

Corrigendum

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The family of the parasitoid *Eulophus pennicornis* was incorrectly stated in the title of this article as Braconidae. This should, in fact, have read Eulophidae. The correct title of the article should be:

Influence of host size on the clutch size and developmental success of the gregarious ectoparasitoid *Eulophus pennicornis* (Nees) (Hymenoptera: Eulophidae) attacking larvae of the tomato moth *Lacanobia oleracea* (L.) (Lepidoptera: Noctuidae).

The authors apologise for this error.

Influence of host size on the clutch size and developmental success of the gregarious ectoparasitoid *Eulophus pennicornis* (Nees) (Hymenoptera: Braconidae) attacking larvae of the tomato moth *Lacanobia oleracea* (L.) (Lepidoptera: Noctuidae)

H. A. Bell*, G. C. Marris, A. J. Prickett and J. P. Edwards

Central Science Laboratory, Sand Hutton, York, YO41 1LZ, UK

*Author for correspondence (e-mail: h.bell@csl.gov.uk)

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Summary

The relationship between clutch size, host size and progeny survival in the gregarious ectoparasitoid *Eulophus pennicornis* was investigated in a number of scenarios. When naive parasitoids were exposed singly to *Lacanobia oleracea* hosts, clutch size was strongly correlated with the size of the host. However, survival of parasitoid offspring was negatively affected by the size of the host such that, in larger hosts, greater wasp larval and pupal mortality was recorded. As a result, no gain in realised fecundity was achieved through parasitizing *L. oleracea* larvae of mass >0.4 g over hosts of mass between 0.2–0.3 g.

When exposed to populations of mixed stadium hosts (larvae in the fourth, fifth and sixth instars) during the entire lifespan of the wasp, host size and clutch size were correlated in early ovipositions (first three ovipositions). However, as the wasps aged, the relationship was much less apparent. When the parasitoid was restricted to foraging upon populations of sixth instar hosts only, no relationship between host size and clutch size was apparent. Exposure of the parasitoid to mixed and fixed

stadium host populations showed that final (sixth) stadium hosts were the most frequently parasitized (ca. 96% of parasitized hosts) and that the average numbers of eggs laid per wasp, and the number of hosts parasitized, was significantly lower when the parasitoid was provided with fourth or fifth instar hosts only.

The results indicate that the reproductive success of *E. pennicornis* does not increase with increasing host size or greater resource availability above a certain threshold, and that the physiological status of the host at the time of parasitism is the governing factor determining oviposition decisions and parasitoid survival. We conclude that *E. pennicornis* has been selected to preferentially utilize those hosts that maximize progeny survival and to adapt clutch size to the size of such hosts. We hypothesize that the major driver leading to the evolution of this strategy is the ability of the parasitoid to physiologically regulate the host.

Key words: parasitoid–host interactions, oviposition behaviour, host selection, host regulation, *Eulophus pennicornis*, *Lacanobia oleracea*.

Introduction

There has been much interest in the mechanisms that parasitoids use to determine the quality of a potential host and, in the case of gregarious species, oviposit clutches of eggs of sizes that are in proportion with the quality of that host. Theoretically, as the number of eggs oviposited increases per unit of host resource, the fitness of each individual that develops from that clutch will decrease (Charnov and Skinner, 1984). Therefore, successfully identifying the quality of the host, and adjusting clutch size accordingly, has important consequences for the fitness of a gregarious parasitoid (Godfray, 1987). Much recent work has built upon the clutch size hypothesis of Lack (1947), who originally suggested that female birds should produce clutch sizes that optimise the number of offspring that survive. This hypothesis has since been widely applied to studies of the parasitic Hymenoptera,

albeit modified to assert that the ovipositional behaviour of a parasitoid should generate clutch sizes that maximize the parent's fitness gain from a given clutch (Charnov and Skinner, 1984; Godfray et al., 1991).

Many factors can ultimately lead to the developmental success or failure of a parasitoid's brood and affect the fitness of the ensuing progeny. For example, overexploitation of a host through the oviposition of a large clutch in or on a small host may lead to increased sibling competition and high levels of mortality (Charnov and Skinner, 1983; Vet et al., 1994; Beckage and Gelman, 2001). Furthermore, large clutches for a given size of host reduce the unit of resource available to each wasp larva, and can have a significant effect on the ultimate fitness of those offspring that survive (Vinson and Iwantsch, 1980a; Bezemer and Mills, 2003). The fitness of a parasitoid's

progeny has frequently been linked to their size on emergence as adults (Hardy et al., 1992; Visser, 1994; Fidgen et al., 2000), and the ultimate fitness gain of a female parasitoid will be determined by the numbers of a given clutch that ultimately survive and their size (i.e. fitness) as adults (Godfray, 1994).

The factors that determine the clutch size that a female parasitoid will oviposit in or on a host have been extensively researched. Such work has demonstrated the importance of the ability of a female parasitoid to respond to variations in host quality as a means of maximizing her lifetime fitness. Several workers have demonstrated that the clutch sizes of gregarious parasitoids are frequently correlated with the size of the host (Takagi, 1986; Hardy et al., 1992; Zaviezo and Mills, 2002) and that the survival of progeny may be either density-dependent (Le Masurier, 1991; Vet et al., 1994) or density independent (Hardy et al., 1992). Further to this, within the range of host sizes that a parasitoid has the potential to parasitize, the physiological status of the host may differ considerably and thus may complicate the simplistic relationship between host size and clutch size (Vinson, 1976). As a result, critical factors, such as the nutritional and endocrinological status of the host, may come into play. This is an important consideration, especially since many parasitoids actively manipulate the physiological status of the host in order to facilitate the successful development of their progeny (see reviews by Edwards et al., 2001; Beckage and Gelman, 2004). Frequently, the success of such manipulation can be affected by the host's physiological status at the time of parasitism and, ultimately, the overall quality of the host can be seen to be a function of several factors, and not necessarily just one of increasing host size (Vinson and Iwantsch, 1980a; Rivers and Denlinger, 1995; Alleyne and Beckage, 1997; Husni and Honda, 2001).

The gregarious koinobiont ectoparasitoid *Eulophus pennicornis* (Nees) lays its eggs externally on the integument of late stadium larvae of several species of noctuid hosts (Shaw, 1987). Studies into the basic biology of this parasitoid, when parasitizing its usual laboratory host, the tomato moth *Lacanobia oleracea* (L.), have largely used penultimate instar larvae (fifth larval stadium), although final (sixth stadium) hosts were also reported to be readily parasitized (Marris and Edwards, 1995). No studies have elaborated on the ovipositional behaviour of this wasp, however, or investigated how it adapts its egg clutches to the size (and developmental stage) of the host. Despite the fact that this parasitoid has been the subject of a number of biological studies (Bell et al., 1999, 2001), no information is available about the effect of parasitizing different larval stadia (i.e. sizes) of *L. oleracea* on the reproductive success of the wasp, or on how its ovipositional behaviour compares with the strategies of other gregarious parasitoids.

For some ectoparasitoids, there is evidence that greater resource availability (i.e. the size of the host) per egg increases the size, and therefore the fitness, of the progeny that ensue (Hardy et al., 1992; Zaviezo and Mills, 2000). Therefore, attacking large hosts and provisioning the clutch with a greater

unit resource per egg could be considered adaptive if host quality was not deleteriously affected by increased size. Recent work, investigating koinobiont parasitoids, has made it apparent that host size at the time of parasitism may have little bearing on the fitness functions of some species (Harvey, 2000; Harvey et al., 2004). However, little information is available as to whether a similar situation occurs in gregarious koinobiont ectoparasitoids. Furthermore, Harvey and Strand (2002) hypothesized that progeny survival, not adult size, should be the primary selection target of a parasitoid, with all other fitness traits rendered secondary to this tenet. Here we report upon work designed to investigate the oviposition behaviour of *E. pennicornis*, and test elements of this hypothesis by investigating how host size and resource availability affects pre-adult survival and, secondly, how progeny survival reflects host choice in this gregarious ectoparasitoid species.

Materials and methods

Insect cultures

Newly emerged *Eulophus pennicornis* adults were derived from a laboratory culture maintained on late stadium larvae of the tomato moth *Lacanobia oleracea*, at 25°C, 70% RH and 16 h:8 h L:D, according to the methods described by Marris and Edwards (1995). *Lacanobia oleracea* larvae were derived from a laboratory culture maintained at 20°C, 70% RH, 16 h:8 h L:D and reared on an artificial diet (Poitout and Bues, 1974) in plastic sandwich boxes (150 mm×150 mm×75 mm) covered with muslin lids. Procedures for rearing, staging and synchronising larvae have been described previously (Corbitt et al., 1996). Experiments utilized larvae in the fourth, fifth and sixth stadia, hereafter referred to as L4, L5 and L6 hosts. All experiments were carried out at 25°C, 70% RH and 16 h:8 h L:D.

Effects of host size on clutch size

Newly ecdysed L5 and L6 larvae were taken from culture, placed individually into ventilated 250 ml plastic pots (Cryovac Europe, Poole, UK) and provided with a small cube of artificial diet. A single mated adult female *E. pennicornis* (ca. 48 h old), which had had no previous exposure to hosts, was introduced into each pot and a smear of 50% aqueous honey solution applied to the lid to act as a food source. Each host was exposed to the wasp until the latter had completed one oviposition. Any hosts that were not parasitized after 4 days were discarded, as *L. oleracea* typically start to lose mass and take on prepupal characteristics from the fourth day of the sixth stadium onwards. Following oviposition, the parasitoid was removed and the host weighed. The host was subsequently kept at 4°C for approximately 20 min to reduce activity, after which time it was anaesthetized with CO₂. Whilst immobile, the eggs present on each parasitized host were counted under a binocular microscope. Each host was then returned to its original pot and maintained until the parasitoid brood had completed development, left the host and pupated. On pupation, all

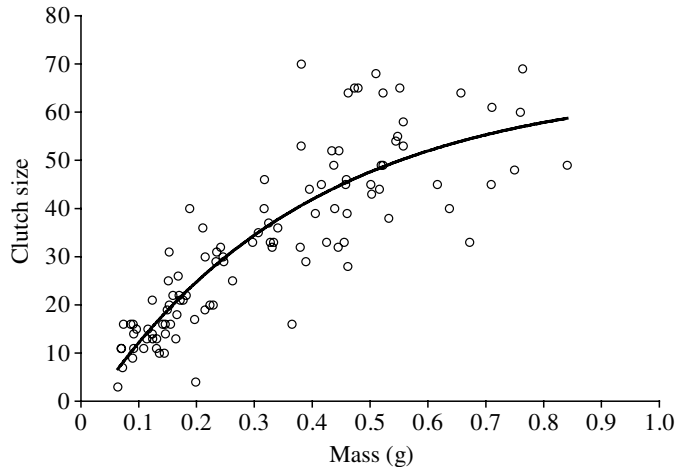


Fig. 1. The relationship between clutch size and host size for the first oviposition in naïve *E. pennicornis* females exposed to a single host larva in either the fifth or sixth stadium. Non-linear regression analysis: $y = 66.29 - (70.62 \times 0.0701^x)$, $r^2 = 0.74$, d.f. = 102.

parasitoid pupae were counted, the host cadaver removed to prevent contamination, and the wasp pupae subsequently monitored for adult emergence. A total of 50–60 parasitized larvae of both the L5 and L6 stadia were monitored in this way.

Host choice experiments

Lacanobia oleracea hosts in the L4, L5 and L6 stadia were removed from culture immediately after moulting from the preceding stadium. Three host larvae from each of the larval stadia were placed in tissue-lined plastic sandwich boxes and provided with artificial diet *ad libitum*. A total of 15 boxes were made up in this way, and a single mated female *E. pennicornis* (48 h post emergence) introduced into each box. The parasitoid was provided with a honey solution food source and allowed to forage until death. Subsequent to the release of the parasitoid, all host larvae were examined daily for parasitism and, on parasitism, were removed from the box. All parasitized larvae were replaced with insects of the same stadium (newly moulted) and, as unparasitized larvae pupated, hosts were replaced with newly moulted L4 caterpillars. This procedure allowed the wasps to be exposed to a mixed stadium population of developing hosts throughout their reproductive lifespans. All parasitized larvae were weighed and then rendered immobile through immersion in ethanol for approximately 1 min. The eggs on the host were counted as they were removed from the hosts with a pair of fine forceps under a binocular microscope. The total number of hosts parasitized, the numbers of eggs laid and the longevity of the ovipositing adult parasitoids was measured in all cases.

Fixed host stadium experiments

To investigate how the availability of a restricted range of host sizes affects the oviposition behaviour of *E. pennicornis*, the above experiment was repeated using host cohorts comprising exclusively L4, L5 or L6 *L. oleracea* larvae. All methods were

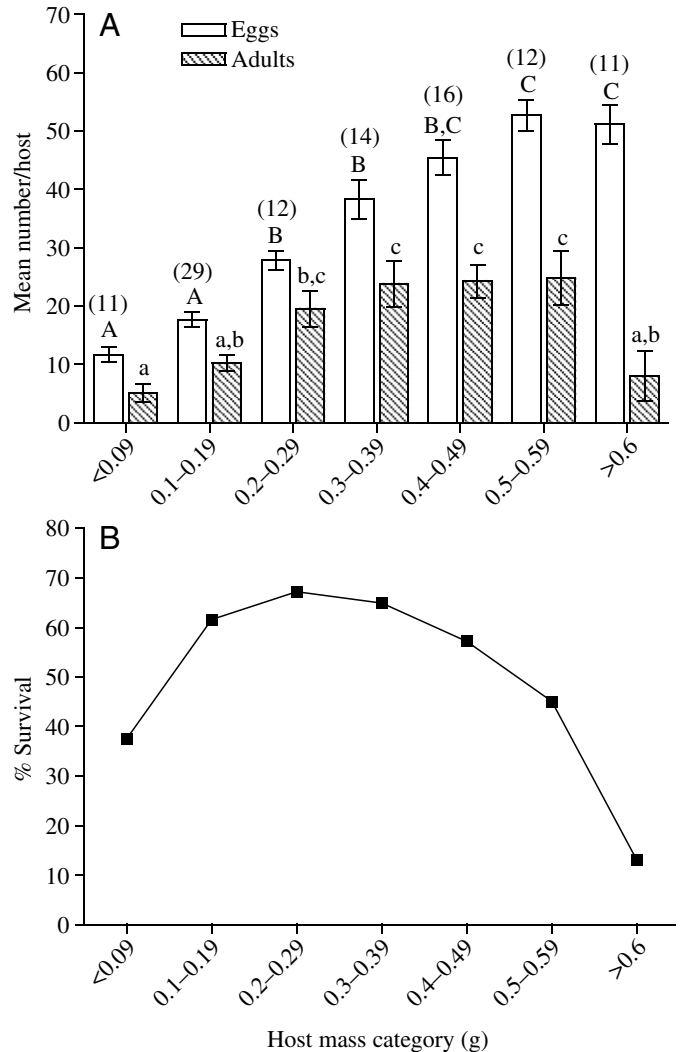


Fig. 2. (A) The numbers of eggs oviposited onto hosts of differing sizes, and numbers of adult wasps that subsequently emerged. Columns for each data series headed by different letters are significantly different (one-way ANOVA, $P < 0.05$). Values are means \pm S.E.M.; numbers in parentheses indicate the N value. (B) The mean percentage survival of clutches developing from egg to adult for parasitized hosts of each mass category.

as above, except that as hosts neared the end of the given stadium, and exhibited the signs of head capsule slippage, they were removed and replaced with newly moulted insects of the same stadium. Parameters measured were as described above.

Host growth following parasitism

To determine the growth of the host following parasitism, L5 and L6 hosts were parasitized individually in tissue-lined 250 ml plastic pots as described above. Non-parasitized hosts of the same developmental stages served as controls. Each host larva was weighed daily until all parasitoid larvae had left the host and pupated. A total of 15–20 hosts were monitored for hosts parasitized on either the first or second day after moulting to the stated stadium.

Statistical analysis

Regression analysis of clutch size data was conducted using the curve-fitting function of GraphPad Prism 4.01. All other statistical procedures were carried out using StatsDirect 2.2.3. Differences between parametric data sets were analysed by one-way analysis of variance (ANOVA) and means separated by Tukey–Kramer HSD *post-hoc* tests. Prior to ANOVA, all

data was subjected to equality of variance tests and, where variances differed significantly ($P < 0.05$), data was log-transformed. Comparisons between two data sets were conducted using Student's unpaired *t*-tests and, in all cases, the accepted level of significance was 5%.

Results

Clutch size, host size and developmental success

The relationship between clutch size and host size for the first oviposition of *E. pennicornis* females exposed to single *L. oleracea* hosts is shown in Fig. 1. The size of a given clutch was significantly correlated with the size of the host, and asymptotic non-linear regression described 74.3% of the variation. It was, however, apparent that whilst clutches oviposited onto smaller hosts (i.e. < 0.3 g) followed the generated trend closely, as hosts became larger the variability of the clutches with respect to host size was much more pronounced.

Parasitized hosts were maintained until the parasitoid broods had pupated and adult wasps had subsequently emerged (Fig. 2A). Each parasitized larva was categorised as belonging to one of seven mass categories ranging from < 0.1 g to > 0.6 g. Increasing mass had a significant effect on both the numbers of eggs laid ($F_{6,99} = 46.72$, $P < 0.01$) and the numbers of progeny that survived to adulthood ($F_{6,99} = 7.69$, $P < 0.01$). The mean number of eggs oviposited increased with the size of the hosts such that caterpillars of 0.5–0.6 g received an average of 52.7 ± 2.66 eggs as opposed to those of < 0.1 g that received 11.7 ± 1.27 eggs. As a result, hosts of > 0.5 g received significantly larger clutches than larvae in all mass categories of < 0.4 g (Tukey's HSD, $P < 0.01$). However, the numbers of parasitoids that emerged as adults did not increase with the increasing size of the hosts above the 0.2–0.3 g category and, for the very highest mass category (> 0.6 g), significantly fewer adults, at 8.1 ± 4.28 per host, emerged than for hosts in categories of less than 0.3 g (Tukey's HSD, $P < 0.05$). The marked decline in the proportion of wasps surviving in larger hosts (Fig. 2B) was a product of both increased larval mortality and an increased failure of adult parasitoids to emerge from the pupal stage. This was best illustrated by the largest hosts (> 0.6 g) where, despite approximately 50% of the eggs ultimately developing to pupae, less than 16% of these gave rise to adults.

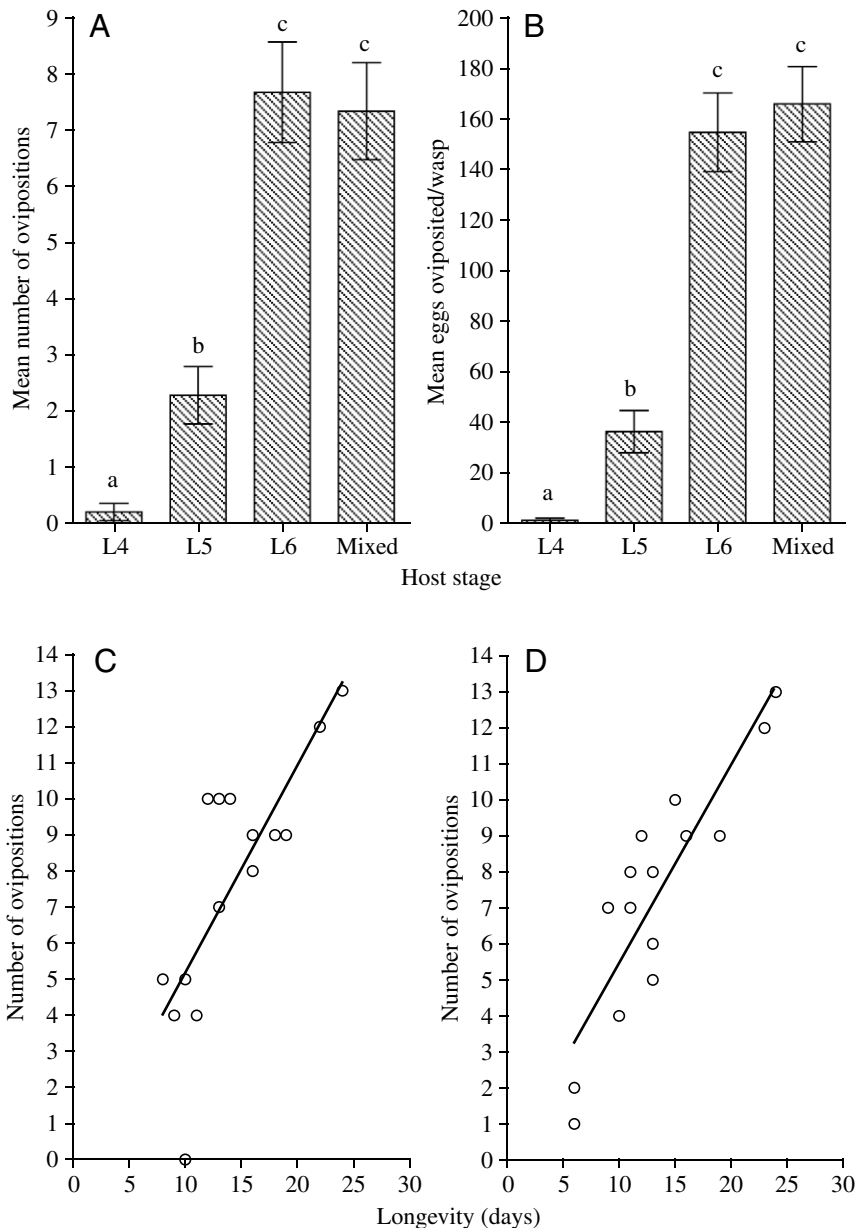


Fig. 3. (A) The number of hosts parasitized when *E. pennicornis* females were provided with populations of L4, L5, L6 (and mixed L4–6 hosts) and (B) the number of eggs laid. Values are means \pm S.E.M. For *N* values, see text. Bars headed by different letters are significantly different (one-way ANOVA of log-transformed data, $P < 0.05$). (C,D) The relationship between longevity and the number of hosts parasitized for *E. pennicornis* females exposed to (C) L6 populations (linear regression analysis: $y = 0.5752x - 0.5772$, $r^2 = 0.62$, $F_{1,13} = 21.56$, $P < 0.001$) and (D) mixed L4–L6 populations (linear regression analysis: $y = 0.5506x - 0.044$, $r^2 = 0.77$, $F_{1,13} = 43.5$, $P < 0.001$).

The unit mass of host per egg laid was seen to vary widely across the range of host sizes parasitized, from as little as 5 mg of host per egg to >20 mg per egg. Overall, with each successive increment in mass category, the average mass of host available to each egg increased. As such, for hosts of <0.1 g an average of 1 egg was laid for every 8 mg of host tissue whilst for hosts >0.6 g this value rose to almost 15 mg of host tissue per egg (results not shown). Regression analysis of the relationship between egg density and parasitoid survival showed that survival was independent of the unit mass of resource available per developing wasp (results not shown).

Brood sizes for parasitoids exposed to mixed and fixed stadium host populations

Host stadium had a significant effect on both the mean number of hosts parasitized ($F_{3,54}=39.25$, $P<0.0001$) and the average number of eggs laid by each parasitoid ($F_{3,54}=54.64$, $P<0.0001$). When female *E. pennicornis* were exposed to L4 hosts only, parasitism was negligible, and only 2/14 parasitoids attacked any hosts (Fig. 3A). The provision of L5 larvae increased the number of hosts parasitized such that, on average, 2.3 hosts were parasitized per wasp ($N=14$). Provision of mixed stadium hosts, or L6 hosts only, resulted in averages of >7.0 hosts being parasitized per wasp with no significant differences apparent (Tukey's HSD, $P>0.05$) ($N=15$). The mean total numbers of eggs oviposited followed the same trend as that of the number of hosts parasitized. Wasps exposed to L4 hosts laid very few eggs, whilst those given L5 hosts oviposited an average of 36.5 ± 8.59 eggs each (Fig. 3B). Parasitoids provided with the mixed host populations, or with L6 larvae only, laid averages of 165.7 ± 14.83 and 154.7 ± 15.52 eggs, respectively. Exposure of *E. pennicornis* females to a mixed population of L4–L6 larvae also provided a measure of the parasitoid's preference for attacking each of the different stages of *L. oleracea*. Results reflected the rates of parasitism recorded above, with the majority of attacks (>96%) occurring on L6 hosts. All remaining attacks were against L5 larvae and no L4 larvae were parasitized. The total number of hosts parasitized was significantly correlated with the longevity of the female *E. pennicornis* when wasps were exposed to either the mixed stage populations or L6 hosts only (Fig. 3C,D). Similarly, the number of eggs oviposited was also correlated with the longevity of the adult wasp, although the relationship was non-linear due to a progressive reduction in clutch sizes as the parasitoids aged (data not shown).

The relationship between clutch size and host size for parasitoids that were exposed to the mixed instar host population, or when restricted to L6

hosts only, is shown in Fig. 4. Exposure to the mixed population initially gave a significant non-linear asymptotic relationship of the type shown in Fig. 1, although non-linear regression explained only 43% of the variation. However, the relationship between host size and clutch size broke down over the reproductive lifetime of the parasitoid. The data were arbitrarily divided into the first three ovipositions for each wasp, and all subsequent egg laying events (Fig. 4A,B). In these experiments, it was apparent that, as the female parasitoids aged, their ability to adapt their clutch sizes to the size of the host declined. When the parasitoid was presented

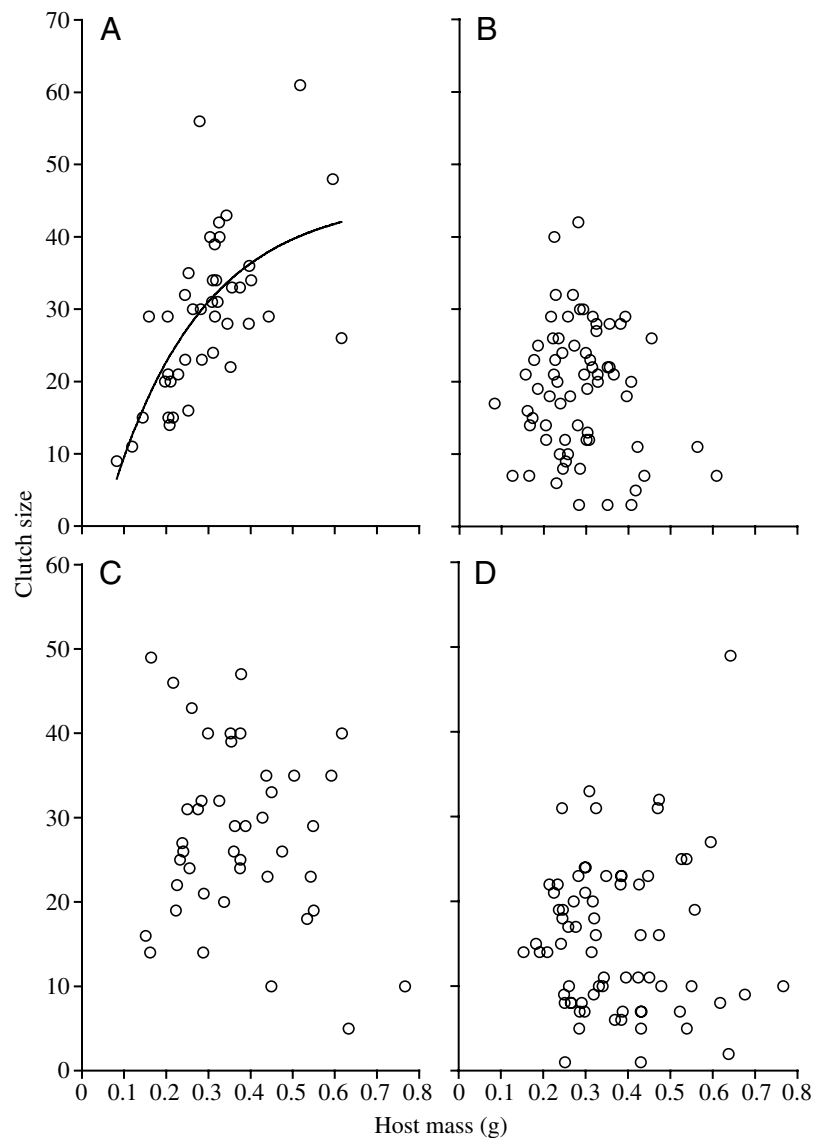


Fig. 4. The relationship between clutch size and host size for *E. pennicornis* females exposed to host populations over their entire lifespans. (A,B) The first three ovipositions of females exposed to (A) mixed stadium hosts [non-linear regression analysis: $y=9.58+(11.75\times 0.027^x)$, $r^2=0.43$, d.f.=39] and (B) all other ovipositions. (C,D) The clutch size–host size relationship for the first three ovipositions in parasitoids exposed to (C) L6 hosts only and (D) all subsequent ovipositions.

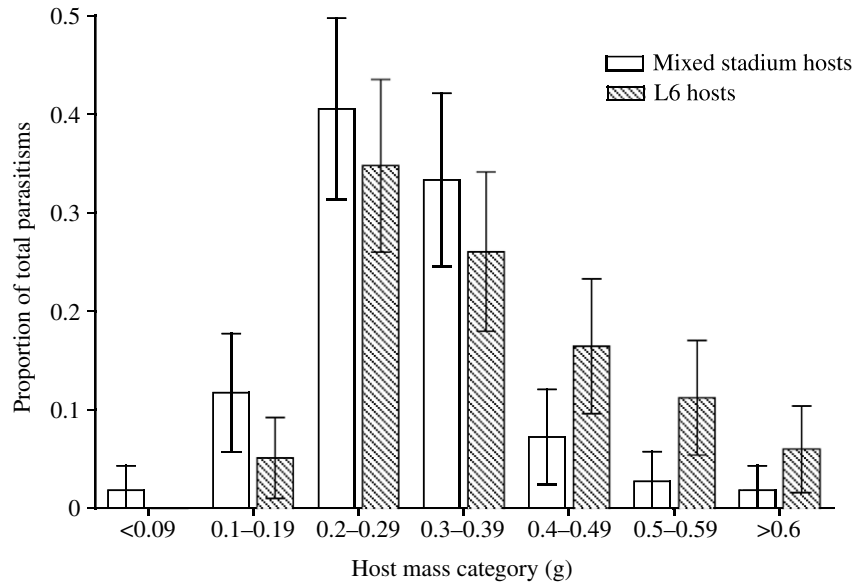


Fig. 5. The proportion of all hosts parasitized by *E. pennicornis*, when provided with either mixed stadium hosts or L6 hosts only, in each mass category. Values are means \pm 95% CL. $N=110$ (mixed hosts) and $N=115$ (L6 hosts).

with a restricted host size range of L6 hosts only, no relationship was apparent at any time (Fig. 4C,D).

When the masses of the parasitized hosts were examined (mixed stage and L6 hosts only), a trend for wasps selecting hosts of mass 0.2–0.4 g was apparent (Fig. 5). Whilst wasps exposed to both types of host population exhibited the same trend, the proportion of larvae parasitized at between 0.2 g and 0.4 g was ca. 74% in parasitoids exposed to the mixed host population, compared to 60% in those exposed to L6 hosts only. Similarly, whilst 34% of attacks were on hosts greater than 0.4 g in the L6 only population, this value was less than 12% in those wasps exposed to cohorts of mixed stage hosts. Due to the restricted host sizes available, wasps provided with only L6 caterpillars parasitized very few hosts that weighed less than 0.2 g, whilst those exposed to the mixed stage population chose these smaller hosts for ca. 14% of ovipositions. As a consequence, when *E. pennicornis* was restricted to L6 hosts, the mean mass of the hosts parasitized, at 0.366 ± 0.015 g, was significantly greater than when the wasp was exposed to mixed host populations (0.286 ± 0.011 g) ($t=4.26$, $P<0.0001$).

When the mean clutch sizes were examined over time, a trend of average clutches in the region of 25–35 eggs for the first two ovipositions was seen, followed by clutches of 20–30 eggs for ovipositions 3–7 (Fig. 6A). This trend was more apparent in wasps exposed to the population of mixed stadium hosts than those provided with L6 hosts only, where clutch sizes were more variable and showed a general decline over time. When related to the mass of each host at oviposition, on average wasps provided with mixed host instars laid a single egg for approximately every 10 mg of host tissue (Fig. 6B). This trend held true for the first five ovipositions, after which time the ratios of host mass to clutch size increased. In the fixed stadium (L6 only) population, however, the trend broke down after the first two attacks, and the ratio of host mass to eggs laid increased to between 15

and 25 mg per egg laid from the third oviposition onwards. The individual clutch sizes over the entire reproductive lifetimes of the females are shown in Fig. 6C,D. For both the mixed stadium and the L6 only populations, the size of the clutches declined with time and became less variable. For mixed stadium hosts (Fig. 6C), clutch sizes ranged from 11–61 eggs over the first 3 days, and 5–22 eggs for clutches laid from 10 days onwards. In the L6 hosts (Fig. 6D) a similar pattern was apparent with clutches of 10–49 eggs oviposited during the first 3 days, whilst clutches laid from the tenth day onwards comprised 11 eggs or fewer.

Host growth following parasitism

Growth of L6 hosts continued for 2 days in hosts parasitized immediately after ecdysis (day 1), and for 1 day when caterpillars were parasitized 1 day later (day 2) (Fig. 7A). For hosts parasitized immediately after ecdysis (day 1), the maximum average mass attained by parasitized hosts was approximately 58% greater than that at the time of parasitism, whilst the mass increase for those hosts parasitized on day 2 was only 30%. Similarly, for L5 hosts parasitized immediately after moulting, hosts typically continued to grow for 2 days and approximately doubled in size. For L5 hosts parasitized 1 day after ecdysis (day 2), however, growth was much less, such that the average maximum mass achieved was only 25% greater than that at the time of parasitism (data not shown). Eggs of *E. pennicornis* typically hatch 2–3 days after oviposition and a gradual decline in mass of the host/parasitoid complex was apparent after the time when the parasitoid larvae had hatched.

Discussion

The principle that clutch size changes in response to variations in the host size has been demonstrated for several gregarious parasitoid species (Purrington and Uleman, 1972; Hardy et al., 1992; Bernal et al., 2001; Zaviezo and Mills, 2000). Furthermore, variations in clutch size for a given host size have been demonstrated to have significant consequences for the survival and for various measures of fitness of parasitoid progeny (Vet et al., 1994; Zaviezo and Mills, 2000; Bezemer and Mills, 2003).

The findings of the current study indicate that *E. pennicornis* similarly has the capacity to evaluate the size of a given host, and lay clutches of eggs that are in proportion to that host's

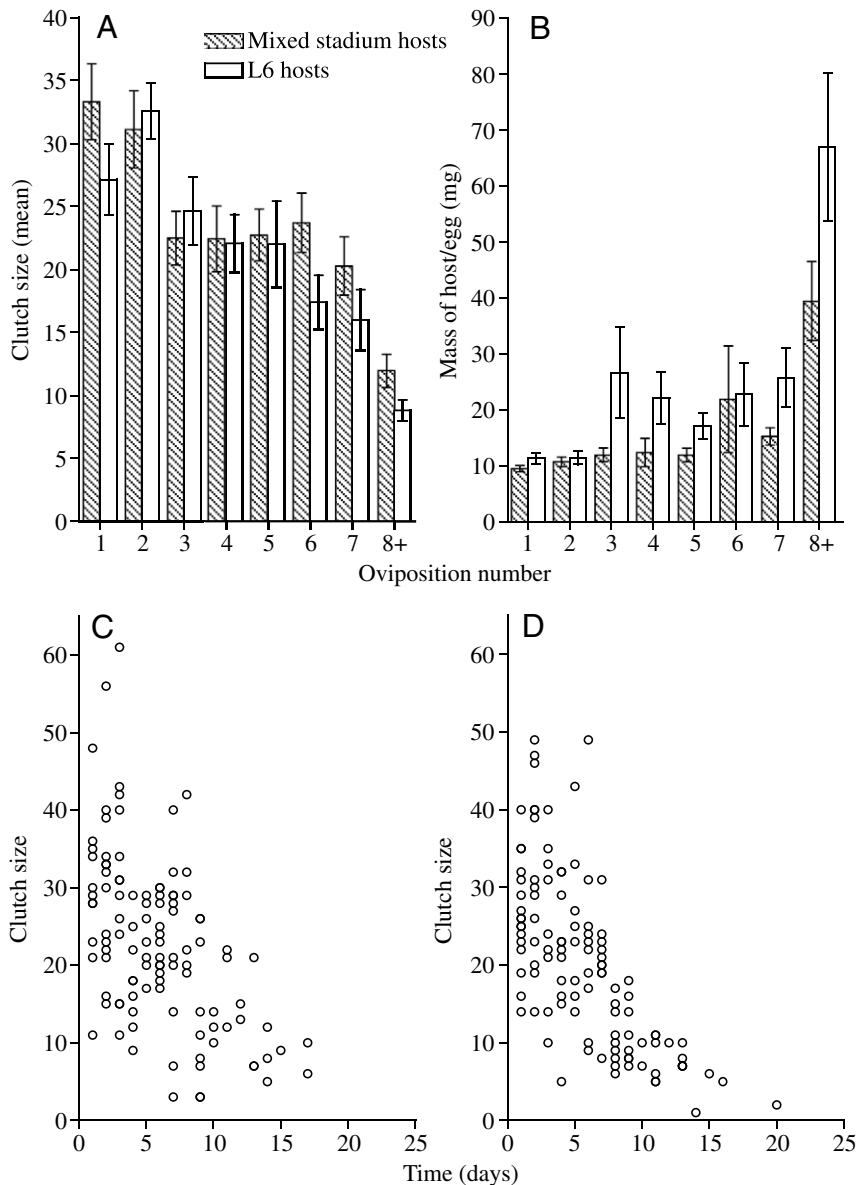


Fig. 6. (A,B) Mean clutch sizes in relation to (A) oviposition number and (B) the eggs:host wet mass (mg) of wasps exposed to mixed stadium and L6 only host populations. Values are means \pm s.e.m., $N=110$ (mixed stadium hosts) and $N=115$ (L6 hosts). (C,D) Clutch sizes relative to time (from exposure to hosts) are shown for wasps exposed to the two host population types (C, mixed and D, L6). All wasps were 2 days old at the start of the experiments.

size. This ability is not a learned response, and females with no previous oviposition experience show the same ability to adjust clutch size in response to host size as those that were allowed to parasitize hosts of mixed stadium populations over extended periods. However, the parasitoid's ability to match clutch size with host size was highly dependent on it being provided with host populations of a range of different sizes (i.e. different larval stadia) and, when given a restricted host range (i.e. L6 caterpillars only), no obvious trend for matching clutch size with host size was apparent.

The host encounter rate, and the variety (size and stadium)

of hosts available, can markedly affect the oviposition behaviour of a parasitoid (Henry and Day, 2000; Elzinga et al., 2005). Here we specifically examined the response of the parasitoid to populations of mixed host stadia (with no host encounter constraints) compared to populations of hosts comprising exclusively larvae of one of the three host stadia usually attacked by this parasitoid. The availability of a range of hosts (of the final three stadia) was seen to have a significant effect on the number of hosts parasitized and the number of eggs oviposited over that seen in wasps restricted to smaller (L4 and L5) hosts. *Eulophus pennicornis* is synovigenic and can oviposit over 200 eggs during its reproductive life (Bell et al., 2004). When the parasitoid was given a choice of host stadia, it almost always chose L6 *L. oleracea* for parasitism. When presented with hosts of a single larval stadium, only parasitoids provided with L6 hosts parasitized comparable numbers of hosts, and laid similar numbers of eggs, to those exposed to the mixed stadium populations. This would suggest that the wasp, in the absence of larger hosts, does not compensate by parasitizing a greater number of smaller insects. This suggests that the relatively long handling times involved in parasitism, and the likelihood of hosts being naturally scarce, may impose time constraints on the utilization of such hosts.

Despite the low rates of parasitism when the wasp was provided with L5 hosts only, the parasitoid frequently identified potential hosts at the end of this stadium, as they neared ecdysis, and stayed with them in order to oviposit immediately after the moult. This strategy effectively led to an extended handling time (at least 24 h) for each of these hosts (which were, in any case, removed prior to moulting to prevent any parasitism of L6 hosts). This runs contrary to the suggestion that L5 larvae are avoided due

to time limitation constraints, and further highlights the fact that *E. pennicornis* shows a preference for hosts that have just entered the sixth larval stadium. Therefore, it would appear that the strategy of waiting for a host to become acceptable is more profitable to the parasitoid than rejecting it in order to search out a host that is immediately suitable for parasitism. This strategy of identifying near acceptable hosts shortly prior to ecdysis has similarly been observed in other Eulophid ectoparasitoids of lepidopteran larvae (Shaw, 1981; van Veen and van Wijk, 1987). The adjustments of clutch size relative to host size observed when *E. pennicornis* was exposed to a

range of sizes of host larvae (L4–L6), where identification of potential hosts just prior to the moult was allowed for, and the absence of such relationships when hosts were restricted to a single stadium, highlights the importance of this behaviour. Therefore, it would appear that identifying hosts at a point prior to ecdysis is adaptive and a major factor in the parasitoid adjusting clutch size to host size over the duration of the parasitoid's life.

Female *E. pennicornis* emerge with approximately 60–70 near-mature eggs within their ovaries. This initial egg load was probably sufficient for the first two or three clutches. Subsequently, batches of eggs were matured and each batch laid sequentially. Average clutch sizes for a given oviposition event were mostly in the region of 20–30 eggs per host although, as the parasitoids aged, the clutch sizes became smaller. This could indicate that the ability of the parasitoid to adjust clutch size in response to host size may become limited by the supply of eggs in older individuals, as is predicted by egg limitation models (Godfray, 1994; Rosenheim, 1999). However, whilst clutch size reduced with female age, the size range of hosts parasitized remained largely unaltered. This was best illustrated by wasps provided with mixed stadium hosts that, despite the declining size of their clutches, continued primarily to choose L6 hosts of between 0.2–0.4 g throughout their reproductive lifespans (over 70% of ovipositions fell within this category). This may, therefore, suggest that choosing the correct developmental stage of host is of greater importance to *E. pennicornis* than accurately adjusting its clutch size to reflect the absolute size of the host. The oviposition strategy of *E. pennicornis* shares characteristics with several other gregarious ectoparasitoids investigated (Uematsu, 1981; Henry ad Day, 2000; Zaviezo and Mills, 2000) and can be contrasted with work showing that some gregarious endoparasitoids maximize reproductive fitness by producing relatively fixed clutch sizes throughout their lives (Harvey, 2000; Elzinga et al., 2005). However, despite the declining clutch sizes observed here, in favourable host encounter conditions, the number of eggs laid per unit mass of host remained, on average, remarkably constant until near the end of the female's life. This would suggest that, although clutch sizes declined as the females aged, *E. pennicornis* has evolved to accurately match clutch size to the size of the host in order to minimize larval mortality through scramble competition, which would potentially occur at high brood densities. Examination of the relationship between egg density and parasitoid survival, however, showed that wasp developmental success was independent from the quantity of

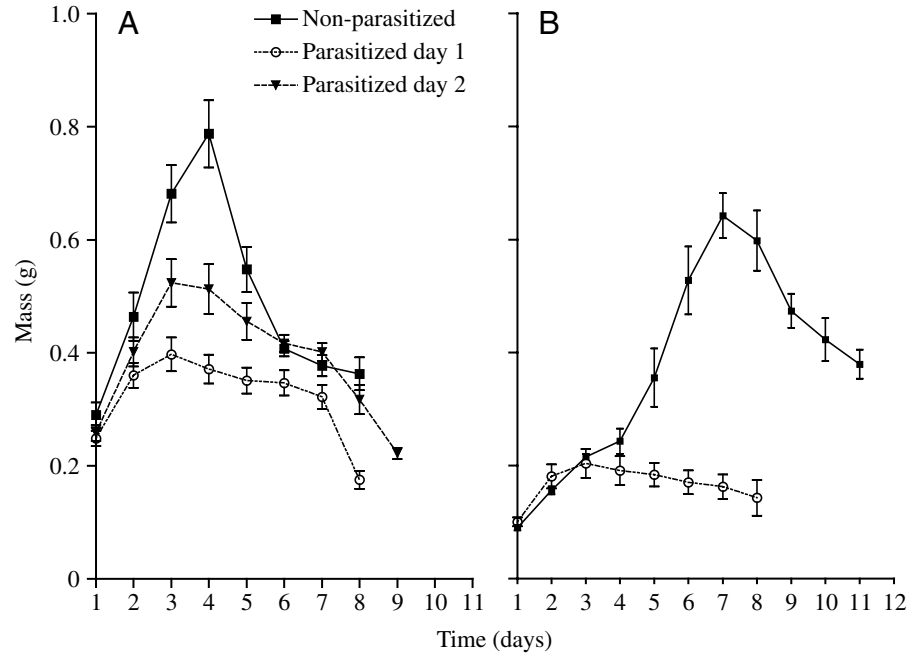


Fig. 7. The growth of hosts following parasitism by *E. pennicornis* in either the fifth or sixth stadium. Growth of L6 hosts parasitized immediately after moulting (day 1) and 1 day later (day 2) (A) and growth of L5 hosts immediately after moulting (B). The growth of hosts parasitized on day 2 was similar to the growth curve plotted and the data is omitted for clarity. Values plotted are the mean mass (\pm S.E.M.) of 10–15 insects.

resource available to each developing parasitoid. This was largely because the effect of host size on wasp survival far outweighed other considerations and the true impact of egg density will probably be only elucidated through the manipulation of clutch sizes upon fixed quality hosts.

Many gregarious koinobiont parasitoids allow for significant growth of the host following parasitism, and the number of progeny produced is frequently correlated with the size that the parasitized host attains before parasitoid egression (Beckage and Riddiford, 1983; Harvey, 2000). For *E. pennicornis*, whilst clutch size was related to the host mass at the time of oviposition, parasitized caterpillars were seen to increase in size markedly following parasitism, although growth was ultimately restricted in L5 larvae by the fact that they were unable to moult following oviposition. Significant growth in both parasitized L5 and L6 hosts was apparent, such that hosts increased in mass by as much as 60% before feeding ceased, an aspect that may have been a factor in the degree of variability observed between the host mass–clutch size relationships. Moreover, this may lead to the possibility that clutch size was not only determined by the size of the host at the time of parasitism, but by the potential for growth subsequent to oviposition. This supposition could, therefore, partially explain the fact that larger hosts (>0.5 g), which have less potential for growth following parasitism, typically received fewer eggs per unit mass than smaller hosts.

The fitness of parasitoid progeny (particularly female wasps) has been equated with various measures of adult size, and such

equations have generally shown that fitness increases with the size of the wasps on reaching adulthood (Waage and Ming, 1984; Ueno, 1999; Fidgen et al., 2000; Rivero and West, 2002). As a result, the primary selection pressure driving the evolution of parasitoids is often seen as one of maximizing the adult size of progeny, and oviposition strategies have been presumed to maximize this trait (Mackauer and Sequiera, 1993; Visser, 1994). This assumption frequently neglects pre-adult parasitoid mortality, however, and has recently been challenged as oversimplistic. Harvey and Strand (2002) and Harvey et al. (2004) have emphasized that, ultimately, the most important feature of parasitoid fitness is progeny survival, and natural selection should drive wasps to accurately identify and parasitized hosts that maximize pre-adult survival of their progeny. Here, we observed a strategy in *E. pennicornis* that substantiates their hypothesis, with host preference very closely reflecting progeny survival. Host quality did not operate as a simple function of increasing host size (i.e. the available resources), a principle similarly reported by Zaviezo and Mills (2000) when investigating the oviposition behaviour of the Eulophid ectoparasitoid *Hyssopus pallidus* (Askew). As a result, absolute fecundity in *E. pennicornis* did not increase when hosts above a certain mass were parasitized – indeed the oviposition of large clutches onto hosts of >0.6 g wasted a large proportion of the eggs laid due to high levels of larval and pupal mortality. Therefore, whilst the available resource per egg remained the same, or increased slightly, with successive increments in host size, parasitoid survival decreased concomitantly and the parasitoid larvae failed to benefit from the additional resource available to them. Whilst this phenomenon could be equated with some inferred nutritional inferiority in larger hosts, as Zaviezo and Mills (2000) have suggested, it is equally plausible that the key element involved in this response to larger hosts is the ability, or failure, of the parasitoid to physiologically regulate the host following oviposition.

The ability of parasitoids to regulate their host's physiology is frequently seen as critical to their reproductive success (Vinson and Iwantsch, 1980b; Beckage, 1985; Edwards et al., 2001). In the case of *Eulophus pennicornis*, manipulation of the host's physiology is achieved through use of a non-paralysing venom that, most obviously, prevents ecdysis in stung L5 larvae through modulation of ecdysteroid levels (Weaver et al., 1997; Marris et al., 2001). Interestingly, in newly ecdysed L6 *L. oleracea* parasitized by *E. pennicornis*, levels of juvenile hormone (JH) increase ~100-fold within 4 days of oviposition (J. Edwards, personal communication). This suggests that the parasitoid manipulates the host's endocrinology to prevent the physiological and behavioural changes associated with pupal commitment that would normally occur if JH is absent during the early sixth stadium (Nijhout, 1994; Edwards et al., 2001). In the current experiments, sixth instar larvae that were more than 2 or 3 days post ecdysis at the time of parasitism were probably already committed to initiate pre-pupation behaviour (burrowing, wandering, cessation of feeding), and these

changes may have been very important factors in reducing the survival of parasitoids developing on these larger hosts. Alternatively, a number of reports have indicated that ectoparasitoid venoms may play an important role in the altering the nutritional composition of the host's haemolymph (Coudron et al., 1998; Nakamatsu and Tanaka, 2004). It is possible that similar processes may occur in the host–parasitoid under investigation here, and such nutritional manipulations could, again, be highly dependent on the time of parasitism. If this is the case, the ability of *E. pennicornis* to successfully regulate the host, and thus generate a nutritionally and physiologically favourable environment for the development of its progeny, may be the principle determinant of host choice and developmental success. In this scenario, the major factor driving selection in this parasitoid therefore becomes the ability to identify, and successfully regulate the physiology of the host immediately after the final larval–larval moult. Thus, in choosing hosts early in the sixth stadium, *E. pennicornis* would appear to utilize the largest possible hosts that allow for complete physiological regulation which, in turn, yield the highest levels of developmental success in its progeny.

The fitness of *E. pennicornis* that developed from different sized hosts, or from differing clutch sizes, was not measured here. As a result, further measures of fitness gain when using different sized hosts, or laying eggs at different densities, remain to be determined. However, it was apparent that *E. pennicornis* preferentially attacked hosts at the start of the sixth stadium and typically laid clutches of 20–30 eggs onto the integument of these hosts. Deviations from this strategy can be explained by the fact that at points during the wasp's lifespan the egg load will be considerably higher than 20–30 eggs (typically when it first commences oviposition), when it may seek out larger hosts. Whilst a proportion of larger and smaller hosts were attacked, and clutch sizes increased or decreased accordingly, developmental success declined with any significant departure from the parasitism of early L6 hosts of around 0.2–0.4 g. The evidence presented here show that newly ecdysed *L. oleracea* larvae at the beginning of the sixth larval stadium represent the physiologically and nutritionally optimal stage for parasitism, and that *E. pennicornis*'s reproductive fitness is maximized through the utilization of hosts that allow for the highest levels of parasitoid survival. Thus, in this case, bigger hosts are not necessarily better hosts, despite the fact that such hosts apparently represent a greater nutritional resource. In the case of *E. pennicornis*, we suggest that the major driver of selection has been for the identification and utilization of hosts that maximize progeny survival. Furthermore, we hypothesize that host choice is based on the requirement to identify the largest sized hosts that are amenable to the range of physiological and nutritional manipulations induced by the parasitoid and necessary for optimising the survival of parasitoid progeny. Thus, the ability to successfully regulate the host can be seen as the major factor governing host choice and oviposition behaviour in this parasitoid, a characteristic that may be commonplace in species

where parasitoid-induced changes in host physiology are a prerequisite for progeny development.

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