

Host suitability, quality and host size preference of *Leptomastix epona* and *Pseudaphycus flavidulus*, two endoparasitoids of the mealybug *Pseudococcus viburni*, and host size effect on parasitoid sex ratio and clutch size

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Accepted: March 2, 2000

Key words: *Leptomastix epona*, *Pseudaphycus flavidulus*, *Pseudococcus viburni*, host size, suitability, quality, preference, sex ratio, clutch size, biological control

Abstract

Five host size classes which mostly consisted of one host stage (first, second, third instar nymph, young adult and preovipositing adult) of the mealybug *Pseudococcus viburni* (Signoret) Fernald, 1903 [Hemiptera: Pseudococcidae] were tested for susceptibility and suitability for the solitary parasitoid *Leptomastix epona* (Walker) Graham, 1969 and the gregarious *Pseudaphycus flavidulus* (Brèthes) De Santis, 1964 [both of them Hymenoptera: Encyrtidae] in no choice experiments. Hosts larger than 1 mm (third instar) were suitable for the development of the parasitoids. Choice experiments with all the host size classes offered simultaneously showed that *L. epona* and *P. flavidulus* are parasitoids of mainly larger hosts (>1 mm, third instar nymphs) therefore the parasitoids exploit the same host range. In *L. epona*, a faster development and a larger size of wasps occurred in host size class 1.83–2.33 mm (young adult mealybugs) indicating a greater host quality compared to other sizes. Proportion of male offspring decreased with the host size following the host size distribution models. In *P. flavidulus* there was no effect of host quality (size at parasitism) on developmental time, sex ratio or parasitoid size and fecundity. Larger clutch sizes of *P. flavidulus* emerged from larger hosts but overall this parasitoid was able to parasitize smaller hosts (<1 mm, second instar nymphs) compared to *L. epona*. Experience of oviposition for 24 h does not seem to affect host size preference or sex ratio of the offspring in *L. epona*. Implications of the findings for mass-rearing and single applications of the parasitoids for the purpose of a biological control programme are discussed.

Introduction

Pseudococcus viburni is a world-wide distributed mealybug and important pest on field agricultural and horticultural plants (citrus, apple, grapevine, stone fruits), ornamentals, tubers of *Dahlia* sp. and potatoes, and in protected cultivation (cacti, *Passiflora* spp., *Kalanchoe* spp., tomatoes) in Northern Europe (Gonzalez, 1991; Ben-Dov, 1994; M. J. Copland, unpubl.). *Leptomastix epona* and *P. flavidulus* are two parasitoid species of the mealybug *P. viburni*. *Leptomastix epona* is a European species geographically distributed within the Palearctic region in Czechoslo-

vakia, Denmark, Finland, Hungary, Poland, Russia, Spain, Sweden, United Kingdom and West Germany, while *P. flavidulus* originates in the Neotropical region in Argentina and Chile (Noyes, pers. comm.).

Host suitability is associated with the successful development and emergence of wasps in the process of parasitism (Mackauer & Sequeira, 1993) and it is directly related to successful parasitism. Costs connected with parasitism may vary between the instars of the same host, hence some stages are more profitable in terms of fitness than others (Godfray, 1994). Such costs may involve increased mortality of the host or parasitoid, increased developmental time and reduced

size or fecundity of wasps (Sait et al., 1997). Host quality describes variations in the state or condition of the host (e.g., size, stage of development) that affect the rates of parasitoid growth and development (Mackauer & Sequeira, 1993). Furthermore, parasitoids become selective of the hosts they attack (host selection behaviour) (Godfray, 1994) and the relative frequency of parasitized host types chosen compared with the frequency of host types available has been defined as host preference (Hopper & King, 1984).

A variable allocation of reproductive resources biased to one sex or the other often occurs in parasitic Hymenoptera. A pattern comprising all sons in small hosts and daughters in large hosts is predicted by host-size distribution models based on the assumption that the amount of resource a developing parasitoid obtains determines its fitness (Charnov et al., 1981; Charnov, 1982).

In gregarious idiobiont species that attack hosts in various instars, smaller hosts often receive single eggs, mid-sized hosts single female eggs and large hosts may receive several eggs (Van Driesche & Bellows, 1996). Sex ratio and selection on clutch size may be interdependent in gregarious parasitoids (reviewed by Godfray, 1994). Low variance in brood sex ratio with production of just enough males to fertilize all their sisters is predicted when mating takes place solely between members of a single clutch (LMC) (Hamilton, 1967). Highly variable brood sex ratios depending on resource availability would be the result of a panmictic mating structure of the population (Williams, 1979).

The host susceptibility, suitability, quality and host size preference of the parasitoids *L. epona* and *P. flavidulus* were studied in 'no choice' or 'choice' experiments in the laboratory with a view to obtain some insights into their potential commercial development and their release singly or simultaneously for the control of *P. viburni*. Sex ratio at emergence of the parasitoids *L. epona* and *P. flavidulus* was also studied in these experiments as it can influence success or failure of parasitoids released to control a target pest, because it is the female that brings about parasitism and death of the pest. The hypothesis of host-size distribution models was tested for the solitary parasitoid *L. epona*. Dependence of clutch size on host size and application of LMC theory on sex composition of the brood at different clutch sizes were examined for the gregarious parasitoid *P. flavidulus*. Lastly, the effect of parasitoid oviposition experience on host preference or sex ratio of the offspring was questioned.

Materials and methods

Mass cultures

The culture of the mealybug *P. viburni* was established in the laboratory from individuals collected in the glasshouses at Wye College. The primary mealybug cultures were maintained on sprouted potatoes of the variety 'Desiree' in plastic sandwich boxes (17.5 × 11.5 × 5 cm) with net covered openings for ventilation. They were kept in a rearing room at 26 ± 1 °C, 50–65 r.h., L16:D8 photoperiod and 3.3 W m⁻² light intensity.

The parasitoids *L. epona* and *P. flavidulus* were reared on *P. viburni* feeding on sprouted potatoes, in sandwich boxes in a rearing room which was similar to the one used for the host but with constant light.

Experiments. Individuals of the pest were transferred from the mass culture on leaf discs of *Passiflora coccinea* (Passifloraceae) up to 24 h before the experiment. The leaf discs were kept with their lower surface upwards on top of a layer of 8 g l⁻¹ Bacteriological Agar (No. 1) (Oxoid Ltd) which had previously been autoclaved, in Petri-dishes of 6 cm diameter. Size classes of the host were used rather than host stages but they were selected so that each size class comprised mostly one stage according to sampled sizes of mealybugs measured after moults. The size classes were 0.3–0.5 mm (crawlers), 0.5–0.9 mm (second instar nymph), 1–1.66 mm (third instar nymph), 1.83–2.33 mm (young adult), 2.33–3.33 mm (preovipositing adult). Only female mealybugs were used in the experiments apart from the two small size classes where males and females could not be distinguished.

Mummified mealybugs were collected from the mass cultures and put into glass vials (length 5.0 cm, diameter 2.5 cm), then kept in an incubator at 26 ± 1 °C under constant light. They were checked twice per day at the same time for parasitoid emergence. Freshly emerged female *L. epona* were put with a male 1–7 days old to mate and observed until mating took place. Female *P. flavidulus* emerging from different hosts that bore gregarious broods with at least one male sibling were assumed to have mated with their brother. After mating, the female wasps of *L. epona* or *P. flavidulus* were fed with 50% honey solution for 24 h.

Two types of experiments, 'no choice' and 'choice' experiments were conducted for both parasitoids. The different host size classes were offered separately

(20 mealybugs/Petri-dish) to the experimental wasps in no choice experiments, whereas they were offered simultaneously (four mealybugs of each size/Petri-dish) in choice experiments. During the experiments, wasps of each parasitoid species were released individually in the Petri-dishes to gain oviposition experience on hosts for 24 h. After their experience, the feeding and release process was repeated.

At the end of the experiment, the female was measured and dissected to confirm that it had been inseminated (de Jong & van Alphen, 1989). The Petri-dishes from the first and the second release of the parasitoids were kept in an incubator at 26 ± 1 °C, constant light and 30–40% r.h. until the mealybugs became mummified. The mummies were collected, measured (tip of the head to the end of the abdomen) and kept individually in 0.5 ml Eppendorf tubes, under the same conditions of temperature and light until parasitoid emergence.

In no choice experiments, the percentage parasitism (mummification) by the parasitoids, the survival of offspring in *L. epona* and the successful parasitism by *P. flavidulus* were recorded. Also secondary sex ratio, size (head width) and developmental time of the offspring of both parasitoids and the clutch size of *P. flavidulus* were noted. Correlation between head width and number of mature eggs in emerging female wasps of *L. epona* was examined as a possible index of wasp size to quantify its fitness in relation to size.

In choice experiments, host preference was measured as the proportion of mealybugs mummified from the total in each size class of the host. The survival of parasitoid offspring for *L. epona* and the sex of emerging offspring of both parasitoids were recorded.

Experimental design and statistical analysis

Leptomastix epona. The five host size classes were the treatments but the smallest and the second smaller size class were excluded from the analysis when no parasitism had occurred.

In no-choice experiments, data resulting from the oviposition of one wasp, thus data from each Petri-dish were considered as one replication. There were twenty replications for each host size class, conducted throughout five different time intervals that were considered as blocks. Data were analysed using General Linear Model (GLM) in SAS (SAS Institute, 1989). The effect of experience on the proportion of parasitism and the proportion of male parasitoid offspring was evaluated using a paired *t*-test.

In choice experiments, twenty-seven Petri-dishes containing all the host size classes where female parasitoids oviposited individually were considered as experimental units. Because observations of host size classes within the same experimental unit were not independent, the twenty-seven observations were divided into three groups and data of a single host size class from each group were compared with another size class from a different group using GLM in SAS. The effect of parasitoid experience on host preference and sex ratio of the offspring was evaluated using orthogonal polynomial contrasts to summarise the results of each Petri-dish and then a paired *t*-test to compare the slope of each response to increasing host size between the two types of experience.

Pseudaphycus flavidulus. In no choice experiments, eight female wasps were tested for each host size class. Data of mummification, successful parasitism and parasitoid survival were analysed with GLM in SAS and each wasp was one replication. GLM and the nested classification of variables in SAS (SAS Institute, 1996) was used to analyse clutch size data where each individual host was one replication. Numbers of male offspring emerged per host size class were analysed with the Generalised Linear Model in GENSTAT (Genstat 5, 1994).

In choice experiments, eleven female wasps were tested but those producing pure male broods were excluded as not inseminated. Data from each experimental wasp (different Petri-dishes) were an independent replication and the effect of separate containers was studied as a block effect. Proportion of mealybugs mummified per host size class was analysed using GLM in SAS. Proportion of male parasitoid offspring emerging per host size class was analysed with the Generalised Linear Model in GENSTAT. Binomial distribution of errors was assumed for the sex ratio data and the effect of host size was fitted in the model after allowing for different Petri-dishes.

Results

Leptomastix epona

Mummification and successful parasitism. The proportion of hosts mummified by *L. epona* differed significantly between the host size classes (GLM, $P = 0.0001$) (Table 1). More larger hosts than smaller hosts were mummified. GLM showed a significant effect of host size on the survival of the offspring of

inexperienced ($P = 0.0110$). However, no significant difference was found between the means when compared with the Tukey test ($P \leq 0.05$) (Table 1).

Sex ratio of parasitoid offspring emerging from different host size classes that were offered separately. The size of host and the sex of offspring in *L. epona* were related: smaller hosts gave a relatively greater proportion of male wasps than larger ones (GLM, $P = 0.0001$ for both inexperienced and experienced wasps) (Table 1). The highest proportion of males emerged from host size class 2 (Table 1).

Parasitoid developmental time, size and fecundity of parasitoid offspring emerging from different host size classes. Table 2 shows that developmental time of parasitoids differed significantly between the host size classes (GLM, $P = 0.0001$ and $P = 0.0001$ for males and females respectively; pooled data of inexperienced and experienced wasps). Both male and female wasps of *L. epona* developed faster in larger than in smaller hosts.

Significantly larger male parasitoids emerged from larger hosts compared with the males from smaller hosts (GLM, $P = 0.0001$; pooled data of inexperienced and experienced wasps) (Table 2). Female parasitoids that emerged from larger hosts were significantly larger than the females from smaller hosts (GLM, $P = 0.0001$; pooled data of inexperienced and experienced wasps) (Table 2). Male parasitoids were smaller than females within each host size class (Table 2).

Size of wasps in *L. epona* was found to be related positively with the host size at and after parasitism (mummified mealybugs). Therefore host size can be a good predictor of size for both male and female parasitoids. The regression line for head width of wasps against host size was $y = 0.192 + 0.071x$, $r^2 = 0.658$, $n = 301$ and $y = 0.244 + 0.080x$, $r^2 = 0.518$, $n = 263$ for males and females respectively. The regression line for head width of wasps against mummy size was $y = 0.147 + 0.169x$, $r^2 = 0.756$, $n = 297$ and $y = 0.194 + 0.176x$, $r^2 = 0.737$, $n = 260$ for males and females, respectively.

Female wasps of *L. epona* which emerged from larger size classes of the host had significantly more eggs in their ovaries than the ones from smaller size classes (GLM, $P = 0.0001$; pooled data of inexperienced and experienced wasps) (Table 2). However, host size before and after parasitism is not a good predictor of the number of eggs in emerging wasps.

The regression line for the number of eggs against host size at parasitism and mummy size was: $y = -11.93 + 9.61x$, $r^2 = 0.423$, $n = 246$ and $y = -9.10 + 4.39x$, $r^2 = 0.258$, $n = 249$, respectively. A strong positive correlation was found between wasp size and the number of ovarian eggs in female *L. epona* upon emergence ($r = 0.637$, $n = 243$).

Host size preference. There was not any mummification in either of the two small size classes of the host, although encounters had been observed for the second size class. The proportion of mealybugs mummified in the two larger classes (adults) was significantly greater than the proportion of mealybugs in size class 3 (GLM, $P = 0.0001$ and $P = 0.0010$ for inexperienced and experienced wasps respectively) (Table 3).

Sex ratio of parasitoid offspring when different host size classes were offered simultaneously. A significantly greater proportion of male *L. epona* emerged from hosts of the size class 3 compared with the two adult size classes (GLM, $P = 0.0295$ and $P = 0.0103$ for inexperienced and experienced wasps respectively) (Table 3). The proportion of male parasitoid offspring from the oviposition of the inexperienced wasps and the oviposition after they had gained experience did not differ significantly (one sample *t*-test on the differences, $P > 0.05$).

Pseudaphycus flavidulus

Mummification and successful parasitism. Table 4 shows that higher proportion of third instar immature mealybugs were mummified by *P. flavidulus* compared to smaller or larger hosts, when different host size classes were offered separately (GLM, $P = 0.0172$ after angular transformation). However, the proportion of mealybugs successfully parasitized at the different host size classes did not differ significantly (GLM, $P = 0.7750$ after angular transformation).

Clutch size, survival and sex ratio of parasitoid offspring emerging from different host size classes that were offered separately. Clutch size of *P. flavidulus* increased with the host size (GLM, $P = 0.0001$) (Table 4). Survival of the parasitoid offspring (mean proportion of offspring emerged from the total produced) did not differ significantly between the size classes of the host (GLM, $P = 0.8581$ after angular transformation) (Table 4). For pooled data of solitary

Table 1. Proportion of mealybugs mummified by *Leptomastix epona*, survival of parasitoid offspring and proportion of male wasps emerged from different host size classes when offered separately in patches of 20 hosts, before and after parasitoid oviposition experience

Host size class (mm)	Proportion of mummified hosts		Survival of offspring		Proportion of male wasps emerged			
	<i>n</i>	$\bar{x} \pm \text{s.e.}$	<i>n</i>	$\bar{x} \pm \text{s.e.}$	<i>n</i>	$\bar{x} \pm \text{s.e.}$	<i>n</i>	$\bar{x} \pm \text{s.e.}$
	Experienced wasps				Inexperienced wasps		Experienced wasps	
0.5–0.9	58/400	0.14 ± 0.03 ^c (20)	37/58	0.60 ± 0.23 ^a (13)	51/52	0.98 ± 0.06 ^a (12)	33/35	0.94 ± 0.09 ^a (10)
1–1.66	169/400	0.42 ± 0.05 ^b (20)	111/169	0.64 ± 0.20 ^a (18)	75/94	0.79 ± 0.13 ^{ab} (16)	75/109	0.70 ± 0.17 ^{ab} (17)
1.83–2.33	240/400	0.60 ± 0.05 ^a (20)	175/240	0.72 ± 0.14 ^a (20)	74/181	0.40 ± 0.14 ^b (20)	65/174	0.37 ± 0.14 ^b (20)
2.33–3.33	245/400	0.61 ± 0.05 ^a (20)	134/245	0.55 ± 0.20 ^a (18)	47/160	0.29 ± 0.13 ^b (18)	28/134	0.20 ± 0.12 ^b (18)
GLM	d.f. = 3, F = 21.33, P = 0.0001		d.f. = 3, F = 4.03, P = 0.0110		d.f. = 3, F = 28.55, P = 0.0001		d.f. = 3, F = 21.91, P = 0.0001	

Figures under \bar{x} are the means and s.e. are the standard errors of the mean per female wasp. Means of male proportion were compared after angular transformation of the data. Values in columns followed by different letters are significantly different (Tukey, $P \leq 0.05$). Figures under *n* indicate total numbers. Numbers in parentheses indicate number of experimental wasps.

Table 2. Mean developmental time, size (head width) and fecundity (number of mature eggs in the ovaries upon emergence) of offspring of the solitary *Leptomastix epona* and the gregarious *Pseudaphycus flavidulus*, when developed in different size classes of *Pseudococcus viburni*

Host size class (mm)	Developmental time (days)		Head width of offspring (mm)				Fecundity	
			<i>Leptomastix epona</i>					
	Males	Females	Males	Females	Males	Females	Females	
	<i>n</i>	$\bar{x} \pm \text{s.e.}$	<i>n</i>	$\bar{x} \pm \text{s.e.}$	<i>n</i>	$\bar{x} \pm \text{s.e.}$	<i>n</i>	$\bar{x} \pm \text{s.e.}$
0.5–0.9	29	27.61 ± 0.66 ^a	–	–	23	0.35 ± 0.01 ^d	1	0.50 ± 0.50 ^b
1–1.66	75	22.9 ± 0.40 ^b	17	27.06 ± 0.95 ^a	43	0.39 ± 0.007 ^c	12	0.48 ± 0.01 ^b
1.83–2.33	73	20.40 ± 0.38 ^c	107	21.51 ± 0.22 ^b	47	0.48 ± 0.005 ^b	54	0.56 ± 0.006 ^b
2.33–3.33	47	21.62 ± 0.36 ^{bc}	113	22.81 ± 0.31 ^b	38	0.55 ± 0.007 ^a	51	0.65 ± 0.006 ^a
GLM	d.f. = 3, F = 17.36 P = 0.0001		d.f. = 2, F = 50.08 P = 0.0001		d.f. = 3, F = 113 P = 0.0001		d.f. = 3, F = 60.53 P = 0.0001	
			<i>Pseudaphycus flavidulus</i>					
	Males and Females	Males	Females	Females	Females	Females	Females	
	<i>n</i>	$\bar{x} \pm \text{s.e.}$	<i>n</i>	$\bar{x} \pm \text{s.e.}$	<i>n</i>	$\bar{x} \pm \text{s.e.}$	<i>n</i>	$\bar{x} \pm \text{s.e.}$
0.5–0.9	16	22.04 ± 0.18	–	–	12	0.26 ± 0.006	11	12.98 ± 3.18
1–1.66	36	20.60 ± 1.07	7	0.25 ± 0.013	26	0.26 ± 0.004	23	12.89 ± 2.54
1.83–2.33	14	19.97 ± 0.90	16	0.24 ± 0.007	19	0.26 ± 0.012	12	13.25 ± 1.38
2.33–3.33	7	19.89 ± 0.59	5	0.25 ± 0.012	21	0.29 ± 0.005	20	17.74 ± 2.02
GLM	P = 0.7023		P = 0.6273		P = 0.2144		P = 0.5436	

Figures under \bar{x} are the means and s.e. are the standard errors. Values in columns followed by different letters are significantly different (Tukey, $P \leq 0.05$). Data of 'inexperienced' and 'experienced' trials of *L. epona* were pooled. Figures under *n* indicate number of parasitoid offspring.

Table 3. Mean proportion of mealybugs mummified by *Leptomastix epona* and *Pseudaphycus flavidulus*, and proportion of male offspring of *L. epona* or sex ratio of both solitary and gregarious broods of *P. flavidulus* emerged from different host size classes when offered simultaneously in patches of 20 hosts, before and after parasitoid oviposition experience

Host size class (mm)	<i>Leptomastix epona</i>							
	Proportion of mummified mealybugs				Proportion of male wasps			
	Inexperienced wasps		Experienced wasps		Inexperienced wasps		Experienced wasps	
<i>n</i>	$\bar{x} \pm \text{s.e.}$	<i>n</i>	$\bar{x} \pm \text{s.e.}$	<i>n</i>	$\bar{x} \pm \text{s.e.}$	<i>n</i>	$\bar{x} \pm \text{s.e.}$	
1–1.66	8/36	0.22 ± 0.06 ^b	14/36	0.38 ± 0.09 ^b	6/6	1.00 ± 0.00 ^a	9/10	0.90 ± 0.14 ^a
1.83–2.33	29/36	0.80 ± 0.10 ^a	27/36	0.75 ± 0.10 ^a	10/21	0.47 ± 0.23 ^{ab}	7/21	0.33 ± 0.19 ^b
2.33–3.33	28/36	0.77 ± 0.08 ^a	32/36	0.88 ± 0.04 ^a	3/14	0.21 ± 0.20 ^b	4/15	0.26 ± 0.17 ^b
GLM	d.f. = 2, F = 13.69, P = 0.0001		d.f. = 2, F = 9.42, P = 0.0010		d.f. = 2, F = 4.31, P = 0.0295		d.f. = 2, F = 5.80, P = 0.0103	

Host size class (mm)	<i>Pseudaphycus flavidulus</i>					
	Proportion of mummified mealybugs			Sex ratio		
	Inexperienced wasps		Experienced wasps	Inexperienced wasps		Experienced wasps
<i>n</i>	$\bar{x} \pm \text{s.e.}$	<i>n</i>	$\bar{x} \pm \text{s.e.}$	<i>n</i>	<i>n</i>	
0.5–0.9	2/32	0.06 ± 0.06 ^b	1/32	0.03 ± 0.03 ^a	1	0: 2
1–1.66	7/32	0.22 ± 0.10 ^{ab}	7/28	0.14 ± 0.07 ^a	–	3 1: 3
1.83–2.33	11/32	0.34 ± 0.08 ^{ab}	3/28	0.11 ± 0.05 ^a	5	1: 2
2.33–3.33	15/32	0.47 ± 0.11 ^a	8/28	0.28 ± 0.10 ^a	6	1: 3.6
GLM	d.f. = 4, F = 5.92, P = 0.0014		d.f. = 4, F = 2.85, P = 0.0457			

Figures under \bar{x} are the means and s.e. are the standard errors of the mean per female wasp (11 and 8 experimental wasps for *L. epona* and *P. flavidulus* respectively). Means were weighted and compared after angular transformation of the data. Values in columns followed by different letters are significantly different (Tukey, $P \leq 0.05$). Figures under *n* indicate total numbers.

Table 4. Mean proportion of mealybugs mummified, successfully parasitized by inexperienced wasps of the gregarious parasitoid *Pseudaphycus flavidulus* in different host size classes when offered separately in patches of 20 hosts, and mean clutch size, proportion of parasitoid offspring emerged (survival) and sex ratio (males:females) of emerging parasitoid offspring from both solitary and gregarious broods

Host size class (mm)	Proportion of mummified hosts		Proportion of successfully parasitized hosts		Clutch size		Survival of offspring		Sex ratio (males:females)	
	<i>n</i>	$\bar{x} \pm \text{s.e.}$	<i>n</i>	$\bar{x} \pm \text{s.e.}$	<i>n</i>	$\bar{x} \pm \text{s.e.}$	<i>n</i>	$\bar{x} \pm \text{s.e.}$	<i>n</i>	
0.5–0.9	29	0.18 ± 0.07 ^b (8)	15/36	0.42 ± 0.70 (4)	15	1.00 ± – ^c	14/15	0.87 ± 0.30 (2)	2	1:12
1–1.66	73	0.46 ± 0.06 ^a (8)	36/84	0.43 ± 0.32 (8)	36	1.42 ± 0.11 ^c	39/51	0.76 ± 0.37 (7)	7	1:3.33
1.83–2.33	35	0.22 ± 0.04 ^{ab} (8)	17/51	0.33 ± 0.23 (8)	14	2.86 ± 0.27 ^b	37/40	0.92 ± 0.12 (5)	5	1:2.36
2.33–3.33	18	0.15 ± 0.06 ^b (7)	9/41	0.22 ± 0.16 (7)	7	4.71 ± 0.71 ^a	28/33	0.85 ± 0.26 (3)	3	1:4.6
GLM	d.f. = 3, F = 4.06 P = 0.0172		P = 0.7750		d.f. = 3, F = 27.39 P = 0.0001		P = 0.8581			

Figures under \bar{x} are the means and s.e. are the standard errors of the mean per female wasp. Means of proportions were weighted and compared after angular transformation of the data. Values in columns followed by different letters are significantly different (Tukey, $P \leq 0.05$). Figures under *n* indicate total numbers. Numbers in parentheses indicate number of experimental wasps.

and gregarious broods, the number of emerged males was unrelated to host size ($n = 17$, deviance ratio = 1.15, d.f. = 3 and residual d.f. = 13). Moreover, host size explained only 18% of the total deviance about the mean.

Parasitoid developmental time, size and fecundity of parasitoid offspring emerging from different host size classes. Developmental time and size of *P. flavidulus* offspring did not differ significantly between the host size classes (GLM, $P = 0.7023$ for developmental time; $P = 0.6273$ and $P = 0.2144$ for size of males and females respectively) (Table 2). Also, fecundity (number of mature eggs in the ovaries upon emergence) of female offspring did not differ significantly between the host size classes ($P = 0.5436$) (Table 2).

Host size preference. Crawlers were attacked by *P. flavidulus* but they were not mummified when different host size classes were offered together. A significantly higher proportion of adult mealybugs were mummified, compared to the smaller second instar nymphs (GLM, $P = 0.0014$, $P = 0.0457$ for inexperienced and experienced wasps respectively) (Table 3).

Sex ratio of parasitoid offspring when different host size classes were offered simultaneously. Host size did not have a significant effect on the proportion of male offspring of *P. flavidulus* that emerged from mealybugs parasitized at mixed size classes. The change in the deviance after allowing for Petri-dishes in the model was 1.705 and 0.379 for inexperienced and experienced wasps respectively, which was not significant at d.f. = 2 (Generalised Linear Model; the approximation χ^2 at d.f. = 2). However, it should be noted that these results are based on small amounts of data.

Discussion

Leptomastix epona

All host stages from second instar to adult are susceptible and suitable for the development of the parasitoid *L. epona*. However, crawlers were never attacked by *L. epona*. Survival of parasitoid offspring in *L. epona* is not affected by the host size which is in agreement with results in *Leptomastix dactylopii* (Howard) (Encyrtidae) (de Jong & van Alphen, 1989).

Both male and female wasps of *L. epona* develop faster in larger hosts than in smaller ones. This re-

sponse of parasitoid wasp development to the host size has often been reported in the literature (King, 1987) and might indicate a lower rate of development in preadult hosts or a developmental delay in some stage of the parasitoid's life (Karamaouna, 1999).

Female wasps of *L. epona* are generally larger than males and larger parasitoid offspring for both sexes emerge from larger hosts. The number of mature eggs in emerging females also increases with the host size, which is in agreement with other parasitoid species (e.g., Harvey & Thompson, 1995). The smaller size of male wasps on average, compared to females emerging from a certain host size, is presumed to correspond with shorter developmental time of males to adulthood (Charnov, 1982). Therefore, males of *L. epona* seem to benefit more from being small, rather than being larger, because they emerge earlier and hence can potentially fertilize more females (King, 1987). Mealybugs of the size class 1.83–2.33 mm (young adult stage) are of a greater quality for the development of *L. epona* in terms of a relatively faster development and production of fit female wasps. Host size preference of *L. epona* is in favour of larger hosts (adult stage) than smaller hosts (third instar nymphs).

The proportion of emerging offspring in *L. epona* is male biased in smaller (1–1.66 mm) compared with larger (1.83–2.33 mm) hosts when different size classes are offered separately or together. This is in agreement with what seems to be the rule for the solitary parasitoids in the Hymenoptera: the proportion of male wasps emerging from large hosts is lower than from small hosts (King, 1987; van den Assem et al., 1989).

The sex ratio (proportion of males) of the parasitoid *L. epona* studied in the experiment was the secondary sex ratio but it is likely that it expresses the primary sex ratio because survival of wasps did not differ with the host size. Consequently, differences in sex ratio with the host size probably suggest selective sex allocation of the eggs on different host sizes by inseminated females of *L. epona*. Hence, *L. epona* appears to support the host-size distribution models (Charnov, 1979) on the assumption that host size has a greater effect on the fitness of female than of male wasps. The findings of these experiments showed that the reproductive success (fitness) of *L. epona* offspring increases with the host size in which they develop. However, the relative effect of host size on the reproductive success of females compared with male wasps was not determined in the experiments.

Pseudaphycus flavidulus

All host stages from crawlers to adult are susceptible to the parasitoid *P. flavidulus*. However, hosts suitable for parasitoid development are larger than 1 mm. Host size does not affect survival of the host *P. viburni* after parasitism by *P. flavidulus*, parasitoid emergence and developmental time. Furthermore, broods in *P. flavidulus* seem to emerge synchronously. Size of the gregarious parasitoid *P. flavidulus* is independent of the host size in which the development of the wasps occurs. Because, clutch size decisions can influence the size of emerging wasps in gregarious parasitoids (Vet et al., 1994), *P. flavidulus* appears to allocate an appropriate number of eggs in different sized hosts in order to produce offspring of similar size independent of the host size. This ability of female wasps suggests that an optimal range in size is important to the reproductive success and population biology of *P. flavidulus* (Bai et al., 1992). Number of ovarian eggs in emerging wasps is not affected by host size. Variation in host size does not seem to affect host quality for the development of *P. flavidulus*. Host size preference of *P. flavidulus* favours hosts larger than 1 mm (third instar nymphs), thus it is broader than that of *L. epona*.

Clutch size of *P. flavidulus* increases with the host size from single in second instar nymphs to an average of 4.7 in adults of the mealybug *P. viburni*. In comparison, *Anagyrus indicus* Shafee et al. (Encyrtidae) varied the number of eggs per host from an average of 1.2 for first instar nymphs to 2.8 for adults, when hosts of the spherical mealybug were presented in a no-choice design (Nechols & Kikuchi, 1985). Both female and male parasitoids of *P. flavidulus* are produced from single broods in small hosts of the mealybug *P. viburni* but most female parasitoids are produced in the larger sizes of the mealybug. The sex ratio (male proportion) produced by *P. flavidulus* is insensitive to host size and in favour of females regardless of whether the mealybug size classes were offered separately or together. The female biased sex ratio in broods of all host sizes and the synchronised emergence of the sexes indicate that in this species LMC occurs.

Influence of oviposition experience on host size selection and sex ratio of the offspring. Oviposition experience on the host by *L. epona* or *P. flavidulus* for 24 hours does not seem to influence the host size preference and sex ratio of the offspring for either parasitoid and the clutch size for *P. flavidulus*. Previous host size experience on either large or small

hosts affected the sex and clutch size response of the parasitoid *Spalangia cameroni* Perkins (Pteromalidae) when ovipositing on large hosts but not on small hosts and in any case the effect was not for more than about one day (King, 1994).

Implications for mass rearing and efficacy of the parasitoids as biocontrol agents. Biological parameters such as successful parasitism, fast development of healthy and fit female wasps and a female biased sex ratio are important qualities for mass rearing a parasitoid and host suppression (DeBach, 1964; van Dijken et al., 1989).

Large hosts (adult mealybugs) seem to be more suitable for a mass-rearing programme of the parasitoid *L. epona* in terms of successful parasitism, a female biased sex ratio and fast development of the offspring. Host size distribution may be the most likely factor potentially affecting sex ratio in rearing populations of *L. epona*. It is tempting when rearing parasitoids to use hosts as young as possible to shorten the turnover of the culture (Kenis, 1996). However, in the case of *L. epona* this would probably result in a male-biased sex ratio and small unfit wasps which may consequently lead to the collapse of the culture (Waage, 1986).

All host stages of *P. viburni* older than the crawlers seem to be suitable for a mass-rearing programme of *P. flavidulus* in terms of production of more female wasps and fast parasitoid development. However, the higher clutch size and higher number of females in larger mealybugs supports a greater reproductive success of *P. flavidulus* in larger compared with smaller hosts. Female wasp crowding in mass cultures of the parasitoid may increase the proportion of male offspring as LMC declines (Charnov, 1982).

For biological control, single applications of either *L. epona* or *P. flavidulus* may gain better profitability of the host and efficacy of the parasitoids to control the host when the young adult stage is in abundance. Hence, field colonization attempts should be made when the host's third nymphal stage is the most abundant, because the period during which preferred and suitable host stages are available would be the longest. However, *P. flavidulus* is more able to parasitize second instar nymphs (0.5–0.9 mm) compared with *L. epona* and broods of *P. flavidulus* in these hosts are female biased. Therefore, *P. flavidulus* could probably be used for control of the mealybug earlier, on smaller host sizes. Allocation of male parasitoids in small hosts by *L. epona* might either stabilize or desta-

bilize the system if hosts are always parasitized as soon as they become large enough to be attacked. Thus the number of females in the next generation will be small and the host population will have an opportunity to recover from parasitism but the number of female parasitoids might be reduced to levels near extinction (Kistler, 1985).

A multiple introduction of *L. epona* and *P. flavidulus* in the same host habitat would make them competitors as both of them are parasitoids of mainly large hosts and consequently exploit the same host range. However, *P. flavidulus* might restrict itself to the second instar nymphs, which are preferred more for oviposition by *P. flavidulus* than *L. epona*. Female biased sex ratios of *P. flavidulus* in small hosts would favour the population built up of the parasitoid towards *L. epona*. Furthermore, a multiple introduction raises the question of the parasitoid coexistence or species displacement because of interactions of other factors like the inherent reproductive activity, the searching capacity, intra- and inter-host discrimination or multiparasitism, external contest and intrinsic competition between the parasitoids.

Acknowledgements

We would like to thank Jan Piet Kaas of 'Biopré' in the Netherlands who provided us with mealybug mummies to start the culture of *L. epona* and Renato Ripa of the 'Centro Nacional de Entomología la Cruz' in Chile who provided the mummies to start the culture of *P. flavidulus*. We also thank Chris Hodgson at Wye College, University of London, U.K. for the identification of the mealybug species and John Noyes at the Natural History Museum, U.K. for confirming the identification of the two parasitoid species. Our thanks are extended to Trudy Watt and Alan Clewer for statistical advice on the design and data analysis of the experiments. Lastly, the first author wishes to acknowledge the Greek State Scholarship Foundation (I.K.Y.) and 'Maria Kasimati Legacy' for financial support during this research.

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