Mutual interference reduces offspring production in a brood-guarding bethylid wasp

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Abstract
Parasitoids have the potential to suppress populations of their hosts and thus may play an important role in influencing the temporal and spatial dynamics of pest arthropods. Behavioural interactions between foraging females, collectively constituting ‘mutual interference’, can reduce host suppression. We use laboratory microcosms to assess the prevalence and consequences of mutual interference behaviour in a bethylid wasp, *Goniozus nephantidis* (Muesebeck) (Hymenoptera: Bethylidae), which is known to brood guard and to engage in agonistic contests for individual hosts and which is also an agent of biological pest control. We hold host and parasitoid numbers constant and vary the degree of female-female contact that can occur. Mutual interference is manifest in a considerable reduction in the number of offspring produced when females are not fully isolated from each other, due to effects operating at the early stages of offspring production. This mutual interference may contribute towards the limited degree of host population suppression achieved when some species of bethylids are deployed as agents of biological pest control and also has clear potential to influence the efficiency of mass rearing of parasitoids prior to field release.
Introduction
Parasitoid wasps are free-living as adults: females search for and attack hosts, usually the
immature stage of another arthropod, laying their eggs on or in these hosts which then form
the only food resource for their developing, parasitic, offspring, leading to the death of the
host (Godfray, 1994; Jervis, 2005). Parasitoid efficiency in host attack, and consequently
offspring production, may be tempered by behavioural interactions with other foraging
females, termed ‘mutual interference’ (Hassell & Varley, 1969; Hassell, 1971, 2000; Rogers
& Hassell, 1974; Beddington, 1975; Free et al., 1977; Begon et al., 1996; Driessen & Visser,
1997; Fellowes et al., 2005; Jervis et al., 2005; Kidd & Jervis, 2005).

Interference interactions can take a variety of forms; seen broadly they include not
only the rate of parasitism of the current generation of hosts but also influences on the size
and sexual composition of the next generation of parasitoids (Visser & Driessen, 1991; Visser
et al., 1999). Examples include time-wasting disruption of foraging for hosts without
explicitly agonistic interactions between foraging females (Hassell, 1971, 2000; Cronin &
Strong, 1993; Field et al., 1998; Wajnberg et al., 2004; Le Lann et al., 2011; Yazdani &
Keller, 2015), aggressive patch or brood guarding (Hassell, 1971; Waage, 1982; Field et al.,
1997; Goubault et al., 2005; Nakamatsu et al., 2009; Venkatesan et al., 2009a,b; de Jong et
al., 2011; Hardy et al., 2013; Mohamad et al., 2015), clutch size and superparasitism
decisions differing in the presence, or anticipated presence, of competitors (van Alphen &
Visser, 1990; Visser & Driessen, 1991; Visser et al., 1992; Visser, 1996; Field et al., 1998;
Goubault et al., 2007a), and sex allocation decisions contingent on the number of ovipositing
‘foundress’ females present (Hamilton, 1967; Waage, 1982; Meunier & Bernstein, 2002;
Irvin & Hoddle, 2006; Ode & Hardy, 2008; Luo et al., 2014).

Empirical investigations of factors contributing to mutual interference have often been
conducted from the perspective of behavioural ecology but there has also been great interest
in their consequences for host–parasitoid population dynamics, in part because this
contributes towards explaining population phenomena on the basis of natural selection
(Hassell & May, 1973, 1989; Anderson, 1989; Cronin & Strong, 1993; Driessen & Visser,
1997; Visser et al., 1999; Bernstein, 2000; Meunier & Bernstein, 2002). Typically, mutual
interference interactions reduce the per-host production of female offspring (the sex that
attacks future generations of hosts) and will also be more prevalent at higher parasitoid
densities: this density-dependent effect may be expected to contribute to the dynamic stability
of host–parasitoid populations, but extreme effects of interference can also lead to predictions
that host populations will not be regulated (sufficiently suppressed) by parasitism (Rogers &
practitioners of biological control, who deploy parasitoids against agricultural pests, the importance of mutual interference is thus that it reduces the likelihood that pest populations will be depressed below thresholds of economic damage (Anderson, 1989; Hassell & May, 1989). Although there have been many successful biocontrol programmes, attempts have also frequently failed because parasitoids have not established and because established parasitoids have not depressed the pest populations sufficiently (e.g., Mills, 2000; Kapranas & Hardy, 2014). In particular, parasitoids are often mass reared and inundatively released at high densities, which may artificially promote the occurrence of economically detrimental mutual interference interactions (Irvin & Hoddle, 2006; Ode & Hardy, 2008).

In this study we use simple laboratory microcosms to assess the prevalence and consequences of mutual interference behaviour in a bethylid wasp, *Goniozus nephantidis* (Muesebeck) (Hymenoptera: Bethylidae). This parasitoid species is known to guard individual hosts and developing broods, to engage in agonistic contests for hosts prior to and following oviposition (Hardy et al., 2013), and is also mass reared for release in biological control programmes against coconut pests (Shameer et al., 2002; Mohan & Shameer, 2003; Venkatesan et al., 2003, 2007, 2009a,b; Lyla et al., 2006). Our purpose is to understand better the competitive and reproductive biology of brood-guarding parasitoids for which prior most investigations have been confined to dyadic interactions (Hardy et al., 2013), and to in turn suggest approaches towards enhancing the efficiency of their mass rearing and field release when utilized as agents of biological pest control. Venkatesan et al. (2009b) have already reported that individual *G. nephantidis* females produced more progeny (per host and also in total) when provided with just one host rather than multiple hosts simultaneously within a single chamber (a glass vial). They also reported that when the number of females within the chamber to which a single host was presented was varied, progeny production was greatest when just one female wasp was present: offspring production was thus greatest (per female and also in total) when one host and one female *G. nephantidis* were present (Venkatesan et al., 2009b). Here, we hold the number of hosts available per female (resource-weighted density; Lewontin & Levins, 1989) constant, at unity, and experimentally vary both spatial density (individuals per unit area) and the degree of physical sub-division between chambers within microcosms (the latter allowing parasitoid behaviour to determine the organism-weighted density; Lewontin & Levins, 1989). The overall hypothesis is that parasitoids will produce fewer offspring when there is more contact possible between reproductive adult females, even though in all cases one host per female is provided.
Biology of Goniozus nephantidis

Goniozus nephantidis is a gregarious larval ectoparasitoid for which many aspects of behaviour and life history have been documented (Hardy et al., 1992; Cook, 1993; Hardy & Cook, 1995; Humphries et al., 2006; Goubault et al., 2007a,b, 2008; Venkatesan et al., 2009a,b). It is naturally associated with the coconut pest, Opisina arenosella Walker (Lepidoptera: Oecophoridae) in the Indian sub-continent (Mohan et al., 2004; Venkatesan et al., 2007, 2009a,b). Opisina arenosella larvae feed on the vascular tissues of coconut fronds by chewing, leading to affected leaves showing a burnt appearance, and also construct protective tunnels and galleries with silk, leaf parts, and frass (Mohan et al., 2004; Venkatesan et al., 2009a). Coconut crop yield losses due to O. arenosella can be as high as 45% and it can take up to 4 years for palms to regain normal yield after heavy infestation (Mohan et al., 2010). Adult G. nephantidis females forage for hosts by entering the galleries. On encountering a host larva, the adult female attacks it by injecting venom whereupon the host becomes paralysed within minutes and their development is arrested (idiobiontism). Eggs are laid onto the host 1-4 days later (Jayaratnam, 1941; Goubault et al., 2007b), the intervening period possibly being used to mature eggs (Stokkebo & Hardy, 2000; but see Goubault et al., 2007b). The number of eggs laid on a host is positively correlated with host size, with clutches ranging up to ca. 18 (Hardy et al., 1992). Eggs hatch about 1 day after oviposition and the larvae begin to feed on the host through punctures in its integument.

During the period between paralysis and oviposition, females remain in close physical association with their hosts, aggressively guarding them against intruding conspecific females which would otherwise utilize the unguarded host (Hardy & Blackburn, 1991; Petersen & Hardy, 1996). Mothers also remain with their broods after laying eggs, typically until the offspring pupate around 7 days later, and during this period guard their eggs and developing larvae against intruders (Hardy & Blackburn, 1991; Goubault et al., 2007b; Venkatesan et al., 2009a). Mothers continue to mature eggs within their abdomens whilst guarding and surviving mothers have an increasing tendency to leave their broods, possibly to forage for further hosts (Goubault et al., 2007b).

Although prior-ownership is an advantage in host-ownership contests, other factors, especially body size differences, contribute to determining the outcomes of agonistic encounters (Petersen & Hardy, 1996; Humphries et al., 2006; Hardy et al., 2013) and prior owners can thus be driven away from hosts they have paralysed (fighting is rarely fatal). Intruders that displace mothers will often destroy the brood and then lay their own eggs, but
the probability of destruction followed by oviposition declines as the developmental stage of
the original brood increases (Hardy & Blackburn, 1991; Goubault et al., 2007b).

Some offspring fail to mature, even in clutches that are not destroyed by intruders,
with mortality of guarded offspring estimated at around 28% (Hardy & Cook, 1995), mainly
due to low juvenile survival during earlier developmental stages. Surviving offspring eclose
as adults approximately 2 weeks after oviposition: males eclose first and mate with their
sisters, then adults disperse from the natal site over the following few days (Hardy et al.,
1999). The sex ratios of broods at eclosion and dispersal are usually female biased (Hardy &
Cook, 1995; Hardy et al., 1999; Venkatesan et al., 2009a,b).

Materials and methods

_Goniozus nephantidis_ cultures were maintained using the rice moth, _Corcyra cephalonica_
Stainton (Lepidoptera: Pyralidae), as a factitious host (following Hardy & Blackburn, 1991;
Hardy et al., 1992; Petersen & Hardy, 1996). _Corcyra cephalonica_ was reared on a diet of
glycerol, honey, corn meal, wheat bran, and yeast (Lizé et al., 2012). All cultures and
experiments were carried out in a climate room at 27 °C, L12:D12, with relative humidity
maintained at ca. 35% by evaporation from a water bath.

We assessed the effects of host and parasitoid density and of spatial sub-division on
parasitoid reproduction using experimental arenas excavated from polyethylene blocks and
with transparent Plexiglas lids, with designs derived from the simpler contest arenas used and
illustrated in Petersen & Hardy (1996) and Goubault et al. (2006). We used four types of
arenas, termed block A-D (Figure 1), all excavated to a depth of 6 mm. Block A contained 25
chambers and each chamber was 1.7 cm in diameter. The total excavated area (floor area)
within the block of the chamber was 56.8 cm². As wasps could not pass between the
chambers, block A served as a control treatment in which interactions between adult females
wasps were prevented. Block B was the same as A except that each chamber was connected
to the others around it by a 1 cm long and 0.1 cm wide slot, through which adult _G.
nephantidis_ can pass with ease. Block B thus had an extra 4 cm² of floor area, an increase of
7% compared to A, but retained the same chamber size as block A and also as in previous
studies (Petersen & Hardy, 1996; Goubault et al., 2006). Block C had the same total
excavated area as A but in the form of a single chamber with no spatial sub-division. Block D
also had a single chamber but with half the total excavated area (28.4 cm²).

We selected larval _C. cephalonica_ weighing between 30-40 mg (mean = 31.4) from
our cultures and presented them individually to an adult female _G. nephantidis_ until the larva
was paralysed (but with no eggs laid on its integument), and then the paralysing female wasp was discarded. Twenty-five paralysed *C. cephalonica* larvae were placed into each replicate block, one per chamber in blocks A and B, and all in the single chamber in C and D. In the latter block types we attempted to arrange the larvae into a regular array within the single chamber. The total weights of the host larvae at the start of the experiment did not differ significantly across block types (ANOVA: $F_{3,44} = 0.54$, $P = 0.66$, $r^2 = 0.035$).

We then selected female *G. nephantidis* aged 3-5 days since emergence as adults in broods containing at least one male (females had thus mated) and which had had no experience of attacking or ovipositing on hosts; we anesthetized them with CO$_2$ gas and then placed them into the chambers within the blocks (one female per chamber in A and B, and all 25 females in the centre of the chamber in C and D). The Plexiglas lid was then placed over the block. Anesthetized wasps regained their activity within 2 min. Each block was inspected daily until adult offspring matured (adult emergence from cocoons).

From each replicate block we recorded the numbers of hosts on which eggs were laid, the clutch sizes laid onto each host, and the numbers of offspring reaching the larval, pupal, and adult stages. Due to ectoparasitic development, immature *G. nephantidis* are observable at all stages; data on numbers of eggs laid are, however, likely to have been underestimates as some eggs could have been eaten between oviposition and observation. Adult female wasps (mothers) were removed from each block once parasitoid offspring had reached the pupal stage; this avoided confusion between generations during subsequent weight measurements.

The numbers of adult male and female offspring were recorded and we obtained measures of the mean dry weight of adult male and of adult female offspring. There were 12 replicates for each of the four block types.

**Statistical analysis**

Data were mainly analysed using generalized linear modelling (GLM) techniques (Crawley, 1993; Zuur et al., 2009). In most analyses, the sampling unit was taken as the summed observations deriving from an individual replicate block, but we also carried out mixed effects modelling (GLMM) on clutch size, in which the replicate within which individual observations derived was fitted as a random factor (Zuur et al., 2009). We adopted quasi-Poisson errors and a log-link function for log-linear analyses of count data and quasi-binomial errors and a logit-link function for logistic analyses of data on proportions, in both cases using empirically estimated scale parameters to correct for overdispersion (Crawley, 1993; Wilson & Hardy, 2002; Zuur et al., 2009; Warton & Hui, 2011). When significant
differences across treatments were found from fitting an initial GLM, the parsimonious
model was identified by aggregation of factor levels (Crawley, 1993). For the analysis of
adult dry weight, errors were non-normally distributed so we employed non-parametric
Kruskal-Wallis ANOVAs (Siegel & Castellan, 1988). All statistical testing was 2-tailed. All
analyses were carried out using the GENSTAT statistical package (v. 15.1; VSN
International, Hemel Hempstead, UK).

Results
The proportion of hosts on which eggs were observed varied significantly between block
types (logistic ANOVA: $F_{3,40} = 8.02, P<0.001$; Figure 2): the probability of a host being
oviposited on was highest in blocks of type A and did not differ significantly among blocks
of the other three types (model simplification by aggregation of factor levels: $F_{2,42} = 0.85, P =
0.43$). Among those hosts on which eggs were observed, clutch sizes varied significantly
across block types (log-linear GLMM: Wald statistic = 70.08, $F_{3,42,8} = 23.36, P<0.001$), with
larger clutches in A [mean (+SE, -SE); A: 7.98 (+0.263, -0.254); B, 5.78 (+0.352, -0.331); C:
4.42 (+0.348, -0.323); D: 4.35 (+0.344, -0.319)]. In terms of the total numbers of eggs,
larvae, pupae, and adults produced per replicate, there were no significant differences among
B, C, and D, but production was significantly greater in A (Table 1). Ultimately, around 4× as
many adults emerged from type A than from the other block types (Figure 3).

The mean (+SE, -SE) proportion of offspring that survived from the egg to the adult
stage was 0.59 (+0.033, -0.032) and was not significantly influenced by block type (logistic
ANOVA: $F_{3,42} = 2.11, P = 0.11$), but note that this estimate is constrained to use the observed
maximum number of eggs per replicate and the true probability of survival in some replicates
could have been lower due to ovicide (see above). However, the probability of (observed)
eggs surviving to the larval stage was affected by block type ($F_{3,42} = 4.05, P = 0.013$) and was
greatest in A and not significantly different among B, C, and D (aggregation of factor levels:
$F_{2,44} = 0.50, P = 0.61$). The probability of larvae surviving to the pupal stage was not
significantly influenced by block type ($F_{3,42} = 1.20, P = 0.32$), nor was the pupal to adult
survival ($F_{3,42} = 2.74, P = 0.055$).

The mean (+SE, -SE) overall sex ratio of adult offspring (proportion that were male)
was 0.32 (+0.021, -0.020) and was not influenced by block type (logistic ANOVA: $F_{3,42} =
1.24, P = 0.31$). The mean dry weights of adult male offspring, female offspring, or males and
females pooled did not differ across the block types (Kruskal-Wallis ANOVA; males: $H =
7.013, P = 0.071$; females: $H = 5.338, P = 0.15$; pooled: $H = 4.717, P = 0.19$; all d.f. = 3).
Discussion

Offspring production by *G. nephantidis* females is negatively affected by direct or indirect contact with conspecific females: only around one quarter as many adults developed under incomplete separation of broods compared to complete separation. Given that one host per female was always provided, the effect is not dependent on resource-weighted density, nor is it due to variation in spatial density (which was either held constant or had non-significant effect), but can be viewed as due to variation in organism-weighted density (Lewontin & Levins, 1989). That is, when not isolated, all females can experience higher parasitoid densities due to behavioural and/or chemical (Goubault et al., 2008) interactions with other females. It is notable that interference interactions occurred even when each female was initially placed in a separate chamber containing a host. A similar regular distribution of females across host patches was observed as the end result of agonistic interactions followed by dispersal of the loser in *Asobara citri* (Fischer), a braconid parasitoid of *Drosophila* spp. larvae, when females were experimentally released at a central point (de Jong et al., 2011).

The fact that *G. nephantidis* females interacted with each other despite being placed in a regular array (block type B) suggests that the perceived host-patch is larger than a single experimental chamber. This accords with prior observation that although brood-guarding *G. nephantidis* females spend most of their time in the close vicinity of their hosts, they also patrol beyond the confines of the chamber (Goubault et al., 2007b) and it is during such patrolling behaviour that they are likely to encounter other females. Direct physical encounters can lead to the release of volatile chemicals (Goubault et al., 2008) which may in turn influence the behaviour of further females indirectly.

The differences in *G. nephantidis* offspring production were principally due to effects operating at the early stages of offspring production: far fewer hosts were oviposited onto and also fewer eggs per host were observed when more than one female had access to each host. We pose three candidate explanations, which are not mutually exclusive. The first, a mechanistic explanation, is that females that were not completely isolated were continually disturbed, and possibly displaced from hosts, prior to and during oviposition. This is supported by observations of mutual attack and disruption of oviposition when one host was presented to several *G. nephantidis* females within a chamber (Venkatesan et al., 2009b). The second, an adaptive explanation, is that game-theoretic reasoning predicts females should lay smaller clutches after having encountered competitors, due to anticipation of a more competitive future environment for their offspring coupled with an increased size of offspring
that develop from smaller clutches on given-sized hosts (Petersen & Hardy, 1996; Mesterton-Gibbons & Hardy, 2004). This gains some support from prior observation of the required effects in G. nephantidis: when females with hosts weighing <30 mg experienced 1-4 competitors for 30 min, their subsequent clutch sizes were reduced by ca. 18% and offspring sizes were consequently enhanced (Goubault et al., 2007a). In our present experiment, the reduction in observed clutch size in response to encountering competitors was more marked (from ca. 8 to ca. 4-6 eggs per host) but so was the likely occurrence of female-female encounters. However, we found no evidence for effects on the size of maturing offspring – we used hosts of 30-40 mg, and Goubault et al. (2007a) also found no effects using hosts >30 mg. The lack of difference in sex ratios across experimental treatments also suggests that mothers did not adaptively adjust their reproductive decisions according to the density of ovipositing females (Hamilton, 1967; Legner & Warkentin, 1988; Irvin & Hoddle, 2006; Ode & Hardy, 2008), probably because aggressive host and brood guarding naturally lead to broods being produced by single foundresses (Hardy & Cook, 1995; Hardy et al., 2013) resulting in little selection for sex-ratio adjustment to foundress number (Herre, 1987).

The third explanation is that oviposition occurred to a similar extent in all experimental conditions but fewer eggs were observed on non-isolated hosts because some were eaten prior to being counted. Ovicidal behaviour is well documented in G. nephantidis, its congeners, and some other bethylids (Venkatraman & Chacko, 1961; Goertzen & Doutt, 1975; Hardy & Blackburn, 1991; Mayhew, 1997; Infante et al., 2001; Pérez-Lachaud et al., 2002; Goubault et al., 2007b; Bentley et al., 2009; Venkatesan et al., 2009b), occurring when clutches of eggs are not attended by a guarding mother, and the lower probability of egg-to-larval survival in blocks of type B, C, and D supports this. As with the mechanistic disruption hypothesis above, females that oviposited may have been intruded upon by such a number of other females that they were unable to defend successfully their clutches (for analogous arguments see Waage, 1982; van Alphen & Visser, 1990; Moore & Greeff, 2003; de Jong et al., 2011). The similar probabilities of the survival of offspring between the larval stages and adulthood, across experimental treatments, accords with prior reports of conspecific larvicide being absent or rare in G. nephantidis and congeners and, if it occurs, to be limited to the smaller larval stadia (Venkatraman & Chako, 1961; Goertzen & Doutt, 1975; Hardy & Blackburn, 1991; Goubault et al., 2007b; Bentley et al., 2009; Venkatesan et al., 2009a). Irrespective of the particular mechanism of mutual interference, the number of G. nephantidis offspring produced is negatively affected by contact between adult females during the host- and clutch-guarding phases. We now consider the potential consequences of
this for biological pest control programmes using *G. nephantidis*, its congeners, and other bethylids. Members of the genus *Goniozus* have been used in several biocontrol programs (Dharmaraju, 1963; Legner & Silveira-Guido, 1983; Gothilf & Mazor, 1987; Legner & Warkentin, 1988; Legner & Gordh, 1992; Mohan & Shameer, 2003; Lyla et al., 2006; Venkatesan et al., 2007, 2009a,b) and others are regarded as having pest control potential (Witethom & Gordh, 1994; Conlong, 1994; Berry, 1998; Abbas et al., 2008; Etiam, 2001; Sadeghi et al., 2012). For instance, estimates of the percentage of *O. arenosella* parasitized by *G. nephantidis* in coconut plantations range from around 4% to nearly 60% (Lyla et al., 2006; Venkatesan et al., 2007, 2009) and almond pests have been shown to be regulated by *Goniozus legneri* Gordh following classical biocontrol introduction (Legner & Gordh, 1992).

Across all biocontrol programmes using bethylids, about half have resulted in parasitoid population establishment and around 10% have achieved partial control of the target pests but records typically indicate that pest suppression is not sufficient to no longer require further control measures (Greathead & Greathead, 1992; Hardy & Goubault, 2007). Mutual interference behaviour could clearly be a contributory factor to a limited suppression of hosts once bethylid populations are established and parasitoid density increases such that foraging females frequently encounter each other at hosts. Even if mutual interference effects in the field are weaker than indicated by our experiment (given that laboratory studies typically over-estimate mutual interference due to the use of artificially high parasitoid densities; Free et al., 1977), our data do suggest that field release of mass-reared *G. nephantidis* females may be more effective (on a per parasitoid basis) when females are released at lower densities, with lower female-female encounter rates, than at higher densities. However, as females spend much time brood-guarding during which they could be foraging for further hosts, their ability to suppress large numbers of hosts will be limited (Venkatesan et al., 2009b). A contrasting suggestion is that, by leading to a regular distribution of parasitism across hosts, female-female fighting behaviour in *A. citri* might promote host population suppression (de Jong et al., 2011). It also contrasts with expectation when deploying bethylids of the genus *Sclerodermus* (Yang et al., 2014): in *Sclerodermus*, the presence of other females can enhance the probability of offspring production because females do not interact with each other aggressively and exhibit cooperative brood care after collectively suppressing and ovipositing on an individual host (Hu et al., 2012; Tang et al., 2014; Kapranas et al., 2016).

Mutual interference interactions are also likely to influence the efficiency of mass rearing of *G. nephantidis* and its congeners. Our data, and the prior results of Venkatesan et al. (2009b), clearly suggest that mass-rearing programmes will not maximize the production
of female offspring if multiple adult females are in contact with each other once presented
with hosts: using one vial for each female wasp plus one host will thus likely be more
effective than using larger communal containers. Irvin & Hoddle (2006) reached the same
conclusion when considering the economics of mass rearing mymarid parasitoids which
adjust their progeny sex ratios when ovipositing in the presence of conspecifics, and several
other studies have similarly concluded that mass production will not be optimized by using
high parasitoid densities (e.g., Luo et al., 2014). In contrast to our results, Abbas (1999)
reported that in the congener *G. legneri* there was no reduction in offspring production when
multiple hosts were provided to multiple females rather than individual females being
presented with hosts separately; we have no ready explanation for this, especially as the
reproductive and competitive behaviours of *G. nephantidis* and *G. legneri* are generally very
similar (Hardy et al., 2013). More in accord with our results is that Legner & Warkentin
(1988) found that for *G. legneri* the production of offspring per adult female ‘foundress’ was
reduced by an increase in foundress number and increased by an increase in the number of
hosts provided. They further found that the largest number of offspring per host was
produced when just one foundress was present (Legner & Warkentin, 1988).

The use of simple laboratory microcosms to suggest population-level consequences of
behaviour has its drawbacks, given that there is neither the fine control achieved by
experiments on the behaviours of one or a few individuals, nor the realism of field-scale
evaluations. Nonetheless, the value of microcosms to examine the summed effects of spatial
complexity and/or agonistic interactions has been recognised at least since the work of
Huffaker (1958; Kareiva, 1989; Hassell, 2000; Infante et al., 2001; Batchelor et al., 2006; de
Jong et al., 2011). Adopting the microcosm approach in this study has shown that interactions
between *G. nephantidis* females extend beyond the immediate vicinity of provided hosts, and
generate increased organism-weighted density when females are not completely separated.
These interactions constitute mutual interference, manifest as a considerable reduction in the
number of offspring produced. This mutual interference may contribute towards the limited
degree of host population suppression achieved when some species of bethylids are deployed
as agents of biological pest control and also has clear potential to influence the efficiency of
mass rearing of female parasitoids prior to field release.

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Table 1 Effects of experimental treatment on total offspring production at progressive life-history stages

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<th>Stage</th>
<th>Log-linear ANOVA</th>
<th>Model simplification$^\dagger$</th>
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<td>$F_{3,44}$</td>
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<td>Eggs</td>
<td>13.65</td>
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<tr>
<td>Larvae</td>
<td>16.65</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Pupae</td>
<td>13.63</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Adult females</td>
<td>9.45</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Adult males</td>
<td>13.75</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Adults (total)</td>
<td>12.28</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

$^\dagger$At every life-history stage, the overall significant difference in offspring production was due to greater numbers in block type A than in the other block types (see Figure 1): model simplification by aggregation of factor levels showed that there were no significant differences between block types B, C, and D.

Figure captions

Figure 1 Illustration of treatment blocks to vary sub-division and parasitoid density.

Figure 2 The effect of treatment on the proportion of hosts within a replicate that had eggs laid onto them. Bars represent standard errors of the means and are asymmetrical due to back transformation from the logit scale. Differences between block types B, C and D were not significant.

Figure 3 The effect of treatment on the number of adult progeny produced per replicate. Bars represent standard errors of the means and are asymmetrical due to back transformation from the log scale. Differences between block types B, C and D were not significant.
Figure 1.

Figure 2.
Figure 3.