Ann. Zool. Fennici 44: 70–80 ISSN 0003-455X
Helsinki 15 March 2007

Development and survival of a specialist herbivore, *Melitaea cinxia*, on host plants producing high and low concentrations of iridoid glycosides

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Received 24 Aug. 2006, revised version received 21 Dec. 2006, accepted 7 Nov. 2006

Saastamoinen, M., van Nouhuys, S., Nieminen, M., O'Hara, B. & Suomi, J. 2007: Development and survival of a specialist herbivore, *Melitaea cinxia*, on host plants producing high and low concentrations of iridoid glycosides. — *Ann. Zool. Fennici* 44: 70–80.

The Glanville fritillary butterfly (*Melitaea cinxia*) in Finland feeds on the plants *Plantago lanceolata* and *Veronica spicata*. These two plant species are distributed heterogeneously and both vary spatially and temporally in iridoid glycoside concentrations. We investigated the associations of plant species and iridoid glycoside (aucubin and catalpol) concentrations with weight, development rate and survival of larvae of the Glanville fritillary under laboratory conditions. In one experiment we compared the performance of split brood groups of larvae feeding on the two plant species collected from natural populations. In the second experiment larvae were fed *P. lanceolata* lines laboratory selected for high and low aucubin and catalpol concentrations. Larvae fed *V. spicata* performed better in terms of survival, weight and growth rate than those feeding on *P. lanceolata*, regardless of iridoid glycoside concentration. However, in the second experiment iridoid glycoside concentration in *P. lanceolata* was positively associated with larval performance. High iridoid glycoside concentrations retarded development rate of the first instar larvae, whereas later on the development was accelerated by higher concentrations of these compounds. The spatial and temporal variation of plant species suitability and iridoid glycoside content, and larval family level effects of plant chemistry on performance convey a dynamic ecological and evolutionary relationship between these host plants and their specialized herbivore.

Introduction

Secondary chemicals produced by plants have shaped the evolution of phytophagous insects (e.g. Ehrlich & Raven 1964, Strong *et al*. 1984, Bowers 1988, Becerra 1997), and the diversity of specialist phytophagous insects (Jaenike 1990, Speight *et al*. 1999) has been explained, at least in part, by adaptation to plant defensive chemicals (e.g. Camara 1997a, Carriere 1998).

While the benefit to plants of being chemically defended against herbivory has been well documented (e.g. Zalucki & Brower 1992, Adler *et al*. 2001), it is also known that chemical defence can be costly (e.g. Zangerl *et al*. 1997, Redman *et al*. 2001, Marak *et al*. 2003) and that the level of secondary chemicals varies greatly among related plant species (e.g. Bolser & Hay 1996, Hartmann 1996, Julkunen-Tiitto *et al*. 1996, Singer & Lee 2000), among individuals of the same species (Bowers & Stamp 1992, Singer *et al*. 2002), and within individual plants over time (e.g. Bowers *et al*. 1992, Nieminen *et al*. 2003). Because of this, even specialised herbivores experience a range of chemical environments. As population and evolutionary dynamics, and community structure depend in part on the extent to which the chemical variation among plants translates (directly or indirectly) to variation in herbivore fitness, more should be known about the ecological consequences of variation of chemical defence in the diet of specialized herbivores.

Checkerspot butterflies in the tribe Melitaeini (Nymphalidae) are primarily oligophagous species feeding on several plant families in the subclass Asteridae. The presence of iridoid glycosides (a group of terpenoids; El-Naggar & Beal 1980, Marak *et al*. 2000) has had a major influence on the evolutionary history of host plant use, and shifts to plants without iridoid glycosides have rarely taken place (Bowers 1983, Wahlberg 2001). Melitaeini butterflies are known to sequester iridoids, catalpol and aucubin particularly, as larvae, and many of them retain the toxic or noxious compounds into adulthood (Gardner & Stermitz 1988, Belofsky *et al*. 1989, Nieminen *et al*. 2003, Suomi *et al*. 2003). Several studies showed the benefits of iridoid glycosides sequestration in defence against insectivorous vertebrates (e.g. Bowers 1980, Bowers & Farley 1990) and invertebrates (Dyer 1995, Dyer & Bowers 1996).

The Melitaeini butterfly *Melitaea cinxia* feeds on several species in the family Scrophulariaceae. In the Åland island of SW Finland *M. cinxia* larvae feed on two host plants, *Plantago lanceolata* and *Veronica spicata* (Kuussaari *et al*. 2000, Nieminen *et al*. 2004). Individual ovipositing *M. cinxia* are known to prefer one plant species over the other. However, their preferences differ throughout the island so neither plant species is on average the preferred host (Kuussaari *et al*. 2000). Large-scale survey data revealed no overall difference in larval survival between the plant species in natural populations, perhaps because mortality due to other factors varies greatly among populations (van Nouhuys *et al*. 2003).

The iridoid glycosides, catalpol and aucubin, are found in high concentrations in both host plant species, and both also contain a few other iridoid glycosides in much lower concentration (Suomi *et al*. 2002). Not surprisingly, catalpol and aucubin are sequestered from the host plants at high concentrations by *M. cinxia* larvae (Suomi *et al*. 2001, 2003). In natural populations the concentrations of these compounds in both host plant species vary spatially and temporally (Bowers *et al*. 1992, Bowers & Stamp 1992, Suomi *et al*. 2002, Nieminen *et al*. 2003). Nieminen *et al*. (2003) found that in natural populations *M. cinxia* eggs were more often found on *P. lanceolata* containing high rather than low concentrations of aucubin and catalpol, indicating that these compounds or correlated plant characteristics affect spatial distribution of the larvae through their association with adult butterfly host plant choice. Alternatively, oviposition induces the production of these compounds, creating heterogeneity of chemical defence.

It is clear from Nieminen *et al*. (2003) and Kuussaari *et al*. (2000) that both plant species and iridoid glycoside content are associated with adult female oviposition behaviour, thus influencing local and regional *M. cinxia* abundances (also *see* Hanski & Singer 2001, Hanski & Heino 2003).

The purpose of the work presented here is to further understand the consequences of these adult oviposition choices for the performance of the larvae. We conducted two laboratory experiments. In one, both host species (*P. lanceolata* and *V. spicata*) were collected from natural populations and the leaves were fed to *M. cinxia* larvae under laboratory conditions. The concentrations of aucubin and catalpol were measured during the experiment. In the second experiment larvae were fed *P. lanceolata* that had been selected in the laboratory over four generations

to produce either high or low aucubin and catalpol concentrations (Marak *et al*. 2000, 2002, Biere *et al*. 2004). Biere *et al*. (2004) showed that this selection did not affect the concentrations of nitrogen or phosphorus in the plants. We do not know what chemicals (nutrients or defensive) or physical characteristics vary along with aucubin and catalpol in the *V. spicata* and *P. lanceolata* collected from natural populations for experiment 1.

The specific questions we address are: (1) Does larval survival, development or growth depend on host plant species (*P. lanceolata vs. V. spicata*)? (2) Do survival, development and growth of larvae vary with the concentrations of iridoid glycosides (or correlated plant traits) in randomly selected *P. lanceolata* and *V. spicata*? (3) How do survival, growth or development of larvae fed *P. lanceolata* artificially selected to produce high and low iridoid glycoside concentrations differ?

Material and methods

Rearing of the larvae

The parents of the larvae used in the experiments were raised in captivity. The parental generation was collected as larvae from a variety of locations around the Åland Islands and mated haphazardly. Therefore the larvae used in this experiment are presumed to be a representative sample from the Åland Islands. Female butterflies laid their eggs between 5 June and 24 June 2001, and larvae from 57 different females were used. Replicates were created by separating ten first instar larvae (three to five days old) from the gregarious larval group using a small paintbrush. Larvae were used instead of eggs because the eggs tend to desiccate easily when separated. Before the separation, larvae fed briefly on either *V. spicata* or *P. lanceolata* depending on which species the female had laid her eggs on. The group of larvae was placed on a 3-cm piece of leaf in a Petri dish on a filter paper. The filter paper was moistened with few drops of water to prevent the larvae and leaf piece from desiccating. The Petri dishes were maintained under natural lighting conditions (14:10 L:D) in the laboratory. The larvae were checked daily and had food available at all times. To obtain the larval development rate the dates when the first larvae of each group moulted to each instar were recorded. When the first larvae of each group had been in the third instar for three days, all larvae in the third instar were weighed and their numbers counted. The larvae were always weighed as a group and the mean individual weight was calculated. Larvae were weighed as a group and counted again when the first individual in a dish had been in the fourth instar for seven days. The experiment ended at this point, just prior to the moult to diapause instar, due to lack of food plants for some of the treatments. The experiments were conducted at the Nåtö biological station in the Åland Islands in southwestern Finland.

Experiment 1: Performance of larvae on natural host plants collected from the field

To determine whether larval performance varied with (1) host plant species, and (2) naturally occurring iridoid glycoside concentrations, 47 *P. lanceolata* and 47 *V. spicata* were collected from a variety of *M. cinxia* habitat patches in the Åland Islands. The sites included pastures and dry meadows on low rocky outcrops. These sites were chosen because some of them contained both host species and, in general, the sites had not been affected by the drought in the early summer of 2001. The plants were collected in the early spring while they were all still small and vegetative. They were transplanted into 10-cm diameter pots, kept in an outdoor common garden, and watered as needed throughout the experiment. Leaves from each plant were fed to groups of ten larvae. Thus each group was reared only on leaves from a single host plant individual. We used larvae from 47 different *M. cinxia* families, splitting each between the *Plantago* and *Veronica* treatments.

Halfway through the experiment samples of middle-aged leaves from each plant were collected and analysed for aucubin and catalpol concentrations. The samples were taken halfway through the experiment because the iridoid concentrations tend to vary throughout the

season (Nieminen *et al*. 2003). The leaves were first air dried and pre-treated by hot water extraction, as described in detail in Suomi *et al*. (2000, 2002) and Nieminen *et al*. (2003). The extracts were analysed using a capillary electromigration technique, micellar electrokinetic capillary chromatography, under the conditions described in Nieminen *et al*. (2003). These methods of iridoid glycoside extraction (Suomi *et al*. 2000) and analysis (Suomi *et al*. 2002) are efficient and effective for many iridoid glycosides. The limits of detection for the iridoid glycosides of interest were low enough to make the analysis of a single leaf possible (Suomi *et al*. 2000). The iridoid glycosides were identified by their UV spectra as well as by spiking the samples with solutions of catalpol and aucubin. The iridoid glycoside standards were donated by Prof. S. R. Jensen (Technical University of Denmark, Lyngby, Denmark).

Experiment 2: Performance of larvae on *P. lanceolata* selected in the laboratory to produce high and low concentrations of iridoid glycosides

A similar procedure to that described above was carried out to study the effects of iridoid glycoside concentration on larval performance using artificially selected lines of *P. lanceolata*. *Melitaea cinxia* larvae were fed *P. lanceolata* selected to produce either high or low iridoid glycoside concentrations. All together 87 first instar larval groups, each containing ten larvae, were formed from 57 larval families. Whenever possible larvae from one family were used in both treatments. Due to the plant availability there were nearly twice the number of high treatments (N = 57) as low treatments (N = 30). We used *P. lanceolata* plants propagated from seeds from family lines selected in the laboratory to produce low iridoid and high iridoid concentration. The divergent *P. lanceolata* lines were developed by A. Biere and H. B. Marak from the Netherlands Institute of Ecology. We used plants from five families of low iridoid lines, and six of high iridoid lines. The iridoid glycoside concentrations varied only slightly among families within selection lines (*see* Marak *et al* 2000). We were unable to analyse the iridoid glycoside concentrations of the plants used in experiment 2. However the concentrations of aucubin and catalpol in the parental plants were previously measured using high performance liquid chromatography (HPLC) (for methods *see* Marak *et al*. [2000]), and the heritability of leaf iridoid glycoside concentration in these plants is known to be high (h^2 = 0.84; Marak *et al*. 2002). It is important to note that the method used to analyse iridoid glycoside concentration in experiment 1 is different than in experiment 2.

Data analyses

The survival of larvae between the diets (experiment 1: *Plantago vs*. *Veronica* diet; experiment 2: high *vs*. low iridoid diet on *Plantago*) was analysed with a generalised linear model, assuming a binomial distribution and a logit link (McCullagh & Nelder, 1989, R-statistical package, 2000). All the factors (treatment, egg cluster and treatment × egg cluster) were tested for overdispersion. F-test was used, as some evidence was found for overdispersion. χ^2-test was used to check whether the effect of treatment varied among egg clusters. There was no need for the egg cluster to be nested with treatment in experiment 1 because all egg clusters were used for both treatments. In experiment 2 however, egg cluster was nested within treatment because only some (more than half) of the egg clusters were used for both treatments due to the lower number of high iridoid treatment plants. Generalised linear model (Statistix 7.0: Analytical software 2000) was used in both experiments to test what factors affected larval weight and development rate (number of days larvae stayed in each instar). Because in some groups not all larvae survived through the entire experiment, the affect of group size was initially included in the model. As group size had no effect it was dropped from the final model. To test whether the number of groups in which all larvae had died by the end of the experiment varied between the two different treatments the data were analysed using χ^2-test. Logistic regression was used in experiment 1 to test whether concentrations of aucubin and catalpol were associated with larval survival. Similarly, generalised linear

model was used to test for the effects of aucubin and catalpol concentrations on larval weight and development rate.

Results

Iridoid glycoside concentrations of the plants used in the two feeding experiments

In experiment 1, comparing the two host plant species, *V. spicata* contained only about half of the concentration of both aucubin and catalpol per unit dry weight as *P. lanceolata* had (Table 1; ANOVA: $F_{1,90} = 29.28$, $P < 0.001$ and $F_{1,90} = 11.44$, $P < 0.001$ for aucubin and catalpol respectively). These values are very similar to those reported by Nieminen *et al*. (2003) for more extensive samples collected from Åland, indicating that the iridoid glycoside concentrations were not changed by transplanting the plants to pots. In experiment 2, the iridoid glycoside concentrations of the parent lines were measured prior to the beginning of the experiment. The mean concentrations of both aucubin and catalpol were almost five times higher in the high iridoid glycoside as compared with that in the low iridoid glycoside lines (Table 1). The iridoid glycoside concentrations cannot be compared between the two experiments because they were measured differently (*see* Table 1 and the method section descriptions of the different methodologies). It is interesting to note that the aucubin: catalpol ratios differ, with apparently relatively more catalpol in the field-collected plants. But again, we cannot tell if this is an actual difference between the plants or an artefact of using different sample pretreatment techniques.

Experiment 1: Performance of larvae on natural host plants collected from the field

The effect of host plant species was highly significant for each measure of larval performance. Larvae fed *V. spicata* weighed more and devel-

Table 1. Aucubin and catalpol concentrations (% dry weight) of plants used in feeding experiments. Data for experiment 1 are for 47 individuals each of *P. lanceolata* and *V. spicata* that were used to feed larvae. Data for experiment 2 are for an average representative individual from 5 (low iridoid) or 6 (high iridoid) lines of *P. lanceolata*. Number of replicates indicates the number of larval families reared on each plant species or line. Standard errors are in parentheses.

Treatment	Families	Aucubin	Catalpol	No. of replicates
Feeding experiment 1				
P. lanceolata		1.0 (0.09)	1.7 (0.17)	47
V. spicata		0.5 (0.04)	1.0 (0.06)	47
Feeding experiment 2*				
Low iridoid	1	0.24	0.17	21
	2	0.24	0.19	8
	3	0.27	0.15	6
	4	0.33	0.21	11
	5	0.33	0.40	11
Mean		0.28 (0.01)	0.23 (0.01)	
High iridoid	1	1.18	1.01	5
	2	1.21	1.10	6
	3	1.37	1.07	5
	4	1.35	1.08	3
	5	1.66	0.98	5
	6	1.41	1.07	6
Mean		1.36 (0.03)	1.05 (0.01)	

* Information provided by Arjen Biere from the Netherlands Institute of Ecology.

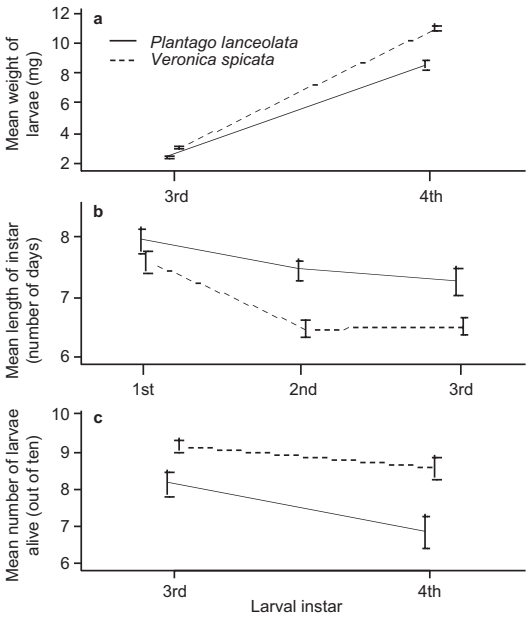

Fig. 1. Differences in the (**a**) mean weight (*see* Table 2), (**b**) instar length (*see* Table 2) and (**c**) survival (*see* Table 3) of larvae fed either *P. lanceolata* or *V. spicata*. Standard errors of mean is given in parenthesis.

oped faster than larvae fed *P. lanceolata* (Table 2; Fig. 1a and b respectively). In both the third and fourth instar, the *M. cinxia* larvae that were fed *V. spicata* also survived better than those that were fed *P. lanceolata* (Table 3 and Fig. 1c). The survival (Table 3) and weight (Table 2) of larvae differed slightly among families.

In this experiment larval weight did not vary with iridoid concentration on either host plant species, though a trend indicated that on *V. spicata*, third instar larval weight increased with concentration of catalpol (Linear Regression Model: coefficient = 4.3×10^{-4}, SE = 2.2×10^{-4}, $P = 0.055$). Larval development rate (duration of larval instars) changed slightly with iridoid glycoside concentration. The durations of the first and second instars of larvae fed *V. spicata* did not change with aucubin and catalpol concentrations. However, in the third instar *M. cinxia* larvae developed faster at higher concentrations of catalpol (Linear Regression Model: coefficient = –1.004, SE = 0.310, $P = 0.002$; Fig. 2). In experiment 1, development rates of the larvae fed *P. lanceolata* did not vary with aucubin or catalpol concentrations. The survival of larvae fed *P. lanceolata* or *V. spicata* did not vary significantly with iridoid glycoside (aucubin and catalpol) concentration.

Table 2. ANOVA table of the effects of instar, treatment (host-plant species), egg cluster and interaction of instar and treatment on larval weight and developmental rate in experiment 1.

Source	d.f.	*F*	*P*
Larval weight			
Instar	1	171.6	< 0.001
Treatment	1	85.13	< 0.001
Egg cluster	45	1.59	0.024
Instar × Treatment	1	25.79	< 0.001
Development rate			
Instar	2	14.13	< 0.001
Treatment	1	23.13	< 0.001
Egg cluster	45	1.20	0.202
Instar × Treatment	1	1.70	0.185

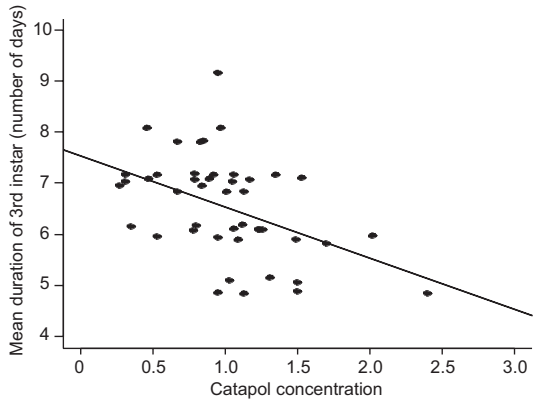

Fig. 2. The relationship between the catalpol concentration (% of dry weight) and mean duration of the third instar of *M. cinxia* larvae fed *V. spicata* ($R^2 = 0.232$, $P = 0.002$).

Experiment 2: Performance of larvae on *P. lanceolata* selected in the laboratory to produce high and low concentrations of iridoid glycosides

In experiment 2, aucubin and catalpol concentrations were positively associated with the weight of the *M. cinxia* larvae. Larvae fed the low iridoid diet weighed significantly less than larvae fed the high iridoid diet (Table 4 and Fig. 3a). A significant correlation between instar and treatment was also found (Table 4), because the difference between the two treatments is greater in

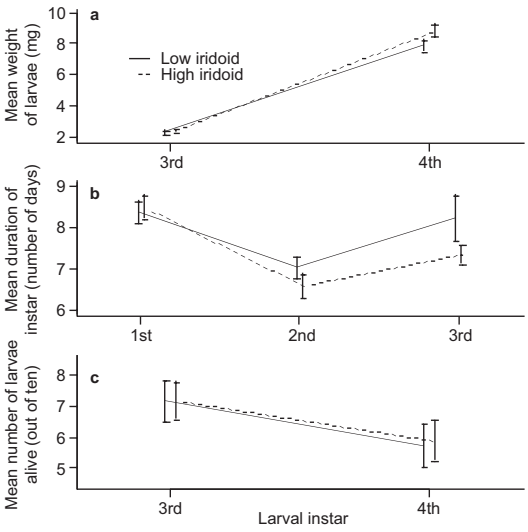

Fig. 3. Differences in the (**A**) mean weight (*see* Table 5), (**B**) instar length (*see* Table 5) and (**C**) survival (*see* Table 3) of larvae fed either low or high iridoid diets. Standard errors of mean are given in parenthesis

Table 4. ANOVA table of the effects of larval instar, treatment (high and low iridoid *P. lanceolata* diet), interaction of instar and treatment, and egg cluster nested within treatment on larval weight and development rate.

Source	d.f.	*F*	*P*
Larval weight			
Instar	1	627.4	< 0.001
Treatment	1	10.66	0.002
Instar × Treatment	1	5.13	0.03
Egg cluster (Treatment)	76	1.51	0.041
Development rate			
Instar	2	15.63	0.004
Treatment	1	1.67	0.198
Instar × Treatment	2	5.30	0.006
Egg cluster (Treatment)	85	0.63	0.989

the fourth than in the third instar (Fig. 3a). Larval development rate (duration of larval instars) also changed with iridoid glycoside concentration, with first instar larvae fed the high iridoid diet developing slowly (Table 4 and Fig. 3b; Bonferroni's pairwise comparison, $P = 0.003$). In contrast, in the second and third instars the pattern changed and larvae fed a high iridoid diet developed faster and the development rate of larvae fed low iridoid diet was reduced. In experiment 2, as in experiment 1, the relationship between survival and iridoid treatment was not significant. However, there was a trend suggesting that survival of larvae fed plants containing the high iridoid concentration was higher than of larvae fed the low iridoid concentration diet (Table 3 and Fig. 3c). Larvae used in the experiment 2

Table 3. Analysis of deviance of the factors affecting larval survival until the third instar and from the 3rd to the 4th instar for feeding experiments 1 and 2.

Trial	Instar	Effect	d.f.	Deviance	*F*	*P*
Feeding experiment 1						
	until 3rd					
		Treatment (PI *vs.* Vs diet)	1	26.20	16.59	< 0.001
		Egg cluster	45	140.38	1.98	0.012
		Treatment × Egg cluster	45	71.06		0.008*
	3rd–4th					
		Treatment (PI *vs.* Vs diet)	1	35.13	26.57	< 0.001
		Egg cluster	15	155.77	2.32	0.003
		Treatment × Egg cluster	44	65.62		0.020*
Feeding experiment 2						
	until 3rd					
		Treatment (high *vs.* low iridoid diet)	1	0.01	0.0024	0.96
		Egg cluster	56	412.41	1.740	0.054
		Treatment* Egg cluster	29	122.86		< 0.001*
	3rd–4th					
		Treatment (high *vs.* low iridoid diet)	1	3.83	2.670	0.063
		Egg cluster	53	316.97	4.170	0.001
		Treatment × Egg cluster	23	32.95		0.082*

* χ^2-test.

came from egg clusters produced by 57 different females collected from various locations in the Åland Islands. The larval weight and survival (but not developmental rate) differed among families (Tables 3 and 4).

Discussion

Our experiments focused on determining the association of iridoid glycoside concentration and host plant species on performance of prediapause *Melitaea cinxia* larvae. Larval size and survival until diapause is known to be one of the key determinants of population size of *M. cinxia* and is important for understanding their metapopulation-wide distribution and dynamics (Nieminen *et al* 2001, Kuussaari *et al*. 2004). More generally, this work contributes to our understanding of the role of host plant variation for the performance of herbivores specialized in feeding on plants that are chemically defended against generalist herbivores.

We demonstrated that iridoid glycosides are clearly not detrimental or toxic to *M. cinxia* larvae. Larvae that fed on plants with a high iridoid glycoside concentration weighed the same as or more than larvae fed on low iridoid plants. This is consistent with previous studies on this and other specialist insect herbivores showing positive relationships between iridoid concentrations and larval weight (e.g. Bowers & Puttick 1988, 1989) or increased development time (Harvey *et al*. 2005). Better growth of larvae fed high iridoid concentrations suggests that they either eat faster or more, or are able to utilize the food more efficiently. Iridoid glycosides are known to be feeding stimulants, increasing the rate of feeding for at least some specialist insect herbivores (for details *see* Bowers [1983] and Bowers & Puttick [1989]).

Naturally, the advantages for the larvae of *M. cinxia* feeding on host plants with high iridoid concentrations should be most apparent when natural enemies are present to cause larval mortality (Bowers & Farley 1990, Camara 1997b, Harvey *et al*. 2005). Since no enemies were included in our experiments we did not expect iridoid glycosides to have positive effects on larval survival. However, a trend suggests that more fourth instar larvae fed high iridoid diet survives than larvae fed low iridoid diet. Bowers (1984) also found that the survival of *Junonia coenia* was positively associated with the iridoid glycoside concentration; larvae grew poorly and had low survivorship on an artificial diet without iridoid glycosides. It has been reported that some iridoids have antimicrobial, antifungal, and/or antioxidant properties (Cameron *et al*. 1984, Davini *et al*. 1986, Ishiguro *et al*. 1986, Marak *et al*. 2002).

In experiment 2 the sharp increase in development rate of larvae fed *V. spicata* with increasing concentrations of catalpol suggests no physiological costs of sequestering iridoid glycosides. However, among larvae reared on *P. lanceolata* we detected an interesting correlation between iridoid glycoside concentration and development rate. The caterpillars fed the high iridoid diet grow slowly in the first instar. In the second and third instars, on the other hand, the larvae develop faster on diets containing high iridoid concentrations. These results thus indicate that at an early stage larvae are sensitive to iridoid glycosides which have slightly negative effects, perhaps on digestion or metabolism. Later on in the development, however, sequestering iridoid glycosides appears inexpensive for *M. cinxia*. Camara (1997a) showed reduced digestibility of diet containing high iridoid glycosides in *J. coenia* indicating that chemical defence does come with a cost. In that study larvae on high iridoid glycoside diets compensated for the reduction in digestibility by consuming more food (Camara 1997a).

The association of iridoid glycosides with insect performance is stronger in experiment 2, where the *P. lanceolata* plants were laboratory-selected to contain either higher or lower iridoid glycoside concentration than in experiment 1, where plants of initially unknown iridoid concentration were collected from the wild. Perhaps this is because a slightly larger range of concentrations is present in experiment 2 (Table 1). Unfortunately the iridoid contents were analysed differently in the two experiments so they cannot actually be compared. In both laboratory selected lines and wild plants iridoid glycoside concentration may correlate with nutrients or other chemicals that affect the nutritional quality

of the plant. These other factors may be stronger in the plants collected from natural populations than in plants bread for several generations in the laboratory. Nevertheless, the significant results in experiment 2 are supported by trends as well as some clear differences detected in experiment 1. While the (correlational) experiments presented here, alone do not demonstrate that iridoid glycosides are the compounds responsible for the observed differences in larval performance, they support the results of feeding experiments on other butterfly species using artificial diets (e.g. Bowers 1983, Camara 1997a).

Overall, we found that the observed and potential benefits of feeding on plants defended with high levels of iridoid glycosides do not have a direct cost in terms of larval performance. Large size is generally considered to increase insect fitness (Roff 1992), and for *M. cinxia* size may be very important for survival during winter diapause, a period of high potential mortality (Kuussaari 1998, Nieminen *et al*. 2001). *Melitaea cinxia* may benefit from short development time both because the growing season is short (Kuussaari *et al*. 2004) and because it decreases the amount of time larvae are vulnerable to predators and parasitoids (van Nouhuys & Lei 2004).

Our results also illustrate that differences among suitable host plant species can have a greater effect on larval performance than iridoid glycoside concentrations. In experiment 1, *P. lanceolata* on average had higher concentrations of iridoid glycosides than *V. spicata*. Because larvae tend to perform better at higher iridoid glycoside concentrations, one might expect larvae to perform best on *P. lanceolata*. Instead, we found that larvae fed *V. spicata* survive better, grow larger and develop faster than larvae fed *P. lanceolata*. Van Nouhuys *et al*. (2003) also found that larvae reared in the laboratory on *V. spicata* weighed more at diapause than larvae reared on *P. lanceolata*. Although concentrations of both of the primary iridoid glycosides, aucubin and catalpol, are lower in *V. spicata* than in *P. lanceolata, V. spicata* does have other iridoids in lower concentration (Suomi *et al*. 2002), some of which could be converted to catalpol by the larvae (Gardner & Stermitz 1988). *Veronica spicata* also may contain other compounds that work as feeding stimulants for the larvae or increase the nutritional quality of the plant. Alternatively, *P. lanceolata* may contain toxic compounds or have a lower nutrient content, retarding growth or lowering survival of the larvae. The high performance of the larvae on *V. spicata* is unlikely to be caused by them being the progeny of locally adapted parents. Firstly, the larvae used in the experiment were the progeny of many females collected from many habitat patches. In some patches, *V. spicata* was the more common host, and in other patches *P. lanceolata* was predominantly used. Furthermore, previous work on this system has shown that although there is a spatial variation of adult butterfly host plant preference (Kuussaari *et al*. 2000), and variation of larval performance, there is not local adaptation of the larvae to the locally preferred oviposition plant species (van Nouhuys *et al*. 2003).

Interestingly, long term and large scale survey data show no systematic difference in survival among larvae using the two host plants in natural populations (Kuussaari 1998, van Nouhuys *et al*. 2003). The difference in performance under laboratory and natural conditions is important to consider for the interpretation of laboratory based experiments on herbivory, and is perhaps not surprising when one consider the many other factors that contribute to the larval performance in natural populations (Kuussaari *et al*. 2004). In the wild, larval survival and development are influenced by factors other than food quality such as food availability, predation, parasitism and weather conditions (Kuussaari *et al*. 2004, van Nouhuys & Hanski 2004, Harvey *et al*. 2005), which they did not experience in our experiment.

Finally, the performance of larvae differs slightly among egg clusters. Variation in performance at the family (egg cluster) level was found in other studies of *M. cinxia* larvae (Laine 2004, van Nouhuys *et al*. 2003). If the difference in performance among egg clusters is genetic, then some families might be better adapted to feeding on one or the other plant species, or be more or less sensitive to iridoid glycoside concentration. This variation may reflect historical or present heterogeneity in the importance of chemical defence, or the evolutionary history of *M. cinxia* using plants with variable levels of iridoid glycosides. Whatever the reasons, the host plant related variation in performance indicates

both ecological complexity in the relationship between plant secondary chemistry and herbivory, and potential for evolution in response to host plants in this system.

Acknowledgements

The authors would like to thank Arjen Biere from the Netherlands Institute of Ecology for seeds for experiment 2. We also thank Ilkka Hanski and two anonymous referees for comments on an earlier draft of the paper. The assistance of Johanna Ehrnsten, Hanna Koskinen and Veera Kuparinen was extremely valuable. This study was supported by the Academy of Finland (Finnish Centre of Excellence Programme 2000–2005, grant number 44887 to I. Hanski).

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