

BIOLOGY OF *EURYTOMA SIVINSKII*, AN UNUSUAL EURYTOMID  
(HYMENOPTERA) PARASITOID OF FRUIT FLY  
(DIPTERA: TEPHRITIDAE) PUPAE

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ABSTRACT

The recently described Mexican parasitic wasp *Eurytoma sivinskii* Gates and Grissell (Hymenoptera: Eurytomidae), attacks *Anastrepha obliqua* (Diptera: Tephritidae) pupae in the soil. The life cycle (egg to adult) is completed in 23.1 ( $\pm$  2.1) d (mean  $\pm$  S.E.) at 27  $\pm$  2°C. Females were capable of superparasitism and laid 1-8 eggs per host (2.59  $\pm$  1.56, mean  $\pm$  S.E.), but invariably only 1 adult parasitoid emerged. Oviposition occurred primarily in the medial and posterior portions of the host. *Eurytoma sivinskii* is ectoparasitic since 100% of the eggs are laid within the internal cavity of the puparium and on the surface of the pupa of the host fly. In no case were first and second instars parasitized. However, 1 third-instar out of 625 fly pupae exposed, yielded a single parasitoid per host. Eight-day-old pupae yielded the most parasitoids although females laid eggs in 1-d- to 14-d-old pupae. There were no significant differences in rates of parasitism among female *E. sivinskii* of different ages. Adults derived from eggs laid in the posterior region developed more rapidly, but adult sex ratio and percent of emergence were the same in both posterior and medially laid eggs. Regardless of oviposition location, adults were more likely to emerge through the middle of the puparium.

Key Words: *Anastrepha*, *Eurytoma*, Eurytomidae, Hymenoptera, natural history, parasitoid, Tephritidae

RESUMEN

La avispa parasitoide *Eurytoma sivinskii* Gates y Grissell (Hymenoptera: Eurytomidae), recientemente descrita en México, ataca pupas de *Anastrepha obliqua* (Diptera: Tephritidae) en el suelo. Este parasitoide completa su ciclo de vida (huevo a adulto) en 23.1 ( $\pm$  2.1) d (media  $\pm$  E.E.) a 27  $\pm$  2°C. Las hembras son capaces de superparasitar a sus huéspedes, ovipositando 1-8 huevos por huésped (2.59  $\pm$  1.56, media  $\pm$  E.E.), aunque invariablemente solo emerge 1 parasitoide adulto. La oviposición ocurre principalmente en las porciones media y posterior del huésped. *Eurytoma sivinskii* es claramente ectoparasítico, ya que 100% de los huevos fueron puestos dentro de la cavidad interna del pupario y sobre la superficie de la pupa de la mosca huésped. En ningún caso hubo parasitismo del primer y segundo instar de la larva. Sin embargo, de 1 larva de tercer instar expuesta a hembras de *E. sivinskii* (de un total de 625 larvas expuestas), emergió 1 parasitoide. De las pupas de 8 d de edad se obtuvieron la mayoría de los parasitoides, aunque las hembras pusieron huevos en pupas de 1 a 14 d de edad. No hubieron diferencias significativas en la tasa de parasitismo entre hembras de *E. sivinskii* de diferentes edades. Los adultos que provinieron de huevos puestos en la región posterior de la pupa se desarrollaron más rápidamente, pero tanto la proporción sexual como el porcentaje de emergencia fueron similares entre los huevos colocados en las partes posterior y media de la pupa. Independientemente de la localización de la oviposición, los adultos emergieron principalmente de la parte media del pupario.

Translation provided by the authors.

*Eurytoma sivinskii* Gates and Grissell, an unusual eurytomid pupal parasitoid of Diptera, was recently discovered in the vicinity of Tejería, Veracruz, Mexico, where it was recovered

from pupae of the West Indian fruit fly, *Anastrepha obliqua* (Macquart) (Diptera: Tephritidae) (Gates & Grissell 2004). Most species of the large and widespread parasitoid genus *Eu-*

*rytoma* attack gall-forming Cynipidae (Hymenoptera) and Diptera (Tephritidae and Cecidomyiidae) (DiGiulio 1997), and numerous other arthropod taxa and plants. In this case, instead of attacking hosts hidden within a plant structure, *E. sivinskii* forages for hosts (i.e., pupae) in and on the soil (Mena-Correa 2005).

Because of *E. sivinskii*'s peculiar foraging behavior for tephritid hosts of economic importance, its potential as a biological control agent is under investigation (J. Mena-Correa, J. Sivinski, R. Ramírez-Romero, M. Gates & M. Aluja, unpublished). Some basic parameters of the parasitoid oviposition behavior and development in pupae of the Mexican fruit fly *Anastrepha ludens* (Loew) are determined herein. Type of parasitism (ecto- or endoparasitism), number of emerged adults per host, duration of immature stages, host stage attacked, parasitism related to female age, and propensity to superparasitize were studied. The locations of eggs laid by the parasitoid on the host pupae, location of adult parasitoid emergence through the host puparium, and how these locations are related to survival, developmental rate, competition among ovipositing females, and sex of the resulting adult were analyzed.

## MATERIALS AND METHODS

### Insect Cultures and Experimental Conditions

This study was carried out at the Instituto de Ecología A.C., Xalapa, Veracruz, Mexico. Environmental conditions were kept at  $27 \pm 2^\circ\text{C}$ ,  $75 \pm 5\%$  RH, with a 12:12 h photoperiod. The *E. sivinskii* colony was maintained on 2-d-old pupae from an *Anastrepha ludens* colony kept for > 200 generations (Aluja et al. 2008). Pupae were placed on the surface of a layer of unsterilized clay soil and exposed to parasitoids for 6-8 d. This soil was gathered from the area where *E. sivinski* was originally discovered in Tejería, Veracruz. Adults of *E. sivinskii* were placed in Plexiglas cages ( $30 \times 30 \times 30$  cm) after emergence and fed *ad libitum* with honey and water. To avoid prior oviposition experience, parasitoids to be used in experiments were not exposed to host pupae.

Parasitoids were transferred from emergence cages to experimental cages ( $23 \times 23 \times 23$  cm) by capturing them in glass vials. The experimental cages were prepared 24 h before the beginning of tests to minimize insect stress. Host pupae were always manipulated with flexible forceps to avoid damage. After pupae were parasitized, they were placed in plastic cups (200 mL) containing vermiculite moistened with water mixed with sodium benzoate at 2 g/L which prevented fungal contamination. Cups were covered with a lid.

### Duration of Immature Stages

Development stages of *E. sivinskii* have been described elsewhere (Gates et al. 2008). Recorded images with Image Pro-Plus® software were examined to determine duration of each stage. All specimens were monitored until d 23 when adult females began to emerge as described by (Mena-Correa 2005).

Specimens used for the determination of the duration of immature stages stemmed from the 47-49<sup>th</sup> generations of our colony. To rear the parasitoids, we exposed 300 mL of *A. ludens* pupae (ca. 7000 pupae) to 3 *E. sivinskii* cohorts kept in  $30 \times 30 \times 30$  Plexiglas cages (200 females and 100 males per cage). Twenty-four h after exposure to parasitoids, pupae were removed and placed in 500 mL plastic vials with humidified vermiculite and covered with a lid. That same day (i.e., 24 h after exposure to parasitism) and from then on every 24 h over a 23-d period pupae were dissected individually in search of immature stages of the parasitoid. Twenty six eggs, 169 larvae (all stages represented), 40 prepupae, and 127 pupae were recovered. All specimens were placed in recently prepared Carnoy fixing solution (60 mL absolute alcohol, 30 mL chloroform and 10 mL acetic acid) for 24 h. Subsequently, they were washed and preserved in 70% EtOH in a hermetic glass flask until needed for evaluations. All dissections were made in a physiological solution to avoid tissue contraction (Martínez 2002).

### Determination of the Host Stage Attacked

First-, second- and third-instars, as well as 3, 8, and 14-d-old *A. ludens* pupae at different developmental stages were exposed to *E. sivinskii* adults inside 200-mL plastic cups. Twenty-five *A. ludens* larvae or pupae were presented to *E. sivinskii* cohorts of 10 females and 5 males (8-d-old) for 5 h. Each treatment was replicated 5 times.

Rates of parasitism among treatments were compared via a one-way analysis of variance (Zar 1998) with developmental stage as the independent factor. Consecutively, we compared the rates of parasitism in host pupae alone with a one-way analysis of variance (Zar 1998) with pupal age as the independent factor. Data were arc-sin  $\sqrt{x}$ -transformed prior to analysis (Zar 1998).

### Parasitism Related to Female Age

To obtain *E. sivinskii* adults of every age, 150 mL of *A. ludens* pupae (3-to 5-d-old) were introduced daily into colony cages for 24 h. After this period, each batch of pupae was placed in a separate cage and newly emerged *E. sivinskii* adults were recovered every day and age-classified. Then, we tested 1-to 27-d-old females to determine if there was an age effect on ability to para-

sitize pupae. Individuals (10 females and 5 males) within each age class were exposed to twenty 3-d-old *A. ludens* pupae over a 15-d-period. Each age-treatment was replicated 5 times, and the number of parasitoids recovered from each treatment was recorded.

Rates of parasitism among treatments were compared via a one-way analysis of variance (Zar 1998) with female age as the independent factor. When significant differences were found, a Tukey's test was performed (Zar 1998). Data were arc-sin  $\sqrt{x}$ -transformed before analysis (Zar 1998).

#### Determination of Superparasitism

Different host pupal densities (i.e., 1, 5, and 10 pupae) were exposed for 5 h to adult parasitoid cohorts of 10 females and 5 males (6- to 9-d-old). After exposure, hosts were dissected with saline solution and a stereo-microscope and the number of eggs per host was recorded. Dissections were performed either the same day or 24 h after parasitoid exposure. Each treatment had 5 replicates.

We analyzed with a two-way ANOVA test the mean number of eggs per parasitized pupae, the rate of parasitized pupae (attacked pupae / exposed hosts), and the rate of pupae with more than 1 parasitoid egg (pupae with more than 1 egg / attacked pupae), with host density and exposure time as independent factors. The rates of parasitized pupae and of pupae with more than 1 egg were arc-sin transformed prior to analysis (Zar 1998). In addition, the randomness of egg distributions in 5- and 10-pupae lots was determined by Chi-square tests, following the generation of expected egg/pupae frequencies through Poisson distributions (Zar, 1998). The nature of the distributions, random, dispersed or clumped, were then identified by dividing the variances by the means and comparing these ratios to a random distribution where the ratio = 1.0 (Zar, 1998).

#### Locations of Eggs and Adult Emergence from the Puparium

After determining that multiple eggs were deposited in a single host, we ran an additional experiment to detect if the initial position of eggs in the host body influenced the parasitoid emergence, rate of parasitism, and sex ratios. Four-d-old *A. ludens* pupae were exposed to 2 *E. sivinskii* cohorts (total of 50 and 150 six- to 9-d-old females and males, respectively). Exposed host pupae were recovered after 24 h and examined under saline solution 24 h later to identify the number of parasitoid eggs and their position in the host body. Based on the number of parasitoid eggs and their position, host pupae were classified and then placed individually in plastic containers (diameter: 4 cm; height: 2 cm) with humidified ver-

miculite and covered with a lid that allowed air-flow. Pupae were observed daily for 30 d and the number of parasitoids that emerged per host, their sex and the site of emergence on the host body (anterior, medium, or posterior) recorded.

The number of days it took adults to emerge was compared by a two-way ANOVA, with the number of eggs per host and the initial location of the eggs in the pupae as independent factors. Prior to this, data were mean-rank transformed. We compared the rates of emergence among emergence positions (i.e., location of emergence hole), considering the initial position of eggs and the number of eggs per host by chi-square tests. When significant differences were found, multiple two-by-two comparisons were performed with a significant threshold level, which was corrected according to the Dunn-Sidak method (Sokal & Rohlf 1995). We compared parasitoid sex-ratios against a 1:1 ratio with a chi-square goodness-of-fit test. In addition, for each set of experiments, parasitoid sex-ratios were compared among pupae with different numbers of eggs and egg positions in the pupae, based on a chi-square test (Zar 1998). Finally, a possible correlation between the location of adult emergence (i.e., emergence hole), egg location, and number of eggs was tested by an *r* Spearman test (Zar, 1998).

## RESULTS

#### Duration of Immature Instars and Stages

The life cycle (egg to adult) was completed in 23.1 ( $\pm$  2.1) d (mean  $\pm$  S.E.) at 27  $\pm$  2°C. Minimum and maximum durations for the egg stage were 0 (where 0 < 24 h) and 4 d. The larval stage lasted between 9 and 18 d. Minimum and maximum durations for the prepupal stage were 0 and 13 d, respectively, and those for the pupal stage were 8 and 14 d.

Minimum and maximum periods for larval instars were 1 and 4 d (first instar), 2 and 7 d (second and third instars), 0 and 8 d (fourth instar) and 4 and 13 d (fifth instar), respectively. The minimum and maximum periods of the pupal instars were 0 and 9 d (first instar), 8 and 14 d (second instar), 0 and 6 d (third instar), 4 and 7 d (fourth instar) and 0 and 3 d (fifth instar). Male emergence began on the 20<sup>th</sup> d following parasitoid exposure. Females started to emerge 23 d after exposure.

#### Host Stage Attacked

Parasitism of *E. sivinskii* in pupae was higher than in larvae ( $F = 839.31$ ;  $df = 1$ ;  $P < 0.001$ ). In no case were first and second instar larvae parasitized. However, 1 third-instar larva out of 625 exposed yielded a single parasitoid. When rates of parasitism at different pupal ages were com-

pared, significant differences were observed ( $F = 24.43$ ;  $df = 2$ ;  $P < 0.001$ ). Eight-d-old pupae exhibited the highest rate of parasitism ( $98.1 \pm 4$ ) (Fig. 1).

#### Parasitism Related to Female Age

There were no differences in rates of parasitism by female *E. sivinskii* of different ages ( $F = 1.284$ ;  $df = 26$ ;  $P = 0.181$ ) (Fig. 2).

#### Type of Parasitism

*Eurytoma sivinskii* was clearly ectoparasitic since 100% of the eggs were laid within the internal cavity of the puparium and on the surface of the pupa.

#### Determination of Superparasitism

*Eurytoma sivinskii* will lay more than 1 egg per host. In the first experiment, of the total parasitized pupae, 25.42% contained 1 egg, 32.20% had 2, 16.94% had 3, 13.56% had 4, 6.77% had 5, 3.39% had 6, and 1.70% had 8 eggs. Host density did not affect the number of eggs per pupae ( $F = 0.277$ ;  $df = 2$ ;  $P = 0.763$ ), the rates of parasitism ( $F = 3.062$ ;  $df = 2$ ;  $P = 0.084$ ), or the rates of pupae with more of 1 egg ( $F = 0.205$ ;  $df = 2$ ;  $P = 0.817$ ) (Table 1). The numbers of eggs / pupae were not randomly distributed in either the 5 or 10 pupae lots ( $\chi^2(5) = 15.9$ ,  $df = 2$ ,  $P < 0.005$ ;  $\chi^2(10) = 12.6$ ,  $df = 4$ ,  $P < 0.01$ ). These non-random distributions were due to clumping as evidenced by variance/mean ratios of 1.9 and 1.6, respectively. Eggs were oviposited on the posterior and middle re-

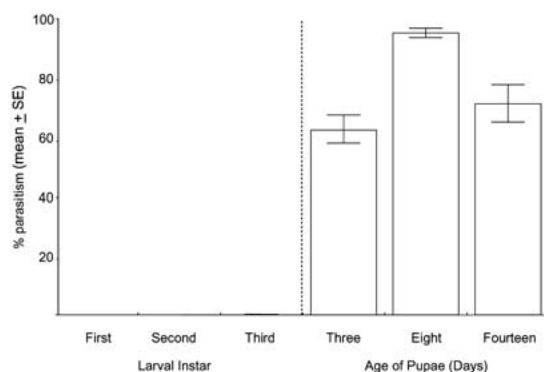


Fig. 1. Parasitism by *Eurytoma sivinskii* related to *Anastrepha ludens* immature stage. Rates of parasitism were compared among the different host stages. In no case were first and second-instar larvae parasitized, but notably one third-instar larva out of 625 exposed yielded a single parasitoid. Significant differences were observed when comparing rates of parasitism among pupal ages ( $F = 24.43$ ;  $df = 2$ ;  $P < 0.001$ ). Eight-day old pupae exhibited the highest rate of parasitism ( $98.1 \pm 4$ ).

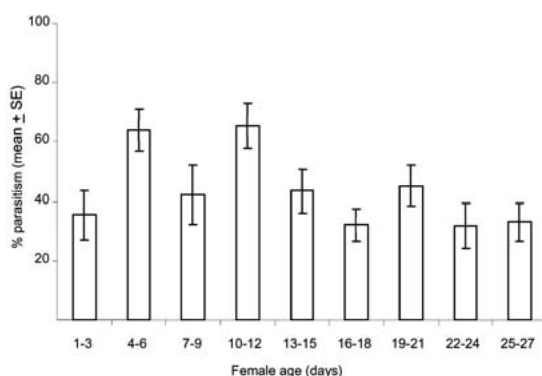


Fig. 2. Parasitism of *Anastrepha ludens* by female *Eurytoma sivinskii* related to female age. No significant differences in rates of parasitism were observed ( $F = 1.284$ ;  $df = 26$ ;  $P = 0.181$ ).

gions of the host. No oviposition was ever detected in the anterior region.

#### Locations of Eggs and Adult Emergence through the Puparium

In these experiments, the maximum number of eggs per host was 5. Thus, the following combinations of egg number vs. location in host were statistically compared: (a) 1 egg-posterior, (b) 2 eggs-posterior, (c) 3 eggs-posterior, (d) 4 eggs-posterior, (e) 5 eggs-posterior, (f) 1 egg-middle, (g) 2 eggs-middle, (h) 3 eggs-middle, (i) 4 eggs-middle and (j) 5 eggs-middle.

The number of eggs ( $F = 6.468$ ;  $df = 4$ ;  $P < 0.001$ ) and their location ( $F = 3.958$ ;  $df = 1$ ;  $P = 0.048$ ) had a significant influence of on the time of adult emergence. In pupae containing 5 eggs, adult emergence was significantly delayed when compared to pupae containing 1, 2, or 3 eggs. In addition, eggs oviposited in the middle of the pupae resulted in more rapid development than eggs in the posterior (Table 2). Peak female and male eclosion were observed at 23.9 ( $\pm 1.8$ ) and 21.4 ( $\pm 1.6$ ) d, respectively. Adults were significantly more likely to emerge from the middle of the host puparium, independent of the initial position of the eggs (i.e., medium or posterior) or the number of eggs (i.e., 1, 2, or 5 eggs).

When sex-ratios were compared against a 1:1 distribution, we observed a female-bias when host pupae contained 2, 4, and 5 eggs ( $\chi^2 = 4.00$ , 6.760, and 18.614;  $df = 1$ ;  $P < 0.05$ ) but not when host pupae contained 1 and 3 eggs ( $\chi^2 = 1.666$  and 0.104;  $df = 1$ ;  $P > 0.05$ ). Regarding the initial position of oviposited eggs, sex-ratio was female-biased in both middle ( $\chi^2 = 4.312$ ,  $df = 1$ ;  $P < 0.05$ ) and posterior ( $\chi^2 = 14.720$ ,  $df = 1$ ;  $P < 0.001$ ) positions. No relationship was observed between the emergence location and the number of eggs ( $R = 0.047$ ;  $P = 0.558$ ) or the location of eggs ( $R = 0.005$ ;  $P = 0.948$ ).

TABLE 1. MEAN NUMBER ( $\pm$  S.E.) OF OVIPOSITED EGGS PER PUPAE, RATES OF PARASITISM, AND OF PUPAE CONTAINING MORE THAN 1 EGG AFTER EXPOSURE OF DIFFERENT DENSITIES OF *ANASTREPHA LUDENS* PUPAE TO *EURYTOMA SIVINSKII* FEMALES.

Host density	Mean number of eggs per pupae <sup>a</sup>	Mean parasitism rate <sup>b</sup> (%)	Mean number of pupae with more of one egg <sup>c</sup> (%)
1	2.20 $\pm$ 1.3	100 $\pm$ 0.0	60.0 $\pm$ 54.8
5	1.96 $\pm$ 1.5	60.0 $\pm$ 6.9	56.0 $\pm$ 51.8
10	2.54 $\pm$ 0.9	78.0 $\pm$ 16.4	77.8 $\pm$ 15.7

<sup>a</sup>Non significant differences ( $F=0.277$ ; 2  $df$ ;  $P=0.763$ ).

<sup>b</sup>Non significant differences ( $F=3.062$ ; 2  $df$ ;  $P=0.084$ ).

<sup>c</sup>Non significant differences ( $F=0.205$ ; 2  $df$ ;  $P=0.817$ ).

### DISCUSSION

*Eurytoma sivinskii* is a solitary ectoparasitoid of fruit fly pupae, particularly those midway in development. Its females could superparasitize, under laboratory conditions, by ovipositing up to 8 eggs per host. Adults emerged in a shorter period of time when hosts originally contained a single parasitoid egg, although there was no lower likelihood of adult emergence when multiple eggs were present. However, longer development could inflict other costs such as increased exposure to pathogens, predators, or hyperparasitoids (Benrey & Denno 1997).

Eggs were commonly laid in the middle and posterior region of the host, but not in the anterior third. Adult parasitoids derived from eggs laid in the posterior region developed more rapidly, but adult sex ratio and percent of emergence were the same in both posterior and medially laid eggs. Oviposition sites might be influenced both by local differences in the resistance offered by the puparium and by advantageous placement of the egg on the pupa itself. *Spalangia endius* Walker (Pteromalidae) females are as likely to drill in the anterior or posterior portions of young

*Musca domestica* L. pupae, but as the puparium toughens with age, parasitoids are more likely to drill in the posterior, where the vulnerable anal spiracles are located, and these drilling are more likely to be successful (King 2001). For *S. endius* larvae, there appears to be no developmental advantage to an initially posterior or anterior placement, which may be due to the capacity of the hatchlings to wander to optimal feeding sites (King 2001). However, initial location does seem to matter in another pupal parasitoid of Diptera, *Nasonia vitripennis* Walker (Pteromalidae) (Rivers & Yoder 1996), where oviposition in the posterior portion of *Sarcophaga bullata* Parker (Sarcophagidae) puparia results in increased larval oxygen consumption, weight, and lipid content. Parasitized *S. bullata* have elevated lipid levels and the effect on the parasitoid was most pronounced when eggs were laid in the posterior portion (Rivers & Yoder 1996). At the same time, envenomation in the anterior parts of host leads to more rapid death and greater developmental disruption (Rivers & Denlinger 1994), which might also have nutritional consequences for the parasitoid. Unlike *E. sivinskii*, oviposition position in *N. vitripennis* has no effect on developmental rate.

TABLE 2. LIFE HISTORY PARAMETERS OF *EURYTOMA SIVINSKII* DEVELOPED IN *ANASTREPHA LUDENS* PUPAE CORRELATED WITH THE INITIAL POSITION OF OVIPOSITED EGGS AND THE NUMBER OF OVIPOSITED EGGS.

Initial position of eggs	Mean ( $\pm$ S.E.) time to adult emergence	Proportion (%) of adult emergence in relation to position in pupae			Total adult emergence (%)	Sex-ratio (% males)
		Anterior	Medium	Posterior		
Medium	23.5 $\pm$ 2.6 a	25.4 a	49.3 b	25.4 a	41.9 a	33.3 a
Posterior	22.7 $\pm$ 1.6 b	14.0 a	65.6 b	20.4 a	58.1 b	30.1 a
No of eggs						
1	23.4 $\pm$ 1.7 a	8.6 b	74.3 a	17.1 b	21.9 a	42.9 a
2	22.6 $\pm$ 1.7 a	16.7 b	75.0 a	8.3 b	22.5 a	33.3 ab
3	22.8 $\pm$ 2.0 a	28.9 a	28.9 a	42.1 a	23.8 a	41.2 a
4	23.6 $\pm$ 2.4 ab	24.0 a	52.0 a	24.0 a	15.6 a	24.0 ab
5	24.4 $\pm$ 2.2 b	15.4 b	65.4 a	19.2 b	16.3 a	7.7 bc

Different letters for each parameter within treatment indicate significant differences at ( $P < 0.05$ ).

The exit sites of adult *E. sivinskii* are concentrated in the middle of the *A. ludens* puparium. This contrasts strongly with the exit sites of *S. endius* which are mostly in the anterior end of the host (King 2001), where the puparium is likely to have weak cleavage lines that would have allowed the fly host itself to emerge. The reasons that *E. sivinskii* leaves from the middle are not entirely clear, but other parasitoids concentrate their penetration activities on the middle of the tephritid puparium. In the fruit fly specialist *Coptera haywardi* (Ogloblin) (Diapriidae), nearly all ovipositions are in the middle regions of *Anastrepha suspensa* (Loew) puparia, while the generalist chalcid, *Dihrinus himalayanus* Westwood, frequently oviposits in both the middle and the posterior (Sivinski et al. 1998). Perhaps the relatively strong preference for the middle by the tephritid specialist reflects a structural weakness in the *Anastrepha* puparial design that is also exploited by emerging adults.

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