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Mitigation of egg limitation in parasitoids: immediate hormonal response and enhanced oogenesis after host use

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Abstract. Synovigenic insects (i.e., insects emerging with few ripe eggs and maturing more eggs during the course of their lifetime) may suffer from transient egg limitation due to the stochastic nature of encounters with patchy hosts and the low availability of ripe eggs at any given time point. Egg limitation also affects the stability of host–parasitoid models. Thus, quantification of the behavioral decisions influencing egg maturation, identification of the underlying physiological mechanisms, and determination of the rate of egg maturation are highly relevant to both parasitoid behavioral ecology and host–parasitoid population dynamics. The aim of this study was to identify, in a highly controlled setting, the physiological processes responsible for egg manufacture after varying host use by a synovigenic parasitoid. We quantified the time course of the reproductive hormonal response and subsequent egg production in the host feeding bruchid parasitoid, *Eupelmus vuilleti* (Hymenoptera: Eupelmidae) for three treatments: (1) host examination without further host use, (2) host feeding, and (3) host feeding followed by oviposition. We carried out continuous behavioral observations with single hosts, enzyme immunoassays for quantifying ecdysteroids, and ovary dissection. Ecdysone levels increased within two minutes of contact with a host, the fastest hormonal response reported for any insect. Even simple contact with a host, without further host use, triggered an increase in hormone levels, leading to the maturation of a single egg, using body reserves only. Feeding on the host caused a much larger increase in ecdysone levels and was followed by a marked increase in oogenesis. Oviposition had a weak effect on hormone levels, but increased oogenesis. We discuss the mechanisms responsible for these rapid responses, the source of ecdysteroids, and the implications of our results for the population dynamics of host–parasitoid systems and the behavioral ecology of synovigenic species.

Key words: Callosobruchus maculatus; *ecdysteroids;* Eupelmus vuilleti; *host feeding; host handling; host–parasitoid interactions; ovarian hormonal response; population dynamics; synovigeny; time and egg limitation.*

Introduction

Parasitic wasps were among the preferred organisms in early tests of optimality models, including rate maximization models, because it was assumed that "host encounters equals fitness increment" (van Baalen and Hemerik 2007). Many of the early models and tests assumed that species were proovigenic, with the females emerging with their whole complement of eggs mature (Ellers and Jervis 2004, Bernstein and Jervis 2008). However, more than 95% of parasitoid species are synovigenic, the females emerging with only a few ripe eggs (Jervis et al. 2001) and subsequently maturing a few eggs at a time over an extended portion of their lifetime. Synovigenic species may also be subject to time limitation, but are particularly prone to egg limitation, depending on the time of death, time of day, and the stochasticity of encounters (Minkenberg et al. 1992, Rosenheim 1999, Casas et al. 2000, Ellers et al. 2000, Rosenheim et al. 2000). The stochastity of encounters is a particularly important factor, as most hosts are patchily distributed in the environment (van Baalen and Hemerik 2007), implying that a discovered host is likely to be located near other hosts, in both space and time. However, synovigeny is an appropriate strategy only if the physiological machinery can accelerate as a function of egg load and host availability. Several authors therefore called for a swift move to more theoretical and experimental work based on the behavioral ecology of synovigenic species, as the link between host handling behavior and fitness is not as straightforward as first thought (Ellers and Jervis 2004, Bernstein and Jervis 2008). Egg limitation, particularly in terms of the time between food intake and the

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manufacture of new eggs, has also been shown to affect theoretical host–parasitoid population models. The most complete host–parasitoid population dynamics model published to date shows that this type of egg limitation may increase the instability of interactions (Shea et al. 1996). This instability is generated by the inability of the parasitoid to lay eggs, or to feed on the host (when the gut is full), or both, at certain times. It can be minimized by decreasing the time lag between host feeding and the manufacture of new eggs. Thus, the behavioral decisions related to reproductive strategy and the physiological processes of egg maturation are important for both parasitoid behavioral ecology and host–parasitoid population dynamics.

The relationship between reproductive decisions and fecundity has long been studied in the context of host handling. Weak but positive correlations between host handling and fecundity have been reported repeatedly since the 1960s for synovigenic herbivores and parasitoids (reviewed in Papaj 2000). Most of these studies were observational in nature, few continuously recorded animal behavior, and even fewer considered the detailed physiological mechanisms at work. A notable exception is provided by one study on burying beetles, in which host handling behavior, hormonal response, and the ovarian dynamics were recorded (Trumbo et al. 1995). Females displayed a significant increase in juvenile hormone levels within 10 minutes of the discovery of a host carcass. Cadaver handling, rather than cadaver consumption, triggered the hormonal response in this beetle. Thus, based on current knowledge of synovigeny in this and other species, we predict that synovigenic species require rapid hormonal responses to host stimuli to enhance oogenesis rapidly enough for the female to attack nearby hosts, thereby minimizing the risk of egg limitation.

Little is known about hormonal signaling in parasitoids, and the hormonal basis of reproduction, in particular, was little studied until very recently. This situation contrasts with the behavioral ecology of reproductive strategies, which have been studied very intensively since the 1940s (Flanders 1935; see Bernstein and Jervis [2008] and Jervis et al. [2008] for the latest reviews). The first hormone involved in the reproductive system of a parasitoid was identified in the synovigenic, host-feeding parasitoid, *Eupelmus vuilleti* (Hymenoptera Eupelmidae). Using in vitro ovarian culture and in vivo bioassays, Bodin et al. (2007) identified ecdysone as the main ecdysteroid found in females and produced by the ovaries. Bodin's results, showing the release of significant amounts of ecdysone into the culture medium by the ovaries, suggested that ecdysone functioned as a hormone in the control of vitellogenesis. This conclusion is consistent with many studies showing that ecdysone stimulates vitellogenesis in female insects, not only in higher dipterans (reviewed in Raikhel et al. [2004]), but also in more primitive insects (Girardie and Girardie 1996, Girardie et al. 1998), although this function is often controlled by juvenile hormone. Moreover, as some ecdysteroids are stored in the eggs of *E. vuilleti* (Mondy et al. 2006), they could also function as precursors for the subsequent control of meiotic reinitiation and embryonic molt cycles (Lagueux et al. 1977, Lanot et al. 1987). Raikhel et al. (2004) have provided a general review of the numerous and variable relationships between ecdysteroids and oogenesis, and Bodin et al. (2007) have compared the role played by these hormones in our system with that in other groups of insects, including blood-feeding mosquitoes in particular.

Unlike hormonal signaling, egg maturation has been the focus of many studies on various synovigenic parasitoids (Rivero and Casas 1999). Feeding gain is not a discrete event occurring shortly after feeding, as assumed in most models of parasitoid behavior (but see Heimpel et al. 1998). Instead, it is spread throughout the lifetime of the parasitoid. This implies, in principle, that host-feeding gain, the latent period, and the rate of egg maturation can be measured without bias only at end of the parasitoid's life. For example, Collier (1995) showed that the host-feeding gain of *Aphytis melinus* females was ~1.5–2 eggs in two days. By contrast, when host-feeding gain was calculated on the basis of lifetime fecundity, Heimpel et al. (1997) found lifetime host-feeding gain to be twice as high: four eggs. The rapid incorporation of trace nutrients into eggs is, however, not incompatible with integration over the entire lifetime of the animal, as demonstrated in a manipulative study with a synovigenic parasitoid species. Radioactive compounds were found in laid eggs within two hours of the intake of labeled food, but over 50% of the nutrients were incorporated into the remaining 30 eggs after peak incorporation (Rivero and Casas 1999). The generation of a complete egg from scratch is, therefore, a time-consuming process involving more than the mere addition of trace amounts of certain constituents.

The aim of this study was to quantify the relationship between the host handling behavior of synovigenic parasitoids, the dynamics of ecdysteroid levels in the body of the female, and ovarian dynamics, to test the hypothesis described previously. This required a dynamic approach in which hormonal and ovarian conditions before stimulation, the time point at which the stimulus was applied, and the duration and intensity of the stimulus were controlled. It was essential to ensure that the hormonal and ovarian responses observed were due entirely to the stimulus, and not to other factors, within or outside the female. Given the difficulty of quantifying with certainty the degree of maturation of an egg in parasitic wasps, measuring the intermediate variable "hormones" precisely is an effective way to assess egg maturation, as it provides a natural link between the onset of the stimulus and the appearance of mature eggs.

We recorded the host handling behavior of *E. vuilleti* and quantified the ecdysteroid response over time, after host examination without further host use, and after full

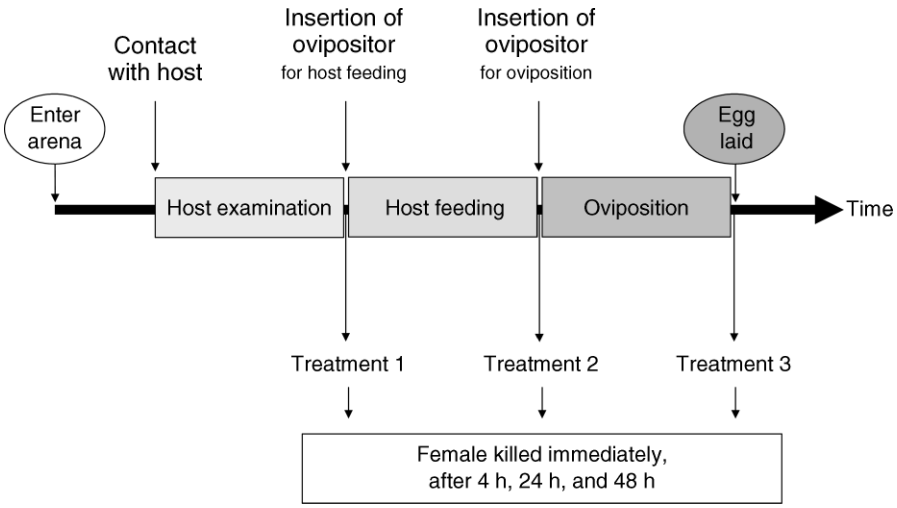

FIG. 1. Experimental setup and definition of the behavioral categories for *Eupelmus vuilleti*, a hymenopteran parasitoid of the bruchid beetle *Callosobruchus maculatus*. The categories are defined as: (1) host examination, from initial contact to first insertion of the ovipositor; (2) host feeding, which also includes the time spent building the host-feeding tube; and (3) the complete sequence, from initial contact with the host to oviposition.

attack on a single host. Such attacks may lead to feeding on the host alone or to feeding on the host followed by oviposition. We also dissected females and counted the number of mature eggs. Thus, the experimental design covered the entire cascade, from behavior to hormone physiology to the effect on oogenesis, as suggested by Papaj (2000), who called for in-depth mechanistic studies integrating resource stimuli, resource use, and ovarian dynamics.

MATERIALS AND METHODS

Experimental conditions

Eupelmus vuilleti (Crawford) (Hymenoptera: Eupelmidae) is a tropical solitary host-feeding synovigenic ectoparasitoid of third- and fourth-instar larvae of *Callosobruchus maculatus* (F.) (Coleoptera: Bruchidae) infesting *Vigna unguiculata* (Fabaceae) pods and seeds. All culture and experimental procedures were carried out in a controlled room with a 13 h light:11 h dark regime, a temperature cycle of 33°C (light):23°C (dark), and constant 75% relative humidity.

L4 instar hosts were provided to females after extraction from the *V. unguiculata* seeds and insertion into a gelatin capsule, as described by Gauthier and Monge (1999). This system has no effect on the natural behavior of females or their life expectancy (Giron et al. 2002), and made it possible to control for the number and developmental stage of the hosts. It also facilitated recording of the number of eggs laid and the number of host-feeding events. A pin was used to make a series of small holes in the capsule before the insertion of the host. This facilitated gaseous exchanges with the exterior and host kairomone perception by female wasps. Host feeding involves the puncturing of the host, the construction of a tube from host or parasitoid compounds, and imbibitions of exudates.

Newly emerged females were kept in isolation in Petri dishes (diameter 9 cm) with access to only water for six days, to ensure that their ecdysteroid content was very low. The control treatment (Treatment 0) consisted of maintaining females under these conditions. For the experiments (Fig. 1), a single female was placed in a Petri dish with a gelatin capsule containing a host and observed continuously. In the contact-only treatment (Treatment 1), females were allowed to examine capsules (typical antennal tapping and examination of the small holes in the capsule), but were removed from the dish as soon as they displayed the arching body posture typical of ovipositor insertion. As the females were hungry, they invariably tried to feed on the host before attempting oviposition. The host-feeding-only treatment (Treatment 2) involved removal of the female as soon as she tried to insert her ovipositor a second time, having spent some time licking the exudates. This "break point," selected so as to avoid confusion with oviposition, may have led to a censoring of both the time spent in host feeding and the expected amount of food obtained. In the host-feeding and oviposition treatment (Treatment 3), we terminated the experiment as soon as the female had been observed to lay an egg. Thus, all females in this treatment laid a single egg. Females that did not engage in oviposition were discarded for this treatment, leading to a potential bias in our sampling of ovarian states. Females were frozen at −20°C, either immediately at the end of the experiments (0 h), or 4, 24, or 48 h after the experiments. The behavior of females in Treatments 1, 2, and 3 was continuously recorded and timed. Times shorter than one minute were rounded up to one minute.

TABLE 1. Host handling behavior by *Eupelmus vuilleti*, a hymenopteran parasitoid of the bruchid beetle *Callosobruchus maculatus*.

Treatment	*N*	Total time (min)		Examining time, E (min)		Feeding time, HF (min)		Ovipositing time, OV (min)	
		Mean ± SE	Range	Mean ± SE	Range	Mean ± SE	Range	Mean ± SE	Range
1) Contact	32	1.4 ± 0.2	1–7	1.4 ± 0.2	1–7	...	...	...	...
2) Contact + HF	27	41.2 ± 3.7	9–70	6.6 ± 1.7	1–42	34.6 ± 3.1	1–53	...	...
3) Contact + HF + OV	16	48.8 ± 4.8	28–88	1.2 ± 0.1	1–2	28.1 ± 2.1	4–39	19.6 ± 4.3	5–65

Notes: Duration of the total time spent with a host, the time spent examining the host (E), time spent feeding on the host (HF), and time spent laying an egg on the host (OV). The individuals observed (*N*) are those analyzed for ecdysteroids at the completion of the experiment.

Ecdysteroid determinations

Enzyme immunoassay (EIA) was used for ecdysteroid determination, as described by Bodin et al. (2007), according to the method of Porcheron et al. (1989), with modification for the use of a peroxidase tracer, conjugated to 20-hydroxyecdysone (De Reggi et al. 1992, Pascual et al. 1995). The ecdysone-specific L2 polyclonal antibody (courtesy of M. De Reggi, Centre d'Immunologie de Marseille, France) was used in all experiments. This antibody is highly sensitive to ecdysone and 2-deoxyecdysone, but is only about one-sixth as sensitive to 20-hydroxyecdysone. The amounts of ecdysteroids are expressed in ecdysone equivalents (E-eq., expressed in units of pg/female parasitoid), as this compound was used for reference curves.

Due to the detection limit of the EIA, each sample from *E. vuilleti* analyzed by EIA was prepared from two females subjected to the same protocol. They were first sonicated in 500 μL methanol and left overnight. The samples were centrifuged and the supernatant evaporated in a Speed-vac (Thermosavant, Holbrook, New York, USA). The pellet was then resuspended in 120 μL of buffer solution (0.1 mol/L phosphate; see Bodin et al. 2007), and the sample was analyzed in duplicate.

We analyzed the amounts of ecdysteroids ingested by parasitoids during host feeding by preparing a pool of 10 individual samples of 1 μL hemolymph from L4 bruchid larvae for a single quantification of ecdysteroids, according to the same protocol and with the same antibody. Irrespective of the nature of the ecdysteroids present in bruchid larvae, the results are expressed as E-eq. to facilitate comparison with the values obtained in *E. vuilleti*.

Ovary dissection

Females were dissected under a binocular microscope at 40× magnification. Eggs were considered ripe if they were ovoid, whitish, and translucent, and lacked the typical granular appearance of immature eggs. All dissections were carried out by the same person, to overcome the problem of inter-observer variability.

Statistical analyses

In total, we analyzed 285 samples from 570 females by enzyme immunoassay. This total includes the emergence ($N = 30$ individuals), control ($N = 29$, 20, 19, and 19 individuals for a time of death 0, 4, 24, and 48 h after the experiment), and experimental groups. For Treatment 1 (contact) we studied 89 individuals (with 34, 35, 10, and 10 individuals for a time of death of 0, 4, 24, and 48 h after the experiment, respectively). For Treatment 2 (host feeding) we used 40 individuals (with 10 females for each time of death), and for Treatment 3 (host feeding followed by oviposition) we used 39 individuals (with 10, 10, 10, and 9 individuals for a time of death of 0, 4, 24, and 48 h after the experiment, respectively). In total, 39 females were dissected at 24 and 48 h (10 for the control, nine for Treatment 1, 10 for Treatment 2, and three for Treatment 3).

Multiple comparisons between treatments would not be appropriate in this study as there is a clear ordering of the treatments (Miller 1997), with time spent in contact with the host and behavioral complexity increasing from the control to the host feeding and oviposition treatments. We therefore followed the advice of several statisticians (Perry 1986, Webster 2007) by presenting the main statistics together with a graphical display of the data distributions as box plots. The use of one-tailed tests is also highly debatable for ordered treatments (van Belle 2002). We therefore opted for conservative two-tailed tests.

RESULTS

Host handling

The total time spent dealing with a host, and the time spent in the different behavioral categories of a typical sequence are listed in Table 1 (see Fig. 1 for their definition).

Ecdysone response

The levels of ecdysone immediately after the experiments were completed (0 h) were much higher for the host feeding only and host feeding followed by oviposition treatments than for the control (Fig. 2). These two treatments did not differ in terms of mean values, but the box plots show a clear upward shift in the lower half of the distribution ($N1 = 10$; $N2 = 10$; Mann-Whitney $U = 28.5$; $P = 0.104$; distributions highly non-normal). A slight increase in ecdysone levels was also observed after simple contact with the host (contact: $N =$ 34 individuals; ecdysone level 3.52 ± 0.23 pg E-eq./female [mean ± SE]); and this difference from control

values was significant (control: $N = 29$ individuals; ecdysone level 2.45 ± 0.42 pg E-eq./female; separate variance $t = -2.072$, df = 51.8, $P = 0.043$, distributions tested for normality). This slight increase in mean values was confirmed by the upward shift of the whole distribution (Fig. 2). The time series for the ecdysone content of females for all treatments over 48 h is shown in Fig. 3. The ecdysone levels in parasitoids subjected to Treatments 2 and 3 declined rapidly, and at about the same speed.

The amount of ecdysone released may be a function of the time spent dealing with the host. The observed large difference in peak levels may therefore be due to the large difference in time spent handling the host. We tested this hypothesis by carrying out a regression analysis based on the host feeding only and host feeding followed by oviposition treatments, for which the total time was great enough and for which host examination corresponded to only a very small percentage of the total time (at most 16%; Table 1). We had data for individual numbering in experiments involving biochemical analysis and time in the different behavioral categories for only 15 females. The relationship between the amount of ecdysone and the total time spent handling the host is negative ($N = 15$; amount of ecdysone = 41.27 pg, which is 0.6 × total time on host [min]; $P = 0.04$, adjusted $R^2 = 0.23$). Thus, longer handling times (inspecting and host feeding) result in lower ecdysone levels.

The ecdysteroid level at emergence is 22.4 ± 0.42 (mean ± SE) pg E-eq./female ($N = 30$ individuals), so handling a single host essentially brings ecdysteroid levels back to their original value. For comparison, the mean ecdysteroid content of 1 μL of host hemolymph was 0.485 ± 0.094 pg E-eq./female ($N = 6$ individuals). The mean amount of host hemolymph consumed during host feeding is 0.258 μL (Giron et al. 2002); the amount of ecdysteroids obtained therefore amounts to about 0.13 pg, expressed as ecdysone equivalents per female (Fig. 4).

Oogenesis

We dissected females once the levels of hormones had all returned to basal levels after the surge triggered by handling a host. This ensured that all treatments, with their different starting levels, were treated equally. This steady state was clearly attained by 24 h (Fig. 3). We pooled the data obtained from dissections at 24 h and 48 h, to ensure that the samples studied were sufficiently large (Fig. 4). The mean number of mature eggs at the time of the experiments was 0.62 ± 0.6 mature eggs ($N =$ 24 individuals). This approximately half an egg must be taken into account when calculating the number of eggs matured during the time to dissection. The control group had no remaining egg (mature eggs in ovaries: $N =$ 10 ovaries; 0 ± 0 eggs) and therefore resorbed the 0.62 egg present at the start of the experiments. The contact group had one egg ($N = 9$ ovaries; 1 ± 0.29 eggs); the host feeding group had two eggs ($N = 10$ ovaries; 2.3 ± 0.85 eggs). The host-feeding and oviposition group also had a mean of two mature eggs in the ovaries ($N = 10$ ovaries; 2.3 ± 0.33 eggs), to which the laid egg must be added when estimating the effect of oviposition on egg maturation. The difference between the control and contact groups was significant (likelihood ratio $\chi^2 =$ 12.24; df = 2; $P = 0.02$).

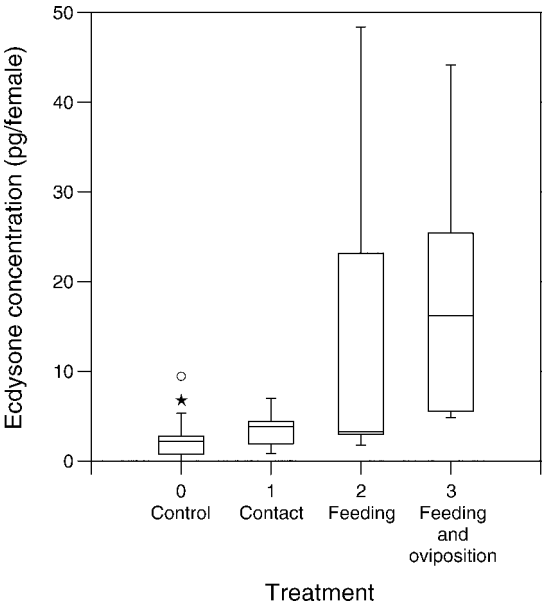

FIG. 2. Immediate ovarian hormonal response. Box plots of ecdysone content of female parasitoids *Eupelmus vuilleti* immediately after the completion of the experiments. The control group (treatment 0) had no access to hosts; the contact-only group (treatment 1), the host-feeding-only group (treatment 2), and the host-feeding and oviposition group (treatment 3) each had access to a single host per parasitoid. The length of each box plot shows the range within which the central 50% of the values fall; the center line marks the median, and box edges (hinges) are at the 25th and 75th empirical quartiles. Whiskers are drawn for the highest and lowest observations at a distance of 1.5 times the interquartile range. Any observations lying beyond the whiskers (outliers) are marked by stars (values between the inner and outer fences) or open circles (values beyond the outer fences).

DISCUSSION

We will first consider our hypothesis that hormonal responses to host stimuli should be rapid to enable the parasitoid to exploit a discovered host patch efficiently. We will then consider the different sources of the hormone and enhanced oogenesis. Finally, we will close the discussion by considering the implications of our results for the population dynamics of host–parasitoid systems and the behavioral ecology of synovigenic insects.

Rapidity of the ecdysteroid response

Ecdysteroid levels began to rise almost immediately on contact with the host, providing the most rapid example of a response to reproductive hormones ever

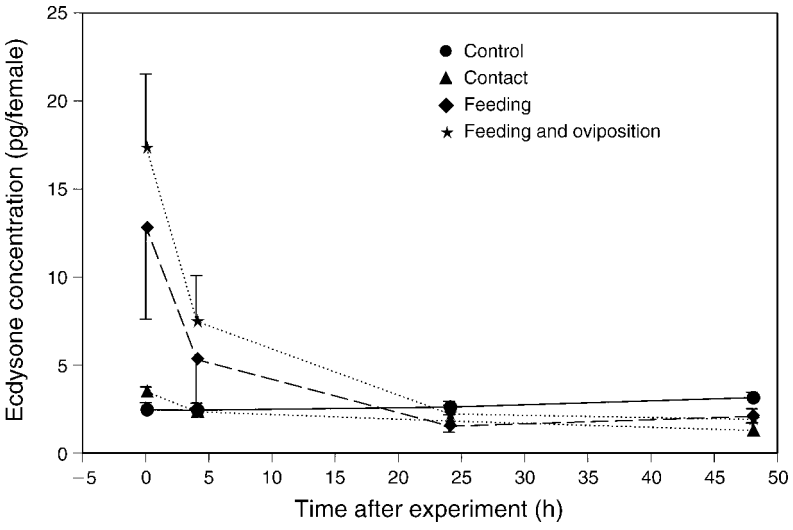

FIG. 3. Ovarian hormonal response over two days showing the time course of ecdysone content (mean and SE) of females immediately after the completion of the experiment (time 0), and 4 h, 24 h, and 48 h after completion of the experiment. The control group (treatment 0; circles, solid line) had no access to hosts, the contact-only group (treatment 1; triangles, dotted line), the host-feeding-only group (treatment 2; diamonds, dashed line), and the host-feeding and oviposition group (treatment 3; stars, dotted line) each had access to a single host per parasitoid.

recorded in insects. Indeed, a small but significant increase in ecdysteroid levels due to host examination was observed within two minutes. Moreover, the complete endocrine sequence leading to oviposition occurs in less than 50 minutes. In most insects, an ecdysteroid peak is observed within hours or days after treatment, a time lapse similar to that observed with juvenile hormones (see the numerous contributions in Volumes 1 and 3 of Gilbert et al. [2004]; in particular Raikhel et al. [2004]). The most rapid response previously reported was that of a burying beetle, which displays a significant increase in juvenile hormone within 10 minutes of discovering a host carcass (Trumbo et al. 1995). Fluctuations in ecdysteroid levels in female insects are generally thought to be controlled by cephalic neuropeptide hormones, as shown in locusts (Charlet et al. 1979, Girardie and Girardie 1996), mosquitoes (Hagedorn et al. 1979, Brown et al. 1998), and flies (Manière et al. 2004). However, neuroendocrine regulation of this type takes time and it would therefore seem likely that the very rapid ecdysteroid response observed following host handling by parasitoids and carcass handling by burying beetles is due to a more rapid reaction involving direct nervous stimulation. Further work is required to investigate this possibility. In conclusion, our results confirm the first part of our prediction: the hormonal response is almost immediate.

Sources of ecdysteroids

Both the marked increase in ecdysone concentration due to host feeding and the speed at which the hormonal system responded raise questions about the source of the ecdysteroids. Ecdysone levels were similar whether the parasitoids simply fed on the host or both fed and laid eggs on the host. We therefore conclude that host feeding, rather than oviposition, is principally responsible for the observed peak of ecdysone concentration in these two treatments. Ecdysone might be obtained directly through feeding, or synthesized from precursors acquired through feeding. Direct use of the ecdysone of the host has been demonstrated for the *Varroa* mite, which is parasitic on bees. This mite uses the host's juvenile hormone to match its own ovarian dynamics (Hänel and Koeniger 1986). However, the amount of ecdysone obtained per host-feeding event was, at 0.13 pg (expressed as ecdysone equivalent), much smaller than the observed surge. Alternatively, the parasitoid may acquire sterols, some of which could be used as

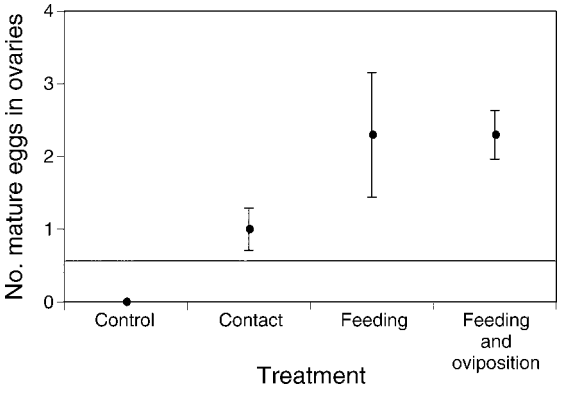

FIG. 4. Oogenesis as function of host use. Mature eggs in ovaries of females (mean ± SE) were recorded once the hormone levels had returned to basal values (24 h and 48 h), for all treatments. The horizontal line indicates the mean egg load at the time of the experiments.

precursors of ecdysteroids, through host feeding. This hypothesis opens up new possibilities for host assessment by parasitoids. In particular, it would be interesting to investigate whether parasitoids can modify their behavior or egg production as a function of the hormonal content of their host. The total amount of steroids obtained per host-feeding event has been estimated at 0.13 μg (based on host hemolymph content, 0.4 μg/μL, as determined by Mondy et al. [2006]), much greater than required for the observed surge if entirely converted to ecdysteroids. Thus, this parasitoid may use some of the host sterols for its own production of ecdysone, rather than using host ecdysteroids directly. Evidence for a requirement for sterol acquisition through host feeding and its use for the reproductive system is provided by the complete failure of egg production, from the fifth day, if females are provided with a sterol-free diet (Mondy et al. 2006). However, the slight increase observed following simple contact with a host is a clear indication that at least some ecdysone is produced endogenously. Thus, we can reasonably conclude that the ecdysone produced probably results from a combination of endogenous production and the acquisition of precursors through host feeding.

Enhanced oogenesis

The ranking of the treatments in terms of hormone levels was maintained in terms of oogenesis. Complete attack of the host enhanced oogenesis markedly and the mere discovery of a single host only slightly increased the number of newly matured eggs. Such positive effects of host encounter on oogenesis and, more generally of host-associated stimuli, have long been suspected and have been clearly demonstrated in some cases (see Papaj [2000] for a review). The most illustrative examples include the studies by Rivero-Lynch and Godfray (1997), Alonso-Pimentel et al. (1998), Lachman and Papaj (2001), Papaj (2005), and Wu and Heimpel (2007). The complete nutrient and energy budget of *E. vuilleti* can be used to assess the sources of investment in these new eggs (Casas et al. 2005). The simple discovery of, and contact with, a host led to completion of the maturation of a single egg, entirely from metabolic reserves. We show here that a single host-feeding event leads to the production of almost two new eggs. However, calculations based on the acquisition of nutrients through host feeding suggest that only 1.5 eggs should be produced in the short term (Giron et al. 2004), consistent with the results obtained for other parasitoids, such as *Aphytis melinus* (1.5 eggs/host-feeding event; Collier 1995, Heimpel et al. 1997) or *Aphelinus albipodus* (1.82 eggs/host-feeding event; Wu and Heimpel 2007). Thus, the parasitoid seems again to invest its own metabolic reserves into the maturation of about half an egg. Finally, oviposition enhanced the production of additional eggs, in contrast to recent findings for fruitflies and other parasitoids (Alonso-Pimentel et al. 1998, Lachman and Papaj 2001, Wu and Heimpel 2007). However, it remains possible that the additional egg observed in the third treatment results from a sampling bias caused by self-selected females willing to oviposit or from a combination of factors dependent on host feeding followed by oviposition. Other experimental studies in which oviposition occurs in the absence of prior host feeding are required to clarify this issue. In any case, our results clearly confirm the second part of our prediction: oogenesis is enhanced, even after simple contact with a host.

The potential number of matured eggs is not reached after 24 hours, as explained in the *Introduction*, but the ranking among treatments is unlikely to change. However, parasitoids were invariably offered additional hosts and food after the experiments in published studies, providing an incentive for further investment in the reproductive system. This may account for the difference in host-feeding gains observed after 24 hours and at the end of the animal's lifetime. Regardless of the problems inherent in the concept of a finite delay between host encounter and the appearance of mature eggs, we show here that the hormonal response is immediate and that it involves the acquisition of nutrients from the fat body for use in egg production. The time at which the next fully formed egg appears, which is of prime importance to the female, then depends primarily on the state of the fat body and ovaries. A well-fed female may divert nutrients rapidly from her fat body rather than waiting for digestion to deliver them. A female with ovaries containing many oocytes in various degrees of maturation may be able to mature some of these oocytes very rapidly. In our study, the females were kept in severe conditions before the experiments: no food and no hosts for six days, to ensure that ecdysteroid levels were minimal and that the ovarian system was either halted or already engaged in oosorption (R. Richard and J. Casas, *personal observations*). We even observed the oosorption of half an egg between the start of the experiment and dissection, for the control group. The time lag of 24 hours before the appearance of mature eggs may therefore be considered a maximum, and this time lag is probably much shorter in normal conditions. Thus, for the third and last part of our prediction, oogenesis seems to occur over a short enough time interval for the female to exploit a host in its vicinity.

Host–parasitoid population dynamics and behavioral ecology of synovigenic insects

Our results have two broader implications, for host–parasitoid population dynamics and for the behavioral ecology of synovigenic insects. The time between host feeding and the appearance of mature eggs is so short that it is likely to cause only slight instability in the interaction. Furthermore, we might expect a small increase in stability simply due to the encounter with a host and oviposition, as these events tend to reduce egg limitation by shortening the time to the appearance of

the next mature egg. We do not call into question the validity of the results obtained by Shea et al. (1996) on increases or decreases in the stability of the interaction due to the different physiological processes of egg limitation. However, the overall influence of this effect is probably smaller than those of other physiological and developmental delays, as explained by Murdoch et al. (2003). We provide here an experimental basis for the theoretical considerations of these authors.

The immediate response of the reproductive hormonal system, its ability to "bounce back" to high levels after a single host-feeding event, and the subsequent enhancement of oogenesis are highly adapted responses to a major problem faced by synovigenic species: the potential mismatch between the highly stochastic rate of host encounters and the small number of mature eggs available at any given time point, leading to missed opportunities (Rosenheim 1999). The strong analogies we observe with burying beetles (Trumbo et al. 1995) are not fortuitous. Indeed, other ecological similarities between burying beetles and this synovigenic species include the highly unpredictable distribution of scare resources in both space and time, the increase in ovarian mass within a day or two of host discovery, and the need to outcompete congeners and other competitors. The rapid and highly adapted responses to an encounter with a host, or a carcass, raise symmetrical questions about the rate of decline of egg manufacture and the onset of egg resorption after a period without encounters. Studies focusing on these processes and the costs of carrying too many eggs are unfortunately rare for insects, with the notable exception of the work of Berrigan (1991) on flies, and no such studies have been carried out for parasitoids. Given the widespread occurrence of ecological conditions and physiological processes similar to those described for this parasitoid and for burying beetles, we believe that our findings may also apply to many other synovigenic insect species.

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