

Intraspecific interference between native parasitoids modified by a non-native parasitoid and its consequence on population dynamics

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Abstract. 1. The number of natural enemies that should be introduced to control a pest is a controversial subject in biocontrol. A previous semi-mechanistic model parameterised using a laboratory system consisting of two parasitoid wasps, *Anisopteromalus calandrae* and *Heterospilus prosopidis*, parasitising a pest beetle, *Callosobruchus chinensis*, indicated that the introduction of the non-native parasitoid *H. prosopidis* decreases the level of intraspecific interference between native *A. calandrae* females. The model also suggested that this decrease was the main factor destabilising the population dynamics of the host–parasitoid system, resulting in chaos.

2. To test this population-level decrease and host density independence in the interference of *A. calandrae*, we observed individual behaviours to quantify the level of intraspecific interference between two *A. calandrae* females in the presence or absence of *H. prosopidis* at two different host densities.

3. When *H. prosopidis* was present, the number of direct antagonistic interference events between *A. calandrae* females, sting duration, host feeding events (but not stinging events), and patch residence time were reduced. However, the presence of *H. prosopidis* decreased the patch residence time and the proportion of hosts parasitised by *A. calandrae* only when the host density was low.

4. The reduction in intraspecific interference between *A. calandrae* females by *H. prosopidis* and its host density independence support the population-level prediction, whereas the observed reduction in host-feeding behaviours in *A. calandrae* by *H. prosopidis* was not predicted. Overall pest control by the native parasitoid was unaffected by the non-native parasitoid as host density increased.

Key words. Biological control, competition, introduced alien parasitic wasp, one host–two parasitoids, patch time allocation, seed predatory azuki bean weevil.

Introduction

The introduction of single versus multiple natural enemies is a classical and controversial topic in the biological control of pest (Pedersen & Mills, 2004). Tuda and Shimada (2005) suggests that the introduction of a non-native biocontrol agent in the presence of a native parasitoid can destabilise population

dynamics (with increased Lyapunov exponents, i.e. divergence of population trajectories) and result in extinction of the species involved, which would not be considered an efficient biological control strategy. If a behavioural assessment of interference is capable of predicting changes in population dynamics induced by the introduction of a second control agent, this would support the need for including behavioural tests in advance of risk assessments as part of the decision-making process for the introduction of biocontrol agents.

Species in food webs interact as predator and prey or as parasite and host (Casula *et al.*, 2006). Interactions between

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multiple species may lead to irregular and complex population dynamics. Understanding such species-specific interactions is crucial to elucidate mechanisms of population dynamics in nature. To accomplish this, two contrasting but complementary modelling strategies can be applied: top-down and bottom-up approaches. A top-down approach describes and draws inferences about population dynamics at the food web or community levels by developing models with a suite of assumptions about the mechanisms of population dynamics that incorporate mean population values (e.g. Dennis *et al.*, 1995; Kendall *et al.*, 1999; Jost & Arditi, 2001; Kristoffersen *et al.*, 2001; Turchin & Hanski, 2001; Tuda & Shimada, 2005). By contrast, a bottom-up approach sums each individual-level behaviour or trait by incorporating individual variation to explain phenomena at higher levels of organisation, such as at the food web or population level (e.g. Moody & Ruxton, 1996; Nakamichi *et al.*, 2008; Tyutyunov & Titova, 2018). To sort out multiple candidate mechanisms and to test for the primary mechanism in these models, predictions made by both top-down and bottom-up approaches must be subjected to experimental testing for a better understanding of the complex phenomena observed at higher levels of organisation.

Three-species models are one of the simplest systems that can provide insight into the complex population dynamics of multi-species assemblages (Holt, 1977; Sih *et al.*, 1985; Briggs *et al.*, 1993; Klebanoff & Hastings, 1994; Fussmann & Heber, 2002). Predator–prey or parasitoid–host systems are prime examples, and the species in a three-species predation or parasitism system are likely to participate in a variety of direct and indirect interactions. The stable oscillatory dynamics of the azuki bean beetle *Callosobruchus chinensis* and its native parasitoid *Anisopteromalus calandrae* are destabilised by the addition of a third species, the non-native parasitoid *Heterospilus prosopidis* (Tuda & Shimada, 2005). This particular system has been known to produce irregular population dynamics (Utida, 1957) characterised by non-linear demographic responses for the three interacting species (Kristoffersen *et al.*, 2001) and by parasitoid foraging behaviour dependent on host distribution (Shimada, 1999). By comparing the dynamics of two- and three-species population models, Tuda and Shimada (2005) demonstrated that, among all estimated model parameters [*C. chinensis*: number of eggs per individual, density-dependent mortality of eggs and larvae, and adult survival rate; *A. calandrae*: searching efficiency, host feeding, and ‘mutual interference’ (Hassell & Varley, 1969; Free *et al.*, 1977); *H. prosopidis*: searching efficiency and handling time], only intraspecific mutual interference between *A. calandrae* females decreased when *H. prosopidis* was present. This suggests that behavioural changes in *A. calandrae* might actually be triggered by the presence of *H. prosopidis*, leading to destabilised population dynamics.

Mutual interference is caused by direct antagonistic interactions between conspecific individuals over a common resource. Hassell and Varley (1969) assume that mutual interference is independent of host density, formulating parasitoid searching efficiency as $a = qP^{-m}$, where q is the intrinsic host searching efficiency (in the absence of conspecific parasitoids), P is the parasitoid density and m is the level of mutual interference.

Mutual interference is a stabilizing factor in host–parasitoid systems when it has a value close to 1.0 (Hassell & Varley, 1969). This parameter may be estimated at the individual level by observing antagonistic encounters (Cresswell, 1998; Wajnberg *et al.*, 2004; Goubault *et al.*, 2005) or at the population level either by experimentally controlling parasitoid density (Chantarasaard *et al.*, 1984; Shimada, 1999; Sagarra *et al.*, 2000; Chong & Oetting, 2006; Tahriri *et al.*, 2007; Chen *et al.*, 2008; DeLong & Vasseur, 2011; Skovgard & Nachman, 2015) or by fitting models to population dynamics (Kristoffersen *et al.*, 2001; Tuda & Shimada, 2005; Liljestrom *et al.*, 2013; Yazdani & Keller, 2015). However, the results obtained from different biological levels of organisation are rarely linked.

In the present study, we test whether direct intraspecific interference, parasitism, and host-feeding behaviour of the native parasitoid is affected by an introduced non-native parasitoid species, based on the previous prediction from population dynamics data by Tuda and Shimada (2005). At the food web level, only intraspecific interference was predicted to decrease after the introduction of the non-native parasitoid and all parameters of the parasitoids were assumed to be independent of host density. We also test the host density independence of these behavioural traits.

Materials and methods

Insects

Our model system consisted of three species with a host, *Callosobruchus chinensis* (Coleoptera: Chrysomelidae: Bruchinae), and two of its parasitoids, *Anisopteromalus calandrae* (Hymenoptera: Pteromalidae) and *Heterospilus prosopidis* (Hymenoptera: Braconidae). *C. chinensis* is a worldwide pest of bean stock, which makes it a convenient model organism for experimental studies in ecology where dispersal is limited as in closed storage (Utida, 1941; Fujii, 1968; Tuda, 1993; Tuda & Shimada, 1995; Shimada & Tuda, 1996). The *C. chinensis* strain we used originated from Japan. After *C. chinensis* eggs hatch on the surface of pods or stored dry beans, the larvae burrow into the beans to feed and develop, with adults emerging from the beans after pupation. A comprehensive description of bruchine ecology can be found in Tuda (2007). We reared *C. chinensis* on azuki beans (*Vigna angularis*) under laboratory conditions (25 °C, 50% RH and 16L:8D). For the experiment, black-eyed beans (*Vigna unguiculata*) were used because their white seed coat allows for easy observation of the parasitoids.

The parasitoid *A. calandrae* is an important natural enemy of bruchines and other stored product pest beetles. *H. prosopidis*, a parasitoid native to the southern United States and Mexico, is also used in biological control programmes against bruchines (Clausen, 1978). The *A. calandrae* strain we used originated from Japan, and the *H. prosopidis* strain originated from Hawaii, which was previously introduced from the southern United States. Female *A. calandrae* are synovigenic and engage in non-destructive host feeding, whereas *H. prosopidis* are pro-ovigenic (or very weakly synovigenic, Vamosi *et al.*, 2011) and do not exhibit host feeding behaviour. Both

species are idiobiont ectoparasitic wasps that parasitise the third and the final (fourth) instar larvae and pupae of bean beetles (Tuda & Shimada, 2005). Both parasitoid species were reared on final instar larvae of *C. chinensis* under the previously described laboratory conditions.

Host preparation

We prepared two treatments of host density—one or two host larvae (or pupae) per bean—to investigate the influence of host density on the intraspecific interference behaviour between *A. calandreae* females. For this, *C. chinensis* females were allowed to lay eggs on black-eyed beans, and then beans on which only one hatched egg was attached were used to produce the hosts in the experiments. Hosts were allowed to develop, and only those in the late final larval instar or pupal stage were exposed to parasitoids. Because parasitoid behaviour could not be observed from above if parasitoids were to crawl underneath a bean, the beans were cut in half and the two halves were glued next to each other on a Petri dish (diameter, 9 cm; height, 2 cm) using a water-soluble adhesive (Konishi, Japan). We refer to these two halves as a 'host patch'. In the treatment with one host, only one of the two bean halves contained a host larva or pupa, while both bean halves contained a host larva or pupa in treatments with two hosts.

Parasitoid preparation

One female and two males of each parasitoid species were randomly selected from the stock culture within 24 h of emergence and allowed to mate for 24 h in a glass test tube. This time is sufficient for a male to mate and inseminate a female (Bressac *et al.*, 2009). In the experiments, two mated *A. calandreae* females were released together, and we focused on the behaviour of a single individual—the 'focal female'—by marking this parasitoid with a small dot of water-based white paint (primacolor permanent white, Pébéo, Gémenos, France) on the thorax between the bases of the wings. This mark is not known to influence behavioural interactions. Female parasitoids were not provided with hosts prior to the experiment, and each parasitoid was used only once.

Behavioural observations

We prepared four treatments consisting of the presence or absence of a female *H. prosopidis* at host densities of one or two *C. chinensis* per bean. Each treatment was replicated 8–12 times for a total of 40 focal females for the experiment. All behavioural observations were done at 28.7 °C and 30.7% RH. Observations were conducted via a stereo microscope (WILD M7, WILD heerbrugg, Gais, Switzerland) positioned above the beans and connected to a video camera (SSC-DC58AP, Sony, Japan), with an illuminator PL3000 (Photonic, Wien, Austria). Within 1 min after introducing the parasitoid wasps—first the focal and non-focal *A. calandreae* and then *H. prosopidis*—and before any stinging or interference behaviour started, we began

recording the behavioural events of all parasitoids. Recordings lasted for a total of 60 min or until the focal female left the host patch for more than 60 s using the event recorder JWatcher 1.0 (Blumstein & Daniel, 2007). Video-recorded data were analysed to count the number of interference, stinging and host feeding events and to measure the duration of each sting as well as the total time the focal female spent in the host patch.

Interference behaviours between *A. calandreae* females and between *A. calandreae* and *H. prosopidis* females were observed. The interference behaviours exhibited by two females in direct competition consist of pushing, running on the back, mounting, and wing flapping. Direct interspecific interactions initiated by *H. prosopidis* were antennation of *A. calandreae*. When the focal female directly interfered with an antagonist, whether actively or passively, it was counted as one interference event regardless of the duration of this behaviour (which was ≤ 1 sec in all cases).

The behaviour of the focal female was scored as 'stinging' when her ovipositor was inserted through the bean seed coat, irrespective of behaviours that followed, and recorded as 'host feeding' when feeding behaviour was observed (mouthparts attached to or head position locked at the sting site). Patch residence time was calculated by subtracting the duration of excursions away from the host patch. If the focal female was still on the host patch when the recording period ended, the patch residence time for that replicate was censored (9 replicates out of 40) (see Bressers *et al.*, 1991). All experimental parasitoids were removed from each Petri dish after the behavioural observations ended and the Petri dishes with beans were stored in a climatic chamber (25 °C, 50% RH and 16L:8D) to rear the host and parasitoids in the beans. The numbers of emerged *A. calandreae* and *H. prosopidis* offspring were counted 4 weeks later. The proportions of hosts parasitised by *A. calandreae* or by both parasitoid species were calculated as the number of emerged parasitoid offspring divided by host density.

Statistical analyses

We analysed the effects of each treatment (host density and the presence or absence of *H. prosopidis*) and the interaction between these two factors on the number of observed events of interference, stinging, and host feeding behaviour for the focal *A. calandreae* females using generalised linear models (McCullagh & Nelder, 1989) with a Poisson distribution and a log link function. We tested the absence of overdispersion. If there was overdispersion, an overdispersion parameter was estimated along with the model fit. Differences in the duration of stinging behaviour were analysed using a parametric survival analysis (Collett, 2015) with replicate as a random effect and with a best-fit log-normal distribution (among Weibull, log-normal, exponential, Frechet and log-logistic distributions). The variation in patch residence time for the focal females was compared using a parametric survival analysis with a best-fit exponential distribution. This model allowed for the correct handling of censored data (Bressers *et al.*, 1991). Spearman's rank correlations were tested among different behavioural variables. The proportion of hosts parasitised was analysed using a logistic regression model on the frequencies of parasitised and unparasitised hosts

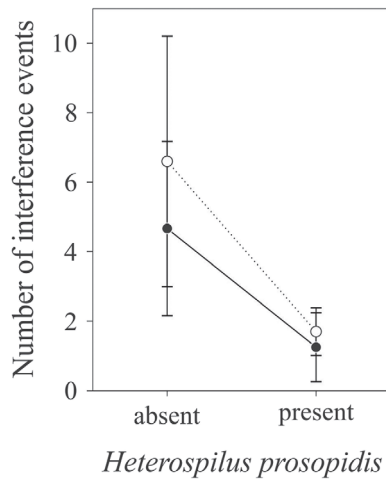


Fig 1. Effects of *Heterospilus prosopidis* presence and host density on the number of direct interference events of focal *Anisopteromalus calandrae* females (*H. prosopidis* presence, $P = 0.043$; host density, $P = 0.659$; *H. prosopidis* presence \times host density interaction, $P = 0.979$). Filled circles connected with solid lines indicate data at a host density of 1 and open circles with dotted lines show data at a host density of 2. Mean \pm SE.

with a logit link function. We used JMP 12.2.0 for all statistical analyses (SAS Institute Inc., Cary, North Carolina).

Results

Frequency and duration of behavioural events

The number of intraspecific antagonistic direct encounters by the focal *A. calandrae* female was reduced by the presence of *H. prosopidis* ($\chi^2_1 = 4.11$, $P = 0.043$) but not affected by host density ($\chi^2_1 = 0.19$, $P = 0.659$) (Fig. 1). There was no significant effect of interaction between the presence of *H. prosopidis* and host density ($\chi^2_1 = 0.001$, $P = 0.979$, Fig. 1).

Direct interspecific antagonistic encounters were infrequently observed (11.1% of replicates with *H. prosopidis* present). The number of interspecific antagonistic encounters increased with host density ($\chi^2_1 = 5.88$, $P = 0.015$, for data in the presence of *H. prosopidis*). These interspecific antagonistic encounters were not correlated with the number of host feeding events or with patch residence time (Table 1). However, they were positively associated with the numbers of intraspecific interference and stinging events and with the mean duration of stinging behaviours per female (Table 1).

For the number of stinging events, there were no significant effects of the presence of *H. prosopidis* ($\chi^2_1 = 0.65$, $P = 0.419$), host density ($\chi^2_1 = 2.22$, $P = 0.136$) and the interaction between the presence of *H. prosopidis* and host density ($\chi^2_1 = 1.29$, $P = 0.257$) (Fig. 2a). The duration of stinging behaviour was reduced by the presence of *H. prosopidis* ($\chi^2_1 = 5.24$, $P = 0.022$) but increased with host density ($\chi^2_1 = 4.04$, $P = 0.045$), with no significant interaction between the two main effects ($\chi^2_1 = 1.48$, $P = 0.224$) (Fig. 2b).

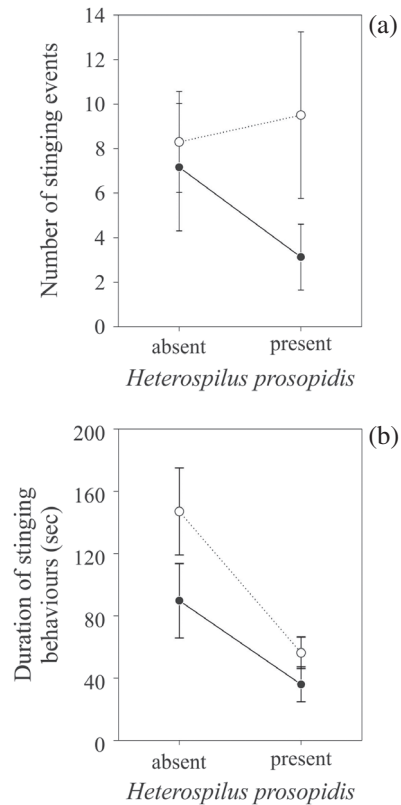


Fig 2. Effects of *Heterospilus prosopidis* presence and host density on the (a) number of stinging events (*H. prosopidis* presence, $P = 0.419$; host density, $P = 0.136$; *H. prosopidis* presence \times host density interaction, $P = 0.257$) and (b) duration of stinging behaviours (*H. prosopidis* presence, $P = 0.022$; host density, $P = 0.045$; *H. prosopidis* presence \times host density interaction, $P = 0.224$) of focal *Anisopteromalus calandrae* females. Filled circles connected with solid lines indicate data at a host density of 1 and open circles with dotted lines show data at a host density of 2. Mean \pm SE.

The presence of *H. prosopidis* contributed to a significant decrease in the number of host feeding events ($\chi^2_1 = 10.43$, $P = 0.001$), whereas the effects of host density ($\chi^2_1 = 3.58$, $P = 0.058$) and the interaction between the presence of *H. prosopidis* and host density ($\chi^2_1 = 2.76$, $P = 0.097$) were absent (Fig. 3).

There was a significant positive correlation between the number of interference and stinging events but not between the numbers of interference and host feeding events or the mean duration of stinging behaviours per female, irrespective of presence/absence of *H. prosopidis* (Table 1). The numbers of stinging and host feeding events as well as the mean duration of stinging behaviours were also positively correlated with each other in the absence of *H. prosopidis* (Table 1). In the presence of *H. prosopidis*, however, the positive correlation between the numbers of stinging and host feeding events became non-significant (Table 1).

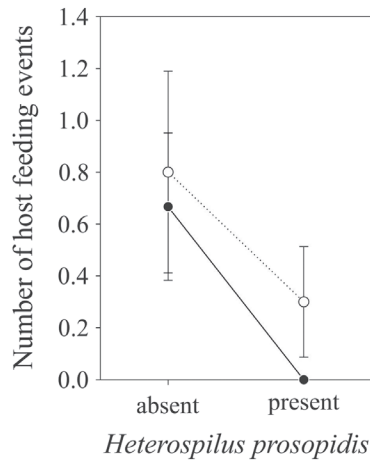


Fig 3. Effects of *Heterospilus prosopidis* presence and host density on the number of host-feeding events (*H. prosopidis* presence, $P = 0.001$; host density, $P = 0.058$; *H. prosopidis* presence \times host density interaction, $P = 0.097$). Filled circles connected with solid lines indicate data at a host density of 1 and open circles with dotted lines show data at a host density of 2. Mean \pm SE.

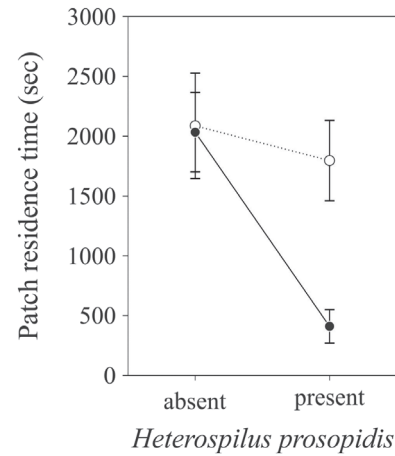


Fig 4. Effects of *Heterospilus prosopidis* presence and host density on the patch residence time of focal *Anisopteromalus calandrae* females (*H. prosopidis* presence, $P = 0.002$; host density, $P = 0.013$; *H. prosopidis* presence \times host density interaction, $P = 0.053$). Filled circles connected with solid lines indicate data at a host density of 1 and open circles with dotted lines show data at a host density of 2. Mean \pm SE.

Patch residence time

The patch residence time of focal *A. calandrae* females decreased in the presence of *H. prosopidis* ($\chi^2_1 = 9.77$, $P = 0.002$) and increased with host density ($\chi^2_1 = 6.17$, $P = 0.013$), and there was a marginally significant interaction between these two factors ($\chi^2_1 = 3.76$, $P = 0.053$) (Fig. 4): The decrease in patch residence time in the presence of *H. prosopidis* was more pronounced at the lower host density. Total patch residence time was positively correlated with the number and duration of stinging behaviours but not with the number of intraspecific interference events, irrespective of the presence/absence of *H. prosopidis* (Table 1). There was also a positive correlation between total patch residence time and the number of host-feeding events in the absence but not in the presence of *H. prosopidis* (Table 1).

Proportion of hosts parasitised

For the proportion of hosts parasitised by *A. calandrae*, the effect of the interaction between the presence of

H. prosopidis and host density was significant ($\chi^2_1 = 4.78$, $P = 0.029$) (Fig. 5). There were no significant effects of the presence of *H. prosopidis* ($\chi^2_1 = 2.54$, $P = 0.111$) and host density ($\chi^2_1 = 0.02$, $P = 0.894$). The numbers of parasitised hosts (mean \pm SE) were $0.92 \pm 0.08/0.38 \pm 0.18$ in the absence/presence of *H. prosopidis* at host density 1 and $1.40 \pm 0.22/1.40 \pm 0.22$ in the absence/presence of *H. prosopidis* at host density 2. The probabilities of hosts parasitised were $0.917/0.375$ in the absence/presence of *H. prosopidis* at host density 1 and $0.700/0.700$ in the absence/presence of *H. prosopidis* at host density 2.

For the proportion of hosts parasitised by both parasitoids, there were no significant effects of the presence of *H. prosopidis* ($\chi^2_1 = 1.03$, $P = 0.311$) and host density ($\chi^2_1 = 0.10$, $P = 0.751$), whereas the effect of the interaction between the two main effects was slightly significant ($\chi^2_1 = 3.87$, $P = 0.049$) (Fig. 5). The numbers of parasitised hosts (mean \pm SE) were $0.92 \pm 0.08/0.50 \pm 0.19$ in the absence/presence of *H. prosopidis* at host density 1 and $1.40 \pm 0.22/1.50 \pm 0.22$ in the absence/presence of *H. prosopidis* at host density 2. The probabilities of hosts parasitised were $0.917/0.500$ in the

Table 1. Spearman rank correlations among different behaviours in the presence ($n = 18$, below the diagonal) and absence ($n = 22$, above the diagonal) of non-native parasitoid *Heterospilus prosopidis*.

| | Interspecific antagonistic encounters | Intraspecific interference | Stings | Stinging duration | Host feedings | Patch residence time |
|----------------------------|---------------------------------------|----------------------------|---------------------------|----------------------|----------------------|----------------------|
| Intraspecific interference | 0.540 (0.021) | – | 0.433 (0.044) | 0.266 (0.231) | 0.156 (0.488) | 0.308 (0.163) |
| Stings | 0.525 (0.025) | 0.500 (0.035) | – | 0.448 (0.037) | 0.543 (0.009) | 0.644 (0.001) |
| Stinging duration | 0.540 (0.021) | –0.040 (0.875) | 0.591 (0.010) | – | 0.657 (0.001) | 0.456 (0.033) |
| Host feedings | –0.125 (0.622) | –0.271 (0.277) | 0.208 (0.407) | 0.493 (0.038) | – | 0.480 (0.024) |
| Patch residence time | 0.450 (0.061) | 0.395 (0.105) | 0.807 (<0.0001) | 0.643 (0.004) | 0.446 (0.064) | – |

P values in parentheses. Results with $P < 0.05$ are in bold. For interspecific interference, correlations only in the presence of the non-native parasitoid are shown.

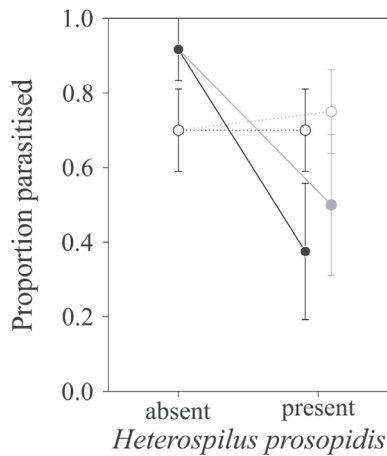


Fig 5. Effects of *Heterospilus prosopidis* presence and host density on the proportion parasitised by *A. calandrea* (in black) (*H. prosopidis* presence, $P = 0.111$; host density, $P = 0.894$; *H. prosopidis* presence \times host density interaction, $P = 0.029$) and by both *A. calandrea* and *H. prosopidis* (in gray) (*H. prosopidis* presence, $P = 0.311$; host density, $P = 0.751$; *H. prosopidis* presence \times host density interaction, $P = 0.049$). Filled circles connected with solid lines indicate data at a host density of 1 and open circles with dotted lines show data at a host density of 2. Mean \pm SE.

absence/presence of *H. prosopidis* at host density 1 and 0.700/0.750 in the absence/presence of *H. prosopidis* at host density 2.

Discussion

This study tested previous predictions from population dynamics (Tuda & Shimada, 2005), i.e. whether direct intraspecific interference, parasitism, and host-feeding behaviour of the native parasitoid are affected by an introduced non-native parasitoid species and whether there is host density dependence in intraspecific interference and other behavioural traits. The presence of the non-native *H. prosopidis* reduced the frequencies of intraspecific interference and host feeding behaviour, sting duration, and patch residence time for focal native *A. calandrea* females. The proportion of parasitised hosts was affected by the non-native parasitoid presence only when the host density was low. No host density dependence was found in intraspecific interference and host feeding. The responses tended to be correlated, making it difficult to determine which response was the targeted response *per se*.

Interestingly, direct interspecific interactions between the native and the non-native species were rarely observed, which contrasts with observations in other systems (e.g. Batchelor *et al.*, 2005; Cusumano *et al.*, 2011; Mohamad *et al.*, 2011, 2015; Hardy *et al.*, 2013). This result indicates that, in the interspecific interactions with the introduced (non-native) species *H. prosopidis*, (i) different parasitoids exhibit different types and frequencies of behaviours and that (ii) factors other than direct physical contacts, such as visual, acoustic, or olfactory cues, might play a role in the reduction of

behaviours associated with interference and reproduction in *A. calandrea* (Howard & Baker, 2003; Tamò *et al.*, 2006).

The behavioural differences exhibited by the resident species in response to the presence or absence of the non-native species that were detected in the present study are consistent with the predictions of Tuda and Shimada (2005), who detected a decrease in the level of mutual interference in *A. calandrea* following the introduction of *H. prosopidis*. This provides evidence that a top-down approach can effectively predict behavioural changes. However, we also obtained unexpected results from our population-level analysis: the presence of *H. prosopidis* affected *A. calandrea*'s patch residence time and proportion of parasitised hosts at a lower host density only, indicating that *A. calandrea* more frequently engages in reproductive behaviours under higher host availability when the non-native parasitoid is present. Host feeding behaviour was instead reduced.

Another unexpected positive relationship was detected between the number of direct interspecific interactions and interference/stinging behaviour. This indicates that reproductively active females engaging in reproduction and direct intraspecific interference are more likely to encounter direct interspecific antagonism. Overall, when compared to females in the absence of *H. prosopidis*, reproduction and direct intraspecific interference behaviours were reduced.

Differences in the life histories of these parasitoids might account for the observed patterns of interference for *A. calandrea*. As with most synovigenic parasitoids, *A. calandrea* must engage in host feeding to mature eggs (Jervis *et al.*, 2001). This strengthens intraspecific competition in this species.

Since the work presented here was performed under laboratory conditions, these results may not fully hold with the complex and weaker interactions present in natural systems. In the field, rates of infestation and parasitism can be lower. For example, the density of *C. chinensis* is approximately 1.1 larvae per infested seed under natural conditions (Shinoda & Yoshida, 1985), and the infestation rate usually ranges from 4–8% (Tuda *et al.*, 2004) to 14% (Shinoda & Yoshida, 1985; Shinoda *et al.*, 1992). Also, parasitism rates by *A. calandrea* and *H. prosopidis* can be as low as about 15% (Ngamo *et al.*, 2007) and 11% (Silva *et al.*, 2020), respectively. However, in closed storage, densities of stored product pests become extremely high and locally attract numerous *A. calandrea* by kairomones (Onodera *et al.*, 2002). Our results would be most relevant in such situations of high pest densities.

Links to population-level outcomes

In this study, we provided evidence that interference between *A. calandrea* females decreases in the presence of *H. prosopidis*. Other behavioural changes, such as the frequency of host feeding, can be considered as consequences of changes in overall host searching efficiency (a). Note that the overall searching efficiency is a function of both intrinsic searching efficiency and mutual interference (in the formulation of Hassell & Varley, 1969). Although our experimental design did not allow for independent estimates of decreased mutual interference

and decreased intrinsic searching efficiency, these parameter changes are predicted to induce complex population dynamics (chaos and quasiperiodicity, respectively) in this three-species system (Tuda & Shimada, 2005).

The dynamical consequences of flexible foraging behaviour, such as predators switching between prey species, are still poorly understood, and influential behaviours suggested by theory have received little empirical attention (Abrams, 2010). In contrast to the well-studied food webs consisting of two competitors attacked by a single predator or parasitoid (e.g. Bonsall & Hassell, 1997; Ohgushi, 2005; Tack *et al.*, 2011), mechanisms for the variety of population dynamics in food webs with two co-occurring parasitoid (or predator) species competing for single host (or prey) species are less understood (Matsumoto *et al.*, 2003; Borer *et al.*, 2004; Bader *et al.*, 2006; Boivin & Brodeur, 2006; Tuda *et al.*, 2006; Peri *et al.*, 2014; Pekas *et al.*, 2016). In this respect, our results provide empirical evidence bridging top-down and bottom-up approaches to describe one host–two parasitoid food web dynamics. The asymmetric interaction between two parasitoid species sharing the same host (e.g. Sjaarda, 1989; Mackauer *et al.*, 1992; Mohamad *et al.*, 2011; Peri *et al.*, 2014; Bili *et al.*, 2016) can alter dynamic properties (e.g. stability or mean population density) via behavioural change in one of the two parasitoid species.

In terms of mutual interference, the model-detected parameter change was supported by our behavioural experiment. However, we also observed behavioural modifications in response to host density that were not addressed by the population model. This indicates a possible limitation of the top-down approach in food webs and that such an approach can miss emergent properties at the behavioural level. In this respect, the reciprocal application of top-down and bottom-up approaches appears to be needed to ensure a more complete understanding of the mechanisms of complex food web dynamics.

Implications for biological control

What do our findings mean for the single versus multiple natural enemies controversy? In our system, the introduction of a non-native biocontrol agent destabilised existing host–parasitoid population dynamics (Tuda & Shimada, 2005). The present behavioural study demonstrates that this change is induced as the introduced biocontrol agent reduces the population-stabilizing behaviour of the native agent, irrespective of host densities (although the ranges of host densities between the population dynamics and this study are different). Therefore, the native biocontrol agent alone could provide stable control of the pest, without further introduction of the second agent. This is contradictory to the perspective that encourages the introduction of multiple natural enemies even with moderate antagonistic interspecific interactions among them (Pedersen & Mills, 2004). Our study suggests that, even when direct antagonistic behaviours are at a low frequency between species, the presence of other natural enemies (i.e. predators or parasites) can disrupt stable interspecific interactions between pests and their natural enemies, ultimately disrupting stable biological control and increasing the frequency of pest outbreaks. However, the

context dependency of the inferences from this laboratory study remains to be tested.

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Author contributions

MT and YN conceived the idea, YN and EW designed the experiment, YN performed the experiment, MT and EW performed statistical analyses and MT, YN and EW wrote the manuscript.

Data availability statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

References

- Abrams, P.A. (2010) Implications of flexible foraging for interspecific interactions: lessons from simple models. *Functional Ecology*, **24**, 7–17.
- Bader, A.E., Heinz, K.M., Wharton, R.A. & Bogran, C.E. (2006) Assessment of interspecific interactions among parasitoids on the outcome of inoculative biological control of leafminers attacking chrysanthemum. *Biological Control*, **39**, 441–452.
- Batchelor, T.P., Hardy, I.C.W., Barrera, J.F. & Perez-Lachaud, G. (2005) Insect gladiators II: competitive interactions within and between bethylid parasitoid species of the coffee berry borer, *Hypothenemus hampei* (Coleoptera: Scolytidae). *Biological Control*, **33**, 194–202.
- Bili, M., Cortesero, A.M., Outreman, Y. & Poinot, D. (2016) Host specialisation and competition asymmetry in coleopteran parasitoids. *Oecologia*, **182**, 111–118.
- Blumstein, D.T. & Daniel, J.C. (2007) *Quantifying behavior the JWatcher way*, p. 211. Sinauer Associates Inc, Sunderland, MA.
- Boivin, G. & Brodeur, J. (2006) Intra- and interspecific interactions among parasitoids: Mechanisms, outcomes and biological control. *Trophic and guild interactions in biological control*, Vol. 3 (ed. by J. Brodeur and G. Boivin), pp. 123–144, Progress in Biological Control. Springer, Dordrecht.
- Bonsall, M.B. & Hassell, M.P. (1997) Apparent competition structures community assemblages. *Nature*, **388**, 371–373.
- Borer, E.T., Murdoch, W.W. & Swarbrick, S.L. (2004) Parasitoid coexistence: linking spatial field patterns with mechanism. *Ecology*, **85**, 667–678.
- Bressac, C., Khanh, H.D.T. & Chevrier, C. (2009) Effects of age and repeated mating on male sperm supply and paternity in a parasitoid wasp. *Entomologia Experimentalis et Applicata*, **130**, 207–213.
- Bressers, M., Meelis, E., Haccou, P. & Kruk, M. (1991) When did it really start or stop: the impact of censored observations on the analysis of duration. *Behavioural Processes*, **23**, 1–20.
- Briggs, C.J., Nisbet, R.M. & Murdoch, W.W. (1993) Coexistence of competing parasitoid species on a host with a variable life cycle. *Theoretical Population Biology*, **44**, 341–373.

- Casula, P., Wilby, A. & Thomas, M.B. (2006) Understanding biodiversity effects on prey in multi-enemy systems. *Ecology Letters*, **9**, 995–1004.
- Chantarasard, S., Hirashima, Y. & Miura, T. (1984) Ecological studies on *Anagrus incarnatus* Haliday (Hymenoptera: Mymaridae), an egg parasitoid of the rice planthoppers. I. Functional response to host density and mutual interference. *Journal of Faculty of Agriculture Kyushu University*, **29**, 59–66.
- Chen, R.X., Zhang, F., Huangfu, W.G., Yao, H.Y., Zhou, J.B. & Kuhlmann, U. (2008) Reproductive attributes of the eulophid *Oomyzus sokolowskii*, a biological control agent of diamondback moth, *Plutella xylostella* (Lepidoptera: Plutellidae). *Biocontrol Science and Technology*, **18**, 753–765.
- Chong, J.H. & Oetting, R.D. (2006) Functional response and progeny production of the Madeira mealybug parasitoid, *Anagyrus* sp. nov. nr. *sinope*: the effects of host and parasitoid densities. *Biological Control*, **39**, 320–328.
- Clausen, C.P. (1978) *Introduced parasites and predators of arthropod pests and weeds: a world review*, p. 545. United States Department of Agriculture, Agriculture Research Service, Washington, DC.
- Collett, D. (2015) *Modelling survival data in medical research*, 3rd edn. Chapman and Hall/CRC, London.
- Cresswell, W. (1998) Variation in the strength of interference competition with resource density in blackbirds, *Turdus merula*. *Oikos*, **81**, 152–160.
- Cusumano, A., Peri, E., Vinson, S.B. & Colazza, S. (2011) Intraguild interactions between two egg parasitoids exploring host patches. *BioControl*, **56**, 173–184.
- DeLong, J.P. & Vasseur, D.A. (2011) Mutual interference is common and mostly intermediate in magnitude. *BMC Ecology*, **11**, 1.
- Dennis, B., Desharnais, R.A., Cushing, J.M. & Costantino, R.F. (1995) Nonlinear demographic dynamics: mathematical models, statistical methods, and biological experiments. *Ecological Monographs*, **65**, 261–281.
- Free, C.A., Beddington, J.R. & Lawton, J.H. (1977) On the inadequacy of simple models of mutual interference for parasitism and predation. *Journal of Animal Ecology*, **46**, 543–554.
- Fujii, K. (1968) Studies on interspecies competition between the azuki bean weevil and the southern cowpea weevil. III. Some characteristics of strains of two species. *Researches on Population Ecology*, **10**, 87–98.
- Fussmann, G.F. & Heber, G. (2002) Food web complexity and chaotic population dynamics. *Ecology Letters*, **5**, 394–401.
- Goubault, M., Outreman, Y., Poinot, D. & Cortesero, A.M. (2005) Patch exploitation strategies of parasitic wasps under intraspecific competition. *Behavioral Ecology*, **16**, 693–701.
- Hardy, I.C.W., Goubault, M. & Batchelor, T.P. (2013) Hymenopteran contests and agonistic behaviour. *Animal Contests* (ed. by I. C. W. Hardy and M. Briffa), pp. 147–177. Cambridge University Press, Cambridge.
- Hassell, M.P. & Varley, G.C. (1969) New inductive population model for insect parasites and its bearing on biological control. *Nature*, **223**, 1133–1137.
- Holt, R.D. (1977) Predation, apparent competition, and structure of prey communities. *Theoretical Population Biology*, **12**, 197–229.
- Howard, R.W. & Baker, J.E. (2003) Morphology and chemistry of Dufour glands in four ectoparasitoids: *Cephalonomia tarsalis*, *C. waterstoni* (Hymenoptera: Bethylinidae), *Anisopteromalus calandrae*, and *Pteromalus cerealellae* (Hymenoptera: Pteromalidae). *Comparative Biochemistry and Physiology B-Biochemistry & Molecular Biology*, **135**, 153–167.
- Jervis, M.A., Heimpel, G.E., Ferns, P.N., Harvey, J.A. & Kidd, N.A.C. (2001) Life-history strategies in parasitoid wasps: a comparative analysis of ‘ovigeny’. *Journal of Animal Ecology*, **70**, 442–458.
- Jost, C. & Arditi, R. (2001) From pattern to process: identifying predator-prey models from time-series data. *Population Ecology*, **43**, 229–243.
- Kendall, B.E., Briggs, C.J., Murdoch, W.W., Turchin, P., Ellner, S.P., McCauley, E. et al. (1999) Why do populations cycle? A synthesis of statistical and mechanistic modeling approaches. *Ecology*, **80**, 1789–1805.
- Klebanoff, A. & Hastings, A. (1994) Chaos in three species food chains. *Journal of Mathematical Biology*, **32**, 427–451.
- Kristoffersen, A.B., Lingjaerde, O.C., Stenseth, N.C. & Shimada, M. (2001) Non-parametric modelling of non-linear density dependence: a three-species host-parasitoid system. *Journal of Animal Ecology*, **70**, 808–819.
- Liljestrom, G.G., Cingolani, M.F. & Rabinovich, J.E. (2013) The functional and numerical responses of *Trissolcus basalus* (Hymenoptera: Platygasteridae) parasitizing *Nezara viridula* (Hemiptera: Pentatomidae) eggs in the field. *Bulletin of Entomological Research*, **103**, 441–450.
- Mackauer, M., Bai, B., Chow, A. & Danyk, T. (1992) Asymmetric larval competition between two species of solitary parasitoid wasps: the influence of superparasitism. *Ecological Entomology*, **17**, 233–236.
- Matsumoto, T., Itioka, T. & Nishida, T. (2003) Rapid change in the settling behavior of the arrowhead scale *Unaspis yanonensis* as an avoidance mechanism against introduced parasitoids, *Aphytis yanonensis* and *Coccobius fulvus*. *Entomologia Experimentalis et Applicata*, **107**, 105–113.
- McCullagh, P. & Nelder, J.A. (1989) *Generalized linear models*, 2nd edn. Chapman and Hall/CRC, London.
- Mohamad, R., Monge, J.P. & Goubault, M. (2011) Agonistic interactions and their implications for parasitoid species coexistence. *Behavioral Ecology*, **22**, 1114–1122.
- Mohamad, R., Wajnberg, E., Monge, J.P. & Goubault, M. (2015) The effect of direct interspecific competition on patch exploitation strategies in parasitoid wasps. *Oecologia*, **177**, 305–315.
- Moody, A.L. & Ruxton, G.D. (1996) The intensity of interference varies with feed density: support for behaviour-based models of interference. *Oecologia*, **108**, 446–449.
- Nakamichi, Y., Toquenaga, Y. & Fujii, K. (2008) Persistent host-parasitoid interaction caused by host maturation variability. *Population Ecology*, **50**, 191–196.
- Ngamo, T.S.L., Kouninki, H., Ladang, Y.D., Ngassoum, M.B., Mapongmestsem, P.M. & Hance, T. (2007) Potential of *Anisopteromalus calandrae* (Hymenoptera: Pteromalidae) as biocontrol agent of *Callosobruchus maculatus* (F.) (Coleoptera: Bruchidae). *African Journal of Agricultural Research*, **2**, 168–172.
- Ohgushi, T. (2005) Indirect interaction webs: herbivore-induced effects through trait change in plants. *Annual Review of Ecology, Evolution, and Systematics*, **36**, 81–105.
- Onodera, J., Matsuyama, S., Suzuki, T. & Fujii, K. (2002) Host-recognizing kairomones for parasitic wasp, *Anisopteromalus calandrae*, from larvae of azuki bean weevil, *Callosobruchus chinensis*. *Journal of Chemical Ecology*, **28**, 1209–1220.
- Pedersen, B.S. & Mills, N.J. (2004) Single vs. multiple introduction in biological control: the roles of parasitoid efficiency, antagonism and niche overlap. *Journal of Applied Ecology*, **41**, 973–984.
- Pekas, A., Tena, A., Harvery, J.A., Garcia-Mari, F. & Frago, E. (2016) Host size and spatiotemporal patterns mediate the coexistence of specialist parasitoids. *Ecology*, **97**, 1345–1356.
- Peri, E., Cusumano, A., Amodeo, V., Wajnberg, E. & Colazza, S. (2014) Intraguild interactions between two egg parasitoids of a true bug in semi-field and field conditions. *PLoS ONE*, **9**, e99876.
- Sagarra, L.A., Vincent, C. & Stewart, R.K. (2000) Mutual interference among female *Anagyrus kamali* Moursi (Hymenoptera: Encyrtidae)

- and its impact on fecundity, progeny production and sex ratio. *Biocontrol Science and Technology*, **10**, 239–244.
- Shimada, M. (1999) Population fluctuation and persistence of one-host-two-parasitoid systems depending on resource distribution: from parasitizing behavior to population dynamics. *Researches on Population Ecology*, **41**, 69–79.
- Shimada, M. & Tuda, M. (1996) Delayed density dependence and oscillatory population dynamics in overlapping-generation systems of a seed beetle *Callosobruchus chinensis*; projection matrix model. *Oecologia*, **105**, 116–125.
- Shinoda, K. & Yoshida, T. (1985) Field biology of the azuki bean weevil, *Callosobruchus chinensis* (L.) (Coleoptera: Bruchidae). I. Seasonal prevalence and assessment of field infestation of Aki-azuki, autumn variety of *Phaseolus angularis* W. *Japanese Journal of Applied Entomology and Zoology*, **29**, 14–20 in Japanese.
- Shinoda, K., Yoshida, T. & Igarashi, H. (1992) Population ecology of the azuki bean beetle, *Callosobruchus chinensis* (L.), (Coleoptera: Bruchidae) on two wild leguminous hosts, *Vigna angularis* var. *nipponensis* and *Dunbaria villosa*. *Applied Entomology and Zoology*, **27**, 311–318.
- Sih, A., Crowley, P., McPeck, M., Petranka, J. & Strohmeier, K. (1985) Predation, competition, and prey communities: a review of field experiments. *Annual Review of Ecology and Systematics*, **16**, 269–311.
- Silva, J.O.S., Costa, M.L.E., Paixao, B.S., Macedo, J.D.B., Rodrigues, P.M.S. & Lins-Neto, E.M.F. (2020) Natural vs managed habitat: effect over the seed-predator *Pachymerus nucleorum* and its natural enemies. *Neotropical Entomology*, **49**, 131–138.
- Sjaarda, N. (1989) *Interactions between the harlequin bug Murgantia histrionica Hahn (Hemiptera: Pentatomidae), and the egg parasitoids, Trissolcus murgantiae Ashm. (Hymenoptera: Scelionidae) and Ooencyrtus johnsonii How. (Hymenoptera: Encyrtidae): Factors affecting patterns of parasitism in a southern California coastal sage habitat*. PhD thesis, University of California, Davis, USA.
- Skovgard, H. & Nachman, G. (2015) Effect of mutual interference on the ability of *Spalangia cameroni* (Hymenoptera: Pteromalidae) to attack and parasitize pupae of *Stomoxys calcitrans* (Diptera: Muscidae). *Environmental Entomology*, **44**, 1076–1084.
- Tack, A.J.M., Gripberg, S. & Roslin, T. (2011) Can we predict indirect interactions from quantitative food webs?—an experimental approach. *Journal of Animal Ecology*, **80**, 108–118.
- Tahriri, S., Talebi, A.A., Fathipour, Y. & Zamani, A.A. (2007) Host stage preference, functional response and mutual interference of *Aphidius matricariae* (Hym.: Braconidae: Aphidiinae) on *Aphis fabae* (Hom.: Aphididae). *Entomological Science*, **10**, 323–331.
- Tamò, C., Roelfstra, L.L., Guillaume, S. & Turlings, T.C.J. (2006) Odour-mediated long-range avoidance of interspecific competition by a solitary endoparasitoid: a time-saving foraging strategy. *Journal of Animal Ecology*, **75**, 1091–1099.
- Tuda, M. (1993) Density dependence depends on scale; at larval resource patch and at whole population. *Researches on Population Ecology*, **35**, 261–271.
- Tuda, M. (2007) Applied evolutionary ecology of insects of the subfamily Bruchinae (Coleoptera: Chrysomelidae). *Applied Entomology and Zoology*, **42**, 337–346.
- Tuda, M. & Shimada, M. (1995) Developmental schedules and persistence of experimental host-parasitoid systems at two different temperatures. *Oecologia*, **103**, 283–291.
- Tuda, M. & Shimada, M. (2005) Complexity, evolution and persistence in host-parasitoid experimental systems, with *Callosobruchus* beetles as the host. *Advances in Ecological Research*, **37**, 37–75.
- Tuda, M., Wasano, N., Kondo, N., Horng, S.-B., Chou, L.-Y. & Tateishi, Y. (2004) Habitat-related mtDNA polymorphism in a stored-bean pest *Callosobruchus chinensis* (Coleoptera: Bruchidae). *Bulletin of Entomological Research*, **94**, 75–80.
- Tuda, M., Matsumoto, T., Itioka, T., Ishida, N., Takanashi, M., Ashihara, W. *et al.* (2006) Climatic and inter-trophic effects detected in 10-year population dynamics of biological control of the arrowhead scale by two parasitoids in southwestern Japan. *Population Ecology*, **48**, 59–70.
- Turchin, P. & Hanski, I. (2001) Contrasting alternative hypotheses about rodent cycles by translating them into parameterized models. *Ecology Letters*, **4**, 267–276.
- Tyutyunov, Y.V. & Titova, L.I. (2018) From Lotka-Volterra to Arditi-Ginzburg: 90 years of evolving trophic functions. *Zhurnal Obshchei Biologii*, **79**, 428–448.
- Utida, S. (1941) *Studies on experimental population of the azuki bean weevil, Callosobruchus chinensis*. I. The effect of population density on the progeny population, Vol. **48**, pp. 1–30. Kyoto, Japan: *Memoirs of the College of Agriculture, Kyoto Imperial University*.
- Utida, S. (1957) Population fluctuation: an experimental and theoretical approach. *Cold Spring Harbor Symposia on Quantitative Biology*, **22**, 139–151.
- Vamosi, S.M., den Hollander, M.D. & Tuda, M. (2011) Egg dispersion is more important than competition type for herbivores attacked by a parasitoid. *Population Ecology*, **53**, 319–326.
- Wajnberg, E., Curty, C. & Colazza, S. (2004) Genetic variation in the mechanisms of direct mutual interference in a parasitic wasp: consequences in terms of patch-time allocation. *Journal of Animal Ecology*, **73**, 1179–1189.
- Yazdani, M. & Keller, M. (2015) Mutual interference in *Dolichogenidea tasmanica* (Cameron) (Hymenoptera: Braconidae) when foraging for patchily-distributed light brown apple moth. *Biological Control*, **86**, 1–6.

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