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Notes on the anatomy and histology of the female reproductive system of *Brachymeria intermedia* (Nees) (Hymenoptera Chalcididae) reared *in vivo* and *in vitro*. (*)

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INTRODUCTION

Brachymeria intermedia is a solitary polyphagous endoparasitoid of lepidopterous pupae, which is well distributed throughout southern Europe, northern Africa and some Asian regions bordering or close to the Mediterranean sea (Iraq, Turkey) (Dowden, 1935; Martelli & Arru, 1958). In Italy this chalcidid is very important as a member of the parasitoid complex of the lepidoptera which defoliate cork oaks in Sardinia [*Lymantria dispar* (L.), *Malacosoma neustria* L. and *Tortrix viridana* L.] (Martelli & Arru, 1958; Luciano & Prota, 1984; Delrio *et al.*, 1983; 1988).

In North America *B. intermedia* was repeatedly introduced from Europe from 1908 onward as a biological control agent for *L. dispar* (Howard & Fiske, 1911), but became established only in the '60s (Leonard, 1966). The results of augmentative release to suppress populations in *L. dispar* were inconclusive (Grimble *et al.*, 1976; Blumenthal *et al.*, 1979).

The mass production of *B. intermedia* for use in large scale IPM programmes requires, however, an improvement of the relevant rearing techniques. Research aimed at setting up artificial diets for this species is therefore of interest for its possible practical applications. At least theoretically, the *in vitro* rearing of parasitoids may in fact permit to eliminate the host and thus reduce production costs (Mellini, 1975; Nettles, 1990).

Recently, Dindo *et al.* (1994 a, b) cultured *B. intermedia* from the egg up to the adult stage on artificial oligidic diets based on different types of commercial

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meat homogenates for babies. The results were encouraging, but of scarce practical interest owing to the low adult yields obtained (33% on the most suitable diet) and to the fact that the substrates had all been added with a considerable amount of host material (20%). However, the adults which were actually obtained on all of the diets (including those which produced the lowest yields) were comparable in size to those usually obtained in the factitious host *Galleria mellonella* L. Moreover, these parasitoids normally mated and the females regularly oviposited in *G. mellonella* pupae producing offspring of both sexes. Nevertheless, the low yields obtained even in the best diets indicate that the artificial substrates employed are not perfectly suitable for *B. intermedia*. The fecundity of the females reared in such diets may therefore be lower as compared to that of *in vivo* reared females. The low number of parasitoids thus far obtained *in vitro* has not permitted to adequately investigate these issues yet. As a preliminary step, an anatomical and histological comparison was made between the reproductive system of females reared on the factitious host *G. mellonella* and *in vitro*.

Materials and methods

A laboratory colony of *B. intermedia* was maintained on *G. mellonella* pupae at 26°C±1°C, 60% r.h. and 16:8 (L:D) photoperiod. *G. mellonella* larvae were fed on the artificial diet developed by Campadelli (1973, 1986).

The parasitoid females oviposit inside the host pupae according to the behavioural pattern described in detail by Tucker & Leonard (1977).

The observations described here were carried out on 6 *in vivo* reared females and 4 females obtained *in vitro* according to the techniques described by Dindo *et al.* (1994 a,b). The artificial diet employed comprised 80% commercial veal homogenates for babies at the beginning of weaning (Plasmon®) and 20% extract of *G. mellonella* pupae.

Immediately after emergence, the females were placed in individual cages each containing two males, the adults being fed on a water-honey solution.

B. intermedia is a synovigenic species (that is, ovogenesis is not yet completed before oviposition begins, but continues throughout the life of the female, except under diapause conditions, see Flanders, 1950). In particular, *B. intermedia* eggs are not yet mature during the first two days following emergence, while oviposition begins from the third day onwards (Barbosa *et al.*, 1986). According to Drost & Cardé (1992), *B. intermedia* is capable of producing mature eggs regardless of host availability as well as of reabsorbing them in case of prolonged host deprivation and/or of adverse environmental conditions. It was thus decided to daily give each female two *G. mellonella* pupae beginning from the third day following emergence.

According to Barbosa *et al.* (1986), the greatest number of eggs is deposited by *B. intermedia* between the fifth and eleventh day. In the present study, therefore, the females (weighing between 10 and 12 mg) were dissected 7 to 8 days after emergence for the purpose of examining their reproductive system, which was studied both anatomically as a whole and in histological sections.The anatomical examination was carried out after the females had been killed by

lateral compression of the thorax. The genitalia were then removed in saline solution.The part being examined was placed on a slide and stained with two drops of 1‰ methylene blue, after which it was rinsed and finally a cover slide was placed over it for observation.

The histological sections were prepared according to the double inclusion technique. The reproductive system was isolated and the ovipositor removed (a necessary step as the ovipositor is too thick to cut). The first inclusion was then made in several drops of 1.3% agar suspension. When solidification occurred, a parallelepiped of gelatin was cut under the stereomicroscope in such a way that its longitudinal axis corresponded to the longitudinal axis of the embedded reproductive system. This small block was then immersed in Bouin's fixative fluid for two hours and then washed for several times in alcohol at 70° before carrying out the usual procedures for embedding in paraffin. As the paraffin is transparent and therefore the agar parallelepiped visible, the block could be appropriately orientated during cutting with the microtome so as to obtain cross or longitudinal sections of the embedded organ as desired. Finally, sections were cut at 8 μ thickness and stained with hemalum and eosin.

Results

All the females, whether reared *in vivo* or *in vitro*, produced offspring of both sexes, which demonstrates that fecundation had regularly occurred (as is known, chalcids display arrhenotoky, see Clausen, 1940).

Visual observations did not reveal any apparent differences between the reproductive system of the *in vivo* and *in vitro* reared females. This system is typically hymenopteran (D'Rozario, 1942), containing two ovaries each of which is comprised of three polytrophic ovarioles repeatedly folded on themselves (as already consistently observed by Dowden, 1935; Barbosa & Frongillo, 1979; Drost & Cardé, 1992).

Eight to ten follicles progressively increasing in size toward the calyx may be observed in each ovariole. The follicular epithelium surrounding the previtellogenetic and vitellogenetic oocytes is made up of relatively thick cuboidal-cylindrical cells (Fig. II, 2) (indicative of an intense synthesis and transport activity), while it becomes flattened and apparently inactive upon the eggs attaining maturity (Fig. II, 3).

One or two mature eggs were found in each ovariole, up to a maximum of 12 eggs per individual, in both the *in vivo* and *in vitro* reared females. Contrary to the reports of Dowden (1935) and Barbosa & Frongillo (1979), the observations made by Drost & Cardé (1992), according to whom each *B. intermedia* ovariole may contain more than one mature egg, were thus confirmed.

Eggs at a more or less advanced stage of reabsorption were found in some of the ovarioles of all the females examined. This is in line with the situation generally encountered in synovigenic hymenopterous parasitoids in which oogenesis and oosorption occur synchronously when hosts are absent or scarce (Flanders, 1942). Obviously, therefore, the two host pupae which were daily supplied to the

females examined by us were not sufficient to prevent oosorption. This finding would seem to be in line with the observations performed by King & Richards (1968), who showed that in *Nasonia vitripennis* (Walker) (Hymenoptera Pteromalidae) the egg which is not deposited within 24 hours begins to be reabsorbed.

Oosorption may also be observed by routine staining (hemalum and eosin) which reveals an irregular hue affinity of the egg cytoplasm and a progressive loss of eosinophilia. The histological observations of the basal portion of the ovarioles of both the *in vivo* and *in vitro* reared females revealed the presence of up to two follicles containing ovular cytoplasm residues crushed by the mature egg. Oosorption seems therefore to be a rather slow process. Indeed, in some cases a mature egg was found to have ruptured the oocyte being reabsorbed (Fig. II, 4).

The epithelium surrounding the degenerating oocyte is made up of cuboidal cells which in appearance are similar to those surrounding the developing oocyte; this apparently indicates that the epithelium has become once more active, albeit in reverse (King & Richards, 1968).

The trophocyte chamber is surrounded by a squameous epithelium, consisting of flat cell, among which some very thick cells are distinguishable. The epithelium has the same appearance throughout all layers of the ovariole. Vice versa, the thickness of the trophocytes gradually increases until the egg has almost reached the end of vitellogenesis, after which it rapidly declines.

No substantial differences were observed in the shape and histological structure of the spermatheca of both *in vivo* and *in vitro* reared females either. The spermatheca is of the type represented by ichneumonids, braconids and chalcidoids (Flanders, 1939), that is, the spermathecal gland empties into the lumen of the sperm duct (Fig. I; II, 5) (whereas in the type represented, for example, by *Apis mellifica* L., the gland discharges directly into the sperm capsule).

A delicate basement membrane forms the outermost coat of the spermathecal gland. The epithelium is very thick, made up of two layers and with a radial configuration. The cells of the external layer are thick and rich in vacuoles, while those of the internal layer outside the intima are much smaller.

The epithelium of the spermatheca is made up of a single layer of cells and the intima is very thin. The two colleterial glands are shaped like a flask and their respective ducts, which are linked to the common oviduct, are very short. The glands of all the specimens examined were found to be full of a secretion homogeneous in appearance, typical of a fluid substance which has been coagulated by the fixative (Fig. II, 6). The wall consists of a basement membrane, of a single-layered epithelium with cuboidal cells (the nuclei of which are located towards the periphery, indicative of an intense secretive activity) and of a very thin intima. The intima of the ducts is slightly ticker. The epithelium outside the intima consists of cylindrical cells which are as high as the cuboidal ones typical of the glands but thinner. The ovipositor anatomy is typical of terebrants in general.

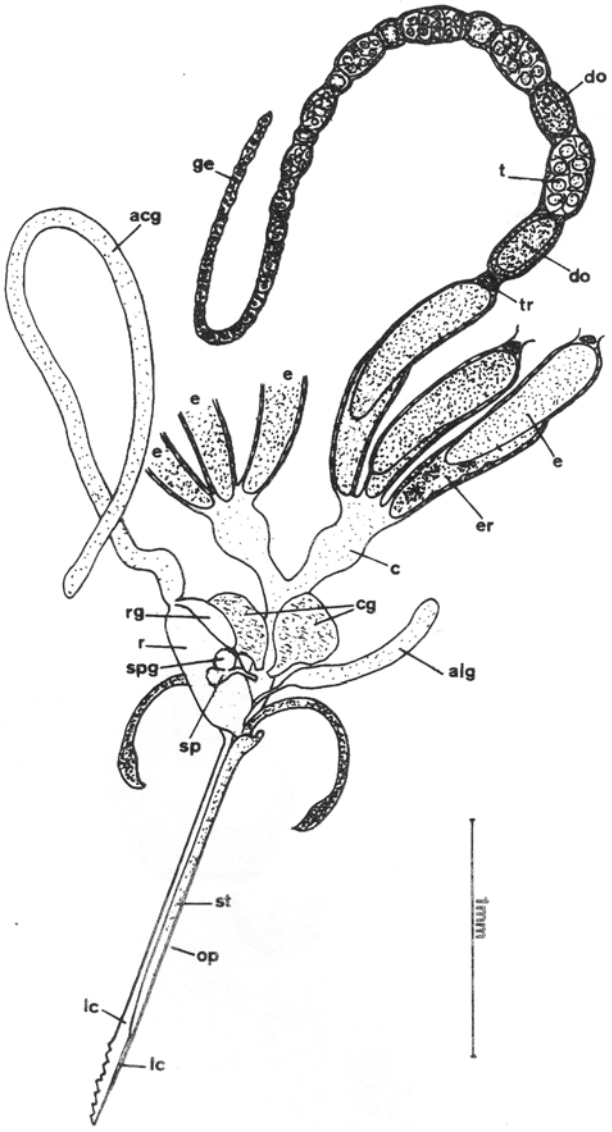
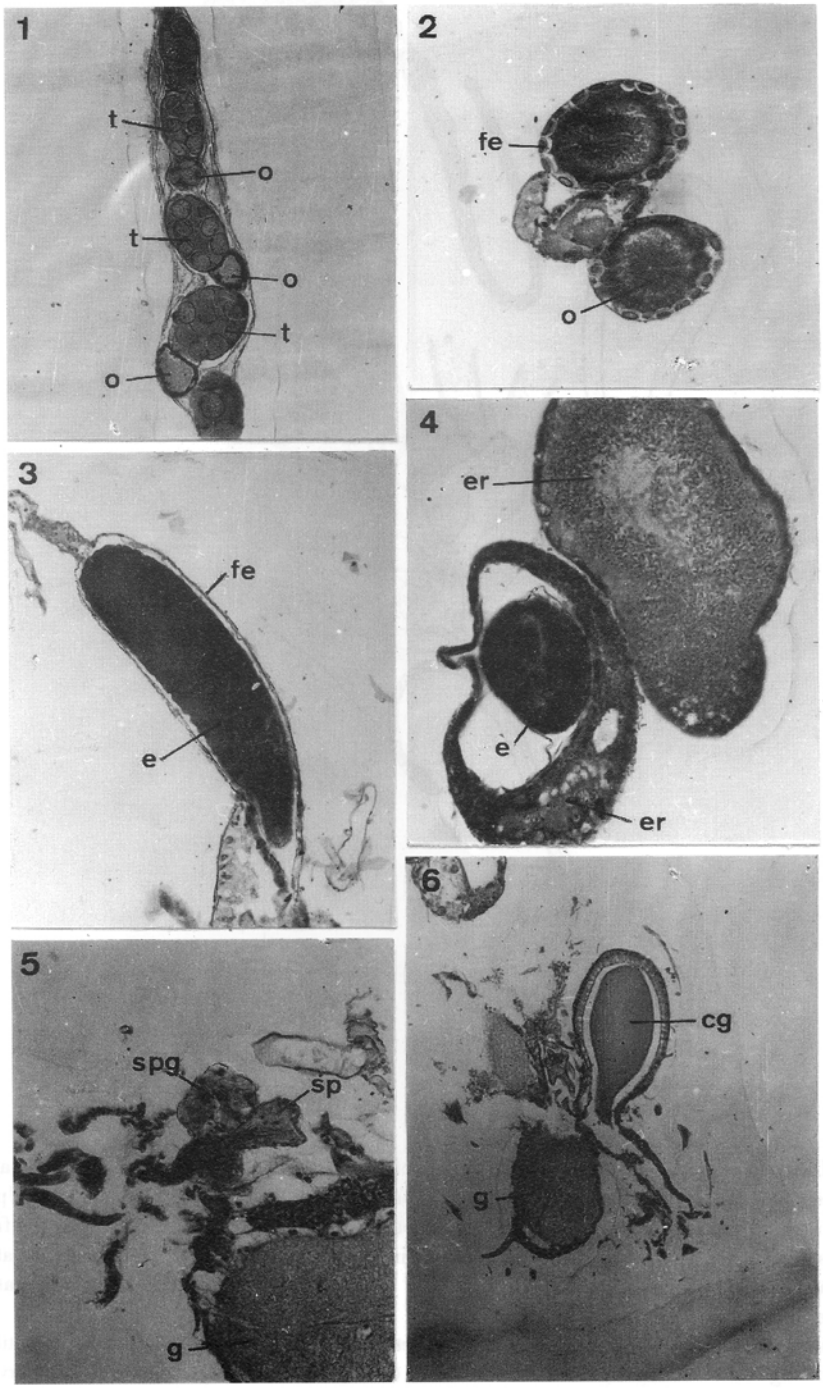


Fig. I. Diagram of the female reproductive system of *Brachymeria intermedia* at 8 days of imago life. Oogenesis proceeds in females deprived of host pupae and consequently unable to oviposit. Upon two eggs per ovariole reaching maturity, if the first is not deposited the membrane separating the first two follicles is ruptured. The two eggs become enclosed in a single follicle, one on top of the other, at which point the old oocyte degenerates and is reabsorbed. In some cases the younger oocyte penetrates into the older one as the latter begins to degenerate.

agc, acid gland; *alg* alkaline gland; *c*, calyx; *cg*, colleterial glands; *do*, developing oocyte; *e*, mature egg; *er*, egg being reabsorbed; *ge*, germarium; *lc*, lancet (Snodgrass, 1935); *op*, ovipositor; *r*, reservoir; *rg*, reservoir gland; *sp*, spermatheca; *spg*, spermathecal gland; *st*, stylet (Snodgrass, 1935); *t*, trophocytes; *tr*, trophocyte residue.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Conclusion

Increasing importance is being given to the quality control of mass-reared arthropods intended for use in biological and IPM programmes (Nicoli *et al.*, 1993). Notwithstanding, studies specifically aimed at evaluating the effectiveness of *in vitro* reared arthropods (in terms of longevity, fecundity, host and prey selection capacity) are still lacking. Such issues are bound to become the subject of studies which will attract increasing attention in the near future. Interest will be particularly focused on species for which mass *in vitro* production is efficient and economical. In this regard, *Trichogramma* spp. (Gao *et al.*, 1982; Liu *et al.*, 1985) and the tachinids *Exorista larvarum* (L.) (Mellini *et al.*, 1994) and *Eucelatoria bryani* (Sabr.) (Bratti & Nettles, 1992) currently appear to be the most promising.

With regard to *B. intermedia* (for which, owing to the low adult yields thus far obtained the practical future applications appear to be remote), the fecundity of the females reared on the diet developed by Dindo *et al.* (1994 a, b) may be considered to be comparable to that of those reared on the factitious host *G. mellonella*. In fact, no substantial anatomical and histological differences were observed in the reproductive system, and in particular in the ovaries, of the females obtained *in vivo* and *in vitro*. Moreover, the number of mature eggs (1 or 2 per ovariole) were not seen to substantially differ between the *in vivo* and *in vitro* reared females. These results however cannot yet be considered conclusive given the low number of specimens examined. Moreover, research specifically aimed at comparing the fecundity of females obtained *in vivo* and *in vitro* need to be carried out. Studies are currently being conducted on oligidic diets based on commercial veal homogenates added with ingredients other than pupal extract in an attempt to both reduce, if not to eliminate, this latter expensive component and to obtain higher adult yields so as to permit a more accurate investigation into the effectiveness on the adults obtained.

Fig. II. 1. Longitudinal section of a portion of ovariole close to the germarium of an *in vivo* reared female. Note the regular sequence of trophocyte chambers and of developing oocytes (90x). 2. Cross section of the ovary of an *in vitro* reared female with two oocytes close to maturation and a follicle with residues of oviposition (or oosorption?). Note the crown of rather thick follicular cells around the oocytes and the presence of yolk in the peripheral cytoplasm (90x). 3. Longitudinal section of an ovariole of an *in vitro* reared female close to the calyx. Note the presence of an egg ready for oviposition, surrounded by a rather thin follicular epithelium (90x). 4. Cross section of two ovarioles of an *in vivo* reared female close to the calyx. Note an egg at an early stage of oosorption (cytoplasm with irregular hue affinity) and another egg at an advanced stage of oosorption pierced by a mature egg (180x). 5. Cross section at the spermatheca level. 6. Section of the colleterial gland of an *in vivo* reared female (90x).

gc, colleterial gland; *e*, mature egg; *er*, egg being reabsorbed; *fe*, follicular epithelium; *g*, ganglion; *o*, oocyte; *sp*, spermatheca; *spg*, spermathecal gland; *t*, trophocytes.

SUMMARY

We compared the anatomy and histology of the reproductive system of female *Brachymeria intermedia*, a pupal parasitoid of Lepidoptera, reared on the factitious host *Galleria mellonella* and *in vitro*. The females obtained *in vitro* were cultured on artificial diet composed of 80% commercial veal homogenate for babies and 20% extract of *G. mellonella* pupae. Mated females received two host pupae per day from the third day after emergence and were dissected on day 7-8 of adult life. No apparent difference was noted in the reproductive system of specimens obtained *in vivo* and *in vitro*. This system is typically hymenopteran and consists of two ovaries each with three polytrophic ovarioles. Each ovariole contains 8-10 follicles and 1 or 2 ripe eggs. In all females partially absorbed eggs were observed. In several cases the younger ripe egg pierced through the older egg in absorption.

As the reproductive system of the females obtained *in vitro* and *in vivo* did not show any evident difference, it can be assumed that the fecundity of the former is comparable to that of the latter.

Alcune osservazioni sull'anatomia e l'istologia dell'apparato genitale di femmine di *Brachymeria intermedia* (Nees) (Hym. Chalcididae) allevate *in vivo* e *in vitro*.

RIASSUNTO

È stato eseguito un confronto, di tipo anatomico e istologico, fra apparati genitali di femmine di *Brachymeria intermedia* allevate, rispettivamente, sull'ospite di sostituzione *Galleria mellonella* e su una dieta artificiale costituita per l'80% da omogeneizzato commerciale di vitello per bambini e per il 20% da estratto di crisalide di *Galleria*. Le femmine, inseminate e fornite quotidianamente di due crisalidi ospiti a partire dal terzo giorno successivo allo sfarfallamento, sono state sezionate a 7-8 giorni di vita immaginale. Le osservazioni effettuate non hanno posto in evidenza palesi differenze tra l'apparato genitale degli esemplari ottenuti *in vivo* e *in vitro*. Tale apparato presenta caratteristiche comuni agli imenotteri in generale. Gli ovari sono costituiti da 3 ovarioli contenenti, ciascuno, 8-10 camere ovocitarie. Tutte le femmine esaminate presentavano 1 o massimo 2 uova mature per ovariolo, nonchè uova in fasi più o meno avanzate di riassorbimento. All'esame istologico è risultato che in più casi l'uovo maturo più giovane aveva trapassato l'uovo in degenerazione.

Non essendo state riscontrate evidenti differenze tra gli apparati genitali delle femmine ottenute *in vivo* e *in vitro*, si può presumere che la fecondità di queste ultime sia paragonabile a quella delle prime.

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