

The roles of foraging environment, host species, and host diet for a generalist pupal parasitoid

J.H. Reudler^{1,2*}  & S. van Nouhuys^{1,3}

¹Department of Biosciences, University of Helsinki, FIN-00014 Helsinki Finland, ²Department of Biological and Environmental Sciences, University of Jyväskylä, FIN-40014 Jyväskylä Finland, and ³Department of Entomology, Cornell University, Comstock Hall, Ithaca, NY 14853, USA

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Abstract

Even for parasitoids with a wide host range, not all host species are equally suitable, and host quality often depends on the plant the host feeds on. We compared oviposition choice and offspring performance of a generalist pupal parasitoid, *Pteromalus apum* (Retzius) (Hymenoptera: Pteromalidae), on two congeneric hosts reared on two plant species under field and laboratory conditions. The plants contain defensive iridoid glycosides that are sequestered by the hosts. Sequestration at the pupal stage differed little between host species and, although the concentrations of iridoid glycosides in the two plant species differ, there was no effect of diet on the sequestration by host pupae. The rate of successful parasitism differed between host species, depending on the conditions they were presented in. In the field, where plant-associated cues are present, the parasitoid used *Melitaea cinxia* (L.) over *Melitaea athalia* (Rottemburg) (Lepidoptera: Nymphalidae), whereas more *M. athalia* were parasitised in simplified laboratory conditions. In the field, brood size, which is partially determined by rate of superparasitism, depended on both host and plant species. There was little variation in other aspects of offspring performance related to host or plant species, indicating that the two host plants are of equal quality for the hosts, and the hosts are of equal quality for the parasitoids. Corresponding to this, we found no evidence for associative learning by the parasitoid based on their natal host, so with respect to these host species they are truly generalist in their foraging behaviour.

Introduction

Parasitoids must find a host in a habitat, choose to parasitise it, and successfully evade host defence. Those with narrow host ranges are known to use specific plant and host cues to identify host species (Quicke, 2015), and parasitising a non-host species is very costly (Condon et al., 2014). Parasitoids with a wide host range do not need to be so specific, yet still must find hosts, assess their suitability, and successfully parasitise them. The cues used and their relative importance determine the position of a generalist parasitoid in an insect community. The host provides the sole nutritional and physiological environment during development of a parasitoid. So host quality is a

major component in host selection, even for parasitoids with a wide host range (Vinson, 1975; Roitberg et al., 2001; Harvey et al., 2013). One important factor responsible for variation in host quality is the plant it consumes (Barbosa et al., 1982; Hopkins et al., 2009), both the nutritional quality of the plant and whether the plant contains toxins that are either detrimental to or sequestered by the host (Harvey et al., 2005; Smilanich et al., 2009; Lampert, 2012; Erb & Robert, 2016). In addition, a herbivore may be more attractive, suitable, or accessible to a parasitoid on one host plant species over another (Poelman et al., 2014; Feng et al., 2015).

Host size, which may differ between species (Wang & Messing, 2004) as well as within a species (Barbosa et al., 1982), is also an important measurement of quality, as it determines the amount of resources available for the developing parasitoid. It is especially critical for gregarious species, as host size can determine the weights of parasitoids developing in it as well as the size of the brood

*Correspondence: J.H. Reudler, Department of Biosciences, University of Helsinki, FIN-00014 Helsinki, Finland.
E-mail: jhtalsma@hotmail.com

(Waage & Godfray, 1985; Harvey et al., 2013; Kraft & van Nouhuys, 2013; Rosa et al., 2017).

Allelochemicals in the diet of hosts can either have positive or negative effects on their natural enemies. Hosts in poor condition due to ingested toxins may contain limited nutritional resources for the developing parasitoid, but may also have a weakened cellular defence system which would be advantageous to the parasitoid (Vinson, 1990; Ode, 2006; Smilanich et al., 2009). Further positive effects occur when allelochemicals slow the development rate of the host, extending the interval during which the herbivore is vulnerable to parasitism (Clancy & Price, 1987). A host that is strongly adapted to a chemically defended food plant may perform well, making it a high-quality recourse for a well-adapted parasitoid (Harvey et al., 2005). On the other hand, adapted hosts can actively sequester plant chemicals in their haemolymph as a defence that is detrimental to some parasitoid species (Bowers, 1981; Barbosa et al., 1986; Harvey, 2005; Reudler et al., 2011; Poyet et al., 2017).

Independent of the innate quality of a host, its apparency and accessibility will depend on the plant species that it is on, due to factors such as plant structural complexity (Andow & Prokrym, 1990) and herbivore-induced attractive volatiles (Kessler & Baldwin, 2001). Finally, a parasitoid may learn cues associated with a given host or habitat, making it likely to prefer its natal host species or habitat (Vet & Groenewoud, 1990; Hastings & Godfray, 1999; Morris & Fellowes, 2002). These cues are learnt during emergence, through antennation of the surrounding, and in association with parasitism (Vet & Groenewoud, 1990; van Emden et al., 1996).

In this study, we compared oviposition choice and offspring performance of the generalist pupal parasitoid *Pteromalus apum* (Retzius) (Hymenoptera: Pteromalidae), on two butterfly species, *Melitaea cinxia* (L.) and *Melitaea athalia* (Rottemburg) (Lepidoptera: Nymphalidae), reared on two plant species, *Plantago lanceolata* L. and *Veronica spicata* L. (Plantaginaceae). These two hosts are in the normal host range of the parasitoid (Shaw et al., 2009) and co-occur in the study area (Reudler Talsma et al., 2008b) where they are parasitised by *P. apum* at a high rate (van Nouhuys & Kraft, 2012). Both butterfly species sequester iridoid glycosides (IGs) from their chemically defended host plants (Suomi et al., 2003; JH Reudler, pers. obs.). The IGs aucubin and – to a greater extent – catalpol are toxic or deterrent to generalist herbivores (Puttick & Bowers, 1988; Bowers, 1991). At the same time, these compounds serve as feeding and oviposition stimulants for some specialist herbivores, including *M. cinxia* (Bowers, 1984; Nieminen et al., 2003; Reudler Talsma et al., 2008a).

The specific questions we address are (1) what is the effect of host plant species and host herbivore species on parasitism, and (2) will plant diet and host species affect life-history traits of parasitoid offspring (e.g., brood size, sex ratio, and weight). To examine these questions, we first conducted an experiment in which naturally occurring parasitoids must find and parasitise hosts placed in association with their food plants in the field. Second, we performed a laboratory choice test, in which host species and diet were manipulated, and the rearing history of each wasp was known (host species and host diet), but the direct effects of host plant, such as odour, structure, and size, were absent. We predicted that (1) more IGs would be sequestered from *P. lanceolata* than from *V. spicata*, which would lead to decreased parasitism and offspring performance in hosts fed *P. lanceolata*; (2) sequestration by *M. cinxia* and *M. athalia* would differ because they are different species, and that would influence parasitism and offspring performance; (3) under field conditions rate of parasitism would be related to host and plant species; and (4) under laboratory conditions, wasps would prefer to parasitise hosts under their natal conditions based on associative learning. Such variation in host use by a generalist parasitoid would affect the community composition in Åland where all five species commonly live sympatrically, and parasitism by *P. apum* is high. More broadly, this study focuses on the behaviour of a generalist parasitoid presented with the normal array of variation in a natural population. Generalist parasitoids, in contrast to specialists, increase food web complexity by indirectly linking prey species together. There are not many studies conducted about generalist parasitoids in the context of multiple plant-host interactions, and their sensitivity to variation dictates the extent to which they fill this role.

Material and methods

Study organisms

Parasitoids. The parasitoid *P. apum* is a gregarious idiobiont ectoparasitoid of Lepidoptera pupae. It has primarily been reared from Nymphalidae, but is known from other hosts, even as diverse as the pupae of megachiline bees (Askew & Shaw, 1997; O'Connor & Ronayne, 2003; Shaw et al., 2009). In the Åland islands, southwest Finland, *P. apum* is a frequent parasitoid of pupae of *M. cinxia* and *M. athalia* (Lei et al., 1997; van Nouhuys & Kraft, 2012). The number of individuals developing on a single host (brood size) ranges from just a few up to 100. Such high brood sizes are the result of superparasitism (Kraft & van Nouhuys, 2013). The parasitoids local use of other hosts is unknown. However, preliminary data indicate that they are somewhat

restricted because locally abundant related Nymphalidae *Aglais urticae* (L.), *Inachis io* (L.), *Polygonum c-album* (L.), *Vanessa atalanta* (L.), and *Vanessa cardui* (L.) are found to be parasitised by a congener, *Pteromalus puparum* (L.), instead of *P. apum*, under natural conditions (S van Nouhuys, pers. obs.).

Butterflies. The butterfly hosts *M. cinxia* and *M. athalia* co-occur in the Åland islands. Both have a Eurasian distribution and are restricted in their diet to feed on plants that contain IGs (Wahlberg, 2001; Reudler Talsma et al., 2008b). *Melitaea cinxia* lives in open meadows, feeding as caterpillars on *V. spicata* and *P. lanceolata* (Kuussaari et al., 2004). Its congener, *M. athalia* inhabits meadows, clearings, and sparse forest, and has a broader host and habitat range (Warren, 1987; Wahlberg, 1997; Reudler Talsma et al., 2008b). In Åland both species commonly occur together in open meadows, where they feed on *P. lanceolata* and *V. spicata* (Reudler Talsma et al., 2008b). Both herbivore species sequester IGs as caterpillars from both host plants (Suomi et al., 2001, 2003; JH Reudler, A Biere, JA Harvey & S van Nouhuys, unpubl.). In the study area, both butterfly species have one generation per year. They lay eggs in clusters on host plants in June and the caterpillars feed until September, after which they diapause for the winter. In April, they resume feeding and pupate inconspicuously in the litter next to host plants or hanging under host plant leaves in May (Wahlberg, 1997; Kuussaari et al., 2004; van Nouhuys & Kraft, 2012).

Plants. *Plantago lanceolata* and *V. spicata* are common meadow species in the study area. *Plantago lanceolata* occurs in practically all meadows suitable for the butterfly species, whereas *V. spicata* is abundant only in the northwestern part of the study area (Kuussaari et al., 2004). The two plant species are nearly equally suitable for larval development of both host species (Saastamoinen et al., 2007), and both are readily used for oviposition (Nieminen et al., 2003; Reudler Talsma et al., 2008b). Furthermore, both plants contain aucubin and catalpol as their main direct defence compounds, with more catalpol than aucubin. There is a higher concentration of both IGs in *P. lanceolata* (Nieminen et al., 2003; Reudler Talsma et al., 2008b).

Rearing of the host caterpillars. Caterpillars of *M. cinxia* and *M. athalia* – 20 and 16 families, respectively – were reared to their last instar in the laboratory. Before diapause (instars 1–5) they were reared on a mixture of the two host plants. After diapause (instars 5–7), when most of the growth occurs, half the families were fed fresh field-

collected *P. lanceolata* leaves, the other half was fed fresh field-collected *V. spicata* leaves. Gregarious caterpillars were reared in family groups in plastic boxes covered with mesh in a climate-controlled chamber.

Field experiment

Last-instar caterpillars were put on potted plants grown from seeds collected in the field. This was done in the last instar rather than at the pupal stage to allow the pupal parasitoids to use odours associated with feeding and pupation as cues (Godfray, 1994). This also allowed the caterpillars to pupate in their natural position, which can influence their vulnerability to parasitism (van Nouhuys & Kraft, 2012). The caterpillars were put on the plant species that matched their post-diapause diet. We placed the host plants in the field in May 2011 in habitat patches spread over the Åland islands where the butterflies and food plants occur naturally (Figure 1). Once in the field we covered the plants with a loose mesh net, sized to prevent the caterpillars from escaping but allow parasitoids to enter freely. The pots were put in the field in groups of four, 30 cm apart. One last-instar caterpillar was placed on each host plant. Within a habitat patch three replicate sets of four pots were placed at least 30 m apart. This was replicated in 10 habitat patches for both butterfly species, a total of 60 *P. lanceolata* with *M. cinxia* and 60 *V. spicata* plants with *M. cinxia*, 60 *P. lanceolata* with *M. athalia*, and 60 *V. spicata* with *M. athalia*. Both butterfly species inhabited all 10 habitat patches. In half the habitat patches *P. lanceolata* was the only natural host plant. In the other half, both plants were present but *V. spicata* was dominant (main surrounding plant of the patch) (Figure 1). When the hosts had been in the field for 2 weeks, we checked to see whether the caterpillars had pupated. Those that pupated were taken back to the laboratory 4 days later. This was repeated until all pupae had a chance to be parasitised. The date of pupation varied depending on weather and the microclimate of the pot. Pupae are susceptible to parasitism by *P. apum* from the day of pupation through day 6 (van Nouhuys & Kraft, 2012).

Laboratory experiment

This experiment was designed to compare the same factors as in the field experiment (host species and diet) under simple controlled conditions (no direct effect of the host plant structure, volatiles, or size). Female wasps that egressed from the field experiment parasitise host pupae in the laboratory experiment. Thus, wasps' development history (host species and host diet) was known and therefore associative learning could be tested. Wasp individuals from each of the four developmental histories (*M. cinxia* × *P. lanceolata*, *M. cinxia* × *V. spicata*,

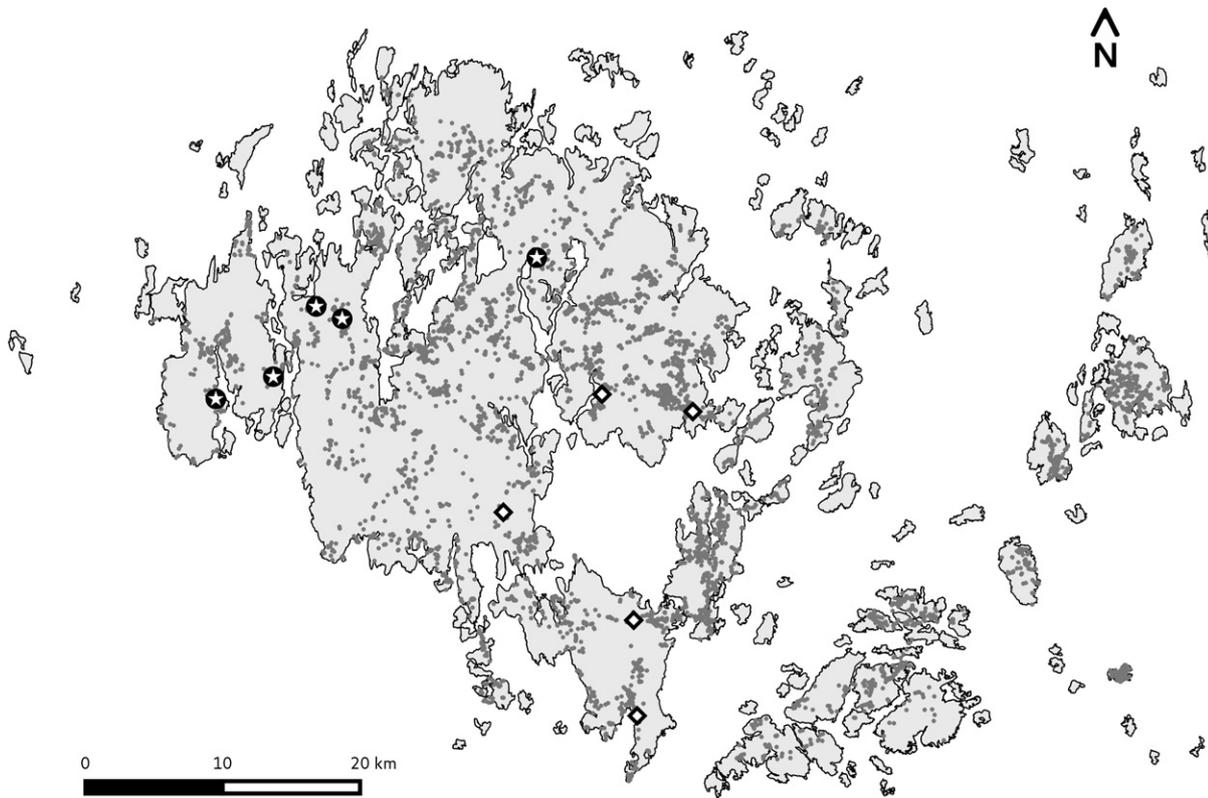


Figure 1 Map of Åland with the natural habitat patches of *Melitaea cinxia* shown as small dark grey dots. Five habitat patches used for the experiment had *Veronica spicata* as most abundant natural plant (marked with a star), and five had *Plantago lanceolata* as most abundant natural plant (marked with a diamond).

M. athalia × *P. lanceolata*, and *M. athalia* × *V. spicata*) were distributed evenly among the treatment replicates. The host pupae were from the same origin as described above ('Rearing of the host caterpillars'), but were kept in larval diapause for three extra weeks in the early spring to delay their development.

Mated female wasps were offered two pupae of the same host species reared on the same host plant, or on two different host plant species in a Petri dish. Wasps were given a choice only between host food plant species *P. lanceolata* (P) or *V. spicata* (V), but not between host butterfly species. In total we had three treatments per host species: two pupae reared on *P. lanceolata* (PP), two pupae reared on *V. spicata* (VV), or one reared at *P. lanceolata* and one reared at *V. spicata* (PV). For *M. athalia* pupae, 22 replicates were used for all three treatments (PP, PV, or VV). Fewer *M. cinxia* pupae were available so we had 13 PP, 14 PV, and 7 VV replicates for *M. cinxia*. After 30 h the wasp was removed from the Petri dish.

Data collection. For both experiments, after exposure to parasitism we weighed each butterfly pupa, and kept them

in individual mesh-topped cups at room temperature until egression of a butterfly or parasitoids. We assessed parasitism (yes/no), brood size, and sex ratio of the brood. The wasps were too small to weigh individually, so for each brood, we counted and pooled the females and weighted them together, and pooled the males and weight them together. We opened all host pupae after the wasps egressed to count undeveloped and dead wasps left in the pupa. Hosts that remained as pupae after 25 days were opened and scored as parasitised (dead developing wasps inside), or not parasitised (dead developing butterfly inside).

Iridoid glycosides in pupae

We used high-performance liquid chromatography (HPLC) analysis to determine whether sequestered IGs in the pupae differed between butterfly species and diets. Thirty-two pupae [10 reared on *P. lanceolata* × *M. athalia* pupae (PA), three reared on *P. lanceolata* × *M. cinxia* pupae (PC), 11 reared on *V. spicata* × *M. athalia* pupae (VA), and eight reared on *V. spicata* × *M. cinxia* pupae (VC)] were frozen and

freeze dried, and then ground to a fine powder by hand. The ground material was extracted in 5 ml of 70% MeOH and left overnight. The crude extract was filtered using Whatman no. four filter paper and the filtrate was diluted 10× with Milli-Q water. The concentrations of the IG aucubin and catalpol were analysed by HPLC using a Bio-Lc (Dionex, Sunnyvale, CA, USA) equipped with a GP40 gradient pump, a CarboPac PA 1 guard (4 × 50 mm) and analytical column (4 × 250 mm), and an ED40 electrochemical detector for pulsed amperometric detection (PAD) equipped with a disposable gold electrode using carbohydrate waveform A (see Rohrer, 2013). Isocratic flow of 70 mM NaOH (flow rate 0.25 ml per min) was used for the elution. Columns were cleaned after each sample with alkaline (100 mM NaOH) 300 mM sodium acetate solution. Retention times were 3.5 and 5.3 min for aucubin and catalpol, respectively. Concentrations were analysed using Chromeleon Client v.6.50 SP10a Build 1065 (Dionex).

Statistical analysis

Statistical analyses were conducted in JMP Pro v.10.0.2 (SAS, Cary, NC, USA) and IBM SPSS v.22 (IBM, Armonk, NY, USA).

Field experiment. We tested whether diet and host species influenced post-diapause survival and pupal weight of the host caterpillar before they were placed in the field using univariate ANOVA with plant diet and butterfly species as fixed factors and percentage survival (of the gregarious caterpillar family) as dependent. This analysis was done because caterpillar survival to pupation may be associated with quality of the surviving pupae, and therefore also the success of parasitism. We also analysed the effects of rearing diet and host species on pupal weight using univariate ANOVA with plant and butterfly species as fixed factors and host pupa weight, after it had been retrieved from the field, as dependent.

We modelled parasitism success (0/1) using logistic regression (GLM with a binomial error structure) with a nested design. Host species was nested in diet which was nested in patch. Only those patches with pupae that received parasitism were included in the analyses. Because only a small fraction of pupae were parasitised the grouping of pupae on plants within the patch was left out of the model. Surrounding plant species and patch could also not both be included in a model so they were evaluated in separate models (each with all other factors), and the best fitting model, based on AIC values, was presented. To test whether there was an effect of host species or diet on wasp brood size we used a standard least squares model with patch as a random factor, diet nested in patch, and host

species nested in diet and patch. Pupal weight was also included as a factor. The same model was used for average weight of the wasps, with brood size also included as an explanatory factor. Because male *P. apum* are significantly smaller than females, we conducted separate tests for each sex. Non-significant interactions were removed from the models. The brood sex ratio was analysed using logistic regression (GLM with a binomial error structure) with a nested design, with host species nested in diet nested in patch. Brood size and pupal weight were included in the model.

Laboratory experiment. We modelled successful parasitism (0/1) using binary logistic regression (GLMs) with host species, diet, and neighbour diet as factors. To determine the effect of the origin of the mother wasp, we included the natal host and plant species of the mother. The age of the mother wasp and the host pupal weight and age were included as covariates. To determine the main effect on brood size and sex ratio, we used univariate ANOVA models with host species, diet, neighbour diet, natal wasp diet, and host as factors, and wasp age, host pupa age, and weight as covariates. For analysis of sex ratio, brood size was included as a factor. To determine which factors influence the average weight of the wasps in a brood, we conducted a univariate ANOVA with plant, host species, wasp plant origin, and wasp host origin as factors, and brood size, pupa weight and age, wasp age, and brood sex ratio as covariates. Again, the males and females were analysed separately. The factors that related to parasitoid development time were determined by a univariate ANOVA, with host species, diet, and host diet as factors, and brood size, sex ratio, wasp age, host size, and age as covariates. Non-significant interactions were removed from the models.

Iridoid glycosides in butterfly pupae. We conducted a univariate ANOVA to investigate whether the percentage of dry weight of the IGs aucubin and catalpol differed between host species and food plant species. All IG values were \sqrt{x} transformed prior to analyses.

Results

Field experiment

Survival of host caterpillars, survival of pupae, and pupal weight. Survival of caterpillars before they were put into the field was higher on *V. spicata* (84.2%) than on *P. lanceolata* (66.7%; $F_{1,31} = 9.992$, $P = 0.004$). Survival of *M. athalia* caterpillars (89.0%) was also greater than survival of *M. cinxia* (60.3%; $F_{1,31} = 26.484$, $P < 0.001$). There was no interaction between the survival of the

caterpillars and the diet they were fed ($F_{1,30} = 2.354$, $P > 0.1$).

Of the 240 caterpillars put in the field, 174 pupated (59 PA, 38 PC, 53 VA, and 24 VC). Five of these pupae were partly eaten by predators, three caterpillars were still in the larval stage at the end of the experiment, 53 of the caterpillars died before pupating (0 PA, 18 PC, 4 VA, and 31 VC), and 11 caterpillars disappeared from the plants due to predation (2 PA, 4, PC, 1 VA, and 4 VC). More caterpillars reared on *P. lanceolata* pupated, and a larger fraction of *M. athalia* caterpillars pupated (Table 1). *Melitaea cinxia* pupae were larger than *M. athalia* pupae ($F_{1,166} = 48.045$, $P < 0.001$), and pupae reared on *P. lanceolata* were larger than those reared on *V. spicata* ($F_{1,166} = 5.76$, $P = 0.017$). There were no interactions with respect to pupal size, between butterfly species and diet ($F_{1,165} = 0.217$, $P > 0.1$).

Parasitism. Twenty-three pupae were parasitised by *P. apum* (13.2%), which is a lower rate of parasitism than in previous studies (van Nouhuys & Kraft, 2012). None of the pupae were parasitised by other parasitoid species. Whether or not a pupa was parasitised was independent of the plant diet; however, relatively more *M. cinxia* (20%) than *M. athalia* pupae (10%) were parasitised ($\chi^2 = 30.36$, $P = 0.004$; Tables 1 and 2). There was no interaction between host pupa and plant species ($P > 0.1$), and parasitism was independent of both pupal weight after parasitism and the main surrounding plant of the patch.

Brood size and sex ratio. Parasitoid brood sizes ranged from 8 to 49, with on average (\pm SE) 28.26 ± 2.33 individuals. Neither diet ($F_{1,17.98} = 0.011$, $P = 0.91$), nor host pupa species ($F_{1,16.91} = 9.913$, $P = 0.35$) were associated with brood size. However, there was a significant interaction between diet and host species ($F_{1,12.29} = 11.236$, $P = 0.005$; Figure 2, Table 1). The sex

Table 2 Binary logistic model (type III) of the rate of parasitism in the field experiment

Source	χ^2	d.f.	P
Model fit	56.665 ^c	26	0.0005
Patch	0.000029	6	1.0
Diet (patch)	8.417	7	0.30
Host (diet, patch)	30.36	13	0.0042

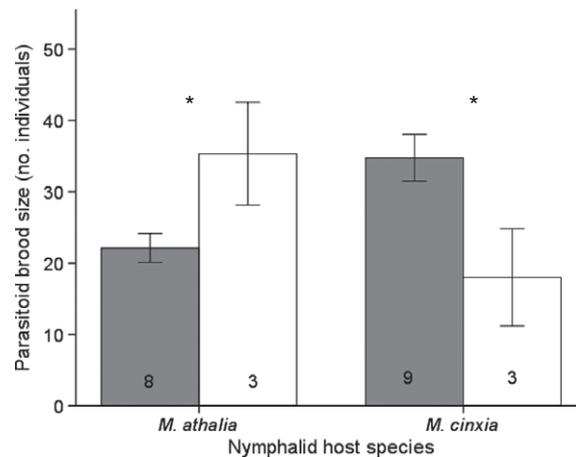


Figure 2 Mean (\pm SE) *Pteromalus apum* brood size (no. individuals) per nymphalid host pupa species on *Plantago lanceolata* (grey) and *Veronica spicata* (white) plants in the field experiment. The number of parasitised hosts is indicated inside the bars. Asterisks indicate significant differences between treatments (standard least squares model: * $P < 0.05$).

ratio of the broods ranged from 7 to 89% male (mean \pm SE = $25 \pm 3.7\%$), and was independent of diet or host pupa species, as well as brood size and host pupal weight (all $P > 0.1$; Table 1).

	Plant	<i>V. spicata</i>		<i>P. lanceolata</i>	
		<i>M. cinxia</i>	<i>M. athalia</i>	<i>M. cinxia</i>	<i>M. athalia</i>
Survival to pupation	Plant	–		+	
	Host	–	+	–	+
Fraction parasitised	Plant	ns		ns	
	Host	+	–	+	–
Brood size	Plant	ns		ns	
	Host	–	+	+	–
Sex ratio	Plant	ns		ns	
	Host	ns	ns	ns	ns
Male weight	Plant	ns		ns	
	Host	ns	ns	ns	ns
Female weight	Plant	ns		ns	
	Host	–	+	–	+

Table 1 Overview of the effect of diet plant (*Veronica spicata* vs. *Plantago lanceolata*) and nymphalid host species (*Melitaea cinxia* vs. *M. athalia*) on the pupation, parasitism, brood size, sex ratio, and weight of *Pteromalus apum* wasps in the field experiment. Factors in rows with positive or negative signs have significant differences between treatments. 'ns' indicates that there is no effect of diet eaten by the host species

Table 3 ANOVA for the average weight of male and female *Pteromalus apum* wasps in a brood in the field experiment

Source		d.f.	F	P
Males	Plant (patch)	2,12.06	2.58	0.12
	Host species (diet, patch)	3,11.14	1.24	0.34
	Pupal weight	1,8.198	5.12	0.053
	Sex ratio	1,12.67	2.10	0.17
	Brood size	1,12.81	8.09	0.014
Females	Plant (patch)	2,8.26	1.44	0.29
	Host species (diet, patch)	3,8.91	6.15	0.015
	Pupal weight	1,12.63	16.07	0.002
	Sex ratio	1,9.54	8.91	0.014
	Brood size	1,10.83	7.63	0.019

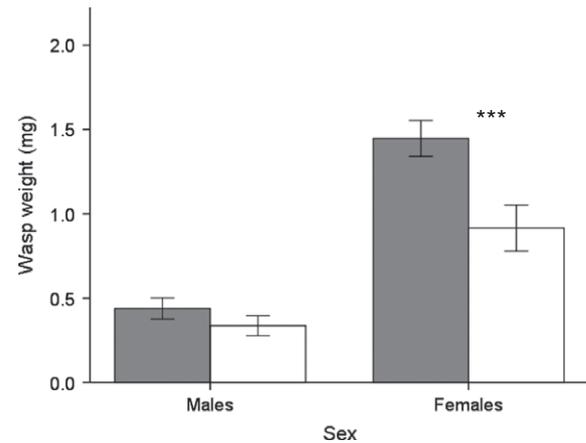
Variance component estimates, males: patch 0.002 (10.2% of total), residual 0.016 (89.8% of total), $R^2 = 0.781$ (adjusted $R^2 = 0.650$); females: patch 0.069 (77.7% of total), residual 0.029 (22.3% of total), $R^2 = 0.950$ (adjusted $R^2 = 0.918$).

Offspring wasp weight. The average weight of the wasps in a brood was dependent on sex, host species, host pupal weight, sex ratio, and brood size. As expected, female wasps (mean \pm SE = 1.18 ± 0.1 mg) were significantly heavier than male wasps (0.39 ± 0.043 mg; t-test: $t = -7.14$, d.f. = 12, $P < 0.001$). For the male wasps, weight decreased with brood size and increased with host pupal weight (Table 3), regardless of host diet and species. However, the average weight of female wasps depended on host species, with those from *M. athalia* pupae heavier than those from *M. cinxia* pupae (Table 3, Figure 3). Average female weight also increased with host pupa weight after parasitism, and decreased with size of the brood.

Laboratory experiment

Parasitism. In total 132/209 hosts were successfully parasitised (63.2%). A smaller fraction of the *M. cinxia* pupae (41/77) was successfully parasitised than of the *M. athalia* pupae (91/132), and wasps originating from *M. cinxia* pupae parasitised 1.2 \times more pupae (of both species) than wasps originating from *M. athalia* pupae. The interaction of diet host pupa diet and neighbour diet was also significant (Table 4).

Brood size and sex ratio. Wasp brood size ranged from one to 42, with an average (\pm SE) size of 15.24 ± 0.76 individuals. There was no direct effect of host diet or host species on the brood size (Table 5). There was a significant interaction between wasp host origin and neighbour diet, with wasps originating from *M. athalia* hosts having larger broods when the neighbour host diet was *V. spicata*. The opposite was true for wasps originating from

**Figure 3** Mean (\pm SE) *Pteromalus apum* wasp weight (mg) by sex on *Melitaea athalia* (grey) and *M. cinxia* (white) host pupa. Asterisks indicate significant differences between treatments (univariate ANOVA: *** $P < 0.001$).**Table 4** Binary logistic model (type III) of the rate of parasitism in the laboratory experiment

Source	Wald χ^2 (d.f. = 1)	P
(Intercept)	0.314	0.58
Diet	2.058	0.15
Host species	12.604	<0.001
Neighbour diet	0.397	0.53
Wasp plant origin	0.048	0.83
Wasp host origin	6.829	0.009
Host weight	0.683	0.41
Wasp age	2.848	0.091
Host age	0.375	0.54
Diet*neighbour diet	3.686	0.055

M. cinxia hosts. Furthermore, there was an association between natal wasp plant and natal wasp host species, with wasps originating from 'host on *V. spicata* diet' having a larger brood size on *M. athalia* pupae, and wasps originating from 'host on *P. lanceolata* diet' having a larger brood size on *M. cinxia* pupae (Table 5).

The brood sex ratio ranged from 0 to 100% male and was on average $45.2 \pm 3.9\%$. There was no effect of host species or diet on sex ratio. However, wasp plant origin and wasp age had a significant effect. Wasps originating from *V. spicata* produced more male-biased broods than did wasps originating from *P. lanceolata*, the difference on *M. athalia* hosts was larger than on *M. cinxia* hosts. Older wasps produced less male-biased broods (Table 6).

Offspring wasp weight. The average weight of the wasp offspring was dependent on sex, host species, sex ratio,

Table 5 ANOVA for brood size in the laboratory experiment

Source	SS	d.f.	MS	F	P
Corrected model	1618.425 ¹	10	161.842	2.311	0.016
Intercept	151.383	1	151.383	2.161	0.14
Diet	157.687	1	157.687	2.251	0.14
Host species	40.280	1	40.280	0.575	0.45
Neighbour diet	110.556	1	110.556	1.578	0.21
Wasp plant origin	252.820	1	252.820	3.609	0.060
Wasp host origin	68.559	1	68.559	0.979	0.32
Host weight	122.953	1	122.953	1.755	0.19
Wasp age	1.873	1	1.873	0.027	0.87
Host age	218.903	1	218.903	3.125	0.080
Neighbour diet*wasp host origin	415.536	1	415.536	5.933	0.016
Wasp diet origin*wasp host origin	272.991	1	272.991	3.897	0.051
Error	8475.295	121	70.044		
Total	40731.000	132			
Corrected total	10093.720	131			

¹R² = 0.160 (adjusted R² = 0.091).

Table 6 Analyses of variance of the sex ratio in the laboratory experiment

Source	SS	d.f.	MS	F	P
Corrected model	37257.203 ¹	10	3725.720	2.310	0.017
Intercept	7587.908	1	7587.908	4.705	0.032
Diet	30.373	1	30.373	0.019	0.89
Host species	0.480	1	0.480	0.000	0.99
Neighbour diet	8.545	1	8.545	0.005	0.94
Wasp plant origin	13038.036	1	13038.036	8.085	0.005
Wasp host origin	37.803	1	37.803	0.023	0.88
Host weight	405.093	1	405.093	0.251	0.62
Wasp age	12664.077	1	12664.077	7.853	0.006
Host age	18.210	1	18.210	0.011	0.92
Brood size	2319.251	1	2319.251	1.438	0.23
Wasp plant origin*wasp host origin	5939.774	1	5939.774	3.683	0.058
Error	175784.955	109	1612.706		
Total	458551.503	120			
Corrected total	213042.158	119			

¹R² = 0.175 (adjusted R² = 0.099).

host weight, host age, food plant origin of the mother wasp and brood size, and there was a significant interaction between diet and host species (Table 7). Females (mean ± SE) = 1.25 ± 0.066 mg) were heavier

Table 7 ANOVA of the mean male and female parasitoid weight in the laboratory experiment

	Source	SS	d.f.	MS	F	P
Males	Corrected model	2.599 ¹	10	0.260	12.917	<0.001
	Intercept	0.000	1	0.000	0.005	0.94
	Diet	0.013	1	0.013	0.639	0.43
	Host species	0.093	1	0.093	4.600	0.035
	Wasp plant origin	0.145	1	0.145	7.230	0.009
	Wasp host origin	0.010	1	0.010	0.518	0.47
	Host age	0.125	1	0.125	6.205	0.015
	Host weight	0.123	1	0.123	6.114	0.015
	Wasp age	0.009	1	0.009	0.436	0.51
	Brood size	0.001	1	0.001	0.052	0.82
	Sex ratio	1.407	1	1.407	69.899	<0.001
	Diet*host species	0.127	1	0.127	6.305	0.014
	Error	1.690	84	0.020		
	Total	17.721	95			
	Corrected total	4.290	94			
Females	Corrected model	8.130 ²	9	0.903	6.296	<0.001
	Intercept	0.052	1	0.052	0.365	0.55
	Diet	0.021	1	0.021	0.150	0.70
	Host species	0.644	1	0.644	4.488	0.039
	Wasp plant origin	0.780	1	0.780	5.436	0.024
	Wasp host origin	0.143	1	0.143	0.996	0.32
	Host age	0.221	1	0.221	1.541	0.22
	Host weight	2.010	1	2.010	14.012	<0.001
	Wasp age	0.010	1	0.010	0.068	0.80
	Sex ratio	0.869	1	0.869	6.055	0.017
	Brood size	4.104	1	4.104	28.604	<0.001
	Error	7.173	50	0.143		
	Total	108.679	60			
	Corrected total	15.304	59			

¹R² = 0.606 (adjusted R² = 0.559).

²R² = 0.531 (adjusted R² = 0.447).

than males (0.38 ± 0.022 mg; t-test: t = -12.49, d.f. = 154, P<0.001). Both male and female wasps that developed in *M. athalia* pupae were slightly heavier (male

mean 0.40 mg, female 1.27 mg) than those from *M. cinxia* pupae (male 0.33 mg, female 1.21 mg). Males developing in *M. athalia* individuals reared on *P. lanceolata* were heaviest. The opposite is true for male wasps developing in *M. cinxia* pupae, as they gained more weight when reared on *V. spicata* (Figure 4). Wasps developing in larger pupae were heavier, which was most pronounced for female wasps. Male wasps developing in younger pupae were also larger; however, larger broods produced smaller female wasps. Finally, wasps originating from *V. spicata* pupae produced heavier offspring than those originating from *P. lanceolata* pupae (male 0.42 vs. 0.34 mg, female 1.28 vs. 1.22 mg).

Development time. The development time (from parasitism until egression) of the parasitoids ranged from 15 to 28 days (mean \pm SE = 20.1 \pm 2.5 days) and was independent of host pupa species or their diet. Development time increased with host size and decreased with brood size. Wasps developed 1.5 days faster if their mother originated from *V. spicata* and the development time increased with the number of males in the brood (Table 8).

Iridoid glycosides in host pupae

Aucubin and catalpol made up 0.06–3.32% dry weight of the butterfly pupae, with just a tiny fraction of that being aucubin (Figure 5). In that small fraction, *M. athalia* contained more aucubin than in *M. cinxia* pupae ($F_{1,29} = 5.966$, $P = 0.021$), but the amount of aucubin was the same for both plant species ($F_{1,29} = 0.630$, $P = 0.43$). The amount of catalpol did not differ between the host species ($F_{1,29} = 2.358$, $P = 0.14$), but there was a trend towards more catalpol in hosts fed *V. spicata* than in hosts fed *P. lanceolata* ($F_{1,29} = 2.886$, $P = 0.1$).

Discussion

Host food plant

Host plant species is known to affect the quality of a host for development of parasitoid offspring. For example, development and survival of parasitoids within tobacco hornworms or fall armyworms depends on whether their hosts fed on nicotine-free or nicotine-containing diets. The effects of nicotine were more severe for the relatively less-adapted parasitoid *Hyposoter annulipes* (Cresson) than for the specialist parasitoid *Cotesia congregata* (Say) (Barbosa et al., 1986). Plant chemistry is well known to affect plant quality, limiting growth and reproduction in herbivores. On the other hand, many herbivores can use plant secondary chemicals in defence against their own natural enemies (Ode, 2006). *Melitaea cinxia* and

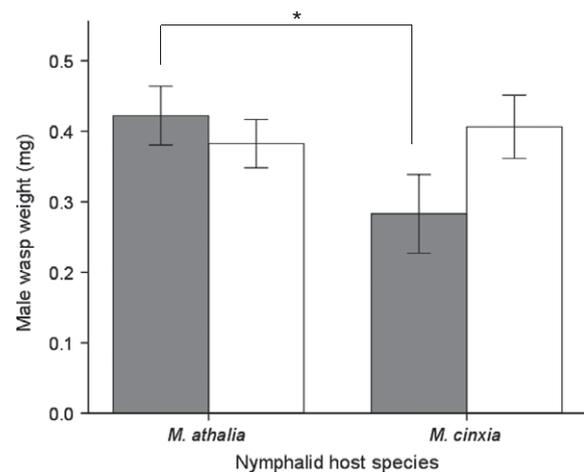


Figure 4 Mean (\pm SE) weight (mg) of male *Pteromalus apum* wasp offspring on *Plantago lanceolata* (grey) and *Veronica spicata* (white) diets in the laboratory experiment, separated by host species. Asterisk indicates significant difference between treatments (univariate ANOVA: * $P < 0.05$).

Table 8 ANOVA of the development time in the laboratory experiment

Source	SS	d.f.	MS	F	P
Corrected model	399.115 ¹	9	44.346	13.715	<0.001
Intercept	722.020	1	722.020	223.306	<0.001
Diet	0.966	1	0.966	0.299	0.59
Host species	0.011	1	0.011	0.003	0.95
Wasp plant origin	31.334	1	31.334	9.691	0.002
Wasp host origin	0.213	1	0.213	0.066	0.80
Host weight	53.300	1	53.300	16.485	<0.001
Wasp age	3.005	1	3.005	0.929	0.34
Host age	0.045	1	0.045	0.014	0.91
Brood size	224.439	1	224.439	69.414	<0.001
Sex ratio	14.678	1	14.678	4.540	0.035
Error	349.199	108	3.233		
Total	48309.000	118			
Corrected total	748.314	117			

¹ $R^2 = 0.533$ (adjusted $R^2 = 0.494$).

M. athalia sequester IGs from their host plants. We found that they retained IGs as pupae, which has been shown in other herbivores adapted to IGs (Bowers & Puttick, 1986). Host pupae reared on the two plant species differed slightly in sequestered defensive chemistry, with hosts reared on *P. lanceolata* containing less catalpol than those reared on *V. spicata*. Catalpol is the more toxic and

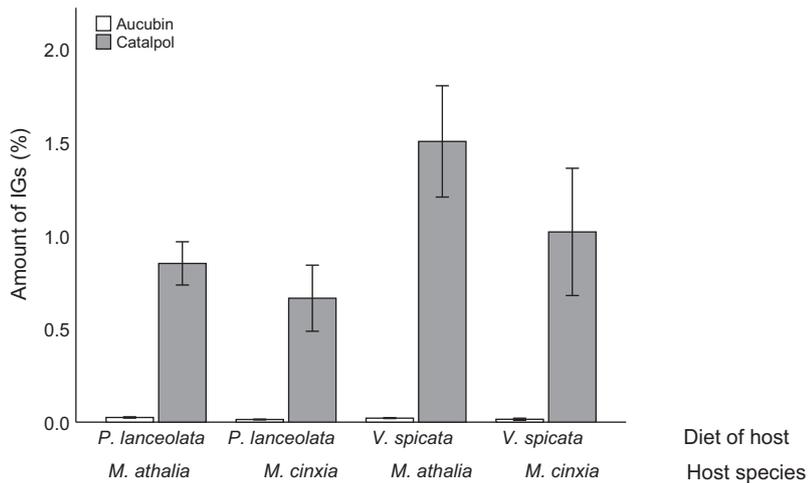


Figure 5 Mean (\pm SE) amount (%) of the iridoid glycosides aucubin (white) and catalpol (grey) present in *Melitaea cinxia* and *M. athalia* pupae reared on diets of *Plantago lanceolata* and *Veronica spicata*.

deterrent of the two IGs (Puttick & Bowers, 1988; Bowers & Puttick, 1989; Bowers, 1991), and catalpol has been shown to have a negative effect on some parasitoids (Nieminen et al., 2003; Singer & Stireman, 2003; Laurentz et al., 2012). However, in the laboratory, we found no direct association of brood size or development time with food plant, which suggests that the higher catalpol in hosts fed *V. spicata* was not detrimental.

Whether a host is parasitised or not can depend on the ability of a parasitoid to locate or access a host on a plant. Plants differ in volatile attractants (Kessler & Baldwin, 2001) qualitatively and quantitatively, according to plant and herbivore species, thus providing specific information for carnivorous arthropods (Pierre et al., 2011). The physical structure of plant species also differs, which can influence the vulnerability, visibility, and accessibility of hosts to parasitoids (Andow & Prokrym, 1990; Feng et al., 2015). Such a pattern was found for the generalist leaf miner parasitoid *Apanteles polychrosidis* Viereck (Wist et al., 2015). *Plantago lanceolata* and *V. spicata* have different volatile emissions (JH Reudler, D Mofikoya, JK Holopainen & S van Nouhuys, unpubl.), and the two specialist parasitoids of *M. cinxia* larvae are more attracted to hosts on *V. spicata* than to hosts on *P. lanceolata* (van Nouhuys & Hanski, 2004; Castelo et al., 2010). We found no direct effect of plant species on whether a pupa became parasitised by *P. apum* in the field, where the attractiveness of the plant and the accessibility of the host were part of the experiment. However, brood size did differ, depending on host species, which could be related to the differing accessibility of the two hosts on each plant species because superparasitism is common. Therefore, for this generalist parasitoid, there is not an overall difference in host quality, apparency, or accessibility between the two plant species –

though their suitability for the wasp may depend on host species.

Host species

Most parasitoids use multiple host species, preferring and performing better on one over another (Ode, 2006; Harvey et al., 2015). We found that a larger fraction of *M. cinxia* than of *M. athalia* that were placed in the field became parasitised, suggesting that the *M. cinxia* is the preferred or more accessible host. In contrast, more *M. athalia* pupae were parasitised under simplified conditions in the laboratory experiment, suggesting that *M. athalia* is the higher quality host. However, in the laboratory experiment, all pupae that died (no wasp or butterfly developed from them) were *M. cinxia*, so the surviving pupae may have been of particularly low quality in the laboratory. In a previous field study in this system (van Nouhuys & Kraft, 2012), *M. athalia* was parasitised at a higher rate than *M. cinxia*. In our study density of pupae was low, and overall parasitism rate in the field was low (13.2%) whereas in the previous experiment host density was high and the average parasitism rate was 40% with a high rate of superparasitism (van Nouhuys & Kraft, 2012; Kraft & van Nouhuys, 2013). The apparently different behaviour of *P. apum* under these three conditions (laboratory, and high vs. low density in the field) demonstrates that there is not an absolute preferred host species, and that foraging conditions influence host species use (Hassell et al., 1991; Spataro & Bernstein, 2007).

Once a pupa is parasitised its quality can influence the performance of the parasitoid offspring. The brood sizes in the field were significantly larger than in the laboratory experiment, which could have been caused by individuals laying larger broods, or by superparasitism. In the field

experiment, we found larger broods in *M. cinxia* than in *M. athalia* pupae on *P. lanceolata*, and the opposite pattern on *V. spicata*. This may have been due to differences in accessibility of the two species on each plant affecting the frequency of superparasitism (Kraft & van Nouhuys, 2013). Alternatively, brood size in *M. athalia* may be reduced by increased sequestration of aucubin. Sequestration of plant defensive chemicals such as IGs by insect herbivores can reduce parasitoid success (Lampert et al., 2008). To our knowledge there are not yet known examples of a negative association of sequestration with the brood size of a gregarious parasitoid.

Wasp origin

Parasitoids use associative learning based on their natal site (host species or plant) to find hosts (Corbet, 1985; Vet & Groenewoud, 1990; Hastings & Godfray, 1999). In the laboratory study we did not find that wasps parasitised their natal host species, or hosts with their natal diet more readily than the unfamiliar hosts. This suggests that they are truly generalist in their foraging behaviour, at least with respect to these host species.

We did, however, find that both host species and host food plant origins influenced the performance of *P. apum* in the laboratory experiment. Individuals reared from *M. cinxia* parasitised more pupae than those reared from *M. athalia*, this difference was mostly because wasps from *M. cinxia* more often parasitised both pupae in a dish (26 vs. 15). So, there was no difference in the number of individual wasps that parasitised, but in the number of pupae they parasitised. Surprisingly, females originating from *M. cinxia* pupae were smaller than females from *M. athalia* pupae, which we would expect to be associated with lower rather than higher fertility (Sagarra et al., 2001). Furthermore, we found that wasps originating from hosts on *V. spicata* produced larger offspring, in larger male-biased broods that developed faster than the progeny of wasps originating from hosts fed *P. lanceolata*. This, again, is not associated with female size, and it was unrelated to the number of pupae they parasitised. The wasps used in the laboratory experiment came from wild patches in areas dominated by either *P. lanceolata* or *V. spicata*. It is thus possible that they are from distinct populations that have slightly different life-history traits, though they are not locally adapted in the classic sense (Kawecki & Ebert, 2004). However, spatial population structure cannot explain the differences in offspring performance based on the host species the mother was reared from, independent of host plant species, because both host pupa species were present at each site. A possible explanation would be host-associated differentiation (Stireman et al., 2005), with sympatric

P. apum populations parasitising the two host species. These are open questions left for future research.

Conclusion

We expected to find that the generalist pupal parasitoid *P. apum* would use one host species over the other, and that hosts feeding on one plant species would be most suitable for their offspring development. As the hosts sequester plant allelochemicals we expected variation in sequestration between host and host plants, with a negative effect of high sequestration on the parasitoid. Finally, we also expected individuals to preferentially parasitise hosts under their natal conditions due to associative learning. Instead, we found the parasitoid to have conditional host use, depending on the plant, and only minor differences in offspring weight and brood size associated with host and plant species. They also exhibited no associative learning. That the wasps do not distinguish systematically between host species makes sense because the hosts turned out to be of nearly equal quality, though it is possible that reduced brood size is associated with higher aucubin concentration in *M. athalia*.

Many insect species that are considered generalists are found, upon closer inspection, to have more narrow host ranges locally, or even to consist of a complex of host- or host plant-associated species (e.g., Hambäck et al., 2013; Loxdale & Harvey, 2016). We found instead that *P. apum* behaves as a generalist, and the relative use of each host species depends only somewhat on their resident plants. Therefore, the relative rate of parasitism of each host depends on which plant species are present and used by each host species in the meadows. The conditionality of this behaviour is increased by the fact that *M. athalia* uses related plant species in addition to *P. lanceolata* and *V. spicata* that are present in the landscape (Reudler Talsma et al., 2008a), and host plant use by *M. cinxia* butterflies locally adapted in Åland (Kuussaari et al., 2000).

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