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In vitro rearing of *Pseudogonia rufifrons* Wied.
(Dipt. Tachinidae) and *Brachymeria intermedia* (Nees)
(Hym. Chalcididae) on oligidic diets (*)¹

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INTRODUCTION

The tachinid *Pseudogonia rufifrons* is a solitary larval-pupal endoparasitoid of Lepidoptera dependent on the host physiology. The first-instar larvae moult to the second instar only when they are triggered by the host's ecdysteroids (generally at host pupation) (Baronio and Campadelli, 1978). This parasitoid was cultured from first-instar larva to adult on *Galleria mellonella* L. pupae homogenate (Bratti and Monti, 1988). Subsequently, Mellini *et al.* (in press) reared *P. rufifrons* from first- and early-second-instar larva on bovine serum-based diets. Several parasitoids reached the adult stage, although the adults failed to completely emerge from the puparia.

The chalcidid *Brachymeria intermedia*, a solitary pupal endoparasitoid of Lepidoptera, was reared from egg to pupa on meridic diets (Thompson, 1980) and from egg to adult both on *Galleria mellonella* pupae homogenate (Dindo, 1990) and on diets based on beef homogenate for babies (Plasmon) (Dindo and Campadelli, 1992).

The following is a description of the artificial culture on oligidic diets of both *P. rufifrons* and *B. intermedia*.

MATERIAL AND METHODS

P. rufifrons first-instar larvae, collected from previously parasitized hosts, were cultured on three media containing: (a) bovine serum alone; (b) bovine serum ($\approx 80\%$) and extract from *Galleria mellonella* pupae ($\approx 20\%$); (c) bovine serum (78-80%), host pupae extract (18-20%), trehalose (1-2%) and chicken egg yolk (1-2%).

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The pupae extract was obtained by the Bratti method (1989). The respiratory requirements of the second- and third-instar larvae, which depend on atmospheric oxygen to survive, were satisfied by setting the diets in 1% agar.

B. intermedia was cultured from egg on four media. The first (A₁) contained the same ingredients as diet (c) above. The second (A₂) contained, in addition to the ingredients used in diet (c), lyophilized bovine serum ($\approx 3.5\%$). The third (B₁) contained beef-based homogenate (Plasmon) alone and the fourth (B₂) beef-based homogenate ($\approx 80\%$) and host pupae extract ($\approx 20\%$). All diets were set in 1-2% agar. The parasitoid's eggs were obtained by the method described by Dindo and Campadelli (1992).

In order to prevent bacterial contamination, all media were supplemented with gentamycin sulphate (0.006%) (Bratti and Monti, 1988).

P. rufifrons larvae and *B. intermedia* eggs were placed individually on small amounts of diet (≈ 0.4 cc), which had previously been pipetted into wells of Multidish Nunc tissue culture plates, which featured 24 wells in all. The plates were sealed, placed in the dark at 25-28 °C and observed daily.

Materials were sterilized by autoclaving for 12 minutes at 120 °C and 1 bar. The bovine serum was sterilized by passing it through a 0.45 μ Millipore filter. All operations, including visual examinations, were performed in a laminar flow hood.

RESULTS

In vitro culture of *Pseudogonia rufifrons*.

On diet (a), most larvae failed to moult to the second instar. On diet (b), in most cases the larvae moulted to the second instar and a few of them reached the pupal stage. On diet (c), more than 50% larvae reached the third instar and more than 30% of the latter pupated. The time for completion of larval development on diet (c) was approximately one week longer than in the host. The puparia were of a similar size to host-reared individuals. Most pupae moulted to the adult stage, but the adults failed to completely emerge from the puparia (see Mellini *et al.*, in press).

In vitro culture of *Brachymeria intermedia*.

The bovine serum-based diets were inadequate for *B. intermedia*. On diet (A₁) no larva reached maturity and eliminated faeces. On diet (A₂) an undersized pharate pupa was obtained. Anyway most larvae died before reaching maturity.

On the opposite, the beef homogenate-based diets proved to be potentially effective for *B. intermedia*, as on diet (B₂) about 40% larvae reached maturity and defecated. Two normal pupae were obtained. Two adult females emerged from the two normal pupae, one of which proved to be fecund. However, on diet (B₁), devoid of host materials, no larval development was obtained.

The larval development, up to the elimination of faeces, on diets (A₂) and (B₂) was 6 to 8 days longer than usually obtained *in vivo* at 26-28 °C. However, the pupal development was similar on the media and in the host pupae, requiring approximately 5 days.

CONCLUSIONS

Promising results were obtained, for both *P. rufifrons* and *B. intermedia*, using oligidic diets based on rather inexpensive ingredients, such as bovine serum and beef-based homogenate. It must be emphasized that the potential of bovine serum as the main ingredient of oligidic diets for *P. rufifrons* had been previously shown by Mellini *et al.* (in press).

However, for both species, no development was obtained on media devoid of host pupae extract.

Nettles (1990) showed that *in vitro* development of many parasitoids is strongly dependent on host materials in the artificial diets. *P. rufifrons* first-instar larvae apparently depend on host hormonal factors for moulting to the second instar and Fanti (1990) demonstrated that the parasitoid development to the second instar is promoted by the addition of exogenous ecdysteroids to meridic diets. Moreover, nutritional host components may be essential for this tachinid as well as for *B. intermedia*, the development of which seems to be independent of host hormones. Thompson (1980) showed that β -ecdysone included in meridic diets at a level of 4 and 8 $\mu\text{g/ml}$, the same order of magnitude as reported in *G. mellonella* (Maroy and Tarnoy, 1978), had no observable effect on *B. intermedia*.

For both species, the isolation and identification of host chemicals may be very important in order to improve *in vitro* rearing and to develop artificial media devoid of host materials.

SUMMARY

The tachinid *Pseudogonia rufifrons* - a larval-pupal parasitoid of Lepidoptera fully dependent on the host physiology - and the chalcidid *Brachymeria intermedia* - a polyphagous parasitoid of lepidopterous pupae - were reared *in vitro* on oligidic diets based on rather inexpensive compounds. *P. rufifrons* was cultured from first instar larva, whereas *B. intermedia* was cultured from egg.

On a diet containing bovine serum (78-80%), host pupae extract (18-20%), trehalose (1-2%) and chicken egg yolk (1-2%), more than 50% larvae of *P. rufifrons* reached the third instar and more than 30% of the latter pupated. Most pupae moulted to the adult stage, but the adults failed to completely emerge from the puparia, which were, however, of a similar size to host-reared individuals.

Bovine serum-based diets were inadequate for *B. intermedia*. On the opposite, a diet containing beef-based homogenate for babies (Plasmon) (80%) and host pupae extract (20%) proved to be potentially effective for this chalcidid. On such diet, two adult females were obtained, one of which proved to be fecund.

Allevamento *in vitro* di *Pseudogonia rufifrons* Wied. (Dipt. Tachinidae) e di *Brachymeria intermedia* (Nees) (Hym. Chalcididae) su diete oligidiche.

RIASSUNTO

Il tachinide *Pseudogonia rufifrons* - un parassitoide larva-pupale a sviluppo totalmente dipendente dalla fisiologia dell'ospite - e il calcidide *Brachymeria intermedia* - un parassitoide polifago solitario di pupe di Lepidotteri - sono stati allevati *in vitro* su diete oligidiche a base di ingredienti di costo relativamente basso. L'allevamento di *P. rufifrons* è stato effettuato a partire da larve di prima età, mentre quello di *B. intermedia* è stato eseguito a partire dall'uovo.

Su una dieta contenente siero bovino (78-80%), estratto di crisalide dell'ospite di sostituzione *Galleria mellonella* (18-20%), trealosio (1-2%) e tuorlo d'uovo (1-2%), più del 50% delle larve di *P. rufifrons* ha raggiunto la terza età e più del 30% di queste si è impupato. Nella maggioranza dei casi, si è avuta la formazione degli adulti, i quali, però, non sono riusciti a liberarsi completamente dei pupari. Comunque, questi ultimi erano di peso tendenzialmente analogo a quanto si ottiene normal-

mente *in vivo*, in crisalidi di media taglia.

Le diete a base di siero bovino si sono dimostrate inadeguate per *B. intermedia*. Al contrario, una dieta contenente omogeneizzato per bambini a base di manzo (Plasmon) (80%) e estratto di crisalide (20%) si è dimostrata potenzialmente efficace per questo calcidide. Su tale dieta, sono state ottenute due femmine adulte, una delle quali si è dimostrata feconda.

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