

# The predator within the parasitoid: Host-killing behaviour, parasitism and survival of the host-feeding codling moth parasitoid *Liotryphon caudatus* at different nutritional conditions

Helen Anna Christine Pfitzner<sup>1</sup>, Annette Herz<sup>1</sup>

<sup>1</sup> Julius Kühn Institute (JKI), Federal Research Centre for Cultivated Plants, Institute for Biological Control, Schwabenheimer Str. 101, 69221 Dossenheim, Germany

Corresponding author: Annette Herz ([annette.herz@julius-kuehn.de](mailto:annette.herz@julius-kuehn.de))

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## Abstract

*Liotryphon caudatus*, an ichneumonid parasitoid of codling moth (*Cydia pomonella*), is known to perform host-feeding on late-instar host larvae. In this study, we examine the relationship between host-feeding, parasitisation behaviour and nutritional requirements of *L. caudatus* females and the impact on oogenesis and parasitism success. Using a series of laboratory experiments, we demonstrate that female *L. caudatus* lacking access to hosts for host-feeding were not able to develop mature eggs. Females successfully attacked only the immobile stages of codling moth to either host-feed or parasitise. No major differences in host attack behaviour were observed between naïve and experienced females, as both performed host-feeding similarly. However, experienced females exhibited significantly greater parasitisation success. We also found that when fed honey, female longevity increased up to fivefold compared to having only host-feeding opportunities or to a pure water control. Additionally, success in killing host larvae through host-feeding or parasitism was significantly enhanced with the access to honey. Overall, our findings emphasize that the presence of a carbohydrate source (honey) and a suitable host for host-feeding (immobile larval stages) are essential for the effective parasitisation and host-feeding performance of *L. caudatus*, as well as for the prolonged survival of females. This study provides new insights into the nutritional requirements, host preferences, and behavioural strategies of *L. caudatus* and underscores the importance of host-feeding for the reproductive success and survival of this parasitoid.

## Key Words

Apple orchard, *Cydia pomonella*, destructive host-feeding, nutrition ecology, synovigenic parasitoid

## Introduction

Some hymenopteran parasitoid species engage in host-feeding, defined as the consumption of host haemolymph and tissues by adult females (Jervis and Kidd 1986; Jervis et al. 2008). This behavior is typically observed in synovigenic species, which, unlike proovigenic ones, do not emerge with mature eggs and therefore rely on nutrients from host material and other sources for egg maturation. Host-feeding is generally classified as either destructive, where the host is killed, or non-destructive, where feeding occurs without lethal damage (Jervis and Kidd 1986). It plays a key role in egg development,

especially in idiobiont species, which often require protein intake after emergence to generate eggs. For instance, within the Ichneumonidae, and particularly in the subfamily Pimplinae, host-feeding is considered common, with species often alternating between parasitizing and killing hosts through feeding (Bartlett 1963; Ueno 1999; Zijp and Blommers 2001; Shaw 2009; Shaw et al. 2011).

Thus, the effect of parasitoids on their host populations is likely to be underestimated when only considering parasitoid offspring production, because host-feeding can cause additional host mortality (Kidd and Jervis 1989). Host-feeding has been shown for over 140 species belonging to 17 hymenopteran parasitoid

families (Johnston 1915; De Bach 1943; Campbell 1963; Jervis and Kidd 1986; Bernardo 2006; Zhang et al. 2022). But comparative or systematic studies on how host-feeding relates to age, experience or nutritional status of females are still scarce.

The codling moth parasitoid *Liotryphon caudatus* (Ratzeburg) (Hymenoptera: Ichneumonidae, Pimplinae) was also observed doing host-feeding (Sandanyaka et al. 2016). This parasitoid is a solitary ectoparasitoid (Mills et al. 1996) and usually attacks the pre-pupal, already cocooned larvae of the codling moth, *Cydia pomonella* (L.), found in the bark of trees (Mills 2005). In laboratory experiments, *L. caudatus* females were able to lay on average 150 eggs during lifetime (Davis et al. 2016). Parasitoid larvae hatch up to seven days after oviposition and feed on their host's body content. They molt once and spin a tan transparent cocoon within the host's cocoon. The adult wasp hatches after three to four weeks. *L. caudatus* overwinters as larva or a cocooned pupa (Bartlett 1978). According to Kuhlmann and Mills (1999), the wasp mainly parasitises *Cydia* sp. and other Tortricid species, but can attack also other Lepidopteran species, which produce thin cocoons and reside under the bark of trees (Cushman 1913).

*Liotryphon caudatus* is a regularly observed natural enemy of *C. pomonella* (Athanasov et al. 1997; Mills 2005) and has been introduced as classical biological control agent in North America and New Zealand (Mills 2005; Cole and Walker 2011). In Germany, it occurs mainly in extensive meadow orchards (personal observation), but its regulatory role and its specific habitat requirements requires further clarification and more knowledge on its life history. For instance, the role of the observed host-feeding in egg maturation, survival, and parasitism success of this parasitoid species as well as for additional host mortality has not yet been explored.

In this laboratory study, we examine the relationship between host-feeding, parasitisation behaviour and nutritional requirements of *L. caudatus* females. Specifically, we investigated whether host-feeding is preferentially performed on certain stages of the codling moth's life cycle, and whether it contributes to increased mortality of cocooned codling moths. We also explored whether naïve and experienced female parasitoids tend to use hosts differently for host-feeding or parasitisation and attempted to clarify the importance of host-feeding for the oogenesis of the females. Additionally, we assessed the effects of host-feeding on fecundity and longevity of the parasitoid under different diet treatments.

## Materials and methods

### Rearing of *Cydia pomonella*

Codling moths were held in cylinder-like cages made of plastic frame covered with gauze (volume app. 3 l) in a rearing room at  $23\text{ °C} \pm 2\text{ °C}$  under long-day (16 h light:

8 h darkness) conditions. Adults were supplied with honey and water. Oviposition took place on plastic foil, which was removed and exchanged regularly. After hatching, neonate larvae were put singly into medication cups (10 ml) filled with a layer of artificial diet. When larvae reached the 5<sup>th</sup> instar, they were transferred to corrugated cardboard for pupation and for further codling moth rearing. Alternatively, a part of the 5<sup>th</sup> instar larvae were used for rearing of *L. caudatus*.

### Rearing of *Liotryphon caudatus*

Rearing of *L. caudatus* was established during summer 2016. Wasps came from several meadow apple orchards in Germany. Species identification was done according to Fitton et al. (1988) and confirmed by molecular barcoding (COI-Analysis performed by AIM GmbH, Leipzig, Germany). Adult wasps were maintained in one cage (50 cm × 30 cm × 30 cm (height × depth × width)) built out of Perspex with two flap doors, allowing easy access to provide feeding and host larvae. The rearing was maintained at room temperature (about 20 °C, not regulated) and under long-day (16 h light: 8 h darkness) conditions. Parasitoids had always access to water and honey. Three times a week, 5<sup>th</sup> instar codling moth larvae ready for spinning their cocoon were added into the rearing cage in cardboard rolls and exposed to the parasitoids for one week. Then parasitised larvae were removed out of the cage and put into an incubator ( $23\text{ °C} \pm 2\text{ °C}$ ; 16 h light: 8 h darkness) to allow further development. Adult parasitoids hatched approximately four weeks later and were collected for rearing or using in experiments.

### Acceptance of different developmental stages of the host

This experiment tested whether *L. caudatus* females accept different stages of the host for parasitisation and/or predation. From ten to one day before the experiment, codling moth larvae (5<sup>th</sup> instar) were placed individually in transparent plastic drinking straws (diameter: 0.5 cm, length: 2.5 cm). This procedure was repeated to obtain codling moth larvae in four developmental stages (early mobile 5<sup>th</sup> instar, immobile 5<sup>th</sup> instar, cocooned larvae, pupa) in these straw pieces. On the day of the experiment, straw pieces with codling moth of a particular developmental stage were put into petri-dishes and fixed with tape, so that at the end five hosts of the same stage were presented to one *L. caudatus* female, which was added for one hour (non-choice-situation). In total, 60 *L. caudatus* females were tested, thus 15 females on each particular developmental stage of the host. Females of different age (at least several days old, but exact age unknown) had been removed from the rearing cage 48 hours prior to the experiment, so they likely had oviposition experience and mature eggs in their ovaries. Host acceptance behaviour

was assessed by counting contacts on the different host stages which were provided every female parasitoid wasp in one petri dish. As “contact” several behaviours belonging to parasitisation were determined, including examination with antennae, ovipositor probing or host feeding attempts. After exposure, host larvae were transferred into medication cups and placed into an incubator ( $23\text{ }^{\circ}\text{C} \pm 2\text{ }^{\circ}\text{C}$ ; 16 h light: 8 h darkness) until emergence of parasitoid or codling moth. Controls with codling moth larvae of the various developmental stages, which had not been exposed to parasitoids, were also kept at similar conditions.

### Host-feeding and parasitisation behaviour of *L. caudatus* of different age

This trial was performed to determine if there are differences in host-feeding activity of *L. caudatus* females which are naïve (freshly emerged up to one day old, no oviposition experience, without access to hosts or contact to male *L. caudatus*) or experienced (at least one week old, taken out of the rearing with oviposition, host-feeding experience and mating opportunity). All *L. caudatus* had the opportunity to consume honey and water. Two days before the trial, late 5<sup>th</sup> instar codling moth larvae were put individually into drinking straw pieces to allow them to build their cocoon before being exposed to the parasitoid. One day before the trial, *L. caudatus* females were isolated in petri-dishes as described before. This experiment was performed with 10 naïve and 10 experienced parasitoid females. After placing two host larva into the petri dish with the female, observation took place for three hours, then these host larva were exchanged by two new larva. In total, we conducted this host exposure four times per parasitoid female (meaning in total 12 hours of observations per female). All observations were documented by a video camera (software Noldus Media Recorder®, Version 5.0) placed above the petri-dish to allow subsequent video analysis of the behavioural pattern of *L. caudatus* during host exposure (software Noldus Observer® XT, Version 10). The following behavioural steps were defined: (1) resting, (2) moving, (3) grooming, (4) drinking water/eating honey, (5) searching: antennal tapping on substrate, (6) repeatedly jabbing the larvae with ovipositor (before parasitisation or injure for host-feeding), (7) host-feeding, (8) parasitisation including oviposition. For statistical analysis the behaviours (1), (2), and (4) were summarized as “for parasitisation or host-feeding not relevant”. In contrast, behaviours (3), (5), (6), (7), (8) were considered as behaviours identified as “host-killing relevant” and were analyzed as single behaviours. After exposure to the female parasitoid, host larvae were put into medication cups and further incubated ( $23\text{ }^{\circ}\text{C} \pm 2\text{ }^{\circ}\text{C}$ ; 16 h light : 8 h darkness). Parasitism was assessed by counting the number of emerged *L. caudatus* males and females from exposed host larvae. The number of successfully emerged codling moth was determined as well.

### Development of eggs in *L. caudatus* females depending on host-feeding

The trial was set up to determine if host-feeding is essential for oogenesis and egg maturation in *L. caudatus*. Freshly hatched *L. caudatus* males and females were paired in 200 ml plastic cups with water and honey. Ten pairs were created, with five of them receiving five codling moth larvae weekly (treatment “host-feeding”), the others were kept without hosts. Pairs were held with water and honey at room temperature (about  $20\text{ }^{\circ}\text{C}$ ; 16 h light : 8 h darkness). Females of both treatments were killed and dissected one, three, seven, 14, 21, or 28 days of the experiment (thus one, three, seven, 14, 21, or 28 days after hatching) to examine ovaries for mature eggs (size and number) under the stereo microscope. Ovaries of freshly hatched females were also studied. Egg lengths in ovaries were visually compared to freshly laid eggs on hosts to assess egg ripening status. Due to limited availability of females from the rearing, we could conduct these dissections only with one female per time point and treatment.

### Survival and parasitism capacity of *L. caudatus* at different nutritional conditions

This trial aimed to assess the effect of host-feeding in comparison to other nutritional sources on survival of *L. caudatus* females. Four feeding conditions were considered: (W) survival when provided only water, (WL) survival when provided water and host larvae in corrugated cardboards (5<sup>th</sup> instar, cocooned), (WH) survival when provided water and honey, (WHL) survival when provided water, honey and five codling moth larvae (5<sup>th</sup> instar, cocooned) in corrugated cardboards. Freshly hatched *L. caudatus* males and females were put in pairs into Perspex cylinders (1 l volume), covered with petri-dish lids and held at room temperature (about  $20\text{ }^{\circ}\text{C}$ ; 16 h light: 8 h darkness) and exposed to one of these feeding treatments. Water was provided as dental cotton roll soaked with water. Honey was provided as thin strikes on parafilm. All provisions were exchanged in weekly intervals. Number of replicates varied from 21 to 24 parasitoid females per treatment. Host larvae which had been exposed to *L. caudatus* for parasitisation were placed into an incubator ( $23\text{ }^{\circ}\text{C} \pm 2\text{ }^{\circ}\text{C}$ ; 16 h light : 8 h darkness) and hatched hosts, parasitoids and dead, probably killed, larvae were counted in 10 (WL) and 11 (WHL) repetitions. Control mortality was assessed by incubation of codling moth larvae (5<sup>th</sup> instar) without being exposed to *L. caudatus* at the same conditions.

### Statistical analysis and manuscript preparation

Data analysis was performed using R (R Core Team, 2022). Prior to analysis, all experimental data were plotted and tested for normality and homogeneity of variance.

When the assumptions for ANOVA were not met, the non-parametric Kruskal-Wallis test to detect differences in treatments was applied. Differences in the duration of behaviours between experienced and naïve females were assessed using t-tests after performing a log transformation of the data. The parasitism rates of experienced and naïve females were compared using General Linear Models (GLM) with a binomial distribution. Survival analysis was conducted using Kaplan-Meier analysis following a Log-Rank test. Parasitism rates were compared between the two treatments where host larvae had been exposed using GLM with a binomial distribution.

Some parts of the manuscript were edited using Language Editing Software DeepL Pro Translator. Also, ChatGPT (GPT-4, OpenAI) was used to assist with the development of R scripts for data analysis. It helped to clarify programming syntax, suggest code structures, and helped identify and correct errors in the R code.

## Results

### Acceptance of different developmental stages of the host

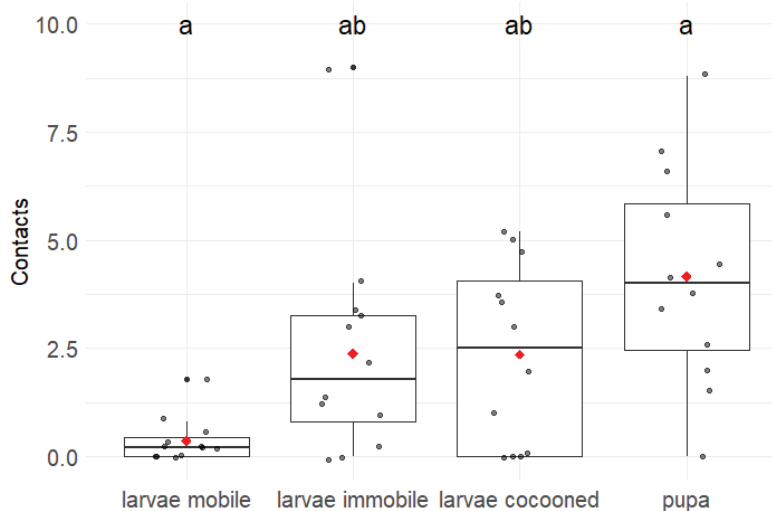
Females of *L. caudatus* exhibited variable acceptance of different host stages (Kruskal-Wallis test:  $\chi^2 = 14.35$ ,  $df = 3$ ,  $p = 0.0025$ ). Immobile host stages (immobile, cocooned, pupa) were more contacted than the mobile ones. However, post-hoc pairwise comparisons using Dunn's test with Bonferroni correction indicated significant differences only between mobile host larvae and the pupa ( $p = 0.0009$ ) (Fig. 1)

In total, 300 host larvae had been exposed to the parasitoid females. Of these, 84% successfully emerged as living adult codling moths. In contrast, 51 dead codling moths (dead 5<sup>th</sup> instar larvae or dead pupae) were counted, of which 41 larvae (80%) showed signs of mold growth and some also injuries, probably from host-feeding or

oviposition attempts. Notably, only one male *L. caudatus* emerged. In the control group, larval mortality was similar (15%), so no clear conclusions could be drawn regarding the influence of host-feeding or parasitisation on the host mortality.

### Host-feeding and parasitisation behaviour of *L. caudatus* of different age

According to the video observation, the parasitisation and searching behaviour of *L. caudatus* females included six different steps: (1) Host searching: the wasp examined the (host) substrate by antennal tapping on the surface (drinking straw and surroundings). If it found tiny holes in the substrate, it inserted its head and, if necessary, its thorax, followed by antennae movements to check whether a host is present. (2) Contact and paralysis: Once a host was found, the parasitoid tried to penetrate the host larva with its ovipositor. To do this, its abdomen bended. (3) Oviposition: If host contact was successful, the wasp's abdomen began to tremble and then went into regular abdominal contractions. During this time, the egg was laid on the paralyzed host. (4) Grooming: After successful oviposition, the wasp groomed itself. (5) Host-feeding: If the female decided to host-feed, parasitisation was interrupted and it withdrew its ovipositor from the host. Subsequently, it positioned itself (head first) into the drinking straw until it contacted the site of host injured by the paralysis. Then, host-feeding began directly at the site, or the host was pulled out from the cocooning site using the mouthparts, and host-feeding took place outside the tube. During host-feeding, the female consumed the leaking haemolymph of the injured host. A further behavior, which was observed during the experiment, was categorized as (6) attempt (attacking without host): the female performed behaviour (1), followed by abdomen bending and penetrating movements though there was no host larva inside the drinking straw. Moreover, no

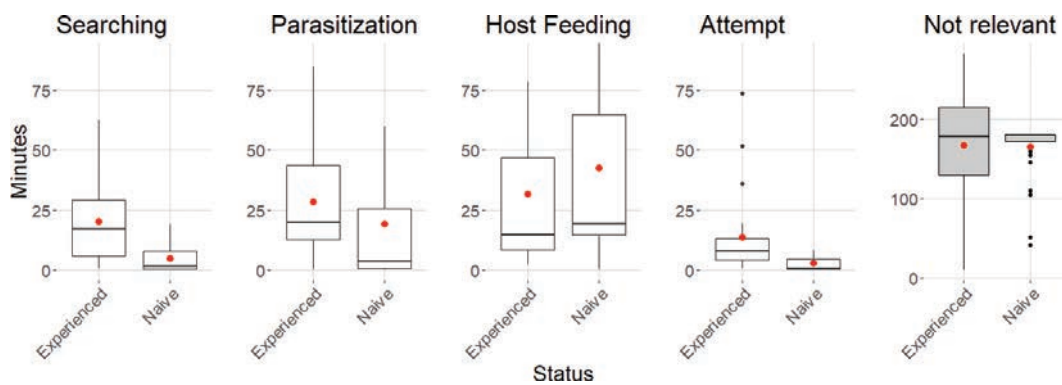


**Figure 1.** Number of parasitising contacts performed by *L. caudatus* females ( $n = 15$  for each treatment, in total  $n = 60$ ) on different developmental stages of codling moth (different letters indicate significance for  $p < 0.05$  in all comparisons). Box plot: median: black line, 25<sup>th</sup> and 75<sup>th</sup> percentile, red dot = mean value.

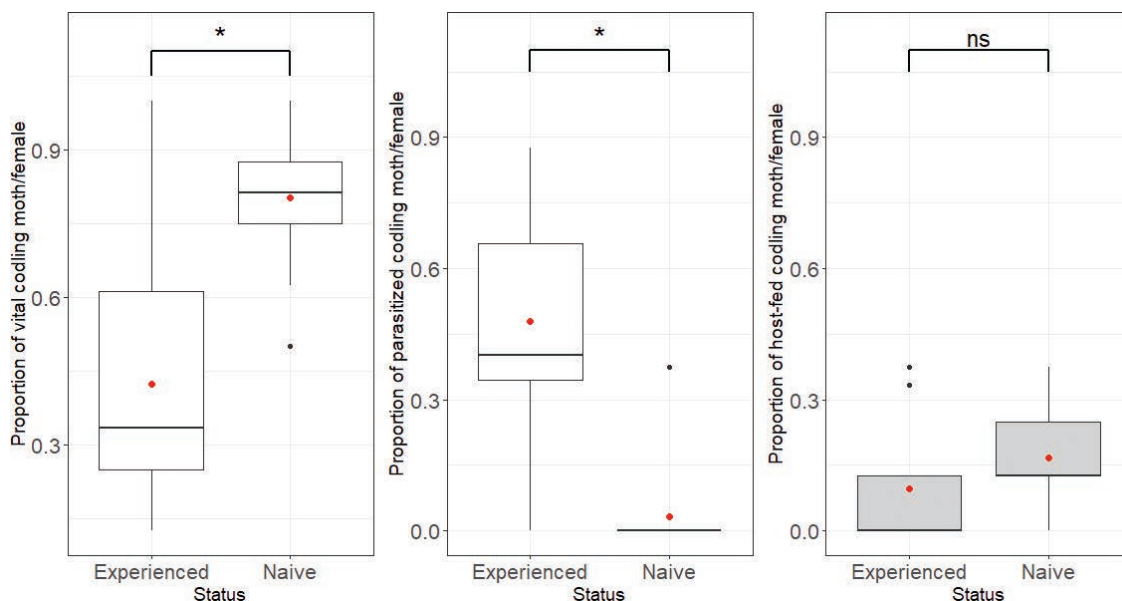
abdominal contractions were observed here. Behaviours which did not affect parasitisation or host-feeding were resting or walking. When a female wasp was resting, it just did nothing at a random place in the petri-dish. When a wasp was walking, it walked without noticeable antennal movement somewhere in the petri-dish. Also drinking and eating honey were behaviours, which could not be associated with parasitisation nor host-feeding. Therefore, resting, walking, and drinking/eating was summarized as a group “not relevant” for host parasitisation or host-feeding. Behaviours (1) - searching, (3) – parasitisation, (5) host-feeding, (6) – attempt were further analyzed by measuring the duration of these different steps as well as the total time duration for “not relevant behaviour” by Noldus Observer®. Parasitisation was defined as a sequence of contact and paralysis, oviposition, and grooming behaviours, as these consistently occurred in succession. “Not relevant behaviour” was conducted by naïve females more often

than by experienced ones ( $t = -7.65, p < 0.0001$ ). Periods of “searching” were shorter than parasitisation in both female groups (naïve:  $t = 4.7, p < 0.0002$ , experienced:  $t = 7.0, p < 0.001$ ). Host-feeding was performed significantly longer than parasitisation by naïve ( $t = 3.41, p < 0.0266$ ), but not by experienced females. Experienced females tended to spend more time in parasitisation than naïve ones, but this difference was not significant. Host-feeding was observed only eight times and took on average 38 min per event (ranging from 30 sec to 114 min). Naïve females ( $n = 5$ ) tended to invest more time in this behaviour (on average 42 min), but the difference was not significant from experienced females ( $n = 3$ ) (Fig. 2).

Parasitism rate of exposed hosts (expressed as proportion) was significantly higher in the group of experienced females than of naïve ones (Fig. 3, GLM with binomial data distribution,  $\chi^2 = 34.8, p < 0.001$ ). On average,  $0.7 \pm 0.28$  male parasitoids hatched from



**Figure 2.** Time [min] that was allocated by *L. caudatus* females (naïve or experienced) to different behaviours when exposed to host larvae (*Cydia pomonella*). Total number of observed behaviours: naïve females: attempt  $n = 3$ , host-feeding  $n = 5$ , not relevant  $n = 40$ , parasitisation  $n = 18$ , searching  $n = 26$ ; experienced females: attempt  $n = 21$ , host-feeding  $n = 3$ , not relevant  $n = 37$ , parasitisation  $n = 31$ , searching  $n = 35$ . Time of observation for each individual wasp (10 naïve and 10 experienced females) was 12 hours. Box plot: median: black line, 25<sup>th</sup> and 75<sup>th</sup> percentile, red dot = mean value.



**Figure 3.** Proportion of vital codling moth (left), parasitized codling moth (middle) and of host-fed host larvae (right) per parasitoid female of experienced and naïve *L. caudatus* after exposure of eight host larvae for 12 h. Box plot: median: black line, 25<sup>th</sup> and 75<sup>th</sup> percentile, red dot = mean value, \* indicates significance and ns no significance for  $p < 0.05$  in all comparisons).

the eight hosts exposed to each of the experienced *L. caudatus* female in contrast to  $0.25 \pm 0.17$  males from hosts, offered to naïve females. Female progeny was generated only by experienced *L. caudatus* with  $2.9 \pm 0.49$  female progeny/parasitoid female on average. In both groups, dead host larvae (5<sup>th</sup> instar) occurred which neither had developed to parasitoids nor to adult codling moths (in total, 16 dead host larvae in the exposure to naïve females; seven dead host larvae in the exposure to experienced females). Assuming that this mortality of hosts was due to host-feeding, the host-feeding rate was higher in naïve females, but not significantly (naïve females:  $1.3 \pm 0.37$  dead hosts/female; experienced females:  $0.7 \pm 2.28$  dead hosts/female; GLM with binomial data distribution,  $\chi^2 = 2.17$ ,  $p = 0.141$ ; Fig. 3). The proportion of completely developed vital codling moth was significantly lower (GLM with binomial data distribution,  $\chi^2 = 6.026$ ,  $p < 0.05$ ; Fig. 3) when host larvae had been exposed to experienced females (naïve females:  $6.4 \pm 0.4$  vital hosts/female; experienced females:  $3.2 \pm 0.28$  vital hosts/females).

### Development of eggs in *L. caudatus* females depending on host-feeding

According to the dissections, *L. caudatus* females had three ovarioles per ovary where eggs were surrounded by follicle cells (Fig. 4, Day 1). Females which had the possibility for host-feeding were able to form eggs which had a length about 300  $\mu\text{m}$  after 3 days. The eggs became bigger in female wasps that were dissected at a later time (about 800  $\mu\text{m}$  egg size after 7 days) until they reached a final length about 1700  $\mu\text{m}$  after 14 days (Fig. 4). Eggs of this size were found also in ovaries of older *L. caudatus* females (21 days, 28 days) and match the size of mature, already deposited *L. caudatus* eggs on the host (own observation). In contrast, females with no opportunity for host-feeding did not produce any mature eggs and eggs remained at a size of 240  $\mu\text{m}$  after seven days and of 470  $\mu\text{m}$  after three weeks. After 14 days, no eggs were found inside the ovaries and in the individual dissected after 28 days the ovary was collapsed (Fig. 4).

### Survival and parasitism capacity of *L. caudatus* at different nutritional conditions

Survival probability of female wasps was significantly influenced by diet (Kaplan Meier:  $\chi^2 = 89.3$ , d.f. = 3,  $p$ -value  $< 0.0001$ , Fig. 5). On average, starving females lived about five days ( $5.5 \pm 2.2^{\text{a}}$  days) with water only. Those females that had the opportunity for host-feeding did not live significantly longer ( $7.1 \pm 5^{\text{a}}$  days with water and host larvae). As soon as *L. caudatus* females had access to honey, they lived about five times longer than without sugar source ( $31 \pm 15.3^{\text{b}}$  days supplied with honey and water;  $31.7 \pm 15.3^{\text{b}}$

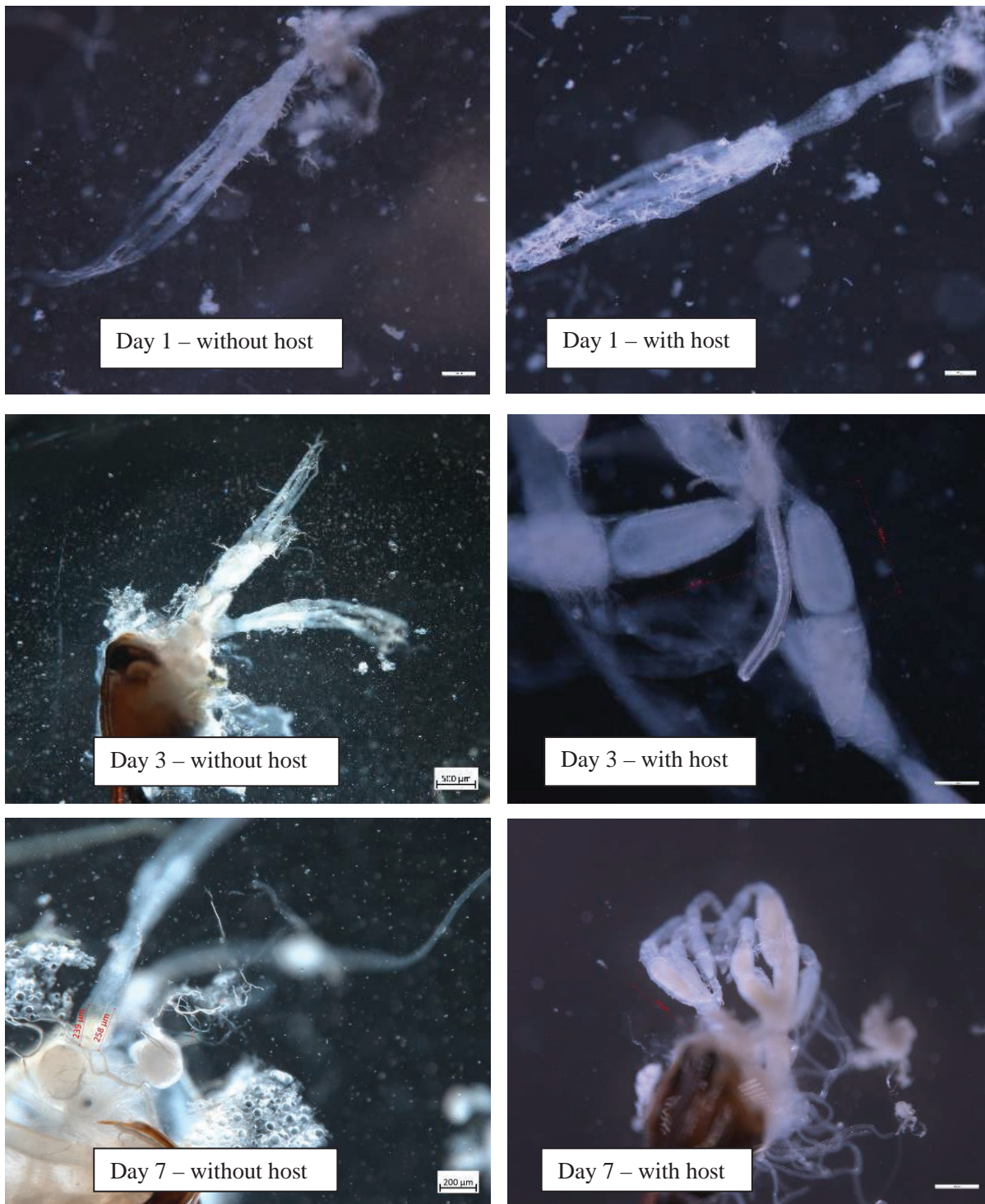
days supplied with honey, host larvae and water (different letters indicate significance according to log-rank test, Bonferroni correction,  $\chi^2 = 1$ ,  $p < 0.05$  in all comparisons).

The parasitisation of offered hosts was significantly higher when females had access to honey (GLM with binomial data distribution,  $\chi^2 = 12.09$ ,  $p < 0.001$ ; WHL:  $14.0\% \pm 1.6\%$ , WL:  $6.7\% \pm 1.3\%$ ) (Fig. 6). The sex ratio (male : female) of fully developed offspring was 1 : 1 for the WL treatment, and 2 : 1 for WHL treatment.

## Discussion

In this study, we investigated the relationship between host-feeding and parasitisation behaviour of *L. caudatus* females, focusing on the impact on oogenesis, parasitism success, parasitoid fitness and codling moth mortality. The species *L. caudatus* is known to conduct host-feeding (Sandanayake et al. 2016), but detailed investigations of the aforementioned characteristics have been lacking. Our findings point to some important life history traits of this parasitoid species regarding the preferred host developmental stage, the importance of host-feeding and other nutritional requirements for reproductive success and parasitoid survival and how experience level affects parasitoid behaviour.

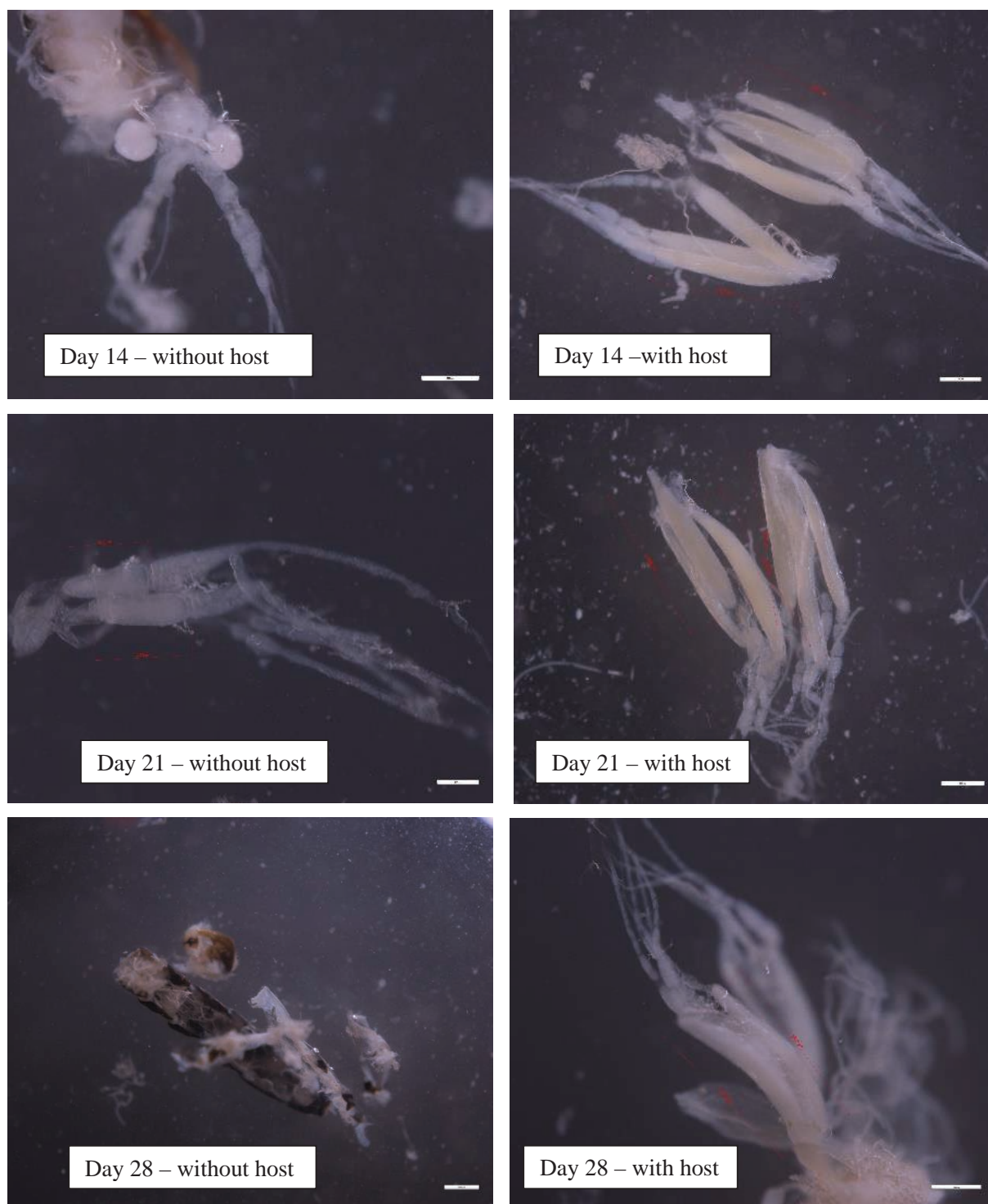
Regarding the preference for host development stages, the *L. caudatus* females contacted immobile host stages more often than mobile ones. These results confirm previous findings that already cocooned, but not yet pupal immobile host stages of codling moth were the main targets parasitised by *L. caudatus* in the field Mills (2005). Parasitisation by *L. caudatus* in the field probably occurs when the host larva is already in its cocoon site in the bark of apple trees, which provides a firm, immobile substrate (own observation). Mobilization and escape of the cocooned host larva from this hiding place is rather unlikely compared to the situation in our experimental set-up. In general, limitations of our study are certainly due to the somewhat non-natural experimental conditions, which might have influenced the behaviour of *L. caudatus* females compared to natural conditions. In addition, Cole and Walker (2011) showed that first instar larvae of *L. caudatus* larvae cannibalised parasitoid eggs and larvae in superparasitism situations, which reduced parasitoid survival by about 50%. Moreover, Ueno (1997) showed for the related *Pimpla nipponica* (Uchida) that the percentage of hosts yielding wasp offspring decreased with increasing host exposure periods. Both events probably happened also in our experiments resulting in high mortality of host larvae without any emerging parasitoid offspring due to repeated oviposition on a single host. In a previous study it has been shown that *L. caudatus* lays its eggs on cocooned 5<sup>th</sup> larval stage (Mills 2005; Davis et al. 2016). This is why we used the cocooned larval stage for further experiments, which also evoked more parasitisation contacts compared to the mobile stage (although not statistically significant). In addition, we cannot exclude



**Figure 4.** Development of ovaries and egg production of *L. caudatus* females which had opportunity for host-feeding (with host) or not (without host). Pictures were taken from one to 28 days after the adult wasp had hatched. Size of the scale bar (sb) varies in the different figures: Day 1 without host: sb = 200  $\mu\text{m}$ ; Day 1 with host: sb = 200  $\mu\text{m}$ ; Day 3 without host: sb = 500  $\mu\text{m}$ ; Day 3 with host: sb = 100  $\mu\text{m}$ ; Day 7 without host: sb = 200  $\mu\text{m}$ ; Day 7 with host: sb = 500  $\mu\text{m}$ ; Day 14 without host: sb = 500  $\mu\text{m}$ ; Day 14 with host: sb = 500  $\mu\text{m}$ ; Day 21 without host: sb = 200  $\mu\text{m}$ ; Day 21 with host: sb = 500  $\mu\text{m}$ ; Day 28 without host: sb = 1000  $\mu\text{m}$ ; Day 28 with host: sb = 500  $\mu\text{m}$ .

that *L. caudatus* may also prefer different host stages for host-feeding or for parasitisation, as it was shown for the parasitoid *Necremnus tuta* Ribes & Bernardo (Hymenoptera: Eulophidae) regarding its host *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) (Zhang et al. 2022) and this requires further study.

According to Jervis and Kidd (1986), different types of host-feeding behaviour occur, depending whether or not host-feeding is performed on the same host individual that is used for oviposition and whether the host is killed by the feeding (destructive) or not. In the related *Ephialtes* species the host-feeding is described as destructive

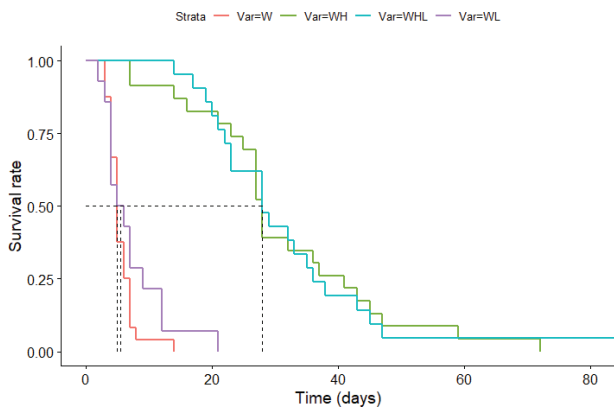


**Figure 4.** Continued.

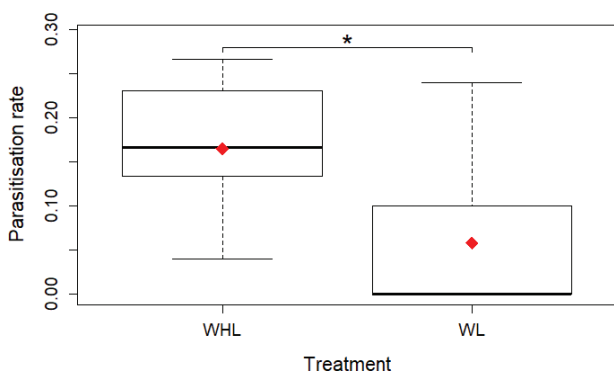
(Jervis and Kidd 1986). According to our results, also *L. caudatus* performs destructive host-feeding, because all codling moth larvae being definitively host-fed (three hosts by experienced and five hosts by naïve parasitoids) did not further develop, thus had obviously been killed.

We had expected that naïve, freshly emerged females may host-feed more frequently due to unsatisfied nutritional requirements for egg maturation. We could not confirm this in our observational experiment, because the different

behaviours occurred only in small numbers and the short observation period covered only 12 hours in the lifetime of the parasitoids. Nevertheless, in this one-day experiment, naïve and not mated females indeed parasitised less in comparison to experienced females and they also spent significantly more time on host-feeding relative to their parasitisation efforts. This suggests that naïve females allocate a greater proportion of their time to host-feeding rather than parasitising hosts confirming assumptions of Heimpel et al. (1994).



**Figure 5.** Survival of *L. caudatus* females provided with water (Treatments: Var = W: water; Var = WL: water and host larvae; Var = WH: water and honey; Var = WHL: water and honey and host larvae. N females: W = 24, WL = 14, WH = 23, WHL = 21.



**Figure 6.** Parasitisation rate of exposed codling moth larvae in the survival experiment. WHL: Female *L. caudatus* had access to water, honey and larvae; n = 11, WL: Female *L. caudatus* had access to water and larvae; n = 10. (Box plot: median: black line, 25<sup>th</sup> and 75<sup>th</sup> percentile, red dot = mean value, \* indicates significance for  $p < 0.05$ ).

In contrast, experienced females showed a more balanced distribution of time between these two behaviours, with parasitisation being more prevalent. This finding highlights the potential role of host-feeding in the nutritional and reproductive strategy of *L. caudatus*, particularly in the context of egg maturation, which may require additional resources before parasitisation can be optimally performed (Heimpel et al. 1994).

In fact, the results obtained from dissecting *L. caudatus* females with and without the possibility for host-feeding suggest that egg maturation was only successful in females with the opportunity for host-feeding. In non host-fed females, the egg maturation occurred much more slowly and eggs also did not reach the full size exceeding 800  $\mu\text{m}$ . These findings are comparable to Leius (1961), who showed in laboratory experiments with the Pimpline *Itopectis conquisitor* (Say) that wasps laid only few eggs without previous host-feeding. This means the formation of mature eggs may be not solely linked to host-feeding, but the number of offspring significantly

increases with host-feeding (Leius 1961; Ueno 1999); probably also true for *L. caudatus*. Most parasitoid Hymenoptera, including all ichneumonids investigated, are unable to synthesize their own lipids *de novo* from carbohydrate sources. Therefore, they need to obtain all fats from their food source or even change host metabolism while developing in the host to obtain more lipids until they emerge as adults (Visser et al. 2010; Visser and Ellers 2011; Visser et al. 2012.). This may depend also on the similarity of lipid profiles from parasitoid and its host. *Itopectis conquisitor* for example has a very similar lipid profile to its host (Leius 1961). Quicke (2015) pointed out, that this might be a general principle of the ichneumonid subfamily Pimplinae being close in protein or lipid profiles to their hosts. *Pimpla nipponica*, for example, is able to produce a relatively small number of eggs (around 20) without host-feeding during the early stage of its life, but is no longer capable of oviposition later on without access to host food. (Ueno 1999). Here the author suggested that these eggs had been produced using stored resources in the female. After these initial phases of egg laying, female wasps need to do host-feeding for further egg production (Ueno 1999). We observed that *L. caudatus* females carry anhydropic yolk-rich eggs, like all ectoparasitoid species do (Quicke 2015), and they developed three ovarioles per ovary, surrounded by follicle cells. Anhydropic eggs are described to be typically large and are produced in parasitoid species that conduct host-feeding (Jervis and Kidd 1986). They contain all the necessary nutrients that are needed for complete embryonic development and females have only limited egg storage capacity (Jervis and Kidd 1986). *Muscidifurax zaraptor* (Kogan and Legner) (Pteromalidae), which also has yolk-rich eggs and three ovarioles per ovary, is capable of resorbing unlaidd eggs, giving the parasitoid greater flexibility in resource allocation for egg production or host searching and maintenance, as reported by Antolin and Williams (1989). Our results suggest that this strategy may also be applicable to *L. caudatus*, being an explanation why ovaries totally collapsed in the female not being host-fed after 28 days in our observation.

Longevity of females was not significantly enhanced when offering host larvae as food source in comparison to starved females (only water), whereas survival of females having access to sugar sources (e.g. honey) increased more than fivefold in comparison. Thus, host-feeding alone did not have a life-extending effect on *L. caudatus*, whereas the provision of honey significantly increased the lifespan of the tested individuals. This was found also in other studies with species performing host-feeding (Leius 1961; Ueno 1997). Parasitisation rates were higher when wasps had access to honey, which is in line with findings for other parasitoid species (Mátray and Herz 2022; Hu et al. 2024). This suggests that not only the availability of a protein source, but also access to sugar resources like floral nectar in the field is crucial for fully exploiting the potential of *L. caudatus*.

## Conclusion

While the observed *L. caudatus* host-feeding behaviour has already been documented (Sandanayake et al. 2016), it has now been confirmed in this study and further emphasize its critical importance for female egg maturation and parasitism success. In addition, this is the first study demonstrating the combined effects of host-feeding and sugar access on parasitism success and survival in *L. caudatus*.

The results underscore the crucial role of both sugar sources and host-feeding opportunities for optimal parasitism performance in *L. caudatus*. Thus, the absence of either resource may significantly impair the parasitoid's parasitism capacity, suggesting that in natural conditions, where these resources may be limited, the efficiency of *L. caudatus* for natural regulation of its most important host *C. pomonella* could be compromised.

Therefore, the access to carbohydrate sources like honeydew or nectar of flowering plants in the orchard ecosystem should be ensured by means of habitat management. For instance, the introduction of flowering strips can serve *L. caudatus* and also other parasitoids without enhancing the pest (Mátray and Herz 2021; Mátray and Herz 2022), and promote pest regulation in apple orchards (Cahenzli et al. 2019). On the other hand, the availability of sufficient or even surplus host larvae in a patch in the apple tree bark located by *L. caudatus* can be expected as a consequence of the known aggregation behaviour of codling moth larvae (Jumenan et al. 2009), which provides opportunities for both host-feeding and parasitism. It would be of high interest to clarify the species-specific ecological relevance of these findings under field conditions.

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## References

Antolin MF, Williams RL (1989) Host Feeding and Egg Production in *Muscidifurax zaraptor* (Hymenoptera: Pteromalidae). *The Florida Entomologist* 72: 129–134. <https://doi.org/10.2307/3494978>

Athanassov A, Charmillot PJ, Jeanneret P, Renard D (1997) Les parasitoïdes des larves et des chrysalides de carpocapse *Cydia pomonella* L.. *Revue Suisse de Viticulture, Arboriculture, Horticulture* 29: 99–106.

Bartlett BR (1963) Patterns in the Host-Feeding Habit of Adult Parasitic Hymenoptera. *Annals of the Entomological Society of America* 57: 344–350. <https://doi.org/10.1093/aesa/57.3.344>

Bartlett BR (1978) Introduced parasites and predators of arthropod pests and weeds: a world review. In: *Agricultural Handbook No. 480*. United States Department of Agriculture, Washington, DC, 294–308.

Bernardo U, Pedata PA, Viggiani (2006) Life history of *Pnigalio soemius* (Walker) (Hymenoptera: Eulophidae) and its impact on a leafminer host through parasitization, destructive host-feeding and host-stinging behaviour. *Biological Control* 37(1): 98–107. <https://doi.org/10.1016/j.biocontrol.2005.11.011>

Cahenzli F, Sigsgaard L, Daniel C, Herz A, Jamar L, Kelderer M, Jacobsen SK, Kruczynska D, Mátray S, Porcel M, Sekrecka M, Swiergiel W, Tasin M, Telfser J, Pfiffner L (2019) Perennial flower strips for pest control in organic apple orchards - A pan-European study. *Agriculture Ecosystems & Environment* 278: 43–53. <https://doi.org/10.1016/j.agee.2019.03.011>

Campbell RW (1963) Some Ichneumonid-Sarcophagid interactions in the gypsy moth *Porthetria dispar* (L.) (Lepidoptera: Lymantriidae). *The Canadian Entomologist* 95(4): 337–345. <https://doi.org/10.4039/Ent95337-4>

Cole LM, Walker JTS (2011) The distribution of *L. caudatus*, a parasitoid of codling moth (*Cydia pomonella*) in Hawke's Bay apple orchards. *New Zealand Plant Protection* 64: 222–226. <https://doi.org/10.30843/nzpp.2011.64.5958>

Cushman RA (1913) The *Calliephialtes* parasite of the codling moth. *Journal of Agricultural Research* 1: 211–237.

Davis VA, Sandanayaka WRM, Charles JG (2016) Longevity and fecundity of laboratory-reared *L. caudatus*, an ectoparasitoid of codling moth. *New Zealand Plant Protection* 69: 318–318. <https://doi.org/10.30843/nzpp.2016.69.5920>

DeBach P (1943) The importance of host-feeding by adult parasites in the reduction of host populations. *Journal of Economic Entomology* 36: 647–658. <https://doi.org/10.1093/jee/36.5.647>

Fitton MG, Shaw MR, Gauld ID (1988) Pimpline Ichneumon-flies, Hymenoptera, Ichneumonidae (Pimplinae). In: Barnard PC, Askew RP (Eds) *Handbooks for the Identification of British Insects*. Royal Entomological Society of London, London 7, 110 pp.

Heimpel GE, Rosenheim JA, Adams JM (1994) Behavioural ecology of host feeding in *Aphytis* parasitoids. *Norwegian Journal of Agricultural Sciences* 16: 101–115.

Hu W, Liu B, Xia S, Ma N, Wang P, Lu Y (2024) Impact of nutritional supplements on the fitness of the parasitoid *Binodoxys communis* (Gahan). *Insects* 15(4): 245. <https://doi.org/10.3390/insects15040245>

Jervis MA, Kidd NAC (1986) Host-feeding strategies in hymenopteran parasitoids. *Biological Reviews* 61(4): 395–434. <https://doi.org/10.1111/j.1469-185X.1986.tb00660.x>

Jervis MA, Ellers J, Harvey JA (2008) Resource acquisition, allocation, and utilization in parasitoid reproductive strategies. *Annual Review of Entomology* 53: 361–385. <https://doi.org/10.1146/annurev.ento.53.103106.093433>

Johnston FA (1915) *Asparagus*-beetle egg parasite. *Journal of Agricultural Research* 4: 303–313.

Jumenan Z, Wood C, Gries G (2009) Frequency distribution of larval codling moth, *Cydia pomonella* L., aggregations on trees in unmanaged apple orchards. *Environ Entomol* 38: 1395–1399. <https://doi.org/10.1603/022.038.0507>

- Kidd NAC, Jervis MA (1989) The effects of host-feeding behaviour on the dynamics of parasitoid-host interactions, and the implications for biological control. *Researches on Population Ecology* 31: 235–274. <https://doi.org/10.1007/BF02513204>
- Kuhlmann U, Mills NJ (1999) Exploring the biodiversity of Central Asia to assess specialized parasitoids for biological control of apple pests in Europe and North America.- *Integrated Plant Protection in Orchards IOBC/wprs Bulletin* 22(6): 9.
- Leius K (1961) Influence of Food on Fecundity and Longevity of Adults of *Itopectis conquisitor* (Say) (Hymenoptera: Ichneumonidae). *The Canadian Entomologist* 93: 711–779. <https://doi.org/10.4039/Ent93771-9>
- Mátray S, Herz A (2021) Do floral resources affect fitness of adult *Cydia pomonella* (Linnaeus 1758) (Lepidoptera: Tortricidae)? *Bulletin of Entomological Research* 111(6): 726–732. <https://doi.org/10.1017/S0007485321000468>
- Mátray S, Herz A (2022) Flowering plants serve nutritional needs of *Ascogaster quadridentata* (Hymenoptera: Braconidae), a key parasitoid of codling moth. *Biological Control* 171: 104950. <https://doi.org/10.1016/j.biocontrol.2022.104950>
- Mills NJ, Dixon L, Hanke R, Buchner R (1996) Integrating parasitism into codling moth control in walnuts. University of California Fruit and Nut Research and Information Centre, Davis, USA, 155–163.
- Mills N (2005) Selecting effective parasitoids for biological control introductions: Codling moth as a case study. *Biological Control* 34: 74–282. <https://doi.org/10.1016/j.biocontrol.2005.02.012>
- Quicke DL (2015) *The Braconid and Ichneumonid parasitoid wasps: biology, systematics, evolution and ecology*. John Wiley & Sons, 681 pp. <https://doi.org/10.1002/9781118907085>
- R Core Team (2022) R: A language and environment for statistical computing R Foundation for Statistical Computing, Vienna, Austria. <https://www.R-project.org/>
- Russel VL (2016) Least-Squares Means: The R Package lsmeans *Journal of Statistical Software* 69(1): 1–33. <https://doi.org/10.18637/jss.v069.i01>
- Sandanayaka WRM, Davis VA, Charles JG (2016) Interspecific competition between *Mastrus ridens* and *L. caudatus*, ectoparasitoids of codling moth *Cydia pomonella*. *New Zealand Plant Protection* 69: 310–217. <https://doi.org/10.30843/nzpp.2016.69.5902>
- Shaw M, Jennings M, Quicke D (2011) The identity of *Scambus planatus* (Hartig, 1838) and *Scambus ventricosus* (Tschek, 1871) as seasonal forms of *Scambus calobatus* (Gravenhorst, 1829) in Europe (Hymenoptera, Ichneumonidae, Pimplinae, Ephialtini) *Journal of Hymenoptera research* 23: 55–64. <https://doi.org/10.3897/jhr.23.1974>
- Shaw MR (2009) Notes on the host-feeding and hyperparasitic behaviours of *Itopectis* species (Hymenoptera: Ichneumonidae, Pimplinae *Entomologist's Gazette* 60: 113–116.
- Ueno T (1999) Reproduction and host-feeding in the solitary parasitoid wasp *Pimpla nipponica* (Hymenoptera: Ichneumonidae). *Invertebrate Reproduction & Development* 35(3): 231–237. <https://doi.org/10.1080/07924259.1999.9652389>
- Ueno T (1997) Effects of superparasitism, larval competition, and host feeding on offspring fitness in the parasitoid *Pimpla nipponica* (Hymenoptera: Ichneumonidae). *Annals of the Entomological Society of America* 90(5): 682–688. <https://doi.org/10.1093/aesa/90.5.682>
- Visser B, Ellers J (2012) Effects of a lipid-rich diet on adult parasitoid income resources and survival. *Biological Control* 60(2): 119–122. <https://doi.org/10.1016/j.biocontrol.2011.11.008>
- Visser B, Le Lann C, den Blanken FJ, Harvey JA, van Allphen JJ, Ellers J (2010) Loss of lipid synthesis as an evolutionary consequence of a parasitic lifestyle. *Proceedings of the National Academy of Sciences* 107(19): 8677–8682. <https://doi.org/10.1073/pnas.1001744107>
- Visser B, Roelofs D, Hahn DA, Teal PE, Marien J, Ellers J (2012) Transcriptional changes associated with lack of lipid synthesis in parasitoids. *Genome Biology and Evolution* 4(8): 864–874. <https://doi.org/10.1093/gbe/evs065>
- Zhang Y, Tian X, Wang H, Castañé C, Arnó J, Wu S, Xian X, Liu W, Desneux N, Wan F, Zhang G (2022) Nonreproductive effects are more important than reproductive effects in a host feeding parasitoid. *Scientific Reports* 12(1): 11475. <https://doi.org/10.1038/s41598-022-15296-2>
- Zhang, Y, Tian X, Wang H, Castañé C, Arnó J, Collatz J, Romeis J, Wu S, Xian X, Liu W, Wan F, Zhang G (2022) Host selection behavior of the host-feeding parasitoid *Necremnus tutae* on *Tuta absoluta*. *Entomologia Generalis* 42(3): 445–456. <https://doi.org/10.1127/entomologia/2021/1246>
- Zijp JP, Blommers LH (2002) Survival mode between the yearly reproduction periods, and reproductive biology of *Scambus pomorum* (Hymenoptera: Ichneumonidae: Pimplinae), a parasitoid of the apple blossom weevil *Anthonomus pomorum* (Coleoptera: Curculionidae). *Entomologia Generalis* 26(1): 29–46. <https://doi.org/10.1127/entom.gen/26/2002/29>