Artificial culture of the pupal parasitoid
*Brachymeria intermedia* (Nees) (Hymenoptera:
Chalcididae) on oligidic diets

M.L. Dindo, R. Farneti & G. Gardenghi

ABSTRACT

The results so far obtained in the in vitro rearing of *Brachymeria intermedia*, a polyphagous solitary endoparasitoid of lepidopterous pupae, are briefly illustrated. This chalcidid was developed from the egg to the adult stage on oligidic media containing commercial veal homogenates for babies as the main ingredient, either with or without host components. Some of the media also contained chicken egg yolk, yeast extract and wheat germ. The percentage yields of adults, based on the number of eggs placed on the diet was as high as 53% on the media integrated with 10% host material and 44% on the host material-free media. Adults were fecund. *B. intermedia* may be comparatively easy to rear in vitro because of the simple physiological interaction existing between this parasitoid and its host.

*Key words:* parasitoid rearing, artificial diets, *Brachymeria intermedia*

INTRODUCTION

Efforts to culture parasitoids from egg or newly-hatched larva to adult in artificial media have so far been successful for more than 30 species. The best results have so far been obtained with several hymenopteran oophagous or pupal parasitoids (Grenier et al, 1994) and with the tachinid *Exorista larvarum* (L.) (Mellini et al., 1996). All these entomophages do not display a high degree of interaction with the living host physiology. Moreover, in vivo their larvae generally exhibit a simple behaviour, likely to be reproduced in artificial diets.

*Brachymeria intermedia* is a solitary pupal endoparasitoid of a wide range of lepidopterous species, including *Lymantria dispar* (L.). Being a pupal parasitoid, this chalcidid kills the host quickly and does not show great dependence upon a specialized environment and, in particular, upon the host physiology. Dindo (1990)
showed that the pupae of *Galleria mellonella* L. die about 50-55 hours after parasitization by *B. intermedia* and that complete parasitoid development can be obtained even in pupae killed before being exposed to parasitoid females, as well as on sub-natural media composed of *G. mellonella* pupal homogenate. In view of these considerations, the development of meridic or oligidic media for rearing *B. intermedia* in vitro was therefore feasible.

In 1980 partial success had been achieved by Thompson, who cultured this parasitoid from egg to pupa on chemically defined media without insect components. In 1981, he also described the complete development on meridic diets of the congeneric species *B. lasus*, which behaves very similarly to *B. intermedia* both in the host and in vitro. In the present paper, the recent results obtained by rearing *B. intermedia* on different oligidic diets (that is, diets composed primarily of chemically undefined components) are briefly illustrated.

**MATERIALS AND METHODS**

All the diets tested contained commercial veal homogenates, a food intended for human babies, as the main ingredient. Most of them also contained host components, mainly extract of *G. mellonella* pupae, prepared as in Bratti (1989). In some tests, the pupal extract was replaced with larval extract, obtained by the same method, or with pupal or larval homogenate, obtained by squeezing pupae in a syringe and then removing the large pieces of cuticle.

In some of the media, host material was partially or totally replaced with different amounts of chicken egg yolk, yeast and wheat germ.

All media were supplemented with 0.006% gentamycin sulphate to inhibit bacterial contamination (Bratti & Monti, 1988) and set in 1.2% agar.

Twenty-four-well plastic plates were used as rearing containers. About 0.4 cc. of diet were pipetted in each well. *B. intermedia* eggs were collected from superparasitized *G. mellonella* pupae and placed singly into the wells. The plates were then sealed with Parafilm® and kept at 26±1 °C under constant darkness, except during daily visual examination.

All operations were performed in a laminar flow hood and instruments and glassware were sterilized by autoclaving for 20 minutes at 120 C.

**RESULTS**

A diet composed of 80% veal homogenate and 20% *G. mellonella* pupal extract gave a mean yield of 33% *B. intermedia* adults (Dindo et al., 1994). Subsequently, on a veal homogenate-based diet containing 10% pupal extract, integrated with chicken egg yolk and yeast extract the percent yield of adults (based on the number of eggs placed on the media) was of about 53%. The development times were similar to those generally observed in vivo. It was also found that the pupal extract may be replaced with an equal amount of extract of last-instar larvae.
Artificial culture of the pupal parasitoid *Brachymeria intermedia* without the adult yields being affected. However, when host extract was replaced with host homogenate, either pupal or larval, only a few adults were obtained on the media.

*B. intermedia* was then developed in vitro, from egg to adult, even in the absence of host material. A diet composed of veal homogenate, chicken egg yolk and yeast gave a mean adult yield of 44%. Adult yields of 50% and 38% were respectively reported for diets integrated and devoid of host material upon half the yeast extract being replaced with an equal amount of wheat germ.

The adults obtained in all the diets were comparable in size to those usually obtained in vivo. They normally mated and the females laid eggs in *G. mellonella* pupae producing a second generation within the host.

An anatomical and histological comparison was made between the reproductive system of females reared on *G. mellonella* and on a diet composed of 80% veal homogenate and 20% pupal extract. No differences were observed. Also, the number of mature eggs (1 or 2 per ovariole) were not seen to differ between the in vivo and in vitro reared females (Dindo et al., 1995).

**DISCUSSION**

The commercial veal homogenate was selected as a component of oligidic diets for *B. intermedia* on the assumption that the basic qualitative nutritional needs of parasitic insects are similar to those of other animals (House, 1977). No parasitoid development was however obtained on media based on the homogenate alone (Dindo & Campadelli, 1992). Moreover, despite the integration with host material, adult yields were still quite low in the diet composed of 80% homogenate and 20% pupal extract. Very little is known about the specific nutritional needs of *B. intermedia* both in terms of the optimal concentration of a nutrient and the optimal balance between nutrients, which is also extremely important (Grenier et al., 1986; 1994). The ingredients utilized to integrate the diets were selected among those commonly employed in artificial media for parasitoids (Bratti, 1990). Compared to the diet composed of homogenate and pupal extract alone, the medium added with chicken egg yolk and yeast extract contained half the host material and gave much higher adult yields. A good adult yield was also obtained on the host material-free medium, composed of homogenate, chicken egg yolk and yeast extract. The yield was however lower than that obtained in diets integrated with 10% pupal extract.

Yeast extract proved to be a key ingredient for several parasitoid species, including *Trichogramma* sp. (Liu & Wu, 1982), *Eucelatoria bryani* Sabr. (Nettles, 1986), *E. larvarum* (Mellini et al., 1996) and *Melittobia* sp. (Fanti et al., unpublished data). This component is however very expensive. In some of the diets employed by us, with or without host material, half the amount of yeast extract was replaced with wheat germ, which is considerably cheaper. Adult yields were slightly lower in the wheat germ-integrated diets than in the wheat germ-free ones.
but, in the case of in vitro mass production, this inconvenience may be offset by the fact that the former diets are more economical than the latter.

More research is needed to better evaluate the actual importance for *B. intermedia* of the ingredients employed by us and the most suitable amounts to be put in the diets. As regards host material, it may be advisable not to completely eliminate it from the diets. Small amounts of host components in the medium may actually reduce the problems possibly associated with continuous multi-generation artificial mass culture, i.e. alteration of host searching behaviour (Mellini et al., 1996). Pupal extract is quite hard to prepare, but it was demonstrated that it can be successfully replaced in the media with extract of last instar *G. mellonella* larvae, though *B. intermedia* is a pupal parasitoid. Larval extract is much easier to prepare than pupal extract as there are no cocoons to remove. Conversely, very low adult yields were obtained in the diets integrated with pupal or larval homogenate. This may have been due to the fact that both of them were sterilized by autoclaving at 120 °C in order to prevent diet contamination by moulds and bacteria, thus denaturing at least part of their nutrients. It ought however to be noted that in spite of autoclaving both pupal and larval homogenates proved to be suitable ingredients in the diets for *E. larvarum* (Mellini & Campadelli, 1994).

The adults obtained in all of the diets were normal in size and fecund. Moreover, no appreciable difference was observed between the reproductive system of in vivo and in vitro reared females (Dindo et al., 1995). The quality of the adults obtained in the media, especially in the host material-free ones, needs however to be better evaluated, in terms of longevity, fecundity and host searching capacity. Yet, the results so far obtained have shown that *B. intermedia* is a promising parasitoid for in vitro mass production.

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REFERENCES


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M.L. DINDO, R. FARNETI & G. GARDENGHI. Istituto di Entomologia "Guido Grandi". Università di Bologna. via Filippo Re, 6, 40126 Bologna, Italy.