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Host preference and offspring performance are linked in three congeneric hyperparasitoid species

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Abstract. 1. The optimisation theory predicts that insect mothers should oviposit on resources on which they attain the highest exclusive fitness. The development of parasitoid wasps is dependent on limited host resources that are often not much larger than the adult parasitoid.

2. In the present study preference and development in three congeneric species of secondary hyperparasitoids attacking cocoons of two congeneric primary parasitoids that differ significantly in size were compared. *Gelis agilis* (Fabricius) and *G. acarorum* (L.) are wingless hyperparasitoids that forage in grassy habitats, whereas *G. areator* (Panzer) is fully winged and forages higher in the canopy of forbs.

3. The three species were reared on cocoons containing pupae of a small gregarious endoparasitoid, *Cotesia glomerata* (L.), and a larger solitary species, *C. rubecula* (Marshall), both of which develop in the caterpillars of pierid butterflies.

4. Adult mass was correlated with initial cocoon mass in all three species, whereas development time was unaffected. Wasps were larger when developing in *C. rubecula*. However, for a given host mass, wasps were larger when developing on the smaller host, *C. glomerata*. This suggests that there is a physiological limit to hyperparasitoid size that was exceeded when *C. rubecula* served as host.

5. All three hyperparasitoids strongly preferred to attack cocoons of the larger species, *C. rubecula*, often avoiding cocoons of *C. glomerata* entirely.

6. Preference and performance are correlated in the three *Gelis* species. However, owing to variation in the distribution and thus abundance of their hosts, it is argued that cumulative fitness may be still higher in the smaller host species.

Key words. Body mass, *Cotesia*, development time, fitness, *Gelis*, host, *Pieris*.

Introduction

The optimisation theory predicts that insect mothers should preferentially oviposit on resources on which they and their progeny attain the highest realised fitness (Roff, 1992). The preference–performance hypothesis has been explored in many insects, with much of the focus being on herbivores (Mayhew, 2001). Because many immature herbivores are largely immobile, the plant selected by the mother ultimately is the one on which her larvae are dependent for their food (Craig *et al.*, 1986; Scheirs *et al.*, 2000; Agosta & Klemens, 2009; García–Robledo & Horvitz, 2012).

Parasitoid wasps have also been used as model insects to test the preference–performance hypothesis because, unlike most herbivores and predators, their development is dependent on limited resources (their hosts) that are often not much larger than the adult parasitoid. In fact, parasitoids are probably more suitable organisms for testing the preference–performance hypothesis, because most parasitoid species are obligated to feed and develop on the host on or in which they hatch (Godfray, 1994). They are thus under strong selection to exploit,

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allocate, and utilise limited host resources for different and potentially competing fitness functions, such as reproduction and survival (Jervis *et al.*, 2008). It is well established that the development of parasitoid wasps varies with a suite of host-related characteristics including the size, stage or age of the parasitised host, the presence of competing organisms inside the host (e.g. other parasitoids and/or pathogens), host nutritional status, and the quality of host diet (Godfray, 1994; Harvey, 2005; Ode, 2006).

Optimal resource allocation to body size has often been considered the main target of selection in parasitoids (King, 1989; Mackauer & Sequeira, 1993), because body size in males and females is often correlated with sperm or egg production and thus lifetime reproductive success (Charnov, 1982; Boivin, 2012). Furthermore, in many parasitoids, adult wasp size has been found to be positively correlated with host size at oviposition (Harvey, 2005). This is particularly true amongst idiobiont parasitoids (Askew & Shaw, 1986) that attack non-growing stages, such as eggs or pupae, or else hosts that are permanently paralysed at oviposition. For idiobionts, host resources remain ostensibly static during parasitoid development, and parasitoid size is often closely tailored to the size of the host at oviposition (Salt, 1940; Arthur & Wylie, 1959; Heaversedge, 1967; Sandlan, 1982; Moratorio*,* 1987; Otto & Mackauer, 1998; Fidgen *et al.*, 2000; Harvey *et al.*, 2006). However, older hosts (at least in the case of pupae) are often of a lower quality than younger hosts because host tissues have undergone some degree of differentiation and sclerotisation making them less palatable for offspring (Mackauer & Sequeira, 1993; Wang & Liu, 2002; Harvey *et al.*, 2006).

Thus far, most studies of preference and development in idiobionts have been based on egg parasitoids (Ruberson & Kring, 1993; Stevens, 1995; Kivan & Kilic, 2002; Luhring *et al.*, 2004). Egg parasitoids are well studied because they are important in biological control programmes and kill the host before it is able to inflict damage on the plant on which the eggs were laid (Godfray, 1994). Considerably fewer studies have examined the link between host choice and offspring development in pupal parasitoids, which is less surprising given that they attack post-feeding hosts. Evidence suggests that pupal parasitoids prefer to oviposit on higher quality hosts (Ueno, 1997; Zhao *et al.*, 2013). Moreover, virtually nothing is known about the link between these parameters in secondary hyperparasitoids, i.e. parasitoids that attack the pupal stages of other parasitoids. Hyperparasitoids can have a negative impact on biological control programmes via the imposition of trophic cascades, although the biology and ecology of most species is poorly known (Sullivan, 1987; Sullivan & Völkl, 1999). In spite of this, the cocoons of some primary parasitoids can harbour several to many hyperparasitoid species (McDonald & Kok, 1991; Poelman, 2008).

Like several other groups in the Hymenoptera, parasitoids exhibit haplo–diploid reproduction whereby male wasps are produced by unfertilised eggs and female wasps by fertilised eggs (Godfray, 1994). Because they can therefore manipulate the sex of their offspring at oviposition, host quality models also generally assume that female parasitoids preferentially lay female eggs in large (=high quality) hosts and male eggs in small (=low quality) hosts (Charnov, 1982). This is because the cost of egg production in female wasps is proportionately higher than the cost of sperm production in males (but see Boivin, 2012). Many published studies provide data that supports these models (see Godfray, 1994 for a discussion) although no, or even opposite effects of host size on female production have also been observed (Harvey *et al.*, 2004).

In this study we compare host preference and performance in three species of solitary secondary hyperparasitoids in the genus *Gelis* (Hymenoptera: Ichneumonidae). Previous studies have shown that species in this genus exhibit very low fecundities and can lay only 1–2 eggs a day, even when host numbers are not limiting (Harvey, 2008; Harvey *et al.*, 2011; Visser *et al.*, 2014). Species of *Gelis* are known to exhibit extremely broad host ranges, including spider eggs sacs and moth pupae, as well as parasitoid cocoons (Bezant, 1956; Cobb & Cobb, 2004). However, cocoons of primary parasitoids in the genus *Cotesia* appear to be particularly susceptible to hyperparasitism by species of *Gelis*. In North America, for instance, cocoons of *Cotesia melanoscela* (Ratzeburg), an introduced biological control agent of the gypsy moth *Lymantria dispar* (L.), are attacked by several *Gelis* species in tree habitats (Weseloh, 1978; Wieber *et al.*, 1995, 2001). Here we compare the preference and performance of two wingless *Gelis* species, *G. agilis* (Fabricius) and *G. acarorum* (L.) and the winged *G. areator* (Panzer) (Fig. 1). All three species are common in the western Palearctic, although the last two have been little studied. The wingless *Gelis* species are restricted to grassy habitats, whereas *G. areator* prefers to forage higher up in the canopy of forbs (Harvey *et al.*, 2014). *Gelis agilis* reproduces asexually and has been recorded as a hyperparasitoid of *Cotesia glomerata* (L.) and *C. melitaerum* W as well as non-parasitoid hosts (Schwarz & Shaw, 1999; van Nouhuys & Hanski, 2000; Harvey, 2008; van Nouhuys *et al.*, 2012) whereas the other two species reproduce sexually (Visser *et al.*, 2014) but have been little studied and their actual host ranges are poorly known. The three *Gelis* species must feed on host blood in order to produce eggs.

Preference and performance of the three *Gelis* species were compared by giving females a choice between cocoons of the gregarious *C. glomerata* and the solitary *C. rubecula* (Marshall), the latter of which is 20–50% heavier than the former. Hyperparasitoids were allowed to choose between cocoons of the two species and two fitness correlates – adult size and egg-to-adult development time – were compared in recently emerged wasps. Offspring sex ratios were also compared in *G. acarorum*. Our main hypothesis is that the three *Gelis* species will preferentially oviposit in cocoons of the larger *C. rubecula*, as they will attain bigger sizes thereby attaining a higher fitness, and that in *G. acarorum*, offspring sex will favour the production of females in larger host.

Methods and materials

Insects

Hosts and parasitoids were maintained at 25 ± 1 °C under a LD 16:8 h regime. Cultures of *Pieris brassicae* (L.), *P. rapae* (L.), *C. glomerata*, and *C. rubecula* were obtained from insects reared

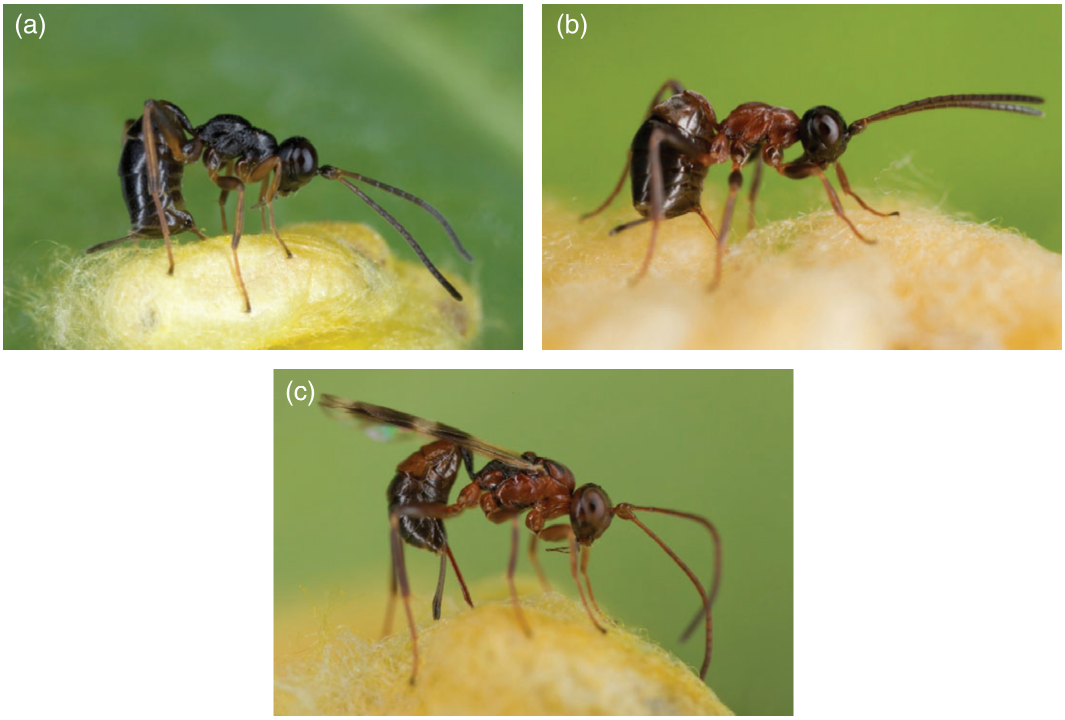

Fig. 1. The three *Gelis* species compared in this study. (a) *Gelis agilis*; (b) *G. acarorum*; and (c) *G. areator*.

at Wageningen University (WUR), the Netherlands. These had been originally collected from agricultural fields in the vicinity of the University. All pierid larvae used in these experiments had been maintained on *Brassica oleracea* var. Cyrus L. (Brussels sprouts) at WUR.

Cotesia glomerata and *C. rubecula* were reared according to the following protocol. Adult female *C. glomerata* wasps typically oviposit 10–40 eggs into first (L1) to third (L3) instars of *P. brassicae*. During their development the parasitoid larvae feed primarily on host haemolymph and fat body. When mature, the larvae emerge from the host caterpillar late during its final instar, and immediately spin cocoons on the host plant adjacent to the host, which perishes within a few days. *Cotesia rubecula* lays single eggs into L1–L2 larvae of *P. rapae*, but larvae feed and develop in a similar way as larvae of *C. glomerata* except that they egress from L4 hosts. In the laboratory both parasitoid species were reared in separate cages on their respective hosts themselves reared on cabbage (cv. Cyrus) plants. Once weekly a cabbage leaf containing L1 caterpillars of either *P. brassicae* or *P. rapae* was placed into a cage containing 50–100 *C. glomerata* or *C. rubecula* wasps, respectively, which were allowed to parasitise the caterpillars over the course of 15–30 min. The caterpillars were then removed and placed separately in cages with 3–4 cabbage plants according to host species and parasitoid until parasitoid egression and cocoon formation. These cocoons were collected in the morning (within 1–2 h of formation) and then used in the experiments (below).

Adult wasps from the three *Gelis* species were originally obtained from cocoons of *C. glomerata* attached to stems of black mustard plants adjacent to the Institute of Ecology in Wageningen. In all three species egg production is contingent on host-feeding activity by the adult female parasitoids. Before gelines are able to produce eggs, adult females deliberately masticate pupae with their ovipositor, and then sip haemolymph that oozes from the wound. Proteins obtained from the haemolymph are utilised by the parasitoids for egg production and possibly maintenance (Harvey, 2008; Visser *et al.*, 2014). Once mature eggs are available, the wasps lay single eggs onto the surface of host (pre)pupae inside their cocoons. In culture all *Gelis* species were maintained exclusively on 1- to 2 day-old cocoons of *C. glomerata*. After emergence, hyperparasitoids were kept in large (20 cm diameter) Petri dishes at 10 °C. Each hyperparasitoid species was kept separately in insect rearing cages.

Experimental protocol

Fresh cocoon clusters of *C. glomerata* were teased apart using forceps and a caecum and pooled in Petri dishes to randomise broods. Cocoons of *C. rubecula* were also kept in separate Petri dishes after collection from rearing cages. Fresh (<24 h old) cocoons of each species were weighed individually, and placed in rows of four in Petri dishes. Two cocoons of each *Cotesia* species were placed in the dishes and positions were alternated across replicate dishes, after which single females of *G. agilis, G. acarorum* or *G. areator* were introduced. Only one female of one hyperparasitoid species was released into a single Petri dish with four cocoons; thus each hyperparasitoid female had a choice of four cocoons, two of *C. rubecula* and two of *C. glomerata*. Each hyperparasitoid female was allowed to parasitise any of the four cocoons over 24 h. These were then removed and placed in separate marked vials according to species and female number. Each of the hyperparasitoid species has a very low reproductive potential. A total of at least 30 females of each species were used to generate data sets for the development and preference assays. The fate of each cocoon was ultimately determined under the following categories: dead cocoon (neither a primary parasitoid nor a hyperparasitoid emerged); *C glomerata* or *C. rubecula* (primary parasitoid) emerged; and *Gelis* sp. (hyperparasitoid) emerged. Newly emerged hyperparasitoids were sexed, anaesthetised using CO_2 and then weighed on a Mettler Microbalance (accuracy ±1 μg). Development time was recorded as the number of days between initial access to cocoons and adult eclosion. Only a small number of females emerged in hosts parasitised by *G. areator*, but many more in hosts parasitised by *G. acarorum*. Therefore, in the latter species, offspring sex ratios were compared in mothers that produced male and female progeny in cocoons of both hosts.

Statistical analyses

Six variables were considered in the statistical analysis of the cocoon preference bioassays: the number of eclosed *Gelis* wasps, the number of eclosed *Cotesia* wasps, and the number of dead cocoons for each of the two *Cotesia* species out of a total of two cocoons. Data were analysed using a generalised linear model (GLM) with a binomial distribution and logit link function with *Gelis* species as main factor. In addition, the relative success of the three *Gelis* wasp species on *C. glomerata* and *C. rubecula* was compared. Here the response variable is the number of *Gelis* wasps emerging from *C. rubecula* cocoons out of the total number of wasps emerging from *C. glomerata* and *C. rubecula* combined. The effects of cocoon and hyperparasitoid species and their interaction on body mass and development time were also analysed with a GLM model, with cocoon mass as a covariate. Data points that deviated > 3 standard deviations from the mean were omitted from the data set (four data points on development time). Data were analysed for females and males separately. If any of the main factors in the model was significant, Tukey–Kramer multiple comparison tests were conducted to reveal differences among the means. To compare offspring sex ratios in *G. acarorum*, a paired *t*-test was performed. The data were analysed in SAS version 9.3 (SAS Institute Inc., Cary, NC, USA).

Results

Host preference of three Gelis species

Irrespective of hyperparasitoid species ($\chi^2_2 = 0.02$, $P = 0.99$), more *Gelis* wasps successfully eclosed from *C. rubecula* than from *C. glomerata* cocoons (Fig. 2). Approximately 80% of the *Gelis* wasps emerged from *C. rubecula* and 20% from *C. glomerata* cocoons. However, the absolute number of eclosing adult hyperparasitods differed with *Gelis* species on *C. rubecula* ($\chi^2_2 = 35.1$, $P < 0.001$) and marginally on *C. glomerata* cocoons ($\chi^2_2 = 5.6$, $P = 0.061$). In general, *G. agilis* females parasitised a smaller percentage of the four available cocoons in individual arenas than *G. areator* and *G. acarorum*. The relative number of dead cocoons also depended on the parasitising *Gelis* species (*C. rubecula*, $\chi^2_2 = 25.5$, $P < 0.001$; *C. glomerata,* $\chi^2_2 = 8.6$, $P = 0.014$) and was also different for each of the two host species (Fig. 2). More than 60% of *C. glomerata* cocoons had died compared with approximately 30% of *C. rubecula* cocoons when exposed to *G. acarorum*, whereas these numbers were almost reversed for the interaction with *G. agilis*, where mortality of *C. glomerata* was less than that of *C. rubecula* cocoons. When exposed to *G. areator*, mortality was more similar on the two host species and varied between 40% on *C. rubecula* and 47% on *C. glomerata* cocoons (Fig. 2). The number of cocoons that survived and developed into healthy *C. rubecula* wasps was low and different when exposed to the three hyperparsitoid species ($\chi^2_2 = 14.0$, $P < 0.001$). None of the *C. rubecula* cocoons exposed to *G. acororum* developed into healthy primary wasps, whereas a small number of the cocoons (<10%) developed into healthy *C. glomerata.* More cocoons developed into healthy *C. glomerata* wasps. Survival of *C. glomerata* cocoons depended on the *Gelis* species to which it had been exposed ($\chi^2_2 = 21.6$, $P < 0.001$), with approximately twice as many *C. glomerata* wasps eclosing when the cocoons were previously exposed to *G. agilis* and *G. areator*, respectively, than to *G. acarorum* (Fig. 2).

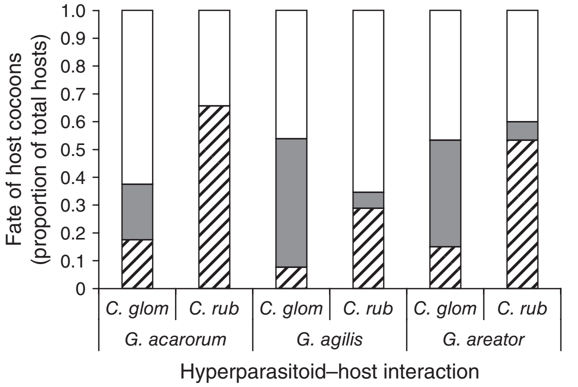

Fig. 2. Fate of two host cocoon species *Cotesia glomerata* and *C. rubecula* when exposed to one of three different congeneric hyperparasitoid species, *Gelis acarorum*, *G. agilis* or *G. aerator*, in a dual choice bioassay. The fate of the cocoon was ultimately recorded as dead cocoon (white bar) *C glomerata* or *C. rubecula* emerged (shaded bar) emerged or *Gelis* sp. emerged (hatched bar). Number of tested individuals was 30 for *G. areator*, 52 for *G. agilis* and 83 for *G. acarorum.*

The relationship between host cocoon mass and offspring sex in *G. acarorum* was not significant ($t_{10} = 0.18$, $P = 0.86$). Female wasps that laid eggs in both host species ($n = 11$) chose cocoons of almost identical mass in which to lay male or female offspring: males = 41.5 mg, females = 4.23 mg. In almost half (=5) of the arenas the mothers laid male eggs into larger hosts than female eggs.

Fitness correlates of the three Gelis species when developing on two different host species

The adult mass of the three parasitoid species was positively correlated with the mass of host cocoons

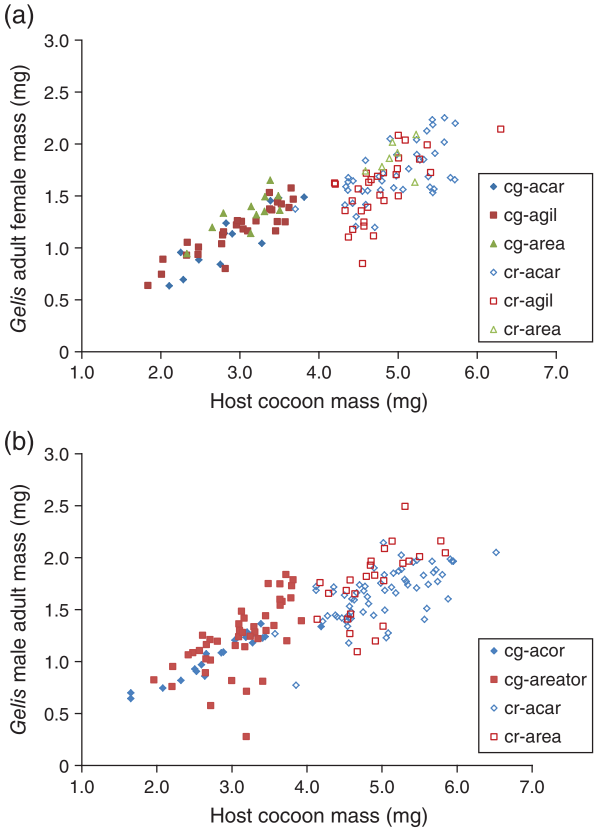

Fig. 3. Adult mass of females (a) and males (b) of three different congeneric hyperparasitoid species, *Gelis acarorum* (diamonds), *G. agilis* (squares), and *G. areator* (triangles) developing from two different host species, *Cotesia glomerata* (closed symbols) or *C. rubecula* (open symbols) in relation to host cocoon size. NB: *G. agilis* is asexual. Data were obtained from the experiment described in Fig. 1.

(females, $F_{1,123} = 129$, $P < 0.001$, males $F_{1,148} = 291$, $P < 0.001$; Fig. 3a,b) with *C. rubecula* producing heavier cocoons than *C. glomerata* (Fig. 3a,b). When cocoon size was accounted for, there was still a significant effect of host species on female body mass ($F_{1,123} = 10.2$, $P = 0.002$), but not on male body mass ($F_{1,148} = 2.10$, $P = 0.15$, only *G. acarorum* and *G. areator* produce males). For a given host size wasps were smaller when emerging from *C. rubecula* than from *C. glomerata*. However, there was no overlap in the cocoon masses of the two species. Therefore, the intercept between the two regressions were significantly different whereas the slopes were not. For both males and females adult mass differed with *Gelis* species. For a given cocoon mass *G. areator* was significantly heavier (~9%) than *G. agilis* (females only) and *G. acarorum* (Tukey–Kramer multiple comparison tests $P < 0.05$). The last two species attained similar body masses for a given cocoon mass (Tukey–Kramer tests, $P > 0.05$).

Egg-to-adult development time did not covary with cocoon mass (females, $F_{1,122} = 1.29$, $P = 0.26$, males $F_{1,145} = 0.93$, $P = 0.34$), or with host species (females, $F_{1,122} = 0.03$, $P = 0.87$, males $F_{1,145} = 1.08$, $P = 0.30$). There was only a significant effect of *Gelis* species on development time (females, $F_{2,122} = 5.44$, $P = 0.005$, males $F_{1,145} = 105$, $P < 0.001$), with *G. acarorum* developing *c.* 10% more slowly than *G. areator* and *G. agilis* (females only) (Fig. 4a,b).

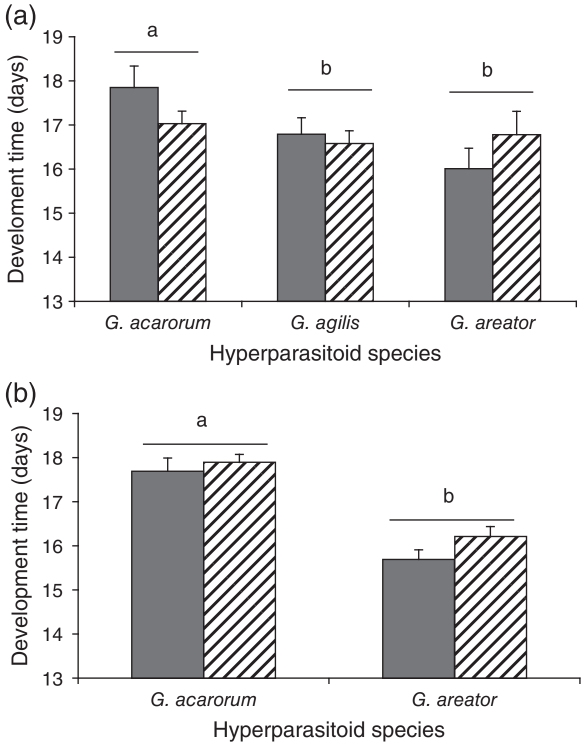

Fig. 4. Mean (+SE) egg-to-adult development of females (a) and males (b) of three different congeneric hyperparasitoid species, *Gelis acarorum*, *G. agilis*, and *G. aerator* developing on two different host species, *Cotesia glomerata* (shaded bars) or *C. rubecula* (hatched bars). NB: *G. agilis* is asexual.Data were obtained from the experiment described in Fig. 1. Bars with different letters are significantly different ($P < 0.05$; Tukey–Kramer tests).

Discussion

The results of the current investigation reveal that all three *Gelis* species significantly preferred to oviposit in cocoons of *C. rubecula* over cocoons of the smaller congener *C. glomerata*. This preference was tailored with performance, as wasps were larger when developing on the larger cocoons of *C. rubecula*, although intra-specifically development time did not differ. Cocoons of *C. rubecula* wasps are generally some 20–40% larger than adult *C. glomerata* wasps (Brodeur *et al.*, 1998; Harvey *et al.*, 1999), and thus provide more resources for the immature hyperparasitoids, which consume them piecemeal before pupation. However, for a given host cocoon size, adult hyperparasitoids were larger when developing in the smaller host species, *C. glomerata*. Two factors could account for this discrepancy. First, the per capita quality of resources of *C. rubecula* may have been lower than in *C. glomerata*. Second, *C. rubecula* may contain excess resources for the three hyperparasitoids to be able to successfully consume and assimilate. In this scenario, the wasps have an upper-size threshold that is genetically determined and any excess resources are excreted with their faeces.

Offspring sex ratios in the two sexually reproducing *Gelis* species showed no evidence of female bias in large hosts as predicted by optimality models (e.g. Charnov, 1982). In *G. areator*, few females were produced in either host species, whereas *G acarorum* did not exhibit a clear preference to lay male or female eggs in cocoons of the larger host. In terms of cocoon fate, significantly more *C. glomerata* survived to eclosion than *C. rubecula*. Many of the cocoons of both host species, however, failed to yield adult hyperparasitoids, and cocoon mortality was higher for *C. glomerata* than for *C. rubecula* cocoons. This could be because of destructive host-feeding behaviour in which many of the hosts were killed in order for the adult females to imbibe fluids used for egg production (Jervis & Kidd, 1986). In some parasitoid species, up to 80% of the host mortality in the field can be attributed to host-feeding (Flanders, 1947). Our results indeed suggest that female hyperparasitoids choose to oviposit on the larger, higher quality host (*C. rubecula*) and to host-feed on the smaller, lower quality hosts (*C. glomerata*). In the field, they may also discriminate in this way, thus 'bet-hedging' on finding more profitable large hosts later.

Links between preference and performance – described in one study as 'optimal synchronization' – (Reznik *et al.*, 1992) have been shown in some parasitoids (van Alphen & Drijver, 1982; Nechols & Kikuchi, 1985; Hopper, 1986; Eben *et al.*, 2000; Videla *et al.*, 2006; Gols *et al.*, 2009) but not in others (Reznik *et al.*, 1992; Chau & Mackauer, 2001a,2001b; Henry *et al.*, 2005). Several vitally important context and trait-dependent factors may affect host selection in parasitoids, but these have been little explored. In the field, host abundance may vary dramatically in space and time, and this in turn will affect a foraging parasitoid's perception of environmental quality. When high-quality hosts are scarce, or else when the physiological condition of a parasitoid is low or deteriorating (as in older individuals or when food is scarce), then discrimination can break down leading to the acceptance of hosts that would otherwise be avoided by young, healthy wasps in a host-rich environment (van Alphen & Visser, 1990). Furthermore, host discrimination may be much less apparent in parasitoids that produce numerous, tiny 'hydropic' eggs (e.g. eggs with a low yolk content).

Many parasitoids attack relatively scarce host types, or, in the case of secondary hyperparasitoids, late stages of primary parasitoid hosts in the third trophic level. Previous work has shown that egg production in *G. agilis* is extremely low – females only produce a maximum of 1–2 eggs per day – and even under optimal conditions with excess hosts, lifetime progeny production is still often less than 30 (Harvey, 2008). Moreover, like many ectoparasitic pupal parasitoids, the three *Gelis* species studied here all combine host-feeding behaviour with the production of very large anhydropic eggs (e.g. eggs that are fully-yolked). The per capita amount of resources invested into each egg is very high relative to body mass (Jervis *et al.*, 2001, 2008), meaning that each egg represents a very valuable resource. Parasitoids exhibited traits such as the production of very small numbers of large eggs, extended host handling times, a limited egg storage capacity, and extended longevities (Mayhew & Blackburn, 1999). Whereas many koinobionts can lay an egg in a second or less, it often takes 30 min or longer for *Gelis* spp. to lay a single egg; in extreme cases handling times up to 24 h has been observed. The three gelines under study here exhibit developmental and reproductive strategies favouring metabolic investment towards extended longevity and low fecundity.

Taking all of the above criteria into consideration, it is not surprising that the three *Gelis* species strongly preferred to attack *C. rubecula* cocoons, as the wasps were much larger when developing on this host than on *C. glomerata*. Clearly eggs in species of *Gelis* are a valuable resource and it pays the wasps to optimise the host in which they are laid. However, for a given host cocoon size, *C. rubecula* was marginally inferior to *C. glomerata* in terms of host expolation efficiencies, indicating that the differences between the hosts on parasitoid development are mainly quantitative, but there are some qualitative differences as well. The three hyperparasitoid species also appeared to differ in host exploitation efficiencies. For a given cocoon mass *G. areator* was significantly heavier than the other two species *G. agilis* and *G. acarorum* which had similar conversion efficiencies. Moreover, both *G. areator* and *G. agilis* completed development into adults more rapidly than *G. acarorum*. We have recently found that the relative metabolic rate of *G. areator* is significantly higher than that of *G. agilis* and *G. acarorum* (B. Visser *et al.*, unpublished). This could be because the possession of wings increases metabolic activity and is a pre-requisite for foraging in the canopy of forbs in early- and mid -successional grassland habitats.

Although parasitoids were larger when developing on the larger host, *C. rubecula*, fitness for adult females of the three hyperparasitoids may be higher if they encounter the smaller host species, *C. glomerata*. This is because *C. rubecula* is solitary and whereas *C. glomerata* is gregarious and up to 40 cocoons are tightly clustered together. This allows the hyperparasitoids to exploit more than a single cocoon when encountering a cluster. However, there are important caveats. The daily maximal reproductive potential of the three gelines is low: *G. agilis* produces a maximum of only one or two offspring per day, whereas *G. areator* and *G. acarorum* can only lay 4–5 eggs per day (Harvey, 2008; Harvey *et al.*, 2011; B. Visser *et al.*, unpublished). The pre-pupae and pupae of *C. glomerata* remain suitable for the hyperparasitoids for about 4–5 days, after which sclerotisation and differentiation of the host parasitoids into pharate adults renders them unpalatable. Harvey *et al.* (2011) found that the maximum number of *C. glomerata* cocoons that *G. agilis* could parasitise over 4–5 days under optimal conditions (mature hyperparasitoids given unlimited access to fresh cocoon clusters) was 7; more typically fewer progeny were produced. This reveals limitations in the functional responses of *G. agilis* and in all likelihood the other *Gelis* species. However, given that *G. areator* females are fully winged and can produce up to 130 progeny in their lifetimes, the *per capita* profitability of *C. glomerata* as hosts is probably balanced by their greater ability to disperse and thus cover a larger habitat foraging arena than in the case of the wingless *Gelis* species.

In summary, this study reports that three species of closely related congeneric secondary hyperparastoids clearly preferred to oviposit onto hosts on which their progeny were larger. This provides further empirical support for the 'mother knows best' principle which has formed the basis for many plant–herbivore and herbivore–parasitoid studies. However, we must point out that the experiments were carried out under fairly simplified conditions in which the hyperparasitoids had a choice between cocoons of a large and a small host that were in very close proximity. In nature, of course, the availability of host cocoons will almost certainly be different and choices based on significant differences in size and hence quality will either not be available or may be diffused within patches. Moreover, it was also be emphasised that *C. rubecula* is solitary whereas *C. glomerata* is gregarious. Consequently, a female hyperparasitoid may gain incrementally in terms of fitness by parasitising several *C. glomerata* cocoons compared with a single cocoon of *C. rubecula*, even when their progeny are larger on the solitary parasitoid. This is especially relevant for the two wingless gelines, which are clearly much more limited in their dispersal capability than *G. areator*. Given that most parasitoids are extremely time, rather than egg limited (Jervis *et al.*, 2001, 2008) the rejection of a host for oviposition based solely on size is extremely unlikely. However, the extent to which such evolutionarily refined decisions are made in parasitoids under natural conditions is currently unknown, and will be the focus of future studies.

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