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DIET QUALITY AFFECTS WARNING COLORATION INDIRECTLY: EXCRETION COSTS IN A GENERALIST HERBIVORE

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Aposematic herbivores are under selection pressure from their host plants and predators. Although many aposematic herbivores exploit plant toxins in their own secondary defense, dealing with these harmful compounds might underlay costs. We studied whether the allocation of energy to detoxification and/or sequestration of host plant defense chemicals trades off with warning signal expression. We used a generalist aposematic herbivore *Parasemia plantaginis* (Arctiidae), whose adults and larvae show extensive phenotypic and genetic variation in coloration. We reared larvae from selection lines for small and large larval warning signals on *Plantago lanceolata* with either low or high concentration of iridoid glycosides (IGs). Larvae disposed of IGs effectively; their body IG content was low irrespective of their diet. Detoxification was costly as individuals reared on the high IG diet produced fewer offspring. The IG concentration of the diet did not affect larval coloration (no trade-off) but the wings of females were lighter orange (vs. dark red) when reared on the high IG diet. Thus, the difference in plant secondary chemicals did not induce variation in the chemical defense efficacy of aposematic individuals but caused variation in reproductive output and warning signals of females.

KEY WORDS: **Aposematism, iridoid glycosides, polymorphism, reproductive costs, tritrophic interactions, warning signals.**

Aposematic animals are unprofitable (e.g., toxic or unpalatable) for their predators and advertise that, for example, with bright coloration (Cott 1940). At present, much is known about how predation pressure selects for certain signal traits such as conspicuousness of the signal (Gamberale-Stille and Tullberg 1999; Lindström et al. 1999; Lindstedt et al. 2008), and for high level of chemical defense (Leimar et al. 1986; Skelhorn and Rowe 2006; Ihalainen et al. 2007; Rowland et al. 2007). At the same time new questions have emerged (e.g., Endler and Mappes 2004; Mappes et al. 2005; Ojala et al. 2007; Sandre et al. 2007; Speed and Ruxton 2007); in addition to predator psychology, studies are starting to focus on the costs and benefits of warning displays, and on the range of selection pressures that affect the optimization of defenses (e.g., Grill and Moore 1998; Speed and Ruxton 2007; Ojala et al. 2007; Blount et al. 2009). Even though selection by predators would increase the fitness of those individuals that are highly conspicuous (Gamberale-Stille and Tullberg 1999; Riipi et al. 2001; Lindstedt et al. 2008) and very unprofitable (Leimar et al. 1986; Skelhorn and Rowe 2006; Rowland et al. 2007; but also see Ihalainen et al. 2007) to their predators, possible costs of production and maintenance of defenses could explain some of the within-species variation often observed in warning coloration (e.g., Brakefield, 1985; Brakefield and Liebert 1985; Sword 1999; Sandre et al. 2007) and in chemical defense (Dyer and Bowers 1996; Camara 1997a; Tullberg et al. 2000; Willinger and Dobler 2001; Despland and Simpson 2005).

Optimization of energy and resources between defense and other traits could be especially challenging for an aposematic

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herbivore. Although many aposematic herbivores exploit the plant toxins in their own secondary defense (Nishida 2002), processing these hazardous chemicals can harm the insects, and detoxification of these compounds may incur energetic costs (Berenbaum and Zangerl 1993; Després et al. 2007). Even though examples exist where the sequestration of plant chemicals for antipredator defense has been shown to be cost-free (e.g., Holloway et al. 1991; Holloway et al. 1993; Camara 1997b; del Campo et al. 2005), trade-offs between strength of the insect's chemical defense and other fitness traits (such as growth rate, weight or reproductive effort) have been repeatedly found (Bowers 1990; Björkman and Larsson 1991; Berenbaum and Zangerl 1993; Dobler and Rowell-Rahier 1996; Fordyce and Nice 2008; see also Longson and Joss 2006). However, whether the allocation of energy to detoxification and/or sequestration of host plant's defense chemicals trades off with warning signal expression has rarely been tested (but see Bezzerides et al. 2007 for predatory *Harmonia axyridis* Asian ladybird). Many of the aposematic insect species are herbivorous and therefore forced to balance between the host plant and the predator (e.g., Nishida 2002). To advance the theory of aposematism, it is essential to study whether variation in host plant defense chemical content affects the possible fitness costs of warning signals and chemical defenses, and whether trade-offs within these defense traits induce variation in them (see e.g., Speed and Ruxton 2007).

The aposematic wood tiger moth (*Parasemia plantaginis*) is an excellent study species to investigate the interactions between warning signal expression, chemical defense, host plant quality, and their effects on the fitness of an individual. *Parasemia plantaginis* larvae show continuous genetic and phenotypic variation in the size of their warning signal (an orange patch against a black body; Ojala et al. 2007; Lindstedt et al. 2009). Considering the high heritability of the signal size (Lindstedt et al. 2009), predation should decrease the variation and favor expression of larger signals, because predators learn to avoid larvae with a large orange signal (proportion of orange $> 60\%$) faster than larvae with a small signal (proportion of orange $< 34\%$; Lindstedt et al. 2008). However, variation in signal size persists indicating that factors other than predation pressure (e.g., diet quality, thermoregulation and parasitism) likely maintain the variance (Ojala et al. 2007; Lindstedt et al. 2009). Because *P. plantaginis* moths are capital breeders (i.e., the adults do not feed) the larval diet is critical for the fitness of the adults as well. In addition, the larvae are polyphagous (e.g., Chinery 1993; Ojala et al. 2005) and therefore sequestration and/or detoxification of plant toxins can be especially costly for them if they need to maintain several different detoxification mechanisms simultaneously.

Adult *P. plantaginis* have conspicuous coloration that varies both locally and geographically (e.g., Watson and Goodger 1986; Chinery 1993). Coloration of the male hind wings is polymorphic; the most typical color morphs are black and yellow or black and white. In females, the coloration of the body and hind wings varies more continuously from orange to red with black patterns. Females are unpalatable to predators in general, and females with red coloration are attacked less (H. Eager, E. Ihalainen, A. Kahilainen, and C. Lindstedt, unpubl. ms.). Therefore, predation should decrease the variation in female coloration. Predation should also act to decrease variability in male coloration because yellow males are attacked less than white males (O. Nokelainen, C. Lindstedt, J. Mappes, and J. H. Reudler Talsma, unpubl. ms.).

We conducted a rearing experiment with two selection lines for larval coloration (small and large warning signal) and with two diets (ribwort plantain, *Plantago lanceolata*) with low or high iridoid glycoside (IG) content. We studied how variation in the host plant IG content affects (1) the level of chemical defense, (2) the expression of warning coloration of *P. plantaginis* larvae and adults, and (3) life-history choices of larvae and adults. Because the detoxification and/or sequestration of plant's defense chemicals requires energy, we expected to find trade-offs between the detoxification/sequestration costs and (1) production of warning coloration and (2) life-history choices. Furthermore, because the production and maintenance of warning coloration and storage of defense chemicals could possibly compete for resources within the individual (e.g., Leimar et al. 1986; Speed and Ruxton 2007; Blount et al. 2009), we also tested whether allocation to effective warning coloration interacts with chemical defense level per se. If the defense chemical content and warning color efficacy are negatively correlated and there is a trade-off between them, it could explain some of the variation observed in the warning coloration of this species.

Methods

PARASEMIA PLANTAGINIS

The larvae of the moth *P. plantaginis* (Arctiidae) are hairy in all instars but their coloration changes from (cryptic) greenish gray in the first two instars to orange-black from the third instar onward. Altogether the larvae have five to seven instars (Ojala et al. 2007). This species usually has only one generation per year in Finland and it overwinters as a larva. In the laboratory, *P. plantaginis* moths can produce two generations per year and the second generation overwinters.

The *P. plantaginis* laboratory stock was established in 2003 (see details in Lindstedt et al. 2009). We reared larvae under laboratory conditions in a greenhouse at the University of Jyväskylä in Central Finland. As larval signal size varies continuously (Ojala et al. 2007) and its heritability and response to selection are rather high (Lindstedt et al. 2009), we have been able to artificially select for the extremes of this signal size continuum (selection lines and criteria for selection are described in more detail in Lindstedt et al.

2009). The larvae used in this experiment were derived from these two selection lines for small (proportion of orange consisting of four body segments or less, $\leq$ 34%) and large orange warning signal (proportion of orange consisting of six segments or more, $\geq$ 60%) from the sixth generation after selection started.

PLANTAGO LANCEOLATA

Ribwort plantain (*P. lanceolata*) is a perennial herb with a worldwide distribution and high ecological amplitude (Sagar and Harper 1964). The distribution ranges of *P. lanceolata* and *P. plantaginis* overlap, and *P. plantaginis* larvae are known to use *P. lanceolata* as a host plant (Bellman 2007; Pabis 2007). Ribwort plantain contains two main IGs—aucubin and catalpol (Duff et al. 1965). Aucubin is the biosynthetic precursor of catalpol (Damtoft et al. 1983). These IGs play an important role in plant–insect interactions as chemical defense compounds: many herbivores are known to sequester and use them for their own defense against predators and parasites (Dyer and Bowers 1996; Camara 1997a; Willinger and Dobler 2001; Nieminen et al. 2003). They are also known to be unpalatable to birds and to taste bitter to humans (e.g., Bowers 1991). Especially catalpol appears to be a strong deterrent to potential predators (Bowers and Collinge 1992; De La Fuente et al. 1994/1995; Dyer and Bowers 1996).

In wild populations, the IG levels in *P. lanceolata* range from undetectable to approximately 9% of the plant's dry weight (Bowers 1991). This variation in IG levels is both genetic and phenotypic (Bowers and Stamp 1992, 1993; Adler et al 1995; Marak et al. 2000). The plants used for this study were derived from an artificial selection experiment in which plants were selected on the basis of high and low concentrations of total leaf IG for four generations (Marak et al. 2000). The average leaf IG levels differ approximately fourfold between the lines but also vary considerably within the lines (Marak et al. 2000, 2003). Seeds resulting from crosses of plants within the high line and plants within the low line were used to grow the plants for this experiment. The seeds were germinated in water agar in a greenhouse (25°C). After 14 days, seedlings were transplanted in pots with 50% potting soil and 50% peat. They were fertilized once a week (Biolan including 1% N, 1% K). The IG content of the diet treatments differed significantly (df = 34, $t = -2.535$, $P = 0.016$). The mean IG level in low treatments was 1.93% (aucubin 0.77%, catalpol 1.17%) and in high treatment 3.29% (aucubin 1.09%, catalpol 2.20%) of dry weight (see Chemical Analyses). Water and nitrogen contents of the leaves do not correlate with the IG level (Bowers and Stamp 1992; Reudler Talsma 2007); thus, the nutritional value of plant treatments did not differ. We used 19 genotypes for the low treatment and 17 genotypes for the high treatment.

EXPERIMENTAL PROCEDURE

The experiment was a two-factorial design in which the factors were the signal selection line (small and large) and the IG content of the diet (low and high). For the first six days after hatching, larvae were reared in groups and fed with a mixed diet of lettuce and *Taraxacum* spp. leaves. At the age of seven days, we randomly selected one individual per family (families originating from either the small or the large signal line) for rearing on ribwort plantain. In total, we used 80 individuals from the small and 80 individuals from the large signal line. Within the signal lines, individuals were further divided into the low and high IG treatments. In total, we had 40 individuals from the small signal line on the low IG diet and 40 on the high IG diet. From the large signal line, we had 39 individuals on the low IG diet and 41 on the high IG diet.

During the experiment, the larvae were reared individually on Petri dishes until they reached the adult stage. Individuals were reared in a greenhouse at the University of Jyväskylä in Central Finland (62°N, 26°E) during June and July 2007. The temperature in the greenhouse varied between 20°C and 30°C (day is of approx. 20 h) and during the night (approx. 4 h) it decreased to 15–20°C. The larvae were checked daily and fresh leaves were added ad libitum while removing old ones. Leaves given to the larvae were randomly selected and mixed among the genotypes within the diet treatment. During the rearing, life-history traits and color measurements were recorded for both larvae and adults. In addition, we measured the reproductive output of adults (see below).

LIFE-HISTORY MEASUREMENTS

Larvae were weighed before they were divided to the diet treatments to minimize differences in weights between the treatment groups. At this point larvae still had their cryptic coloration, thus, we could not select experimental individuals based on their signal size. The individuals were also weighed on the day of their pupation. Growth rate was calculated as ln pupal mass (mg)/larval development time (days) to pupal stage (see e.g., Ojala et al. 2005). We also recorded the number of individuals that survived to pupal and adult stage. After the adults emerged, they were mated within the selection lines and treatments to test whether the number of eggs and number of larvae hatched differed between the treatments or selection lines. Altogether we had eight pairs in the small signal line and seven in the large signal line for the low IG diet and seven in the small signal line and 14 in the large signal line for the high IG diet.

CHEMICAL ANALYSES

Leaf samples of the plants were taken at the beginning of the experiment. For the analyses, we took one leaf from each plant that was used for the diet. The leaves were frozen at −80°C and then freeze-dried. Leaves were ground with a Mikro-dismembrator U (B. Braun Biotech International, Allentown, PA). For the extraction and HPLC analyses, we followed the protocol described in

Reudler Talsma et al. (2008). To test if the IG from the diet were present in the herbivores, we analyzed different stages of the herbivore. We analyzed 10 last instar larvae (two larvae from small and three from large signal line in low and high IG diets, respectively), 121 pupal skins, and 120 adults (12 and 14 females from small signal lines and 9 and 14 females from large signal lines; 19 and 15 males from small signal line and 19 and 18 from large signal line in low and high IG diet, respectively; wings and body separately). To measure the amount of aucubin and catalpol in the herbivores, the same method was employed as for the leaves, except that we ground the insects by hand in an Eppendorf tube and extracted them in 5 mL 7% MeOH.

COLOR MEASUREMENTS

The proportional size of the warning signal was measured similarly to Lindstedt et al. (2009) from the last instar larvae by counting the number of body segments that were covered with orange hairs. Because larvae always have 13 segments, this measure estimates the proportional size of the orange patch in the larval body.

The female hind wing color varies continuously from orange to dark red, so we used calibrated digital photographs (Stevens et al. 2007) to measure reflectance of the orange/red color. Dead specimens were photographed with an ultraviolet sensitive Fujifilm Finepix S3 Pro UVIR camera with a UV transmitting lens (Coastal Optical Systems, Jupiter, FL) under a light bulb emitting both visible and UV wavelengths (Arcadia Reptile D3, Salfords, UK). Two photographs were taken of each individual: a "human-visible light" photo (spanning 400–700 nm approx.) taken with a UV and infrared blocking filter (Baader UV/IR Cut, Baader Planetarium, Mammendorf, Germany), and a UV photo taken with a filter (Baader U) transmitting only in the UV region (300–400 nm approx.). UV reflectance was included in the analysis as avian predators are sensitive to UV wavelengths (Cuthill 2006). The response of the camera's RGB (red, green, and blue) channels to increasing light intensity (radiance) was measured as nonlinear, and we first linearized the response of each of these channels (see Stevens et al. 2007). A gray standard (Labsphere Spectralon diffuse reflectance standard reflecting 50% of all light across the avian visual spectrum) was included in every photo, allowing the digital images to be converted to reflectance data using a custom MATLAB program (see Stevens et al. 2007). From the photos we sampled (using GIMP software) three red/orange areas of the left hind wing of 54 females (11 females from the small signal/low IG diet, 10 from the small signal/high IG, 16 from the large/low and 17 from the large/high; six of the females were left out from color analysis because the photos failed). From these samples, we recorded mean values of reflectance in the long, medium, short, and ultraviolet reflectance images.

Overall, the orange/red areas reflected, primarily, long and medium wavelengths [63.6 ($\pm$ SE 0.7) and 17.5 ($\pm$ SE 0.5) percent, respectively] whereas reflectance in the short and UV regions was lower [10.4 ($\pm$ SE 0.2) and 8.5 ($\pm$ SE 0.5) percent]. We used two measures of coloration. As a measure of overall "brightness" or "achromatic intensity" of the orange/red pattern elements, we used the mean reflectance over the whole spectrum ((LW+MW+SW+UV)/4). Therefore, "brightness" is not a measure of conspicuousness or contrast with the black pattern elements. To analyze the "hue" of the orange/red areas (excluding the variation in brightness), we standardized each LW, MW, SW, and UV reflectance value into a proportion of the total (e.g., proportional value for LW = LW/(LW+MW+SW+UV). As a measure of female hue, we then extracted first (describing mainly the variation in long and medium wavelengths) and second principal components (describing mainly the variation in UV wavelengths) from these four values (PC1 explained 50.8% of variation in hue, with an eigenvalue of 2.032 and PC2 explained 30.1% of variation in hue, with an eigenvalue of 1.203). We used PC1 and PC2 as variables in statistical analyses. Correlation between PC1 and brightness was 0.781 ($P < 0.001$) and between PC2 and brightness it was -0.163 ($P = 0.239$).

Yellow and white color morphs of adult males are easy to separate by the human eye, thus we classified males either as yellow or white. We had altogether 20 males from the small and 16 from the large signal line in low IG diet and 23 males from the small and 18 from the large signal line in high IG diet.

DATA ANALYSES

We used analysis of variance (ANOVA) to test the effect of signal line and diet on the larval signal size, adult female color (brightness, PC1 and PC2), egg number, hatchability, and offspring number. Because gender has previously been shown to have an effect on pupal size (Ojala et al. 2005), we included gender as a fixed factor in the models when we analyzed the effect of signal line and diet on pupal weight, growth rate (ln pupal mass/larval development time in days), and chemical content of the individuals. However, gender was excluded as a nonsignificant factor from the final model of the growth rate ($F_{1,119} = 0.117$, $P = 0.733$). Pupal weight correlates positively with fertility in *P. plantaginis* (Ojala et al. 2005); as a result, we ran analysis of covariance (ANCOVA) for the effect of signal line and diet on number of eggs, hatchability (number of larvae hatched/number of eggs), and number of larvae that hatched. Pupal weight of females was included as a covariate in the model. We arcsine transformed the hatchability prior to the ANOVA analysis.

We used a binary logistic regression to analyze the effect of signal line, diet, and their interaction on the survival of individuals to adulthood and on the male color morph frequencies. Spearman correlations were used to analyze the monotonic

Table 1. The effects of iridoid glycoside content of the diets (low vs. high) on life-history traits of *P. plantaginis* from either small or large signal selection line.

Source of variation	df	MS	*F*	*P*
Pupal weight				
Diet	1	1517.15	1.459	0.229
Signal line	1	1251.17	1.204	0.275
Sex	1	158066	152.051	<0.001
Diet × signal line	1	1271.1	1.223	0.271
Diet × sex	1	949.183	0.913	0.341
Signal line × sex	1	2986.39	2.873	0.093
Diet × signal line × sex	1	831.347	0.8	0.373
Error	123	1039.56		
Growth rate				
Diet	1	<0.001	0.051	0.823
Signal line	1	0.001	1.613	0.206
Diet × signal line	1	0.004	4.656	0.033
Error	128	0.001		
Egg number				
Diet	1	10049.4	1.23	0.227
Signal line	1	13304.5	1.629	0.212
Diet × signal line	1	8136.73	0.996	0.327
Female pupal weight (covariate)	1	334.64	0.041	0.841
Error	29	8169.33		
Hatchability				
Diet	1	1.217	12.168	0.002
Signal line	1	0.023	0.226	0.638
Diet × signal line	1	0.135	1.349	0.255
Female pupal weight (covariate)	1	0.141	1.41	0.245
Error	28	0.1		
Offspring number				
Diet	1	2.233	17.109	<0.001
Signal line	1	<0.001	<0.001	0.988
Diet × signal line	1	0.378	2.894	0.101
Female pupal weight (covariate)	1	0.164	1.258	0.272
Error	26	0.131		

relationships between larval signal size, female brightness (i.e., mean reflectance), and defense chemical content of adults (percentage of chemical from dry weight). Linear relationships between life-history traits and defense chemical content were analyzed with Pearson correlation. To test possible differences in the defense chemical content of yellow and white male morphs, we used an independent sample *t*-test. All the tests were performed on SPSS, inc. 15.0.

Results

LIFE-HISTORY TRAITS

Survival did not differ between the signal lines (Wald = 0.006, P = 0.937) or diet treatments (Wald = 0.027, P = 0.870). Neither were there interactions between the signal lines and diet treatments (Wald = 0.061, P = 0.518).

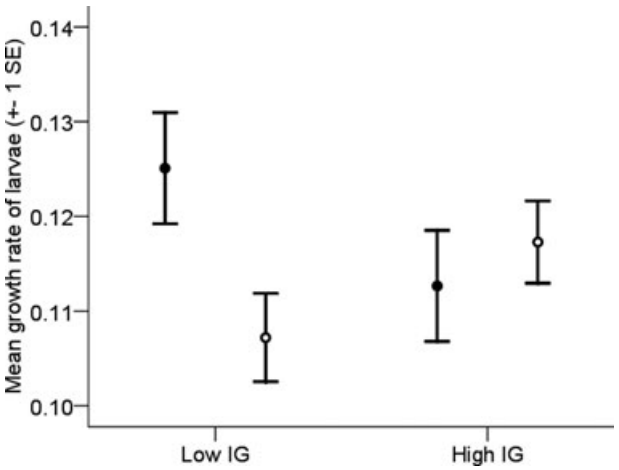

Figure 1. The mean growth rate of larvae from small (filled circles) and large (open circles) signal lines on low and high IG diets. Error bars show standard error of mean.

There was an interaction between signal line and diet for growth rate (Table 1). Separate analyses for the diet treatments showed that larvae on the low IG diet with small signals grew faster than larvae with large signals ($F_{1,65}$ = 5.754, P = 0.019) but in the high IG diet the growth rates did not differ between the signal lines ($F_{1,65}$ = 0.559, P = 0.457; Fig. 1).

IG content of the diet, signal line, and gender, or their interactions, did not affect the pupal weight of the larvae (Table 1). However, pupal weight of males was negatively correlated with the body IG content as an adult (Pearson r = −0.303, n = 71, P = 0.011). Even though the correlation was nonsignificant, the pupal weight and adult IG content of the females tented to be also negatively correlated (Pearson r = −0.560, n = 41, P = 0.087). Thus, the lower the pupal weight, the higher the IG content was in the adult body.

The number of eggs laid did not differ between the signal lines or the diet treatments (Table 1), and there was no interaction either. However, the hatchability (number of larvae hatched/number of eggs) of offspring was higher for parents that were reared on the low IG diet compared to parents reared on the high IG diet (Table 1). Also, the number of offspring was higher for parents that were reared on the low IG diet compared to parents reared on the high IG diet (Table 1, Fig. 2). The signal line or its interaction with the diet did not affect the hatchability or number of offspring (Table 1).

CHEMICAL DEFENSE

Only catalpol was found in the larvae (Table 2). Both catalpol and aucubin were found in adult bodies, but there were no traces in adult wings or pupal skins. In general, the IG concentration (including both aucubin and catalpol) of both larvae and

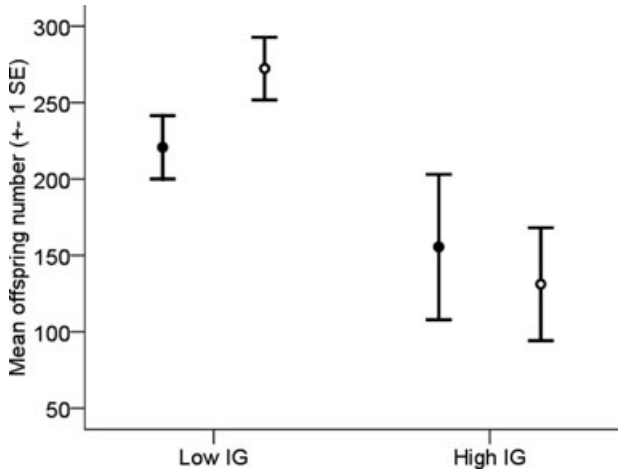

Figure 2. The mean number of offspring of parents reared on either low or high IG diet. Filled circles show the mean number of offspring of parents from small signal line and open circles show the mean number of offspring of parents from large signal line. Error bars show the standard error of mean.

adults was quite low in comparison to the IG content of the diet (Table 2).

IG content or content of catalpol alone in the adult bodies did not differ between the diet treatments, signal lines, or between the sexes (Table 3). Neither were there interactions between them. However, although aucubin content of the adults did not differ between the diet treatments or signal lines, it was higher in males than in females. There were no interactions (Table 3).

COLORATION

Orange signals of the larvae were larger in the large signal line compared to the small signal line but the IG level of the diet did not affect signal expression and there was no interaction (Table 4). Thus, IG content of the diet did not affect larval coloration.

Signal line did not affect brightness (i.e., mean reflectance) of the adult female hind wings. However, the females were brighter on the high IG diet than on the low IG diet (Table 4). Signal line did not affect the two principal components of hue (i.e., PC1 and PC2) of the adult female hind wings (Table 4), but diet affected PC1 (which also correlated positively with mean reflectance, see Methods). Thus, to a human eye, females were lighter orange (higher mean reflectance and higher PC1) on the high IG diet than on the low IG diet (where females were darker red) (Fig. 3, Table 4).

However, because there was a marginally nonsignificant interaction between diet and signal line with regard to female brightness (Fig. 3, Table 4), we ran a more detailed analysis of brightness and also of PC1 separately within the selection lines. We found that on the small signal line brightness did not differ between the diet treatments ($F_{1,27} = 0.972$, $P = 0.334$) but on the large signal line the females from the high IG diet were brighter than females from the low IG diet ($F_{1,27} = 7.683$, $P = 0.010$). Similar to brightness, within the small signal line hue (PC1) did not differ between diet treatments ($F_{1,27} = 1.049$, $P = 0.316$) but within the large signal line, the females from the high IG diet had higher values of PC1 than females from the low IG diet ($F_{1,27} = 6.806$, $P = 0.015$). Therefore, the overall color difference between diets was mostly due to the females from the large signal

Table 2. Mean iridoid glycoside contents of diet, larvae, and adults reported as percentage of dry weight. Values in brackets indicate the ±SD.

	Diet		Larvae		Males		Females	
	Low	High	Low	High	Low	High	Low	High
IG content								
Small signal line	1.93	3.29	–	–	0.01	0.01	0.007	0.01
	(1.03)	(2.12)			(0.011)	(0.011)	(0.007)	(0.013)
Large signal line	1.93	3.29	–	–	0.009	0.007	0.01	0.009
	(1.03)	(2.12)			(0.016)	(0.008)	(0.008)	(0.009)
Catalpol								
Small signal line	1.17	2.20	0.02	0.05	0.007	0.006	0.005	0.009
	(0.63)	(1.36)	(0.004)	(0.038)	(0.010)	(0.008)	(0.007)	(0.011)
Large signal line	1.17	2.20	0.03	0.02	0.006	0.003	0.009	0.009
	(0.63)	(1.36)	(0.020)	(0.005)	(0.012)	(0.006)	(0.009)	(0.009)
Aucubin								
Small signal line	0.77	1.09	–	–	0.002	0.002	0	0.001
	(0.51)	(0.89)			(0.004)	(0.006)	(0.000)	(0.004)
Large signal line	0.77	1.09	–	–	0.003	0.001	0	0
	(0.51)	(0.89)			(0.005)	(0.003)	(0.000)	(0.000)

Table 3. The effects of iridoid glycoside content of the diets (low vs. high) on the iridoid glycoside contents of adult moths from either small or large signal selection line.

Source of variation	df	MS	*F*	*P*
Total IG content				
Diet	1	6.350	0.061	0.805
Signal line	1	71.348	0.688	0.409
Sex	1	0.020	0.000	0.989
Diet × signal line	1	104.644	1.008	0.317
Diet × sex	1	111.188	1.071	0.303
Signal line × sex	1	14.188	0.137	0.712
Diet × Signal line × sex	1	113.019	1.089	0.299
Error	114	11830.692		
Catalpol				
Diet	1	11.951	0.165	0.686
Signal line	1	35.949	0.496	0.483
Sex	1	70.552	0.972	0.326
Diet × signal line	1	37.267	0.514	0.475
Diet × sex	1	86.585	1.193	0.277
Signal line × sex	1	24.832	0.342	0.560
Diet × signal line × sex	1	122.138	1.683	0.197
Error	114	72.556		
Aucubin				
Diet	1	0.878	0.073	0.788
Signal line	1	6.008	0.499	0.482
Sex	1	68.222	5.661	0.019
Diet × signal line	1	17.015	1.412	0.237
Diet × sex	1	1.536	0.128	0.722
Signal line × sex	1	1.480	0.123	0.727
Diet × signal line × sex	1	0.177	0.015	0.904
Error	114	12.051		

line. Diet did not affect the second principal component (PC2) (Table 4).

The signal line (Wald = 1.514, $P = 0.218$), diet treatment (Wald = 0.174, $P = 0.677$), or their interaction (Wald = 0.381, $P = 0.537$) did not explain the frequencies of yellow and white male color morphs in different treatment groups.

DEFENSE CORRELATES

To investigate whether the high sequestered IG content trades off with warning color expression, we measured the relationship between coloration and the amount of defense chemicals in the larvae and adults, respectively. Correlation between the defense chemical content of individual larvae and their signal size was nonsignificant ($r_S = -0.123$, $P = 0.734$). Furthermore, the signal size as a larva did not correlate with the defense chemical content as an adult (IG: $r_S = -0.078$, $P = 0.391$, catalpol: $r_S = -0.084$, $P = 0.353$, aucubin: $r_S = -0.086$, $P = 0.345$). The total IG, catalpol, and aucubin contents were similar between the male color morphs (*t*-test: all *P*-values > 0.455). Also, female hind

Table 4. The effect of iridoid glycoside content of the diets (low vs. high) on the warning color expression of *P. plantaginis* moths from either small or large signal selection line.

Source of variation	df	MS	*F*	*P*
Signal size of larvae				
Diet	1	1.047	1.437	0.232
Signal line	1	364.516	500.254	<0.001
Diet × signal line	1	0.131	0.180	0.672
Error	152	0.729		
Brightness of females				
Diet	1	269.525	7.589	0.008
Signal line	1	74.550	2.099	0.154
Diet × signal line	1	77.556	2.184	0.146
Error	50	35.514		
Hue of females (PC1)				
Diet	1	5.975	6.555	0.014
Signal line	1	0.096	0.105	0.748
Diet × signal line	1	1.106	1.214	0.276
Error	50	0.912		
Hue of females (PC2)				
Diet	1	0.823	0.792	0.378
Signal line	1	0.153	0.147	0.703
Diet × signal line	1	0.069	0.066	0.798
Error	50	1.038		

wing brightness (mean reflectance) and the total amount of IGs in the body (percentage of dry weight) were not correlated (IG: $r_S = 0.259$, $P = 0.073$, catalpol: $r_S = 0.160$, $P = 0.272$, aucubin: $r_S = 0.063$, $0 = 0.668$).

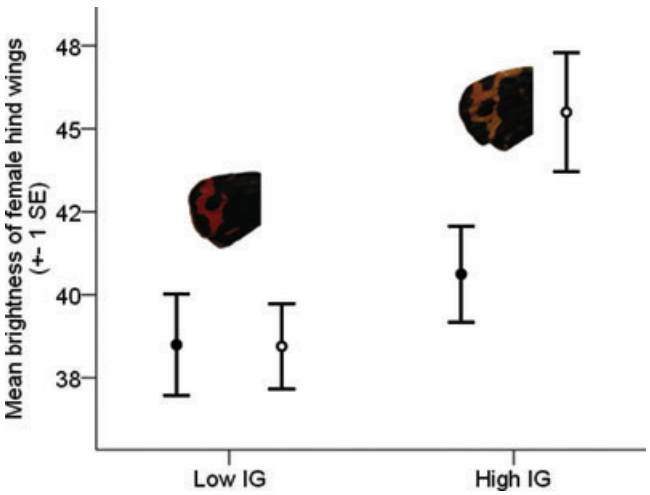

Figure 3. The brightness (i.e., mean reflectance) of female hind wings from small (filled circles) and large (open circles) signal lines on low and high IG diets. Error bars show the standard error of mean. The female hind wing examples above the bars illustrate the color differences (high values indicate light orange, low values indicate dark red).

Discussion

If an effective defense is costly to produce and maintain, the costs can have an impact on signal expression and chemical defense of an aposematic individual (see Endler 1991; Speed and Ruxton 2007). Here, we show that the defense chemical content of the host plant can have several consequences on the fitness and defense traits of aposematic insects. Therefore, variation in the host plant's toxin content can maintain variation in warning coloration directly as shown in adult female coloration, but also indirectly through fitness costs and more complex interactions between larval coloration, host plant toxicity, and adult coloration. We did not find evidence for a linkage between warning color production and chemical defense per se.

It is not evident that generalist herbivores can always exploit the host plant's defense chemicals (Bowers and Farley 1990; Willinger and Dobler 2001). In addition, the toxin content of the host plant does not always reflect the toxin content of the herbivore. For example, Bowers and Stamp (1997a) observed substantial variation in the sequestration ability of IGs among generalist arctiid species. Alternatively, even though sequestration would be efficient, storage of defense chemicals can be inefficient (Brückmann et al. 2000). The low IG content of *P. plantaginis* across the diets in our study suggests that, rather than accumulating the IGs for defense purposes, individuals seemed to dispose of them quite effectively (J. H. Reudler Talsma, unpubl. ms.). The excretion of the IGs was costly for the individual as high IG content of the diet decreased the moth's reproductive output shown as a lower hatchability and lower number of offspring (Fig. 2). Pupal weights did not explain the hatchability or offspring number and thus the decrease in fertility is not explained by the decrease in adult size on the high IG diet. Even if disposing of IGs was costly, it may have been more beneficial than storing higher amounts of harmful chemicals in the body, because pupal weights of males were negatively correlated with the defense chemical content of the body. However, the evidence is not very strong, because we did not find the same effect for females.

Even though the overall quantities of IGs per individual were relatively low, the quantity of chemical defenses varied between the different life stages. Larvae had higher IG content in comparison to adults although there was no aucubin found from the larvae. It is suggested that metamorphosis could constrain the defense chemical content of adults (Nishida 2002). For instance, in many Lepidopteran species only the larvae and pupae contain IGs (Bowers and Stamp 1997b). In some species this difference also reflects their defense strategies: the warning colored larvae contain IGs but the cryptically colored adults are free of plant chemicals (Bowers 1990). However, because males and females of *P. plantaginis* species are rather conspicuously colored, they probably benefit from the defense chemicals they contain. Although we did not detect larval aucubin in the present experiment, we have found it in other studies (J. H. Reudler Talsma, unpubl. ms.). The lack of larval aucubin may be by chance as our sample for the chemical analyses of larvae was rather small; 20% of adult individuals had no aucubin detected in their bodies either. On the other hand, it is possible that IGs could have some other functions in addition to defense. For instance, in several lineages of tiger moths the defense chemicals such as pyrrolizidine alkaloids (PAs) can be used as components of the males' courtship pheromones (Weller et al. 1999). Because there was more aucubin in adults than in larvae and because the aucubin levels were significantly higher in males than in females, it may indicate that aucubin has a role in the courtship behavior of *P. plantaginis*. Males and females probably differ also in the proportion of fat, muscles, and other tissues, possibly explaining the difference.

Energetic costs of IG excretion could explain the finding that a high IG content of the host plant caused differences in the coloration of adult *P. plantaginis* females. On the high IG diet the females who had allocated to a large warning signal as a larva had more orange (as opposed to red) and brighter (i.e., higher mean reflectance) hind wings than females from the small signal line on the same diet (Fig. 3). Because predators attack the redder *P. plantaginis* females less (Eager et al., unpubl. ms.) and because predation should favor signal uniformity (Müller 1879; Joron and Mallet 1998; Kapan 2001; Beatty et al 2004; Rowland et al. 2007), costs associated with dealing with the toxins in the diet could induce variation in the warning color expression of adult females and decrease their fitness. In addition, it seems that larval coloration and female coloration are somehow linked. The high IG diet affected coloration more clearly in females from the large signal line than from the small signal line. Therefore, the energetic costs of producing the red pigment combined with excretion costs of IGs are higher for the individuals that have a large orange signal as a larva. IG content of the diet did not have any effects on male coloration.

We did not find evidence for a phenotypic linkage between coloration and toxicity (see e.g., Summers and Clough 2001; Darst et al. 2006; Blount et al. 2009). Our study indicates that variation in the defense traits of *P. plantaginis* is more strongly influenced by the costs associated with each of the defense traits separately instead of trade-offs between defense traits within an individual. Thus, selection for warning signal efficacy appears not to affect the strength of the chemical defense directly in this species. Other studies have also suggested that linkage between coloration and chemical defense is not necessarily very tight (e.g., Grill and Moore 1998; Grill 1999). For example, in an aposematic beetle, *H. axyridis*, allocation to chemical defense has shown to be costly in terms of growth rate and survival (Grill and Moore 1998), but use of a chemical defense (i.e., reflex bleeding) has no

(Grill 1999) or only minor effects (Grill and Moore 1998) on the brightness and hue of its coloration.

We found complex interactions among diet, signal line, and growth rate of the larvae, which could possibly affect their signal expression (Fig. 1). Contrary to what we would have expected in the light of the "costly defenses" hypothesis, large signal larvae grew slower than small signal larvae on the low IG diet but on the high IG diet the growth rates did not differ. There is no straightforward explanation for this result. According to Ojala et al. (2007) *P. plantaginis* larvae with large signals develop faster and live longer indicating that a large signal is less costly to produce than a small signal. However, in that particular experiment by Ojala et al. (2007) the diets of the larvae did not include IGs or any other highly toxic defense chemicals. Therefore, based on our result, it seems that growth rate is not only related to the larval signal size but also to the quality and concentration of defense chemicals in the diet. Although resolving the actual mechanism behind this interaction demands further study, we can already conclude that variation in the defense chemical content of the host plant can have an impact on warning signal expression through the trade-offs between growth and signal expression. The overall benefit of higher growth rate can be important as it decreases the period when larvae are vulnerable to predation and parasites, and therefore increases the probability that the individual survives until the reproductive life stage (Damman 1987; Teder and Tammaru 2001; but see Benrey and Denno 1997).

It is often assumed that different chemical contents in plants induce variation in the chemical defense efficacy of aposematic individuals but not in their coloration (e.g., Guilford 1990; Ruxton et al. 2004; Speed and Ruxton 2007). Our results provide an interesting alternative; that variation in chemical contents of host plants can induce variation in coloration as well. Although the difference in IGs was clear between the diet treatments, it did not reflect the chemical content in the aposematic *P. plantaginis* larvae. Instead, differences in diet chemistry caused variation in reproductive output and adult female coloration. A possible mechanism behind this finding is that the maximum amount of IGs that *P. plantaginis* can tolerate and store is low and they can sequester this amount from a diet of low IG concentration. It follows that the cost of energy and time allocated to detoxification and excretion of the excess chemicals in the diet would constrain life-history traits or expression of warning signals. Nevertheless, it is worthwhile to note that because *P. plantaginis* moths are polyphagous they can probably exploit other defense chemical groups in addition to IGs. If there are differences in the sequestration and excretion efficacy among these groups, chemical defense and its efficacy can vary as well. Moreover, if the defense chemical repertoire of *P. plantaginis* is dependent on the chemicals in the host plant, availability of host plants can induce variation in qualitative and possibly also in quantitative chemical defense. Thus, to elucidate the selective factors maintaining the diversity shown in aposematic defense strategies (see references in Endler and Mappes 2004), it is important to extend the study to multiple trophic interactions taking into account the individual's selective environment as a whole, in addition to predator–prey interaction.

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LITERATURE CITED

Adler, L. S., J. Schmitt, and M. D. Bowers. 1995. Genetic-variation in defensive chemistry in *Plantago lanceolata* (Plantaginaceae) and its effects on the specialist herbivore *Junonia coenia* (Nymphalidae). Oecologia 101:75–85.

Beatty, C. D., K., Beirinckx, and T. N. Sherratt. 2004. The evolution of Müllerian mimicry in multispecies communities. Nature 431:63–67.

Bellman, H. 2007. Vlinders, rupsen en waardplanten. Tirion uitgevers B.V., Baarn.

Benrey, B., and R. F. Denno. 1997. The slow-growth—high mortality hypothesis: a test using the cabbage butterfly. Ecology 78:987–999.

Berenbaum, M. R., and A. R. Zangerl. 1993. Furanocoumarin metabolism in *Papilio polyxenes*: biochemistry, genetic variability, and ecological significance. Oecologia 95:370–375.

Bezzerides, A., K. MacGraw, R. Parker, and J. Husseini. 2007. Elytra colour as a signal of chemical defense in the Asian ladybird beetle *Harmonia Axyridis*. Behav. Ecol. Sociobiol. 61:1401–1408.

Björkman, C., and S. Larsson. 1991. Pine sawfly defense and variation in host plant resin acids: a trade-off with growth. Ecol. Entomol. 16:283–289.

Blount, J. D., M. P. Speed, G. D. Ruxton, and P. A. Stephens. 2009. Warning displays may function as honest signals of toxicity. Proc. R. Soc. Lond. B. 276:871–877.

Bowers, M. D. 1990. Recycling plant natural products for insect defense. Pp. 353–387 *in* D. L. Evans and J. O. Schmidt, eds. Insect defense: adaptive mechanisms and strategies of prey and predators. State Univ. of New York Press, New York.

———. 1991. Iridoid glycosides. Pp. 297–325 *in* G. A. Rosenthal, and M. R. Berenbaum, eds. Herbivores: their interaction with secondary plant metabolites. Academic Press, San Diego, CA.

Bowers, M., and S. Farley. 1990. The behaviour of grey jays, *Perisoreus Canadensis*, towards palatable and unpalatable Lepidoptera. Anim. Behav. 39:699–705.

Bowers, M. D., and S. K. Collinge. 1992. Fate of iridoid glycosides in different life stages of the buckeye, *Junonia coenia* (Lepidoptera, Nymphalidae). J. Chem. Ecol. 18:817–831.

Bowers, M. D., and N. E. Stamp. 1992. Chemical variation within and between individuals of *Plantago lanceolata* (Plantaginaceae). J. Chem. Ecol. 18:985–995.

———. 1993. Effects of plant-age, genotype, and herbivory on *Plantago* performance and chemistry. Ecology 74:1778–1791.

———. 1997a. Fate of host-plant iridoid glycosides in Lepidopteran larvae of Nymphalidae and Arctiidae. J. Chem. Ecol. 23:2955–2964.

———. 1997b. Effect of hostplant genotype and predators on iridoid glycoside content of pupae of a specialist insect herbivore, *Junonia coenia* (Nymphalidae). Biochem. Syst. Ecol. 25:571–580.

Brakefield, P. M. 1985. Polymorphism Müllerin mimicry and interactions with thermal melanism in ladybirds and a soldier beetle: a hypothesis. Biol. J. Linn. Soc. 26:243–267.

Brakefield, P. M., and T. G. Liebert. 1985. Studies of colour polymorphism in some marginal populations of the aposematic jersey tiger moth *Callimorpha quadripunctaria*. Biol. J. Linn. Soc. 26:225–241.

Brückman, M., J. R. Trigo, M. A. Foglio, and T. Hartman. 2000. Storage and metabolism of radioactively labeled pyrrolizidine alkaloids by butterflies and larvae of *Mechanitis polymnia* (Lepidoptera: Nymphalidae, Ithomiinae). Chemoecology 10:25–32.

Camara, M. 1997a. Predator responses to sequestered plant toxins in buckeye caterpillars: are tritrophic interactions locally variable? J. Chem. Ecol. 23:2093–2106.

———. 1997b. Physiological mechanisms underlying the costs of chemical defense in *Junonia coenia* Hübner (Nymphalidae): a gravimetric and quantitative genetic analysis. Evol. Ecol. 11:451–469.

Chinery, M. 1993. Collins guide to insects of Britain and Western Europe. 3rd ed. Harper-Collins. London.

Cott, H. B. 1940. Adaptive colouration in animals. Methuen, London.

Cuthill, I. C. 2006. Vol. I: Mechanisms and Measurements. Pp. 3–40, *in* G. E. Hill, and K. J. McGraw, eds. Bird colouration. Harvard Univ. Press, Cambridge, MA.

Damman, H. 1987. Leaf quality and enemy avoidance by the larvae of a pyralid moth. Ecology 68:88–97.

Damtoft, S., S. R. Jensen, and B. J. Nielsen. 1983. The biosynthesis of iridoid glycosides from 8-epi-deoxyloganic acid. Biochem. Soc. Trans. 11:593–594.

Darst, C. R., M. E. Cummings, and D. C. Cannatella. 2006. A mechanism for diversity in warning signals: conspicuousness versus toxicity in poison frogs. Proc. Natl. Acad. Sci. USA 103:5852–5857.

De La Fuente, M., L. A. Dyer, and M. D. Bowers. 1994/1995. The iridoid glycoside, catalpol, as a deterrent to the predator *Camponotus floridanus* (Formicidae). Chemoecology 5:13–18.

Del Campo, M. L., S. R. Smedley, and T. Eisner. 2005. Reproductive benefits derived from defensive plant alkaloid possession in an arctiid moth (*Utetheisa ornatrix*). Proc. Natl. Acad. Sci. USA 38:13508–13512.

Despland, E., and S. J. Simpson. 2005. Food choices of solitarious and gregarious locusts reflect cryptic and aposematic antipredator strategies. Anim. Behav. 69:471–479.

Després, L., J.-P. David, and C. Gallet. 2007. The evolutionary ecology of insect resistance to plant chemicals. Trend. Ecol. Evol. 22:298–307.

Dobler, S., and M. Rowell-Rahier. 1996. Reproductive biology of viviparous and oviparous species of the leaf beetle genus Oreina. Entomol. Exp. Appl. 80:375–388.

Duff, R. B., J. S. D. Bacon, C. M. Munide, V. C. Farmer, J. D. Russell, and A. R. Forrester. 1965. Catalpol and methylcatalpol: naturally occurring glycosides in *Plantago* and *Buddleia* species. Biochem. J. 96:1–5.

Dyer, L., and M. Bowers. 1996. The importance of sequestered iridoid glycosides as a defense against an ant predator. J. Chem. Ecol. 22:1527–1539.

Endler, J. A. 1991. Interactions between predators and prey. Pp. 169–196, *in* J. R. Krebs and N. B. Davies, eds. Behavioural ecology: an evolutionary approach. Blackwell Science, Cambridge.

Endler, J. A., and J. Mappes. 2004. Predator mixes and the conspicuousness of aposematic signals. Am. Nat. 163:532–547.

Fordyce, J. A., and C. C. Nice. 2008. Antagonistic, stage-specific selection on defensive chemical sequestration in a toxic butterfly. Evolution 62:1610–1617.

Gamberale-Stille, G., and B. S. Tullberg. 1999. Experienced chicks show biased avoidance of stronger signals: an experiment with natural colour variation in live aposematic prey. Evol. Ecol. 13:579–589.

Grill, C. 1999. Development of colour in an aposematic ladybird beetle: the role of environmental conditions. Evol. Ecol. Res. 1:651–652.

Grill, C., and A. Moore. 1998. Effects of a larval antipredator response and larval diet on adult phenotype in an aposematic ladybird beetle. Oecologia. 114:274–282.

Guilford, T. 1990. The evolution of aposematism. Pp. 23–63, *in* D. L. Evans and J. O. Schmidt, eds. Adaptive mechanisms and strategies of prey and predators. New York Press, New York.

Holloway, G. J., P. de Jong, P. M. Brakefield, and H. de Vos. 1991. Chemical defense in ladybird beetles (Coccinellidae). I. Distribution of coccinelline and individual variation in defense in 7-spot ladybirds *(Coccinella septempunctata)*. Chemoecology 2:7–14.

Holloway, G. J., P. W. de Jong, and M. Ottenheim. 1993. The genetics and cost of chemical defense in the 2-spot ladybird (*Adalia bipunctata* L.). Evolution 47:1229–1239.

Ihalainen, E., L. Lindström, and J. Mappes. 2007. Investigating Müllerian mimicry: predator learning and variation in prey defenses. J. Evol. Biol. 20:780–791.

Joron, M., and J. Mallet. 1998. Diversity in mimicry: paradox or paradigm. Trend. Ecol. Evol. 13:461–466.

Kapan, D. 2001. Three-butterfly system provides a field test of Müllerian mimicry. Nature 409:338–340.

Leimar, O., M. Enquist, and B. Sillén-Tullberg. 1986. Evolutionary stability of aposematic colouration and prey unprofitability: a theoretical analysis. Am. Nat. 128:469–490.

Lindstedt, C., L. Lindström, and J. Mappes. 2008. Hairiness and warning colours as components of antipredator defense: additive or interactive benefits? Anim. Behav. 75:1703–1713.

———. 2009. Thermoregulation can constrain effective warning signal expression. Evolution 63:469–478.

Lindström, L., R. V. Alatalo, J. Mappes, M. Riipi, and L. Vertainen. 1999. Can aposematic signals evolve by gradual change? Nature 379:249–251.

Longson, C. G., and J. M. P. Joss. 2006. Optimal toxicity in animals: predicting the optimal level of chemical defenses. Funct. Ecol. 20:731–735.

Mappes, J., N. Marples, and J. A. Endler. 2005. The complex business of survival by aposematism. Trend. Ecol. Evol. 20:598–603.

Marak, H. B., A. Biere, and J. M. M. van Damme. 2000. Direct and correlated responses to selection on iridoid glycosides in *Plantago lanceolata* L. J. Evol. Biol. 13:985–996.

———. 2003. Fitness costs of chemical defense in *Plantago lanceolata* L.: effects of nutrient and competition stress. Evolution 57:2519–2530.

Müller, F. 1879. *E. Ituna* and *Thyridia*; a remarkable case of mimicry in butterflies. Trans. Ent. Soc. Lond. xx–xxix.

Nieminen, M., J. Suomi, S. Van Nouhuys, P. Sauri, and M. L. Riekkola. 2003. Effects of iridoid glycoside content on oviposition host plant choice and parasitism in a specialist herbivore. J. Chem. Ecol. 29:853–844.

Nishida, R. 2002. Sequestration of defensive substances from plants by Lepidoptera. Annu. Rev. Entomol. 47:57–92.

Ojala, K., R. Julkunen-Tiitto, L. Lindström, and J. Mappes. 2005. Diet affects the immune defense and life-history traits of an Arctiid moth *Parasemia plantaginis*. Evol. Ecol. Res. 7:1153–1170.

Ojala, K., L. Lindström, and J. Mappes, 2007. Life history constraints and warning signal expression in Arctiid moth. Funct. Ecol. 21:1162–1167.

Pabis, K. 2007. New species of Lepidoptera for the Biogradska Gora National Park, Montenegro. Glas. Republ. Zavoda Zašt. Prirode podgorica 29–30:167–169.

Reudler Talsma, J. H. 2007. Costs and benefits of iridoid glycosides in multitrophic systems (PhD-thesis), Pp. 152. Wageningen Univ., The Netherlands.

Reudler Talsma, J. H., K. Torri, and S. van Nouhuys. 2008. Host plant use by the Heath fritillary butterfly, *Melitaea athalia:* plant habitat, species and chemistry. Arthropod-Plant Interactions 2:63–75.

Riipi, M., R. V. Alatalo, L. Lindström, and J. Mappes. 2001. Multiple benefits of gregariousness cover detectability costs in aposematic aggregations. Nature 413:512–514.

Rowland, H. M., E. Ihalainen, L. Lindström, J. Mappes, and M. P. Speed. 2007. Co-mimics have a mutualistic relationship despite unequal defense levels. Nature 448:64–66.

Ruxton, G. D., T. N. Sherratt, and M. P. Speed. 2004. Avoiding attack. Pp. 249, *in* G. D. Ruxton, T. N. Sherratt, and M. P. Speed, eds. Evolutionary ecology of crypsis, warning signals and mimicry. Oxford University Press.

Sagar, G. R., and J. L. Harper. 1964. Biological flora of the British isles. *Plantago major*, L., *Plantago media* L., and *Plantago lanceolata* L. J. Ecol. 52:189–221.

Sandre, S.-L., T. Tammaru, T. Esperk, R. Julkunen-Tiitto, and J. Mappes. 2007. Carotenoid-based colour polymorphism in a moth species: a search for fitness correlates. Ent. Exp. Appl. 124:269–277.

Skelhorn, J., and C. Rowe. 2006. Prey palatability influences predator learning and memory. Anim. Behav. 71:1111–1118.

Speed, M., and G. Ruxton. 2007. How bright and how nasty: explaining diversity in warning signal strength. Evolution 61:623–635.

Stevens, M., A. Párraga, I. C. Cuthill, J. C. Partridge, and T. Troscianko. 2007. Using digital photography to study animal colouration. Biol. J. Linn. Soc. 90:211–237.

Summers, K., and M. E. Clough. 2001. The evolution of coloration and toxicity in the poison frog family (Dendrobatidae). Proc. Natl. Acad. Sci. USA 98:6227–6232.

Sword, G. 1999. Density-dependent warning colouration. Nature 397:217.

Teder, T., and T. Tammaru. 2001. Large larvae of a flush-feeding moth (*Epirrata autumnata*, Lepidoptera: Geometridae) are not at a higher risk of parasitism: implications for the moth's life-history. Eur. J. Entom. 98:277–282.

Tullberg, B. S., G. Gamberale-Stille, and C. Solbreck. 2000. Effects of food plant and group size on predator defense: differences between two co-occuring aposematic Lygaeinae bugs. Ecol. Entomol. 25:220–225.

Watson, A., and D. T. Goodger. 1986. Catalogue of the Neotropical tiger-moths. Trustes of British Museum (Natural History), London. 71 p.

Weller, S., N. Jacobson, and W. Conner. 1999. The evolution of chemical defenses and mating systems in tiger moths (Lepidoptera: Arctiidae). Biol. J. Linn. Soc. 68:557–578.

Willinger, G., and S. Dobler. 2001. Selective sequestration of iridoid glycosides from their host plants in Longitarsus flea beetles. Biochem. Syst. Ecol. 29:335–346.

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