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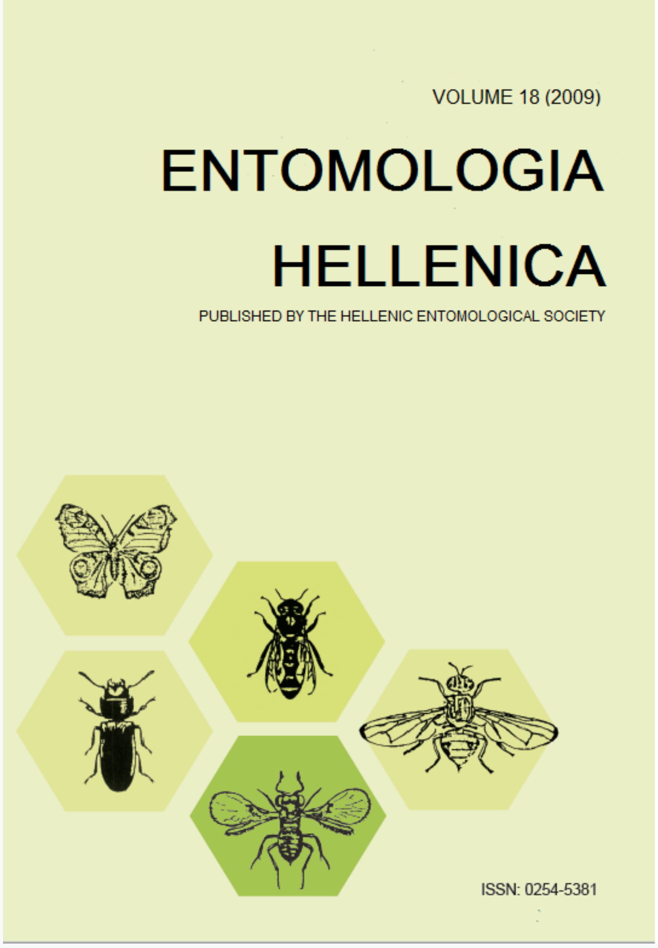

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Conspecific and heterospecific host discrimination in two parasitoid species of the mealybug *Pseudococcus viburni*, the solitary *Leptomastix epona* and the gregarious *Pseudaphycus flavidulus*

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ABSTRACT

Leptomastix epona (Walker) and *Pseudaphycus flavidulus* (Brèthes) (Hymenoptera: Encyrtidae) are endoparasitoids of the mealybug *Pseudococus viburni* (Signoret) (Hemiptera: Pseudococcidae). *Leptomastix epona* is a solitary parasitoid originating from Europe and *P. flavidulus* is a gregarious parasitoid from South America. Conspecific and heterospecific host discrimination was examined between unparasitised female adult mealybugs and others already parasitized, at different time intervals between the primary and the following oviposition. Female wasps of *L. epona* discriminate between unparasitized hosts and hosts parasitized 0-96 hours previously by a conspecific selecting more often the first over the latter for oviposition. Females of *P. flavidulus* discriminate between unparasitized hosts and hosts parasitized prior to 0-72 hours by a conspecific. Conspecific superparasitism also occurs in both parasitoid species. The secondary sex ratio of *L. epona* and the clutch size of *P. flavidulus* are not affected by superparasitism whereas the secondary sex ratio of *P. flavidulus* in superparasitized hosts is more male biased than in single parasitized hosts. The solitary parasitoid *L. epona* does not discriminate between unparasitized hosts and hosts parasitized by *P. flavidulus* 0-3 or 24 hours previously. Nevertheless the gregarious parasitoid *P. flavidulus* discriminates between unparasitized hosts and hosts parasitized by *L. epona* 0-3 hours earlier, but it oviposits without discrimination in hosts heterospecifically parasitized 24 hours beforehand. When multiparasitism occurs at 0-3 hours after the first oviposition, *L. epona* is a superior competitor regardless of which species oviposits first. However, when the time interval between the two ovipositions is 24 hours, the probability of the offspring of *P. flavidulus* winning the competition with *L. epona* increases when either species oviposits first. The potential effect of multiparasitism on parasitoid coexistence is discussed in relation to prospects for multiple-species introductions or augmentative releases for the biological control of the mealybug.

KEYWORDS: conspecific, interspecific, host discrimination, multiparasitism, multiple-species introduction, parasitoid competition, superparasitism.

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Introduction

Intraspecific host discrimination and superparasitism are parasitoid traits which may result from an adaptive strategy depending on the environment (host abundance) or the physiological state (egg supplies) of the ovipositing parasitoid (van Lenteren 1978, Godfray 1994, Potting et al. 1997). Furthermore, the rate at which parasitoids avoid superparasitism can vary with the length of the time interval between the first egg being laid and the host being subsequently re-encountered by the same or a different wasp (Chow and Mackauer 1986, Hubbard et al. 1987, Micha et al. 1992, Ueno 1999).

In solitary parasitoids conspecific superparasitism may cause a substantial reduction of fitness through the loss of reproductive potential or the waste of time from searching in patches with parasitized hosts, which is undesirable in a biological control programme (Mackauer et al. 1990). However, superparasitism is suggested to be a functional behaviour in host selection if the offspring of the second-parasitizing female has a probability higher than zero of surviving to the adult stage (van Alphen et al. 1987, Hubbard et al. 1987, Mackauer et al. 1990, van Baaren and Nenon 1996).

In gregarious parasitoids the optimal primary clutch size in the case of conspecific superparasitism depends on the rate of finding unparasitised and parasitised hosts and on the form of function relating clutch size to individual fitness, and it may be smaller, the same or even larger than in unparasitised hosts (van Dijken and Waage 1987). Local Mate Competition (LMC) Theory predicts that in most cases, the second ovipositing wasp lays a smaller clutch of eggs and produces a more male-biased sex ratio (Hamilton 1967, Suzuki and Iwasa 1980, Werren 1980). The optimal sex ratio of the second wasp is considered sensitive to the ratio of the two clutch sizes and, less strongly, to the sex ratio of the first clutch. Godfray (1994) refers to relevant studies supporting this theory and Dorn and Beckage (2007) suggest a species-specific relationship between the sex ratio and clutch size in gregarious species subject to superparasitism. Nevertheless, a review by Shuker and West (2004) concludes that the secondary sex ratio in superparasitized hosts by gregarious parasitoids is less female biased than after a single oviposition.

When two parasitoid species have to compete for the same host, multiparasitism can reduce the overall impact of parasitism on the host population the same way as conspecific superparasitism. Heterospecific (interspecific) host discrimination brings no benefit to a superior competitor that always wins intrinsic competition with another parasitoid species (van Alphen and Visser 1990). Inferior competitors may benefit from host discrimination, as it prevents the wastage of their eggs in hosts parasitized by the superior parasitoid (Pedata et al. 2002, Wang and Messing 2004). The competitor which has the higher probability to win the intrinsic competition could change with the time interval between ovipositions (Wai and Fujii 1990).

The impact of a multiple parasitoid introduction on the suppression of a host and the interaction between the parasitoids has been widely argued (Smith 1929, Turnbull and Chant 1961, De Bach 1964, May and Hassel 1981, Ehler and Hall 1982, Kakehashi et al. 1984, Hassel and Waage 1984). May and Hassel (1981) predicted accumulative parasitism in case of introduction of two parasitoids with no niche overlap; Ehler and Hall (1982) suggested that natural enemies capable of effective control of the target pest may fail to establish in multiple introductions as a result of competitive exclusion; Kakehashi et al. (1984) showed that overall parasitism depends on the degree of niche overlap and other parameters (i.e. intrinsic rate of increase of the host, intrinsic and extrinsic competition between the parasitoids); Hassel and Waage (1984) proposed that interspecific competition brings about complementary species attributes often observed in parasitoid communities (i.e. high rates of increase and dispersal opposed to lower rates of increase and higher larval competitiveness).

Pseudococcus viburni (Signoret) (Hemiptera: Pseudococcidae) is a world-wide distributed mealybug and important pest on citrus, apple, grapevine, stone fruits, field crops, ornamentals and in protected cultivation in Northern Europe (Williams 1962, Panis 1986, Gonzalez 1991, Phillips and Sherk 1991, Ben-Dov 1994). *Leptomastix epona* (Walker) and *Pseudaphycus flavidulus* (Brèthes) (Hymenoptera: Encyrtidae) are endoparasitoids of *P. viburni*. Regarding *L. epona* it is a solitary parasitoid which originates from Europe whereas *P. flavidulus* is a gregarious parasitoid and originates from the Neotropical region (Argentina and Chile) (Noyes, personal communication). Both parasitoids have been used as biological control agents in practice in single species releases or together during the last years (Daane et al. 2002, EPPO 2002, Daane et al. 2004). A mass release programme of both *L. epona* and *P. flavidulus* in Central Coast vineyards in California for the control of the obscure mealybug (June 1997 to May 2000: 5,500 *L. epona* and 194,000 *P. flavidulus*) resulted in establishment of the two species during the release period (Daane 1999, Daane et al. 2004) but only *P. flavidulus* was recovered in surveys in 2001 (Daane et al. 2002) and neither species was recovered in 2004 (Daane et al. 2004). However, this finding was not regarded as conclusive evidence that the parasitoids, especially *P. flavidulus*, had disappeared from the area of release because many parasitized mealybugs might have escaped monitoring due to their tendency to move down to the trunk of the vine before mummifying (Daane et al. 2004).

These experiments examined conspecific and heterospecific host discrimination by the parasitoids *L. epona* and *P. flavidulus* at various time intervals after the primary oviposition in the host. The secondary sex ratio of the parasitoid offspring (proportion of males out of total emerging wasps) and the clutch size (gregarious species) were investigated in superparasitized hosts *vs.* once parasitized hosts. As both parasitoid species exploit the same host resources (2nd instar nymphs and mainly 3rd instar nymphs and female adult mealybugs) and *L. epona* shows a preference in adult mealybugs compared to 3rd instar nymphs (Karamaouna and Copland 2000a, b) it is worth to know, in case the parasitoids would have to compete for the same host in a biological control programme, whether host multiparasitism occurs and which species - the solitary or the gregarious- is the intrinsically superior competitor. The results of the experiments will offer an insight to the consequences of a multiple introduction or inoculative release of the parasitoids for the control of the mealybug.

Materials and Methods

Cultures

Mass rearing of the mealybug *P. viburni* was established in the laboratory from individuals collected on the plant *Passiflora coccinea* (Passifloraceae) in the glasshouses at Imperial College at Wye. The mealybug cultures were maintained on sprouted potatoes of the variety "Desiree" in plastic sandwich boxes (17.5x11.5x5cm) with net covered openings for ventilation. They were kept in a rearing room at 26±1°C, 50-65% R.H., under a photoperiod of 16:8 (L:D) and 3.3 Watts/m^2

light intensity. The parasitoids *L. epona* and *P. flavidulus* were reared on mealybugs of *P. viburni* feeding on sprouted potatoes, in sandwich boxes in a rearing room which was similar to the one with the host but with continuous light. The reason of continuous lighting was to avoid the change of infra red radiation when the lights go on and off and thereby keeping the parasitoids under a constant temperature both by ambient and through diurnal warming by absorption of infra red.

Experiments

Female parasitoids, 0-24 hours old after emergence, were mated in glass vials and then kept in them individually provided with 50% honey solution and deprived of hosts for 24 hours. Mating of female wasps of *L. epona* was succeeded by introduction of a male (1 female: 1 male) in the glass vials where it was observed and retained until insemination. Only female wasps of sex-mixed broods of *P. flavidulus* were used in the experiments, which were assumed to have mated with the males of the same brood after emergence. Feeding was obtained from vials containing the honey solution, which were inserted into the glass vials containing the female wasps through a hole in their cover, via embedded cotton wool used as a closure of the feeding vials. The mated and fed female wasps were offered adult female mealybugs (1.83-3mm) in order to have oviposition experience in the host. Females of *L. epona* were offered five mealybugs, one after the other, and were observed for oviposition in each host; *P. flavidulus* were given three hosts for one hour. The two species were given different oviposition experience exposure regimes before the experiment because the solitary *L. epona* deposits its egg in the host within a few seconds whereas it takes 10-15 minutes to the gregarious *P. flavidulus* to deposit its egg load (Karamaouna 1999). Afterwards, the wasps were returned to vials with the feeding apparatus and kept for another 24 hours before being used in the experiments. Female wasps 48-72 hours old after emergence, which were mated and experienced, were used in the experiments.

In the experiments, the female parasitoids of either species were given a choice between a parasitized and an unparasitized host of adult female mealybugs (1.83-3mm), which were size-matched of approximately the same size. One host of each quality (parasitized-unparasitized) was put in a 6cm diameter Petri-dish where an equal sized leaf-disc of the host plant *Passiflora coccinea* was placed (lower surface upwards) on a layer of 8 g/l Bacteriological agar.

To examine superparasitism, female wasps of either *L. epona* or *P. flavidulus* were offered an unparasitized host and a host 0-3, 24, 48, 72 and 96 hours (only *L. epona*) previously parasitized by a conspecific. To examine multiparasitism two sets of tests were conducted: a) female wasps of *L. epona* were offered an unparasitized host and a host 0-3 or 24 hours previously parasitized by *P. flavidulus* and b) females of *P. flavidulus* were offered an unparasitized host and a host 0-3 or 24 hours previously parasitized by *L. epona*. The tests were repeated fifteen times in each set and for each time interval using different ovipositing wasps (replications).

The wasps were observed under a stereo-microscope (x 30) at room temperature (22^{o}C) and a record of the first antennation and the first oviposition on either host was kept. In the case of *L. epona,* insertion of the ovipositor was classified as 'oviposition' only when it was followed by a pumping movement of the host's abdomen (de Jong and van Alphen 1989, Karamaouna and Copland 2000b). In the case of *P. flavidulus,* insertion of the ovipositor was recorded as 'oviposition' unless the parasitoid retracted her ovipositor after a probe (van Lenteren 1976).

Observations continued even after the first oviposition until a possible oviposition in the other host in order to be able to study possible superparasitism or multiparasitism. The maximum duration of the tests was five minutes for *L. epona* and 30 minutes for *P. flavidulus* due to the longer duration of egg deposition in the latter (Karamaouna 1999). Hosts from each Petri-dish were incubated at 26°C until parasitoid emergence, so that the sex of the offspring in the case of superparasitism or the species of the offspring in the case of multiparasitism could be recorded.

Statistical analysis

Statistical analysis was performed using MINITAB 10.51 Xtra for Windows (Minitab Inc. 1995). The number of previously parasitised hosts which were selected for first antennation or first oviposition over unparasitised hosts by either a conspecific or a heterospecific wasp was analysed using the goodness of fit χ^2 test for each time interval elapsed after parasitism by the first ovipositing wasp. The effect of time interval on the number of first antennations or first ovipositions on previously parasitised hosts was analysed with the contingency χ^2 test.

The probability of emergence of male offspring from once parasitized or superparasitized hosts by *L. epona* was analysed with the one-sample binomial test for each time interval. The effect of time interval on the number of male progeny emerged from hosts superparasitized by *L. epo*na was analysed with the Kruskal-Wallis test.

Proportions of male offspring and clutch size of *P. flavidulus* emerged from once parasitized and superparasitized mealybugs were compared using two sample t-test over all the time intervals. The effect of time interval on proportion of male parasitoids emerged from hosts superparasitized by *P. flavidulus* at different time intervals was analysed using ANOVA.

The probability of emergence of *L. epona* offspring from hosts previously parasitized by *P. flavidulus* or vice versa was analysed with the one-sample binomial test for each time interval.

Results

Conspecific host discrimination

The number of first antennations and first ovipositions by females of *L. epona* did not differ between unparasitized hosts and hosts parasitized 0-3, 24, 48, 72 and 96 hours previously by conspecifics (Table 1). The effect of time interval on the number of first antennations and first ovipositions of *L. epona* in already parasitized hosts by conspecifics was not found significant (Table 1). However, when data of all time intervals were pooled, then the total number of first antennations or ovipositions by the parasitoids was significantly higher in unparasitized hosts compared with the parasitized hosts (Table 1).

The number of first antennations by female wasps of *P. flavidulus* in unparasitized hosts and already parasitized hosts by conspecifics did not differ significantly at all time intervals (0-72 hours) between the primary and the following oviposition (Table 2). The effect of time interval on the number of first antennations of *P. flavidulus* on already parasitised hosts by conspecifics was not found significant (Table 2). The number of first ovipositions by female wasps of *P. flavidulus* was significantly higher in unparasitized hosts than in hosts parasitized 0-3 and 72 hours previously by conspecifics but it did not differ significantly between unparasitized hosts and hosts parasitized 24

TABLE 1. Number of first antennations and ovipositions by females of *L. epona* in unparasitized hosts and hosts parasitized 0-3, 24, 48, 72 and 96 hours previously by conspecifics.

First Antennation

	Time interval between primary and following oviposition (hours)					Pooled data	Total Time effect
Quality of Host	0-3	24	48	72	96	0-96	
Previously parasitized	4	6	4	6	7	27	
Unparasitized	11	10	11	8	7	47	
n	15	16	15	14	14	74	
χ^2	3.267	1	3.267	0.285	0	5.405	2.604
df	1	1	1	1	1	1	4
P	0.070	0.317	0.070	0.592	1	0.02	0.626*

First Oviposition

Previously parasitized	5	5	6	6	5	27	
Unparasitized	10	11	9	8	9	47	
n	15	16	15	14	14	47	
χ^2	1.667	2.25	0.600	0.286	1.143	5.405	2.608
df	1	1	1	1	1	1	4
P	0.197	0.134	0.438	0.593	0.285	0.02	0.626*

Analysis of data in columns with goodness of fit χ^2 test; n = number of observations.
*Analysis for time effect with contingency χ^2 test, based on data of all time intervals.

TABLE 2. Number of first antennations and ovipositions by females of *P. flavidulus* in unparasitized hosts and hosts parasitized 0-3, 24, 48 and 72 hours previously by conspecifics.

First Antennation

	Time interval between primary and following oviposition (hours)				Pooled data	Total time effect
Quality of host	0-3	24	48	72	0-72	
Previously parasitized	9	7	6	6	28	
Unparasitized	5	6	8	8	27	
n	14	13	14	14	55	
χ^2	1.143	0.077	0.286	0.286	0.018	1.774
df	1	1	1	1	1	3
P	0.285	0.781	0.593	0.593	0.892	0.621*
First Oviposition						
Previously parasitized	3	3	3	3	12	
Unparasitized	11	9	7	11	38	
n	14	12	10	14	50	
χ^2	4.571	3.000	1.600	4.571	13.52	E<5
df	1	1	1	1	1	
P	0.032	0.083	0.206	0.032	0.00024	

Analysis of data in columns with goodness of fit χ^2 test; n = number of observations; *E* = expected counts.

*Analysis for time effect with contingency χ^2 test, based on data of 0-3, 24, 48 and 72 hours time intervals.

and 48 hours previously by conspecifics (Table 2).

Conspecific superparasitism

The number of male offspring of *L. epona* emerged did not differ significantly between once parasitized and superparasitized hosts at 0-3, 24 and 96 hours intervals; results of 48 and 72 h intervals could not be analysed due to small number of replications (One-tailed binomial test, $\alpha = 0.01$; Table 3). The number of male offspring emerged from superparasitized hosts by *L. epona* did not differ significantly between the time intervals (Kruskal-Wallis, $H = 3.27$, $df = 4$, $P = 0.514$).

The mean clutch size of *P. flavidulus* was 5.19 ± 0.54 (n= 36) and 5.06 ± 0.40 (n= 49) in once parasitized hosts and superparasitized hosts respectively over all time intervals and it did not differ significantly between them (two sample t-test, $P = 0.84$, $df = 69$; n = number of replications). The mean proportion of male offspring of *P. flavidulus* out of the total in the same clutch was 0.28 ± 0.06 (n= 29) and 0.44 ± 0.06 (n= 36) in once parasitized and superparasitized mealybugs over all time intervals, being significantly higher in superparasitized hosts (two sample t-test, $P = 0.04$, $df = 62$; n = number of replications) The mean proportion of male parasitoids/clutch emerged from hosts superparasitized by *P. flavidulus* did not differ significantly between the time intervals (ANOVA, $df = 3$, $P = 0.351$) (Table 4).

Interspecific host discrimination

The number of first antennations or first ovipositions by females of *L. epona* in hosts parasitized 0-3 or 24 hours previously by *P. flavidulus* did not differ significantly compared to unparasitized hosts (goodness of fit χ^2 test, $P \geq 0.05$) (Table 5). Significantly fewer female wasps of *P. flavidulus* oviposited in hosts previously parasitized within 0-3 hours by *L. epona* than in unparasitized hosts ($P \leq 0.05$) (Table 5). The small number of observations did not allow statistically reliable results on the number of ovipositions of *P. flavidulus* in hosts parasitized 24 h previously by the solitary species versus unparasitised hosts (Table 5).

Multiparasitism

Significantly more offspring of *L. epona* than *P. flavidulus* emerged from multiparasitized hosts, whatever the species multiparasitizing at 0-3 hours interval. The number of *L. epona* and *P. flavidulus* progeny emerged from multiparasitized hosts in which either of the two parasitoids had oviposited first 24 hours previously did not differ significantly between the parasitoid species (One-tailed binomial test, $\alpha = 0.01$; Table 6).

Discussion

Females of the solitary parasitoid *L. epona* seem to discriminate at antennation between unparasitized hosts and hosts which have been parasitized previously by conspecifics at different time intervals (0-96 hours) after the primary oviposition, and select the former over the latter for oviposition (Table 1). Conspecific host discrimination by *L. epona* was not statistically proved for each time interval however it should be noted that the number of replications was relatively small and that time interval did not affect first antennations or ovipositions on previously parasitized hosts (Table 1). Host discrimination at antennation is possible as *L. epona* seems to use mainly the antennae to examine the host in order to proceed in acceptance or rejection for oviposition (Karamaouna and Copland 2000b).

TABLE 3. Sex ratio (male/female) of parasitoid offspring emerged from once parasitized or superparasitized mealybugs of *P. viburni* by *L. epona,* when hosts were offered in two choice situation tests.

Time interval after primary oviposition (hours)	Parasitoid sex ratio (male/female) Once parasitized: Superparasitized hosts				Male parasitoids emerged from hosts once parasitized or superparasitized by the same wasp		
	n		n		n	once parasitized: superparasitized hosts	*p*
0-3	8	1.7:1	12	0.5:1	8	5:2	0.500
							p (x ≤2; 8)
24	10	0.7:1	11	0.8:1	7	3:2	0.227
							p (x ≤2; 7)
48	11	1.2:1	7	0.7:1	3	0:0	
72	10	2.3:1	6	1:1		1:3	
96	11	0.6:1	10	0.1:1	7	2:1	0.062
							p (x ≤1; 7)

n= number of replications; x= smaller frequency of male offspring in each time interval; binomial test (α= 0.01); H_o: probability of a male offspring from once parasitised host = probability of a male offspring from superparasitised host; *p* (x; n)= One-tailed binomial probability under H_o; *P* (probability of male or female offspring from once parasitised host in each replication) = Q (probability of male or female offspring from superparasitised host in each replication) = 0.5

TABLE 4. Mean proportion of male parasitoid offspring, emerged from once parasitized or superparasitized mealybugs of *P. viburni* by *P. flavidulus,* when hosts were offered in two choice situation tests.

Time interval between primary and following oviposition (hours)	Mean proportion of male parasitoid offspring emerged from hosts				Means of the difference of the proportions	
	Once parasitized		Superparasitized			
	n	$\bar{x}$ ± SE	n	$\bar{x}$ ± SE	n	$\bar{x}$ ± SE
0-3	9	0.22 ± 0.11	11	0.49 ± 0.11	6	0.26 ± 0.27
24	6	0.19 ± 0.14	9	0.75 ± 0.15	3	0.55 ± 0.33
48	5	0.33 ± 0.19	8	0.14 ± 0.13	3	-0.19 ± 0.35
72	9	0.27 ± 0.12	8	0.26 ± 0.13	7	-0.00 ± 0.07

Means and standard errors are not weighted.
n = number of replications, $\bar{x}$ = mean number, SE = standard error

TABLE 5. Number of first antennations and ovipositions by either *L. epona* or *P. flavidulus* in hosts of *P. viburni* which were unparasitized or parasitized 0–3 or 24 hours previously by heterospecifics.

Discrimination by *L. epona* of hosts previously parasitized by *P. flavidulus* or unparasitized				
	Time interval between primary and following oviposition (hours)			
Quality of host	0-3		24	
	Antennations	Ovipositions	Antennations	Ovipositions
Previously parasitized	6	7	8	6
Unparasitized	9	8	7	9
n	15	15	15	15
χ^2	0.600	0.067	0.067	0.600
df	1	1	1	1
P	0.438	0.796	0.796	0.438
Discrimination by *P. flavidulus* of hosts previously parasitized by *L. epona* or unparasitized				
Previously parasitized	7	3	7	4
Unparasitized	7	11	8	5
n	14	14	15	9
χ^2	0	4.571	0.067	$E < 5$
df	1	1	1	
P	1	0.032	0.797	

Analysis of data in columns with goodness of fit χ^2 test; n = number of observations; *E* = expected counts.

*Analysis for time effect with contingency χ^2 test, based on oviposition data of 0-3 and 24 hours time interval.

TABLE 6. Number of multiparasitized hosts of *P. viburni* from which *L. epona* or *P. flavidulus* offspring emerged, when either *L. epona* or *P. flavidulus* multiparasitized.

Number of hosts from which either parasitoid emerged after multiparasitism by *L. epona*		
	Time interval between primary and following oviposition (hours)	
Species emerged	0-3	24
L. epona	12	10
P. flavidulus	2	4
n	14	14
p (x; n)	*p* $(x \leq 2; 14) = 0.006$	*p* $(x \leq 4; 14) = 0.090$
Number of hosts from which either parasitoid emerged after multiparasitism by *P.flavidulus*		
	Time interval between primary and following oviposition (hours)	
Species emerged	0-3	24
L. epona	12	8
P. flavidulus	1	3
n	13	11
p (x; n)	*p* $(x \leq 1; 13) = 0.002$	*p* $(x \leq 3; 11) = 0.113$

n= number of replications; x= smaller frequency of parasitoid offspring in each time interval; binomial test (α= 0.01); H_o: probability of *P. flavidulus* offspring from hosts where multiparasitism occurred by *L. epona* = probability of *P. flavidulus* offspring from hosts where multiparasitism occurred by *P. flavidulus*; *p* (x; n)= One-tailed binomial probability under H_o; *P* (probability of *L. epona* or *P. flavidulus* offspring from hosts where multiparasitism occurred by *L. epona* in each replication) = Q (probability of *L. epona* or *P. flavidulus* offspring from hosts where multiparasitism occurred by *P. flavidulus* in each replication) = 0.5

Anagyrus pseudococci (Hymenoptera: Encyrtidae) is another mealybug parasitoid which is able to discriminate between parasitized hosts by conspecifics and unparasitized hosts of *Planococcus citri* (Hemiptera: Pseudococcidae) rejecting the parasitized hosts more commonly through antennal perception of external markers than during ovipositor probing which could have encountered internal markers. In the case of *A. pseudococci* the antennal/ovipositor rejection relationship changes with increasing time after oviposition (Islam and Copland 2000).

Conspecific superparasitism is also observed in *L. epona* (Table 1) and whether it reveals an adaptive value, when occurring, needs further investigation. More than one eggs which were laid within a 0-24 hours interval by the same individual or a conspecific wasp of *L. epona* in superparasitized hosts of *P. viburni* developed successfully to young larval stages (Karamaouna 1999, Karamaouna and Copland 2002). However, both in the formerly mentioned and in the present experiment single successful emergence followed from the superparasitized hosts and it is not known which of the two conspecific females was the parent of the emerging offspring.

Females of *P. flavidulus* do not discriminate at antennation but do discriminate at oviposition between unparasitized hosts and hosts parasitized 0-72 hours, and especially 0–3 and 72 hours, previously by a conspecific and oviposit preferentially in the unparasitized mealybugs (Table 2). *Pseudaphycus flavidulus* oviposits without discrimination in unparasitized hosts and in hosts parasitized 24 and 48 hours earlier by conspecifics (Table 2). However the effect of time interval on discrimination of already parasitized hosts at oviposition by conspecifics of *P. flavidulus* could not be analyzed due to small number of replications. The lack of host discrimination by *P. flavidulus* at antennation must be related to the fact that the parasitoid uses the antennae or the ovipositor (tapping) in order to examine the host, thus acceptance of the host for oviposition may result after antennation or ovipositor insertion (Karamaouna and Copland 2000b). Host discrimination by *P. flavidulus* in the early (0-3) hours after the primary oviposition prevents overcrowding whereas in 72 hours interval possibly saves the second clutch from being eaten or being unable to feed because the host resources are limited by the first clutch (Potting et al. 1997).

When conspecific superparasitism occurs in *L. epona* (Table 3), it does not influence the secondary sex ratio of the offspring like in the solitary mealybug parasitoid *Epidinocarsis lopezi* (Hymenoptera: Encyrtidae) (van Dijken et al. 1993). However, conspecific superparasitism causes a shift in the overall secondary sex ratio of *P. flavidulus* towards male in superparasitized hosts (Table 4), which is in compliance with the theoretical predictions of Local Mate Competition (LCM) in gregarious parasitoids (Hamilton 1967, Suzuki and Isawa 1980, Werren 1980, Shuker and West 2004). LCM was shown to occur in *P. flavidulus* in single parasitized mealybugs of *P. viburni* based on the female biased secondary sex ratio of the parasitoid in all host sizes and the synchronized emergence of the sexes (Karamaouna and Copland 2000a).

Superparasitism does not affect the secondary clutch size in *P. flavidulus* comparing with the single parasitized hosts. This could be due to a restriction of the second parasitizing female in oviposition in accordance to clutch theory predictions that in general gregarious parasitoids are expected to lay a clutch size which maximizes the gain in fitness from the whole clutch (Godfray 1994) or the increased competition for limited resources among progeny inside the host (van Lenteren 1981, Waage 1986).

Heterospecific host discrimination is not attributed to *L. epona* whereas it is recognised as a behavioural trait of *P. flavidulus* towards hosts freshly (0-3 hours) parasitized by *L. epona* (Table 5). However, females of *P. flavidulus* oviposit without discrimination in unparasitized hosts and hosts parasitized 24 hours previously by *L. epona*. As *L. epona* and *P. flavidulus* have a different origin, the ability of *P. flavidulus* to discriminate between unparasitized hosts and freshly parasitized hosts by *L. epona* could not result as coevolution within this parasitoid complex as suggested for sympatric species (Bokonon-Ganta et al. 1996).

When multiparasitism occurs within 0-3 hours after the first egg is laid, *L. epona* is the superior internal competitor regardless of which species oviposits first (Table 6). The probabilities of *P. flavidulus* offspring to survive from the intrinsic competition and outcompete *L. epona* increase when the time interval between the two ovipositions extends to 24 hours (Table 6). Hence, the time interval between successive ovipositions of *L. epona* and *P. flavidulus* may adversely affect one species or favour the other so that different species will be the intrinsically superior in diverse situations when the parasitoids share the same host habitat. Many laboratory and field studies have shown that heterospecific competition can be important in the dynamics of host-parasitoid communities including cases of competitive displacement of the intrinsically inferior species in multiparasitism (Huffaker and Messenger 1976, Pijls et al. 1990, Gutierrez et al. 1993). The possibility that two parasitoid species will displace one another at low host densities is reduced if the winner of the competition varies under different conditions (Mackauer et al. 1990).

Pseudaphycus flavidulus can overcome intrinsic competition with *L. epona* by discrimination of hosts freshly parasitized by the latter and the consequent avoidance of oviposition. Moreover, *P. flavidulus* escapes intrinsic competition with *L. epona* in the second instar nymphal stage of the host, which is not utilized by the solitary parasitoid for parasitism but for host feeding (Karamaouna and Copland 2000a,b). Even in case that *P. flavidulus* were always the intrinsically inferior species, coexistence with *L. epona* is still favoured if *P. flavidulus* is extrinsically superior in having a greater searching capacity than *L. epona* (Hassel and Waage 1984).

Further studies on parasitoids' searching efficiency and other parameters such as parasitoids' and host's intrinsic rates of increase are necessary in order to determine possible complementary attributes of the two parasitoid species, which will let them sharing the same habitat and to decide which practice, single or multiple -species introduction/ augmentative release, should be undertaken for more effective biological control of the pest (Hassel and Waage 1984, Kakehashi et al. 1984).

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References

Alphen, J.J.M. van, M.J. van Dijken and J.K. Waage. 1987. A functional approach to superparasitism: host discrimination needs to be learnt. Netherlands Journal of Zoology, 37: 167-179.

Alphen, J.J.M. van and M. Visser. 1990. Superparasitism as an adaptive strategy for insect parasitoids. Annu. Rev. Entomol., 35: 59-79.

Baaren, J. van and J.-P. Nènon. 1996. Intraspecific larval competition in the solitary parasitoids, *Apoanagyrus (Epidinocarsis) lopezi* and *Leptomastix dactylopii*. Entomol. Exp. Appl., 81: 325-333.

Ben-Dov, Y. 1994. A Systematic catalogue of the mealybugs of the world (Insecta: Homoptera, Coccoidea: with data on geographical distribution, host Plants, biology and economic importance). Intercept Publications Ltd., Andover, 686 pp.

Bokonon-Ganta, A.H., J.J.M. van Alphen and P. Neuenschwander. 1996. Competition between *Gyranusoidea tebygi* and *Anagyrus mangicola*, parasitoids of the mango mealybug, *Rastrococcus invadens*: interspecific host discrimination and larval competition. Entomol. Exp. Appl., 79:179-185.

Chow, F.J. and M. Mackauer. 1986. Host discrimination and larval competition in the aphid parasite *Ephedrus californicus*. Entomol. Exp. Appl., 41: 243-254.

Daane, K.M., 1999. Investigation of a cooperative based insectary and augmentation program for grape, longtailed and obscure mealybugs. Pest Management Grants Final Report to the Department of Pesticide Regulation, pp.23.

Daane, K.M., R. Malakar-Kuenen, M. Guillén, W.J. Bentley, M. Bianchi and D. González. 2002. Abiotic and biotic pest refuges hamper biological control of mealybugs in California vineyards. In: *The Proceedings of the 1st International Symposium of Biological Control of Arthropods*, January 14-18, 2002, U.S.D.A. Forest Service, Honolulu, Hawai, U.S., pp. 573.

Daane, K.M., M. Battany, E. Nelson, M. Cooper, K. Sime, J.R. Hagler. 2004. Investigation of the grape mealybug complex and its natural enemies to improve biological control. American Vineyard Foundation Annual Research Reports Crop Year 2004, pp.30.

DeBach, P. (ed) 1964. *Biological Control of Insect Pests and Weeds*. Chapman and Hall, London, 844 pp.

Dijken, M.J. van, P. van Stratum and J.J.M. van Alphen. 1993. Superparasitism and sex ratio in the solitary parasitoid *Epidinocarsis lopezi*. Entomol. Exp. Appl., 68: 51-58.

Dijken, M.J. van and J.K. Waage. 1987. Self and conspecific superparasitism by the egg parasitoid *Trichogramma evanescens*. Entomol. Exp. Appl., 43: 183-192.

Dorn, S. and N.E. Beckage. 2007. Superparasitism in gregarious hymenopteran parasitoids: ecological, behavioural and physiological perspectives. Physiol. Entomol., 32: 199-211.

Ehler, L.E. and R.W. Hall. 1982. Evidence for competitive exclusion of introduced natural enemies in biological control. Environ. Entomol., 11: 1-4.

EPPO Standards on Phytosanitary Measures – Safe Use of Biological Control. 2002. List of Biological Control Agents Widely Used in the EPPO Region [PM 3/(2)]. EPPO.

Godfray, H.C.J. 1994. Parasitoids: Behavioural and Evolutionary Ecology. Princeton University Press, Princeton, 473 pp.

Gonzalez, R.H. 1991. Mealybugs (Hom. Pseudococcidae), a new pest problem of japanese plums in Chile. Revista Fruticola, 12: 3-7.

Gutierrez, A.P., P. Neuenschwander and J.J.M. van Alphen. 1993. Factors affecting biological control of cassava mealybug by exotic parasitoids: a ratio dependent supply-demand driven model. J. Appl. Anim. Ecol., 30: 706-721.

Hamilton W.D. 1967. Extraordinary sex ratios. Science 156: 477-488.

Hassell, M.P. and J.K. Waage. 1984. Host-parasitoid population interactions. Annu. Rev. Entomol., 29: 89-114.

Hubbard, S.F., G. Marris, A. Reynolds and G.W. Rowe. 1987. Adaptive patterns in the avoidance of superparasitism by solitary parasitic wasps. J. Anim. Ecol., 56: 387-401.

Huffaker, C.B. and P.S. Messenger. 1976. Theory and Practice of Biological Control. Academic Press, New York, 788 pp.

Islam, K.S. and M.J.W. Copland. 2000. Influence of egg load and oviposition time interval on the host discrimination and offspring survival of *Anagyrus pseudococci* (Hymenoptera: Encyrtidae), a solitary endoparasitoid of citrus mealybug *Planococcus citri* (Hemiptera: Pseudococcidae). Bull. Entomol. Res., 90: 69-75.

Jong, P.W. de and J.J.M. van Alphen. 1989. Host size selection and sex allocation in *Leptomastix dactylopii*, a parasitoid of *Planococcus citri*. Entomol. Exp. Appl., 50: 161-169.

Kakehashi, N., Y. Suzuki and Y. Iwasa. 1984. Niche overlap of parasitoid systems: its consequence to single versus multiple introduction controversy in biological control. J. Anim. Ecol., 21: 115-131.

Karamaouna, F. 1999. Biology of the parasitoids *Leptomastix epona* (Walker) and *Pseudaphycus flavidulus* (Brèthes) and behavioural interactions with the host mealybug *Pseudococcus viburni* (Signoret). Ph.D. Thesis, University of London, 333 pp.

Karamaouna, F. and M.J.W. Copland. 2000a. 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. Entomol. Exp. Appl., 96: 149-158.

Karamaouna, F. and M.J.W. Copland. 2000b. Oviposition behaviour, influence of experience on host size selection, and niche overlap of the solitary *Leptomastix epona* and the gregarious *Pseudaphycus flavidulus*, two endoparasitoids of the mealybug *Pseudococcus viburni.* Entomol. Exp. Appl., 97: 301-308.

Karamaouna, F. and M.J.W. Copland. 2002. Larval development of the endoparasitoid *Leptomastix epona* (Hymenoptera: Encyrtidae) and factors influencing egg and larval mortality by encapsulation in the mealybug *Pseudococcus viburni* (Hemiptera: Pseudococcidae), In: Abstracts of the VIIth European Congress of Entomology, October 7-13, 2002, Thessaloniki, Greece. p. 93.

Lenteren, J.C. van 1976. The development of host discrimination and the prevention of superparasitism in the parasite *Pseudeucoila bochei* Weld (Hym.:

Cynipidae) Netherlands Journal of Zoology, 26: 1-83.

Lenteren, J.C. van 1981. Host discrimination by parasitoids. In: D.A. Nordlund, R.L. Jones & W.J. Lewis (eds). Semiochemicals, their Role in Pest Control. Wiley, New York, pp. 153-180.

Lenteren, J.C. van, K. Bakker and J.J.M. van Alphen. 1978. How to analyse host discrimination. Ecol. Entomol., 3: 71-75.

Mackauer, M., L.E. Ehler and J. Ronald. 1990. Critical Issues in Biological Control. Intercept, Andover, 330 pp.

May, R.M. and M.P. Hassell. 1981. The dynamics of multiparasitoid-host interactions. American Naturalist, 117: 234-261.

Micha, S.G., P.W. Wellings and R. Morton. 1992. Time-related rejection of parasitized hosts in the aphid parasitoid *Aphidius ervi*. Entomol. Exp. Appl., 62: 155-161.

Minitab Inc. 1995. Minitab User's Guide: Release 10 Xtra for Windows and Macintosh. State College, Pennsylvania.

Panis, A. 1986. Biological features of *Pseudococcus affinis*, Maskell (Homoptera: Pseudococcidae) as a guideline of its control in water-sprinkled citrus orchards. In: Cavallor and E. DiMartino (eds) Integrated Pest Control in Citrus Groves. Balkema, Rotterdam. pp. 59-65.

Pedata, P.A., M. Giorgini and E. Guerrieri. 2002. Interspecific host discrimination and within competition between *Encarsia formosa* and *E. pergandiella* (Hymenoptera: aphelinidae), two endoparasitoids of whiteflies (Heniptera: Aleyrodidae). Bull. Entomol. Res., 92: 521-528.

Phillips, P.A. and C.J. Sherk. 1991. To control mealybugs, stop honeydew-seeking ants. California Agriculture, 45: 26-28.

Pijls, J.W.A.M., K.D. Hofker, M.J. van Staalduinenand and J.J.M. van Alphen. 1990. Interspecific host discrimination and competition by *Apoanagyrus (Epidinocarsis) lopezi* and *A. diversicornis*, parasitoids of the cassava mealybug, *Phenacoccus manihoti*. Mededelingen van de Faculteit Landbouwwetenschappen van deRijksuniversiteit Gent, 55: 405-415.

Potting, R.P.J., H.M. Snellen. and L.E.M. Vet. 1997. Fitness consequences of superparasitism and mechanism of host discrimination in the stemborer parasitoid *Cotesia flavipes*. Entomol. Exp. Appl., 82: 341-348.

Shuker, D.M. and S.A. West. 2004. Information constraints and the precision of adaptation: Sex ratio manipulation in wasps. Proceedings of the National Academy of Sciences of the United States of America, 101: 10363-10367.

Smith, H.S. 1929. Multiple parasitism: its relation to the biological control of insect pests. Bull. Entomol. Res., 20: 141-149.

Suzuki Y. and Y. Iwasa. 1980. A sex ratio theory of gregarious parasitoids. Res. Popul. Ecol., 22: 366-382.

Turnbull, A.L. and D.A. Chant. 1961. The practice and theory of biological control of insects in Canada. Can. J. Zool., 39: 697-753.

Ueno, T. 1999. Multiparasitism and host feeding by solitary parasitoid wasps (Hymenoptera: Ichneumonidae) based on the pay-off from parasitized hosts. Behaviour, 92: 601-608.

Waage, J.K. 1986. Family planning in insect parasitoids. In: J.K. Waage and D. Greathead (eds). Insect Parasitoids. Academic Press, London. pp. 63-95.

Wai, K.M.and K. Fujii. 1990. Intraspecific larval competition among wasps parasitic of bean weevil larvae. Res. Popul. Ecol., 32: 85-98.

Wang, X.G. and R.H. Messing. 2004. Two different life-history strategies determine the competitive outcome between

Dirrhinus giffardii (Chalcididae) and *Pachycrepoideus vindemmiae* (Pteromalidae), ectoparasitoids of cyclorrhaphus Diptera. Bull. Entomol. Res., 94: 473-480.

Werren, J.H. 1980. Sex ratio adaptations to local mate competition in a parasitic wasp. Science 208: 1157-1159.

Williams, D.J. 1962. *The British Pseudococcidae*. Bulletin of British Museum (Natural History), London, No 12. pp. 1-79.

Αλληλεπιδράσεις μεταξύ των παρασιτοειδών *Leptomastix epona* και *Pseudaphycus flavidulus* στον παρασιτισμό του ψευδόκοκκου *Pseudococcus viburni*

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ΠΕΡΙΛΗΨΗ

Τα είδη *Leptomastix epona* (Walker) και *Pseudaphycus flavidulus* (Brèthes) (Hymenoptera: Encyrtidae) είναι ενδοπαρασιτοειδή του ψευδόκοκκου *Pseudococus viburni* (Signoret) (Hemiptera: Pseudococcidae). Το *L. epona* είναι μονήρες παρασιτοειδές που κατάγεται από την Ευρώπη και το *P. flavidulus* είναι πολλαπλό παρασιτοειδές από τη Νότια Αμερική. Μελετήθηκε η ικανότητα των παρασιτοειδών να αναγνωρίζουν μη παρασιτισμένα ενήλικα θηλυκά (ξενιστές) του ψευδόκοκκου από άλλα που έχουν παρασιτιστεί προηγουμένως από άτομα του ίδιου ή του άλλου παρασιτοειδούς (host discrimination), σε διάφορα χρονικά διαστήματα μεταξύ της αρχικής και της επόμενης ωοτοκίας. Ακμαία θηλυκά του *L. epona* αναγνωρίζουν μη παρασιτισμένους ξενιστές του ψευδόκοκκου από άλλους που έχουν παρασιτιστεί 0-96 ώρες νωρίτερα από διαφορετικό άτομο του ίδιου παρασιτοειδούς επιλέγοντας συχνότερα τους πρώτους έναντι των δεύτερων για ωοτοκία. Ακμαία θηλυκά του *P. flavidulus* αναγνωρίζουν τους μη παρασιτισμένους ξενιστές από εκείνους που έχουν παρασιτιστεί 0-72 ώρες πριν από άλλο θηλυκό του ίδιου είδους. Επιπλέον παρατηρείται επιπαρασιτισμός από διαφορετικά άτομα του ίδιου είδους (conspecific superparasitism) και στα δύο παρασιτοειδή. Η δευτερογενής αναλογία φύλου (ποσοστό αρσενικών απογόνων) του *L. epona* και ο αριθμός των απογόνων/ξενιστή του *P. flavidulus* δεν επηρεάζονται από τον επιπαρασιτισμό. Αντίθετα η δευτερογενής αναλογία φύλου του *P. flavidulus* είναι μεγαλύτερη στους ξενιστές όπου έχει γίνει επιπαρασιτισμός σε σχέση με αυτούς που έχουν παρασιτιστεί μία μόνο φορά. Το μονήρες παρασιτοειδές *L. epona* δεν αναγνωρίζει μη παρασιτισμένους ξενιστές από άλλους που έχουν παρασιτιστεί από το *P. flavidulus* 0-3 ή 24 ώρες νωρίτερα. Αντίθετα το πολλαπλό παρασιτοειδές *P. flavidulus* αναγνωρίζει μη παρασιτισμένους ξενιστές από άλλους παρασιτισμένους από το *L. epona* 0-3 ώρες νωρίτερα αλλά ωοτοκεί χωρίς διάκριση σε ξενιστές που έχουν παρασιτιστεί από το *L. epona* 24 ώρες πριν. Όταν λαμβάνει χώρα πολυπαρασιτισμός (multiparasitism) 0-3 ώρες μετά την πρώτη ωοτοκία, το *L. epona* υπερέχει στον εσωτερικό ανταγωνισμό ανεξάρτητα από το ποιο παρασιτοειδές έχει ωοτοκήσει πρώτο. Ωστόσο, όταν το διάστημα μεταξύ δύο ωοτοκιών είναι 24 ώρες, η πιθανότητα του απογόνου του *P. flavidulus* να κερδίσει τον ανταγωνισμό με το *L. epona* αυξάνεται οποιοδήποτε παρασιτοειδές και να ωοτοκήσει πρώτο. Συζητείται η πιθανή επίδραση του πολυπαρασιτισμού στην συνύπαρξη των δύο παρασιτοειδών στην προοπτική της εισαγωγής ή μαζικής απελευθέρωσης των δύο ειδών για τη βιολογική αντιμετώπιση του ψευδόκοκκου.