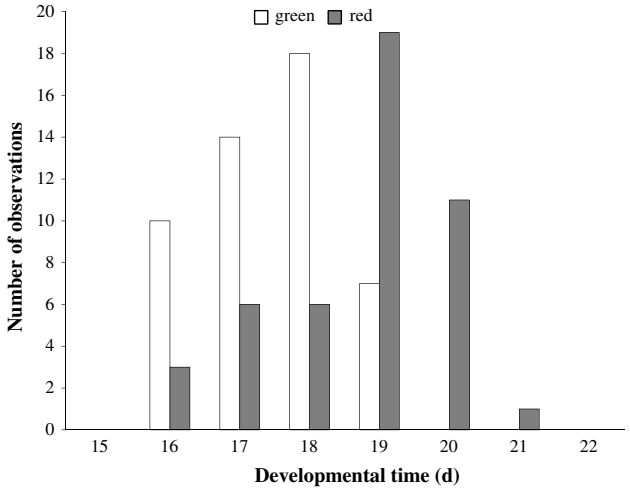

Fig. 1 Frequency distributions of developmental time in larvae feeding on either of the two cabbage variants at 24 °C. Completion of development on red cabbage leaves was significantly delayed ($P < 0.001$; $N = 49$ and 46 for green and red cabbage treatments, respectively)

growth and metabolic rates. Unless stated otherwise, statistical analyses were performed using SPSS version 19.0.

Results

Survival, development and growth patterns

No significant difference ($P = 0.66$) was found in the survival rates of third instar larvae in the two food treatments, where 87 % (49/56) and 77 % (46/60) of the larvae pupated successfully on green and red cabbage, respectively. With the exception of one individual, all pupating larvae eventually eclosed, with 1:1 sex ratios (23♂:25♀ and 23♂:23♀ in the green and red cabbage treatment, respectively). However, total larval developmental time from egg hatching to pupation was significantly affected by the food treatment ($\chi^2_5 = 66.4$, $P < 0.001$; Figs. 1, 2). Mean (±SE) egg to pupa developmental durations were 17.4 ± 0.1 and 18.7 ± 0.1 days in the green and red cabbage treatments, respectively. A considerably delayed growth in larvae feeding on red cabbage is evident in three out of the four cohorts (*r*1, *r*3 and *r*4; Fig. 2). Overall, treatment effect on developmental time was significant ($F_{1,87} = 39.3$, $P = 0.045$) with no significant cohort effect ($F_{3,87} = 3.6$, $P = 0.16$). Despite the longer developmental time of larvae feeding on red leaves, there was no effect of diet on maximal larval mass, typically recorded a day before larval pupation ($F_{1,87} = 1.5$, $P = 0.30$).

It is evident from Fig. 2 that larvae that fed on red cabbage leaves not only completed development later, but also exhibited lower growth rates in earlier larval stages. Mean larval body mass was significantly lower in the red cabbage treatment on the second day of the fourth ($F_{1,39} = 40.3$, $P < 0.001$) and fifth ($F_{1,39} = 21.8$, $P < 0.001$) instars. A significant cohort effect ($P < 0.01$ for both instars) was found, but it did not interact with the main treatment effect ($P > 0.98$ for both instars). Molting to the fifth and last larval instar was also delayed and occurred 13.7 ± 0.2 days after hatching in the red cabbage treatment, compared with 12.2 ± 0.1 days in the green cabbage treatment ($F_{1,87} = 22.2$, $P = 0.017$; $F = 5.5$, $P = 0.1$ for cohort effect).

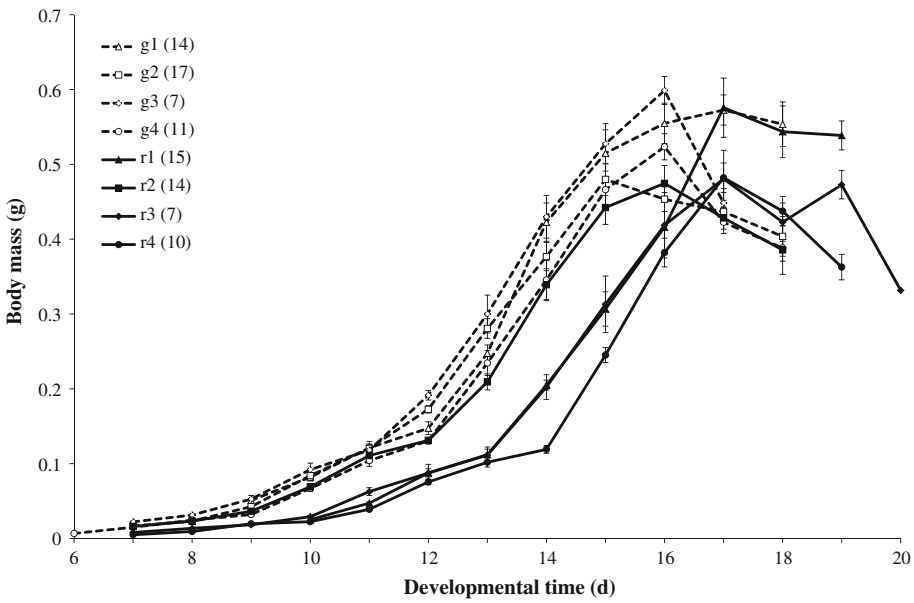

Fig. 2 Larval body mass (mean ± SE) across cohorts as a function of developmental time (from egg hatching) at 24 °C in the two feeding treatments (*g* and *r* for green and red cabbage leaves, respectively). Note that values on the *x*-axis start at six as daily measurements of larval mass began during the third larval instar (*N* for each treatment × cohort combination in brackets)

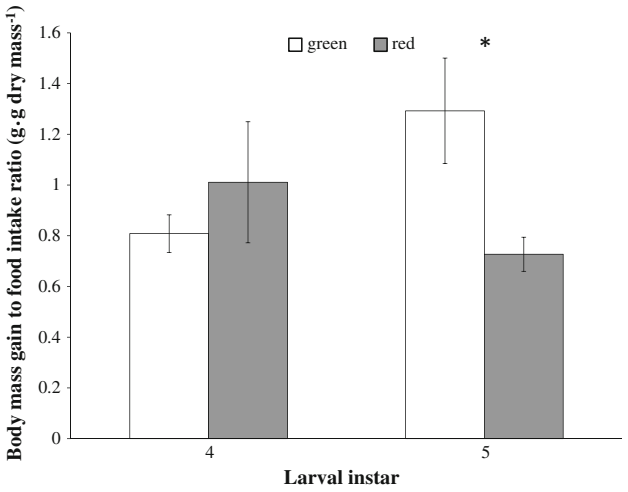

Fig. 3 Food dry mass to wet body mass conversion ratios (mean ± SE) in larvae feeding on green and red cabbage leaves. Calculations are based on food consumption and growth rates during the second day following molting to each of the last two larval instars. The *asterisk* denotes a statistically significant difference ($P < 0.05$; $N = 24$ and 23 for the green and red cabbage treatments, respectively)

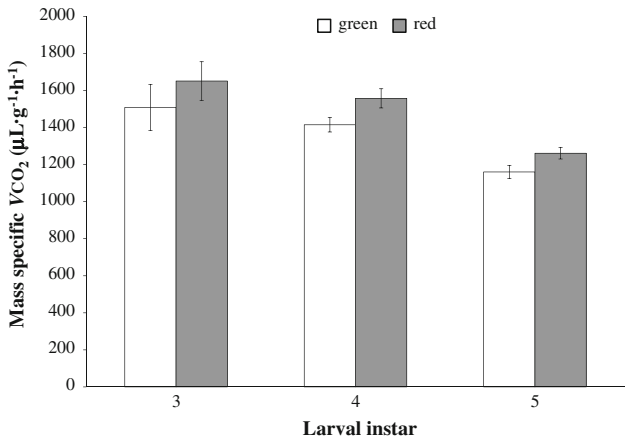

Fig. 4 Larval mass-specific metabolic rates (measured as CO_2 emission rates; mean ± SE) at 24 °C ($N = 12$–14 in each food type × larval instar sample). Food treatment had a significant effect on larval metabolic rates ($P < 0.05$, see text)

Food intake

We found no significant food treatment effect on food consumption rates in the fourth (ANCOVA, $F_{1,44} = 0.81$, $P = 0.38$) and fifth ($F_{1,44} = 0.85$, $P = 0.85$) larval instars. A significant difference was found in the water content of the two cabbage variants (ANCOVA with dry leaf mass as a covariate; $F_{1,11} = 25.8$, $P = 0.001$). Dry matter accounted for only 6.7 ± 0.3 % of the green cabbage leaves compared with 8.3 ± 0.2 % in the red cabbage, and therefore, dry mass food intake rate was calculated as well. No significant treatment effect was found in fourth instar larvae (ANCOVA, $F_{1,44} = 0.16$, $P = 0.69$), but intake rate of dry food mass was significantly higher in the red cabbage treatment in the fifth instar (0.17 g compared to 0.13 g in green cabbage; $F_{1,44} = 7.9$, $P = 0.007$). Despite a significantly higher dry matter intake, the increase in body mass measured between the second and third day of fifth instar larvae was significantly lower in the red compared with the green cabbage treatment (ANCOVA, $F_{1,44} = 4.9$, $P = 0.032$). Together, this indicates a significantly lower body mass gain to dry food intake ratio in fifth instar larvae feeding on red cabbage leaves ($F_{1,44} = 6.4$, $P = 0.015$) (Fig. 3).

Metabolic rates

Analysis of respirometry data from a total of 75 larvae (38 and 37 fed on green and red cabbage, respectively) revealed that both developmental stage ($F_{2,68} = 4.81$; $P = 0.011$) and food treatment ($F_{1,68} = 4.46$; $P = 0.038$) significantly affected larval metabolic rates. Mass-specific metabolic rates decreased with advancing larval instars (Fig. 4). Importantly, larval diet significantly affected metabolic rates ($F_{1,68} = 4.5$, $P = 0.038$), with metabolic rates for larvae feeding on red cabbage ~10 % higher than those measured in the green cabbage treatment in each of the last three larval instars (Fig. 4).

Discussion

Based on behavioral assays that demonstrated that female *Pieris* avoid laying eggs on red host plants (Radcliffe and Chapman 1966; Metspalu et al. 2003), we hypothesized that development on red foliage incurs a cost to larvae and perhaps in turn reduces adult fitness. In this study, as in preliminary experiments (data not shown), there was no significant food treatment effect on larval survival rates (see also Markwick et al. 2013). Although we did not determine the number of first instar larvae, or that of eggs damaged during egg allocation to the two treatments, the similar number of larvae molting to third instar in the two treatments suggests similar survival rates earlier in development as well. Adult insects do not grow, and their body size is determined by resource accumulation during larval development (Nijhout 2003). Thus, larger larval size translates to larger adult body size, which has been shown to be positively correlated with adult mating success (Tigreros 2013) and fecundity (Jones et al. 1982) in *Pieris*. In our experiment, larvae from the two food treatments reached a similar maximal larval size, suggesting that

feeding on red foliage does not carry a fitness cost in terms of smaller adult size.

Nevertheless, this study provides evidence for other fitness costs of development on red cabbage leaves, which could support the hypothesis that red foliage color is a reliable indicator of poor-quality substrate for larval development. Larvae feeding on anthocyanin-rich red leaves exhibited significantly longer developmental time, which appeared to be characterized by slower growth rates throughout their development. Although insect larvae attain much of their maximal body mass in the final larval instar, our results indicate delayed growth long before this stage of rapid resource accumulation. This may reflect the susceptibility of earlier developmental stages to plant defense mechanisms, which is often overlooked (Zalucki et al. 2002). The lower body mass of larvae feeding on red cabbage leaves in both the fourth and fifth larval instars and indeed their delayed growth in earlier developmental stages (Fig. 2) provide evidence for the lower quality of the red compared with the green cabbage leaves.

Intake rates of fresh leaf mass and maximal larval body masses did not differ between the two treatments. Hence, the longer developmental time required for reaching maximal larval body mass in the red cabbage treatment attests to the lower quality of this food source. Furthermore, fifth instar larvae in the red cabbage treatment exhibited lower growth rates despite significantly higher rates of dry matter intake. This resulted in a significantly lower ratio of larval mass gain to food dry matter intake early in the fifth instar (Fig. 3). Dry larval body mass was not measured in this study, but a recent report confirms the lower conversion efficiency of ingested (and digested) red cabbage compared with the green cabbage leaves (Mehrkhou et al. 2013). This finding further highlights the lower quality of this substrate compared with the green variant. In a previous study, no difference was found in nitrogen levels between red and green cabbage variants (Rosen et al. 2005), indicating a possible role of secondary metabolites in the observed variability in larval performance.

Anthocyanin levels, and potentially those of other secondary metabolites sharing its biosynthetic pathway, are considerably higher in red compared with green cabbage leaves (Gerchman et al. 2011). Red cabbage leaves also have significantly higher levels of a range of *Pieris*-favored glucosinolates (Rosen et al. 2005; Fritz et al. 2010). Glucosinolate levels typical of wild *Brassica* species inhibit larval growth and development of *P. rapae* in a dose-dependent manner (Agrawal and Kurashige 2003). This could explain an evolved avoidance of red foliage by gravid *Pieris* females (Radcliffe and Chapman 1966), despite the existence of a glucosinolate-specific detoxification mechanism in the gut of *P. rapae* larvae (Wittstock et al. 2004). We consider this explanation unlikely on two grounds. Firstly, *P. brassicae* larvae possessing the same resistance mechanism not only tolerate but preferentially feed on high glucosinolates concentration substrate, on which they exhibit higher growth rates (Smallegange et al. 2007). Secondly, *P. rapae* larval mass was not significantly affected by the ecologically relevant glucosinolate levels in eight domesticated cabbage cultivars (Poelman et al. 2008). Therefore, the role of red foliage color as a reliable indicator of poor host quality for *P. brassicae* development is more likely to be associated with other allelochemicals. Anthocyanin is generally not considered toxic to higher animals (Lee et al. 1987). Therefore, the developmental consequences of larval feeding on red leaves in *P. brassicae* are more likely to result from high concentrations of flavonoids sharing its biosynthetic pathway than from variation in levels of anthocyanin itself (Schaefer and Rolshausen 2006). Still, recent evidence attributes some anti-herbivory role for natural concentrations of anthocyanins in *Petunia* (Johnson et al. 2008).

In this study, only larval wet mass was determined, and thus, it is possible that variation in water retention is responsible for the observed differences in mass gain. However, we noticed that larval excretions in the red cabbage treatment tended to be drier, and therefore, it is unlikely that lower mass gain in this treatment resulted from lower water retention rate. An alternative explanation for the low mass gain to food intake ratio in larvae feeding on red cabbage is that the suggested high concentration of secondary metabolites in this substrate incurs a metabolic cost of detoxification. Developing larvae expend energy for growth and for maintenance of existing tissues. Metabolic demands of detoxification mechanisms could compromise both processes at the expense of larval fitness. Compromised homeostatic mechanisms could include the immune system with reduced resistance to pathogens, whereas slower larval growth means increased predation risk prior to reproduction (e.g., Gotthard 2000).

While measurements of food consumption rates and larval body mass indicated a cost for feeding on low-quality substrate toward the end of development, measurements of larval metabolic rates revealed consistently higher values in the red cabbage treatment from as early as the third larval instar (Fig. 4). The significantly higher mass-specific metabolic rates in the red cabbage treatment are indicative of a metabolic cost for feeding on the red anthocyanin-rich leaves. Interestingly, in contrast to our findings, a previous study reported similar developmental times and body masses in *P. brassicae* larvae growing on green and red cabbage at 19 °C (Metspalu et al. 2003). We cannot rule out an effect of differences in secondary metabolites among the cabbage cultivars used in the two studies (see Poelman et al. 2008). However, we suggest an

alternative explanation for the conflicting conclusions regarding food effects on larval development. In the present study, larvae were reared at 24 °C, at which tissue metabolic demands are expected to be considerably higher at typical Q_{10} values of 2–3. Despite higher diffusion rates, the ability of the tracheal system to maintain adequate supply of oxygen relative to tissue demand diminishes with increasing temperatures (Frazier et al. 2001). This may suggest that the inhibited larval development reported in this study in larvae feeding on red cabbage results from metabolic costs of detoxification when sufficient oxygen delivery is further challenged at the higher experimental temperature. Furthermore, this may indicate that development on red foliage at yet higher temperatures may result in variation in larval size, or even survival rates.

It is important to note that red color in cabbage is an artificially selected trait, and therefore, extrapolation of our results to the evolution of red coloration in plants should be made with caution. However, our results provide additional support to the surprisingly scarce and often equivocal evidence for plant red coloration as a visual cue for its chemical anti-herbivory capacities. Anthocyanin (and leaf redness) levels were found to correlate with total phenolic levels in senescing but not young leaves (Karageorgou et al. 2008). Similarly, red leaf margins were not an accurate predictor for total phenolic content or reduced herbivory in five species of *Veronica* (Hughes et al. 2010), but correlated with levels of the primary defense compound and incurred reduced insect herbivory in the New Zealand pepper tree, *Pseudowintera colorata* (Cooney et al. 2012). Whether a result of insect–plant coevolution, or that of pleiotropic effects of pigmentation, the anti-herbivory effect of red plant material is based on color being an honest indicator of plant quality for larval growth. A recent study demonstrated that red leaf margins provided an honest visual signal for plant quality, which affected larval herbivory (Cooney et al. 2012; see also Markwick et al. 2013). Our results provide evidence for negative effects on fitness correlates involved in the development of *P. brassicae* larva on red compared to green cabbage leaves. Together with the documented effects of plant pigmentation on reproductive behavior in *Pieris* (Irwin et al. 2003), this supports a more general role for red color in plant anti-herbivory defense. Slower growth and development despite higher food intake rates, and increased metabolic rates, suggest an increased metabolic load incurred by developing on low-quality red substrate. Increased predation risk as larval development prolongs (Gotthard 2000, but see Williams 1999) and potential effects on larval size and survival at high temperatures imply that red color is a reliable indicator for low foliage quality as a substrate for insect development.

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