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## Preliminary studies on the artificial culture of *Brachymeria intermedia* (Nees) (Hym. Chalcididae) on oligidic diets(\*).

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### INTRODUCTION

One of the purposes of the studies on *in vitro* rearing of entomophagous insects is to culture parasitoids on oligidic diets, that is, diets composed primarily of chemically undefined, crude and possibly unexpensive components (Mellini, 1975).

Many more or less successful attempts have so far been made to culture hymenopterous parasitoids on oligidic media containing components of animal origin, i.e. raw beef (Simmonds, 1944), pork liver (Bronskill and House, 1957), foetal bovine serum (Hoffmann and Ignoffo, 1974; Greany, 1980; 1981; 1986; Liu and Wu, 1982), pork serum (Liu *et al.*, 1979) as well as egg yolk and powdered cow's milk, extensively utilized in diets for egg parasitoids (see Bratti, 1990).

The chalcidid *Brachymeria intermedia*, a polyphagous solitary endoparasitoid of lepidopterous pupae, was reared from egg to pupa on meridic diets (Thompson, 1980a) and from egg to adult on homogenate of *Galleria mellonella* L. pupae (Dindo, 1990).

The following is a description of the preliminary results obtained from the rearing of *B. intermedia* on oligidic diets containing bovine serum or beef-based homogenate for children (Plasmon) as the main ingredient.

Partial success had been previously achieved by Mellini *et al.* (1992) by rearing *Pseudogonia rufifrons* Wied. (Dipt. Tachinidae) on bovine serum-based media similar to those utilized in this study.

### MATERIALS AND METHODS

Stock colonies of *Brachymeria intermedia* were maintained on the laboratory host *Galleria mellonella*.

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Four diets were tested. The first (A<sub>1</sub>) contained bovine serum (78-80%), *G. mellonella* pupae extract (18-20%), trehalose (2-3%) and chicken egg yolk (1-2%). The pupae extract was obtained by the Bratti method (1989). In addition to the above ingredients, the second diet (A<sub>2</sub>) also contained lyophilized bovine serum (≈3.5%), while the third (B<sub>1</sub>) contained beef-based homogenate alone, and the fourth (B<sub>2</sub>) beef-based homogenate (≈80%) and host pupae extract (≈20%). All media were supplemented with gentamycin sulphate (0.006%) in order to prevent bacterial contamination (Bratti and Monti, 1988). As no development was achieved on sub-natural substrates without gelling agents (Dindo, 1990), all diets were set in 1-2% agar.

As is well known, the components of mammal serum comprise water (90%), proteins (6-8%) and several inorganic and organic compounds (2-4%), including low amounts of free amino-acids, which, according to several authors, are essential for survival and growth of parasitic early-instar larvae.

The nutrient content of the beef-based homogenate is reported in Table I.

*B. intermedia* eggs were obtained by dissecting superparasitized *G. mellonella* pupae. Collected eggs were held in sterile physiological saline, surface-sterilized for 2 minutes in 0.03% sodium hypochlorite and rinsed with sterile physiological saline. Eggs were then placed individually on small quantities of diets, approximately 0.4 cc, which had previously been pipetted into wells of Multi-dish Nunc tissue culture plates, which featured 24 wells in all. Some wells in the outer row were partially filled with sterile distilled water in order to maintain high humidity levels. The plates were sealed, placed in the dark at 25-28°C and observed daily.

All procedures were carried out in a laminar flow hood and the materials used were autoclaved at 120°C and 1 bar for 12 minutes. The bovine serum was sterilized by passing it through a 0.45 μ Millipore filter.

## RESULTS

The results are summarized in Table II.

### 1) Bovine serum-based diets.

Diet A<sub>1</sub> was tested twice. In the first test, 5 larvae reached at least the third instar, as 9 pairs of spiracles were distinguishable, 1 pair each on the meso-

Tab. 1 - Nutrient content (/50 g) of the beef-based homogenate for children used in diets B<sub>1</sub> and B<sub>2</sub>.

Dry residue	≥	10.75 g
Proteins (Nx6.25)		4.75 g
Lipids	≤	3.00 g
Minerals		0.25 g
Carbohydrates		3.2 g

Tab. 2 - *In vitro* rearing of *Brachymeria intermedia* on diets (A<sub>1</sub>), (A<sub>2</sub>), (B<sub>1</sub>), (B<sub>2</sub>).

Diet	Test	Eggs hatched	Mature larvae*	Larval development time (days)	Dead while pupating	Pharate pupae	Normal pupae	Adults
A <sub>1</sub>	I	11/20 55%	—	—	—	—	—	—
A <sub>1</sub>	II	17/20 85%	—	—	—	—	—	—
A <sub>2</sub>	III	15/20 75%	2/15 13%	13	—	1/15 6.7%	—	—
B <sub>1</sub>	I	17/27 63%	—	—	—	—	—	—
B <sub>2</sub>	II	20/26 77%	4/20 20%	18 ±4.4	4/20 20%	—	—	—
B <sub>2</sub>	III	13/15 87%	4/13 31%	15.3±2.3	—	1/13 7.6%	1/13 7.6%	1/13 7.6%
B <sub>2</sub>	IV	18/20 90%	9/18 50%	14.6±3.7	3/18 16.6%	3/18 16.6%	1/18 5.5%	1/18 5.5%

\* which eliminated faeces

thorax and the metathorax and 1 pair on each of the first 7 abdominal segments (Dowden, 1935). This was observed in 1 larva after 5 days, in 2 larvae after 6 days and in other 2 larvae after 7 days. One larva certainly moulted further by the 8th day, as at that time the cast integument was clearly detectable. In the following week all larvae died.

In the second test, 9 pairs of spiracles were distinguishable in 12 larvae after 10 days. Three larvae moulted further, two of which by the 11th and the last one by the 12th day. As in the previous test, no larva reached maturity.

On diet A<sub>2</sub>, which was tested once, 9 pairs of spiracles were observed in 5 larvae, in four of which after 7 days and in the last one after 10 days. One undersized pharate pupa was obtained. Furthermore, a larva matured and defecated, but failed to complete ecdysis to last instar. Faeces, therefore, remained under the penultimate instar integument. This larva, which was smaller than host-reared mature larvae, survived approximately one month in all.

Generally, in concurrence with *B. intermedia* larval growth, the substrate darkens. This phenomenon was observed in the host and on sub-natural media (Dindo, 1990), as well as on meridic diets (Thompson, unpublished). Melanization of artificial media also accompanied feeding by the parasitoid *Exeristes roborator* (Fab.) (Hym. Ichneumonidae) and was found to be due to phenyloxidase activity in the salivary secretion of the developing larvae (Thompson, 1980b). *B. intermedia* salivary secretion may also contain phenyloxidase activity. However, on diet A<sub>2</sub> such darkening did not occur, notwithstanding the fact that two individuals completed larval development.

2) Beef homogenate-based diets.

Diet B<sub>1</sub> was tested once. In one larva, which survived 2 weeks in all, 9 pairs of spiracles were distinguishable after 9 days. The rest of the larvae failed to grow and died within a few days. Diet B<sub>2</sub> was tested three times. In the first test 10 fully grown larvae were obtained. In concurrence with larval growth the substrate darkened (Fig. 1). The beginning of darkening was observed in 1 larva after 8 days, in 3 larvae after 9 days, in 4 larvae after 13 days and in 2 larvae after 22 days. Four mature larvae eliminated faeces, but died while pupating.

In the second test, one undersized pharate and one normal pupa were obtained. The development of the latter from egg to pupa required 15 days. After eliminating faeces, the larva moulted to pupa in about 7 hours and the pupa darkened within two days. After three days, an adult female emerged.

In the third test, 16 fully grown larvae were obtained. The substrate darkened in concurrence with larval growth. Seven larvae died prior to eliminating faeces, whereas 9 larvae defecated. Three pharate and one normal pupa were obtained. From the latter, an adult female emerged, which proved to be fecund. This female, the weight of which was 11.4 mg, was comparable in size to host-reared individuals, as the mean weight of 10 newly-emerged females cultured *in vivo* was  $11.52 \pm 1.12$  mg. The development from egg to adult required 20 days.

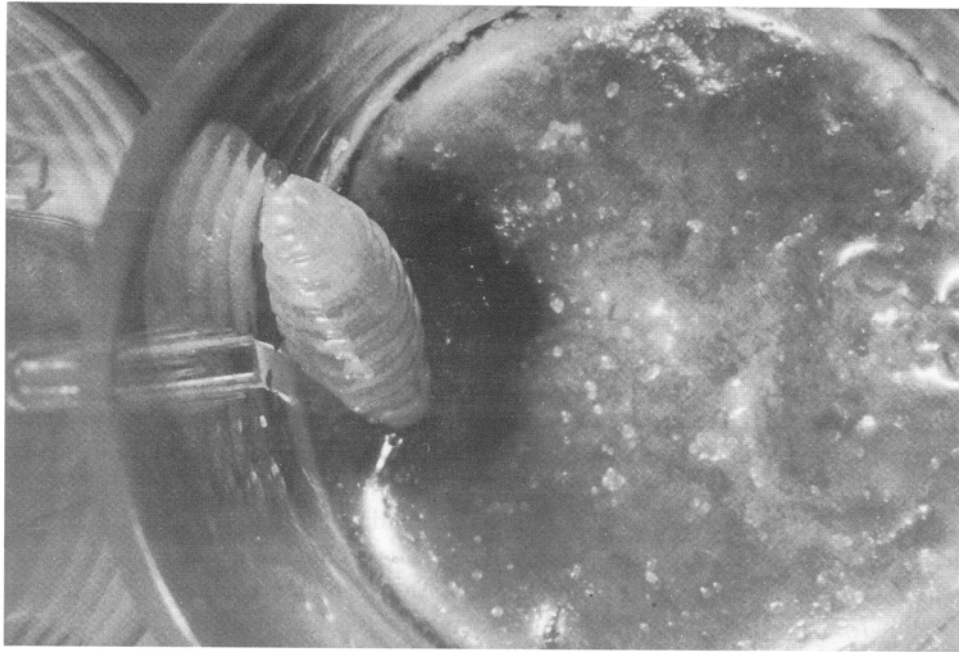


Fig. 1 - *Brachymeria intermedia* larva on diet B<sub>2</sub>.

## CONCLUSIONS

The bovine serum-based media, though integrated with host pupae extract, were inadequate for *B. intermedia*. Viceversa, on similar diets the tachinid *P. rufifrons* reached the adult stage, but the adults failed to completely emerge from the puparia (Mellini *et al.*, 1992).

The beef-based homogenate proved to be a potentially effective component of oligidic diets for *B. intermedia*. The time for completion of larval development on diet B<sub>2</sub> (up to the elimination of faeces) was approximately twice as long as that in the host, which generally requires 6 to 8 days at 26-28°C, whereas the pupal development was similar on the diet and in the host pupae, requiring approximately 5 days.

However, no development was obtained on beef-based homogenate devoid of host pupae extract (diet B<sub>1</sub>). Nettles (1990) showed that *in vitro* development of many parasitoids is strongly dependent on host materials in the artificial diet. For *B. intermedia*, nutritional host components may be essential. Therefore, the isolation and the identification of host chemicals may be very important in order to improve *in vitro* rearing of this chalcidid and to develop artificial media devoid of host materials.

## SUMMARY

The chalcidid *Brachymeria intermedia*, a polyphagous solitary endoparasitoid of lepidopterous pupae, was cultured from egg on four oligidic diets. The first diet (A<sub>1</sub>) contained bovine serum (78-80%), host pupae extract (18-20%), trehalose (2-3%) and chicken egg yolk (1-2%). In addition to the above ingredients, the second diet (A<sub>2</sub>) also contained lyophilized bovine serum (≈3.5%), while the third (B<sub>1</sub>) contained beef-based homogenate for children (Plasmon) alone, and the fourth (B<sub>2</sub>) beef-based homogenate (≈80%) and host pupae extract (≈20%). All diets were supplemented with gentamycin sulphate (0.006%) and set in 1-2% agar.

The bovine serum-based diets proved to be inadequate for *B. intermedia*. On diet A<sub>1</sub>, all larvae died prior to maturing. Most larvae died before maturing on diet A<sub>2</sub> as well, even though one undersized pharate pupa was obtained.

On diet B<sub>1</sub>, devoid of host materials, no larval development was achieved. On diet B<sub>2</sub>, several larvae matured and eliminated faeces. Some pharate and two normal pupae were obtained. Two adult females emerged from the two normal pupae, one of which proved to be fecund.

The time for completion of larval development on the artificial media (up to the elimination of faeces) was approximately twice as long as that in the host, which generally requires 6 to 8 days at 26-27°C. However, the pupal development was similar on the medium and in host pupae, requiring approximately 5 days.

In conclusion, beef-based homogenate for children proved to be a potentially effective component of oligidic diets for *B. intermedia*, although much research is still to be carried out in order to eliminate host-derived materials from the media.

Primi risultati riguardanti l'allevamento di *Brachymeria intermedia* (Nees) (Hym. Chalcididae) su diete oligidiche.

## RIASSUNTO

L'imenottero calcidide *Brachymeria intermedia*, endoparassitoide polifago solitario di pupe di Lepidotteri, è stato allevato a partire dallo stadio di uovo su quattro diete oligidiche. La prima

dieta (A<sub>1</sub>) era costituita da siero bovino (78-80%), estratto di crisalide di *Galleria mellonella* (18-20%), trealosio (2-3%), tuorlo d'uovo (1-2%) e la seconda (A<sub>2</sub>) dai medesimi ingredienti integrati con siero bovino liofilizzato (≈3.5%). La terza dieta (B<sub>1</sub>) era costituita da solo omogeneizzato per bambini a base di carne di manzo (Plasmon), mentre la quarta (B<sub>2</sub>) era costituita dal suddetto omogeneizzato (≈80%) e da estratto di crisalidi (≈20%). Le diete sono state addizionate con solfato di gentamicina (0.006%) e agarizzate (concentrazione finale di agar 1-2%).

Le diete a base di siero bovino si sono dimostrate inadeguate per *B.intermedia*. Sulla dieta A<sub>1</sub>, tutte le larve sono morte prima di raggiungere la maturità, così come la maggior parte delle larve allevate sulla dieta A<sub>2</sub>. Comunque, sulla dieta A<sub>2</sub>, è stata ottenuta una pupa farata e sottodimensionata.

Sulla dieta B<sub>1</sub>, priva di materiali derivati dall'ospite, le larve praticamente non si sono accresciute, mentre sulla dieta B<sub>2</sub> diverse larve hanno raggiunto la maturità ed eliminato gli escrementi. Sono state ottenute alcune pupe farate e due pupe normali. Queste ultime hanno lasciato sfarfallare due femmine adulte, una delle quali si è dimostrata feconda.

I tempi di sviluppo larvale sul substrato artificiale (fino all'eliminazione delle feci) sono stati all'incirca doppi rispetto a quanto si verifica normalmente *in vivo*; viceversa, il tempo intercorso tra la formazione della pupa e lo sfarfallamento dell'adulto (di 5 giorni circa) è stato analogo a quello abitualmente riscontrato nell'ospite.

In conclusione, l'omogeneizzato di manzo per bambini si è dimostrato un componente potenzialmente valido di diete oligidiche per *B. intermedia*. Comunque, ulteriori, approfondite ricerche sono necessarie per eliminare dal substrato materiali derivati dall'ospite.

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