

Offspring production and self-superparasitism in the solitary ectoparasitoid *Spalangia cameroni* (Hymenoptera: Pteromalidae) in relation to host abundance

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Abstract

Parasitoid fitness strongly depends on the availability and quality of hosts, which provide all resources required for larval development. Several factors, such as host size and previous parasitism, may affect host quality. Because self-superparasitism induces competition among a female's offspring, it should only occur if there is an imperfect recognition of self-parasitized hosts or if there is a fitness advantage to self-superparasitism. Against this background, we investigated self-superparasitism and offspring production in *Spalangia cameroni* (Hymenoptera: Pteromalidae) in relation to the abundance of a novel host, *Ceratitis capitata* (Diptera: Tephritidae). Individual pairs of parasitoids were provided with either two (low host abundance) or ten (high host abundance) pupae per day. Under high host abundance, lifetime fecundity (number of eggs laid), offspring number, number of pupae parasitized and hosts killed were greater than under low host abundance, whereas the number of eggs per host was lower; and the proportion of hosts that did not produce offspring tended to be lower. The latter suggests the occurrence of ovicide, when hosts are scarce due to an at least imperfect recognition of previously self-parasitized hosts. Offspring production per parasitized pupa was higher when hosts were scarce and levels of self-superparasitism high, suggesting the existence of beneficial effects of self-superparasitism.

Keywords: egg production, host abundance, medfly, parasitic wasp, sex ratio

(Accepted 18 July 2011; First published online 31 August 2011)

Introduction

Parasitoid fitness strongly depends on the availability and quality of hosts because hosts provide all resources required for larval development (Caron *et al.*, 2010). Host quality may, for instance, affect parasitoid oviposition rate, offspring survival, longevity, sex ratio, body size and fecundity (King,

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2000; Silva-Torres & Matthews, 2003; Silva-Torres *et al.*, 2009). Consequently, a female's fitness returns depend on her ability to identify suitable hosts, *viz.* on the process of host selection during which host quality is assessed (Hassell, 2000; Caron *et al.*, 2010). Several factors may determine the quality of a host, with a particularly important one being host size, as larger hosts provide more food to developing larval parasitoids (Hardy *et al.*, 1992; Zaviezo & Mills, 2000; Silva-Torres *et al.*, 2009). Another important factor affecting host quality is whether a given host has been parasitized previously (Silva-Torres *et al.*, 2009). Larvae developing in superparasitized hosts may compete for limited resources, which may result in a fitness reduction for each individual through various mechanisms, including smaller size and lower fecundity (Charnov & Skinner, 1984; van Alphen & Visser, 1990; Godfray, 1994).

To avoid detrimental effects of superparasitism, many parasitoid species seem to have evolved the ability to discriminate between parasitized and unparasitized hosts (Ueno & Tanaka, 1994; Bell *et al.*, 2005; Silva-Torres *et al.*, 2009). In solitary parasitoids, surplus eggs or larvae are often even killed by physical attack or physiological suppression (Hubbard *et al.*, 1987; Godfray, 1994; Mackauer & Chau, 2001). Therefore, superparasitism in solitary species was historically considered as a failure to discriminate between parasitized and unparasitized hosts (van Lenteren, 1976). Parasitoids may avoid superparasitism by means of early patch leaving (Rosenheim & Mangel, 1994), transient paralysis (Desneux *et al.*, 2009) or by detecting scent marks (Hubbard *et al.*, 1999). However, the ability to discriminate may not necessarily result in an avoidance of superparasitism (Montoya *et al.*, 2000, 2003; Burton-Chellew *et al.*, 2008); and, at least in some parasitoids, host discrimination is only poorly developed (Caron *et al.*, 2010). Further, superparasitism may, under certain circumstances, increase offspring number (Mackauer & Chau, 2001; Gu *et al.*, 2003; Silva-Torres & Matthews, 2003; Keasar *et al.*, 2006). Thus, detrimental effects on individual offspring may be counterbalanced by higher offspring numbers, resulting in a net fitness gain for female parasitoids (Vet *et al.*, 1994; Silva-Torres *et al.*, 2009). Consequently, superparasitism may actually be advantageous to females, at least when hosts are scarce and when egg load is concomitantly high (Weisser & Houston, 1993; Yamada & Miyamoto, 1998; Mackauer & Chau, 2001; Silva-Torres *et al.*, 2009). Superparasitism is predicted to increase with decreasing host abundance (Hubbard *et al.*, 1999) and increasing egg load (Sirot *et al.*, 1997). If females are time-constrained and face a high risk that not all eggs can be deposited, discriminating between parasitized and unparasitized hosts may not pay off (Hughes, 1979).

The occurrence of self-superparasitism (i.e. depositing an egg on a host that has been previously parasitized by the same female) is even more difficult to understand, as it will inevitably induce competition among siblings. Self-superparasitism may result from not discriminating between parasitized and unparasitized hosts, or alternatively from females laying multiple-egg clutches (Rosenheim & Hongkham, 1996; Mackauer & Chau, 2001). Even self-superparasitism (or laying multiple egg clutches) may be beneficial to female parasitoids, as long as the overall fitness gain is higher in hosts receiving more than one compared to those only receiving one egg (Ito & Yamada, 2005). This may even be the case in solitary parasitoids, if (i) more eggs increase the probability of host rejection by conspecific females, thus protecting a female's offspring from competition, if (ii) the chance that a female's offspring

succeed in competition with conspecific offspring increases with the number of eggs laid, or if (iii) the risk that all offspring are killed by a conspecific female or the host's immune response decreases with increasing egg number (van Alphen & Visser, 1990; Godfray, 1994; Rosenheim & Hongkham, 1996; Mackauer & Chau, 2001).

Against the above background, we here explore self-superparasitism in relation to host abundance in the solitary parasitic wasp *Spalangia cameroni* (Hymenoptera: Pteromalidae). This species is used worldwide for biological control of house and stable flies (Skovgard & Nachman, 2004; Birkemoe *et al.*, 2009). Specifically, we predicted that self-superparasitism will increase with decreasing host abundance. By scoring lifetime offspring production, we further investigated the fitness consequences of depositing more than one egg per host in this species, thus testing whether self-superparasitism may exert beneficial effects in a solitary parasitoid, as has been predicted by some researchers (van Alphen & Visser, 1990; Ito & Yamada, 2005). As host, we used an economically important pest species of fruits, the Mediterranean fruit fly *Ceratitis capitata* (Diptera: Tephritidae) (Fimiani, 1989; Liquido *et al.*, 1991).

Materials and methods

Study organisms

The parasitoid *S. cameroni* was originally described from the Hawaiian Islands, but has a worldwide distribution owing to intentional introductions to control filth fly populations (Boucek, 1963). Females are synovigenic (eclosing with 12–40 mature eggs), and destructive host feeding is required to mature additional eggs (Gerling & Legner, 1968; King, 2002). This species is considered a solitary ectoparasitoid, as the larvae develop on the host inside the puparium of various dipteran species, with only one larva completing development per host (Boucek, 1963; Gerling & Legner, 1968). Adults probably feed on nectar or pollen because they readily accept honey in laboratory experiments (e.g. Legner & Gerling, 1967; King, 2000; Tormos *et al.*, 2009).

The experimental host, *C. capitata*, has a wide distribution, including the Mediterranean area, where it attacks more than 250 commercially-grown fruits (Fimiani, 1989; Fletcher, 1989; Liquido *et al.*, 1991). It has up to five generations per year in favourable areas of Spain (Muñiz & Gil, 1984) and produces usually about 300 (but up to 1000) eggs per female (Weems, 1981; Fletcher, 1989). We are currently studying *S. cameroni* as a potential biological control for this economically important pest (Pérez-Hinarejos & Beitia, 2008; Tormos *et al.*, 2009, 2010). The parasitoids and hosts used here originated from colonies maintained at the Instituto Valenciano de Investigaciones Agrarias (IVIA) in Moncada (Valencia, Spain). The parasitoid colony was founded in 2003 with individuals that had emerged from pupae of *C. capitata* that had been collected in the field near Bétera (Valencia) (Falcó *et al.*, 2004). The *C. capitata* host colony was established in 2002 by collecting attacked fruits at various locations in the province of Valencia.

Experimental design

Throughout all experiments, parasitoids were reared in a climate cabinet at 24.5 ± 0.5°C, 60 ± 10% relative humidity, and a 16h:8h light-dark cycle. They were maintained in translucent plastic boxes (1 litre) covered by muslin for ventilation.

Table 1. Results of a general linear models analysis testing for the effects of lifetime exposure of *S. cameroni* to two vs ten pupae of *C. capitata* per day (treatment) and experimental block (random effect, nested within treatment). Variables examined were numbers of eggs laid, hosts parasitized, the proportion of dead eggs, eggs per host (all data from experiment B), offspring produced and hosts killed in addition to those successfully parasitized (data from experiment A). Significant *P*-values are given in bold. Significant block effects indicate trait variation over time.

Trait	Source	df _(num)	df _(den)	F	<i>P</i>
Eggs laid	Treatment	1	4	8.1	0.0463
	Block [treatment]	4	52	3.3	0.0176
Hosts parasitized	Treatment	1	4	44.2	0.0027
	Block [treatment]	4	52		0.0422
Additionally killed hosts	Treatment	1	4	2.7	0.0069
	Block [treatment]	4	51	3.3	0.0185
Offspring produced	Treatment	1	4	59.6	0.0015
	Block [treatment]	4	51	0.8	0.5247
Eggs per host	Treatment	1	4	19.6	0.0114
	Block [treatment]	4	52	6.9	< 0.0001
Proportion of dead eggs	Treatment	1	4	6.5	0.0634
	Block [treatment]	4	52	5.6	0.0008

Honey and mineral water were provided for adult feeding *ad libitum*. For experiments, only freshly eclosed parasitoids (<20h) were used. Host pupae were offered in Petri dishes (Ø 60mm) located in the centre of each box. All host pupae used were 3–5 days old in order to minimize confounding effects of host age (King, 1998). To further minimize effects of host size on sex ratio (King & King, 1994), only pupae of similar size and colour (as judged visually) were used. All material (Petri dishes, boxes, etc.) used in the experiments was either new or washed in de-ionized water prior to use.

To investigate self-superparasitism in relation to host abundance, two different experiments (A and B) were carried out as detailed below. Both experiments used one parasitoid pair (i.e. one male and one female) per box and two treatments, with parasitoid pairs being provided either two (treatment 2P) or ten host pupae (10P) per day. While experiment A focused on the number of *S. cameroni* offspring produced, experiment B focused on the number of eggs laid per host. Carrying out two experiments was necessary because counting parasitoid eggs is impossible without dissecting the hosts and, thus, harming parasitoid offspring. In experiment A, Petri dishes with host pupae were removed daily, tightly sealed, checked ten days later for the number of eclosed (=surviving) hosts and additionally 40 days later for the number and sex of eclosed parasitoids. The latter time span is sufficient to allow all individuals to finish development (Moon *et al.*, 1982; Tormos *et al.*, 2009). Additionally, the number of dead host pupae which had not died from parasitism (but e.g. from probing or host feeding) was recorded. Experiment B used the same setup as above, but here all host pupae were dissected soon after removal from the boxes (typically within 24h) in order to identify (i) the proportion of pupae parasitized, (ii) the number of eggs per pupa, and (iii) the numbers of intact (being regularly shaped) and dead eggs (being more irregular) per pupa.

Thirty replicate boxes (=parasitoid pairs) were used per treatment and experiment, resulting in a total of 120 boxes (2 experiments × 2 treatments × 30 replicates). Due to space limitations within the climate cabinet, only 40 boxes, though, could be used at a time. Therefore, experiments were divided into three consecutively analyzed blocks, with each block containing ten replicates per treatment and experiment. Throughout, females were provided with host pupae until day 28 of adult life or death (if females died before day 28).

A time span of 28 days was chosen because no later egg-laying was observed in the 20 females used in the first block (experiment B). Therefore, our data reflect lifetime reproductive investment throughout. Males that had died before females were replaced to assure egg fertilisation. Throughout both experiments, baseline mortality of host pupae was scored using 20 pupae not having had any contact to parasitoids per day.

Statistical analyses

From the 120 replicates in total (2 experiments × 2 treatments × 10 replicates × 3 time blocks), five had to be excluded from further analyses, as either no offspring (experiment A, three replicates) or no eggs (experiment B, two replicates) were produced during the entire female lifespan. Differences in the number of offspring, parasitized hosts, hosts killed additionally, total number of eggs per female, eggs per pupa, and the percentage of dead eggs across treatment groups were tested using general linear models, with treatment as a fixed factor and block (nested within treatment) as a random factor. Note that using generalized models resulted in qualitatively identical results. Differences in treatments over time were tested by repeated measures ANOVAs, using the first 20 days of the oviposition period (a longer period would lead to an increasing reduction in sample size through death). Obtained offspring sex ratios were tested against even sex ratios using chi-square tests. Throughout the text, means are given ±1 SE. All statistical tests were calculated using SPSS (version 12.0; Inc., 2004; Chicago, IL, USA) or JMP (version 7.0.1; SAS Institute, 2007; Cary, NC, USA).

Results

Females provided with ten host pupae per day laid significantly more eggs (63.7 ± 3.8 vs 41.2 ± 2.8), parasitized significantly more hosts (49.4 ± 3.0 vs 17.9 ± 0.9), killed significantly more hosts in addition to parasitism (22.6 ± 1.6 vs 7.8 ± 0.7) and produced significantly more offspring (27.3 ± 1.7 vs 13.8 ± 0.9) compared to females provided with two pupae per day (table 1). Treatment effects were strikingly pronounced during the first ten days of the oviposition period, as indicated by significant interactions between treatment and time in repeated measures ANOVAs (number of eggs

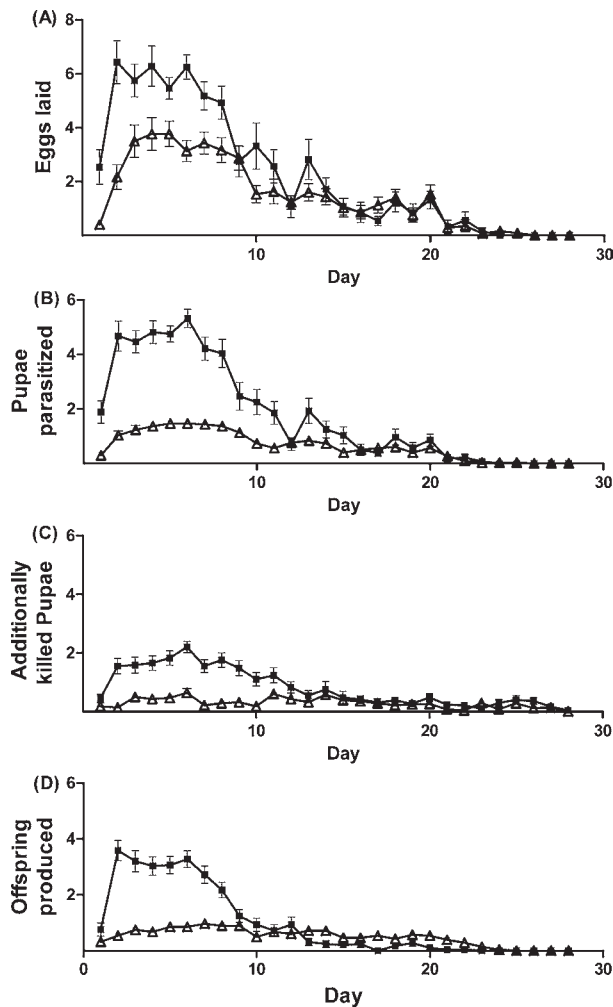


Fig. 1. Number of (a) eggs laid, (b) pupae parasitized, (c) additionally killed pupae and (d) offspring produced (means \pm 1 SE) by *Spalangia cameroni* over time in relation to host abundance (2P, two host pupae per day; 10P, ten host pupae per day) (—■—, 10P; —▲—, 2P).

laid: $F_{19,33}=3.2$, $P=0.0018$; pupae parasitized: $F_{19,33}=9.5$, $P<0.0001$; additionally killed pupae: $F_{19,36}=3.8$, $P=0.0003$; offspring produced: $F_{19,36}=8.7$, $P<0.0001$; fig. 1). Offspring sex ratio (M/F) was female-biased in both treatments (1:1.7 each; treatment 2P: $\chi^2=24.9$, $P<0.0001$; treatment 10P: $\chi^2=13.4$, $P=0.0002$). The mean number of eggs per pupa (1.3 ± 0.03 vs 2.1 ± 0.08) was significantly lower when provided ten compared to two host pupae, and there was an according tendency for the proportion of all eggs that was dead ($9.0 \pm 1.2\%$ vs $18.1 \pm 1.2\%$; table 1). Although superparasitism (defined here as the presence of >1 egg per pupa) remained higher throughout the oviposition period in females being provided two host pupae per day (non-significant interaction between treatment and time in repeated measures ANOVA: $F_{19,56}=0.26$, $P=0.99$), differences in the proportion of dead eggs were particularly pronounced during oviposition days 4–11 (interaction: $F_{19,51}=1.8$, $P=0.0209$; fig. 2). Host mortality in the controls without contact to parasitoids was low and

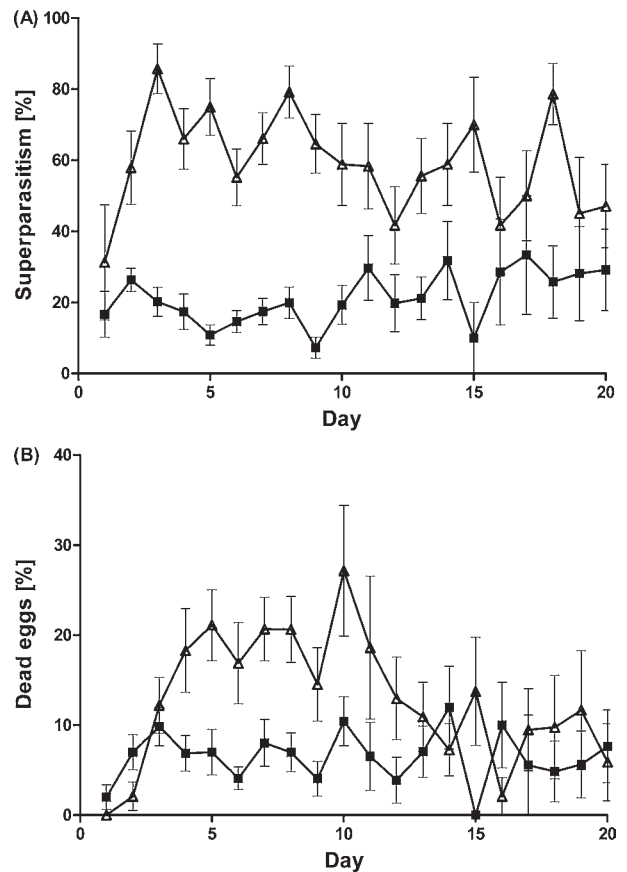


Fig. 2. Percentage of self-superparasitism: (a) more than one parasitoid egg per host pupa; and (b) dead parasitoid eggs (means \pm 1 SE) for *Spalangia cameroni* over time in relation to host abundance (2P, two host pupae per day; 10P, ten host pupae per day). Data after day 20 were excluded due to low sample size (—■—, 10P; —▲—, 2P).

constant over time ($3.0\% \pm 0.4\%$). In contrast, host mortality (in addition to successfully parasitized pupae) in experiment A was much higher ($23.2\% \pm 1.3\%$ in treatment 10P; $54.7\% \pm 2.0\%$ in treatment 2P; table 1).

Note that, based on the above mean values, offspring production per egg laid was slightly higher in the 10P compared to 2P treatment (0.43 vs 0.33 offspring per egg). However, offspring production per pupa parasitized was higher in the 2P (0.77 offspring per pupa) compared to the 10P treatment (0.55). As the data on offspring produced (experiment A) and eggs laid per pupae parasitized (B) stem from different experiments, a formal statistical comparison of the above ratios is difficult. Using chi square tests based on the total numbers of offspring produced, eggs laid and pupae parasitized for illustrative purposes here reveals significant differences in the proportion of offspring per egg laid ($\chi^2_1=23.2$, $P<0.0001$) and of offspring per pupa ($\chi^2_1=8.2$, $P=0.0041$).

Discussion

As expected and in accordance with earlier findings on parasitoids, our results show that lifetime fecundity (egg laid),

the number of pupae parasitized and offspring numbers were all positively affected by host abundance (Legner, 1969; He *et al.*, 2006; Dannon *et al.*, 2010; Mahmoudi *et al.*, 2010). Female-biased offspring sex ratios as found here have also been repeatedly documented for *S. cameroni* and other parasitoids (e.g. Moon *et al.*, 1982; King, 1989; Carleton *et al.*, 2010; Peters, 2010). Regarding parasitisation rates, it should be noted that, in both treatments, eggs were distributed unevenly across hosts, i.e. on some pupae more than one egg was deposited while others were not used at all for oviposition. Thus, an obvious question is why all available hosts were not used by *S. cameroni*? One explanation is that *S. cameroni* needs hosts not only for parasitisation but also for host feeding (alimentation). Host feeding is essential for triggering egg production but is destructive and, therefore, excludes using the same host for oviposition (non-concurrent destructive host feeders: Gerling & Legner, 1968; Shi *et al.*, 2009; Zang & Liu, 2010). Consequently, females are forced into a trade-off between alimentantion and reproduction when hosts are scarce (Heimpel & Collier, 1996). This notion is supported by the fact that approximately four times more pupae were killed without being parasitized, most likely caused by host feeding, when hosts were abundantly available. At the same time, higher levels of alimentantion, facilitated by the higher host abundance, may have contributed to the increased egg numbers in the 10P treatment.

Interestingly, a substantial proportion of parasitoid eggs was found to be dead, especially early in the oviposition period (i.e. when many eggs were laid) and in the 2P treatment. Although there are alternative explanations (e.g. host immune defence or egg resorbition, with the latter resulting in the deposition of the resorbed (= dead) eggs: Gerling & Legner, 1968). However, egg resorbition is expected to decrease rather than increase with lower host abundance (Quezada *et al.*, 1973; Rosenheim *et al.*, 2000), although the results of Richard & Casas (2009) indicate that egg resorbition most likely occurs at intermediate host densities. Consequently, not only overall energy gain but also timing seems to play an important role. Thus, in our study, it seems most likely that the eggs were killed by the females themselves (ovicide of own eggs: Godfray, 1994; Yamada & Kitashiro, 2002; Collier *et al.*, 2007). Egg death might occur accidentally while superparasitizing, or could be a deliberate ovicide by the female, following from her inability to recognize her own eggs. As female *S. cameroni* strongly concentrate parasitisation on the dorsum of the abdomen of the host pupae and as the host used here is relatively small (Gerling & Legner, 1968; Tormos *et al.*, 2009), killing of eggs through both above mechanisms is certainly possible.

As expected, the occurrence of more than one egg per pupa increased with decreasing host availability (Sirot *et al.*, 1997; Hubbart *et al.*, 1999; He *et al.*, 2006; Keasar *et al.*, 2006), though it occurred even when hosts were abundant. Note that up to six eggs (deposited by a single female within 24 h) were found in one puparium, while at the same time other hosts remained completely untouched. This suggests that *S. cameroni* either does not distinguish between parasitized and unparasitized hosts (Hubbard *et al.*, 1987; Roitberg & Mangel, 1988; Visser, 1993; but not Wylie, 1972) or that, at least occasionally, multiple-egg clutches are laid by individual females (Rosenheim & Hongkham, 1996; Mackauer & Chau, 2001). Unlike an inability to discriminate between parasitized and unparasitized hosts resulting in superparasitism, multiple-egg clutches represent a response of females to environmental

conditions, such as host scarcity (Mackauer & Chau, 2001). In our case, the lack of a decline in superparasitism with female age, despite a concomitant reduction in egg load thus reducing female time constraints, may favour an inability to recognize parasitized hosts as explanation. The failure to use some of the host pupae provided would then result from host feeding and from variation in host quality, with females repeatedly favouring a given host pupa over others. Note, though, that the ability to discriminate may not necessarily result in an avoidance of superparasitism, especially since self-superparasitism and producing multiple-egg clutches, respectively, may yield fitness gains (Montoya *et al.*, 2000, 2003; Mackauer & Chau, 2001; Keasar *et al.*, 2006; Burton-Chellew *et al.*, 2008; Silva-Torres *et al.*, 2009).

Following up on the question whether laying more than one egg per pupa might be adaptive in *S. cameroni*, it is interesting to note that the ratio between eggs laid and offspring produced was higher in the 10P compared to the 2P treatment. Consequently, the higher offspring number in females with hosts abundantly available is a function of both their higher egg number and an increased survival probability of offspring during development from egg to adult. This is in line with other studies reporting detrimental effects of superparasitism for individual offspring (van Alphen & Visser, 1990; Sousa & Spence, 2000; Ahmad *et al.*, 2002; but not e.g. Mackauer & Chau, 2001). Offspring production per parasitized pupa, in contrast, was higher when hosts were scarce and levels of self-superparasitism concomitantly high. This finding indicates that self-superparasitism may be beneficial here by increasing offspring numbers per pupa (cf. Mackauer & Chau, 2001; Gu *et al.*, 2003; Silva-Torres & Matthews, 2003; Keasar *et al.*, 2006). As in our experiment, there was no interference with conspecific females or other parasitoids; the positive effect documented here may result from an enhanced chance to overcome the host's immune defence (van Alphen & Visser, 1990; Godfray, 1994; Rosenheim & Hongkham, 1996; Mackauer & Chau, 2001), e.g. by injecting venoms (Rivers, 2004). The relatively low overall survival rates of *S. cameroni* on the given host (<50%) indicate that the host's immune response is a limiting factor for offspring development in *S. cameroni*. Consequently, higher egg numbers per host, as found under limited host availability, will evidently result in a higher number of offspring per parasitized pupa.

In summary, our study highlights the importance of host availability on parasitoid egg numbers, offspring production, eggs per pupa, number of hosts attacked and ovicide. The attack and mortality rates suggest that *S. cameroni* should be further investigated as a biological control agent for *C. capitata*. The patterns of self-superparasitism and putative ovicide by *S. cameroni* suggest a lack of discrimination between parasitized and unparasitized hosts combined with an inability of females to recognize their own offspring, or alternatively the deposition of multiple egg clutches as an adaptive strategy. However, we found only weak evidence for negative effects of self-superparasitism. Perhaps depositing more than one egg per pupa is even beneficial when hosts are scarce and survival rates unpredictable, thus forcing females into a trade-off between searching time and oviposition. Future experiments should test whether and how *S. cameroni* females respond to hosts previously used by themselves or by conspecific females, and whether superparasitism results from depositing single eggs repeatedly on the same hosts or from multiple-egg clutches.

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