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

3
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14 **Running head:** *Host diet and host species effect*

15
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17 preference-performance, *Pteromalus apum*, pupal parasitism, *Veronica spicata*,
18 Hymenoptera, Pteromalidae, Lepidoptera, Nymphalidae

19
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1 **Abstract**

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

20

21 **Abbreviated abstract**

22 Even for parasitoids with a wide host range, not all host species are equally suitable, and host
23 quality often depends on the plant the host feeds on. Using field and laboratory experiments
24 we show that for a generalist pupal parasitoid, host species use is context dependent, but
25 independent of host food plant species.

26

27 **Introduction**

28 Parasitoids must find a host in a habitat, choose to parasitize it, and successfully evade host
29 defence. Those with narrow host ranges are known to use specific plant and host cues to
30 identify host species (Quicke, 2015), and parasitizing a non-host species is very costly
31 (Condon et al., 2014). Parasitoids with a wide host range do not need to be so specific, yet
32 still must find hosts, assess their suitability, and successfully parasitize them. The cues used
33 and their relative importance determine the position of a generalist parasitoid in an insect

1 community. The host provides the sole nutritional and physiological environment during
2 development of a parasitoid. So host quality is a major component in host selection, even for
3 parasitoids with a wide host range (Vinson, 1975; Roitberg et al., 2001; Harvey et al., 2013).
4 One important factor responsible for variation in host quality is the plant it consumes
5 (Barbosa et al., 1982; Hopkins et al., 2009), both the nutritional quality of the plant and
6 whether the plant contains toxins that are either detrimental to or sequestered by the host
7 (Harvey et al., 2005; Smilanich et al., 2009; Lampert, 2012; Erb & Robert, 2016).
8 Additionally, a herbivore may be more attractive, suitable, or accessible to a parasitoid on
9 one host plant species over another (Poelman et al., 2014; Feng et al., 2015).

10 Host size, which may differ between species (Wang & Messing, 2004) as well as
11 within a species (Barbosa et al., 1982), is also an important measurement of quality, as it
12 determines the amount of resources available for the developing parasitoid. It is especially
13 critical for gregarious species, as host size can determine the weights of parasitoids
14 developing in it as well as the size of the brood (Waage & Godfray, 1985; Harvey et al.,
15 2013; Kraft & van Nouhuys, 2013, Rosa et al 2017).

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

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

1 In this study we compared oviposition choice and offspring performance of the
2 generalist pupal parasitoid *Pteromalus apum* (Retzius) (Hymenoptera: Pteromalidae), on two
3 butterfly species, *Melitaea cinxia* (L.) and *Melitaea athalia* (Rottemburg) (Lepidoptera:
4 Nymphalidae), reared on two plant species, *Plantago lanceolata* L. and *Veronica spicata* L.
5 (Plantaginaceae). These two hosts are in the normal host range of the parasitoid (Shaw et al.,
6 2009) and co-occur in the study area (Reudler Talsma et al., 2008b) where they are
7 parasitized by *P. apum* at a high rate (van Nouhuys & Kraft, 2012). Both butterfly species
8 sequester iridoid glycosides (IGs) from their chemically defended host plants (Suomi et al.,
9 2003; J.H.R. pers. obs.). The IGs aucubin and – to a greater extent – catalpol are toxic or
10 deterrent to generalist herbivores (Puttick & Bowers, 1988; Bowers, 1991). At the same time,
11 these compounds serve as feeding and oviposition stimulants for some specialist herbivores,
12 including *M. cinxia* (Bowers, 1984; Nieminen et al., 2003; Reudler Talsma et al., 2008a).

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

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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 parasitized by a congener, *Pteromalus puparum* (L.), instead of *P. apum*, under natural conditions (S.v.N 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 (Reudler Talsma et al., 2008b; Wahlberg, 2001). *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; J.H. Reudler, A. Biere, J.A. Harvey and S. van Nouhuys, in prep.). 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).

1 *Plants*

2 *Plantago lanceolata* and *V. spicata* are common meadow species in the study area. *Plantago*
3 *lanceolata* occurs in practically all meadows suitable for the butterfly species, whereas *V.*
4 *spicata* is abundant only in the northwestern part of the study area (Kuussaari et al., 2004).
5 The two plant species are nearly equally suitable for larval development of both host species
6 (Saastamoinen et al., 2007), and both are readily used for oviposition (Nieminen et al., 2003;
7 Reudler Talsma et al., 2008b). Furthermore, both plants contain aucubin and catalpol as their
8 main direct defence compounds, with more catalpol than aucubin. There is a higher
9 concentration of both IGs in *P. lanceolata* (Nieminen et al., 2003; Reudler Talsma et al.,
10 2008b).

11

12 *Rearing of the host caterpillars*

13 Caterpillars of *M. cinxia* and *M. athalia* – 20 and 16 families, respectively – were reared to
14 their last instar in the laboratory. Before diapause (instars 1-5) they were reared on a mixture
15 of the two host plants. After diapause (instars 5-7), when most of the growth occurs, half the
16 families were fed fresh field-collected *P. lanceolata* leaves, the other half was fed fresh field-
17 collected *V. spicata* leaves. Gregarious caterpillars were reared in family groups in plastic
18 boxes covered with mesh in a climate-controlled chamber.

19

20 **Field experiment**

21 Last-instar caterpillars were put on potted plants grown from seeds collected in the field. This
22 was done in the last instar rather than at the pupal stage to allow the pupal parasitoids to use
23 odours associated with feeding and pupation as cues (Godfray, 1994). This also allowed the
24 caterpillars to pupate in their natural position, which can influence their vulnerability to
25 parasitism (van Nouhuys & Kraft, 2012). The caterpillars were put on the plant species that
26 matched their post-diapause diet. We placed the host plants in the field in May 2011 in
27 habitat patches spread over the Åland islands where the butterflies and food plants occur
28 naturally (Figure 1). Once in the field we covered the plants with a loose mesh net, sized to
29 prevent the caterpillars from escaping but allow parasitoids to enter freely. The pots were put
30 in the field in groups of four, 30 cm apart. One last-instar caterpillar was placed on each host
31 plant. Within a habitat patch three replicate sets of four pots were placed at least 30 m apart.
32 This was replicated in 10 habitat patches for both butterfly species, a total of 60 *P. lanceolata*
33 with *M. cinxia* and 60 *V. spicata* plants with *M. cinxia*, 60 *P. lanceolata* with *M. athalia*, and
34 60 *V. spicata* with *M. athalia*. Both butterfly species inhabited all 10 habitat patches. In half

1 the habitat patches *P. lanceolata* was the only natural host plant. In the other half both plants
2 were present but *V. spicata* was dominant (main surrounding plant of the patch) (Figure 1).
3 When the hosts had been in the field for 2 weeks, we checked to see whether the caterpillars
4 had pupated. Those that pupated were taken back to the laboratory 4 days later. This was
5 repeated until all pupae had a chance to be parasitized. The date of pupation varied depending
6 on weather and the microclimate of the pot. Pupae are susceptible to parasitism by *P. apum*
7 from the day of pupation through day 6 (van Nouhuys & Kraft, 2012).

8 9 **Laboratory experiment**

10 This experiment was designed to compare the same factors as in the field experiment (host
11 species and diet) under simple controlled conditions (no direct effect of the host plant
12 structure, volatiles, or size). Female wasps that egressed from the field experiment parasitize
13 host pupae in the laboratory experiment. Thus wasps' development history (host species and
14 host diet) was known and therefore associative learning could be tested. Wasp individuals
15 from each of the four developmental histories (*M. cinxia* × *P. lanceolata*, *M. cinxia* × *V.*
16 *spicata*, *M. athalia* × *P. lanceolata*, and *M. athalia* × *V. spicata*) were distributed evenly
17 among the treatment replicates. The host pupae were from the same origin as described above
18 ('Rearing of the host caterpillars'), but were kept in larval diapause for three extra weeks in
19 the early spring to delay their development.

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

29 30 *Data collection*

31 For both experiments, after exposure to parasitism we weighed each butterfly pupa, and kept
32 them in individual mesh-topped cups at room temperature until egression of a butterfly or
33 parasitoids. We assessed parasitism (yes/no), brood size, and sex ratio of the brood. The
34 wasps were too small to weigh individually, so for each brood we counted and pooled the

1 females and weighted them together, and pooled the males and weight them together. We
2 opened all host pupae after the wasps egressed to count undeveloped and dead wasps left in
3 the pupa. Hosts that remained as pupae after 25 days were opened and scored as parasitized
4 (dead developing wasps inside), or not parasitized (dead developing butterfly inside).

6 **Iridoid glycosides in pupae**

7 We used high-performance liquid chromatography (HPLC) analysis to determine whether
8 sequestered IGs in the pupae differed between butterfly species and diets. Thirty-two pupae
9 [10 reared on *P. lanceolata* × *M. athalia* pupae (PA), three reared on *P. lanceolata* × *M.*
10 *cinxia* pupae (PC), 11 reared on *V. spicata* × *M. athalia* pupae (VA), and eight reared on *V.*
11 *spicata* × *M. cinxia* pupae (VC)] were frozen and freeze dried, and then ground to a fine
12 powder by hand. The ground material was extracted in 5 ml of 70% MeOH and left
13 overnight. The crude extract was filtered using Whatman no. 4 filter paper and the filtrate
14 was diluted 10× with Milli-Q water. The concentrations of the IG aucubin and catalpol were
15 analysed by HPLC using a Bio-Lc (Dionex, Sunnyvale, CA, USA) equipped with a GP40
16 gradient pump, a Carbopac PA 1 guard (4 × 50 mm) and analytical column (4 × 250 mm),
17 and an ED40 electrochemical detector for pulsed amperimetric detection (PAD) equipped
18 with a disposable gold electrode using carbohydrate waveform A. Isocratic flow of 70 mM
19 NaOH (flow rate 0.25 ml per min) was used for the elution. Columns were cleaned after each
20 sample with alkaline (100 mM NaOH) 300 mM sodium acetate solution. Retention times
21 were 3.5 and 5.3 min for aucubin and catalpol, respectively. Concentrations were analyzed
22 using Chromeleon Client v.6.50 SP10a Build 1065 (Dionex).

24 **Statistical analysis**

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

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

1 host pupa weight, after it had been retrieved from the field, as dependent.

2 We modelled parasitism success (0/1) using logistic regression (GLM with a binomial
3 error structure) with a nested design. Host species was nested in diet which was nested in
4 patch. Only those patches with pupae that received parasitism were included in the analyses.
5 Because only a small fraction of pupae were parasitized the grouping of pupae on plants
6 within the patch was left out of the model. Surrounding plant species and patch could also not
7 both be included in a model so they were evaluated in separate models (each with all other
8 factors), and the best fitting model, based on AIC values, was presented. To test whether
9 there was an effect of host species or diet on wasp brood size we used a standard least squares
10 model with patch as a random factor, diet nested in patch, and host species nested in diet and
11 patch. Pupal weight was also included as a factor. The same model was used for average
12 weight of the wasps, with brood size also included as an explanatory factor. Because male *P.*
13 *apum* are significantly smaller than females, we conducted separate tests for each sex. Non-
14 significant interactions were removed from the models. The brood sex ratio was analysed
15 using logistic regression (GLM with a binomial error structure) with a nested design, with
16 host species nested in diet nested in patch. Brood size and pupal weight were included in the
17 model.

18
19 *Laboratory experiment.* We modelled successful parasitism (0/1) using binary logistic
20 regression (GLMs) with host species, diet, and neighbour diet as factors. To determine the
21 effect of the origin of the mother wasp, we included the natal host and plant species of the
22 mother. The age of the mother wasp and the host pupal weight and age were included as
23 covariates. To determine the main effect on brood size and sex ratio, we used univariate
24 ANOVA models with host species, diet, neighbour diet, natal wasp diet, and host as factors,
25 and wasp age, host pupa age, and weight as covariates. For analysis of sex ratio, brood size
26 was included as a factor. To determine which factors influence the average weight of the
27 wasps in a brood, we conducted a univariate ANOVA with plant, host species, wasp plant
28 origin, and wasp host origin as factors, and brood size, pupa weight and age, wasp age, and
29 brood sex ratio as covariates. Again, the males and females were analysed separately. The
30 factors that related to parasitoid development time were determined by a univariate ANOVA,
31 with host species, diet, and host diet as factors, and brood size, sex ratio, wasp age, host size,
32 and age as covariates. Non-significant interactions were removed from the models.

33
34 *Iridoid glycosides in butterfly pupae.* We conducted a univariate ANOVA to investigate

1 whether the percentage of dry weight of the IGs aucubin and catalpol differed between host
2 species and food plant species. All IG values were \sqrt{x} transformed prior to analyses.

3 4 **Results**

5 **Field experiment**

6 *Survival of host caterpillars, survival of pupae, and pupal weight*

7 Survival of caterpillars before they were put into the field was higher on *V. spicata* (84.2%)
8 than on *P. lanceolata* (66.7%; $F_{1,31} = 9.992$, $P = 0.004$). Survival of *M. athalia* caterpillars
9 (89.0%) was also greater than survival of *M. cinxia* (60.3%; $F_{1,31} = 26.484$, $P < 0.001$). There
10 was no interaction between the survival of the caterpillars and the diet they were fed ($F_{1,30} =$
11 2.354 , $P > 0.1$).

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

21 22 *Parasitism*

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

30 31 *Brood size and sex ratio*

32 Parasitoid brood sizes ranged from 8 to 49, with on average (\pm SE) 28.26 ± 2.33 individuals.
33 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

1 associated with brood size. However, there was a significant interaction between diet and
2 host species ($F_{1,12.29} = 11.236$, $P = 0.005$; Figure 2, Table 1). The sex ratio of the broods
3 ranged from 7 to 89% male (mean \pm SE = $25 \pm 3.7\%$), and was independent of diet or host
4 pupa species, as well as brood size and host pupal weight (all $P > 0.1$; Table 1).

6 *Offspring wasp weight*

7 The average weight of the wasps in a brood was dependent on sex, host species, host pupal
8 weight, sex ratio, and brood size. As expected, female wasps (mean \pm SE = 1.18 ± 0.1 mg)
9 were significantly heavier than male wasps (0.39 ± 0.043 mg; t-test: $t = -7.14$, d.f. = 12,
10 $P < 0.001$). For the male wasps, weight decreased with brood size and increased with host
11 pupal weight (Table 3), regardless of host diet and species. However, the average weight of
12 female wasps depended on host species, with those from *M. athalia* pupae heavier than those
13 from *M. cinxia* pupae (Table 3, Figure 3). Average female weight also increased with host
14 pupa weight after parasitism, and decreased with size of the brood.

16 **Laboratory experiment**

17 *Parasitism*

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

24 *Brood size and sex ratio*

25 Wasp brood size ranged from one to 42, with an average (\pm SE) size of 15.24 ± 0.76
26 individuals. There was no direct effect of host diet or host species on the brood size (Table 5).
27 There was a significant interaction between wasp host origin and neighbour diet, with wasps
28 originating from *M. athalia* hosts having larger broods when the neighbour host diet was *V.*
29 *spicata*. The opposite was true for wasps originating from *M. cinxia* hosts. Furthermore, there
30 was an association between natal wasp plant and natal wasp host species, with wasps
31 originating from 'host on *V. spicata* diet' having a larger brood size on *M. athalia* pupae, and
32 wasps originating from 'host on *P. lanceolata* diet' having a larger brood size on *M. cinxia*
33 pupae (Table 5).

34 The brood sex ratio ranged from 0 to 100% male and was on average $45.2 \pm 3.9\%$.

1 There was no effect of host species or diet on sex ratio. However, wasp plant origin and wasp
2 age had a significant effect. Wasps originating from *V. spicata* produced more male-biased
3 broods than did wasps originating from *P. lanceolata*, the difference on *M. athalia* hosts was
4 larger than on *M. cinxia* hosts. Older wasps produced less male-biased broods (Table 6).

6 *Offspring wasp weight*

7 The average weight of the wasp offspring was dependent on sex, host species, sex ratio, host
8 weight, host age, food plant origin of the mother wasp and brood size, and there was a
9 significant interaction between diet and host species (Table 7). Females (mean \pm SE) = $1.25 \pm$
10 0.066 mg) were heavier than males (0.38 ± 0.022 mg; t-test: $t = -12.49$, d.f. = 154, $P < 0.001$).
11 Both male and female wasps that developed in *M. athalia* pupae were slightly heavier (male
12 mean 0.40 mg, female 1.27 mg) than those from *M. cinxia* pupae (male 0.33 mg, female 1.21
13 mg). Males developing in *M. athalia* individuals reared on *P. lanceolata* were heaviest. The
14 opposite is true for male wasps developing in *M. cinxia* pupae, as they gained more weight
15 when reared on *V. spicata* (Figure 4). Wasps developing in larger pupae were heavier, which
16 was most pronounced for female wasps. Male wasps developing in younger pupae were also
17 larger; however, larger broods produced smaller female wasps. Finally, wasps originating
18 from *V. spicata* pupae produced heavier offspring than those originating from *P. lanceolata*
19 pupae (male 0.42 vs. 0.34 mg, female 1.28 vs. 1.22 mg).

21 *Development time*

22 The development time (from parasitism until egression) of the parasitoids ranged from 15 to
23 28 days (mean \pm SE = 20.1 ± 2.5 days) and was independent of host pupa species or their
24 diet. Development time increased with host size and decreased with brood size. Wasps
25 developed 1.5 days faster if their mother originated from *V. spicata* and the development time
26 increased with the number of males in the brood (Table 8).

28 **Iridoid glycosides in host pupae**

29 Aucubin and catalpol made up 0.06-3.32% dry weight of the butterfly pupae, with just a tiny
30 fraction of that being aucubin (Figure 5). In that small fraction, *M. athalia* contained more
31 aucubin than in *M. cinxia* pupae ($F_{1,29} = 5.966$, $P = 0.021$), but the amount of aucubin was the
32 same for both plant species ($F_{1,29} = 0.630$, $P = 0.43$). The amount of catalpol did not differ
33 between the host species ($F_{1,29} = 2.358$, $P = 0.14$), but there was a trend toward more catalpol
34 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 *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 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 parasitized 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 (J.H. Reudler, D. Mofikoya, J.K. Holopainen and S. van Nouhuys, in prep.), 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 parasitized by *P. apum* in the field, where the attractiveness of the plant and the accessibility of the host were part of the experiment.

1 However, brood size did differ, depending on host species, which could be related to the
2 differing accessibility of the two hosts on each plant species because superparasitism is
3 common. Therefore, for this generalist parasitoid there is not an overall difference in host
4 quality, apparency, or accessibility between the two plant species – though their suitability
5 for the wasp may depend on host species.

6 7 *Host species*

8 Most parasitoids use multiple host species, preferring and performing better on one over
9 another (Ode, 2006; Harvey et al., 2015). We found that a larger fraction of *M. cinxia* than of
10 *M. athalia* that were placed in the field became parasitized, suggesting that the *M. cinxia* is
11 the preferred or more accessible host. In contrast, more *M. athalia* pupae were parasitized
12 under simplified conditions in the laboratory experiment, suggesting that *M. athalia* is the
13 higher-quality host. However, in the laboratory experiment all pupae that died (no wasp or
14 butterfly developed from them) were *M. cinxia*, so the surviving pupae may have been of
15 particularly low quality in the laboratory. In a previous field study in this system (van
16 Nouhuys & Kraft, 2012), *M. athalia* was parasitized at a higher rate than *M. cinxia*. In our
17 study density of pupae was low, and overall parasitism rate in the field was low (13.2%)
18 whereas in the previous experiment host density was high and the average parasitism rate was
19 40% with a high rate of superparasitism (Kraft & van Nouhuys, 2013; van Nouhuys & Kraft,
20 2012). The apparently different behaviour of *P. apum* under these three conditions
21 (laboratory, and high vs. low density in the field) demonstrates that there is not an absolute
22 preferred host species, and that foraging conditions influence host species use (Hassell et al.,
23 1991; Spataro & Bernstein, 2007).

24 Once a pupa is parasitized its quality can influence the performance of the parasitoid
25 offspring. The brood sizes in the field were significantly larger than in the laboratory
26 experiment, which could have been caused by individuals laying larger broods, or by
27 superparasitism. In the field experiment, we found larger broods in *M. cinxia* than in *M.*
28 *athalia* pupae on *P. lanceolata*, and the opposite pattern on *V. spicata*. This may have been
29 due to differences in accessibility of the two species on each plant affecting the frequency of
30 superparasitism (Kraft & van Nouhuys, 2013). Alternatively, brood size in *M. athalia* may be
31 reduced by increased sequestration of aucubin. Sequestration of plant defensive chemicals
32 such as IGs by insect herbivores can reduce parasitoid success (Lampert et al., 2008). To our
33 knowledge there are not yet known examples of a negative association of sequestration with
34 the brood size of a gregarious parasitoid.

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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 parasitized 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* parasitized more pupae than those reared from *M. athalia*, this difference was mostly because wasps from *M. cinxia* more often parasitized both pupae in a dish (26 vs. 15). So, there was no difference in the number of individual wasps that parasitized, but in the number of pupae they parasitized. 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 (Sagarraa 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 parasitized. 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 parasitizing 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 of sequestration between host and host plants, with a negative effect of high sequestration on the parasitoid. Finally, we also expected individuals to preferentially parasitize hosts under their

1 natal conditions due to associative learning. Instead, we found the parasitoid to have
2 conditional host use, depending on the plant, and only minor differences in offspring weight
3 and brood size associated with host and plant species. They also exhibited no associative
4 learning. That the wasps do not distinguish systematically between host species makes sense
5 because the hosts turned out to be of nearly equal quality, though it is possible that reduced
6 brood size is associated with higher aucubin concentration in *M. athalia*.

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

17

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1 **Figure captions**

2

3 **Graphic abstract caption:**

4 Design of the study comparing the performance of the generalist parasitoid *Pteromalus apum*
5 (a) on two host species, *Melitaea athalia* (b) and *M. cinxia* (c), fed two plant species,
6 *Plantago lanceolata* (d) and *Veronica spicata* (e). Line drawings by Z. Kolev and
7 photographs by S. van Nouhuys

8

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

13

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

18

19 **Figure 3** Mean (\pm SE) *Pteromalus apum* wasp weight (mg) by sex on *Melitaea athalia* (grey)
20 and *M. cinxia* (white) host pupa. Asterisks indicate significant differences between treatments
21 (univariate ANOVA, F statistic: *** $P < 0.001$).

22

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

27

28 **Figure 5** Mean (\pm SE) amount (%) of the iridoid glycosides aucubin (white) and catalpol
29 (grey) present in *Melitaea cinxia* and *M. athalia* pupae reared on diets of *Plantago lanceolata*
30 and *Veronica spicata*. There are no significant differences.

31

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

	Plant	<i>V. spicata</i>		<i>P. lanceolata</i>	
	Host	<i>M. cinxia</i>	<i>M. athalia</i>	<i>M. cinxia</i>	<i>M. athalia</i>
Survival to pupation	Plant		-		+
	Host	-	+	-	+
Fraction parasitized	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	-	+	-	+

6

7

1 **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`	26	0.0005
Patch	0.000029	6	1.0
Diet (patch)	8.417	7	0.30
Host (diet,patch)	30.36	13	0.0042

2

1 **Table 3** ANOVA for the average weight of male and female *Pteromalus apum* wasps in a
 2 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

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

6

1 **Table 4** Binary logistic model (type III) of the rate of parasitism in in the laboratory
 2 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

3

4

1 **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			

¹ $R^2 = 0.160$ (adjusted $R^2 = 0.091$)

2

3

1 **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			

¹ $R^2 = 0.175$ (adjusted $R^2 = 0.099$)

2

1 **Table 7** ANOVA of the mean male and female parasitoid weight in the laboratory
 2 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			

3 ¹ R² = 0.606 (adjusted R² = 0.559)

4 ² R² = 0.531 (adjusted R² = 0.447)

5

6

1 **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			

2 ¹ R² = 0.533 (adjusted R² = 0.494)

3