Clutch size in a larval-pupal endoparasitoid

1. Variation across and within host species

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Abstract. Clutch size decisions by *Aphaereta minuta* (Nees) (Hymenoptera: Braconidae), a polyphagous, gregarious, larval-pupal endoparasitoid, were studied under laboratory conditions. This parasitoid attacks larvae of Diptera inhabiting ephemeral microhabitats such as decaying plant and animal material. Females oviposit in young larval stages, but the eventual size of the host pupa determines host food availability for competing offspring. The size of the pupa can differ greatly between host species. We questioned how *A. minuta* females deal with this delay between the moment of oviposition and eventual host food availability, and whether they make clutch size decisions that benefit their fitness. It was shown that females indeed vary their clutch size considerably and in an adaptive way: (1) females lay larger clutches in larvae of host species that produce larger pupae, even when the larvae are the same size at the moment of oviposition, and (2) females lay larger clutches in larger larvae than in smaller larvae of the same host species. The latter seems functional as larvae parasitized at an older stage indeed developed into larger pupae compared to larvae parasitized at a younger stage. Furthermore, mortality of parasitized young host larvae was greater than that of both unparasitized larvae and parasitized older larvae. Under field conditions the risk of mortality of young host larvae is expected to be even higher due to the limited period of microhabitat (host food) availability, strong scramble type competition between the host larvae, and the longer period of being exposed to predation.

Key words: Clutch size – Parasitoid – *Aphaereta minuta* – Hymenoptera – Braconidae

For insect parasitoids, the size of the host insect attacked often determines the amount of food available for the development of offspring. The capacity to allocate dif-

ferent numbers of offspring to hosts of different sizes, and therefore different quality, potentially enables polyphagous, gregarious parasitoids to exploit available host resources in an optimal way. Indeed, as predicted by optimality models (reviewed in Godfray 1987; Godfray et al. 1991), there are many literature reports of a positive correlation between clutch size and host size (Salt 1961; Klomp and Teerink 1962; Wylie 1967; Takagi 1986).

For some species it has been shown that the female parasitoid actually measures the size of the host, prior to oviposition, to determine how many eggs to lay (e.g. Schmidt and Smith 1985; Takagi 1986). These examples refer to parasitoids attacking insect eggs or pupae, i.e. sessile host stages that cease to develop after being parasitized (idiobionts), constituting an unchanging, and therefore easily assessed, amount of resource. Parasitoids that attack hosts that continue to develop after parasitization (koinobionts) have a more difficult task in making optimal decisions on the allocation of the number of eggs to hosts, as the eventual size of the host has not yet been reached. This is especially the case for larval-pupal parasitoids that attack young larvae but where the host is killed only after it has pupated.

The parasitoid *Aphaereta minuta* (Nees) (Hymenoptera: Braconidae) is a polyphagous gregarious larval-pupal endoparasitoid of fly species of different size ranging from small Drosophilidae to large Calliphoridae flies, whose larvae feed on ephemeral food sources such as decaying plant and animal material (Sychevskaya 1964; Vet and van Alphen 1985). Young larvae (first and second instar) are preferred for oviposition and 10–15 eggs are deposited in larvae of the flies *Lucilia sericata* and *Calliphora erythrocephala* (Evans 1933). For the sibling species *Aphaereta pallipes* a range of up to 20 eggs are reported for the hosts *Delia antiqua* and *Orthellia caesarion* (Salkeld 1959; Houser and Wingo 1967). Female parasitoids search for hosts by vibrotaxis, detecting vibrations produced by the hosts (Evans 1933; Vet and van Alphen 1985). After paralyzing the host, a number of elongated eggs are laid in the haemocoel. The eggs swell up to 2900 times their initial volume and hatch

during or just after the pupation of the host, after which the entire content of the host puparium is consumed and the host dies (Evans 1933).

Hence, at the moment of oviposition, a larval-pupal parasitoid such as *A. minuta* is faced with a problem of how to estimate the amount of resource that is going to be available for its offspring after pupation of the host. The foraging female parasitoid can encounter and parasitize larvae that greatly differ in age, size and potential to grow further. How does she deal with such variation in resources and how is she able to optimize the allocation of her offspring?

In the present study we analyse clutch size variability in *A. minuta*. We investigate whether clutch size is influenced by the species and size (or instar) of the host larva at the moment of oviposition. With the anthomyiid fly *D. antiqua* as a host we further investigate how parasitization of larvae of different ages affects larval and pupal mortality – and thus clutch survival – and how parasitization itself affects pupal size.

Materials and methods

Parasitoids

The culture of *A. minuta* used in this study originated from females that emerged from onion baits containing *D. antiqua* larvae collected near Wageningen, The Netherlands. *A. minuta* was maintained on first and second instar larvae of the onion fly *D. antiqua*. Some initial studies were conducted with an *A. minuta* strain collected in Greece (mentioned in figure legends when used). This strain was collected from decaying tomatoes containing larvae of various Diptera species and maintained on *Phormia regina*. Parasitoids were maintained under a 16L–8D photocycle regime at 12° C and fed on a sugar solution.

Hosts

Larvae of different ages of *Delia antiqua* (Meigen) (Diptera: Anthomyiidae), *Phormia regina* (Meigen) (Diptera: Calliphoridae) and *Drosophila hydei* Sturt. (Diptera: Drosophilidae) were used as hosts. *Phormia regina* and *Drosophila hydei* larvae of two different ages were used (2 and 4 days after oviposition). For *P. regina* these larvae were first and first/second instar respectively and for *Drosophila hydei* they were first and second/third instar. In the case of *Delia antiqua* we used first, second and third instar larvae (respectively 4, 6 and 9 days after oviposition). *Delia antiqua* was reared on decaying onions at 23° C, 70 % relative humidity, *P. regina* on decaying meat at 23° C and *Drosophila hydei* on a yeast medium at 20° C.

Experiments

Mated 7-day-old females were offered about 20 hosts in Petri dishes (5.5 cm in diameter) containing an agar layer with host rearing medium. One hour before the experiment the parasitoids were acclimatized at 22° C, the temperature at which all experiments were conducted.

To obtain a direct estimate of parasitoid clutch size, parasitoids were observed during oviposition and the number of egg laying bouts (quiverings of the last abdominal segments and the ovipositor sheath) was counted. In the analysis the first three clutches were omitted because of possible effects of egg load and inexperience of

the parasitoids on clutch size. Hosts were dissected directly afterwards and the number of eggs counted.

To analyse the correlation between larval host volume and parasitoid clutch size, parasitized larvae were killed in hot water and length and width (at the widest part) were measured. Others were incubated individually under a 16L–8D photocycle regime at 25° C, relative humidity 50–70%, in a plastic cup (30 ml) containing a surplus of *Delia antiqua* rearing medium consisting mainly of water, carrot-powder and yeast, and their pupal size was measured. A control group of unparasitized larvae was treated identically.

To measure the relation between clutch size and larval host volume for *Delia antiqua* and *Drosophila hydei* larvae of both species and of different sizes were offered together to a female parasitoid. The volume of larvae and pupae was calculated using the formula for the volume of a cylinder: $L \cdot (W/2)^2 \cdot \pi$, where L = larval or pupal length (mm) and W = larval or pupal width (mm).

Results

Determination of clutch size

The relation between the number of eggs found on dissecting the host (*Drosophila hydei* and *P. regina*) immediately after oviposition and the number of egg laying bouts is shown in Fig. 1. There is a very good correlation. Clutch size can therefore be determined by observing the female ovipositing in the host. When referring to clutch size we actually consider the number of egg laying bouts. An egg laying bout lasted on average 10.1 (± 2.1) s ($n = 207$).

Clutch size in hosts of different species and ages

Figure 2 shows the clutch size in 2- and 4-day-old *Drosophila hydei* and *P. regina* larvae. In both species, significantly more eggs were deposited in older larvae. In between-species comparisons, a significant difference was only found in 4-day-old larvae. At this age *P. regina*

Fig. 1. Relation between the number of eggs counted at dissection of *Delia hydei* and *Phormia regina* larvae and the number of egg laying bouts of *Aphaereta minuta* (Greek strain). Linear regression equation: $y = 0.96x - 0.31$, where y = number of eggs counted at dissection and x = number of egg laying bouts ($n = 131$, $r^2 = 0.87$, $P < 0.001$). The number behind the symbol represents the number of observations

Fig. 2. Clutch size of *Aphaereta minuta* (Greek strain) in 2- and 4-days-old larvae of *Delia hydei* and *Phormia regina*. Mean (bars) and SD (lines). Number of observations in parentheses. Bars with the same letter are not significantly different at $P < 0.01$ (multiple comparison test, based on Kruskal-Wallis rank sums)

Fig. 3. Clutch size of *Aphaereta minuta* in *Delia antiqua* larvae of three ages. Mean (bars) and SD (lines). Number of observations in parentheses. Bars with the same letter are not significantly different at $P < 0.01$ (multiple comparison test, based on Kruskal-Wallis rank sums)

larvae were 1.5 times the volume of *Drosophila hydei* larvae. No significant difference was found between larvae of 2 days old, although larvae of *P. regina* were three times the volume of *Drosophila hydei* larvae. Figure 3 shows the number of eggs deposited in *Delia antiqua* larvae of three different ages: 4, 6 and 9 days after oviposition corresponding with first, second and third

Fig. 4. Relation between clutch size of *Aphaereta minuta* and host volume of *Delia antiqua* and *Drosophila hydei* larvae. Regression equations (multiplicative model): *D. antiqua*, $y = 7.587x^{0.367}$ ($n = 46$, $r^2 = 0.54$, $P < 0.01$); *D. hydei*, $y = 3.525x^{0.460}$ ($n = 46$, $r^2 = 0.45$, $P < 0.01$), where y = clutch size and x = host volume (mm^3)

instar larvae respectively. Clutch size significantly increased with host age. When we compare clutch size for the three host species at the age of 4 days, clutch size is highest in *P. regina* (multiple comparison test, based on Kruskal-Wallis rank sums, $P < 0.01$). *P. regina* also forms the largest pupae.

Effect of larval host volume on clutch size

The relation between clutch size and larval host volume for *Delia antiqua* and *Drosophila hydei* is shown in Fig. 4. Clutch size significantly increased with larval host volume in both species ($r^2 = 0.54$ and 0.45 for *Delia antiqua* and *Drosophila hydei*, respectively). *A. minuta* distinguished between species since significantly more eggs were laid in *Delia antiqua* compared to equally sized larvae of *Drosophila hydei*. Figure 5 shows the relation between larval host volume and clutch size on a smaller scale of variability, i.e. within second instar *Delia antiqua* larvae. Clutch size still significantly increased with the volume of second instar larvae, although in this case host volume only explained a small part of the variation ($r^2 = 0.06$).

Effect of parasitization on mortality and pupal size of hosts

Mortality of larvae which have been parasitized in the first instar was higher than for unparasitized larvae (X^2 , $P = 0.03$). When larvae were parasitized in the second or third instar, larval and pupal mortality were not significantly different when compared to unparasitized larvae

Fig. 5. Relation between clutch size of *Aphaereta minuta* and host volume of 6-day-old (second instar) *Delia antiqua* larvae. Linear regression equation: $y = 6.92 + 1.18x$, where y = clutch size and x = host volume (mm^3) ($n = 295$, $r^2 = 0.06$, $P < 0.001$)

Fig. 6. Pupal size of parasitized (at different ages) and unparasitized *Delia antiqua* larvae. Mean (bars) and SD (lines). Number of observations in parentheses. Bars with the same letter are not significantly different at $P < 0.01$ (multiple comparison test, based on Kruskal-Wallis rank sums)

Table 1. Larval, pupal and total mortality (%) of unparasitized and parasitized *Delia antiqua* larvae at three different ages: -, not significantly different; * χ^2 , $P < 0.05$, comparison between unparasitized and parasitized larvae of the same age. Different letters refer to a significant difference (χ^2 , $P < 0.05$) between different ages in the same column and the same treatment (Par., parasitized; Unpar., unparasitized). n = number of observations

Instar	Treatment	n	Larval	Pupal	Total
First	Unpar.	140	12.9 a	7.1 a	20.0 a
	Par.	99	28.3 x	9.1 x	37.4 x
Second	Unpar.	140	12.9 a	10.7 a	23.6 a
	Par.	96	5.2 y	9.4 x	14.6 y
Third	Unpar.	145	13.1 a	16.6 b	29.7 a
	Par.	80	15.0 z	13.8 x	28.8 x

(Table 1). Larval mortality was highest when parasitism occurred in the first instar and lowest when parasitism occurred in the second instar. Pupal size of parasitized and unparasitized larvae is shown in Fig. 6. Parasitized larvae developed into significantly smaller pupae than unparasitized larvae. Pupal sizes of larvae which had been parasitized at different ages were significantly different. When larvae were parasitized at an older age, they developed into significantly larger pupae. However, this increase in pupal size cannot completely account for the larger clutches in older larvae. Figure 7 shows that within each instar, pupal size was positively correlated with clutch size; larvae containing more parasitoid eggs developed into larger pupae. The regression coefficients were significantly different [$P < 0.005$, comparison of regression lines (Sokal and Rohlf 1981)].

Discussion

Clutch size optimality models commonly predict that larger clutches should be placed on higher-quality hosts (Godfray et al. 1991). For parasitoids, the quality of a host will be determined by host size (amount of food available to developing parasitoids). Le Masurier (1987) found that, across parasitoid species, brood size of gregarious larval parasitoids correlates with host size (indirectly measured by length of forewing of adults of the host species). For several parasitoid species there is evidence for a positive relation between clutch size and the size of the host species (Klomp and Teerink 1962), but also between clutch size and the size of the host instar attacked (Neser 1973; Luck et al. 1982; Sato and Tanaka 1984; Dijkstra 1986; Sato et al. 1986; Hardy et al. 1992).

Dijkstra (1986) showed that the positive relation between clutch size and host weight is present within several host instars and thus that clutch size is not directly related to host instar. The data presented here similarly show that clutch size increased with age for three host species. This relationship is not linear (Fig. 4). Within second instar larvae, clutch size also increased with host size (Fig. 5), so host instar is not the only factor that determines clutch size. *Aphaereta minuta* is able to distinguish between equally sized hosts of different species, depositing more eggs in larvae of species which develop into larger pupae. What we do not know, however, is how the wasp makes this distinction. Since Diptera larvae are often covered by the substrate, the only means of contact the parasitoid has is through the ovipositor. It has been shown that chemo- and mechanoreceptors are present on the ovipositor (van Lenteren 1981), with which the female has to assess both size and physiological age of the larvae. So far the cues involved can only be

Fig. 7A–C. Relation between pupal size of *Delia antiqua* larvae when parasitized at different ages and clutch size of *Aphaereta minuta*. Linear regression equations: A first instar: $y = 11.676 + 0.678x$ ($n = 71$, $r^2 = 0.09$, $P < 0.05$); B second instar: $y = 15.392 + 0.293x$ ($n = 139$, $r^2 = 0.13$, $P < 0.01$); C third instar: $y = 13.960 + 0.539x$ ($n = 53$, $r^2 = 0.13$, $P < 0.01$), where y = pupal size (mm^3) and x = clutch size

guessed. Size for instance may be determined by the level of resistance of the cuticle when pierced, or the force of the struggling larvae. Physiological age may be assessed by determining certain chemical cues correlated with larval development such as ecdysone, juvenile hormone (JH), prothoracicotropic hormone, eclosion hormone, bursicon and their derivatives, (Cymborowski 1992). When this concerns fairly general substances (JH and ecdysone, for example), the parasitoid could distinguish between hormone levels and combine this with an estimation of host size.

Although quantitative differences in host volume seem to be important in determining the clutch size, differences in quality per unit of volume may also play a role.

A. minuta generally oviposits into young larvae. As the host larva is only at the beginning of its growth, the parasitoid has to estimate the expected pupal size because host size at parasitization is not a direct determinant of the amount of food available to the developing parasitoids. In addition the parasitoid has to consider several factors that may reduce clutch survival. When parasitizing young larvae she has to account for the relatively high mortality of the host larvae (Table 1). This relatively high mortality was found in the laboratory under optimal conditions (surplus of food, no predators, etc.) and is expected to be much more intense under field conditions. Diptera larvae feed on ephemeral substrates, and food competition can be very intense due to the high number of fly eggs laid and the scramble-type competition. Many young larvae never make it to the pupal stage. Furthermore, the longer period of exposure to predators and other parasitoids imposes an extra mortality risk for younger larvae.

Several studies (Beckage and Riddiford 1983; Sato and Tanaka 1984) revealed that heavily parasitized hosts grew larger than lightly parasitized hosts. The parasitoid larvae are thought to be responsible for this positive correlation between clutch size and final host size. Parasitoids may manipulate their host larva to increase feeding, a phenomenon which occurs frequently in gregarious

parasitoids (Slansky 1986). Our results show a decreasing effect of parasitization on pupal size. Parasitized larvae develop into smaller pupae than unparasitized larvae, probably due to induction of earlier pupation (Salkeld 1959; Zinov'eva 1974). This decreasing effect of parasitization on pupal size was stronger when younger larvae were parasitized. Interestingly however, within instars, pupal size was positively related to clutch size (Fig. 7) suggesting there may also be an effect of host manipulation by developing *A. minuta* larvae, as was found for other gregarious parasitoids (Slansky 1986). There may be an alternative explanation for this positive correlation between pupal size and clutch size in our study. Perhaps the parasitoid larvae do not modify the host's behaviour, but the ability of the ovipositing female to assess host quality is simply underestimated. It is possible that the ovipositing female is able to estimate subtle differences in the potential net host size (pupal size) at the moment of oviposition and allocates more eggs to higher-quality larvae that will develop into larger pupae.

In a second paper (Vet et al. 1993) we analyse clutch size variability in *A. minuta* in a quantitative way. By manipulating the size of the clutch and calculating fitness curves we test how optimal the wasps' clutch size decisions really are.

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