FACULTATIVE EGG-LARVAL PARASITISM OF THE BEET ARMYWORM, *SPODOPTERA EXIGUA* (LEPIDOPTERA: NOCTUIDAE) BY *COTESIA MARGINIVENTRIS* (HYMENOPTERA: BRACONIDAE)

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ABSTRACT

The braconid parasitoid *Cotesia marginiventris* (Cresson) has long been known to be a larval parasitoid of numerous lepidopteran species. Recent field observations, however, indicated that *C. marginiventris* is also capable of functioning as an egg-larval parasitoid of the beet armyworm, *Spodoptera exigua* (Hübner). These field observations were corroborated by laboratory observations, demonstrating that *C. marginiventris* is capable of ovipositing in *S. exigua* eggs, and of successfully developing and emerging from host larvae hatching from stung eggs. The mechanisms used by the parasitoids to locate host egg masses in the field were not determined. These results lend support to phylogenetic hypotheses of the Braconidae that indicate a close relationship between the Cheloninae and the microgastroid taxa.

Key Words: *Cotesia marginiventris*, *Spodoptera exigua*, egg-larval parasitism, parasitoid, Braconidae, Microgastrinae

RESUMEN

Desde hace tiempo se conoce que el bracónido *Cotesia marginiventris* (Cresson) es un parasitoide de numerosas especies de lepidópteros. Sin embargo, observaciones recientes de campo indicaron que *C. marginiventris* es además capaz de funcionar como un parasitoide huevo-larval del gusano de la remolacha, *Spodoptera exigua* (Hübner). Tales observaciones de campo fueron corroboradas en el laboratorio, demostrando que *C. marginiventris* es capaz de ovopositar en huevos de *S. exigua* y emerger exitosamente de las larvas del hospedero eclosionadas de huevos parasitados. Los mecanismos usados por los parasitoides para localizar las masas de huevos del hospedante en el campo no fueron determinados. Estos resultados soportan la hipótesis filogenética de que en los Braconidae hay una estrecha relación entre los Cheloninae y los taxa microgastroides.

The parasitoid *Cotesia marginiventris* (Cresson) is a common and important parasitoid in many agricultural systems (e.g., Kok & McEvoy 1989, McCutcheon et al. 1990, Ruberson et al. 1994). It is capable of attacking a wide range of hosts, chiefly from the lepidopteran family Noctuidae (Krombein et al. 1979), although the suitability of hosts varies with the species and age of the host attacked (A. Datema & J. Ruberson, unpublished data).

Among the braconid subfamily Microgastrinae, to which *C. marginiventris* belongs, larval parasitism is assumed to be the typical behavior, although at least one species, *Cotesia hyphantriae* (Riley), appears to be an egg-larval parasitoid (Tadic'

1958). Several species of known larval parasitoids in this subfamily have been demonstrated in laboratory tests to be capable of facultatively parasitizing host eggs and emerging from the larvae (Johannson 1951, Wilbert 1960). But Johannson (1951), working with *Cotesia glomerata* (L.), observed that the females only did so when held in close proximity to the eggs, and concluded that in nature only first-instar larvae of *Pieris brassicae* L. are attacked by this parasitoid. Thus, it appears that at least some species are capable of successfully parasitizing both host eggs and larvae, although the occurrence of such events in the field has never been documented. *C. marginiventris* has been clearly shown to be a larval parasitoid (e.g., Boling & Pitre 1970, Kunnalaca & Mueller 1979, Braman & Yeargan 1991), but the extent to which this parasitoid could function as an egg-larval parasitoid has not been examined, if indeed it has ever been considered.

Although *C. marginiventris* has historically been considered a larval parasitoid, our recent field observations, supported by laboratory data reported below, indicate that this parasitoid, while typically a larval parasitoid, is also capable of parasitizing eggs of the beet armyworm, *Spodoptera exigua* (Hübner) and emerging from the larvae. This paper presents data from field and laboratory studies demonstrating that *C. marginiventris* is capable of facultatively parasitizing beet armyworm eggs, and that such an event may not be unusual in the field. We will conclude by considering this behavior in light of phylogenetic relationships within the Braconidae.

<center>MATERIALS AND METHODS</center>

Field Collections

Sampling of beet armyworm egg masses was incidentally undertaken as a component of other projects designed to characterize and quantify the impact of the parasitoid complex attacking this pest in cotton (see Ruberson et al. 1994). Most of the collections in these studies focused on larvae, but occasionally egg masses were collected, chiefly to assess the impact of the egg-larval parasitoid *Chelonus insularis* Cresson. At the time egg masses were collected in the respective cotton fields, larval populations of beet armyworms were very low. In 1993, a total of 75 egg masses were collected on 3 different dates from 3 different locations [2 in Tift County (1 northern and 1 southern) and 1 in Laurens County, GA]. In 1995, a single egg mass was collected from a third location in Tift County, GA. After each egg mass had hatched, 30 randomly-selected larvae from each egg mass were individually placed in diet cups with a semisynthetic diet (Burton 1969) and held in the laboratory. These larvae were examined daily for parasitoid emergence.

Laboratory Trial

A single experiment was run in the laboratory to determine whether female *C. marginiventris* would sting eggs of the beet armyworm and if the parasitoid's offspring could successfully develop after oviposition in the host egg. Female parasitoids were obtained from a laboratory culture that had originated from parasitized beet armyworm larvae collected in cotton fields. The culture had been in the laboratory approximately one year at the time of the experiment and had been maintained using beet armyworm larvae as hosts. Three parasitoids were each given 1 egg mass (2-d old) and their behavior observed for 1 hour. The parasitoids were then removed, and the egg masses held until larvae hatched, at which time 30 randomly-selected larvae

from each egg mass were placed individually in diet cups as above. The larvae were examined daily for parasitoid emergence.

RESULTS AND DISCUSSION

Initial parasitoid specimens collected in 1993 appeared to be *C. marginiventris*, based on the appearance of the cocoons and the emerged parasitoids, but were discarded before they could be determined to species. Nevertheless, the identification of the parasitoids was not absolutely certain at that time. Parasitoids emerging from the egg mass collected in 1995 were determined to be *C. marginiventris* by JBW. We assume, therefore, with considerable confidence that the parasitoids reared from egg masses in 1993 were also *C. marginiventris*. Laboratory data (see below) lend further support to the validity of this assumption.

Field sampling indicated that egg-larval parasitism of beet armyworms by *C. marginiventris* may not be an unusual phenomenon in the field (Table 1). In the largest sample taken (19 August 1993 in Tift County), 23% of the collected egg masses yielded larvae parasitized by *C. marginiventris*. Indeed, some parasitism was found in samples of egg masses from all locations. Rates of parasitism within parasitized egg masses ranged from 15.4% to 100%, but typically were on the order of 30-40% (Table 1). These levels of parasitism suggest either that individual female *C. marginiventris* parasitize the egg masses heavily after they locate them, or that multiple females are discovering and exploiting the egg mass (i.e., exhibiting an aggregative response). Beet armyworm egg masses typically consist of approximately 100 eggs, although the number can range from 40 to over 250 (Ruberson et al. 1994); therefore, parasitism rates of 30-40% in large egg masses may represent a substantial reproductive investment by female *C. marginiventris* which Braman & Yeargan (1991) demonstrated to have a realized lifetime fecundity of 362.7 offspring.

In the laboratory test, 2 of the females exhibited considerable interest in their respective egg masses, repeatedly probing the egg masses with their ovipositors during the observation period. The third female showed no interest. Johannson (1951) and Wilbert (1960) observed similar behavior with *C. glomerata* and *Cotesia pieridis* (Bouche) females, respectively. Only 7 larvae from the 2 stung egg masses [5 (16.7%) from one egg mass and 2 (6.7%) from the other] yielded *C. marginiventris* despite the

TABLE 1. PREVALENCE OF EGG-LARVAL PARASITISM BY *COTESIA MARGINIVENTRIS* AMONG FIELD-COLLECTED EGG MASSES OF THE BEET ARMYWORM, *SPODOPTERA EXIGUA*.

Collection Date/Locale	No. Egg Masses Collected	No. Egg Masses Parasitized	Mean % Parasitism within Egg Masses[1]
Tift Co., GA			
29 July 1993 (Site 1)	2	2	32.8 ± 5.80
19 August 1993 (Site 2)	61	14	42.1 ± 22.64
3 July 1995	1	1	26.7
Laurens Co., GA			
4 September 1993	11	2	31.7 ± 7.07

[1]With SD; means are for parasitized egg masses only.

high level of apparent ovipositional activity by the females. Nevertheless, these results demonstrate that *C. marginiventris* is capable of successfully functioning as an egg-larval parasitoid. Age of the host eggs may also influence the success of parasitism—Johannson (1951) noted that only host eggs nearing eclosion were suitable for subsequent development of *C. glomerata*. A similar situation may also occur for *C. marginiventris*, but this was not considered in the present experiments in which eggs of intermediate age (2-d old) were used.

The facultative parasitization of host eggs by *C. marginiventris* raises some interesting questions regarding the foraging behavior of this parasitoid. A body of literature has demonstrated that plant kairomones activated by the feeding of host larvae are key foraging cues for this parasitoid (Loke et al. 1983, Turlings et al. 1989, 1990, 1991). However, such cues are lacking on plants with only egg masses present and are limited or absent in fields with low or no larval populations. The prevalence of parasitism observed in our field collections suggests that the parasitoids were quite successful at locating egg masses, even when cues induced by larval feeding were rare. What cues are being used to locate the host egg masses? It is possible that volatiles are released from the large mass of scales that the ovipositing female beet armyworm deposits on the egg mass; perhaps volatiles similar to those left after oviposition by other lepidopteran species (Beevers et al. 1981, Noldus & Van Lenteren 1983). It may also be a result of beet armyworm sex pheromone remnants on the egg mass or leaf, as Noldus et al. (1991) found with the parasitoid *Trichogramma evanescens* Westwood. Further, the plant itself may respond to some minor disruption of the leaf cuticle resulting from oviposition, by releasing some volatile. Another possible explanation is that female parasitoids were drawn into the field by the feeding of the few larvae present in the field and, thereafter, exploited the egg masses. Whatever the
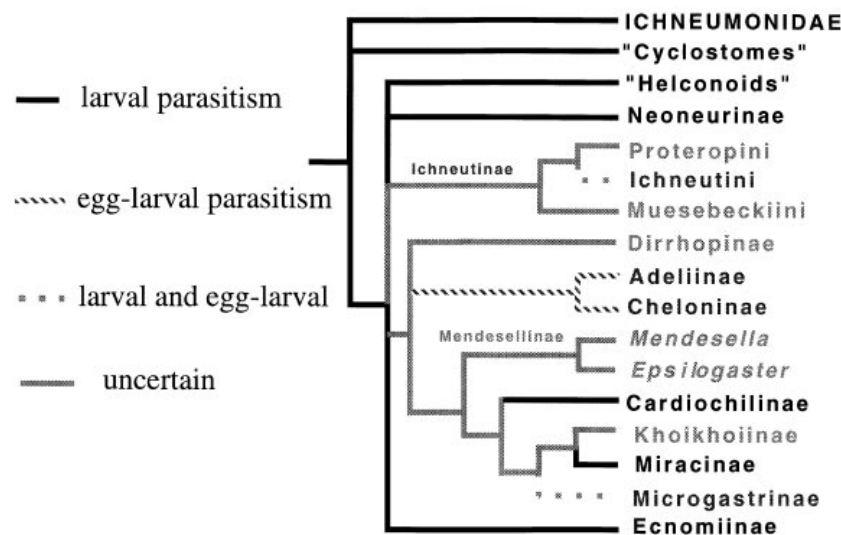

Fig. 1. Phylogeny of the microgastroid lineage of Braconidae, based on morphological data from Whitfield & Mason (1994), with the known distribution of egg-larval parasitism shown. Biological data from Shaw & Huddleston (1991) and Whitfield & Mason (1994).

cues, however, it is quite clear that larval damage is not the only source of cues to which *C. marginiventris* is capable of responding in close-range host location.

The confirmation that at least some *Cotesia* spp. are indeed capable of ovipositing into host eggs is of comparative physiological and phylogenetic interest. It has been suspected for some time that the Microgastrinae (including *Cotesia*) are likely to be closely related to the Cheloninae, the members of which all typically oviposit into host eggs and emerge from the host larvae (Fig. 1). Many other biological similarities (in addition to the structural similarities reviewed by Tobias (1967) between the two subfamilies have now been noted: typically they have three endoparasitic larval instars (Shaw & Huddleston 1991), they both have associations with polydnaviruses for host immune suppression (Fleming 1992, Stoltz & Whitfield 1992), and both groups attack largely overlapping groups of Lepidoptera. Another subfamily, the Adeliinae, has been proposed as being the actual sister-group to the Cheloninae (e.g., Nixon 1965, Dudarenko 1974). Recent phylogenetic work based on comparative morphology (Whitfield & Mason 1994) appears to establish fairly firm sister-group relationship between both Adeliinae and Cheloninae and a close relationship between these two subfamilies and the "microgastroid" complex of subfamilies, including the Microgastrinae (Fig. 1). Ongoing phylogenetic work based on mitochondrial DNA sequences (Whitfield, in prep.) also appears to confirm these relationships.

In the Cheloninae (and predictably perhaps also the Adeliinae, see Wharton 1994), the parasitoid eggs hatch relatively soon after oviposition, but development of the first instar is delayed (Ullyett 1949, Narayanan et al. 1961, Powers & Oatman 1984, Bühler et al. 1985, Kawakami 1985, Shaw & Huddleston 1991). The details of the developmental and immunological interactions and the effects of venoms and polydnaviruses appear to differ to some degree between the studied species of chelonines (Jones 1985, 1987, Leluk & Jones 1989) and microgastrines (reviewed by Lavine & Beckage 1995), but too few species have been studied for firm conclusions to be drawn. It would be interesting indeed to examine in further detail the aspects in which the two groups differ or resemble one another in their physiological responses to the common problem of egg-larval parasitism.

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