



Nest provisioning with parasitized caterpillars by female potter wasps: costs and potential mechanisms



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Females of the potter wasp *Delta dimidiatipenne* collect caterpillars from the surrounding vegetation and place them inside their mud-constructed nest cells to provision their offspring. It has been observed that females frequently collect caterpillars that are already internally parasitized by the larvae of the gregarious parasitoid wasp *Copidosoma primulum*. In such cases, the potter wasp offspring's food supply may become depleted, and they may fail to complete their development, while the *C. primulum* offspring mature but remain trapped and eventually die within the mud cell. This raises the question: why do potter wasp females continue bringing parasitized caterpillars into their nests? We aimed at quantifying the fitness costs of this behaviour, while investigating the potential mechanisms sustaining it. For this, we conducted a field survey of *Heliothis nubigera* caterpillars, the most common prey of *D. dimidiatipenne*, from the nest cells and from the nearby vegetation. These were used to estimate the parasitism rate and, in laboratory experiments, to quantify the cost of feeding and developing on parasitized caterpillars, as well as the response of parasitized versus nonparasitized caterpillars to a simulated predator attack. We found that potter wasp larvae that were provisioned with parasitized caterpillars had reduced developmental success. Early potter wasp larval stages were indeed less likely to feed efficiently when provided with a single parasitized caterpillar compared to a nonparasitized one. Despite these costs, we found that female potter wasps seem to collect parasitized caterpillars more frequently than expected according to their occurrence in the vegetation. We found that parasitized caterpillars reached a higher body mass and were less active in their response to a simulated predator attack. These characteristics might contribute to the presumed attractiveness and higher susceptibility of parasitized caterpillars to the predatory potter wasps.

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Food provisioning is one of the most basic forms of parental care, occurring in a variety of species throughout the animal kingdom (Clutton-Brock, 1991; Royle et al., 2014). In animals in which offspring rely on provisioning, the quantity and quality of the food provided may have profound consequences for their development, survival, body size and reproductive success. For example, the delivery of large food items increased fledgling mass and recruitment in the house sparrow, *Passer domesticus* (Schwagmeyer & Mock, 2008), high milk quality positively affected the overwintering survival of juvenile ground squirrels, *Urocitellus columbianus* (Skibieli & Hood, 2015) and selective provisioning of good-quality food by the mother shortened developmental time and enhanced nymph survival in a shield bug, *Parastrachia japonensis* (Filippi et al., 2000). Hence parents are selected to invest time and

energy in acquiring the best-quality food within their physical and physiological limitations and given certain environmental constraints.

The importance of food quantity and quality may be even more crucial in mass-provisioning insects (mainly bees and wasps), where each offspring is provided with a fixed amount of food in a sealed chamber (Field et al., 2020). This is because in such cases, nutritional deficiencies cannot be compensated for by the offspring receiving or acquiring more food at a later stage of development. Accordingly, the nutritional quality of pollen has been shown to promote offspring developmental success in mass-provisioning bees (Austin & Gilbert, 2021; Roulston & Cane, 2002), while in predatory wasps, the number, size, species and nutritional value of the prey were all shown to have important effects on offspring development (Field, 1992; Judd & Fasnacht, 2017; Strohm, 2000). Mass-provisioning females, in turn, show evidence for preference for high-quality food (Auko et al., 2015; Mapalad et al., 2008).

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One aspect of food quality that has received less attention, in this respect, is the previous infection of the prey by parasites. In particular, many insects are prone to attack by parasitoids whose larvae complete their development inside their body (Godfray, 1994). Parasitoids have been shown to alter their host's development, physiology and even behaviour, to the extent that parasitized individuals have been considered as different 'ecological entities' than their nonparasitized conspecifics (Brodeur & Boivin, 2004). All these effects may have consequences for the quality of the parasitized prey as a food source, as well as its susceptibility to predation. Nevertheless, while considerable evidence suggests that female parasitoids discriminate against already parasitized hosts (Bakker et al., 1985; Godfray, 1994), little is known about the response of insect predators, and more specifically those that mass-provision their nest, to the parasitism status of their prey, as well as the consequences of provisioning their offspring with parasitized prey.

Here we investigated a poorly understood phenomenon, where a predatory wasp frequently provisions its nest with parasitized prey, despite high apparent fitness costs for their offspring's development. The potter wasp *Delta dimidiatipenne* (Hymenoptera, Vespidae, Eumeninae) constructs nests composed of individual mud cells on rock surfaces. Females lay a single egg per cell and provision it with several caterpillars collected from the surrounding vegetation. It has been observed that *D. dimidiatipenne* frequently collects caterpillars that are already parasitized by the gregarious parasitoid wasp *Copidosoma primulum*. In such cases, the parasitoid larvae feed on the caterpillar internally and pupate inside its cuticle, presumably depleting the food required for *D. dimidiatipenne* development. For the *C. primulum*, the prospects do not seem to be more optimistic; being much smaller than *D. dimidiatipenne* (ca. 2 mm versus ca. 2 cm, respectively), adult *C. primulum* parasitoids are apparently unable to break out of the nest cells and are often found dead within them (Segoli et al., 2020).

While several studies have similarly reported the occurrence of parasitized prey in the nests of solitary wasps (Bohart et al., 1982; Buschini & Buss, 2010; Jennings & Houseweart, 1984; Tscharnkte et al., 1998), these were mainly based on rare, anecdotal observations, and their consequences were not evaluated. In contrast, evidence for the occurrence of caterpillars parasitized by *C. primulum* in *D. dimidiatipenne* nests have been documented in 11 of 13 investigated field sites in the Negev Desert, and in ca. 70–80% of recently constructed nest cells. Moreover, the survival and pupal mass of *D. dimidiatipenne* offspring were found to be negatively correlated with the presence and number of parasitized caterpillars inside a cell (Segoli et al., 2020). Hence, the collection of parasitized prey, in this case, seems to occur frequently and to carry high fitness costs. However, these previous results were merely observational, and potential mechanisms for sustaining this behaviour have not been investigated.

Here we took one further step in quantifying the fitness costs and revealing the underlying mechanisms sustaining this behaviour via a combination of field surveys and laboratory experiments. We hypothesized that the collection of already parasitized caterpillars by *D. dimidiatipenne* females would have a negative impact on their offspring. Specifically, we predicted that *D. dimidiatipenne* larvae would be less likely to feed efficiently when provided with a caterpillar parasitized by *C. primulum*, and that larvae developing on parasitized caterpillars would suffer from a lower survival rate and longer developmental time and would mature at a lower body mass. We further hypothesized that due to such costs, female *D. dimidiatipenne* would attempt to avoid collecting caterpillars parasitized by *C. primulum* in the field. Specifically, we predicted that the parasitism rate of caterpillars inside the potter wasp nest cells would be lower than that of those from the surrounding

vegetation, suggesting that the foraging females discriminate against parasitized caterpillars. Alternatively, we hypothesized that some biases or limitations may prevent female *D. dimidiatipenne* from avoiding collecting parasitized caterpillars. Specifically, we predicted that parasitized caterpillars reach a larger body mass than nonparasitized ones, as previously reported for hosts parasitized by *Copidosoma* spp. (Byers et al., 1993; Segoli et al., 2009; Strand, 1989). Furthermore, we predicted that parasitized caterpillars would behave differently than nonparasitized ones (Brodeur & Boivin, 2004) and, specifically, that they would react less to a simulated predator attack. These characteristics might make parasitized caterpillars more attractive or more susceptible to predation by *D. dimidiatipenne* under field conditions.

METHODS

Study Species

The potter wasp *D. dimidiatipenne* is a solitary predatory wasp. The adults are about 25 mm long and feed on nectar. The species is distributed in North Africa and the Canary Islands, the Arabian Peninsula and in various locations in Asia (Augul, 2017; Siddiqui et al., 2015; Srinivasan & Kumar, 2010). In Israel, *D. dimidiatipenne* individuals have been observed in drier areas from the centre to the south of the country (Alon & Kugler, 1989). The wasps are normally active between March and June. Females construct nests composed of ca. 20 cells and provide each cell with a mean of four to five prey items. In the Negev Desert, *D. dimidiatipenne* have been observed to prey primarily on caterpillars of the family Noctuidae (Lepidoptera), mainly the native species *Heliothis nubigera*. Caterpillars of this species are the most abundant prey found in the nests of *D. dimidiatipenne*, as well as in the natural vegetation around their nesting grounds. Female *D. dimidiatipenne* have been observed to forage on *Zygophyllum dumosum*, an abundant shrub in this area on which *H. nubigera* caterpillars are commonly found (S. Leduc, & M. Segoli, personal observation; Segoli et al., 2020).

Copidosoma spp. (Hymenoptera, Encyrtidae) are polyembryonic parasitoid wasps attacking species of Lepidoptera, mainly moths. Females lay an egg inside the egg of their host, and each egg proliferates into multiple, genetically identical embryos (Godfray, 1994; Segoli et al., 2009). The caterpillar host continues developing while the parasitoid larvae feed on it internally until they fully consume it. The parasitoids then pupate inside the caterpillar cuticle and later emerge as adults (ca. 1–2 mm). When the parasitoids pupate, the caterpillar host takes a distinct mummified shape, which is maintained following parasitoid emergence. This enables visual identification of parasitized caterpillars inside potter wasp nest cells during and following *Copidosoma* pupation and adult emergence. The species found in the nests of *D. dimidiatipenne* in the Negev Desert was identified as *C. primulum* (Hymenoptera, Chalcidoidea, Encyrtidae). While this is the first record of this species in Israel, it was previously found in several locations with similarly dry climates, including Spain, Sudan, Turkey and Turkmenistan (Guerrieri & Noyes, 2005); therefore, it is likely to be native to the area. In the Negev Desert, *C. primulum* were found to parasitize caterpillars of *H. nubigera*, the main prey species found in *D. dimidiatipenne* nests (Segoli et al., 2020).

Study Sites

In the springs of 2019 and 2020, we collected the contents of potter wasp nest cells, as well as *H. nubigera* caterpillars from the natural vegetation, in seven sites (see Appendix Table A1) throughout the Negev Highlands. This region is located at an elevation ranging between 370 m and 520 m above sea level. The

climate is characterized by hot, dry summers, with an average maximum daily temperature of around 33 °C in July–August, and cold winters, with an average minimum daily temperature of around 5 °C in January–February. Annual precipitation is ca. 100 mm (data retrieved from the Israel Meteorological Service, <https://ims.gov.il/en>).

Sampling of Caterpillars from Potter Wasp Nest Cells

Caterpillars of *H. nubigera* (the main prey species of *D. dimidiatipenne*) were collected from *D. dimidiatipenne* nest cells by carefully opening the cell walls and exposing their contents. These caterpillars were used for several purposes: (1) for an experiment testing *D. dimidiatipenne* developmental success in relation to the number and parasitism status of caterpillars (see ‘Potter wasp rearing experiment’ below); (2) for an experiment testing *D. dimidiatipenne* larval feeding efficiency in relation to the caterpillar parasitism status (see ‘Potter wasp feeding experiment’ below); (3) to estimate the parasitism rate on caterpillars in *D. dimidiatipenne* nest cells, by dissecting a sample of caterpillars ($N = 380$) under the microscope to detect the presence of *C. primulum* larvae or embryos inside their bodies following Segoli et al. (2009); and (4) to estimate the body mass of parasitized versus nonparasitized caterpillars in the nest cells, and of caterpillars in the nest cells compared to those found on the vegetation (see ‘Caterpillar mass comparisons’ below). Altogether, we took samples in six sites on 11 sampling dates between March 2019 and May 2020. The number of caterpillars retrieved from the nest cells in the different dates and sites and their uses are summarized in Appendix Table A2.

Sampling of Caterpillars from the Vegetation

We collected *H. nubigera* caterpillars from the vegetation, mostly from *Z. dumosum* shrubs, by visual search or by gently beating the branches on a tray. These were also used for several purposes. (1) To estimate the parasitism rate of caterpillars collected from the vegetation, we reared a sample of caterpillars ($N = 69$) individually in small petri dishes while daily providing them with a few leaves of *Z. dumosum*. Parasitism status was determined at the end of development, as nonparasitized caterpillars matured into adult moths, and caterpillars parasitized by *C. primulum* became mummified. Another sample of caterpillars ($N = 81$) were dissected immediately after being collected from the vegetation to determine their parasitism status. (2) We estimated the final mass of parasitized versus nonparasitized caterpillars, and of caterpillars from the vegetation compared with those in the nest cells (see ‘Comparisons of caterpillar mass’ below). (3) We carried out an experiment testing caterpillar response to a simulated predator attack, in relation to their parasitism status (see ‘Caterpillar behavioural experiment’ below). Altogether, we took samples in seven sites on 13 sampling dates between March 2019 and May 2020. The number of caterpillars retrieved from the vegetation in the different dates and sites and their uses are summarized in the supplementary materials (Appendix Table A3).

Potter Wasp Rearing Experiment

This experiment was designed to quantify the cost of potter wasp development on parasitized caterpillars, in terms of developmental success, developmental time and final body mass. The *D. dimidiatipenne* egg or first-instar larvae that were collected from *D. dimidiatipenne* nest cells were each placed in a 50 ml falcon tube with two, four or six randomly assigned caterpillars, collected from the same cells, as prey. The tubes were placed in an incubator at

25 °C in the dark and checked daily. The experiment was repeated 50 times for each treatment, but some replicates were eliminated from the analysis if the egg did not hatch or the vial was infested by other parasites, including several species of parasitoid flies and wasps (for a full list, see Appendix Table A4). The final sample size included 33 tubes with two caterpillars, 43 tubes with four caterpillars and 35 tubes with six caterpillars.

At the beginning of the experiment, the parasitism status of the caterpillars was still unknown and, therefore, could not be directly manipulated. By the end of the experiment, parasitized caterpillars were detected either because they had become fully mummified or via dissection, and their number per tube was quantified. This number might have been an underestimate as some parasitized caterpillars could have been fully consumed before they became mummified. However, we have never observed a nonparasitized caterpillar that was not fully consumed by the end of the experiment, if the potter wasp completed its development.

In addition, we collected the contents of two nests with a total of 44 cells and reared the potter wasp offspring, as above, but without manipulating the cell content. The number of caterpillars per cell was 4.69 ± 1.65 (mean \pm SD, range 2–9). This provided baseline information on developmental success in nest cells naturally supplemented with prey by the mother, for comparison with our experimental manipulation of prey number.

Potter Wasp Feeding Experiment

This experiment was designed to estimate the potential difficulties encountered by a potter wasp larva when feeding on a caterpillar parasitized by *C. primulum*. We offered each *D. dimidiatipenne* larva one caterpillar at a time, to observe its ability to feed on caterpillars that were visibly parasitized (*C. primulum* close to pupation or already pupated) versus visibly nonparasitized (*C. primulum* absent or at an early developmental stage). We used these criteria as some caterpillars were fully consumed and, hence, we could not have confirmed their parasitism status after the trial. The mass of each potter wasp larva was recorded immediately before it was placed into the vial with a single caterpillar for 24 h and again at the end of this period. Cases in which the larva gained mass were considered as efficient feeding events, while cases in which no mass was gained were considered as events of inefficient feeding. We related the occurrence of efficient feeding to the caterpillar’s inferred parasitism status. Larvae that were viable following the first trial were reused. In total, 26 larvae were used for 52 trials.

Caterpillar Mass Comparisons

We compared the mass of caterpillars retrieved from the nest cells ($N = 791$) to that of caterpillars found on the vegetation ($N = 162$), predicting that if foraging *D. dimidiatipenne* females prefer larger caterpillars, those collected from the nest cells would be, on average, heavier than those available on the vegetation. In addition, we compared the mass of parasitized and nonparasitized caterpillars, predicting that parasitized caterpillars would be generally heavier. One comparison was conducted using caterpillars from recently constructed nest cells (i.e. where the potter wasp offspring was still at the egg stage, $N = 210$). These caterpillars had their mass recorded and, immediately after, were dissected to determine their parasitism status. This enabled us to compare the mass of parasitized and nonparasitized caterpillars upon collection by the potter wasp. A second analysis was performed using caterpillars collected from the vegetation that were further reared on *Zygophyllum* leaves in the laboratory ($N = 107$). Their mass was measured daily, and the measure used for this comparison was the last mass recorded a day before the caterpillar pupated or became

mummified, to compare the final mass reached by parasitized versus nonparasitized caterpillars.

Caterpillar Behavioural Experiment

This experiment was designed to compare the behaviour of parasitized versus nonparasitized caterpillars when under attack. We placed each of 48 caterpillars collected from the vegetation in a small Petri dish with a few *Z. dumosum* leaves, which were replaced twice a day. Every 12 h, we observed the reaction of each caterpillar after poking it with pincers to imitate a predator's attack. The reaction was classified either as active (moving away from or attacking the pincer) or as passive (no reaction or curling up). Following the experiment, caterpillars were dissected to determine their parasitism status.

Statistical Analyses

Statistical analyses were all performed using RStudio, version 4.0.2. (RStudio Team, 2020). We used the packages 'car' (Fox et al., 2013) to perform the ANOVA tests and 'lme4' (Bates et al., 2015) to perform the linear and generalized mixed-effect models. We used nonparametric tests in cases where the distribution did not meet the assumptions of a parametric test, even following transformation.

For the rearing experiment, we used a generalized linear model (GLM) with a binomial error distribution and a log-link function to test for treatment effect (two, four or six caterpillars) on potter wasp developmental success. Similarly, we used GLMs to test the effect of the number of parasitized caterpillars on potter wasp developmental success, separately for each treatment group. In the four-caterpillar treatment group, tubes with three or four parasitized caterpillars were pooled for the analysis, and in the six-caterpillar treatment group, vials with five or six parasitized caterpillars were pooled due to small sample sizes in these categories. For cases in which the potter wasp larvae completed their development, we further tested for treatment effect on final body mass using a one-way ANOVA and on developmental time using a Kruskal–Wallis test. We then used linear regression models to test the effect of the number of both parasitized caterpillars and caterpillars that were not detected as parasitized on final body mass and developmental time. These were performed for all treatment groups combined because the number of surviving potter wasps was too small to be analysed separately.

For the feeding experiment, we used a generalized linear mixed-effect model (GLMM) to test the effect of caterpillar mass, parasitism status and the interaction between them on the occurrence of efficient feeding by the potter wasp larva. In this model, we used the identity of the larva as a random effect.

We tested the effect of origin (nest or vegetation) on caterpillars' parasitism status, using a generalized mixed-effect model (GMM), with the study site/date combination as a random effect. We compared the mass of the caterpillars that were collected from the vegetation with that of the caterpillars collected from the potter wasp nest cells, using a Mann–Whitney *U* test. The mass of parasitized versus nonparasitized caterpillars was compared using a linear mixed-effect model (LMM), with site/date as a random factor. This was done separately for caterpillars collected from the vegetation and for those collected from the nest cells. Mass data of caterpillars from the nest cells was square root transformed as it was not distributed normally.

For the behavioural experiments, we compared the reaction of parasitized and nonparasitized caterpillars to a simulated predator attack, using a GLMM, with the identity of the caterpillar as a random effect.

Ethical Note

The research follows the ASAB/ABS Guidelines for the Use of Animals in Research. Sampling permits were obtained from the Israel Nature and National Parks Protection Authority (permit no. 42084).

RESULTS

Potter Wasp Rearing Experiment

We found that 15% of the potter wasp larvae provided with two caterpillars, 34% of the larvae with four caterpillars and 11% of the larvae with six caterpillars, in a tube, completed their development (GLM: $\chi^2 = 7.84$, $N = 111$, $P = 0.02$; Fig. 1). In comparison, the success rate in the tube where the number of caterpillars was not manipulated was 37% ($N = 44$ nest cells). The low developmental success in the six-caterpillar treatment could have been at least partially due to an experimental artefact, as these tubes accumulated more humidity and mould, possibly resulting in the potter wasp's death (S. Leduc, personal observations). Within each treatment group, the success rate decreased as the number of remaining parasitized caterpillars increased (Fig. 1). This difference was significant for the two-caterpillar treatment (GLM: $\chi^2 = 5.28$, $N = 33$, $P = 0.02$) and for the four-caterpillar treatment (GLM: $\chi^2 = 14.50$, $N = 43$, $P < 0.001$). The six-caterpillar treatment showed a similar, but nonsignificant, trend (GLM: $\chi^2 = 2.87$, $N = 35$, $P = 0.09$).

Treatment had a significant effect on adult body mass, with potter wasps that developed in the two-caterpillar treatment maturing at the lowest mass, those in the four-caterpillar treatment having the highest mass and those in the six-caterpillar treatment having intermediate mass (ANOVA: $F_{2,21} = 8.67$, $N = 24$, $P = 0.002$; Fig. 2a). Adult mass was unrelated to the number of parasitized caterpillars in a tube (linear regression: $R^2 = -0.04$, $P = 0.81$; Fig. 2b). However, it was positively associated with the number of fully consumed caterpillars (linear regression: $R^2 = 0.24$, $P = 0.009$; Fig. 2b). The treatment had no significant effect on potter wasp developmental time (Kruskal–Wallis rank sum test: $\chi^2 = 0.19$, $N = 24$, $P = 0.91$). In addition, there was no effect of the number of remaining parasitized caterpillars (linear regression: $R^2 = 0.06$, $P = 0.14$) or of fully consumed caterpillars (linear regression: $R^2 = 0.05$, $P = 0.16$) on developmental time.

Potter Wasp Feeding Experiment

In this experiment, *D. dimidiatipenne* larvae were provided with a single caterpillar at a time for 24 h. The feeding efficiency (efficient = the larvae gained mass during the trial) was not

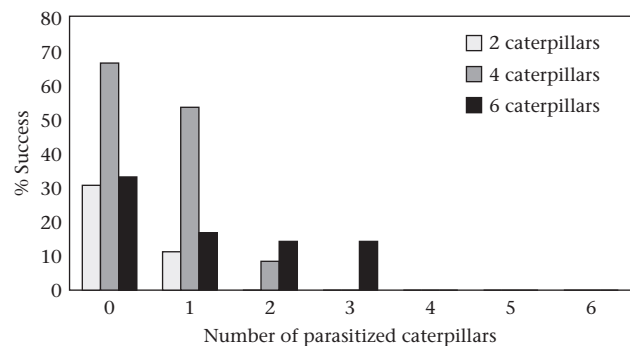


Figure 1. Developmental success (% cases where the potter wasp completed its development) according to the initial number of caterpillars (two, four or six) provided as food, and the number of remaining parasitized caterpillars. $N = 33$ tubes with two caterpillars, 43 tubes with four caterpillars and 35 tubes with six caterpillars.

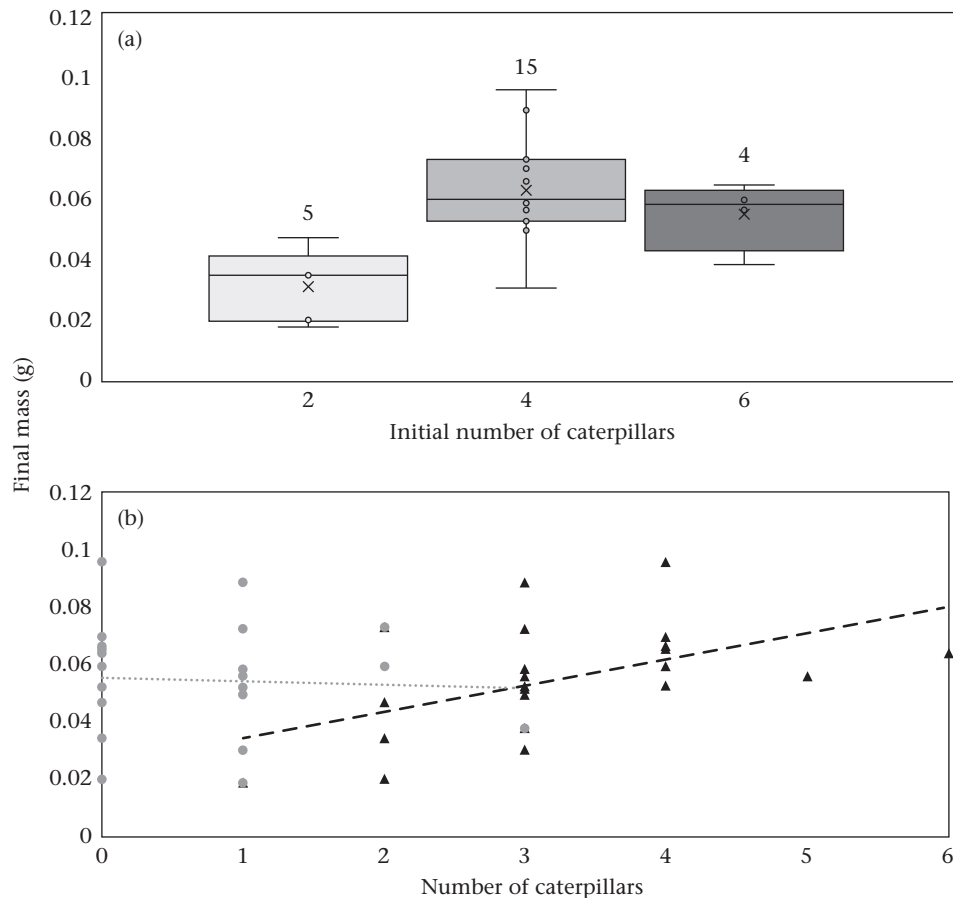


Figure 2. Final body mass (X = mean; line = median; box = first and third quartile; whiskers = range) of the potter wasp in relation to (a) the number of caterpillars initially provided and (b) the number of remaining parasitized caterpillars (grey circles, dotted line) and of fully consumed caterpillars (black triangles, dashed line) (all treatments combined). Numbers above boxes in (a) indicate sample sizes.

significantly related to the parasitism status of the caterpillar (GLMM: $\chi^2 = 1.45$, $N = 52$, $P = 0.23$) or to the initial mass of the potter wasp larva (GLMM: $\chi^2 = 0.05$, $P = 0.82$). However, the interaction between these factors in their effect on the feeding efficiency was significant (GLMM: $\chi^2 = 3.82$, $P = 0.05$). This was probably because smaller larvae were less likely to feed efficiently on a visibly parasitized caterpillar, while the larger larvae fed similarly on caterpillars of both categories (Fig. 3).

Parasitism Rate in the Field

The parasitism rate by *C. primulum* was significantly higher for the caterpillars retrieved from potter wasp nest cells than for those collected from the vegetation (GLMM: $\chi^2 = 14.64$, $N = 530$, $P < 0.001$). This pattern was consistent in all sites and on all dates (Fig. 4).

Caterpillar Mass Comparisons

Caterpillars found in the nest cells were significantly heavier than caterpillars found on the vegetation (Mann–Whitney U test: $W = 2364$, $N = 953$, $P < 0.001$; Fig. 5). For example, no caterpillars of less than 0.05 g were found in the nests, while this mass range was the most common on the vegetation, suggesting the potter wasps ignore caterpillars below this threshold.

In addition, parasitized caterpillars had a significantly higher body mass than nonparasitized ones. This was true both for

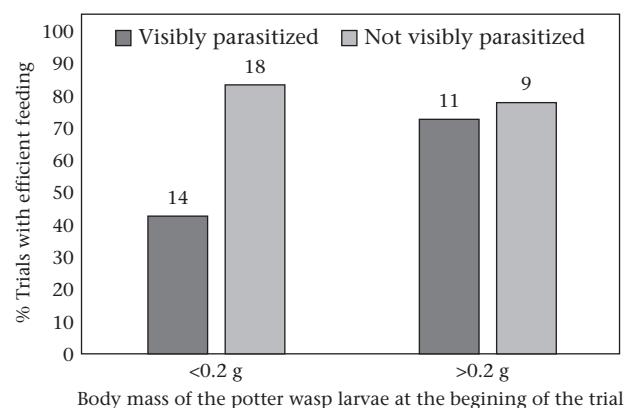


Figure 3. Percentage of trials in which larvae fed efficiently on a caterpillar visibly parasitized or not visibly parasitized, according to their initial mass. Numbers above columns indicate sample sizes.

caterpillars collected from the nest cells (LMM: $\chi^2_1 = 5.91$, $N = 107$, $P = 0.02$; Fig. 6a) and for those collected from the vegetation and further reared until pupation (LMM: $\chi^2_1 = 8.00$, $N = 210$, $P = 0.005$; Fig. 6b).

Caterpillar Behavioural Experiment

Throughout the experiment, nonparasitized caterpillars were more likely to react actively to a simulated predator attack than

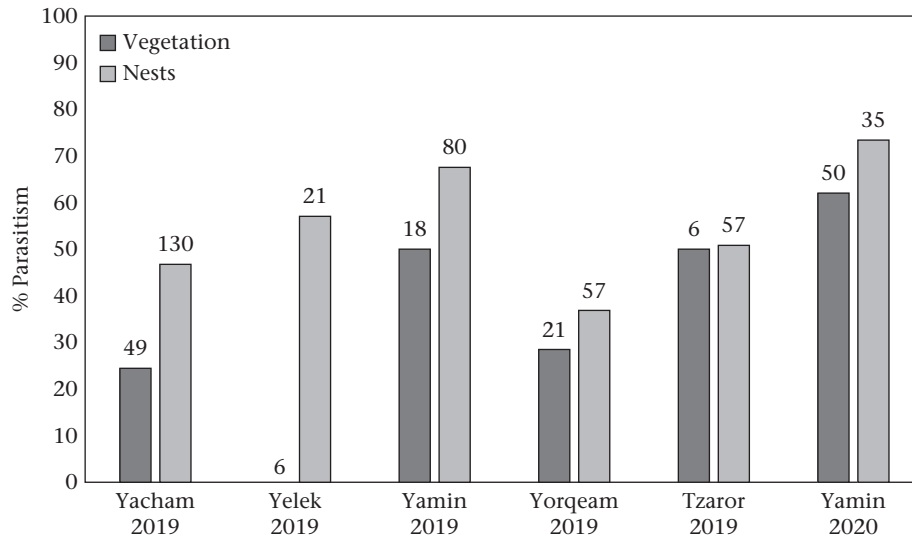


Figure 4. Parasitism rates of *C. primulum* on caterpillars from potter wasp nest cells and from the vegetation at the different sampling sites and dates. Numbers above columns indicate sample sizes.

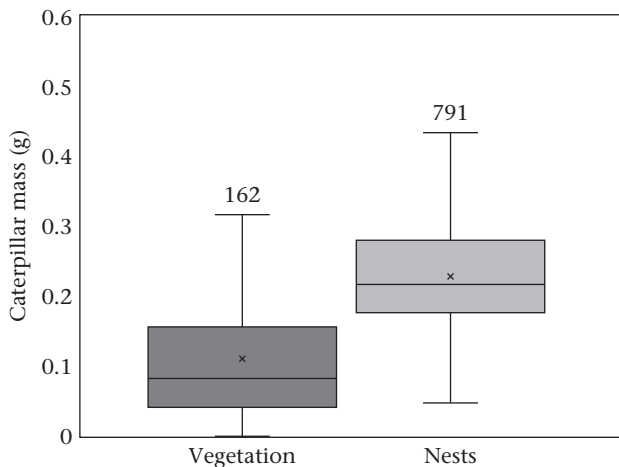


Figure 5. Mass (X = mean; line = median; box = first and third quartile; whiskers = range) of the caterpillars sampled from freshly constructed potter wasp nest cells or from the vegetation, upon sampling. Numbers above boxes indicate sample sizes.

were parasitized ones (GLMM: $\chi^2 = 6.84$, $N = 48$ caterpillars \times 7 trials, $P = 0.009$; Fig. 7). The proportion of caterpillars that reacted actively decreased over the course of the experiment, possibly indicating habituation to the stimulus (GLMM: $\chi^2 = 30.73$, $P < 0.001$; Fig. 7).

DISCUSSION

In this study, we aimed to experimentally quantify the consequences of a seemingly maladaptive behaviour, namely, the collection of already parasitized caterpillars by *D. dimidiatipenne* females, and to explore possible mechanisms for its persistence. We found that potter wasp larvae, developing with parasitized caterpillars, had a lower developmental success, and that young larvae fed less efficiently on parasitized caterpillars than on non-parasitized ones. Nevertheless, parasitized caterpillars were found to be more frequent in newly constructed potter wasp nests than on the vegetation nearby, suggesting that female *D. dimidiatipenne* do not discriminate against them and perhaps are even more likely to

collect them. This can be explained by our further findings that parasitized caterpillars are generally heavier and less responsive to a simulated predator attack, possibly making them more attractive or more susceptible to predation by the potter wasps.

Our experimental results indicated that the success rate of potter wasps developing on four caterpillars was higher than that of those developing on either two or six caterpillars, and it was slightly lower than that of those from cells in which the number of caterpillars was unmanipulated. The lower success rate when provided with two caterpillars was expected and is likely to be due to food shortage. In contrast, the low success rate of larvae provided with six caterpillars was unexpected and is likely, at least partially, to be due to an experimental artefact (i.e. the accumulation of humidity and mould in the experimental tubes; see Results). This interpretation is supported by field data indicating that *D. dimidiatipenne* females almost never collect fewer than three caterpillars into a single cell, but they do often place more than four and even up to nine caterpillars in a cell (with a mean of 4.7 caterpillars), with no evidence of mould or rot development in the cells (S. Leduc & M. Segoli, personal observations, current study). It could be that conditions in the field are generally drier, or that females deposit substances onto the cell walls, as reported in other mass-provisioning insects (Herzner et al., 2013; Kett et al., 2021), preventing such occurrences.

Despite this likely experimental artefact, comparisons within each treatment group strongly suggested that the provision of parasitized caterpillars compromised *D. dimidiatipenne* developmental success. These results are consistent with a previous field study where survival rate and pupal mass were shown to be negatively correlated with the presence and number of mummified caterpillars in a nest cell (Segoli et al., 2020). However, in the previous study, the initial number of caterpillars placed in the cell by the female potter wasp was unknown, making it more difficult to interpret the results, while in the current study, it was experimentally controlled. The strong negative impact was probably mostly due to the number of parasitized caterpillars placed at the expense of nonparasitized ones in our experiment (e.g. within each treatment group). Moreover, the number of fully consumed caterpillars was a better predictor, with a positive effect on offspring final mass, than the number of remaining parasitized ones. Hence, it seems that parasitized caterpillars often contributed poorly to the

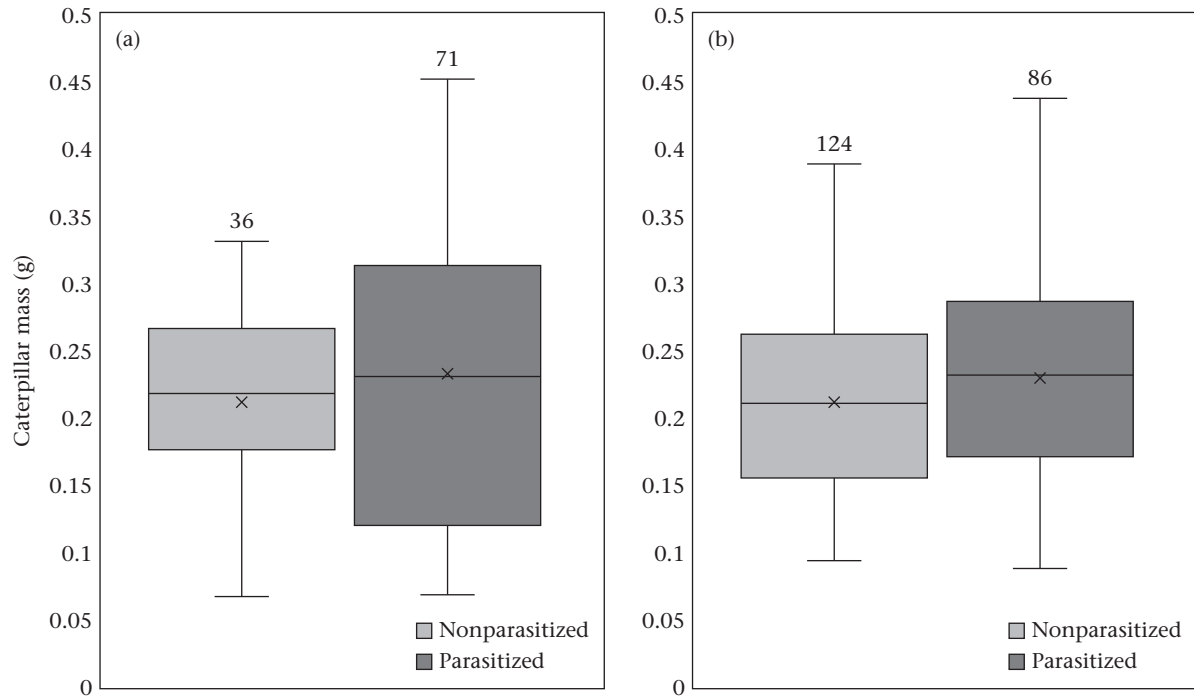


Figure 6. Mass (X = mean; line = median; box = first and third quartile; whiskers = range) of the caterpillars in relation to their parasitism status, for (a) caterpillars collected from the vegetation and reared in the laboratory, taking their final mass before pupation, and (b) caterpillars sampled from newly constructed nest cells and immediately dissected. Numbers above boxes indicate sample sizes.

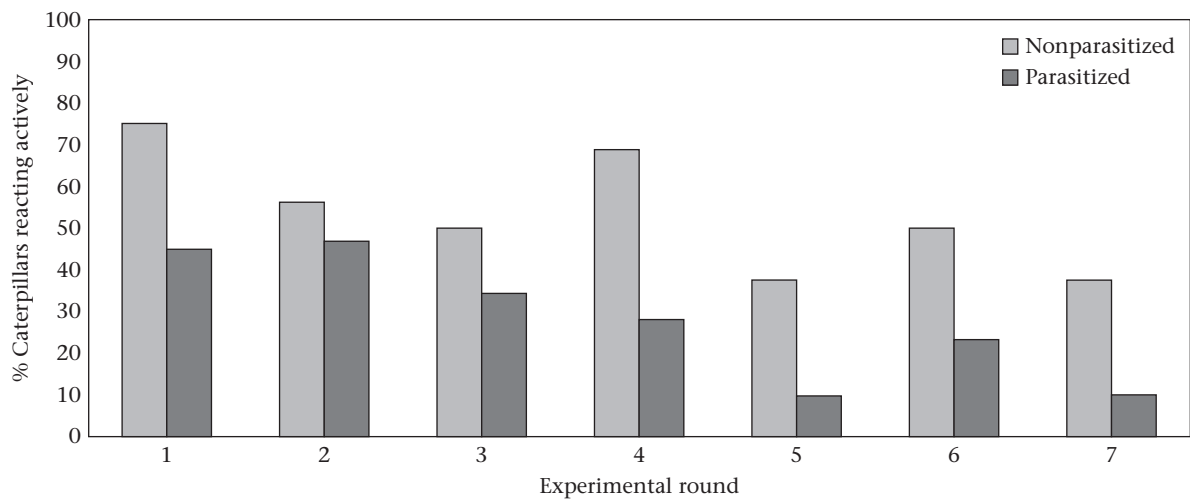


Figure 7. Percentage of caterpillars that actively reacted to a simulated predator attack (by escaping or attacking) for nonparasitized ($N = 16$) and parasitized ($N = 32$) caterpillars in each experimental round (every 12 h).

development and growth of the potter wasp offspring, but their mere presence did not necessarily have subsequent negative effects. Given the finite volume of each nest cell, which is determined prior to prey provisioning, the space occupied by parasitized caterpillars is likely to come at the expense of nonparasitized ones also under natural conditions. Similarly, Bohart et al. (1982) reported on the provision of prey (weevil larvae), out of which 8.7% were already parasitized by an ichneumonid parasitoid, in the nests of the ground-nesting wasp *Odynerus dilectus*. Parasitized prey were ignored by the developing offspring, most likely compromising their development.

While we did not further explore the effect of adult body size on *D. dimidiatipenne* reproductive success, body size is known to correlate with fitness measures in many organisms, as well as in other predatory wasps (Cowan, 1981; Freeman, 1981; O'Neill, 2001). For example, Cowan (1981) found that larger females of the potter wasps *Ancistrocerus adiabatus* and *Euodynerus foraminatus* provisioned more prey to their nest cells than smaller females, and larger males had higher mating success. Therefore, we can consider the smaller body size to contribute to the overall reduction in the potter wasp offspring's reproductive fitness when experiencing food shortage.

In contrast, potter wasp developmental time was not affected by the number of caterpillars, parasitized or not. A possible explanation may be that in potter wasps, as in other mass-provisioning insects, females supply each cell with a fixed amount of food (Field et al., 2020). Hence, delaying maturation may not be advantageous to the developing offspring, as it does not allow them access to additional food resources. In contrast, feeding on low-quality food has been shown to prolong development in insects, where the offspring can continue feeding to compensate for nutrient deficiencies. For example, Kolss et al. (2009) found that *Drosophila melanogaster* larvae had a 70% longer egg-to-adult developmental period when fed with unlimited amounts of poor quality food during the larval stages. Another study found that caterpillars of the moth *Malacosoma disstria*, developing in an environment with poor-quality food, had a longer developmental period and underwent additional larval stages (Jones & Despland, 2006).

Given the strong evidence that developing *D. dimidiatipenne* offspring were disadvantaged when provisioned with parasitized caterpillars, we were somewhat surprised to find that some larvae were able to feed and to gain mass when provided with a single visibly parasitized caterpillar. In fact, in a few cases, such caterpillars were fully consumed by the potter wasp larva, including the *C. primulum* inside them. However, even if fed upon, parasitized prey, and especially those at later stages of parasitism, may constitute a lower-quality food resource (Brodeur & Boivin, 2004; Roger et al., 2001). Moreover, our analysis detected a size-dependent difference in the larva's ability to feed on parasitized caterpillars, with smaller larvae being less likely to feed efficiently on visibly parasitized caterpillars. A possible explanation is that younger larvae need softer food, as their mandibular and head muscles may be less developed (Hochuli, 2001; Pritchard & Mutch, 1984), or have reduced digestive ability (Lundgren & Weber, 2010). This may compromise their ability to consume tougher food items, such as mummified caterpillars that are composed of sclerotized parasitoid pupae and pupal cases. Studies on herbivorous insects have similarly reported that food quality more strongly affected early larval stages. For example, Bezemer and Jones (1998) studied the impact of lower plant quality on the feeding habits of 43 insect herbivores and found that younger larvae were generally more strongly negatively affected. Similarly, Jones and Despland (2006) found that a reduction in leaf quality affected the younger instars of *M. disstria*, leading to reduced adult body mass, while it had no major effect on later instars. The overall observed fitness reduction of potter wasp offspring could therefore be primarily due to the reduced feeding efficiency of the early developmental stages. Indeed, if young larvae are fatally disadvantaged, they would not have the opportunity to develop further, even if at later stages they could potentially feed on the lower-quality resource.

One possible explanation for the high occurrence of parasitized caterpillars in potter wasp nests may be the large overlap in prey use by *D. dimidiatipenne* and *C. primulum* in the field. Nevertheless, some strategies could have evolved to reduce this occurrence, or the costs associated with it. For example, discrimination against already parasitized hosts is commonly reported in parasitoid wasps (Godfray, 1994). In contrast, little is known about the ability of foraging predatory wasps to discriminate against already parasitized prey. To learn about the potential discrimination ability by *D. dimidiatipenne* potter wasps, we compared the parasitism rate of caterpillars from the nest cells to that of those on the nearby vegetation. In contrast to our expectations, we found that caterpillars parasitized by *C. primulum* were more frequent in the nests than on the vegetation. These results suggest that *D. dimidiatipenne* females do not discriminate against, and perhaps even collect, caterpillars parasitized by *C. primulum* more frequently than

expected by chance. This interpretation should be tested by future choice experiments that directly examine female potter wasps' preference for parasitized versus nonparasitized prey.

Notably, the adaptive value of discrimination may depend on environmental conditions, such as the abundance of the prey species in the area, the parasitism rate and the cost of discrimination (Lotem et al., 1995; Van Baaren et al., 1994). For example, if the parasitism rate in the field is low, discrimination might be unnecessary, as the risk of collecting a parasitized caterpillar would be too small to influence the overall fitness. If the parasitism rate is very high or prey abundance is low, discrimination could be unprofitable, as nonparasitized prey would be too difficult to locate. Moreover, as being highly discriminative could result in more time spent foraging away from the nest, the female potter wasps would thereby increase their risk of predation by predators while foraging or the risk of nest parasitism. From our field observations and estimates, it seems that prey availability may be limited, and that the parasitism rate by *C. primulum* may be high (up to ca. 70%), although these factors are likely to fluctuate in time and space. In addition, we found evidence for parasitism by several species of nest parasites (current study, Appendix Table A3; Segoli et al., 2020). It may well be that under such conditions, discrimination against parasitized prey would not be advantageous. Future experiments should be designed to explore potter wasp females' ability and exhibition of discriminative behaviour under varying environmental conditions.

Discrimination ability against parasitized prey may also be compromised if it is mixed with other cues for host quality. One such obvious cue is prey size. Preference for larger prey is common in many organisms, including predatory wasps, at least when accounting for the limits of load that can be carried by the female (Hastings et al., 2010; Hellman & Fierke, 2014; Polidori et al., 2009). By comparing the size of caterpillars from potter wasp nest cells to the size of those on the vegetation, we found evidence that females prefer larger caterpillars and ignore prey below a certain mass threshold. Consistent with this notion, in one instance, we observed a foraging *D. dimidiatipenne* examining a caterpillar that was below the mass range of caterpillars found in the nests, and then abandoning it on the vegetation. At the same time, we found evidence that *H. nubigera* caterpillars parasitized by *C. primulum* reach a higher body mass than nonparasitized ones, as previously documented in other cases of parasitism by *Copidosoma* spp. (Byers et al., 1993; Segoli et al., 2009; Strand, 1989), as well as other parasites (Bernardo & Singer, 2017). The combined evidence suggests that parasitized caterpillars could be more attractive to predatory wasps due to their higher mass. However, the mass differences were not large, and further experiments are necessary to determine whether they are detectable by female potter wasps. Another potential consequence of the preference for larger caterpillars may be that they contain *C. primulum* that are closer to pupation and, hence, are likely to become nonpalatable to the potter wasp larvae sooner. Despite these costs, it is possible that discrimination based on size is easier to execute and, on average, more profitable to *D. dimidiatipenne* foraging females under realistic fluctuating conditions throughout the nesting season.

Another nonmutually exclusive explanation for the frequent collection of parasitized caterpillars is due to some alterations in their defensive behaviour. It is well known that parasitized hosts may behave differently than their nonparasitized counterparts, and this could potentially lead them to being more exposed to predation (Brodeur & Boivin, 2004). For example, in a study on sawflies, parasitized larvae were more susceptible to predation by a predatory bug than nonparasitized ones, due to their location at the periphery of the colony (Tostowaryk, 1971). In another study, parasitized caterpillars of the cabbage butterfly, *Pieris rapae*, were

more vulnerable to predation by ants, possibly due to a reduced ability to defend themselves (Jones, 1987). We found that parasitized caterpillars were consistently less reactive to a simulated predator attack than the nonparasitized ones. This suggests that parasitized caterpillars are less able to resist predation. Furthermore, in the field, we observed some caterpillars dropping off the vegetation when we attempted to collect them. This dropping behaviour is known in other herbivorous insects (Francke et al., 2008) and has also been documented in a caterpillar in response to a predatory wasp (Castellanos & Barbosa, 2011). A field experiment, in which *H. nubigera* caterpillars are disturbed directly on the vegetation to observe their dropping behaviour, which would later be related to their parasitism status, could complement the laboratory experiment conducted here, and give more insights into the significance of the observed responses under natural conditions.

In conclusion, we studied the consequences of *D. dimidiatipenne* females provisioning their offspring with parasitized prey and explored potential mechanisms that could explain its persistence. Our results suggest that although this behaviour seems maladaptive, potential adaptive responses to mitigate it, such as discrimination against parasitized prey, may not be profitable under realistic environmental conditions. We propose that environmental risks and limitations, such as the shortage of high-quality prey, risks while foraging, biases towards larger prey and altered behaviour of parasitized prey, may all contribute to the high occurrence of parasitized caterpillars in potter wasp nests. These insights can be generalized to other systems, suggesting that the occurrence of seemingly maladaptive behaviours can be maintained as the result of limiting and fluctuating environmental conditions, as well as contrasting cues or aspects of resource quality. Our study emphasizes that the adaptive value of foraging decisions, as well as the outcomes of complex biological interactions, cannot be evaluated while ignoring their natural context.

Author Contributions

Sarah Leduc: Conceptualization, Methodology, Investigation, Formal analysis, Writing—Original Draft; **Tamir Rosenberg:** Investigation, Resources; **Alfred Daniel Johnson:** Investigation, Writing—Review & Editing; **Michal Segoli:** Conceptualization, Funding acquisition, Supervision, Writing—Review & Editing.

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Appendix

Table A1
Location of field sites

Site name	Latitude	Longitude
Ammonite wall	30.575826	34.877447
Havarim	30.843180	34.766732
Yacham	30.355308	34.967642
Yamin	30.944038	35.073079
Yelek	30.558482	34.911522
Yorqeam	30.938106	35.040428
Zaror	30.856901	34.822629

Table A2

Sample size of caterpillars that were retrieved from potter wasp nest cells at each site and date and the numbers used in the various parts of the study*

Site	Date	Total no.	Parasitism rate	Potter wasp rearing experiment	Potter wasp feeding experiment	Mass of caterpillars from nest cells (vs vegetation)	Mass of parasitized vs nonparasitized caterpillars
Yacham	24 Mar 2019	83	0	0	0	83	0
Yacham	3 Mar 2019	358	130	187	0	203	73
Yelek	10 Apr 2019	53	21	32	0	17	6
Yamin	14 Apr 2019	238	80	155	0	171	57
Yorqeam	17 Apr 2019	232	57	160	0	155	9
Tzror	1 May 2019	159	57	90	0	88	18
Yelek	25 Apr 2020	110	0	0	9	35	27
Yelek	1 May 2020	90	0	0	5	9	8
Yelek	5 May 2020	60	0	0	20	15	10
Yamin	12 May 2020	46	35	0	6	0	0
Havarim	13.05.20	58	0	0	17	15	8

* Some caterpillars were used for more than one purpose.

Table A3

Sample size of caterpillars retrieved from the vegetation in each site and date and the numbers used in the various parts of the study *

Site	Date	Total no.	Parasitism rate	Mass of caterpillars from vegetation (vs nests)	Mass of parasitized vs nonparasitized caterpillars	Behavioural experiment
Yacham	24 Mar 2019	33	28	31	13	0
Yacham	3 Apr 2019	21	21	0	0	0
Yelek	10 Apr 2019	9	6	9	2	0
Yamin	17 Apr 2019	26	18	26	12	0
Yorqeam	17 Apr 2019	25	21	25	17	0
Tzror	1 May 2019	12	6	11	2	0
Yamin	26 Apr 2020	30	29	30	0	30
Yamin	3 May 2020	30	21	30	0	30
Yelek	18 Feb 2019	17	0	0	17	0
Havarim	7 Mar 2019	2	0	0	2	0
Ammonite wall	20 Mar 2019	28	0	0	28	0
Tzror	20 Mar 2019	11	0	0	11	0
Havarim	31 Mar 2019	3	0	0	3	0

* Some caterpillars were used for more than one purpose.

Table A4
Parasitoids associated with potter wasp nest cells and their likely function

Family	Scientific name	Likely function
Braconidae	<i>Chelonus</i> sp.	Parasitoid of the caterpillar
	<i>Cotesia</i> sp.	Parasitoid of the caterpillar
	<i>Apanteles</i> sp.	Parasitoid of the caterpillar
	<i>Rogas</i> sp.	Parasitoid of the caterpillar
	<i>Phanerotoma</i> sp.	Parasitoid of the caterpillar
Tachinidae	Undetermined	Parasitoid of the caterpillar
Chrysididae	<i>Stilbum</i> sp.	Nest parasite of the potter wasp
Torymidae	<i>Monodontomerus</i> sp.	Nest parasite of the potter wasp
Eulophidae	<i>Melittobia acasta</i>	Nest parasite of the potter wasp