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Diet affects the immune defence and life-history traits of an Arctiid moth *Parasemia plantaginis*

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ABSTRACT

Questions: Does herbivores' diet *per se* affect their immunocompetence? Do other fitness measures vary accordingly? Can the observed differences be explained by the chemical composition of the diets?

Organisms: Full-sib families of *Parasemia plantaginis* (Lepidoptera: Arctiidae).

Methods: We reared larvae in laboratory on five different diets. We tested the encapsulation ability of the larvae with standard artificial implants. We also analysed the phenols, tannins and antioxidants in the diets. We then compared the encapsulation and chemical analysis results to other fitness measurements (pupal mass, development time, growth rate, survival and egg production).

Results: We found that the ability of *P. plantaginis* to encapsulate a foreign object varies depending on the host plant species and that the ability corresponds with the amount of antioxidants in the diet. However, the other fitness measures did not correlate with encapsulation ability. We also found a significant host plant–genotype interaction in encapsulation ability suggesting heritable variation and a possible trade-off in specialization to different host plant species.

Keywords: antioxidant, encapsulation, genotype–environment interaction, immunocompetence, parasitoid resistance, phenolics.

INTRODUCTION

One of the most important factors affecting the fitness of insect herbivores is their diet – that is, the quality of the plant species they eat. Polyphagous herbivores in particular face a challenge, as eating different host plant species can result in differences in life-history traits, such as growth, development time and fecundity. These differences may be due to them having a limited possibility to co-evolve with all of their potential host plants, which have differing chemical (nutritional value, secondary metabolites) and other traits (e.g. mechanical

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defence) that affect the herbivores' life-history traits (Gordon, 1961; Erickson and Feeny, 1974; Cates, 1980; Price *et al.*, 1980; Berenbaum and Zangerl, 1999). Thus, it is likely that generalist herbivores are adapted to the most common secondary metabolites and are more sensitive to defensive compounds that only occur in some plant genera (e.g. Levins and McArthur, 1966). However, plant secondary metabolites are not always harmful to herbivores; some of them are used as feeding cues, especially by specialist herbivores, and some can be beneficial to the herbivore. Carotenoids, for example, are important antioxidants and reduce the harmful effects of stress caused by, for example, ultraviolet radiation or infection (Demming-Adams and Adams, 1996; Ouchane *et al.*, 1997).

It has often been demonstrated that generalist herbivores perform differently on different host plant species (e.g. Price *et al.*, 1980; Bernays and Chapman, 1994). In spite of the performance differences, genetic interactions in the performance of herbivores feeding on different host plants have generally not been found (see, for example, Jaenike, 1990 and references therein). Genetic interactions in herbivores' growth or other general performance measures on different host plant species would suggest that there is a trade-off in the metabolism of allelochemicals between different host plant species. The absence of these interactions has been interpreted to mean that the 'metabolic load' of detoxifying capacity (which is expected to be energy limited) has by itself a trivial effect on larvae (Scriber and Feeny, 1979; Appel and Martin, 1992). Looking for energy costs is perhaps not the best way to seek to understand the feeding costs of herbivores on many different host plant species.

Diet can also have indirect effects on the survival of herbivorous insects. It has been well established that insects' host plant species affects the probability of their being predated and parasitized (Fox *et al.*, 1990; Farrar and Kennedy, 1993; Lill *et al.*, 2002; Mira and Bernays, 2002). Predation pressures on herbivores vary depending on their host plant species. Herbivores may, for example, be more obvious to predators on one plant than on another (Gross and Price, 1988; Ohsaki and Sato, 1994). Moreover, plant-derived compounds in herbivores can make them inedible and provide protection from predators (Rothschild, 1973; Dobler and Rowell-Rahier, 1994; Camara, 1997; Stamp, 2001) and parasitoids (Nieminen *et al.*, 2003; Singer and Stireman, 2003), especially in species that are specialized to plant species that contain harmful substances. In spite of the numerous studies demonstrating significant differences in parasitoid load among insects feeding on different host plants in nature, the mechanisms behind these differences are unclear. Lill *et al.* (2002) presented several hypotheses to explain the differences in parasitoid load in generalists feeding on different host plants: (1) plant volatile-related differences in parasitoid attraction and/or retention rates; (2) variation in the conspicuousness of caterpillars on different host plants; (3) density-dependent foraging by parasitoids; (4) the presence and abundance of other herbivore species on the host plants; and (5) host-plant effects on caterpillar resistance to parasitism. Another factor affecting parasite load, demonstrated by Benrey and Denno (1997), is that (6) low-quality host plants can slow down the development of the herbivore and thus increase the probability of it being parasitized.

The primary insect defence against hymenopteran and dipteran parasitoids (Nappi, 1975; Godfray, 1994) as well as against nematodes (Stoffolano, 1986) and fungi (Vey and Götz, 1986) is encapsulation. An encapsulation reaction is a general response to foreign intrusions: all small inert particles inside an insect are encapsulated (Nappi, 1975; Lackie, 1988). In encapsulation, reaction cells circulating in the haemocoel recognize an object as foreign and attach to it. A closed, blackened capsule is formed around the object and the intruder dies through lack of oxygen and/or nutrients (Nappi, 1975; Godfray, 1994). There are differences in encapsulation reaction among individuals, since it requires resources and has been found to be more efficient in individuals in good general condition (Kraaijeveld and Godfray, 1997). Thus it is likely

that variation in host plants can result in differences in encapsulation ability in herbivorous insects, either through the direct effect of plant chemical composition providing the necessary substances for the herbivore to mount an encapsulation reaction or indirectly via differences in general vigour on different host plants.

Encapsulation reactions create free radicals that can seriously damage the cells of an insect (Nappi *et al.*, 1995; von Schantz *et al.*, 1999). Antioxidants acquired from the diet can prevent damage to the cells (Johnson and Felton, 2001) and thus reduce the cost of immune defence against parasitoids. Different plant species have different amounts of antioxidants and thus the food plant of an herbivore is important, especially if the likelihood of pathogen attack is high. However, not all plant antioxidants are beneficial to herbivores; some can have adverse effects. For example, a common plant phenol and antioxidant chlorogenic acid has been reported to deter some herbivores (Matsuda and Senbo, 1985). Additionally, the same substances that act as antioxidants in some or most situations can be pro-oxidants in different chemical environments. Even though pro-oxidants are generally harmful to the cells of the herbivore, they can also prevent viruses from entering the larva via the gut wall (Hoover *et al.*, 1998).

In this study, we examined whether an insect's ability to fight parasites and parasitoids is affected by their larval diet. More specifically, we tested if Arctiid moth larvae's encapsulation ability varies among their host plants and among families. Using artificial implants in a laboratory, we were able to test specifically whether the differences in parasitoid loads among herbivores on different host plants that have been observed in nature might be dependent directly on the host plants' effects on resistance to parasitism. We chose host plants that we expected to differ in secondary metabolite content, but to be relatively similar in nitrogen content, which has been shown to have an important effect on herbivore growth (Mattson, 1980). To evaluate the effect of plant chemicals to these herbivores, we analysed the chemical constituents of the diets we used in this study. Larvae were fed on a combination of their natural host plant species and an artificial diet. We can expect that parasitoids, and larvae's ability to fight them by encapsulation, are very important to the survival of the larvae and thus to their fitness. We were particularly interested in whether encapsulation is correlated with general larval performance on different host plant species, or if the host plant independently affects the encapsulation rate of *Parasemia plantaginis*. Moreover, we studied the costs of encapsulation by testing possible trade-offs in the encapsulation and other fitness measures among diets, which could affect the evolution of diet choice of herbivorous species. Also, we tested whether there are genotype–environment interactions in Arctiid moths among host plant species in life-history traits and encapsulation ability, as this is an indicator of different feeding specializations among families

MATERIALS AND METHODS

Study species

The Arctiid moth *Parasemia plantaginis* (Arctiinae) is polyphagous, and feeds on numerous herbaceous and arborescent plant species (Marttila *et al.*, 1996). In Finland, this species usually has only one generation per year. *Parasemia plantaginis* overwinters as larvae, but in laboratory conditions larvae keep growing if kept in warm and light conditions and can produce several generations per year.

Arctiid moths are capital breeders – that is, the adults do not feed. The larvae have 5–7 instars and have to collect all the nutrients and other substances the moths need to survive and reproduce, making the larval diet critical to the fitness of the individual. Although the female's choice of host plant for oviposition undoubtedly affects the larval diet, it is clear that the larvae themselves also perform diet choices. First, the larvae are very mobile and, especially the older larvae, encounter many different plant species during their lives and must decide whether to feed or not. Second, the female lays a large egg clutch on one host plant and the larvae are too numerous to be supported by a single host plant. Third, in laboratory conditions, each larva feeds on several plant species successively if given a choice (K. Ojala, personal observation).

Adult *P. plantaginis* females were caught with a butterfly net in late June and early July 2002 from two populations: five females from Jyväskylä in central Finland (62°N, 26°S) and three females from Jomala in the Åland Islands (60°N, 20°S). All the wild-caught females had already mated, and laid eggs in the laboratory. In addition to using wild females, we also used three laboratory-reared females from Jyväskylä, which were all descendants of one female caught in Jyväskylä in 2001. These females were mated with different wild-caught males from the Jyväskylä area.

Larval diets

We used five diets in this study. The first two diets consisted of *Taraxacum* sp. (Cichoriaceae) and *Rumex longifolius* (Polygonaceae) respectively, two natural host plants of *P. plantaginis* and both of which are common in the Jyväskylä area and the Åland Islands. The third diet consisted of *Lactuca sativa* (Cichoriaceae), a cultivated species which has very low levels of plant secondary metabolites, but which is well-liked by the larvae. Larvae on the fourth plant-based diet were offered a mixture of these three plant species. This condition was included in the study because this is a more natural diet, as the larvae of this species often feed on many plant species sequentially (K. Ojala, personal observation). Moreover, it has been shown that many generalist insect herbivores perform best when presented with a mixture of food plant species rather than a single species (Bernays *et al.*, 1994; Hägele and Rowell-Rahier, 1999; but see Bernays and Minkenberg, 1997). By switching between host plant species, larvae may maximize the benefits from different plants by getting a well-balanced diet of, or minimize the costs by diluting, plant chemical defence compounds in the host plant. Thus, it is possible that a mixed diet gives the moths the best possible growth and also enhances fitness by providing all the necessary chemicals for successfully fighting parasitoids by encapsulation. The fifth diet was an artificial insect diet based on semolina and wheat germ, which contains the nutrients needed by the larvae but only traces of plant secondary metabolites [modified from Poitout and Bues (1974) by leaving out ascorbic acid and formaldehyde and adding 0.5% Vanderzant vitamin mixture for insects].

Chemical analyses of the diets

We analysed the artificial diet and dried mature leaves ($n = 5$) of the host plant species for phenolic compounds (flavonoids, phenolic acids). Vacuum-dried material was ground into a fine powder and stored in Eppendorf tubes at room temperature. Eight to ten milligrams of the diets were suspended in 600 μl of methanol (HPLC grade) and homogenized for 1 min with Ultra-Turrax homogenizer (Janke and Kunkel, Ika-Labortechnik, Germany). The

samples were left to stand for 15 min at 4°C and then centrifuged (13,000 rev·min^{-1} for 3 min; Biofuge pico, Heraeus Instruments, Germany). The supernatant was removed, and the residue was further extracted for 1 min three more times. The methanol was reduced from combined extracts under nitrogen flow. The samples were redissolved into methanol and an aliquot of 1 ml was used for high-performance liquid chromatography (HPLC), as published previously (Julkunen-Tiitto and Sorsa, 2001). Condensed tannins were determined by an acid-butanol assay from methanol-extracts according to Julkunen-Tiitto and Sorsa (2001). The total nitrogen content of the plants and the artificial diet were analysed from the same dried samples (10–20 mg) with a LECO nitrogen analyser (FP-528).

We also analysed the antioxidant activity of the diets ($n = 10$ individual plants per species). Powdered material (5–10 mg dry weight) was extracted in 600 μl of methanol with a coarse glass rod for 15 s and kept at 4°C for 15 min. After centrifugation (16,000 *g* for 3 min; Biofuge pico, Heraeus Instruments, Germany), the residue was extracted three more times in 600 μl of methanol. The combined extracts were dried with nitrogen. The methanol extract measures the composite antioxidative activity covered mostly by flavonoids and condensed tannins. Another set of samples of the powdered material (2 mg dry weight) was extracted in 600 μl of acetone for 15 s. After standing for 10 min at 4°C, the sample was centrifuged and the residue was extracted three more times in 600 μl of acetone. Again, the combined extracts were dried under nitrogen. The acetone extract measures the antioxidative activity covered mostly by different carotenoids. The effects of the extracts on DPPH (1,1-diphenyl-2-picrylhydrazyl; Sigma Chemical Co., St. Louis, MO, USA) radicals were determined as in Flores and Galston (1982), with some modifications. Changes in absorbency of the methanolic solution of DPPH (6×10^{-5} M) at 517 nm were measured (Specord 200, Analytik Jena AG, Jena, Germany) before analysis. The dried methanol and acetone extracts were dissolved in methanol, aiming at a concentration of 0.3 mg of material in 50 μl of methanol, which was added to 2 ml of the DPPH solution. After standing in darkness for 20 min, the absorbencies of the resulting solutions were measured at 517 nm. The changes in DPPH absorbance without sample addition were also measured after incubation. The results are expressed as inhibition (%) of DPPH – that is, the loss of radicals in the solution due to the scavenging activity of the extracted compounds.

Rearing

The study was done as a full-sib common garden design. The neonate larvae (total $n = 555$) of *P. plantaginis* were equally divided into diet treatments on the day of hatching, 4–20 larvae per diet per female (mean 10 larvae per family). Larvae were reared in individual containers in a greenhouse, where the temperature and lighting followed outside conditions. The daytime temperature varied between 20 and 30°C and night-time temperature was 15–25°C. Since the nights are very short in July in Jyväskylä, the young larvae were never in total darkness, but as the summer advanced, the daylight hours gradually decreased. Larvae were checked every day, and fresh food was added *ad libitum* if necessary, at least every second day. The day after pupation, individuals were weighed and kept in warm and light conditions, and thus adults normally hatched within 4–18 days. If a moth had not hatched a month after pupation, the pupa was opened and the individual was sexed if possible. Seventy-one of the females were mated immediately after hatching with a non-sibling male and the total number of eggs produced was counted.

Encapsulation assessment

An encapsulation reaction can be quantitatively measured by using a novel and standardized 'parasite' such as nylon monofilament implants (König and Schmid-Hempel, 1995; Ryder and Siva-Jothy, 2000) that mimic the parasitoid inside the insect. The advantage of the implant method is that the 'parasite' is neutral and does not have means to overcome the host's defences. Thus, the outcome of encapsulation is solely dependent on the host, giving a relevant quantitative measure of the insect's ability to fight parasitoids. Moreover, the insect larvae survive the implantation and develop into adults, thus enabling us to measure the life-history parameters of these individuals.

Altogether, 198 larvae were implanted, 26 larvae from the artificial diet, 32 from the *Lactuca* diet, 44 from the *Taraxacum* diet, 53 from the *Rumex* diet and 43 from the mixed diet. We implanted larvae from every family, and because we implanted them when they reached a certain size, they represent mostly the faster-growing larvae. Their minimum weight at implantation was 95 mg (mean 173.6 mg) and the larvae were in the 5th–7th instar. We used nylon implants that were 0.11 mm in diameter and 4 mm long. The larvae were anaesthetized with CO_2 and a small cut was made in the dorsal side of the larva, in the skin immediately behind the first segment behind the head, with a sterilized needle. Two-thirds of the implant was inserted inside the larva. The immune system of the larva was allowed to react to the implant for 5 h. After implantation, we recorded the skin moulting of the *P. plantaginis* larvae, which indicated how many instars the larva has left until pupation.

The implant was then removed, dried and photographed under a microscope with 57× magnification with a Panasonic wv-CL702 video recorder. Three black and white pictures from different angles were taken from each implant to ensure they were seen from all sides (Rantala *et al.*, 2000). The mean grey value of the implant was measured with ImagePro Plus 4.0 (Media Cybernetics) from the 1 mm of the end of the implant that had been inside the larva. We subtracted the grey value of the background from the grey value of the implant to correct for any variation in lighting during photographing. The absolute value of the mean of the three grey values was used in all analyses. Twenty randomly chosen implants were photographed and measured three times to assess the repeatability of this method. The repeatability of this method was very high ($r = 0.941$, $F_{2,18} = 61.602$, $P < 0.001$).

Statistical analysis

We used analysis of variance (ANOVA) in which pupal mass, development time, growth rate, number of eggs produced and encapsulation score were dependent variables, and sex, diet and family were used as factors. We used all the individuals for analysing the life-history traits, thus combining the data from both implanted and non-implanted individuals. We tested the population effect but, since it was not significant for any of the dependent variables, it was removed from the final model. Family was treated as a random factor in all analyses. Other factors (sex and diet) were fixed. All data were checked for normality and homogeneity of variances. Data for pupal mass, development time and number of eggs produced was heteroscedastic and thus we used ranked data for ANOVA. To meet the assumptions of ANOVA, the data for encapsulation ability were log-transformed. Growth rate was calculated using the formula ln pupal mass/larval development time in days. Since survival rates differed on different diets, we calculated Spearman correlations between larval

development time and pupal mass and also between encapsulation score and mass at implantation, pupal mass, development time and growth rate separately for each diet for both females and males. We then used meta-analytical tools to combine these correlations to test the overall effect (Rosenthal, 1991). However, some of the correlations were heterogenic (development time and pupal mass: females, $\chi^2 = 9.537$, d.f. = 4, $P = 0.049$; males, $\chi^2 = 15.05$, d.f. = 4, $P = 0.005$; encapsulation score and mass at implantation: females, $\chi^2 = 10.05$, d.f. = 4, $P = 0.040$; encapsulation score and pupal mass: females, $\chi^2 = 12.08$, d.f. = 4, $P = 0.017$; encapsulation score and development time: females, $\chi^2 = 11.36$, d.f. = 4, $P = 0.023$) and therefore these results should be interpreted with caution. All statistical analyses were performed with SPSS 11.5 (SPSS Inc., 2002).

RESULTS

Chemical constituents of the diets

Nitrogen content was the highest in *Lactuca* leaves, while it did not vary much between *Taraxacum, Rumex* and the artificial diet (Table 1). HPLC analyses revealed 18 different flavonoids for the plant species (see Appendix for a list of all flavonoids). The total flavonoid content was very high in *Taraxacum* and in *Rumex*, while *Lactuca* had less than 20% of the flavonoids in *Taraxacum* (Table 1). The artificial diet had only traces of flavonoids. The level of condensed tannins varied between species, and was markedly higher in *Rumex* leaves than in other diets, which had relatively similar values among them. The amount of carotenoids, measured as their antioxidation activity, was also highest in *Rumex* and very low in the artificial diet and in *Lactuca*. Total antioxidant activity measured from methanolic extracts followed a similar variation, though values were moderately higher than that of carotenoids. Total antioxidant activity was lowest in the artificial diet, relatively low in *Lactuca* and high in *Taraxacum* and *Rumex*.

Life-history traits

Diet, family and sex all had a statistically significant effect on the pupal mass, development time and growth rate of the larvae (Table 2). On average, females were larger than males and had longer development time than males, but their development rates were similar (Table 3). There was a significant positive correlation between development time and pupal mass (meta-analysis containing all diets: females, $r_s = 0.567$, $Z = 8.01$, $P < 0.001$; males, $r_s = 0.650$, $Z = 8.40$, $P < 0.001$; Fig. 1). The total number of eggs produced during the female's lifetime

Table 1. The chemical constituents and antioxidation activity of the diets

Diet	Nitrogen ($mg \cdot g^{-1}$ d.w.)	Flavonoids ($mg \cdot g^{-1}$ d.w.)	Condensed tannins ($mg \cdot g^{-1}$ d.w.)	Carotenoids antioxidant activity (%)	Total phenolics antioxidant activity (%)
Artificial diet	3.40	trace	0.5	4.36	4.21
Lactuca	5.67	10.4	1.4	6.21	20.24
Taraxacum	3.08	58.6	3.1	15.74	55.77
Rumex	3.93	45.0	19.0	46.77	75.19

Table 2. Results of analyses of variance testing for the effects of sex (fixed), diet (fixed) and family (random) on pupal mass (ranked), larval development time (ranked) and encapsulation ability (log-corrected)

Source of variation	d.f	MS	*F*	*P*
Pupal mass				
Sex	1	334 224.700	228.297	0.000
Diet	4	6 941.894	4.742	0.001
Family	10	9 363.967	6.396	0.000
Diet × Family	34	1 387.759	0.948	0.555
Error	256	1 463.993		
Larval growth rate				
Sex	1	0.00008	0.293	0.595
Diet	4	0.004	8.994	0.000
Family	10	0.002	4.019	0.001
Diet × Family	34	0.000	1.649	0.017
Error	252	0.000		
Larval development time				
Sex	1	81.040	151.558	0.000
Diet	4	7.449	10.980	0.000
Family	10	1.549	2.296	0.027
Diet × Family	34	0.709	1.326	0.116
Error	252	0.535		
Encapsulation				
Sex	1	1.057	1.141	0.287
Diet	4	4.813	5.197	0.001
Family	10	1.403	1.515	0.140
Diet × Family	34	1.711	1.848	0.007
Error	145	0.926		

correlated significantly with pupal mass ($r_s = 0.599$, $n = 58$, $P < 0.01$), indicating that bigger females laid more eggs. The number of eggs produced was also affected by the diet (ANCOVA with pupal mass as covariate: $F_{4,54} = 3.485$, $P = 0.013$; Fig. 2).

Pupae were largest on *Rumex* and smallest on *Lactuca*, on which survival was lowest as well. Development time was shortest on the mixed diet, although the development time on *Lactuca* was nearly as short. Although we did not record on which plant species the larvae on the mixed diet fed, based on casual observations they consumed all three species, somewhat preferring *Lactuca*. Development time was longest on the artificial diet (Table 3). Individuals that fed on *Rumex* or the mixed diet produced the highest number of eggs and individuals fed on *Lactuca* the lowest. Survival from newly hatched larvae to adulthood was lowest on *Lactuca* and highest on *Rumex* (38.4% on *Lactuca*, 69.7% on artificial diet, 74.6% on *Taraxacum*, 78.3% on mixed diet, 82.7% on *Rumex*; $\chi^2 = 73.947$, $P < 0.001$).

Table 3. The mean and standard error of pupal mass, larval development time from hatching to pupation, larval growth rate and encapsulation scores for males and females and diet treatments of *Parasemia plantaginis*

Diet	Pupal mass (mg)		Development time (days)		Larval growth rate ($\ln mg \cdot day^{-1}$)	Encapsulation score
	Female	Male	Female	Male		
Lactuca	248.29 ± 7.84^{a}	176.01 ± 7.57^{a}	53.17 ± 1.61^{abd}	45.06 ± 1.67^{a}	0.106 ± 0.0027^{ab}	29.90 ± 3.62^{abc}
Artificial diet	277.47 ± 8.20^{bc}	213.93 ± 5.41^{b}	71.65 ± 2.59^{c}	65.68 ± 3.36^{bd}	0.078 ± 0.0021^{c}	25.10 ± 3.09^{ab}
Taraxacum	275.24 ± 8.09^{b}	212.78 ± 8.03^{b}	59.18 ± 1.99^{ab}	59.89 ± 2.49^{bc}	0.095 ± 0.0018^{a}	41.28 ± 4.01^{ac}
Rumex	288.35 ± 5.96^{c}	215.03 ± 7.06^{b}	60.12 ± 1.74^{ab}	54.74 ± 2.31^{cde}	0.010 ± 0.0019^{b}	45.43 ± 4.04^{c}
Mixed diet	276.37 ± 9.18^{bc}	208.84 ± 6.48^{b}	50.10 ± 2.19^{ad}	51.90 ± 1.83^{ae}	0.110 ± 0.0025^{b}	32.05 ± 3.85^{abc}

Note: Within each column, values with different superscript letters are significantly different (LSD, $P < 0.05$).

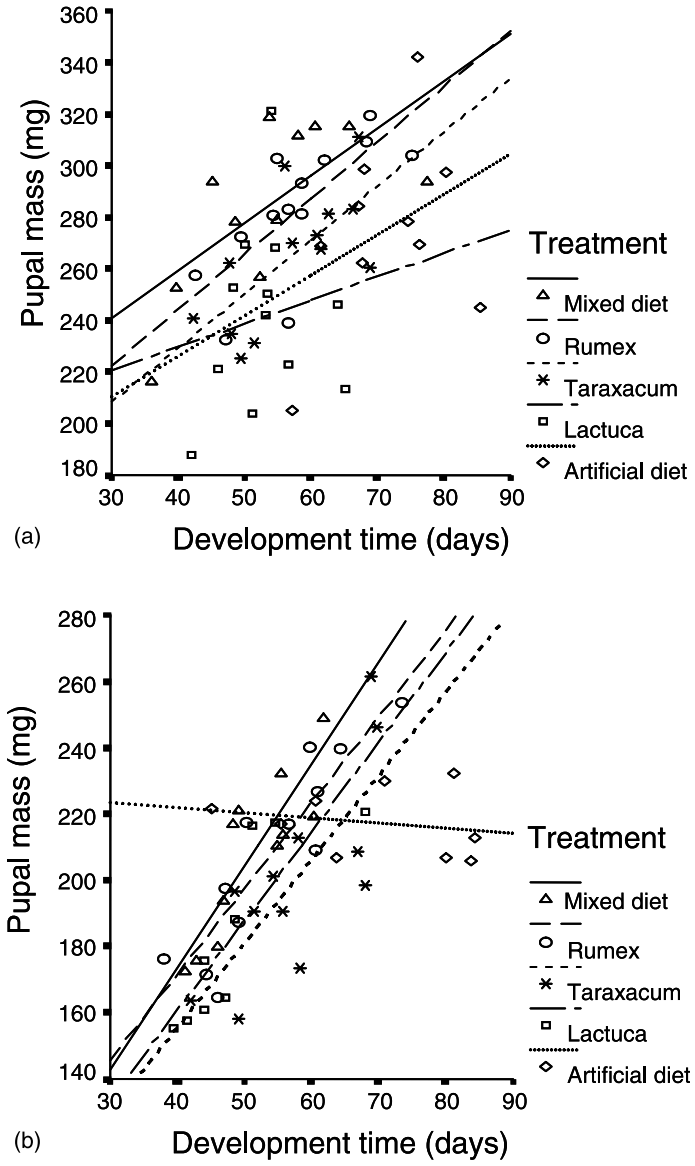

Fig. 1. Scatterplot of development time and pupal mass. The dots represent family means on different diets. (a) females, (b) males.

Encapsulation

Diet significantly affected the encapsulation rate of larvae. A diet of *Rumex* resulted in the strongest encapsulation reactions and an artificial diet in the weakest (Tables 2 and 3, Fig. 3). There was a significant diet × family interaction, indicating that different diets were not equally beneficial for the encapsulation ability of individuals from different families. Encapsulation ability did not correlate with pupal mass (Fig. 4) or mass at implantation,

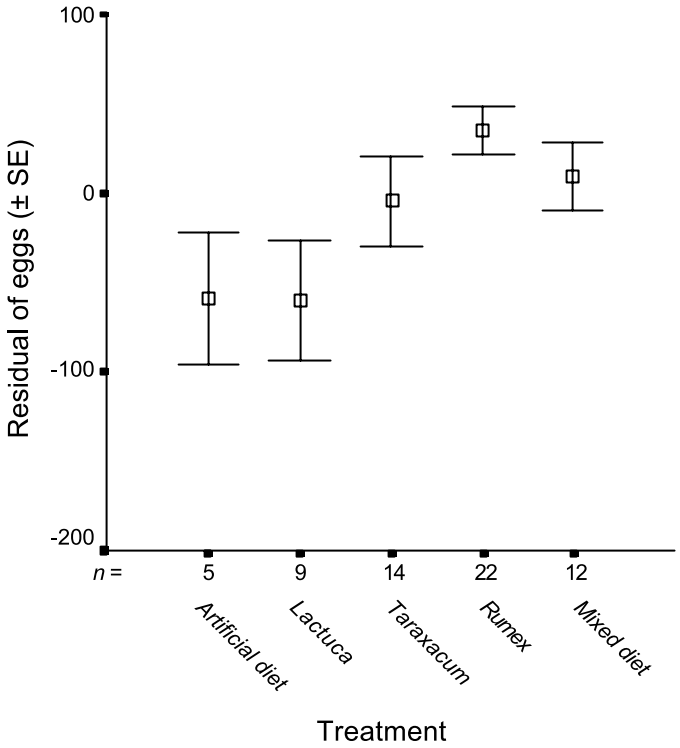

Fig. 2. Mean (± standard error) standardized residuals for number of eggs and pupal mass for females on different diets.

development time or the number of larval instars left before pupation (meta-analyses containing all diets, Fisher's Z, all P-values > 0.07).

DISCUSSION

Diet and encapsulation ability

Encapsulation ability was found to depend strongly on the diet of the larvae but varied somewhat independently of the studied life-history parameters. The size of the individual and the development time did not correlate with encapsulation ability (Figs. 3 and 4). This suggests that diet *per se* affects encapsulation and the high scores for encapsulation reactions are not attributable to the better condition of the larvae. Furthermore, the lack of correlation between developmental rate and encapsulation ability suggests that *Parasemia plantaginis* larvae do not directly trade growth rate for encapsulation ability. Unfortunately, there were not enough families in this study to analyse the genetic interactions among the diets.

Surprisingly, the mixed diet, which was the best diet when considering life-history parameters (fast development and large pupae), resulted in relatively low encapsulation ability. A possible explanation for the poor encapsulation ability is that the larvae on the mixed diet appeared to eat relatively more *Lactuca* than other plant species, and *Lactuca* is a

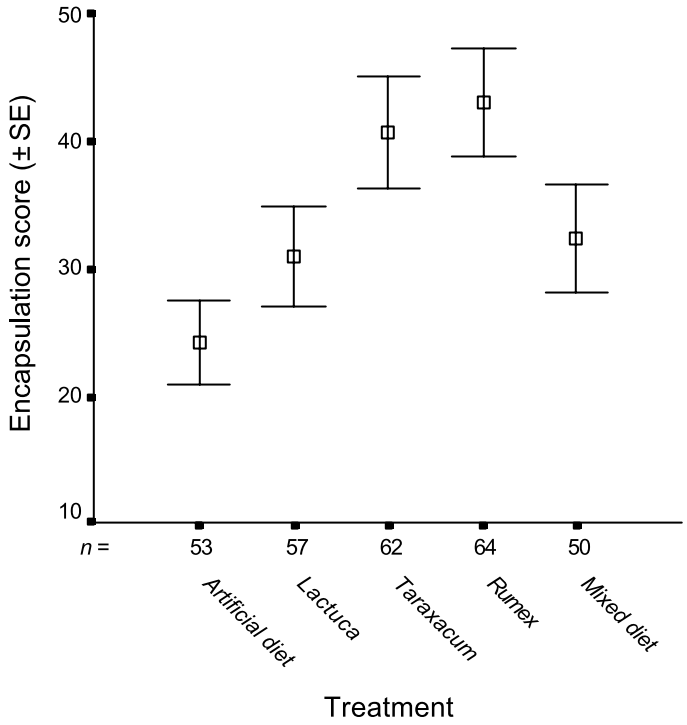

Fig. 3. Mean (± standard error) encapsulation scores on different diets.

poor diet for encapsulation ability. This lack of correlation between life-history parameters and immunocompetence is at odds with the results of previous studies. Herbivorous insect larvae have, in general, been found to be better able to resist parasitoid infestation when they are in overall good health. For example, Salt (1964) found that wax moth larvae in good dietary condition were able to encapsulate an ichneumonid parasite, whereas starved larvae could not do so. Also, Benrey and Denno (1997) reported that encapsulation rates in *Pieris rapae* were higher for rapidly developing larvae than for slow developing larvae on two host plant species.

We suggest that, in *P. plantaginis*, the host plant species affects the encapsulation ability via plant secondary metabolites that affect herbivores' growth but also enable the larvae to produce successful encapsulation. These substances might also be important in herbivore resistance to pathogens such as bacteria and fungi, since many plant secondary chemicals have been found to inhibit bacterial and fungal growth (Seigler, 1998; Talley *et al.*, 2002). This effect is likely, since larvae feeding on *Lactuca*, which has very low levels of secondary metabolites but relatively high levels of nitrogen, had a very high mortality presumably due to disease.

The effect of diet on life-history parameters

This study has demonstrated that diet affects the fitness of *P. plantaginis* by altering the speed of growth, body mass accumulation and egg production. Pupal mass is a good fitness measure in *P. plantaginis*, especially in females in whom egg production and mass at

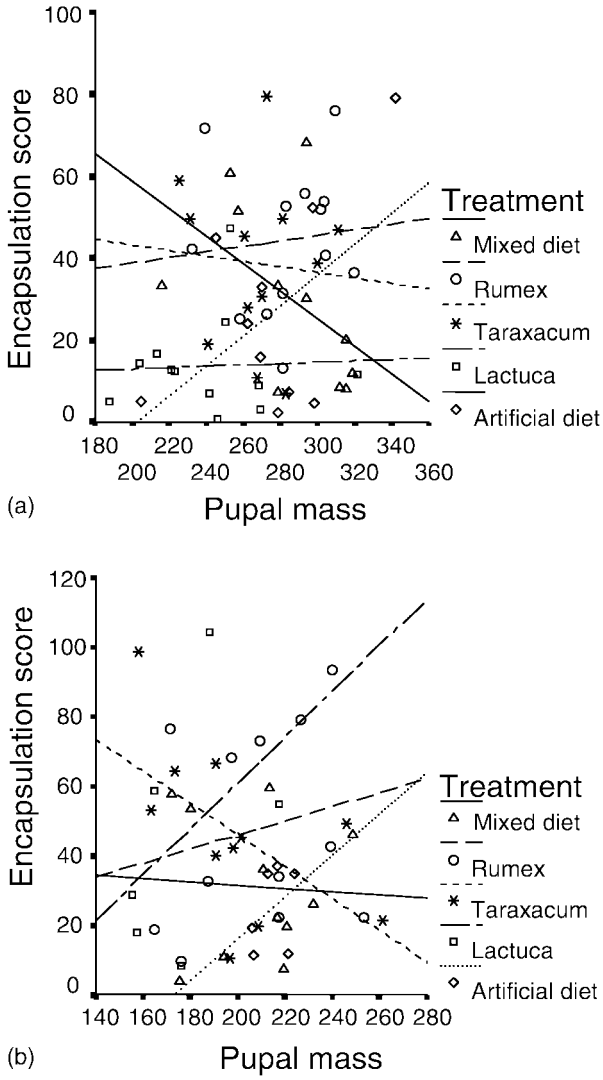

Fig. 4. Pupal mass and encapsulation score. Each dot is a family mean on different diets. (a) females, (b) males.

pupation are tightly linked. For males, the relationship between size and fitness is not as clear but it is likely that, in nature, larger males have better survival and larger flight muscles and are therefore better able to find the more stationary females. However, it is less clear what fitness consequences the developmental time has for *P. plantaginis* populations that overwinter as larvae, and have only one generation per year. Our observations suggest that relatively large individuals have a better winter survival than small ones (K. Ojala, personal observation), and thus relatively fast development would be beneficial for at least pre-diapause larvae. It is also important for *P. plantaginis* individuals in temperate regions to be able to complete development within the time constraints seasonality imposes.

The diets used in this study varied in nutritional value and in the amount of secondary metabolites (Table 1). The amount of nitrogen between diets varied relatively little and nitrogen did not seem to be a limiting factor for larval growth. Many flavonoids have traditionally been considered to be feeding deterrents for herbivorous insects (Seigler, 1998) and to have evolved as a defence against herbivory (Fraenkel, 1959). In contrast, many flavonoids and, in particular, carotenoids function as antioxidants and thus are potentially beneficial to insects needing to reduce oxidative damage, especially during periods of infection and stress (Johnson and Felton, 2001). Tannins, on the other hand, are considered digestibility-reducing substances, as they bind to proteins and hinder their ingestion and thus reduce growth (Swain, 1979; Seigler, 1998). In this study, the amounts of digestibility-reducing tannins and potentially beneficial flavonoids and carotenoids of the diets co-varied positively. Thus, eating the non-tannin diet meant that the moth larvae received much fewer antioxidants and possibly also much less other substances beneficial to successful immunodefence. The larvae reared on *Lactuca* showed very high mortality rates, which could be partly because of low resistance to pathogens. Although the other measures of performance were relatively low on *Lactuca*, development time was the shortest, and the growth rate on *Lactuca* was nearly as high as on the mixed diet, which produced the highest growth rate. *Lactuca* had the highest amount of nitrogen in this experiment, and this could offer an explanation for the larvae's short development time on this diet. *Lactuca* also had a very low level of (possibly growth-retarding) secondary metabolites.

When larvae were offered a choice of the three host plant species (mixed diet treatment), they had the shortest development time, and their egg production was the best. This is in accordance with the results of previous studies (Bernays *et al.*, 1994; Hägele and Rowell-Rahier, 1999). There are several possible explanations why host plant mixing can be beneficial: (1) the larvae obtained all the necessary nutrients, vitamins, and so on from the mixed diet but did not when there was only one plant species; (2) the plant species had different secondary metabolites and thus there were not enough of any of them to inhibit the development of the larvae; (3) the physical constitution of the food was better since *Lactuca* has a high water content and can also keep the other plant species moist and more edible.

Both *Rumex* and *Taraxacum* were beneficial for larval growth. Both of these host plant species can result in intermediate to high growth of the larvae consuming them. Interestingly, eating an artificial diet produced large females at the cost of a very long development time. Long development time might in part be explained by the larvae eating less on the artificial diet than they would on their natural food plants, possibly because it lacks the plant substances used as feeding cues.

Cost of parasitoid resistance

In insects, trade-offs have been found between, for example, foraging activity and immunodefence in bumblebees (Doums and Schmid-Hempel, 2000). We did not find any indication of direct trade-offs between *P. plantaginis* encapsulation ability and other fitness traits within treatments – that is, encapsulation ability was not correlated to pupal mass or development time either at an individual or a family level (Fig. 4). However, since in this study the larvae always had food available, the larvae that invested more in encapsulation ability were probably able to compensate the possible costs by eating more. Kraaijeveld and Godfray (1997) and Kraaijeveld *et al.* (2001) found that a line of *Drosophila melanogaster* that was artificially selected for high encapsulation ability consisted of poorer larval competitors

under conditions of resource scarcity. The basis of this ability was the greater density of haemocytes in the high resistance line. When competition for food was not as fierce, trade-offs were not detected.

Thus, when larvae make a decision what to eat, if they make that decision solely based on optimal egg production ability, they might have to compromise their immunodefence. Also, the effects of certain chemicals can vary depending on, for example, other chemical concentrations in the larvae's environment. Research by Hoover *et al.* (1998) has demonstrated that antioxidants actually hinder moths' resistance to viruses while pro-oxidants aid resistance. A possible explanation for this is that viruses enter moth larvae via the gut wall and free oxygen radicals destroy gut cells that have been infected and thus prohibit further infection. If this is a general phenomenon, insect herbivores would have a trade-off to consider when choosing their diet, since the best possible feeding strategy might depend on the specific enemy community in their environment.

In our study, the optimal feeding strategy for *P. plantaginis* seems to be eating a mixture of plant species if fitness is calculated by pupal mass, development time, egg production and survival in the laboratory. But when resistance to parasitoids, which is likely to be a very important fitness trait in nature, is taken into account, the picture is somewhat different; the larvae eating a mixture of plant species have a lowered encapsulation ability compared with those eating *Rumex* or *Taraxacum* only (Fig. 3). Thus, it is likely that the optimal diet choice for a grazing insect herbivore varies in time and place depending on, for example, the enemy community and time available for development. When parasitoids are common, *P. plantaginis* should eat relatively more plants that have a high concentration of plant secondary metabolites and might slow their development, but give them greater potential to defend themselves against parasites.

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APPENDIX

A list of individual phenolics in the diets detected by HPLC-DAD

Compound	Retention time (min)	Content ($mg \cdot g^{-1}$ d.w.)
Lactuca		
chlorogenic acid der.1	7.84	0.788
chlorogenic acid	8.06	0.490
chlorogenic acid der.2	12.98	0.354
chlorogenic acid der.3	18.79	6.905
chlorogenic acid der.4	19.10	0.612
chlorogenic acid der.5	19.80	0.979
quercetin-glycoside	20.24	0.256
Taraxacum		
chlorogenic acid der.1	7.78	6.53
chlorogenic acid	8.04	1.07
rhododendrin-der.	12.369	0.271
chlorogenic acid der.2	12.96	0.469
luteolin-7-glucoside	17.97	0.172
luteolin-glycoside 1	18.23	0.133
chlorogenic acid der.3	18.74	46.9
chlorogenic acid der.4	19.06	2.79
rhododendrin-der	19.45	0.317
Rumex		
neochlorogenic acid	4.09	2.98
chlorogenic acid der.1	7.92	2.02
hyperin	18.09	7.62
quercetin-3-glucoside + glucuronide	18.62	3.17
quercetin-3-arabinoside	20.21	1.86
quercetin-3-rhamnoside	21.36	25.8
kaempferol-3-glucoside	23.38	0.259
monocoumaroyl-astragalin der	32.99	1.65

Abbreviation: der = derivative.