

## Influence of honey and maternal age on egg load of lab-cultured *Cotesia marginiventris*

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**Abstract** Laboratory experiments were conducted to determine the impact of feeding status and maternal age on egg load of *Cotesia marginiventris* (Cresson) (Hymenoptera: Braconidae), a solitary, koinobiont endoparasitoid of noctuid pests. Egg load was defined as the number of mature (i.e., fully-chorionated) eggs found in the ovaries and oviducts. Significantly more mature eggs were stored in honey-fed than starved females. For honey-fed females, egg load increased within several days of isolation from hosts. This study suggests that *C. marginiventris* is weakly synovigenic because females emerge with a considerable number of mature eggs and are capable of maturing many more eggs. Feeding on a suitable source of carbohydrate should increase the egg load (i.e., potential fecundity) of this insect within 3–4 days in an in vivo rearing system.

**Keywords** Beet armyworm · Biological control · Egg load · Endoparasitoid · Rearing

### Introduction

*Cotesia marginiventris* (Cresson) (Hymenoptera: Braconidae) is an important solitary, koinobiont endoparasitoid of pestiferous noctuids (Novoa and Luna 1996) and has been considered for augmentative biological control of these caterpillars on vegetables (Urbaneja et al. 2002). Knowledge of the oviposition behavior of *C. marginiventris* may provide clues to designing efficient systems for rearing this parasitoid. Research has revealed that *C. marginiventris* females parasitize more hosts and produce more female progeny within the first several days of adult life (Tillman 2001). Recently-emerged, 1–2 day-old females have routinely been used for stinging hosts in a rearing system (see Riddick 2004). In order to more-fully

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understand the oviposition behavior of lab-cultured *C. marginiventris*, I examined the egg load of young females in relation to food (i.e., honey) and maternal age.

Egg load has been defined as the number of mature oocytes (i.e., fully-chorionated eggs) found within the ovaries and oviducts of an insect at any given moment in its lifetime; it has been used as an indirect measure of fitness in a number of insects (Jervis and Ferns 2004). The ability of parasitoids to mature additional eggs after emergence is linked to their life history. Emergence with a high percentage of mature eggs would seem advantageous to short-lived species that expend much of their egg load early-on in adult life; whereas, emergence with a low percentage of mature eggs would seem advantageous to species that oviposit a limited number of eggs over a longer period of time (Ellers and Jervis 2003). Species that emerge with a full complement of mature eggs are strictly proovigenic and those that emerge with few or no mature eggs are strictly synovigenic (Jervis et al. 2001). There is likely a continuum of ovigenic strategies in the parasitic Hymenoptera, ranging between the extremes of strict proovigeny and synovigeny. Strictly proovigenic and synovigenic species have an ovigeny index of 1 and 0, respectively (Jervis et al. 2001).

If parasitoids emerge with a full complement of mature eggs and dispense them into hosts within a few days, feeding on sugar would seem inconsequential (see Riddick 2003). Species emerging with few mature eggs would more likely have to feed to extend lifespan so that immature eggs could recommence development. Feeding on carbohydrates is known to increase longevity and fecundity of many parasitic wasps (Wäckers 2003). The stimulatory effect of carbohydrates on egg maturation in purportedly synovigenic species that do not host-feed is equivocal (Olson et al. 2000; Lee et al. 2004; Bezemer et al. 2005). [Parasitoids that do not host-feed typically produce yolk-deficient (i.e., hydropic) eggs, which do not require proteinaceous substances for continued maturation (Flanders 1942).] Carbohydrates may simply help to maximize the lifespan of a parasitoid (Siekman et al. 2001) rather than stimulate egg maturation directly.

Whether or not maternal age affects egg load could be subject to the feeding status of a parasitoid (Eliopoulos et al. 2003). Naturally, fed synovigenic females would have more opportunities to expand their egg load as they aged, up to a limit. In other words, food and maternal age might be intimately linked to egg production in some synovigenic species (see Harvey et al. 2001; Roberts and Schmidt 2004). In this study, I tested the hypothesis that honey and maternal age have no influence on the egg load of young, lab-cultured *C. marginiventris* females.

## Materials and methods

*Cotesia marginiventris* was reared at the USDA-ARS, Biological Control and Mass Rearing Research Unit (BCMRRU), Mississippi State, MS, USA for more than 150 continuous generations. The original parasitoids were from a colony maintained at an USDA-ARS facility in Tifton, GA, USA. The culture at BCMRRU was maintained by exposing late first to second instar beet armyworms, *Spodoptera exigua* (Hübner) (Lepidoptera: Noctuidae), reared on a meridic diet, to recently-emerged (i.e., 1–2 day-old) *C. marginiventris* females for 24 h in a ‘sting’ box (45.7 × 66.0 × 8.9 cm, W × L × H; 18.9 l clear plastic) provisioned with pure honey

and sterile water, in cotton pads. Parasitized larvae were placed within polystyrene rearing trays (with meridic diet) and held in an environmental room (27–28°C, 60–70% RH, and 16L:8D cycle). After 2 weeks, trays were removed and *C. marginiventris* cocoons (i.e., pupae) were harvested.

An experiment was designed to determine the importance of feeding status (i.e., honey-fed versus starved) on egg load of young females. Approximately 200 *C. marginiventris* cocoons, of the same generation, were harvested from rearing trays in September 2004 and randomly subdivided and placed at the base of one of two clean Petri dishes (1.0 cm depth × 3.5 cm diameter). Each Petri dish was positioned at the bottom (i.e., floor) of a white, polypropylene emergence cage (30 × 30 × 30 cm) in an environmental room (27–28°C, 60–70% RH, and 16L:8D cycle). One cage was provisioned with pure honey (streaked on the sides of the cage) and sterile water on cotton pads; the other cage was provisioned with sterile water only. Cages were checked daily, at approximately the same time, for emergence of adults; all un-emerged cocoons were placed into clean cages with or without honey and sterile water on pads. This procedure continued until wasp emergence ended. One day after all wasps had emerged, living females in treatment cages were collected and placed in glass vials for storage in a lab-top freezer (at –20°C), until dissection. The treatments consisted of honey-fed versus starved 1, 2, and 3 day-old females. Initial sample size (for dissection) was 2, 16, and 15; and 2, 24, and 5 females for 1, 2, and 3 day-old honey-fed versus starved females, respectively. Because of small sample sizes on the first and last days (in the fed versus starved treatment groups, respectively), data were pooled across female ages. Comparisons of egg load were made only between fed versus starved females in this experiment. Individual females were considered as sampling units.

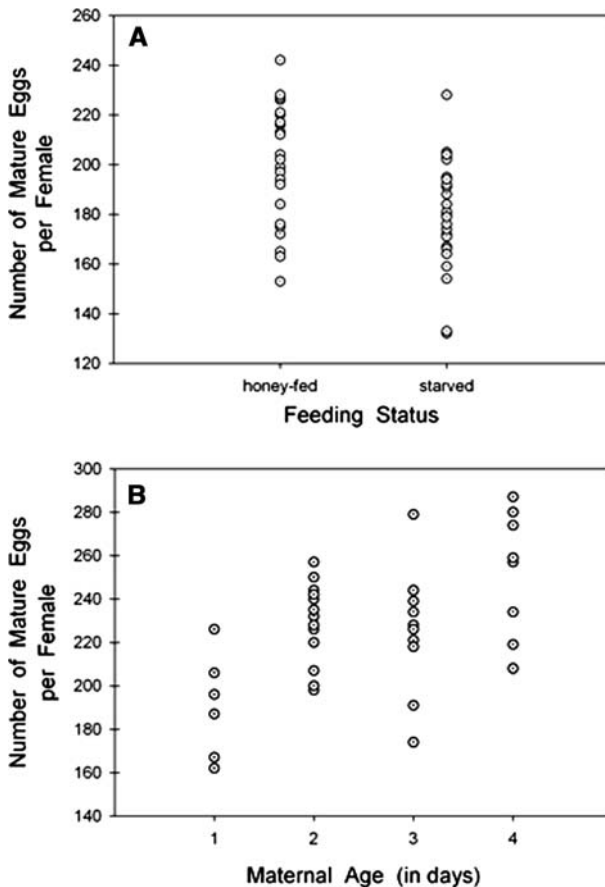
This experiment was essentially repeated (on 4 October 2004); but, this time, only wasps emerging in food (honey) treatment cages, and still alive at the end of the emergence sequence, were used to assess the effect of maternal age on egg load. Living females in treatment cages were collected and placed in glass vials for storage in a lab-top freezer. Sample size (for dissection) was 6, 15, 11, and 8 females for 1, 2, 3, and 4 day-old (honey-fed) females, respectively. Individual females were considered as sampling units.

For dissection, each female was placed on a glass microscope slide in several drops of saline solution (i.e., 6.5 gm NaCl per liter) and observed under an Olympus SZ11 zoom stereo microscope (60–90×). Using two # 0 insect micropins, the abdomen of each female was teased apart and the reproductive system exposed. A glass coverslip was placed over the specimen and the mature eggs stored in the ovaries and lateral oviducts were counted. Immature eggs were not counted; they were barely discernable in the distal portions of the ovarioles.

Data were analyzed following a completely randomized design. A Student's *t*-test was used to determine the significance of feeding status (honey-fed versus starved) on egg load and a one-factor analysis of variance (ANOVA) tested the significance of maternal age on egg load of honey-fed females. Data were not transformed prior to analysis because the requirements of normality and homogeneity of variances were met for both experiments. Means were considered significantly different when  $P < 0.05$ . The Holm–Sidak method was used to separate means after the ANOVA. SigmaStat (2004) software was used for the analyses.

## Results and discussion

Each *Cotesia marginiventris* female possessed two ovaries with two ovarioles per ovary. Mature eggs were found in the ovaries and lateral oviducts; usually the same number of mature eggs was found in each ovary/lateral oviduct. Honey-fed rather than starved females contained significantly more mature eggs ( $t = 4.6$ ;  $df = 62$ ;  $P < 0.001$ ). The mean  $\pm$  SEM egg load per female was  $203.4 \pm 3.8$  and  $179.1 \pm 3.7$  mature eggs for honey-fed and starved females, respectively. Egg load ranged from 153 to 242 and 132 to 228 mature eggs in honey-fed and starved females, respectively (Fig. 1A). Maternal age had an effect on egg load. Significantly fewer mature eggs were found in 1 day-old than in 2, 3, or 4 day-old, honey-fed females ( $F = 7.3$ ;  $df = 3, 36$ ;  $P < 0.001$ ). The mean  $\pm$  SEM egg load of 1–4 day-old females was  $190.7 \pm 9.8$ ,  $227.9 \pm 4.9$ ,  $226.2 \pm 8.2$ , and  $252.2 \pm 10.3$  mature eggs, respectively. Egg load ranged from 162 to 226, 198 to 257, 174 to 279, and 208 to 287 mature eggs per 1, 2, 3, and 4 day-old (honey-fed) females, respectively (Fig. 1B). These results suggest



**Fig. 1** Number of mature eggs found in the ovaries and lateral oviducts of lab-cultured *C. marginiventris* females in relation to feeding status (A) and maternal age (B). Sample size ( $n$ ) was 64 and 40 females in the feeding and age experiments, respectively

that egg load can increase significantly within several days away from hosts. It also highlights the fact that the oviducts can store a large number of mature eggs. When given access to hosts, honey-fed females that were 1 week old, or less, produced significantly more progeny (including more females) than cohorts that were 2 weeks old (Riddick 2004). Likewise, Tillman (2001) indicated that most progeny of normal sex ratios were produced during the second through fifth day of exposure to hosts.

Egg production of females of a host-deprived braconid, *Microplitis croceipes* Cresson, was greatest during the third through sixth day after emergence (Navasero and Elzen 1992). The rate of parasitism was greatest for 1–6 day-old *M. croceipes* females; it was strongly correlated with egg production. Eliopoulos et al. (2003) indicated that females of a synovigenic ichneumonid, *Venturia canescens* (Gravenhorst), did not experience an increase in egg load with maternal age under conditions of food and host deprivation. When given access to food but deprived of hosts, *V. canescens* increased its egg load (up to 160 mature eggs) within 6 days (Harvey et al. 2001); when given continuous access to hosts and food, maternal females produced more progeny within the first week of adult life than at any other time.

Rate of egg maturation in another host-deprived braconid, *Macrocentrus grandii* (Goidanich), was slightly less in fed than in starved females; egg load increased from 33 to 85 vs. 33 to 130 eggs in sucrose-fed versus starved females, respectively, within the first five days of adult life (Olson et al. 2000). Neither floral nectar from buckwheat nor aphid honeydew affected the egg load of a host-deprived ichneumonid, *Diadegma insulare* Cresson (Lee et al. 2004). Egg maturation in a synovigenic braconid, *Fopius arisanus* (Sonan), occurred regardless of food supply and the act of ovipositing into hosts increased the rate of egg maturation (Wang and Messing 2003). Egg maturation in another host-deprived ichneumonid, *Mastrus ridibundus* (Gravenhorst), remained static during the first several days after emergence in the absence of honey (Bezemer et al. 2005). These studies indicate that the effects of food on egg maturation may vary depending on the species.

Using data from a previous study, an ovigeny index for *C. marginiventris* was calculated. The average egg load at emergence was 149 mature eggs (Riddick 2006). If the maximum egg load of 4 day-old females (in this study) is used as an estimate of lifetime potential fecundity, the ovigeny index is 0.52 (i.e., 149/287) or 52%. This index suggests that lab-cultured *C. marginiventris* females are weakly synovigenic.

In conclusion, feeding should increase the egg load (i.e., potential fecundity) of this parasitoid within 3–4 days in an in vivo rearing system. Maximizing the egg load may be beneficial, because not all eggs laid result in progeny. Superparasitism of hosts has been documented and may be quite common under rearing conditions. Consequently, wastage of some eggs is expected to occur. Based on knowledge of the egg load and oviposition behavior of *C. marginiventris*, I envisage that efficient rearing of this parasitoid will involve exposing recently-emerged females to food and an abundance of new hosts each day for up to a week.

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