Journal of Insect Physiology 55 (2009) 694–699

Contents lists available at ScienceDirect

Journal of Insect Physiology

journal homepage: www.elsevier.com/locate/jinsphys

Could hosts considered as low quality for egg-laying be considered as high quality for host-feeding?

Sébastien Lebreton, Eric Darrouzet *, Claude Chevrier

Institut de Recherche sur la Biologie de l'Insecte, UMR 6035 CNRS, Université François Rabelais, 37200 Tours, France

ARTICLE INFO

Article history:
Received 26 February 2009
Received in revised form 28 April 2009
Accepted 29 April 2009

Keywords:
Host-feeding
Egg maturation
Haemolymph composition
Pteromalidae
Anisopteromalus calandrae

ABSTRACT

When parasitoid females encounter a host, they can either lay an egg and thus invest in current reproduction or feed on the host and thus invest in future reproduction. However, hosts could have different values according to their parasitized status. Whereas already parasitized hosts represent poor quality for egg-laying, they could have a high nutritive value for feeding. Moreover, the optimal strategy adopted generally depends on the females' physiological state. In this study, the impact of the females' physiological state on their reproductive strategies was investigated in the solitary parasitoid *Anisopteromalus calandrae*. We analysed how their age and diet influenced (i) the use of hosts (feeding vs. oviposition) and (ii) host selection (previously parasitized vs. unparasitized). Our results show that both age and diet influence the reproductive strategy of *A. calandrae* females: old females fed with the poorer diet laid fewer eggs and made more host-feeding than others. Females also showed a preference for already parasitized hosts for feeding. This strategy cannot be explained by the nutritive value of haemolymph, as parasitized hosts carry fewer lipids. However, as parasitized hosts are also paralyzed, it could be less costly to feed on them than on unparasitized hosts.

1. Introduction

In order to maximize lifetime reproductive success, females constantly need to balance their investment between laying eggs, i.e. in current reproduction, and searching for food, i.e. in future reproduction. In some cases, the same resource can have a reproductive and a nutritive value. Parasitoid females lay their eggs in or on hosts, and after hatching, larvae develop at the expense of hosts. Adult females can also feed directly on hosts by puncturing and consuming host haemolymph through a feeding tube (Giron et al., 2002). Feeding tubes are made by parasitoid females with secretion from their ovipositor inserted into the host (Fulton, 1933). A clear fluid flows from the ovipositor and congeals around it to form the feeding tube (Pupedis, 1978). The females then turn and use the feeding tube to extract the host haemolymph with their mouthpart (Giron et al., 2002). Depending on whether the host is used for feeding or for oviposition, parasitoid females obtain an immediate or a potential future fitness benefit.

Host-feeding could provide adult females with nutritional resources to increase their life expectancy (Giron et al., 2002), egg maturation (Phillips, 1993; Ueno, 1999; Burger et al., 2005; Rivero and West, 2005; Ferracini et al., 2006), or both (Collier, 1995; Heimpel et al., 1997; Giron et al., 2004). Host-feeding can be classified into different types: concurrent (when both host-feeding and oviposition occur on the same host), non-concurrent, destructive (when host-feeding kills the host and thus makes the host unsuitable for parasitoid development), and non-destructive (Jervis and Kidd, 1986; Krivan, 1997). Non-concurrent/destructive and concurrent/non-destructive have been shown to be the feeding patterns which maximize parasitoid fitness (Krivan, 1997) and to be the most common in parasitoid species (Jervis and Kidd, 1986).

An important feature of the trade-off between current and future reproduction is the quality of hosts encountered by females. They prefer to feed on hosts of poor quality for larval development, while saving hosts of higher quality for oviposition and offspring development (Burger et al., 2004). *Anisopteromalus calandrae* females tend to choose large hosts for oviposition and small hosts for feeding (Choi et al., 2001). Another host quality predictor is the parasitized status of the host, already parasitized hosts usually being considered as lower quality for oviposition than unparasitized ones (Godfray, 1994). In solitary species, only one adult can emerge from a given host, whatever the number of eggs laid, due to lethal larval fights (Ueno, 1997; van Baaren et al., 1999). Thus, by laying an egg on a parasitized host, females reduce the survival rate of their progeny (Lebreton et al., 2009). However, already parasitized hosts could be more profitable for feeding. In idiobiont species of pupal and larval parasitoids, females inject venom into the host before ovipositing or feeding, inducing the

---

* Corresponding author.
E-mail address: eric.darrouzet@univ-tours.fr (E. Darrouzet).

0022-1910/$ – see front matter © 2009 Elsevier Ltd. All rights reserved.
doi:10.1016/j.jinsphys.2009.04.017

paralysis of the host and arresting its growth (Mackauer and Sequeira, 1993). Feeding on these paralyzed hosts could be advantageous for females because less time and energy are required. Moreover, the venom could induce changes in the haemolymph composition of the hosts (Cox, 1970), such as an increase in the amount of haemolymph lipids (Cox, 1970; Rivers and Denlinger, 1995; Nakamatsu and Tanaka, 2003). Feeding on already parasitized hosts could thus provide the females with an additional supply of nutrients. This aspect has never been investigated in studies dealing with the trade-off between current and future reproduction.

However, several recent theoretical studies have investigated this trade-off (Collier et al., 1994; Heimpel et al., 1994; Sirot and Bernstein, 1996, 1997; Burger et al., 2004). Overall, they indicate that the optimal strategies depend on both the physiological state of female parasitoids and host availability. According to this theory, female parasitoids give preference to host-feeding when they are young, when their egg load and their nutritional state are low and host availability is high (Heimpel et al., 1994). These theoretical models have been supported by experimental studies. For example, females of the parasitoid *Aphytis melinus* invest more in host-feeding than in oviposition when they have lower egg load and fewer nutritional reserves (Heimpel and Rosenheim, 1995). While the value allocated to hosts by parasitoid females depends on their strategy (feeding or egg-laying), host selection could be influenced by the females' physiological state.

The main purpose of this study was to investigate the trade-off between current and future reproduction in the solitary ectoparasitoid *A. calandrae* Howard (Hymenoptera, Chalcidoidea, Pteromalidae). We investigated whether females' reproductive strategies (oviposition or feeding) were influenced by the host's parasitized status (parasitized or unparasitized) and/or by their physiological state (age and diet). The cost and benefits of feeding according to host status are then discussed, in relation to the amount of nutrients available in host haemolymph.

2. Materials and methods

2.1. Rearing conditions

A. calandrae is a parasitoid of the larvae and pupae of the bruchid *Callosobruchus maculatus* (Coleoptera, Bruchidae) from the tropical zones of West Africa. Both *C. maculatus* and *A. calandrae* originated from Ivory Coast (collected in 2000) and were reared in the laboratory under conditions close to their area of origin: 12 h light at 30 °C, 12 h dark at 22 °C, and 71% r.h.

2.2. General methods

A. calandrae females were allowed to lay on an artificial seed system composed of gelatine capsules (Gauthier and Monge, 1999; Darrouzet et al., 2003). The bruchid L4 larvae were placed inside the capsules after removal from seeds by dissection. The size of larvae was homogenized to avoid a bias in the females' strategy due to this factor. The gelatine capsules mimic the bruchid pupal chamber in the seed and are accepted for oviposition by females. To obtain parasitized hosts, five gelatine capsules each containing one *C. maculatus* L4 larva were offered to a group of 10 virgin *A. calandrae* females confined to an arena (2.5 cm high × 8 cm in diameter). Hymenoptera parasitoids reproduce by arrhenotokous parthenogenesis, whereby fertilized (diploid) eggs develop into females and unfertilized (haploid) eggs into males. Thus, by using virgin females, only male eggs were obtained, thereby preventing a bias due to the sex of eggs presented to the females. The injection of *A. calandrae* venom induces the paralysis of *C. maculatus* larvae (Lebreton, personal observations). To ensure that females injected venom during oviposition, the paralyzed status of the parasitized hosts was controlled. These hosts were then used in choice tests. The survival rate of an egg laid on an already parasitized host decreases sharply with the time after the first parasitism event (Lebreton et al., 2009). In order to avoid a bias due to these survival probability differences, hosts were only offered for 2 h after oviposition; the survival rate of the second egg laid remains stable at about 50% during this time interval (Lebreton et al., 2009).

2.3. Preparation of experimental females

To investigate the influence of the physiological state of mated females on their reproductive strategies, we looked at parameters of both age and nutritional state. Mated females were obtained by placing a 2-h-old virgin female with a 24-h-old virgin male in a Petri dish. Mating was confirmed by direct observation. Two age categories (3 and 15 days old) of females were investigated. Under our laboratory conditions, less than 25% of females remain alive after 23 days (Do Thi Khanh et al., 2005); 15-day-old females could therefore be considered as old. Furthermore, *A. calandrae* is a synovigenic species, i.e. females emerge without mature eggs; the experiment therefore took place after 3 days to ensure that the young females were bearing mature eggs. These two categories of age were thus selected to compare the reproductive strategy of young and old females. Before the experiments, all the females were fed with a solution of sucrose replaced every 2 days. Three days before the beginning of the experiment one group of females in each age category was allowed to feed on *C. maculatus* larvae. In order to avoid a bias due to previous oviposition experience, the larvae offered to females were first removed from their seed, preventing the females from laying eggs on them, without stopping them from feeding (Lebreton, personal observation).

2.4. Impact of age and diet on reproductive strategies and host selection

Females of each category (3 days old (3d; $n = 56$), 3 days old allowed to feed on host (3d-fed; $n = 56$), 15 days old (15d; $n = 75$), and 15 days old allowed to feed on host (15d-fed; $n = 53$)) were then given a host-choice test consisting of two capsules, one containing an unparasitized host, the other containing a previously parasitized host. Choice tests took place in a climatic chamber (22 °C, 71% r.h.), and all the behavioural sequences were observed. The selected host, the strategy employed by females (feeding and/or oviposition) and the time taken were noted. The test ended as soon as females left the selected capsule, with a maximum time limit of 2 h. The proportion of active females (females which host-fed and/or oviposited) was then calculated. To check the impact of host-feeding on egg survival, each egg laid on an unparasitized host was deposited with its associated host in a separate cell in a Plexiglas sheet until emergence of parasitoid adults (Darrouzet et al., 2003). After emergence, the proportion of eggs laid after host-feeding and which reached the adult stage was compared to those of eggs laid without host-feeding. The strategy employed by females (host-feeding and/or oviposition) and the mortality of eggs developed on hosts used for host-feeding allowed us to determine the host-feeding pattern (concurrent or not, and destructive or not) used by *A. calandrae* females. To analyse the consequences of age and diet on the viability of the eggs, the proportion of offspring laid by females (3d, $n = 34$; 3d-fed, $n = 21$; 15d, $n = 30$ and 15d-fed, $n = 34$) reaching adulthood was calculated and compared.

2.5. Impact of age and diet on egg maturation and resorption

To analyse the impact of both age and diet on egg maturation and resorption, random females of each category (3d, $n = 9$; 3d-fed,

$n = 9$; 15d, $n = 13$; and 15d-fed, $n = 12$) were dissected in a drop of saline solution (128.3 mM NaCl, 4.7 mM KCl, 2.3 mM $CaCl_2$), and the number of mature eggs was recorded.

2.6. Analysis of the haemolymph of parasitized and unparasitized hosts

Haemolymph was extracted from unparasitized and parasitized hosts 2 h after parasitism. Extractions were carried out by inserting a microcapillary connected to a manual pump in the mid-lateral side of their body (Giron et al., 2002). The fluids collected (ca. 0.5 μl) were immediately pumped out in a 0.5 ml Eppendorf tube placed on ice. Haemolymph of 10 hosts of the same status were pooled in the same tube in order to obtain a sufficient quantity of fluids, and the tube was then frozen at −20 °C.

To quantify the amount of proteins, the Bradford dye-binding microassay (Bradford, 1976) procedure was used. For each sample ($n = 9$ for both parasitized and unparasitized hosts), 1 μl was added to 500 μl of a solution containing Bradford Reagent (Bio-Rad Laboratories, Munich, Germany). Each sample was left to react for 5 min at room temperature and then transferred to a microcuvette and read in a spectrophotometer at 595 nm. For the sugar analysis, 1 μl of each sample ($n = 8$ for parasitized hosts and $n = 10$ for unparasitized hosts) was added to 1 ml of a solution of anthrone reagent. The whole solution was then heated at 90 °C for 10 min, placed at room temperature for 5 min and read in a spectrophotometer at 625 nm. The amount of lipids was analysed using a sulfo-phospho-vanillin reaction (Van Handel, 1985). First, 1 μl of each sample ($n = 9$ for parasitized hosts and $n = 11$ for unparasitized hosts) was completely evaporated at 90 °C. Next, 40 μl of sulfuric acid was added and samples were heated at 90 °C for 10 min. After 5 min at room temperature, 960 μl of a solution of vanillin reagent was added, samples were left to react for 10 min at room temperature and then read at 525 nm.

Calibration curves were obtained using bovine serum albumin (BSA; Sigma–Aldrich) for protein analysis, glucose for sugar analysis, and a standard vegetable oil for lipid analysis.

2.7. Statistical analysis

To analyse female activities and both host selection and the strategies used among these hosts, a χ^2-test was performed. A χ^2-test was also used to analyse egg survival. We used Student's *t*-tests to compare (1) the time needed to feed and/or lay eggs on parasitized and unparasitized hosts, and (2) the hosts' haemolymph composition. To analyse the number of mature eggs in the ovaries, only females of the same age or diet needed to be compared, and therefore, rather than a global test we used a non-parametric Mann–Whitney *U*-test with a Bonferroni correction method (threshold level of $P < 0.0125$). For all other tests, a threshold level of $P < 0.05$ was used. Analyses were carried out with R software (R 2.1.1, R Development Core Team, Free Software Foundation Boston, MA, USA).

3. Results

3.1. Impact of age and diet on reproductive strategies and host selection

When *A. calandrae* females fed on *C. maculatus* larvae before the host-choice test (3d-fed and 15d-fed), they were less active during the test than the other groups (Fig. 1A; $\chi^2 = 22.72$, d.f. = 1, $P < 0.001$). Fewer eggs were laid and more host-feeding observed with active 15d than 15d-fed ($\chi^2 = 19.22$, d.f. = 3, $P < 0.001$) and 3d females ($\chi^2 = 13.80$, d.f. = 3, $P = 0.003$). At the same time, the same number of eggs were laid and similar host-feeding was carried out by active 3d as 3d-fed females ($\chi^2 = 1.65$, d.f. = 3, $P = 0.65$) and by 15d-fed as 3d-fed ($\chi^2 = 2.56$, d.f. = 3, $P = 0.46$). Age and diet also had an effect on host selection and on how strategies were allocated among these hosts ($\chi^2 = 47.06$, d.f. = 15, $P < 0.001$), but there was no effect of age on these factors when females fed on larvae before the tests (3d-fed and 15d-fed) (Fig. 1B; Fisher test, $P = 0.53$). When looking at females irrespective of categories, 60%

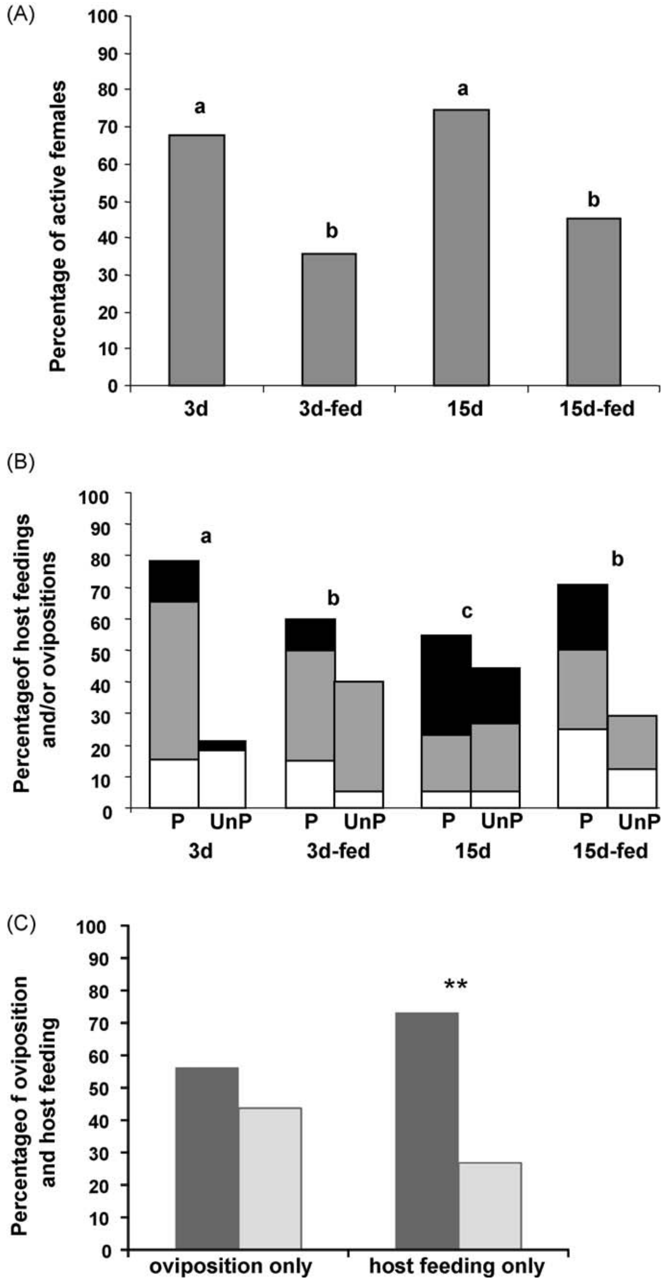

Fig. 1. Activity and strategy of females during the choice test. (A) Percentage of active females during the test (females who selected a host for feeding and/or egg-laying) in each category: 3-day-old females with (3d-fed) or without (3d) access to hosts before the test, and 15-day-old females with (15d-fed) or without (15d) access to hosts. (B) Reproductive strategies of active females (oviposition in white, host-feeding in black, or both in grey) on each category of host (parasitized: P, or unparasitized: UnP) for each category of females. Statistical tests (χ^2-test) compare the global distribution of the three possible strategies on parasitized and unparasitized hosts between female categories. Statistical differences between female categories are shown by different letters (a, b, and c). (C) Percentage of active females which performed oviposition only or host-feeding only on unparasitized (light grey) or parasitized (dark grey) hosts irrespective of their physiological state. $^{**}P < 0.01$ (χ^2-test).

of active females made a feeding tube and then laid an egg on the same host, with no difference between parasitized and unparasitized hosts (58.3% vs. 67.6%, $\chi^2 = 0.84$, d.f. = 1, $P = 0.36$). Moreover, they fed more on already parasitized than unparasitized hosts (Fig. 1C; $\chi^2 = 8.80$, d.f. = 1, $P = 0.003$), but the oviposition rate was the same on the two host categories (Fig. 1C; $\chi^2 = 0.5$, d.f. = 1, $P = 0.48$). Most previously parasitized hosts were paralyzed during the first parasitism (92% of parasitized hosts). The same time was needed for a female to perform host-feeding and oviposition on an unparasitized (67.8 ± 5.3 min) and an already parasitized host (71.2 ± 3.4 min; $t = 0.56$, d.f. = 60, $P = 0.58$). Moreover, there was no difference in the time needed only to host-feed (76.4 ± 6.4 min) and that needed to feed and oviposit (70.1 ± 2.8 min; $t = 0.99$, d.f. = 76, $P = 0.33$). There was no difference between the survival rate of eggs laid after host-feeding (87.5%) and those laid without host-feeding (88.2%; Fisher test, $P = 1.0$). Neither age nor diet had an effect on the survival rate of the eggs produced (3d: 83%, 3d-fed: 67%, 15d: 80%, 86%; $\chi^2 = 3.02$, d.f. = 3, $P = 0.39$).

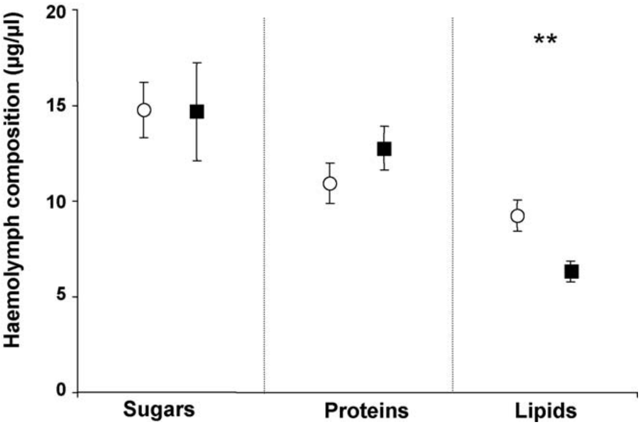

Fig. 3. Amount of sugars, proteins and lipids (mean ± SE) in haemolymph of unparasitized (○) and 2-h-parasitized hosts (■). $^{**}P < 0.01$ (Student *t*-tests).

3.2. *Impact of age and diet on egg maturation and resorption*

The quantity of mature eggs was affected by both diet and age. For the same age category, 3d and 15d females carried fewer mature eggs than 3d-fed ($U = 8.5$, $P = 0.003$) and 15d-fed females ($U = 23$, $P < 0.001$; Fig. 2). For the same diet, 15-day-old females carried fewer mature eggs than 3-day-old females (unfed: $U = 20.5$, $P = 0.009$; fed: $U = 0.5$, $P < 0.001$).

3.3. *Analyses of the haemolymph of parasitized and unparasitized hosts*

Haemolymph from unparasitized and 2-h-parasitized hosts did not differ in the amount of sugar ($t = 0.66.0$, d.f. = 15, $P = 0.52$) and protein ($t = 1.17$, d.f. = 16, $P = 0.26$) (Fig. 3), but the haemolymph of parasitized hosts carried fewer lipids than those of unparasitized hosts ($t = 2.90$, d.f. = 18, $P = 0.009$; Fig. 3).

4. Discussion

Our results demonstrate that the trade-off between current and future reproduction in *A. calandrae* females is strongly influenced by their physiological state (age and diet). Diet influenced their activity: when females had access to hosts in order to feed prior to the experiment, the probability of selecting a host during the choice test decreased. In fact, these females were less active during the choice tests, i.e. a higher proportion selected no host. This could be explained by a decrease in the proportion of females actively searching a host to feed on to increase their nutritional reserves. A theoretical study has suggested that when the survival rate is high (i.e. when energy reserves are high) females will be more selective towards the hosts they encounter at the expense of current reproduction (Sirot et al., 1997). This observation could also be linked to a satiation factor. Moreover, it is also possible that host pre-exposure itself influences their activity. Under our experimental conditions, as females were confronted with unparasitized and parasitized hosts in the same patch, it is possible that they waited for unparasitized hosts before ovipositing. This could explain the low activity rate of females with high reserves in our study.

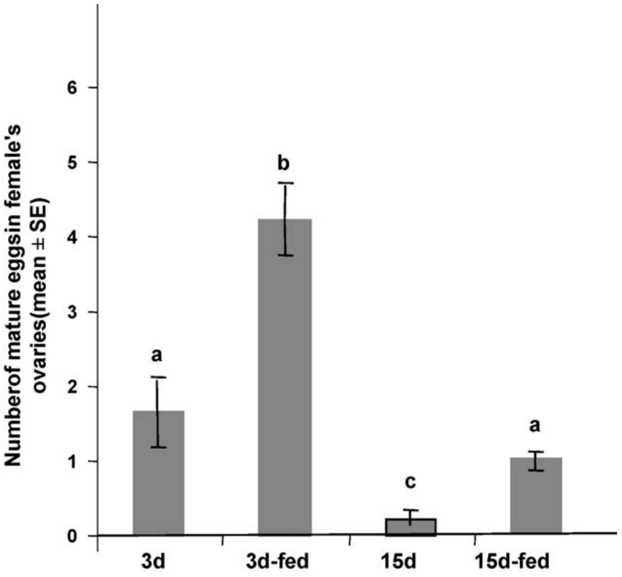

Fig. 2. Number of mature eggs (mean ± SE) carried by 3- and 15-day-old females with both diet treatments before the choice test. Statistical differences (Mann–Whitney *U*-test) are shown by different letters (a, b, and c).

The female's expectancy to feed and/or to oviposit depends on her egg load. Our results show that females who carry the least number of mature eggs in their ovaries invest more in future reproduction by host-feeding. This result is in line with previous theoretical (Collier et al., 1994; Heimpel et al., 1994) and experimental studies (Heimpel and Rosenheim, 1995). At the same time, when they have eggs in their ovaries, females keep the same host-feeding rate whatever their diet, suggesting that the number of eggs is not important in the host-feeding rate as long as they do have eggs. This is supported by theoretical models which predict that if mortality is dependent on nutritional state, animals will tend to maintain high energy reserves (Sirot and Bernstein, 1996). This prediction supports our results which show that the majority of females who laid eggs first fed on the host. This strategy seems to be adaptive, particularly as prior host-feeding did not influence the survival rate of an egg laid on the same host. *A. calandrae* females therefore perform a concurrent and non-destructive pattern of behaviour, one of the two patterns which maximize their fitness.

Haemolymph consumption provides females with nutrients that could increase egg production (Phillips, 1993; Collier, 1995; Ueno, 1999; Rivero et al., 2001). Our results indicate that when females have access to hosts for feeding, they increase their egg load. Although haemolymph consumption is probably the main factor involved in egg production, it is possible that host exposure alone can increase egg maturation (Casas et al., 2009). When females were deprived of hosts, they resorbed their mature eggs. Egg resorption could provide them with nutritional reserves for their survival (Rosenheim et al., 2000; Santolamazza Carbone et al., 2008). In this way, they increase their life expectancy and thus the probability of encountering hosts again (Rosenheim et al., 2000). Moreover, when females were allowed to feed on hosts for 3 days, old females (15 days old) increased their egg load by just one egg, whereas the egg load of young females (3 days old) increased by more than 2.5 eggs. This result suggests that egg maturation is impaired in old females. This hypothesis is in line with the study of Hegazi et al. (2007) who observed that the fecundity of females decreased with age when they were host-deprived for a long time. While parasitoid females have been shown to reduce their investment in egg quality with age (Giron and Casas, 2003), in our study neither age nor diet affected egg viability.

Physiological state also influences host selection and the strategy employed towards these hosts. However, when females had access to a richer diet, they allocated their strategy among hosts similarly, whatever their age. This result suggests that, among other physiological factors, nutritive reserves could play an important role in this trade-off and host selection. However, by being exposed to hosts, these females are no longer naïve, and this exposure could also be an explanation of the strategy allocation observed. In fact, prior experience has already been shown to influence host selection in *A. calandrae* (Ghimire and Phillips, 2008). When looking at females irrespective of physiological state, under our experimental conditions they selected as many unparasitized as parasitized hosts for reproduction only, whereas for feeding they selected more hosts which had been parasitized for 2 h. A number of studies have observed that host haemolymph composition is modified after parasitism. These studies often observed an increase in the amount of lipids in the haemolymph (Rivers and Denlinger, 1995; Nakamatsu and Tanaka, 2003), probably due to the lysis of host fat body by the venom components (Nakamatsu and Tanaka, 2004). However the venom composition of most parasitoid species and their action on host metabolism remains unknown (Quicke, 1997; Moreau and Guillot, 2005). In our study, fewer total lipids were carried by parasitized than unparasitized hosts in their haemolymph. Lipids are important in egg maturation (Giron et al., 2004; Casas et al., 2005) or viability (Mondy et al., 2006). However, in the parasitoid wasp *Eupelmus vuilletti*, females acquire most lipids during their larval stages (Casas et al., 2005). In this case, the smaller gain of lipids generated by feeding on already parasitized hosts probably has a low impact on the females' fertility and longevity. Furthermore, modifications in the host's lipid metabolism after venom injection could vary among host species. For example, the *Nasonia vitripennis* venom induces an increase in lipids in the haemolymph of three host species, but a decrease in a fourth (Rivers and Denlinger, 1995). *A. calandrae* is a generalist parasitoid and it is therefore possible that its venom induces an increase in the amount of haemolymph lipid in the majority of its natural hosts, in contrast to our findings in *C. maculatus*. Moreover, the selection of parasitized hosts for feeding could be explained by the higher cost of making a feeding tube on an unparasitized host; feeding tubes are very brittle, and if hosts are not totally paralyzed before females feed, only a small movement by the host will break them (Lebreton, personal observation). Females thus have to paralyze the host before making the feeding tube. The paralysis is likely to be energy-costly for a female: she has to invest in venom production, host stinging and venom injection. Feeding on parasitized hosts, i.e. already paralyzed hosts, thus avoids this energy investment. Further studies are however required to determine precisely the cost of venom production and injection. Nevertheless, paralysing a host is not time-costly for an *A. calandrae* female, because there is little difference in the time taken to feed on unparasitized and already parasitized hosts.

The time spent on the host does not vary whether females feed and lay an egg or whether they only feed on the host, whatever the parasitized status of the host. Thus, under our experimental conditions, many females laid an egg after feeding, even on already parasitized hosts. However, in solitary species, the survival rate of an egg laid under superparasitism conditions (on an already parasitized host) is reduced (Lebreton et al., 2009). How could this strategy be advantageous for females? Under our conditions, hosts were parasitized 2 h before the experiment. In this case, an egg laid under superparasitism conditions has about a 50% chance of survival (Lebreton et al., 2009), which is not negligible. We saw that females did not invest more time in laying an egg after feeding, and they could thus optimize the time invested in feeding by laying an egg. This strategy would be optimal if females were more time- than egg-limited. Actually, *A. Calandrae* females are synovigenic, and our data show that they have only a few mature eggs at any given moment. At the same time, they reproduce on aggregates of hosts, suggesting that under natural conditions, hosts are not a limited resource. This indicates that, in this species, females should be more egg- than time-limited. However, under our experimental conditions, females were allowed to encounter only a few hosts and could be thus considered as time-limited. Moreover, the females in this study had never previously laid eggs. It is possible that they subsequently modified their oviposition strategies according to their nutritional reserves. In fact, when females were confronted with parasitized and unparasitized hosts for 1 week, they preferentially oviposited on the latter (Lebreton et al., 2009).

To conclude, the present work highlights the strong link between the trade-off between current and future reproduction, host selection and the females' physiological state (age, diet and egg load) in a solitary parasitoid species. *A. calandrae* females resorb their eggs when they are deprived of hosts for a long time. To allow their eggs to mature, or just to maintain high nutritive reserves, they perform host-feeding when they encounter a host again. Females preferentially feed on already parasitized hosts, which possibly avoids them having to invest energy in paralyzing hosts prior to feeding. This indicates that the quality allocated by parasitoids to hosts depends on the strategy employed; hosts considered as having low reproductive qualities do not necessarily have low nutritive values.

Acknowledgments

We would like to thank J. Casas, D. Giron and two anonymous reviewers for providing useful comments on the manuscript.

References

Bradford, M.M., 1976. A rapid and sensitive method for the quantification of microgram quantities of protein utilising the principle of protein-dye binding. Analytical Biochemistry 72, 248–254.

Burger, J.M.S., Hemerik, L., Lenteren, J.C., Vet v., L.E.M., 2004. Reproduction now or later: optimal host-handling strategies in the whitefly parasitoid *Encarsia formosa*. Oikos 106, 117–130.

Burger, J.M.S., Kormany, A., Lenteren, J.C., Vet, L.E.M., 2005. Importance of host feeding for parasitoids that attack honeydew-producing hosts. Entomologia Experimentalis et Applicata 117, 147–154.

Casas, J., Pincebourde, S., Mandon, N., Vannier, F., Poujol, R., Giron, D., 2005. Lifetime nutrient dynamics reveal simultaneous capital and income breeding in a parasitoid. Ecology 86, 545–554.

Casas, J., Vannier, F., Mandon, N., Delbecque, J.-P., Giron, D., Monge, J.-P., 2009. Mitigation of egg limitation in parasitoids: immediate hormonale response and enhanced oogenesis after host use. Ecology 90, 537–545.

Choi, W.I., Yoon, T.J., Ryoo, M.I., 2001. Host-size-dependent feeding behaviour and progeny sex ratio of *Anisopteromalus calandrae* (Hym., Pteromalidae). Journal of Applied Entomology 125, 71–77.

Collier, T.R., 1995. Host feeding, egg maturation, resorption, and longevity in the parasitoid *Aphytis melinus* (Hymenoptera: Aphelinidae). Annals of the Entomological Society of America 88, 206–214.

Collier, T.R., Murdoch, W.W., Nisbet, R.M., 1994. Egg load and the decision to host-feed in the parasitoid, *Aphytis melinus*. Journal of Animal Ecology 63, 299–306.

Cox, F.E.G., 1970. Changes in the composition of host haemolymph after attack by an insect parasitoid. Nature 227, 191–192.

Darrouzet, E., Imbert, E., Chevrier, C., 2003. Self-superparasitism consequences for offspring sex ratio in the solitary ectoparasitoid *Eupelmus vuilleti*. Entomologia Experimentalis et Applicata 109, 167–171.

Do Thi Khanh, H., Bressac, C., Chevrier, C., 2005. Male sperm donation consequences in single and double matings in *Anisopteromalus calandrae*. Physiological Entomology 30, 29–35.

Ferracini, C., Boivin, G., Alma, A., 2006. Costs and benefits of host feeding in the parasitoid wasp *Trichogramma turkestanica*. Entomologia Experimentalis et Applicata 121, 229–234.

Fulton, B.B., 1933. Notes on *Habrocytus cerealellae*, a parasite of Angourmois grain moth. Annals of the Entomology Society of America 26, 536–553.

Gauthier, N., Monge, J.P., 1999. Could the egg itself be the source of the oviposition deterrent marker in the ectoparasitoid *Dinarmus basalis*? Journal of Insect Physiology 45, 393–400.

Ghimire, M.N., Phillips, T.W., 2008. Effects of prior experience on host selection and host utilization by two populations of *Anisopteromalus calandrae* (Hymenoptera: Pteromalidae). Environmental Entomology 37, 1300–1306.

Giron, D., Casas, J., 2003. Mothers reduce egg provisioning with age. Ecology Letters 6, 273–277.

Giron, D., Pincebourde, S., Casas, J., 2004. Lifetime gains of host-feeding in a synovigenic parasitic wasp. Physiological Entomology 29, 436–442.

Giron, D., Rivero, A., Mandon, N., Darrouzet, E., Casas, J., 2002. The physiology of host feeding in parasitic wasps: implications for survival. Functional Ecology 16, 750–757.

Godfray, H.C.J., 1994. Parasitoids, Behavioral and Evolutionary Ecology. Princeton University Press, Princeton, NJ, USA.

Hegazi, E.M., Abd El-Aziz, G.M., El-Shazly, A.Y., Khafagi, W.E., 2007. Influence of host age and host deprivation on longevity, egg load dynamics and oviposition rate in *Microplitis rufiventris*. Insect Science 14, 485–495.

Heimpel, G.E., Rosenheim, J.A., 1995. Dynamic host feeding by the parasitoid *Aphytis melinus*: the balance between current and future reproduction. Journal of Animal Ecology 64, 153–167.

Heimpel, G.E., Rosenheim, J.A., Adams, J.M., 1994. Behavioral ecology of host feeding in *Aphytis melinus* parasitoids. Norwegian Journal of Agricultural Sciences 6, 101–115.

Heimpel, G.E., Rosenheim, J.A., Kattari, D., 1997. Adult feeding and lifetime reproductive success in the parasitoid *Aphytis melinus*. Entomologia Experimentalis et Applicata 83, 305–315.

Jervis, M.A., Kidd, N.A.C., 1986. Host-feeding strategies in Hymenopteran parasitoids. Biological Reviews 61, 395–434.

Krivan, V., 1997. Dynamical consequences of optimal host feeding on host-parasitoid population dynamics. Bulletin of Mathematical Biology 59, 809–831.

Lebreton, S., Labarussias, M., Chevrier, C., Darrouzet, E., 2009. Discrimination of the age of conspecific eggs by an ovipositing ectoparasitic wasp. Entomologia Experimentalis et Applicata 130, 28–34.

Mackauer, M., Sequeira, R., 1993. Patterns of development in insect parasites. In: Beckage, N.E., Thompson, S.N., Federici, B.A. (Eds.), Parasites and Pathogens of Insects. Academic Press, New York, pp. 1–23.

Mondy, N., Corio-Costet, M.-F., Bodin, A., Mandon, N., Vannier, F., Monge, J.-P., 2006. Importance of sterols acquired through host feeding in synovigenic parasitoid oogenesis. Journal of Insect Physiology 52, 897–904.

Moreau, S.J.M., Guillot, S., 2005. Advances and prospects on biosynthesis, structures and functions of venom proteins from parasitic wasps. Insect Biochemistry and Molecular Biology 35, 1209–1223.

Nakamatsu, Y., Tanaka, T., 2003. Venom of ectoparasitoid, *Euplectrus* sp. near plathypenae (Hymenoptera: Eulophidae) regulates the physiological state of *Pseudaletia separata* (Lepidoptera: Noctuidae) host as a food resource. Journal of Insect Physiology 49, 149–159.

Nakamatsu, Y., Tanaka, T., 2004. Venom of *Euplectrus separatae* causes hyperlipidemia by lysis of host fat body cells. Journal of Insect Physiology 50, 267–275.

Phillips, D.S., 1993. Host-feeding and egg maturation by *Pachycrepoideus vindemia*. Entomologia Experimentalis et Applicata 69, 75–82.

Pupedis, R.J., 1978. Tube feeding by *Sisyridivora cavigena* (Hymenoptera: Pteromalidae) on *Climacia areolaris* (Neuroptera: Sisyridae). Annals of the Entomology Society of America 71, 773–775.

Quicke, D.L.J., 1997. Parasitic Wasps. Chapman & Hall, London.

Rivero, A., Giron, D., Casas, J., 2001. Lifetime allocation of juvenile and adult nutritional resources to egg production in a holometabolous insect. Proceedings of the Royal Entomological Society of London (A) 268, 1231–1237.

Rivero, A., West, S.A., 2005. The costs and benefits of host feeding in parasitoids. Animal Behaviour 69, 1293–1301.

Rivers, D.B., Denlinger, D.L., 1995. Venom-induced alterations in fly lipid metabolism and its impact on larval development of the ectoparasitoid *Nasonia vitripennis* (Walker) (Hymenoptera: Pteromalidae). Journal of Invertebrate Pathology 66, 104–110.

Rosenheim, J.A., Heimpel, G.E., Mangel, M., 2000. Egg maturation, egg resorption and the costliness of transient egg limitation in insects. Proceedings of the Royal Society of London B: Biological Sciences 267, 1565–1573.

Santolamazza Carbone, S., Pestana Nieto, M., Cordero Rivera, A., 2008. Egg resorption behaviour by the solitary egg parasitoid *Anaphes nitens* under natural conditions. Entomologia Experimentalis et Applicata 127, 191–198.

Sirot, E., Bernstein, C., 1996. Time sharing between host searching and food searching in parasitoids: state-dependent optimal strategies. Behavioral Ecology 7, 189–194.

Sirot, E., Bernstein, C., 1997. Food searching and superparasitism in solitary parasitoids. Acta Oecologica 18, 63–72.

Sirot, E., Ploye, H., Bernstein, C., 1997. State dependent superparasitism in a solitary parasitoid: egg load and survival. Behavioral Ecology 8, 226–232.

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, 682–688.

Ueno, T., 1999. Reproduction and host-feeding in the solitary parasitoid wasp *Pimpla nipponica* (Hymenoptera: Ichneumonidae). Invertebrate Reproduction & Development 35, 231–237.

van Baaren, J., Landry, B.L., Boivin, G., 1999. Sex allocation and larval competition in a superparasitizing solitary egg parasitoid: competing strategies for an optimal sex ratio. Functional Ecology 13, 66–71.

Van Handel, E., 1985. Rapid determination of total lipids in mosquitoes. Journal of the American Mosquito Control Association 1, 302–304.