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Basic and Applied Ecology 13 (2012) 241–249

Basic and
Applied Ecology

www.elsevier.com/locate/baae

Performance of secondary parasitoids on chemically defended and undefended hosts

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Received 14 November 2011; accepted 22 March 2012

Abstract

Defensive chemicals produced by plants can travel up the food chain by being sequestered by herbivores, and then in turn being sequestered by their parasitoids. Insect species with wide host ranges are predicted to perform poorly in the face of specific chemical defence. However, a species at a high trophic level is expected to have a wide host range. This creates a conflict for hyperparasitoids, many of which depend on specialized hosts. We studied the performance of two hyperparasitoids, *Lysibia nana* and *Gelis agilis*, both of which have wide host ranges, on two host species, one chemically defended and the other not. We predicted that both hyperparasitoids would perform better using the undefended host *Cotesia glomerata* than the defended host *C. melitaeorum*, which sequesters terpenoid allelochemicals (iridoid glycosides). Furthermore, we expected that the progeny of *G. agilis* collected from an area where hosts defended by iridoid glycosides are absent (the Netherlands) would perform poorly using *C. melitaeorum* in comparison with *G. agilis* collected from an area where *C. melitaeorum* is a common host (Åland, Finland). In a series of laboratory experiments we found that, contrary to prediction, both hyperparasitoids performed well on both hosts, reaching a larger size on *C. glomerata*, but having a higher conversion efficiency and developing more quickly on the chemically defended *C. melitaeorum*. *Lysibia nana* metabolized the plant derived iridoid glycosides, which are chemicals that it does not normally encounter. *Gelis agilis* retained some of the iridoid glycosides. But whereas Finnish *G. agilis* retained both aucubin and catalpol, Dutch *G. agilis* mainly retained aucubin, illustrating that though generalists, local populations still cope differently with toxic allelochemicals.

Zusammenfassung

Von Pflanzen synthetisierte chemische Abwehrstoffe können sich in der Nahrungskette anreichern, indem sie zunächst von Herbivoren und dann von deren Parasitoiden akkumuliert werden. Es wird angenommen, dass Insektenarten mit einem breiten Wirtsspektrum schlecht mit spezifischen chemischen Abwehrstoffen zurechtkommen. Arten in oberen Trophieebenen haben in der Regel ein breites Wirtsspektrum. Dadurch entsteht ein Konflikt für Hyperparasitoide, die häufig von einem spezialisierten Wirt abhängig sind. Wir haben zwei Hyperparasitoide mit breitem Wirtsspektrum, *Lysibia nana* und *Gelis agilis*, auf zwei Wirten untersucht. Einer der beiden Wirte unterliegt einer pflanzlichen chemischen Abwehr. Wir gingen davon aus, dass beide Hyperparasitoide besser auf dem Wirt, der keiner chemischen Abwehr unterliegt (*Cotesia glomerata*) zurechtkommen, als

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auf *C. melitaeorum*, welcher terpenoide Allelochemikalien (Iridoidglycoside) akkumuliert. Des Weiteren erwarteten wir, dass Nachkommen von *G. agilis*, entnommen aus einem Gebiet ohne Vorkommen von Wirten, die durch Iridoidglycoside verteidigt werden (Niederlande), schlechter mit *C. melitaeorum* zurechtkommen, als *G. agilis* aus einem Gebiet, in dem *C. melitaeorum* ein weit verbreiteter Wirt ist (Åland, Finnland). In mehreren experimentellen Studien im Labor zeigte sich, dass sich im Gegensatz zu unseren Vorhersagen beide Hyperparasitoide gut auf beiden Wirten entwickelten. Sie erreichten auf dem größeren Wirt, *C. glomerata*, eine größeren Biomasse, aber der ökologische Wirkungsgrad und die Entwicklungsgeschwindigkeit waren auf dem mit Iridoidglycosiden geschützten *C. melitaeorum*, höher. Weiterhin zeigte sich, dass die pflanzlichen Iridoidglycoside durch *Lysibia nana* metabolisiert werden können, Chemikalien, denen sie normalerweise nicht begegnet. *Gelis agilis* akkumulierte einige der Iridoidglycoside. Individuen aus Finnland konnten sowohl Aucubin als auch Catalpol binden, während Individuen aus den Niederlanden nur Aucubin anreicherten. Dieses Ergebnis zeigt, dass auch lokale Populationen von Generalisten unterschiedlich mit giftigen Allelochemikalien zurechtkommen.

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Keywords: Allelochemical; *Cotesia glomerata*; *Cotesia melitaeorum*; *Gelis agilis*; Host range; Hyperparasitoid; Iridoid glycoside; *Lysibia nana*; Multitrophic level interaction

Introduction

Defensive chemicals produced by plants travel up the food chain by being sequestered by herbivores. Sequestration of plant derived secondary chemicals can reduce predation of herbivores (Bowers 1991; Opitz & Müller 2009). In some cases, these allelochemicals are further sequestered or concentrated by parasitoids of the herbivores, presumably aiding in their own defence (reviewed in Ode 2006). They may also be sequestered or concentrated beyond the third trophic level (Reudler et al. in prep), potentially providing defence for secondary parasitoids.

The effect of sequestered allelochemicals on consumers is thought to be related to their degree of host specialization. Species with wide host ranges are expected to perform poorly in the face of specific defensive chemicals because of the potentially great cost of maintaining multiple detoxification pathways (Bernays & Graham 1988; Karban, Karban, Huntzinger, Pearse, & Crutsinger 2010). We therefore generally think of defensive plant chemicals as effective against consumers with a wide host range, while consumers with a narrow host range overcome and may even benefit from them. This idea is well supported for consumers at the second trophic level (Barbosa et al. 1986; Bowers 1991; Stamp 2001). For consumers at higher trophic levels we have the same expectation, but there are few studies that have addressed associations between consumer host range, and effectiveness of specific defensive metabolites higher up the food chain.

Whether consumer species with wide host ranges indeed perform poorly on prey that sequester defensive compounds is likely to depend on consumer life style. Notably, the idea that higher trophic level generalists are deterred by plant-derived chemicals sequestered by their hosts is well supported for predators (Montllor, Bernays, & Cornelius 1991; Dyer & Bowers 1996; Hristov & Conner 2005; Baden & Dobler 2009), but is less clear for endoparasitoids (Reudler, Biere, Harvey, & van Nouhuys 2011). This may be because regardless of host range endoparasitoids have a more intimate

physiological relationship with their prey than do predators, and thus must be better able to detoxify or avoid plant-derived toxins (Ode 2006; Smilanich, Dyer, Chambers, & Bowers 2009). It also may be that the association is not clear because we have little understanding of the host range of parasitoids (Shaw 1994). For instance, there is growing evidence that presumed generalist parasitoids are actually genetically differentiated populations or even cryptic species groups associated with particular host taxa (Kankare, Stefanescu, van Nouhuys, & Shaw 2005; Smith et al. 2008; Kaartinen, Stone, Hearn, Lohse, & Roslin 2010). Thus, parasitoid species, both primary and secondary, with nominally large host ranges may actually be locally specialized populations or cryptic species groups.

The restricted ability of generalist consumers to deal with sequestered specific allelochemicals in their prey is likely to pose an increasing challenge as we move up the trophic cascade. Insect species at high trophic levels, such as hyperparasitoids, are expected to have inherently wide host ranges because high on the food chain a single prey species is an unreliable resource (Holt & Hochberg 1998; van Nouhuys 2005). This creates a conflict for hyperparasitoids, because many of them depend on a range of host species that may include specialized, chemically defended, hosts.

Based on these considerations, a general, but yet untested, prediction is that hyperparasitoids will perform poorly on those species from within their host range that are able to sequester allelochemicals, unless the hyperparasitoids represent aggregates of locally adapted populations specialized on different host species.

In this paper we test the prediction that hyperparasitoids perform poorly in terms of rate of parasitism, offspring development rate, and size, on host species from within their host range that sequester specific allelochemicals from lower trophic levels. We compare the performance of two hyperparasitoids, *Gelis agilis* (Fabricius) (Hymenoptera: Ichneumonidae) and *Lysibia nana* (Gravenhorst) (Hymenoptera: Ichneumonidae), both with a broad host range (though *G. agilis* uses a more diverse set of host species than does *L.*

nana), on two congeneric parasitoid species. One of the host species is defended by sequestered allelochemicals, *Cotesia melitaeorum* (Wilkinson) (Hymenoptera: Braconidae), and the other, *C. glomerata* (L.) (Hymenoptera: Braconidae) is not. The parasitoid *C. melitaeorum* is specialized on the nymphalid butterfly *Melitaea cinxia* (L.) (Lepidoptera, Nymphalidae), whose larvae feed on plants containing a class of terpenoids known as iridoid glycosides (Wahlberg 2001). These compounds are sequestered by *M. cinxia* (Suomi, Siren, Jussila, Wiedmer, & Riekkola 2002) and further sequestered or concentrated by *C. melitaeorum* (Reudler et al. in prep). We hypothesize that the hyperparasitoids perform better on the undefended *C. glomerata* than on the defended *C. melitaeorum*.

In addition, for one of the parasitoids, *G. agilis*, we investigate whether this species shows adaptation to local host chemical defence, despite its broad host range. Specifically we test whether individuals originating from an area where they use hosts sequestering iridoid glycosides (Finland, FI) perform better on iridoid glycoside-defended *C. melitaeorum* than individuals originating from an area where they are unlikely to encounter such hosts (The Netherlands, NL). While such adaptive responses have been studied extensively in second trophic level consumers (herbivores), and in third trophic level species (predators and primary parasitoids), this study examines for the first time local responses to chemical defence at the fourth trophic level.

Material and methods

Study species

Hyperparasitoids – The parasitoid *Lysibia nana* is an ectoparasitoid of cocoons of Hymenoptera, particularly braconids in the subfamily Microgasterinae (Schwarz & Shaw 2000). It has mostly been reared from gregarious species in the large genus *Cotesia*, and is closely associated with the host *C. glomerata* (Harvey, Wagenaar, & Goles 2011). *Lysibia nana* is also known to use *Cotesia* parasitoids of iridoid glycoside feeding butterflies (Schwarz & Shaw 2000; Stefanescu, Planas, & Shaw 2009). However, in Finland where *C. melitaeorum* is intensively studied, *L. nana* is not found to use *C. melitaeorum* (Lei, Vikberg, Nieminen, & Kuussaari 1997). Likewise, in the Netherlands the butterfly *M. cinxia*, on which *C. melitaeorum* is specialized, is extinct and so *C. melitaeorum* is absent. Further, other nymphalid butterflies that feed on iridoid glycoside producing Lamiales (Wahlberg 2001) are extremely rare in the Netherlands and absent from the collection area.

Gelis agilis is a broad generalist (Schwarz & Shaw 1999), and a ubiquitous secondary parasitoid of *Cotesia* parasitoids of Lepidoptera larvae (Shaw, Stefanescu, & van Nouhuys 2009; Stefanescu et al. 2009). *Gelis agilis* is solitary and the adults are entirely female, wingless and feed on both nectar and hosts (Schwarz & Shaw 1999; Harvey 2008). In Finland

there are multiple host species for *G. agilis*, including both *C. melitaeorum* and *C. glomerata* (van Nouhuys & Hanski 2000; van Nouhuys & Tay 2001). By contrast, in the Netherlands, *G. agilis* has no recent evolutionary history using *C. melitaeorum* or similar hosts for the same reasons as described for *L. nana* above. Where the two secondary parasitoids occur together, *L. nana* is sometimes itself used as a host by the more generalist *G. agilis* (Harvey, Wagenaar, & Bezemer 2009; Stefanescu et al. 2009).

Parasitoids – The chemically defended host, *C. melitaeorum* develops gregariously in the nymphalid butterfly *M. cinxia* which feeds on host plants defended with iridoid glycosides (Wahlberg 2001). Iridoid glycosides deter or inhibit generalist herbivores (Bowers 1991; Camara 1997; Biere, Marak, & van Damme 2004; Harvey, van Nouhuys, & Biere 2005). However the specialist *M. cinxia* is adapted (Nieminen, Suomi, van Nouhuys, Sauri, & Riekkola 2003; Harvey et al. 2005) and sequesters iridoid glycosides (Suomi et al. 2002). Recent work has shown that iridoid glycosides are further sequestered or concentrated by *C. melitaeorum* (Reudler et al. in prep).

The chemically undefended host *C. glomerata* develops gregariously in *Pieris* butterfly (Lepidoptera: Pieridae) larvae that are specialized to feed on cruciferous plants containing glucosinolates as plant secondary compounds that inhibit generalist herbivores (Harvey 2008; Hopkins, van Dam, & van Loon 2009; Shaw et al. 2009). *Pieris* butterflies do not sequester glucosinolates (Müller, Agerbirk, & Olsen 2003). Therefore *C. glomerata* feeding on *Pieris* butterflies do not contain plant-derived glucosinolates.

Insect rearing

The *L. nana* cultures used for the experiments were obtained from wasps that emerged from *C. glomerata* cocoons attached to black mustard plants growing adjacent to the Netherlands Institute of Ecology at Wageningen, NL. The *L. nana* used for the experiment were then reared on cocoons of *C. glomerata* that were in turn hosted by *Pieris brassicae* caterpillars reared on cultivated cabbage plants (Cv Cyrus) at the Institute of Ecology. All *L. nana* experiments were conducted in the Netherlands. For experiments involving the host *C. melitaeorum*, freshly parasitized *M. cinxia* host caterpillars were sent from Finland.

The *G. agilis* cultures used for the experiments originated either from the Netherlands or from Finland. Dutch *G. agilis* were collected from *C. glomerata* cocoons attached to black mustard (*Brassica nigra*) plants near Heteren, NL. For the prior seven years they were reared on *C. glomerata* pupae hosted by *Pieris brassicae* caterpillars. Finnish *G. agilis* were obtained from *C. melitaeorum* cocoons that had come from *M. cinxia* caterpillars collected from six local populations in Åland, FI, in the spring before the experiments were conducted. Upon adult eclosion, the wasps were allowed to host-feed and mature eggs for three days, after which they

were given laboratory reared *C. melitaeorum* cocoons, hosted by *M. cinxia* that had fed on *Plantago lanceolata*. To rear the *G. agilis* used for the experiments, individuals from both origins were given fresh *C. glomerata* cocoons for oviposition and host-feeding every other day for 10 days. After 15–18 days adult wasps from both origins began to eclose. These were host-fed using *C. glomerata*, and then maintained at 10 °C. Before use in an experiment, adult *G. agilis* were allowed to host-feed on a cluster of *C. glomerata* cocoons at room temperature (22–25 °C) for 24 h and had access to honey water (1:3). All *G. agilis* experiments were performed in Åland, Finland. For experiments involving the host *C. glomerata*, fresh *C. glomerata* pupae were sent from the Netherlands, and kept at 11 °C until use.

Set-up of the experiments

We conducted two pairs of experiments. In the first two we studied whether the hyperparasitoids, *L. nana* (Exp. 1) and *G. agilis* (Exp. 2), performed differently in terms of rate of parasitism, offspring development rate and size, on the two host species *C. glomerata* and *C. melitaeorum*. In the second pair of experiments we focused on one of the hyperparasitoids, *G. agilis*, studying whether those originating from the Netherlands (NL) and Finland (FI) performed differently on the two host species, *C. glomerata* (Exp. 3) and *C. melitaeorum* (Exp. 4).

Experiment 1 – Comparison of the suitability of cocoons of the two host species *C. melitaeorum* and *C. glomerata* for *Lysibia nana*. Approximately 110 *C. glomerata* and 74 *C. melitaeorum* cocoons from multiple gregarious broods were teased apart and individually weighed on a microbalance (accuracy 1 mg). Cocoons of both *C. glomerata* and *C. melitaeorum* were then individually presented to individual 5–10-day-old *L. nana* wasps for parasitism. At least 30 different individual wasps were used. Parasitism was verified by observation of insertion of the ovipositor. The exact time of parasitism was recorded, in order to get an accurate measure of egg to adult development time. This experiment was performed in the NIOO laboratory in Wageningen, NL.

Experiment 2 – Comparison of the suitability of cocoons of the two host species *C. melitaeorum* and *C. glomerata* for Dutch *G. agilis*. Cocoons of the host species were obtained as described above. For each replicate of the experiment, three Dutch *G. agilis* (10–20 days old) were placed with three individually weighed ~four-day-old *C. melitaeorum* or *C. glomerata* cocoons in a 9 cm Petri dish for 24 h. The experiment was replicated 44 times for each host species. Depending on the number of cocoons and wasps available, between four and eight replicates of each host species was run per day. Cotton wool soaked with honey water (1:3) was placed in each Petri dish for the wasps to drink. The number of cocoons provided to each wasp was based on the fecundity schedules of *G. agilis* described in

Harvey (2008). The *G. agilis* wasps were removed from the Petri dish after 24 h. This experiment was performed in the laboratory in Finland.

Experiment 3 – Comparison of hyperparasitoid origins: Dutch and Finnish *G. agilis* on *Cotesia glomerata* cocoons. For each treatment replicate, two *G. agilis* females from the Netherlands or Finland were placed in a dish with three *C. glomerata* cocoons. This experiment was replicated 15 times for each wasp origin following the same procedure as described for experiment 2.

Experiment 4 – Comparison of hyperparasitoid origins: Dutch and Finnish *G. agilis* on *Cotesia melitaeorum* cocoons. For each treatment replicate two *G. agilis* females from the Netherlands or Finland were placed in a dish with three *C. melitaeorum* cocoons. This experiment was replicated 22 times for each wasp origin following the same procedure as described for experiment 2.

For all experiments the parasitized *Cotesia* pupae were left to develop at room temperature (22–25 °C) until egression of adult *Cotesia* and *L. nana*/*G. agilis*. If nothing emerged the cocoon was dissected. Adult *L. nana*/*G. agilis* were weighed within 4 h of emergence, and then frozen for later chemical analysis.

Chemical analysis of iridoid glycoside content

The iridoid glycoside content of *G. agilis* and *L. nana* progeny that egressed from *C. melitaeorum* cocoons as well as the iridoid glycosides in the empty silken cocoons, which may be from either the host remains or the meconium of the hyperparasitoid. 38 *G. agilis* adults and cocoon remains from experiments 2 and 4 (26 NL and 12 FI) were each analyzed individually. 16 *L. nana* from experiment 1 were analyzed in groups of 2. The samples were air dried and ground by hand in an Eppendorf tube. The wasps and their empty cocoons were extracted in 0.7 ml 7% MeOH and left overnight at room temperature. The crude extract was filtered on a 13 mm Syringe Filter w/0.2 µm PTFE membrane (VWR International). The concentrations of aucubin and catalpol were analyzed by HPLC using a Bio-LC (Dionex Corp., Sunnyvale, USA) equipped with a GS50 gradient pump, a CarboPac PA 20 guard (3 mm × 30 mm) and analytical column (3 mm × 150 mm). Detection was performed with an ED50 pulsed amperometric detector (PAD) equipped with a disposable gold electrode using carbohydrate Waveform A (Dionex Corporation 1998). Isocratic flow of 70 mM NaOH (flow rate 0.25 ml/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 4.00 min and 6.08 min for aucubin and catalpol, respectively. Concentrations were analyzed using Chromeleon Client version 6.50 SP10a Build 1065 (Dionex Corp., Sunnyvale, USA).

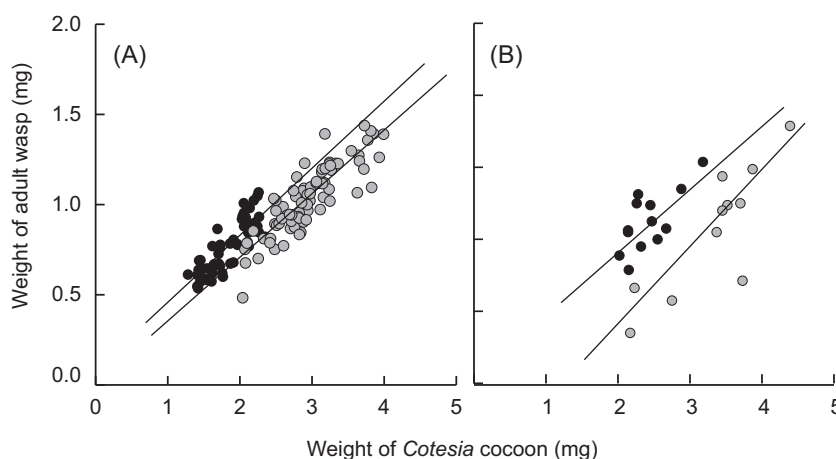


Fig. 1. Relationship between adult *L. nana* (A) (experiment 1) and Dutch *G. agilis* (B) (experiment 2) fresh body mass (mg), and cocoon mass (mg) of *C. melitaeorum* (black circles) and *C. glomerata* (gray circles).

Statistical analyses

Each experiment was analyzed separately using the statistical software JMP (SAS Institute 2007). For experiment 1, two sided *t*-tests were used to compare weight and conversion efficiency (mg of hyperparasitoid adult produced per mg of host cocoon) between hosts for *L. nana*. A Fisher's exact test was used to analyse the rate of successful parasitism. For the *G. agilis* experiments (2–4) successful parasitism (production of an adult *G. agilis*) was analyzed using a GLM logit model with a binomial distribution. Date and replicate were included in the models along with host weight, host origin (Exp. 2) or *G. agilis* origin (Exp. 3 and 4). The effect of host species (Exp. 2) or *G. agilis* origin (Exp. 3 and 4) on development time and conversion efficiency were analysed using GLM with a normal distribution and maximum likelihood estimation. Host weight and the interaction between host weight and host species or *G. agilis* origin were included in the models. One-way ANOVA was used to analyze the association of *G. agilis* origin with iridoid glycoside content (% dry weight catalpol, aucubin and total iridoid glycosides). Where means are presented in the text the value is followed by ± 1 standard error.

Results

Experiment 1 – Comparison of the suitability of cocoons of the two host species *C. melitaeorum* and *C. glomerata* for *Lysibia nana*.

All of the *Cotesia* cocoons were observed to be parasitized by *L. nana*. Parasitism was mostly successful and the rate of parasitism did not differ between the two host species (Fisher's exact test $p=0.31$). Overall, 86% (94/109) of the cocoons of *C. glomerata* produced *L. nana* and 78% (59 out of 74) cocoons of *C. melitaeorum* produced *L. nana*. *Cotesia melitaeorum* were smaller hosts than *C. glomerata* (on average 1.90 mg vs. 3.00 mg; $t=15.33$, $p<0.0001$) and *L.*

nana developing on *C. melitaeorum* were smaller than those developing on *C. glomerata* (on average 0.78 mg vs. 1.05 mg; $t=8.96$, $p<0.0001$) (Fig. 1A). However, the efficiency of conversion was higher for *C. melitaeorum* than for *C. glomerata* hosts; *L. nana* from *C. melitaeorum* reached an average of 0.41 the weight of the host cocoon while *L. nana* from *C. glomerata* were on average only 0.35 the weight of the host cocoons ($t=9.63$, $p<0.0001$; Fig. 2A). Furthermore, *L. nana* developed slightly faster on *C. melitaeorum* than on *C. glomerata*, maturing, on average in 11.0 and 11.5 days, respectively ($t=7.41$, $p<0.0001$; Fig. 2B).

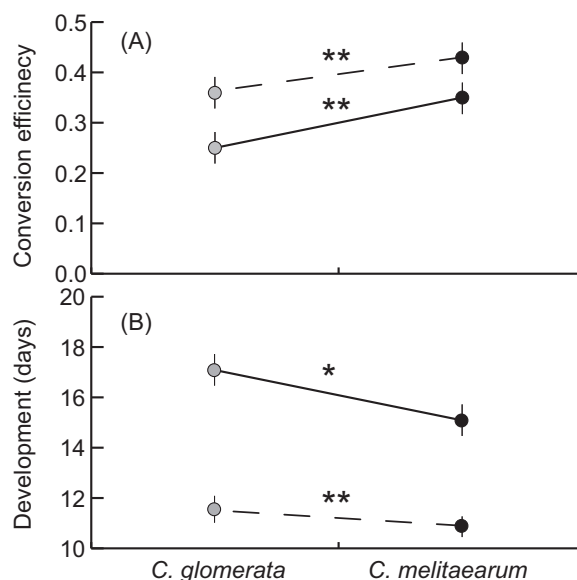


Fig. 2. The mean (\pm SE) conversion efficiency (A) and rate of development (*G. agilis* wt/*Cotesia* wt) (B) of *L. nana* (dashed line, experiment 1) and Dutch *G. agilis* (solid line, experiment 2) on *C. glomerata* (gray circles) and *C. melitaeorum* (black circles). * $p<0.0001$, ** $p<0.005$.

Experiment 2 – Comparison of the suitability of cocoons of the two host species *C. melitaearum* and *C. glomerata* for Dutch *G. agilis*.

Out of the 264 cocoons (132 of each species), only 44 (16.7%) were successfully parasitized by *G. agilis*. There was no significant difference in the rate of parasitism between the two host species, neither for those that made it to adulthood using *C. glomerata* or *C. melitaearum* (11 vs. 13), nor for those that were found fully developed but dead at dissection (12 vs. 8). *Gelis agilis* developed about 2 days faster on *C. melitaearum* than on *C. glomerata* (an average of 15 and 17 days, respectively; $\chi^2(1) = 7.94$; $p = 0.0048$; Fig. 2B). *Cotesia melitaearum* was also used more efficiently than *C. glomerata*. That is, *G. agilis* from *C. melitaearum* were 0.35 the mass of the host cocoon, whereas those from *C. glomerata* were 0.25 the mass of the host on average ($\chi^2(1) = 7.94$; $p < 0.0001$; Fig. 2A). Consequently, though *C. melitaearum* cocoons are on average smaller than *C. glomerata* cocoons, *G. agilis* reached the same average mass, 0.85 mg, on both hosts (Fig. 1B).

Experiment 3 – Comparison of hyperparasitoid origins: Dutch and Finnish *G. agilis* on *C. glomerata* cocoons.

In this experiment 90 *C. glomerata* cocoons were used, 22 (24.4%) of which were successfully parasitized by *G. agilis*. 29% of cocoons offered to Finnish *G. agilis* were parasitized, and 20% of cocoons offered to Dutch *G. agilis* were parasitized. There was no difference in rate of parasitism between the two wasp origins, nor did *G. agilis* origin affect the development rate or efficiency of conversion (0.30 ± 0.32).

Experiment 4 – Comparison of hyperparasitoid origins: Dutch and Finnish *G. agilis* on *C. melitaearum* cocoons.

There were 132 *C. melitaearum* cocoons in this experiment, 35 (26.5%) of which were parasitized by *G. agilis*. Larger host cocoons were parasitized at a higher rate than smaller host cocoons, with parasitized cocoons weighing 2.56 ± 0.07 mg, and unparasitized cocoons weighing 2.33 ± 0.05 mg on average ($\chi^2 = 5.91$, $p = 0.01$). There was no significant difference in the rate of successful parasitism (FI 30.3% and NL 22.7%), development time (14.86 ± 0.27 days), size of *G. agilis* (0.93 ± 0.08 mg) or efficiency of conversion (0.36 ± 0.03) between the two *G. agilis* origins.

Sequestration of iridoid glycosides from *C. melitaearum*

Gelis agilis hyperparasitoids developing in *C. melitaearum* retained small but significant amounts of iridoid glycosides as adults. The pattern of retention differed between Finnish and Dutch *G. agilis* (Fig. 3). *Gelis agilis* from both origins retained similar amounts ($\sim 0.0020\%$ dry wt) of the iridoid glycoside aucubin. However, while Dutch *G. agilis* contained only trace amounts of catalpol, Finnish *G. agilis* retained an average of $0.0032 \pm 0.001\%$ dry weight catalpol (Fig. 3A;

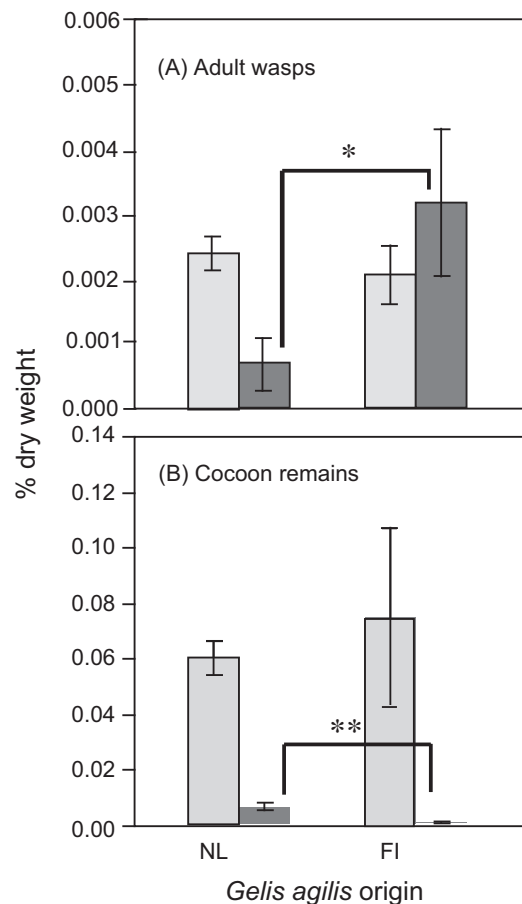


Fig. 3. The mean (\pm SE) percent dry weight of the iridoid glycosides aucubin (light) and catalpol (dark) in Dutch and Finnish *G. agilis* adults (A) and remains of the *C. melitaearum* host after eclosion (B). * t -Ratio = 2.99, $p = 0.005$ ANOVA; ** t -ratio = 2.54, $p = 0.015$.

ANOVA t -ratio (1,37) = 2.99, $p = 0.005$). Wasps from both origins left considerable amounts of aucubin (~ 0.06 to 0.07% dry wt) in the remains of the host cocoon when they became adults (Fig. 3B), but left only small amounts of catalpol in the cocoon remains. Corresponding to the pattern in adult wasps, *G. agilis* of Finnish origin left less catalpol in cocoons than those of Dutch origin (Fig. 3B; ANOVA t -ratio (1,37) = 2.54, $p = 0.015$).

In contrast to *G. agilis*, adult *L. nana* contained only trace amount of catalpol (0.00014–0.00039% dry weight), and even less aucubin when developing in chemically defended *C. melitaearum* cocoons. The amounts of iridoid glycosides retained in adult *L. nana* were significantly lower than those retained in adult *G. agilis*, regardless of whether all wasps were compared ($F(1,44) = 10.41$, $p = 0.002$) or whether only wasps from the same origin (NL) were included in the analyses ($F(1,32) = 12.96$, $p = 0.001$). *Lysibia nana* also left little or no iridoid glycosides in the cocoon remains, with the exception of one out of the six cocoon samples in which 0.015% dry mass catalpol was detected.

Discussion

We tested the hypothesis that two secondary parasitoids, *G. agilis* and *L. nana*, both generalists, but the second primarily associated with hosts in the genus *Cotesia*, would perform better on the chemically undefended host *C. glomerata* than on the chemically defended host *C. melitaearum*. We also predicted that *G. agilis* from the Netherlands, where *C. melitaearum* and potential hosts associated with iridoid glycoside feeding caterpillars are absent, would be less successful using the host *C. melitaearum* than *G. agilis* from Finland, that commonly use *C. melitaearum* as a host. We evaluated the hyperparasitoids with respect to rate of successful parasitism, offspring development time, size, and conversion efficiency. We assume that for most parasitoids and hyperparasitoids, fast development is advantageous because it decreases vulnerability to predation and generation time, and that a larger size is associated with increased longevity, fecundity, and reproductive success (Quicke 1997; Eilers & Jervis 2003; Harvey 2008; Joyce, Bernal, Vinson, & Lomeli-Flores 2009). This study is the first, as far as we know, to have examined local responses to chemical defence at the fourth trophic level.

Contrary to our prediction, we found that the defended host *C. melitaearum* is not less suitable for development of either of the parasitoid species than the undefended *C. glomerata*. The rate of successful parasitism was equal on the two hosts. Both *L. nana* and *G. agilis* reached a greater size on *C. glomerata*, which is a bigger host. However, they both developed faster and had a higher conversion efficiency (measured as hyperparasitoid adult weight/host cocoon weight) on *C. melitaearum*. The high performance on *C. melitaearum* is especially surprising for *L. nana*, first because previous studies have shown that this species is especially well adapted to *C. glomerata* (Harvey et al. 2011), and second because, in the area from which it was collected, *L. nana* has no recent history with iridoid glycoside sequestering hosts. In fact, *L. nana* was much more successful than *G. agilis* on both host species.

The analyses of iridoid glycosides in the hyperparasitoids developing on *C. melitaearum* revealed some interesting results. Insects may excrete, metabolize or sequester iridoid glycosides (Opitz & Müller 2009; Lindstedt, Reudler, Ihalainen, Lindström, & Mappes 2010). HPLC analysis of the iridoid glycoside content of *L. nana* developing from *C. melitaearum* indicates that the iridoid glycosides from *C. melitaearum* were broken down in the gut (Bowers 1991; Dobler 2001) instead of being excreted unmetabolized or retained in the body. In contrast, *G. agilis* both retained iridoid glycosides as adults and left some in the host cocoon unmetabolized (as meconium), or in the host remains. In order to sequester iridoid glycosides an insect must isolate the ingested compounds from the gut glucosidases and transport them to the hemolymph or other body tissue by an unknown mechanism (Pankoke, Bowers, & Dobler 2010). This mechanism probably comes at a cost (Smilanich et al. 2009; Lindstedt et al. 2010), but has evolved in many insect

lineages (Dobler 2001), and may be adaptive (Harvey et al. 2005). The amount of iridoid glycosides experienced by *G. agilis* and *L. nana* is small since *C. melitaearum* cocoons contain on average about 0.04% dry weight iridoid glycosides (Reudler et al. in prep). It is even smaller in comparison to the amounts in host plants in Åland (on average about 3% dry weight: Nieminen et al. 2003), and in the larvae of the host *M. cinxia* (1 to 3% dry weight: Suomi et al. 2002). It is possible that a higher concentration of iridoid glycosides would have a negative effect on these wasps.

The amount of iridoid glycosides retained by *G. agilis* adults is a small fraction of what is in the host. This amount of stored defensive chemicals may be small, but can still have ecological consequences (Lindstedt et al. 2010). Though the need for defence might be presumed to decrease at higher trophic levels, developing *G. agilis* potentially fall prey to tertiary parasitoids, adults may be prey to spiders and other arthropods, and for any animal there is the need for generalized defence against disease. Iridoid glycosides are associated with defence against all of these enemies in lower trophic level species (Theodoratus & Bowers 1999; Biere et al., 2004; Reudler et al. 2011), and should function in the same way at higher trophic levels. Finnish *G. agilis* concentrated more of the iridoid glycoside catalpol than did *G. agilis* from the Netherlands. Interestingly, catalpol has been found to be the more efficiently sequestered of the two iridoid glycosides (Bowers & Collinge 1992; Camara 1997). It is also more deterrent to predators (Bowers & Collinge 1992; Dyer & Bowers 1996; Nieminen et al. 2003). While the consequence of the difference in metabolism of iridoid glycosides between the *G. agilis* origins is not known, it is notable that they cope differently with the chemical defence, and that those originating from an area where the chemically defended host is common, retain more catalpol than those that do not normally encounter iridoid glycosides.

While the host ranges of secondary parasitoids are not well known, it is generally thought that they have broad host ranges because a single host species is an unreliable resource at higher trophic levels (Holt & Hochberg 1998; Brodeur 2000; van Nouhuys 2005). Thus, that a species with a wide host range should be sensitive to plant derived allelochemicals is at odds with the needs of a species living towards the terminal end of the food chain. Our results support the argument that these high trophic level species are able to use a range of host species. Although *L. nana* has mostly been reared from cocoons of *C. glomerata* and is very well suited to that host (Harvey 2008; Harvey et al. 2009, 2011), records of other *Cotesia* host species in the field (Schwarz & Shaw 2000; Stefanescu et al. 2009), and the suitability of *C. congregata* (Harvey, van Dam, Witjes, Soler, & Gols 2007) and now *C. melitaearum* in the lab, suggest that it also possesses physiological mechanisms enabling it to face a potentially diverse array of chemical challenges. *Gelis agilis* is known from host records to be a ubiquitous generalist (Schwarz & Shaw 1999). Here we show that though it can use a range of hosts, there is variation among populations in how the hosts

are used. This variation is not based simply on the ability to parasitize available hosts, but rather in the metabolism of defensive chemicals.

Acknowledgments

We thank I. Merikanto, S. Ehonen and T. Lahtinen for laboratory assistance, the Ålands Naturbruksskola for laboratory facilities in Finland, and Klaus Hvvemeyer and two anonymous reviewers for helpful comments on the manuscript. Academic Academy of Finland grant nos. 213547 and 128508 provided financial support.

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