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Developmental rates of two congeneric parasitoids, *Encarsia formosa* and *E. pergandiella* (Hymenoptera: Aphelinidae), utilizing different egg provisioning strategies

David M. Donnell [a,*], Martha S. Hunter [b]

[a] *Interdisciplinary Program in Insect Science, University of Arizona, Tucson, AZ, 85721, USA*
[b] *Department of Entomology, University of Arizona, Tucson, AZ, 85721, USA*

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Abstract

Endoparasitic Hymenoptera vary in the extent to which they provision their eggs and thus in the degree to which they appear to rely on their hosts for resources during embryonic development. In this study, developmental rates were examined in two congeneric parasitoid species, *Encarsia formosa* and *E. pergandiella*, that provision their eggs to different degrees. *E. formosa* eggs are much larger than *E. pergandiella* eggs. *E. formosa* eggs hatch significantly earlier than the eggs of *E. pergandiella* when deposited in 1st or 4th instar nymphs of a common whitefly host, *Bemisia tabaci*. Both species hatch earlier in 4th instar nymphs, but the delay in hatching in hosts parasitized as 1st instars is much greater in *E. pergandiella*. While *E. formosa* develops more rapidly to the 1st larval instar, *E. pergandiella* emerge as adults significantly earlier, though smaller, than *E. formosa* adults regardless of the host instar parasitized. These findings show that the extent of provisioning in the eggs of these wasps does not strictly determine their order of progression through different stages of development. © 2002 Published by Elsevier Science Ltd.

Keywords: Parasitoids; Aphelinidae; *Encarsia*; Development; Egg provisioning

1. Introduction

Encarsia formosa and *Encarsia pergandiella* (Hymenoptera: Aphelinidae) are solitary, internal parasitoids of whitefly (Homoptera: Aleyrodidae) nymphs with overlapping host ranges and preferences for ovipositing in the 3rd and 4th instars of their hosts (Liu and Stansly, 1996; Boisclair et al., 1990). These parasitoids are remarkably different for congeners in that *E. formosa* produces yolky eggs estimated to be more than six times larger by volume than the apparently yolkless eggs of *E. pergandiella* (Fig. 1). The species also differ in that the eggs of *E. pergandiella* swell markedly upon oviposition (Gerling, 1966) and develop a cellular extraembryonic membrane (Hunter, 1991) while those of *E. formosa* do not exhibit these features (personal observation). These observations suggest that *E. pergandiella* offspring may be relying on their host for nutrients during embryonic development to a much larger extent than *E. formosa*.

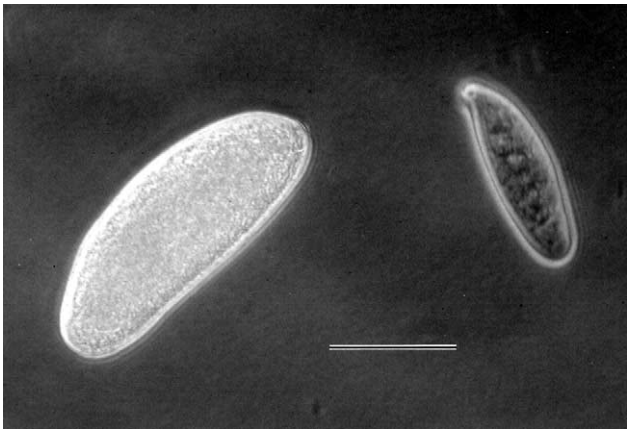

Fig. 1. Eggs of *E. formosa* and *E. pergandiella* within 4 h of oviposition. The egg on the left is that of *E. formosa*. Photograph taken on a Zeiss Axioskop using phase contrast setting. Scale bar=50 μM.

Since whitefly nymphs differ considerably in size

* Corresponding author. Tel.: +1-520-621-1731; fax: +1-520-621-1150.
E-mail address: donnell@ag.arizona.edu (D.M. Donnell).

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between instars (Bethke et al., 1991), the smaller hemolymph volume of 1st instar hosts is likely to provide smaller quantities of readily accessible nutrients to developing wasps than the greater volume of older instars. The eggs of *E. pergandiella* seem more likely to be affected by these differences than those of *E. formosa*. In this paper we report the results of a study conducted to determine if the developmental rates of the two parasitoid species differ when their eggs are deposited in the 1st and 4th (final) instars of a common whitefly host, the sweet-potato whitefly, *Bemisia tabaci*.

2. Materials and methods

2.1. Cultures

The *E. pergandiella* culture was established in 1994 using insects obtained from the USDA/APHIS Mission Biological Control Laboratory, TX (Quarantine No. M94055). This population of *E. pergandiella* was originally collected in Brazil on *B. tabaci*. The *E. formosa* culture was established in 1997 from insects obtained from a commercial insectary (CIBA Bunting, Colchester UK). Both *Encarsia* populations are thelytokous parthenogens and so produce only females. The parasitoids were reared on the greenhouse whitefly, *Trialeurodes vaporariorum*, feeding on green bean plants, *Phaseolus vulgaris* cv. Landmark. Cultures and experiments were conducted in an environmental chamber maintained at 27±1 °C, 35±5% r.h.,with a 16L:8D photoperiod.

2.2. Host preparation

Cotton seedlings, *Gossypium hirsutum* cv. D& PL3415, were grown until the one or two true leaf stage in small pots (diam.=6.2 cm), then were exposed to adult *B. tabaci* (Biotype B=*B. argentifolii* Bellows and Perring). The adult whiteflies were removed after 2 days and their progeny incubated for an additional 7 or 14 days depending on the stage of whitefly desired. After 7 days, most whiteflies were in the sessile phase of their 1st instar; after 14 days they were in their early 4th instar. An arena was prepared by placing a self-adhesive, annular callous pad (Walgreen Co., Deerfield, IL) with a 1.5 cm interior diameter and depth of 0.3 cm on the abaxial side of a leaf over an area containing a large number of nymphs. One- to four-day old adult *E. formosa* or *E. pergandiella* were taken from the culture containers. Two to seven parasitoids of either species were placed into each arena depending on the number of nymphs present to give an approximate 5:1 nymph:parasitoid ratio. A small piece of organdy fabric was used to cover the arena by attaching it to the pad with soft wax. After 4 h, the parasitoids were removed.

2.3. Hatching times

The interval between oviposition and hatching was determined for both parasitoid species in 1st and 4th instar whiteflies. The approximate time of hatching for each of the parasitoid/host instar groups was determined by preliminary observations. Regular dissections of hosts were then made at 4 h intervals starting 8 h prior to the first expected hatching event for each of the groups. The parasitoid eggs found in whitefly nymphs were scored as hatched or not hatched. A minimum of 100 parasitized whiteflies, obtained from no fewer than 17 arenas (and thus containing progeny from no fewer than 17 parasitoids), was dissected for each stage and species combination.

Almost all of the arenas used by *E. pergandiella* contained some whiteflies that yielded multiple parasitoid eggs upon dissection. The presence or absence of a hatched parasitoid larvae in such instances was recorded along with the total number of eggs present and the stage of the whitefly at the time of parasitism to determine the effects of superparasitism on the hatch times of *E. pergandiella*. A few arenas used by *E. formosa* contained whiteflies that yielded multiple parasitoid eggs upon dissection. Since this species will readily kill the eggs of conspecifics before depositing an egg into a previously parasitized host (Netting and Hunter, 2000), the effect of multiple eggs on the hatch time of this species was not investigated and data from such hosts was not tabulated.

2.4. Emergence times

The time from oviposition to adult emergence was determined for both species. Hosts were parasitized as above in their 1st or 4th instars. After 12 days (for hosts parasitized in the 1st instar) and 11 days (for hosts parasitized in the 4th instar), all whitefly nymphs in the arena containing a visible parasitoid pupa were removed to 1.2 ml vials. The vials were stoppered with cork then checked daily for emerged parasitoids for the next 10 days.

2.5. Adult sizes

To determine if adult sizes differed between groups, hind tibial measurements were made on 20 parasitoids taken from two arenas for each group using an ocular micrometer on a compound microscope at 100×.

2.6. Statistical analysis

All analyses were conducted using the JMP IN statistical package (Ver. 4, SAS Corp. 1999). The hatch data for hosts containing a single parasitoid were analyzed by fitting logistic models to the data collected for each

parasitoid/host instar group. The time of dissection (=h post-oviposition) was used as an independent variable and hatching status as a binary response variable (unhatched / hatched) in each analysis. The form of these equations is:

$$\text{Ln } p/q = \beta_0 + \beta_1 T$$

where p=the proportion hatched at a given time, $q = 1-p$, β_0=intercept, β_1=the adjustment parameter for time, and T=Time. The maximum likelihood estimates of the parameters were obtained using a weighted least squares regression analysis. The significance of the parameters was determined using Effect Likelihood Ratio χ^2 Tests (α=0.05). These tests assess whether a parameter significantly reduces the error deviance of the model relative to the model without the parameter. The mean time to hatching was determined for each of the parasitoid / host instar groups using the inverse prediction function. This function allows the user to specify the desired probability of obtaining a success (i.e., the probability of obtaining a 50% hatch), then solves the logistic equation to give the value of the regressor variable (i.e., Time) that would yield the desired probability within specified confidence intervals. The mean time to hatching was compared across all groups by checking for overlap between 95% confidence intervals. Group means whose 95% confidence intervals did not overlap were considered significantly different. The effect of superparasitism on the hatch rate of *E. pergandiella* eggs was determined by fitting logistic regression models to the data for each of the host instars parasitized in the same manner as above using the time of dissection and the number of eggs (one egg/more than one egg) as independent variables and hatching status as a binary response variable.

Significant differences among mean emergence times and mean adult sizes for each of the parasitoid/host instar groups were determined using linear constrasts after performing an ANOVA on the results.

3. Results

3.1. Hatching times

Both species of parasitoid hatched earlier following development in 4th instar hosts as compared to 1st instars, but there were marked differences between the species (Table 1, Fig. 2). The time to 50% hatch for *E. formosa* eggs in the older hosts was 56 h, 7 h shorter than for those deposited in 1st instars (63 h), and hatching extended over a 16–20 h period irrespective of host stage at oviposition. The time to 50% hatch for eggs of *E. pergandiella* in 4th instars was 78 h, 22 h longer than that of *E. formosa* in this instar while hatching extended over an interval similar to that of *E. formosa*. In contrast, the time to 50% hatch of *E. pergandiella* following oviposition into 1st instar hosts was 166 h, more than double the amount of time required for development in 4th instar hosts, and the time to hatching was much more variable, extending over about 60 h.

The effect of increasing the number of eggs in a host on embryonic development of *E. pergandiella* is shown in Fig. 3. Analysis of the time to hatching for *E. pergandiella* eggs deposited in 1st instar hosts indicates that hatching is delayed when more than one egg is present (Table 2). Of the hosts found to contain four or more eggs, only one, observed in a sample of 10 hosts examined at 196 h post-oviposition, contained a parasitoid larva. A similar analysis for eggs deposited into 4th instar hosts revealed no significant effect of egg number on the time of hatching (Table 2).

3.2. Emergence times

Parasitoids emerged sooner from hosts parasitized in the 4th instar than from hosts parasitized in the 1st instar, regardless of species ($t_{1,\ 610}$=26.93; $P<0.0001$; Table 3). The first *E. pergandiella* adults to emerge from hosts parasitized in the 1st instar were observed on day 13 following oviposition and those of *E. formosa* on day 14 (Fig. 4). The daily emergence curves are approximately normal for both species but *E. pergandiella* adults emerged significantly sooner on average than *E. formosa* adults ($t_{1,\ 610}$=4.56 ; $P<0.0001$; Table 3). The first *E. pergandiella* and *E. formosa* adults to emerge from hosts parasitized in the 4th instar were observed on day 12 following oviposition (Fig. 5). More than 75% of the parasitoids from hosts parasitized in the 4th instar emerged during the first two days of the emergence period. This is a much more skewed emergence pattern than was observed for parasitoids emerging from hosts parasitized in the 1st instar. In 4th instar hosts as well, *E. pergandiella* adults emerged significantly sooner than *E. formosa* adults ($t_{1,\ 610}$=3.12; P=0.002; Table 3).

3.3. Adult sizes

Hind tibial measurements demonstrate that *E. formosa* adults are larger than *E. pergandiella* adults regardless of the instar parasitized ($t_{1,\ 76}$=11.87; $P<0.0001$; Table 4). The size of *E. pergandiella* adults did not vary significantly as a function of the host instar parasitized ($t_{1,\ 76}$=−0.96; P=0.34; Table 4), however, *E. formosa* adults emerging from hosts parasitized in the 1st instar were significantly larger than those emerging from hosts parasitized in the 4th instar ($t_{1,\ 76}$=2.33; P=0.02; Table 4).

Table 1
Parameter estimates for the maximum likelihood equations describing the effect of time on hatching and the estimated time to 50% hatch for each of the parasitoid species/host instar groups. The estimates listed are highly significant by Effect Likelihood Ratio χ^2 Tests ($P<0.0001$). Mean hatch times followed by the same letters do not differ significantly ($\alpha=0.05$)

Parasitoid	Host instar	*n*	Parameter estimates			Confidence intervals (95%)	
			Intercept	Time (*T*)	50% Hatch (h)	Lower Limit	Upper Limit
Ef	1sts	139	24.64	−0.39T	63.20a	61.98	64.54
	4ths	126	19.32	−0.34T	56.31b	54.87	57.93
Ep	1sts	539	10.8	−0.06T	166.50c	162.71	169.65
	4ths	164	16.18	−0.21T	78.32d	76.31	80.07

Table 2
Parameter estimates for the maximum likelihood equations describing the effects of the number of eggs in each host and time on hatching of *E. pergandiella* eggs in whiteflies. The estimates listed are highly significant by Effect Likelihood Ratio χ^2 Tests ($P<0.01$). Unlisted estimates did not explain a significant amount of the variance ($\alpha=0.05$)

Host instar	*n*	Parameter estimates			
		Intercept	Time	Number of eggs in host	Egg number×Time
1sts	868	8.75	−0.05	1.91	0.03
4ths	422	15.37	−0.20	n.s.	n.s.

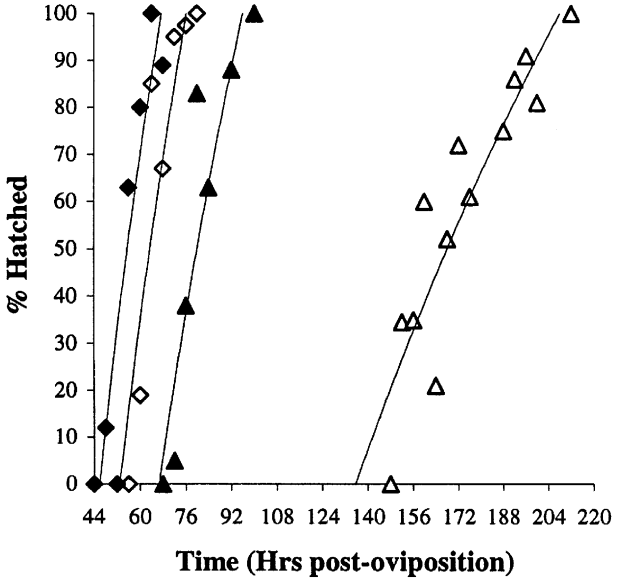

Fig. 2. Hatching as a function of time after oviposition for *E. formosa* (*Ef*) and *E. pergandiella* (*Ep*) eggs in 1st and 4th instar hosts. Each point represents the percentage of eggs hatched in a sample of hosts taken at one time. Each sample contained at least eight hosts. ◇, *Ef* on 1sts (n=139); ◆, *Ef* on 4ths (n=126); △, *Ep* on 1sts (n=539); ▲, *Ep* on 4ths (n=164). Trendlines were drawn for each parasitoid/host instar group using the Excel "Add Trendline" function.

4. Discussion

This work demonstrates that the hatching times of *E. formosa* and *E. pergandiella* eggs are influenced by the host's stage of development. *E. formosa* eggs hatch slightly but significantly sooner in 4th instar hosts than in 1st instar hosts. Hatching of *E. pergandiella* eggs deposited into 4th instar whiteflies lags behind that of *E. formosa* eggs in this stage but is much more delayed in eggs deposited in 1st instar whiteflies. Thus, host stage has a much greater influence on embryonic developmental rate in *E. pergandiella* than in *E. formosa*.

E. formosa deposits larger and presumably more nutrient-rich eggs into its hosts than *E. pergandiella* (Fig. 1). The results of this study suggest that the nutrients in the eggs of *E. formosa* facilitate the rapid development and hatching of the first instar of this species. The much slower development of *E. pergandiella* embryos appears due to an increased reliance on nutrients that must be absorbed from the host hemolymph in the presumed absence of yolk. The marked difference between the hatch times of *E. pergandiella* eggs deposited into the 1st and 4th host instars and the observation that hatch rates are negatively correlated with superparasitism in 1st, but not 4th host instars, suggests that nutrients are especially limiting in the hemolymph of early host stages. The delay in hatching resulting from superpara-

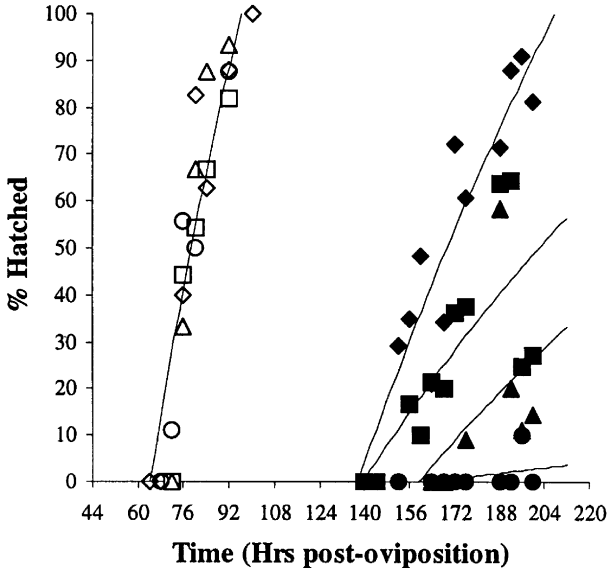

Fig. 3. The effect of superparasitism on the hatching times of *E. pergandiella* eggs deposited in 1st and 4th instar hosts. Each point represents the percentage of eggs hatched in a sample of hosts. Each sample contained at least eight hosts except for three samples in the 1st instar 4+ eggs group (four or more eggs per host) that contained only four hosts. The extent of superparasitism (i.e., the number of eggs found in a host) is shown for each of the host instars. 1st instar hosts: ◆, 1 egg (n=470); ■, 2 eggs (n=162); ▲, 3 eggs (n=71); ●, 4+ eggs (n=49). 4th instar hosts: ◇, 1 egg (n=170); □, 2 eggs (n=110); △, 3 eggs (n=56); ○, 4+ eggs (n=49). Trendlines were drawn for each parasitoid/host instar group using the Excel "Add Trendline" function.

Table 3
Mean emergence times for *E. pergandiella* and *E. formosa* adults emerging from whitefly hosts parasitized in the 1st and 4th instar. Means followed by the same letter do not differ significantly (linear contrasts; α=0.05)

Parasitoid	Whitefly instar	*n*	Mean emergence time (Days)
E. pergandiella	1st	274	16.5±0.1c
	4th	90	13.0±0.1a
E. formosa	1st	148	17.2±0.1d
	4th	102	13.7±0.1b

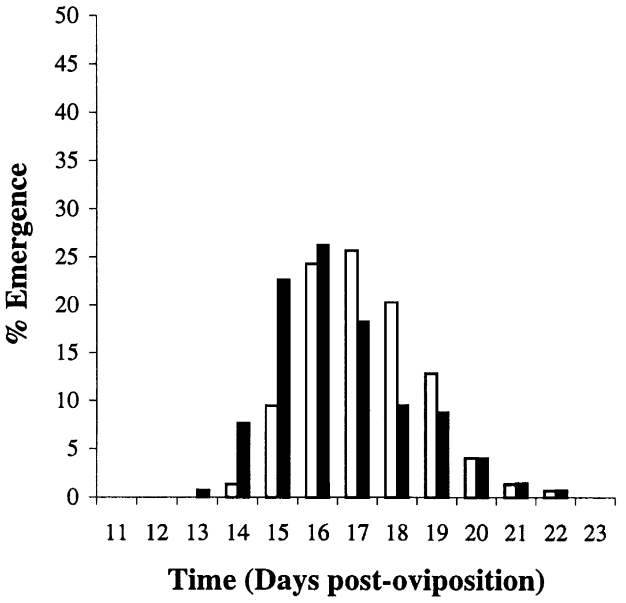

Fig. 4. Daily emergence rates for *E. formosa* (□; n=148) and *E. pergandiella* (■; n=274) emerging from hosts parasitized in the 1st instar.

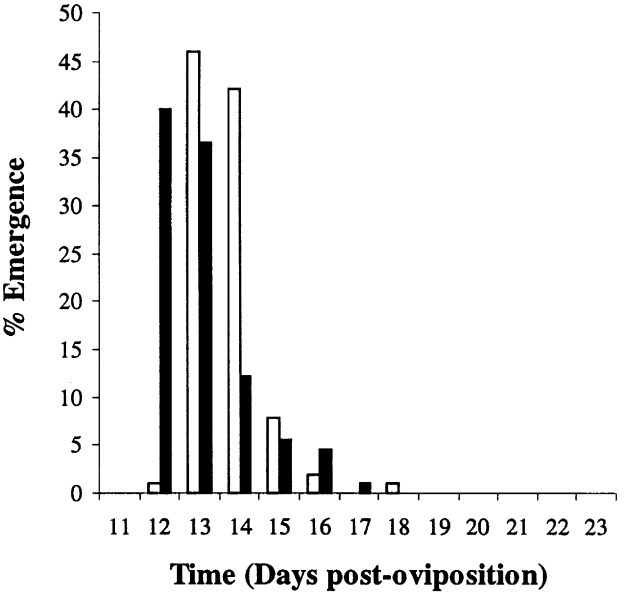

Fig. 5. Daily emergence rates for *E. formosa* (□; n=102) and *E. pergandiella* (■; n=90) emerging from hosts parasitized in the 4th instar.

sitism in 1st instar hosts is, however, not as great as the difference between hatch times of parasitoids in singly parasitized 1st and 4th instar hosts. Thus, nutrient availability may not be the only condition limiting *E. pergandiella* development in 1st instar hosts.

Using a different population of *E. pergandiella*, Liu and Stansly (1996) noted no significant difference in the early developmental rates of parasitized and unparasitized 1st and 2nd instar whiteflies. These authors speculated that the parasitoids deposited into early host instars might be arrested in development as eggs or early larvae until the host 3rd instar (Liu and Stansly, 1996). The data reported here suggest that *E. pergandiella* eggs deposited in the host 1st instar do not hatch until the whiteflies are in the 4th instar. Embryonic development is not completely arrested during the early stages of the host, however, as dissections indicate that the syncytium and extraembryonic membrane of *E. pergandiella* embryos develop prior to the host 3rd instar (DD, unpublished data).

Despite the developmental lead of *E. formosa* larvae at the time of hatching in both of the host instars examined, *E. pergandiella* adults begin emerging before *E. formosa* adults from hosts parasitized at the same time.

Table 4
Measurements of hind tibia for *E. pergandiella* and *E. formosa* emerging from whitefly hosts parasitized in the 1st and 4th instar. Means followed by the same letter do not differ significantly (linear contrasts; $\alpha = 0.05$)

Parasitoid	Host Instar	*n*	Mean length (μM)
Ef	1sts	20	205±18.8a
	4ths	20	195±12.5b
Ep	1sts	20	163±9.8c
	4ths	20	167±9.8c

Thus, post-embryonic development of *E. pergandiella* is faster than that of *E. formosa*. Nechols and Tauber (1977) reported that *E. formosa* larvae did not develop beyond the 1st instar until their hosts enter the 4th instar. The authors suggested a role for host hormones or nutritional state in the delay in *E. formosa* development analogous to that posited by Corbet (1968) in her study of the larvae of the ichneumonid parasitoid, *Venturia* (as *Nemeritis*) *canescens*. Corbet (1968) reported that developmental processes during the early stages of the host caterpillar, *Anagasta* (=*Ephestia*) *kuhniella*, result in low protein concentrations in the hemolymph, which in turn result in a reduced feeding rate by *V. canescens* during these instars. Information regarding changes in whitefly hormones or hemolymph nutrient levels associated with different instars is unavailable. However, it seems likely that earlier whitefly stages will possess smaller nutrient reserves than older whitefly stages. In the case of parasitism of 4th instar whiteflies, however, *E. formosa* would not experience the developmental delay observed in earlier host instars. Nevertheless, *E. pergandiella* still emerge faster than *E. formosa* from hosts parasitized in this instar. Thus, some other explanation must be found to account for the shift in development rates observed in these two species.

Jones and Greenberg (1999) reported that *E. pergandiella* developing in whiteflies parasitized in the 1st instar did not reach the same body length as those developing in hosts parasitized later in development and that hosts parasitized in the 4th instar stopped developing immediately after parasitism. These observations support the possibility that *E. pergandiella* is completing development faster than *E. formosa* because *E. pergandiella* exhausts its food supply sooner. Food availability has been shown to control the onset of metamorphosis in another holometabolous insect, the dung beetle, *Onthophagus taurus* (Shafiei et al., 2001). The tibial measurements in the present study suggest, however, that while significantly smaller than *E. formosa* at emergence, *E. pergandiella* adults emerge the same size regardless of the whitefly instar parasitized (Table 4). This finding suggests that the post-embryonic shift in developmental rates could be due to intrinsic differences in the developmental programs of the two wasps.

One clear difference in the development of the two wasp species is that *E. pergandiella* embryos are surrounded by a cellularized membrane (Hunter, 1991). This membrane becomes quite extensive during the latter stages of embryo development (personal observation). Similar membranes in the eggs of other wasp species have been credited with performing such functions as protecting developing embryos against fluctuations in osmotic pressure and assisting in the acquisition of resources (Vinson and Hegazi, 1998; Quicke, 1997; Pennachio et al., 1994; Gauld and Bolton, 1988; Tremblay and Caltigirone, 1973; Flanders, 1942). The potential role of the extraembryonic membrane in facilitating the development of *E. pergandiella* embryos remains to be investigated.

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