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Artificial culture of the parasitoid *Exorista larvarum* L. (Dipt. Tachinidae) on bovine serum-based diets.*(1)(2)

Introduction

Our research group has been studying for many years the host-parasitoid relationships in insects, with particular regard to Diptera Tachinidae. For some time now, our studies have also been directed to the culture of parasitoid larvae on artificial media. In 1975, Mellini gave an exhaustive overview of the promising, though not brilliant, results which had been achieved until then in the world. Twelve years later, Campadelli and Dindo (1987) reexamined the situation and concluded that progress over the period had not been as remarkable as expected. Notwithstanding, the best results obtained were those with parasitoids living at the expense of motionless and incapable of feeding stages, i.e. eggs and pupae. More recently, Bratti (1990) reviewed the *in vitro* rearing techniques for parasitoids as well as the results achieved by culturing Diptera Tachinidae on artificial media (Bratti, 1993).

Besides these overviews, we have also carried out many laboratory studies on *in vitro* culture of parasitoids, mainly with Tachinidae and, in particular, *Pseudogonia rufifrons* Wied., an olygophagous species attacking the larvae of Lepidoptera Noctuidae in the Far East, Africa and southern Europe. This tachinid was reared, with greater or lesser success, on sub-natural and meridic diets by Bratti and Monti (1988), Bratti (1990), Fanti (1990), Bratti and Benini (1991), Fanti and Bratti (1991). Moreover, some trials were performed with other Tachinidae, i.e. *Palexorista laxa* (Curr.) (Bratti and Nettles, 1988) and *Eucelatoria bryani* Sabr. (Bratti and Nettles, 1992) as well as with a hymenopterous species, the chalcidid *Brachymeria intermedia* (Nees), which was reared from egg to adult on sub-natural (Dindo, 1990) and oligidic diets (Dindo and Campadelli, 1992).

Having verified that these parasitoids are capable of developing out of a host, even on media based on alien-to-host materials, the following, decisive step was to ascertain the possibility of mass-rearing them on inexpensive diets.

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Mellini (1992) considered the possibility of testing mammal serum as the main ingredient of artificial diets for tachinid parasitoids, given that Tachinidae are related to Sarcophagidae and Calliphoridae, which include several species capable of causing myiasis in mammals, and they themselves comprise a few species which have been known to cause accidentally myiasis in man. A first attempt at rearing *P. rufifrons* in a diet consisting of bovine serum (78-80%), host pupae extract (18-20%), small amounts of trehalose and egg yolk was made by Mellini *et al.* (1993). On such diets, quite high yields of pupae were obtained from parasitoid first-instar larvae, collected from host larvae muscles, and second-instar larvae, collected from host prepupae, with the adults, however, failing to completely emerge from the puparia. Slight modifications subsequently made to diet composition and preparation led to low yields of completely emerged adults being obtained (Mellini and Campadelli, unpublished data).

It must be, however, emphasized that *P. rufifrons* belongs to an evolute group of Tachinidae. It is an obligate larval-pupal parasitoid with microtype eggs, the development of which is highly influenced by the host physiology. It was, therefore, decided to test the same diets on *Exorista larvarum* L., a less evolute tachinid with macrotype eggs, which attacks Lepidoptera as well. This species, the development of which is substantially independent of host physiology, behaves gregariously.

Materials and methods

A. Brief remarks concerning the biology of *Exorista larvarum*.

E. larvarum is a very well-known polyphagous parasitoid of Lepidoptera, more than fifteen families having been reported amongst its hosts. It is distributed throughout Europe, Northern Africa and several regions of Asia.

Its biology was thoroughly studied by Hafez (1953) in Egypt in the host *Prodenia litura* F. (Lep. Noctuidae). His data were mostly confirmed by the observations made by us following the breeding of this tachinid on the factitious host *Galleria mellonella* L. (Lep. Galleriidae) under laboratory conditions, at 27°C, 65-70% R.H. and a 16:8 (L:D) photoperiod. Females deposit macrotype dehiscent eggs upon the host body. The incubation period is about 3 days. Hatching takes place by lifting up the convex and dorsal surface of the egg. The line of fracture extends along the anterior half of the egg separating its ventral surface from the dorsal arch. The parasitoid larva can penetrate the host integument directly in front of the egg. In most cases, however, larvae crawl on the host surface before beginning to bore into it; therefore a certain distance is found between the anterior end of the egg and the edge of the entrance hole. After the larva has bored its way into the host body, an integumental primary respiratory funnel is formed.

Successful parasitization only occurs when the eggs of *E. larvarum* are deposited on 1- to 4-day old last instar larvae. In such hosts, the duration of the parasitoid larval development is about 7-8 days. Earlier host stages, the duration of which is usually shorter than that of parasitoid embryogenesis, are generally unsuitable, since most eggs can be removed before hatching, together with the larval exuvia, during moulting.

The larva attains maturity in the mature larva or pupa of the host, depending on the stage of development of the host at which the parasitoid attacks it. Parasitoid development, therefore, appears to be substantially independent of host physiology. In fact, Hafez (1953) showed that about two generations of *E. larvarum* develop for each generation of *P. litura* in field conditions in Egypt. As there were about 7 generations of *P. litura* per year, he assumed that there were about 15 generations of *E. larvarum*.

In most cases, pupation occurs next to host larva remains. In some cases puparia may form within the host body and are orientated the same way as the host. The duration of the pupal stage is about 10 days.

All parasitoids employed for the present study were obtained from a stock-colony maintained in the laboratory using *G. mellonella* as a factitious host. The colony was established in 1992 by our cooperator, Dr. Amadou K. Coulibaly, from a few adult parasitoids reared from larvae of *Hyphantria cunea* Drury (Lep. Arctiidae) which had been collected in the province of Bologna.

B. Diet composition.

Two very similar diets were tested, the composition of which was the following.

Bovine serum	g 7.5
Extract (diet I) or homogenate (diet II) of *G. mellonella* pupae	g 2
Additives	g 0.5

Host homogenate and extract were obtained by the methods described by Bratti and Monti (1988) and Bratti (1989), respectively.

C. Experimental design.

Small amounts of diet, approximately 0.62 cc, were pipetted into wells of Multidish Nunc tissue culture plates featuring a total of 24 wells, 12 of which were supplied with diet I and the other 12 with diet II. Each well had a diameter of 16.5 mm. Diet thickness in the wells was about 3.2 mm.

E. larvarum eggs were collected from *G. mellonella* last instar larvae which were superparasitized by exposing about 10 individuals to about 100-150 adult parasitoids for half an hour. Eggs were removed from the host integument by spatulate pins and placed individually into wells 24 hours after diet preparation. Two eggs were introduced in some of the wells, in order to observe the larval behaviour in a gregarious situation in an artificial environment.

Plates were put into glass Petri dishes in which a little container with sterile distilled water was also placed, in order to maintain humidity at high.

In all of the experiments, the plates were kept in the dark at 25-27°C throughout the test except when they were removed for examinations, which were initially performed every other day and after a week once a day. As soon as the puparia formed, they were removed, weighed and transferred into cages where adult emergence occurred.

Materials (such as instruments and glassware) were sterilized by autoclaving for

12 minutes at 130°C and 2 bars. All procedures, including visual examinations, were performed in a laminar flow hood.

Six replicates, plus 2 preliminary ones, were carried out, each comprising 2 plates (48 wells in total).

Results and discussion

1. Behaviour of *E. larvarum* larvae.

The larvae hatched on the gelled media, independently of the egg position, whether on their side or on their back, i.e. inversely to their natural position on the host.

A quite high percentage of eggs did not hatch, especially if they had been deposited by young females. Moreover, a few eggs were damaged when removed from the host cuticle, to which they were attached by the fixing apparatus studied by Gardenghi *et al.* (1993). The percentages of eggs hatched considerably varied among replicates as well as between treatments of each replicate.

The newly-hatched larvae usually crawled for some time on the diet before boring into it. As a consequence, in most cases they eventually sank into the pabulum on the periphery of the well. Some larvae also dug tunnels deep into the diet, the consistence of which was pasty. These are the main differences observed between larval behaviour in the diet and in the host. In the latter, the newly-hatched larvae usually move away from the egg only for a short distance before penetrating the host integument, where they anchor themselves to the entrance hole with the last abdominal segment. In most cases, however, the larvae assumed the same position in the diet as in the host, i.e. after sinking into the pabulum the posterior pair of spiracles was maintained closely associated with the entrance hole. The oxygen requirements of the larvae in the diet were thus met.

Given the consistence of the diet, both the hole and the last part of the tunnel were gaping. In the latter, the larva last abdominal segments kept moving backwards and forwards. Larvae were seen to exhibit the same behaviour in the host as well, while forming the respiratory funnel.

After sinking into the diet, the larvae grew and developed continuously and rapidly. Before moulting, a slight reddish halo began to appear on the tunnel walls, thus revealing the position of the narrow entrance hole.

About 3 days after hatching the larvae moulted to the second instar. The position of second instar larvae in the diet was the same as that of the first instar larvae. The entrance hole and tunnel increased in length and width, while the reddish pigmentation became more extensive and intense. A sort of rather thick sheath formed, seemingly similar to the respiratory funnel in the host.

After moulting to the third instar, the larvae abandoned the tunnel and dug wide holes deep into the diet, down to the floor of well. As a consequence, even the surface of the pabulum mass was broken up into big and tender fragments. This phase apparently corresponded to the "predatory" phase of the parasitoid development within the host, which occurs after the larva has abandoned the respiratory funnel.

In most cases mature larvae wandered off the media and crawled on to the periphery of the well, thus digging a furrow in the remains of the diet which were finally amassed at the centre of the well. Larvae generally pupated on the remains of the media, rarely in the depth of the upset pabulum. Nevertheless, some larvae abandoned the well or even the plate, in which case they pupated in the Petri dish where the plate was placed.

Two larvae were seen to be capable of developing in a single well without seemingly disturbing each other.

In conclusion, *E. larvarum* larvae exhibited very similar behaviour patterns both in the diet and the host. The main differences concern the wandering behaviour of the newly-hatched larvae and the pupation habits, as *in vivo* mature larvae do not usually wander about for a long time in search of a suitable place where to pupate but can even pupate within the host larva remains.

2. Pupal yields.

On media in which contamination by moulds and/or bacteria was limited, very few larvae died, most of which were of the first instar. Most parasitoids, therefore, completed their larval development. The percentage yields of pupae, based on the number of eggs hatched, were as high as 90%. Anomalous puparia formed in only a very few cases.

3. Weight of puparia.

Unlike what normally occurs, male puparia were generally heavier than female ones. As the differences were very small, however, mean weights were calculated without considering sex.

On diet I, the mean weight of 63 puparia obtained from solitary larvae (a) was 38.44 mg, whereas the mean weight of 32 puparia obtained from 2 larvae reared in a single well (b) was 42.38 mg.

On diet II, the mean weight of 83 puparia (a) was mg 48.77, whereas the mean weight of 26 puparia (b) was 41.62 mg.

The mean weight of puparia obtained from larvae reared individually did not differ from that of larvae reared gregariously. The most likely reason is that the standard quantity of diet was more than sufficient even for two larvae. In fact, a considerable amount of pabulum was not consumed. Vice versa, in *G. mellonella* the puparia from solitary larvae were generally heavier than those obtained from two larvae within a single host.

It must be emphasized that the mean weight of puparia formed *in vitro* was similar to that of puparia formed *in vivo*.

4. Development times.

On media not contaminated, development times were almost as fast as those *in vivo*. As expected, under the same temperature conditions, egg incubation period was the same as in the host. Actually, it might be somewhat longer in eggs laying in the diet on their dorsal side, as in these eggs the aeropyle function might be

partially or totally obstructed.

The duration of larval development *in vitro* was approximately 1-2 days longer than *in vivo*. This was at least partially due to the fact that, on diet, larvae did not start feeding immediately after hatching.

The duration of pupal development was similar *in vivo* and *in vitro*, being equal to approximately 10 days in both situations.

On our artificial diet, therefore, the whole life-cycle of *E. larvarum* was only slightly longer than in the host.

5. Adult emergence.

Adult emergence considerably varied among replicates, depending on diet contamination level by bacteria and fungi, and was apparently not affected by the size of puparia. In fact, even small puparia, with a weight of about 20 mg, were big enough to permit the adult to emerge, while, on the other hand, adults were not necessarily capable of emerging from big puparia having a weight of more than 65 mg.

The percentage yields of emerged adults, based on the number of puparia, were similar in diet I and in diet II, being as high as 70.5 in the former and 71.6 in the latter. Such percentages were calculated without considering the few individuals which emerged from puparia only with their forepart.

Puparia, which did not let the adult emerge, upon dissection revealed the presence of individuals which had died at different stages, from prepupa to completely formed adult.

6. Adult yield.

When calculations of percentage yields of adults were based on the number of eggs put on the diet, an overall yield of 36% was obtained. The greater loss was due to the quite high percentage of eggs which did not hatch (approximately 37%). Moreover, about 10% and 30% of individuals obtained following hatching died as larvae or as pupae respectively.

When calculations of percentage yields were based on the number of larvae obtained following hatching, adult yield was as high as 59%, a value comparable to *in vivo* yields.

This result, as well as the mean weight of puparia, demonstrated that the bovine serum-based media tested were well suitable for *E. larvarum*.

Anyway, in highly contaminated diets adult yields dropped, almost reaching zero in case of very early and widespread contamination by bacteria and moulds.

The adults obtained on the diet were completely efficient. They mated and the females oviposited on *G. mellonella* larvae. The eggs were viable and produced a normal second generation within the host.

7. Comparison between the suitability of diet I and diet II.

Pupal yields were approximately 12% higher in diet II than in diet I. The mean weight of the puparia obtained from solitary larvae was 26% higher in the former

than in the latter diet. Diet II, containing non-sterilized homogenate of pupae, was more easily contaminated by moulds and bacteria. Anyway, especially as compared to the other tachinids we attempted to rear on artificial diets, *E. larvarum* larvae exhibited a very high resistance to contamination. As the latter became more and more widespread on the diet surface, larvae sunk into the pabulum, down to the well floor. They grew very slowly and with difficulty, and in most cases survived for a long time, but eventually died. In several cases, however, undersized puparia formed. It is interesting to note that, *in vivo*, *E. larvarum* larvae developing at the expense of hosts affected by mortal diseases show a similar behaviour.

On contaminated media, the undersized puparia were usually located on diet surface, in the mycelium mass. Sometimes they let the adult emerge, which very soon became entangled in the thick spore layer.

Conclusions

Both diet I and diet II proved to be suitable for *E. larvarum*, notwithstanding the fact that diet II, containing non-sterilized homogenate of pupae, was more easily contaminated by moulds and bacteria. The diets were quite unexpensive and easy to prepare, being almost entirely made up of two ingredients (i.e. bovine serum and extract or homogenate of *G. mellonella* pupae).

E. larvarum appears to be very suitable for mass-production on artificial diet for inundative or augmentative release in IPM programmes, as it is gregarious and quite resistant to contamination and as it develops quite rapidly, independently of host physiology. We are presently continuing our studies in order to make the artificial culture of this tachinid more economical and efficient. First of all, we are trying to diminish the quantity of pupae extract (or homogenate), which is the most expensive component of the media so far tested. Moreover, we are attempting to rear larvae gregariously in glass Petri dishes rather than individually in the expensive plastic multiwell plates, which cannot be re-utilized. In the preliminary trials we performed, 6-cm diameter glass Petri dishes containing 15 cc of diet and 24 eggs gave adult and pupal yields similar to those obtained in the present study using multiwell plates containing the same total amount of diet (0.62cc X 24) and the same number of eggs, which had been placed individually into the wells. The weights of puparia and development times were also similar in the two situations.

Summary

The artificial culture of *Exorista larvarum* on the oligidic media utilized in this study represents one of the most successful attempts made up to now at rearing tachinid parasitoids *in vitro*.

In practice, the artificial media gave pupal and adult yields equal to those obtained by rearing *E. larvarum* on the factitious host *Galleria mellonella* L. *In vitro* and *in vivo* the weights of puparia and development times were also similar.

The oligidic media employed contained bovine serum (75%), *G. mellonella* pupae extract (diet I) or homogenate (diet II) (20%), additives (5%).

The parasitoid larvae exhibited a very similar behaviour in the media and in the host. First-instar larvae sank into the diet digging tunnels deep into it, but keeping the posterior pair of spiracles closely associated with the entrance hole through a pigmented siphon rather similar to the respiratory funnel which is formed *in vivo*. The position of second-instar larvae did not differ from that of first-instar ones. The entrance hole and tunnel progressively increased in length and width. Third-instar

larvae abandoned the tunnel and dug wide holes deep down into the diet, which was eventually broken into big fragments even on the surface.

The adults obtained on the diet were completely efficient. They mated and the females oviposited on *G. mellonella* larvae. The eggs were viable and produced a normal second generation within the host.

Besides being completely suitable for *E. larvarum*, both diet I and diet II were rather cheap and easy to prepare. However, further tests are being carried out in order to be able to rear this parasitoid more efficiently and less expensively.

Allevamento del parassitoide *Exorista larvarum* L. (Dipt. Tachinidae) su diete artificiali a base di plasma bovino.

RIASSUNTO

L'allevamento *in vitro* di *Exorista larvarum*, sulla dieta oligidica impiegata nella presente sperimentazione, costituisce uno dei tentativi meglio riusciti, nell'ambito dei parassitoidi appartenenti alla famiglia dei Ditteri Tachinidi.

In pratica, non sono emerse differenze, nè nella qualità, nè nella resa in adulti, fra l'allevamento su dieta artificiale e quello sull'ospite di sostituzione *Galleria mellonella* L. Infatti, i tempi di sviluppo, i pesi dei pupari e le percentuali di sfarfallamento degli adulti sostanzialmente coincidono nelle due condizioni sperimentali.

La dieta oligidica contiene plasma bovino (75%), estratto (dieta I) ovvero omogeneizzato di crisalidi (dieta II) di *G. mellonella* (20%), additivi (5%).

Il comportamento delle larve nel pabulum mima sorprendentemente quello esibito nell'ospite. In I età, esse penetrano nella dieta, mantenendo però gli stigmi posteriori in superficie tramite un sifone alquanto pigmentato analogo all'imbuto respiratorio indotto *in vivo*; in II età restano nello stesso cunicolo, che ampliano ed allargano progressivamente; in III età se ne allontanano scavando ampie cavernosità nella massa trofica, che alla fine sconvolgono anche in superficie.

Gli adulti sono risultati fecondi e le femmine hanno deposto regolarmente le uova sulle larve di *G. mellonella*, dando origine ad una seconda generazione di adulti efficienti.

La dieta, oltre che pienamente valida, è economica e di facile preparazione. Sono comunque in corso ricerche per abbassarne ulteriormente il costo e per semplificare al massimo le tecniche di produzione del parassitoide *in vitro*.

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