Entomologica, XIX, Bari, 16-XII-1984.

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Functional anatomy of *Dacus oleae* Gmel. female genitalia in relation to insemination and fertilization processes [2]

ABSTRACT

In *Dacus oleae* the spermatozoa are transported free from male to female by direct injection of the semen into an « insemination pocket » located as an atrium at the entrance of the spermathecal ducts on the inner dorsal wall (roof) of the vagina. Also the ducts of the accessory glands debouche into this pocket. Sperm migration to the receptaculum seminis takes place almost passively, helped by injection movements of the endophallum and by peristaltic contractions of the strong muscular sheath of the spermathecal ducts. Some chemotactic action on spermatozoa also seems to be performed by the spermathecal epithelial secretions. Just prior to ovulation, a limited number of spermatozoa are transported from the spermathecae to the « fertilization chamber », located in front of the insemination pocket on the ventral wall (floor) of the vagina. Functional anatomy, sperm movements (insemination and fertilization) and storage, and the conceivable role played in these processes by the secretions of the spermathecal secretory cells and the reproductive accessory glands have been investigated by direct observations in fresh saline (or after fixing and staining) whole mounts as well as through sections (from female just emerged, after copulation and during ovulation-fertilization) examined in light-and transmission and scanning electron microscopy.

CONTENTS

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[2] Presented at the XVII Int. Congr. Entom. (S8.1. Sperm transfer, storage and utilizzation in *Dacus oleae* Gmel.), Hamburg: August 20-26, 1984.

1. Introduction

The sperm-egg encounter occurs in insects only after many sorts of problems have been overcome in a sequence of processes such as sperm transfer from male to female (copulation-insemination), sperm storage for a short or long time, usually in a spermatheca, the release of spermatozoa from the spermatheca (at ovulation, just before an egg is laid), and the entry of spermatozoa into the egg through the micropyle(s) (fertilization, strictly speaking).

The transfer of semen can be effected by various mechanisms, but essentially there is a wide range of solutions between two extreme cases: transport by spermatophore, and direct injection into the spermatheca by means of an extremely long intromittent organ. Even though sperm transfer via spermatophore is considered to be a primitive feature, we can find such totally different mechanisms also among close related species such as (e.g.) *Rhodnius prolixus* and *Oncopeltus fasciatus* (Davey, 1965). Among *Diptera*, only few species of *Nematocera* have been reported to form spermatophores (Engelmann, 1970), while other *Nematocera* (Neumann, 1958) and *Cyclorrhapha* (De Marzo, Nuzzaci, Solinas, 1976; Lefevre, Jonsson, 1962) deposit free sperm deep into the female genitalia.

Our knowledge of the process of sperm migration or transport from the vagina to the spermatheca is very limited (Gupta, Smith, 1969). In some cases the said migration seems to be effected by a chemotactic action of the spermathecal secretions on the spermatozoa (Clements, Potter, 1967; Grodner, Steffens, 1978; Villavaso, 1975a), while in other cases there is evidence that the sperm is pumped into the spermatheca (Dallai, 1975) or at least the presence of a muscular sheath properly arranged around the spermathecal duct(s) suggests such process (Giglioli, 1963; Jones, Fischman, 1970).

Fortunately, there is fairly wide information on the presence and psysiology of a secretory epithelium (or discrete glands) in (or associated with) the spermatheca, whose secretion(s) presumably supply nutrients for the spermatozoa stored there, also in addition to the chemotactic substances above mentioned (Grodner, 1979; Hallberg, 1984; Happ, Happ, 1970; Villavaso, 1975a).

A fairly obscure problem is, thus far, the removing of spermatozoa from the spermatheca at the time of fertilization. In some instances, the said removal seems to be effected simply by contractions of spermathecal muscles (Dallai, 1975; Gupta, Smith, 1969; Villavaso, 1975b).

Also our present understanding of the process of fertilization is very poor and actually limited to the interesting contributions given by Leopold, Degrugillier (1973), Leopold, Meola, Degrugillier (1978) on *Musca domestica*, and Leopold (1980) on *Musca domestica* and other related muscoid

species. These contributions deal with the functional morphology of a « fertilization chamber » and the possible role of the accessory reproductive gland secretion(s) as responsible of the dramatic changes that occur both in the acrosomes of sperm and in micropyle region, when sperm-egg encounter takes place.

In the present work our aim was to contribute new information on the above mentioned open questions in general, as well as to present new findings on the reproductive biology of such an economically important insect as *Dacus oleae*.

2. Materials and Methods

Dacus oleae males and females, emerged from puparia collected directly in the field during October-December, were dissected at emergence or maintained alive on a diet with a mixture of feeding yeast and honey (1:2), while water was given separately. Observations were carried out on fresh saline (or after fixing and staining) whole mounts as well as through sections from females dissected: a) at emergence, b) during and after copulation, c) during ovulation-fertilization. For histological investigations female reproductive organs were fixed in cold solution of 4% glutaraldehyde on 0.1 M phosphate buffer (pH 7.2) containing 5% sucrose for three hours, washed overnight with the same buffer, and post-fixed in 2% Osmium tetroxide in the same phosphate buffer for two hours, washed with the same buffer, dehydrated in ethanol and embedded through propylene oxide in Araldite 502. Thick (0.5-1.0 μm) sections for light microscopy were stained with 1% toluidine blue in 1% aqueous borax for one minute and observed-photographed under a Zeiss III photomicroscope. Thin sections for electron microscopy were stained sequentially with uranyl acetate and lead citrate, and examined in a Zeiss EM 109 T.E.M. Whole mounts of the female internal genitalia, fixed and dehydrated using the above described procedures and gold coated in an Edwards S 150 A sputter coater, were viewed in a Cambridge Stereoscan 100 S.E.M.

Explanation of the symbols used in the Figs:

	English	*Italiano*
AE	aedeagus	edeago
AG	accessory glands	ghiandole genitali accessorie
AL	autophagosome	autofagosoma
AV	anterior vagina (enlarged part)	tratto anteriore (ingrossato) della vagina
BM	basement membrane	lamina basale
CC	cuticulogenic cell	cellula cuticologena
CO	common oviduct	ovidutto comune
CP	cuticular microprocesses	microprocessi cuticolari
DI	distal valve	valvola distale
DU	ductule	ductulus
E	insemination pocket entrance	ingresso della tasca d'inseminazione

EA	anterior extrinsic muscles	muscoli estrinseci anteriori
EC	epithelial cells	cellule epiteliali
EG	eggs	uova
EM	extrinsic muscles of fertilization chamber	muscoli estrinseci della camera di fecondazione
EN	endapparatus	apparato terminale di raccolta del secreto
EP	posterior extrinsic muscles	muscoli estrinseci posteriori
EPH	endophallus	endofallo
FB	fat body capsule	capsula adiposa
FC	fertilization chamber	camera di fecondazione
G	Golgi complex	apparato di Golgi
GD	accessory gland duct	dotto di ghiandola accessoria
GP	gonopore	gonoporo
HY	hypopygium	ipopigio
IL	insemination pocket lobe	lobo della tasca d'inseminazione
IM	intrinsic muscles	muscoli intrinseci
I	intima	intima
IP	insemination pocket	tasca d'inseminazione
IR	injection ram	pistone iniettore
IS	integumental swelling	ispessimento tegumentale
L	lumen	lume
LO	lateral oviduct	ovidutto laterale
M	mitochondria	mitocondri
MA	muscle attachements	attacchi muscolari
MC	microvillar cavity	cavità intermicrovillare
MF	muscular fiber	fibra muscolare
MP	micropyle	micropilo
MS	muscular sheath	tunica muscolare
MV	microvilli	microvilli
N	nucleus	nucleo
NU	nucleolus	nucleolo
OP	ovipositor	ovopositore
OV	ovary	ovario
P	pumping device	dispositivo pompante
PR	proximal valve	valvola prossimale
PV	posterior vagina (tabular part)	tratto posteriore (tubolare) della vagina
RE	rough endoplasmic reticulum	ergastoplasma
RS	receptaculum seminis	ricettacolo seminale
SA	spermiophore alveoli	alveoli spermiofori
SC	secretory cell	cellula secretrice
SD	spermathecal duct	dotto di spermateca
SE	septate junctions	desmosomi settati
SZ	spermatozoa	spermatozoi
TR	tracheole	tracheola
V	vesicles	cisterne
VIII	eighth abdominal segment	parte dell'ottavo urite

3. Results and Discussion

3.1 - Gross morphology of the female internal reproductive system.

The female internal genitalia of *Dacus oleae* (Fig. 1) consist of: paired ovaries (small and bud-shaped when immature, large and spindle-shaped in ovigerous specimens) with meroistic polytrophic ovarioles; two short lateral

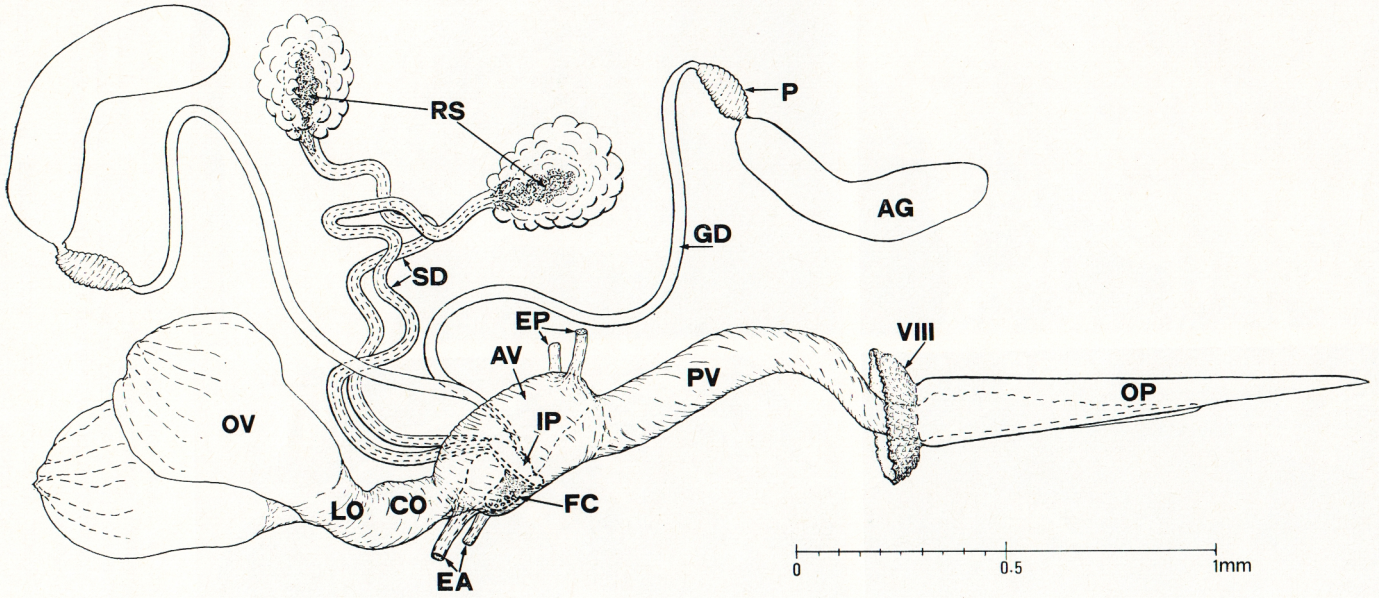

Fig. 1 - Genitalia of newly emerged female (semi-schematic lateral view).
Apparato genitale di femmina neosfarfallata (disegno semischematico).

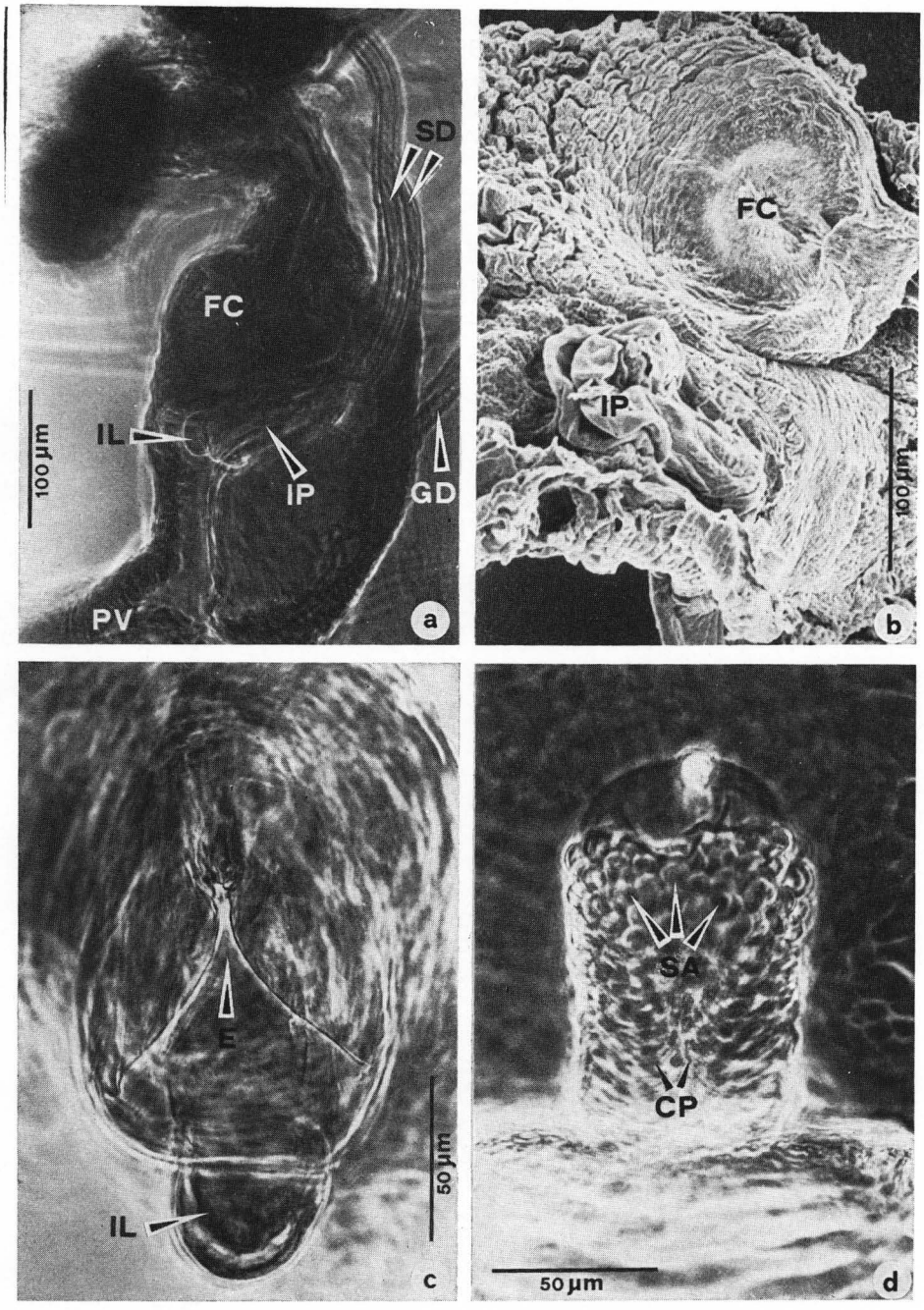

Fig. 2 - Anterior vagina: a) mesal optic section (left side); b) scanning electron micrograph showing (after a longitudinal incision along left side) the inner ventral wall (floor) with fertilization chamber entrance (FC), and the inner dorsal (roof) with insemination pocket; c) photomicrograph of insemination pocket viewed from the vaginal lumen; d) frontal optic section of fertilization chamber.

Tratto anteriore della vagina: a) sezione ottica sagittale mediana; b) micrografia elettronica a scansione mostrante le pareti interne (ventrale con camera di fecondazione e dorsale con tasca di inseminazione) dell'organo aperto longitudinalmente ad arte; c) tasca d'inseminazione vista dal lume vaginale; d) sezione ottica frontale della camera di fecondazione.

oviducts, and a short common oviduct followed by a long vagina. In addition to these primary parts, there are a pair of spermathecae and paired accessory glands.

In particular, the vagina exhibits two distinct parts: a posterior tube-shaped tract, and an anterior (Fig. 1, AV) one swollen, ovoid, receiving the ducts of spermathecae and accessory glands through its dorsal wall, and having peculiar structures related to copulation (i.e. « insemination pocket »: Fig. 1, IP) and fertilization (« fertilization chamber »: Fig. 1, FC). Moreover, two pairs of prominent muscle bands project from the anterior ventral (Fig. 1, EA) and posterior dorsal (Fig. 1, EP) surfaces of the anterior vagina and attach to the body wall at the seventh abdominal segment. The anterior vagina is actually a bursa copulatrix fit to house the « distal piece » of the aedeagus during copulation in order to put in close contact male gonopore and spermathecal duct entrances.

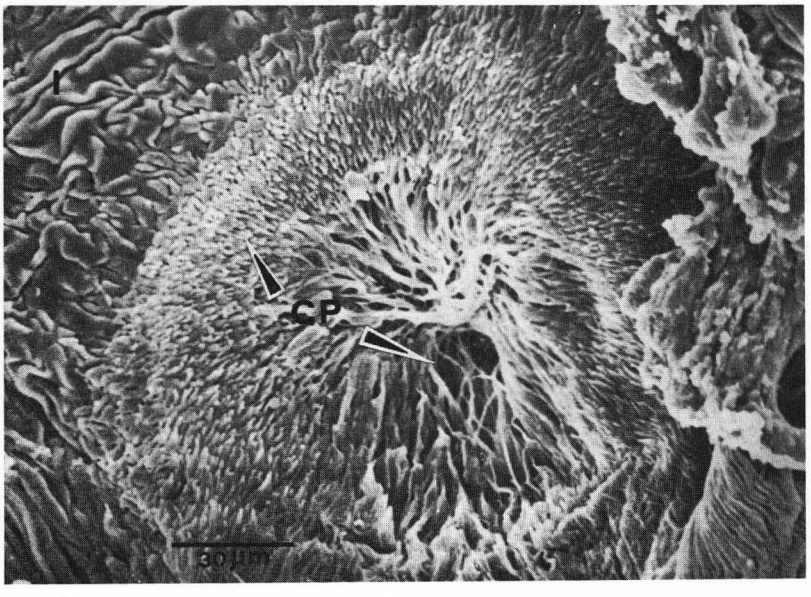

Fig. 3 - Scanning electron micrograph of the fertilization chamber entrance.
Micrografia elettronica a scansione dell'ingresso della camera di fecondazione.

The insemination pocket (« camera dell'ovidutto »: BERLESE, 1902; « dorsal valve »: DEGRUGILLIER, LEOPOLD, 1973) consists of a thick and soft (inner texture spongy) integumental structure (Figs 1-4: IP) which gradually arises

on the dorsal wall (roof) of the anterior vagina, and on the thickest point forms a cavity partially covered by an integumental fold (Fig. 2, c). Within that cavity the ducts of the spermatheca and accessory glands open.

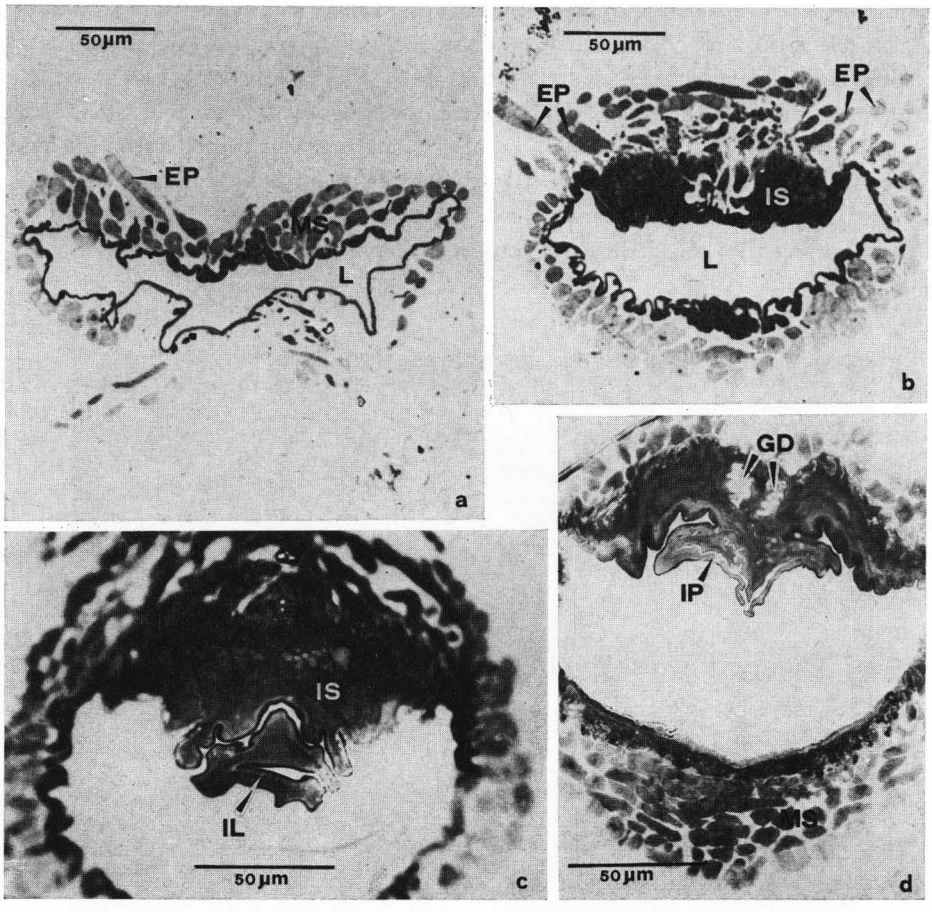

Fig. 4 - Anterior vagina, successive cross sections made: a) near the organ posterior end; b) just behinde the insemination pocket, and showing the integumental swelling (IS) on which the latter onwards arises; c) through the insemination pocket lobe (IL); d) through insemination pocket, near the debouches of the accessory gland ducts.

Tratto anteriore della vagina, sezioni trasversali successive effettuate: a) presso l'estremità posteriore dell'organo; b) appena dietro la tasca d'inseminazione, mostrante l'ispessimento tegumentale (IS) sul quale più avanti si differenzia quest'ultima; c) all'altezza del lobo (IL) della tasca d'inseminazione; d) presso gli sbocchi dei dotti delle ghiandole accessorie (GD) nella tasca d'inseminazione.

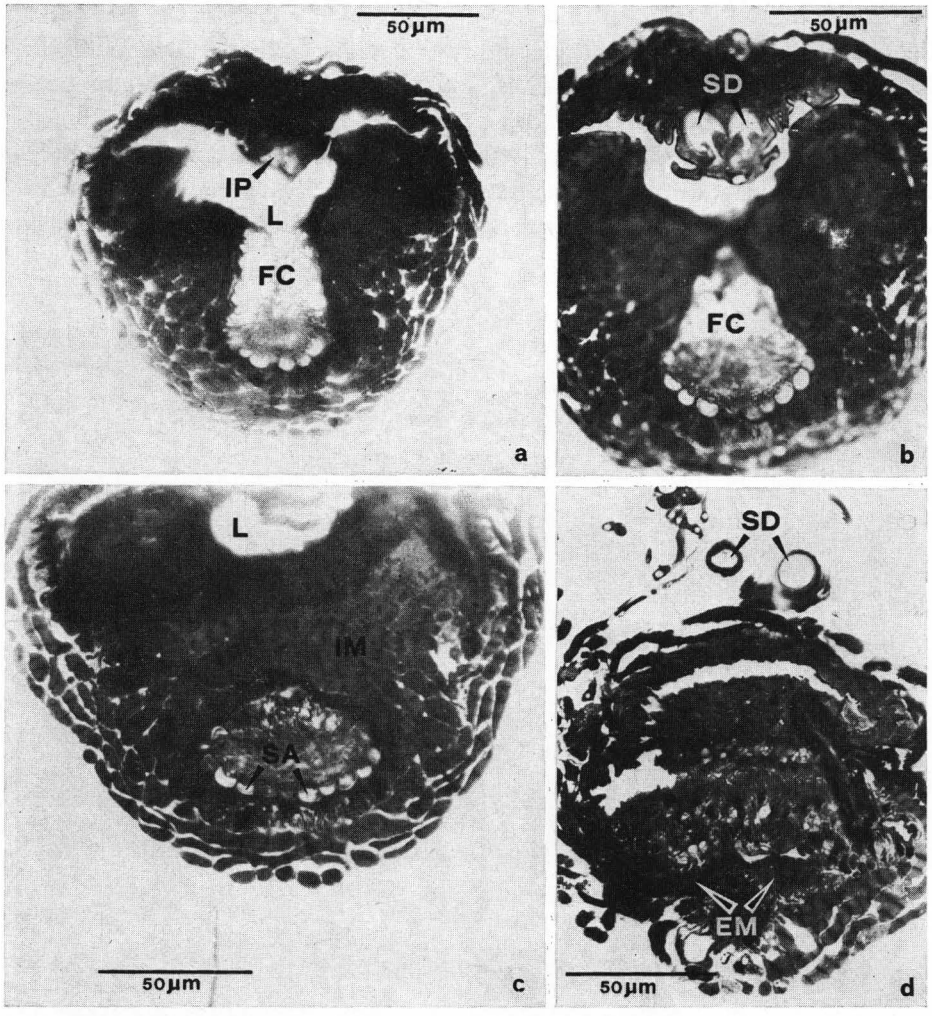

Fig. 5 - Anterior vagina, successive cross sections made: a) where the insemination pocket entrance and fertilization chamber entrance (at rest) come into close contact with each other; b) through insemination pocket (and fertilization chamber) near the spermathecal duct debouches (proximal valve is visible); c) through the anterior half of the fertilization chamber; d) near the vagina anterior end.

Tratto anteriore della vagina, sezioni trasversali successive effettuate: a) dove combaciano (in posizione di riposo) l'ingresso della tasca d'inseminazione con quello della camera di fecondazione; b) presso gli sbocchi dei dotti delle spermateche nella tasca d'inseminazione; c) all'altezza della metà anteriore della camera di fecondazione; d) presso il confine anteriore della vagina.

The fertilization chamber (Fig. 1: FC) (« anterior chamber »: DEGRUGILLIER, LEOPOLD, 1973; « fertilization chamber »: LEOPOLD, MEOLA, DEGRUGILLIER, 1978; « ventral receptacle »: LEFEVRE, JONSSON, 1962) consists of a posteriorly directed sacklike structure surrounded by a strong muscular sheath (Figs 5, 20: IM) and located on the anterior ventral wall (floor) of the vagina. Said chamber is lined by a widely deformable intima smooth at the bottom and dorsal anterior walls and bearing about 40 irregularly round pits (about 3 μm deep and on

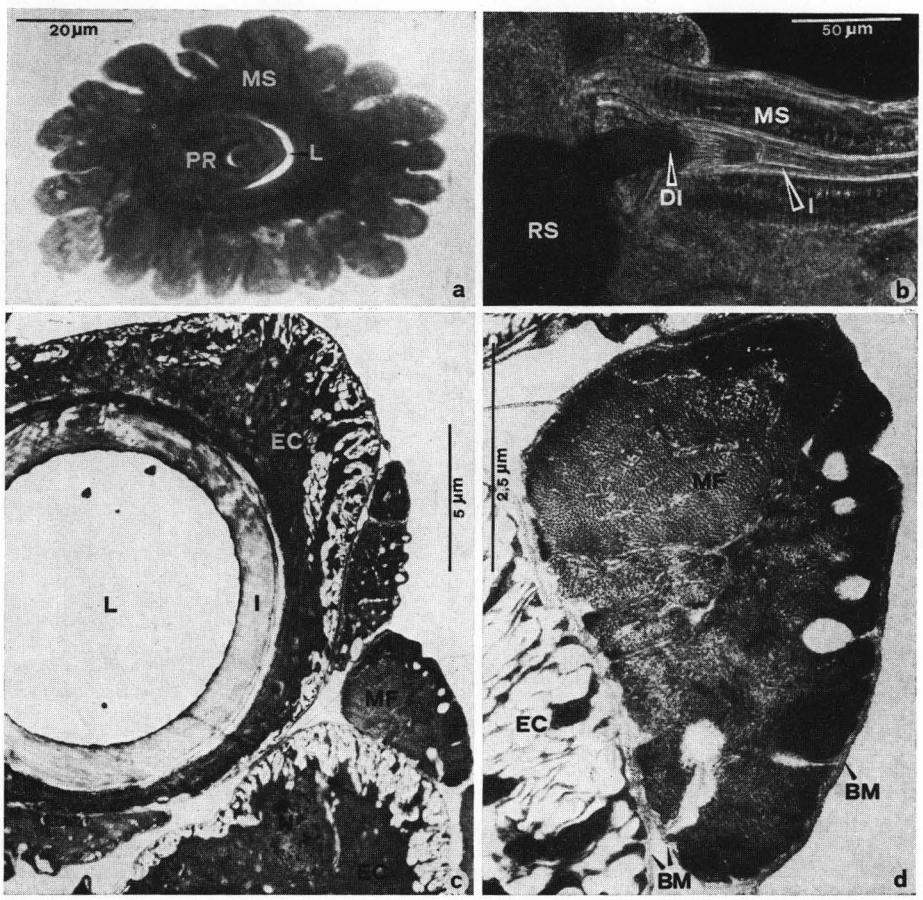

Fig. 6 - Spermathecal duct details: a) cross section through the proximal valve; b) longitudinal optic section through the distal valve; c) transmission electron micrograph of intermediate cross section; d) detail of the latter.

Spermateca, particolari del dotto: a) sezione trasversale della valvola prossimale; b) sezione ottica longitudinale della valvola distale; c) micrografia elettronica a trasmissione di sezione trasversale intermedia; d) particolare di quest'ultima.

average 6 μm in diameter), here named « spermiophore alveoli » (Figs 2, 4, 20: SA), on the ventral anterior wall. The rest of the chamber intima is covered with cuticular microprocesses (Figs 2, 3, 20: CP) particularly abundant all around the chamber entrance.

Each spermatheca (Figs 1, 8) consists of: a) a receptaculum seminis (Figs 1, 8, etc.: RS) made of an irregular, coiled, sclerotized, not deformable, dark brown cuticular tube, bounded by epithelial cuticulogenic and secretory cells, the whole embedded in adipose tissue; and b) a ductus spermathecae (Fig. 1: DS), or spermathecal duct, about 1500 μm long, with lumen lined by strong and elastic intima (Fig. 6), rather regular and about 10 μm in diameter, ending with two valves (a proximal: PR, Fig. 6; and a distal: DI, Figs 6, 8), invested in a strong muscular sheath made of only a single layer of longitudinal, twisted fibers (Figs 6, 7: MS, MF). Given their peculiar arrangement, these muscular fibers, when contracting, may cause a radial extension of the duct lumen and, consequently, a

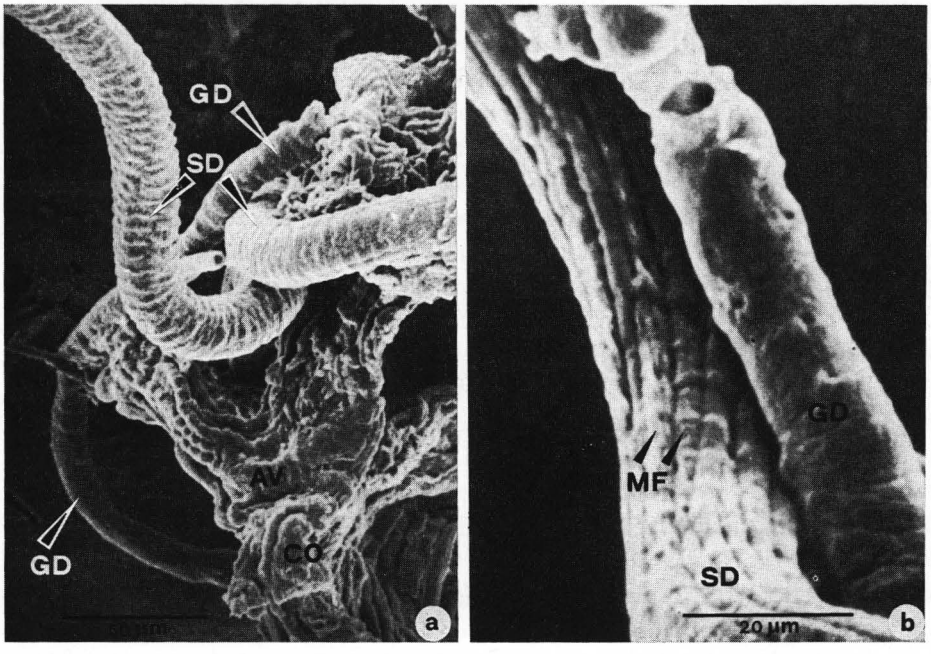

Fig. 7 - Scanning electron micrograph showing: a) anterior vagina (dorsal view) with proximal tract of the spermathecal ducts and of the ducts of the accessory glands; b) detail of a spermathecal duct and an accessory gland duct.

Micrografia elettronica a scansione mostrante: a) il tratto anteriore della vagina (dal dorso) con la parte prossimale dei dotti delle spermateche e delle ghiandole accessorie; b) particolare di un dotto di spermateca e di uno di ghiandola accessoria.

partial vacuum in it. While the two mentioned valves may ensure one-way flow of the sperm along the ducts towards the receptaculum during insemination, and vice versa just prior to fertilization.

Each accessory gland (Figs 1, 13, 17: AG) consists of: a) a sacklike body relatively slender in newly emerged females and much more distended and full of secretion in ovigerous females; b) a pumping device spindle shaped, invested in a very strong muscular sheath (Figs 1, 16: P), situated at the outlet of the gland body; and d) an efferent duct (Figs 1, 7: GD) very simple, a little longer and thinner than spermathecal ducts.

3.2 - Histology, ultrastructure and physiology of the spermathecal epithelium.

The epithelium bounding the receptaculum seminis consists of a single layer of cuticulogenic and secretory cells together (Fig. 9).

The cuticulogenetic cells are very thin and they lie between intima and secretory cells, but frequently separating the latter from one another by very thin folds (Figs 9, 10). They exhibit a relatively large nucleus rather irregular in shape (Fig. 10), and are tightly packed with ribosomes (Fig. 10, b) and mitocondria (Fig. 12, b), so that these cells have a much higher contrast than secretory ones. The cell surface bounding the cuticle often appears to be considerably folded because of somewat compressed microvilli (Fig. 12, b). These cuticulogenic cells are connected with one another by septate junctions (Fig. 10, b) as usual in epithelial cells. The same cuticulogenic cells produce the intima of both the receptaculum seminis and secretory cell ductules described below (Fig. 9, d). The intima inner surface is normally thrown into folds (Figs. 9, a; 10, b; 12, b) resembling irregular tracheal taenidia.

The single secretory cells behave like rather isolated units; in fact, there are only loose junctions between one another (Figs 11, a; 12, a), and the same happens between secretory cells and cuticulogenics (Figs. 10, a; 12, a) with the exception of the boundaries around the ductules, were septate junctions (Fig. 12, c) are frequent between secretory and cuticulogenic cells. Each secretory cell has a basal region (Figs. 9, a, b; 10; 11) containing nucleus and typical cytoplasmic organelles, and an apical region (Figs 9, a, c, d; 12) with a large endapparatus connected with a ductule (Figs. 9, d; 12, c, d) leading to the spermathecal lumen (Figs. 9, d; 12, c). The nucleus is of an irregular oval shape and displays a prominent nucleolus (Figs 9, b; 11, a). The endoplasmic reticulum (mainly rough) is very dilated and forming numerous large cisternae which give this region a spongy aspect (Figs 9, 12). There are many Golgy complexes located

among the cisternae (Figs 9, b; 11, a, b, c), and relatively few mitochondria. Autophagosomes are relatively abundant within secretory cells of only sexually mature females (Fig. 11, c, d). The said apical region exhibits a bulb-like, 2-3 μm wide, sclerotized (intima about 1 μm thick) endapparatus (Figs 9, 12) connected with receptaculum seminis through an efferent ductule (Figs 9, d; 12 c, d) also well sclerotized, 3-4 μm long and about 1 μm in diameter. The endapparatus is surrounded by microvilli which are relatively few and almost all abuting it in

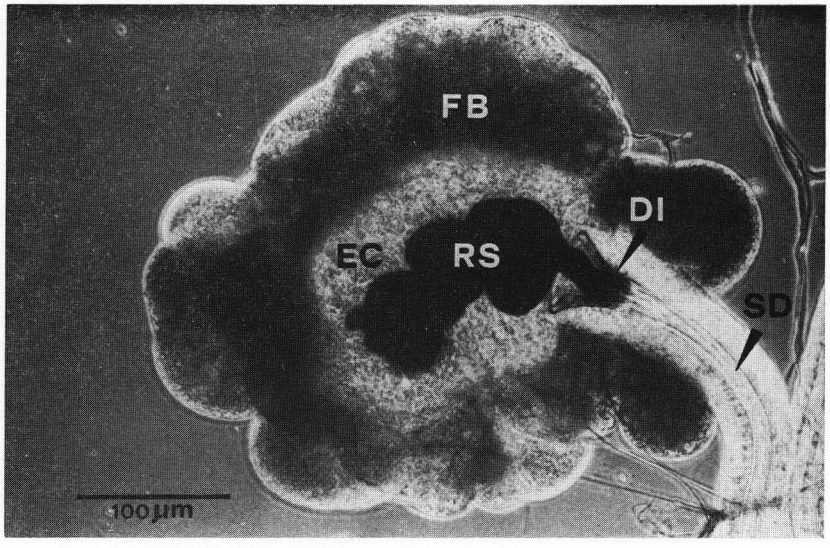

Fig. 8 - Phase contrast microphotograph of a fresh spermatheca (slightly pressed under a coverglass).

Microfotografia in contrasto di fase di spermateca fresca (appena schiacciata lateralmente sotto il coprioggetto).

newly emerged females (Fig. 9, a, c), while in sexually mature ones (Fig. 12) they are very numerous, tight packed together, sometimes abuting the endapparatus and sometimes not because of the more or less developed microvillar cavity (Fig. 12). A flocculent electrondense material seems to be the same in the microvillar cavity, endapparatus, ductule, and spermathecal lumen, both in newly emerged and mated females (Figs 9, a, d; 12).

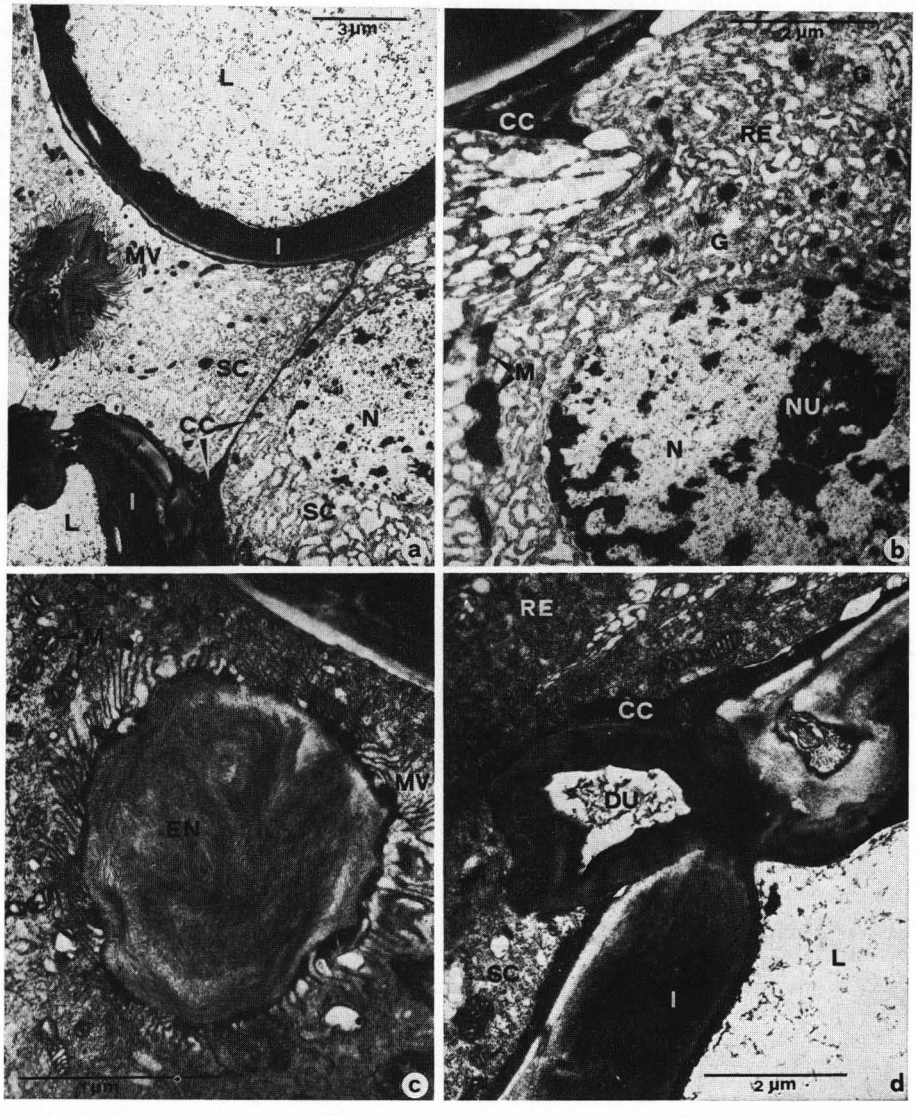

Fig. 9 - Spermatheca, receptaculum seminis of a newly emerged female. Transmission electron micrographs showing details explained in the text.

Spermateca, receptaculum seminis, di femmina neosfarfallata. Micrografie elettroniche a trasmissione illustranti particolari spiegati nel testo.

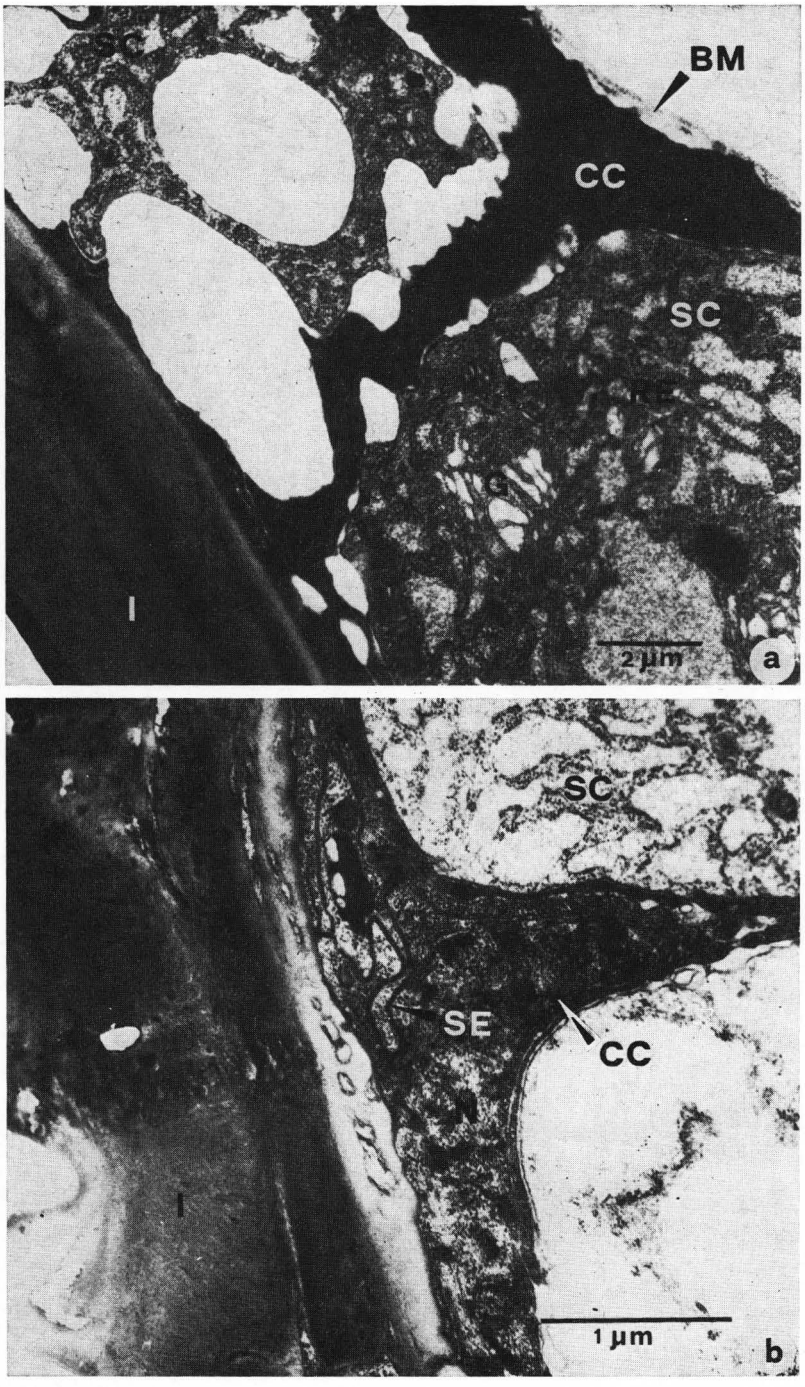

Fig. 10 - Spermatheca, receptaculum seminis of a newly emerged female. Transmission electron micrographs showing details explaned in the text.

Spermateca, receptaculum seminis di femmina neosfarfallata. Micrografie elettroniche a trasmissione di particolari spiegati nel testo.

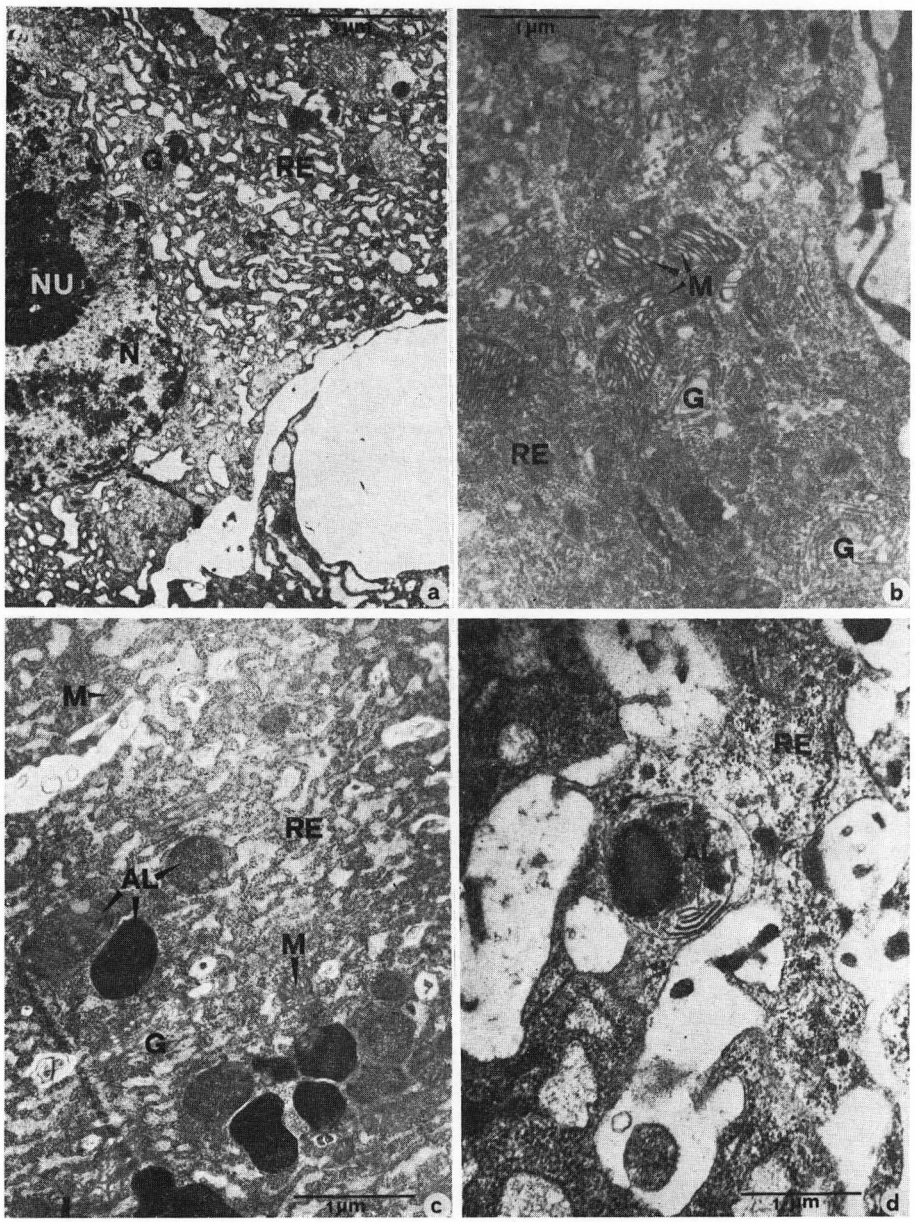

Fig. 11 - Spermatheca, receptaculum seminis of a female dissected after copulation. Transmission electron micrographs showing secretory cell details explained in the text.

Spermateca, receptaculum seminis di femmina sacrificata appena dopo la copula. Micrografie elettroniche a trasmissione di particolari di cellula secretrice spiegati nel testo.

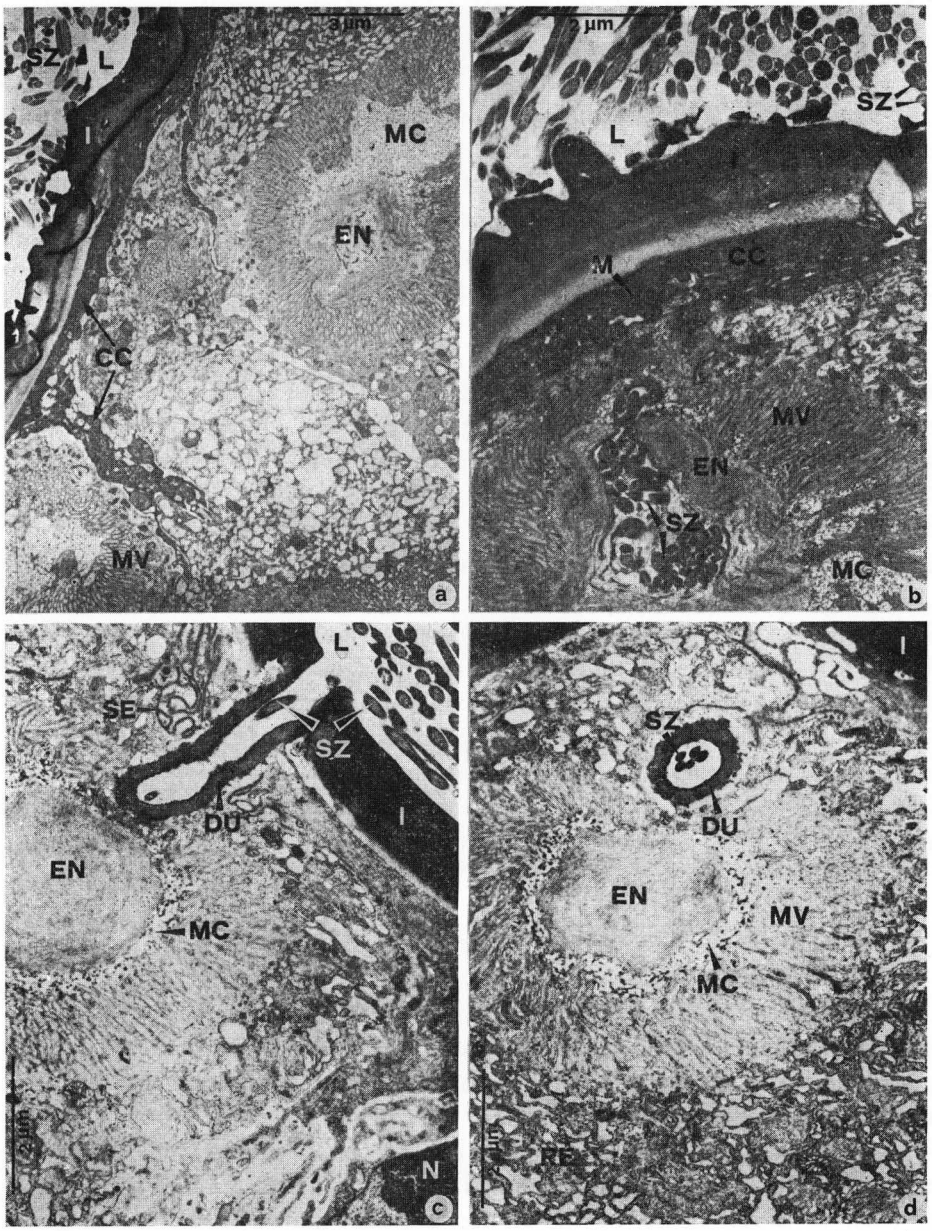

Fig. 12 - Spermatheca, receptaculum seminis of a female dissected after copulation. Transmission electron micrographs showing details of secretory and cuticulogenic cells explained in the text.

Spermateca, receptaculum seminis di femmina sacrificata dopo la copula. Micrografie elettroniche a trasmissione di particolari di cellule secretrici e cuticologene spiegati nel testo.

3.3 - Histology, ultrastructure and physiology of the reproductive accessory glands.

These glands are typical integumentary glands and consist of a single layer of cylindric secretory cells sandwiched between a thick basement membrane and a thin layer of cuticulogenic cells (Fig. 14: a, b). The latter line the gland lumen intima without extending themselves between the secretory cells. There is a rather distinct demarcation line (mainly septate junctions: Figs 14, a; 15, c) between the latter and the cuticulogenic cells which produce a relatively thin intima.

The single secretory cells are rather isolated units. They display a relatively large ovoid nucleus with a prominent nucleolus (Figs 13; 14, a; 15, a, b). The endoplasmic reticulum (almost only rough: Figs 14; 15, b, c) is very extensive and compact; there are relatively few Golgi complexes (Fig. 15) and mitochondria (Figs 14; 15); many vesicles filled with varied material (Fig. 15, b, d) are irregularly present. The cell apical region appear quite different in newly emerged females and in mated ones because of the great development of the microvillar cavity in the latter (Fig. 15), while in the former it is still very limited (Fig. 14). The endapparatus is almost spherical in shape, shows a very thin intima (Fig. 15, d), and is connected with an efferent ductule about 4-5 μm long which leads to the gland lumen. Mated female microvillar cavity and gland lumen are filled with a very much electron-dense homogeneous secretion (Fig. 15). Moreover, there are few secretory cells which display an enormously developed microvillar cavity (Fig. 16) filled with a secretion much less electrondense than in the neighbours.

3.4 - Insemination process and sperm storage.

During mating, the aedeagus apical piece (Figs 17; 18, a) is housed within anterior vagina with endophallus plugged in the insemination pocket and male gonopore facing the entrance of spermathecal ducts; so that spermatozoa are deposited in said pocket and presumably pushed into spermathecal ducts by the injection-ram-shaped apex (Figs 17; 18, b: IR) of the endophallus. The filling of receptaculum seminis is accomplished mainly by passive transport of sperm through peristaltic action of the strong muscular sheath of the spermathecal ducts. Nevertheless, an active sperm migration seems to be important. In fact, beyond the visual impression through direct observation in vivo under light microscope, a chemotactic role of the spermathecal secretions is also supported by the sperm invasion of ductules and even endapparatus of the spermathecal

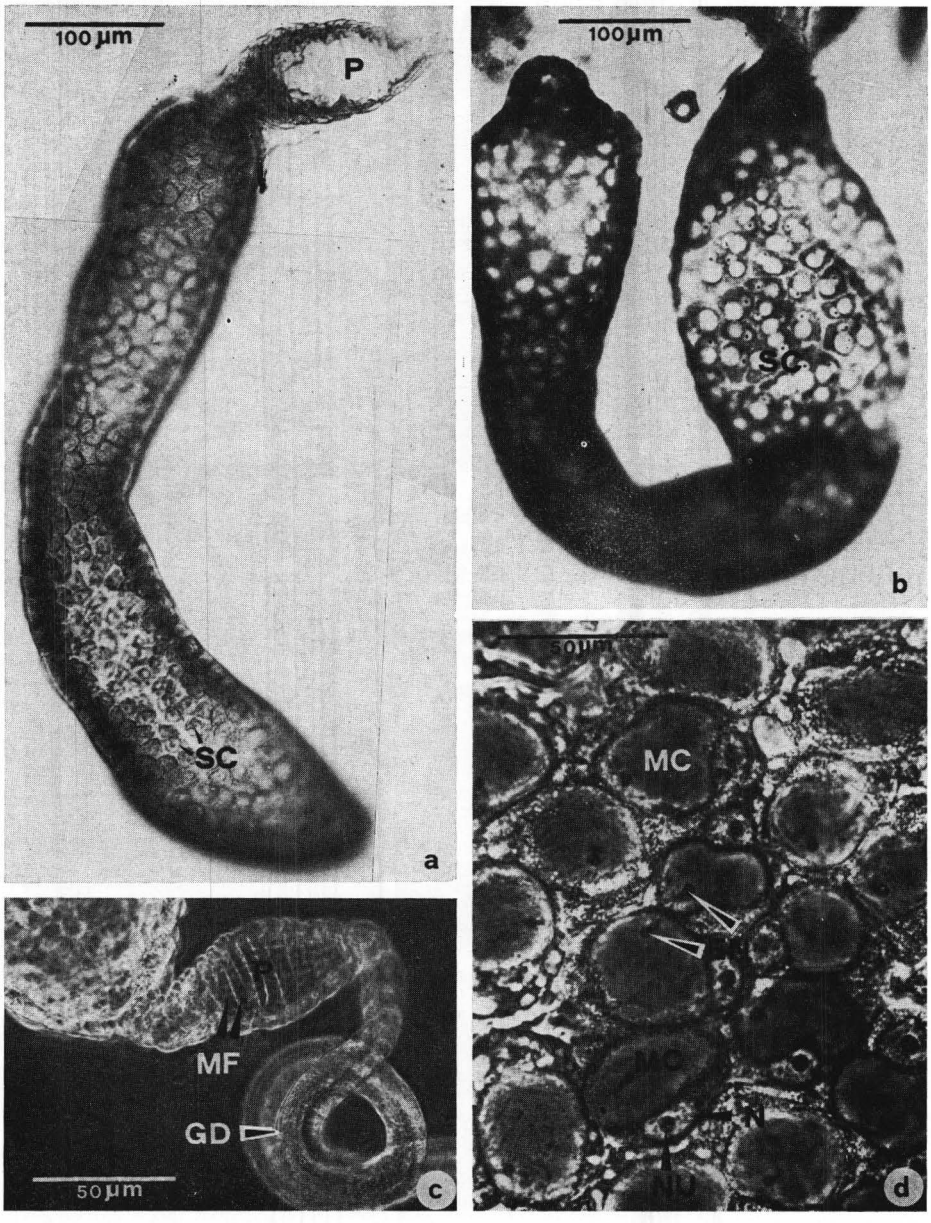

Fig. 13 - Reproductive accessory gland: a) of a newly emerged female; b) of a female dissected after copulation; c) detail showing the pumping device; d) detail of (b).

Ghiandola genitale accessoria: a) di femmina neosfarfallata; b) di femmina sacrificata dopo la copula; c) particolare mostrante il dispositivo pompante; d) particolare di (b).

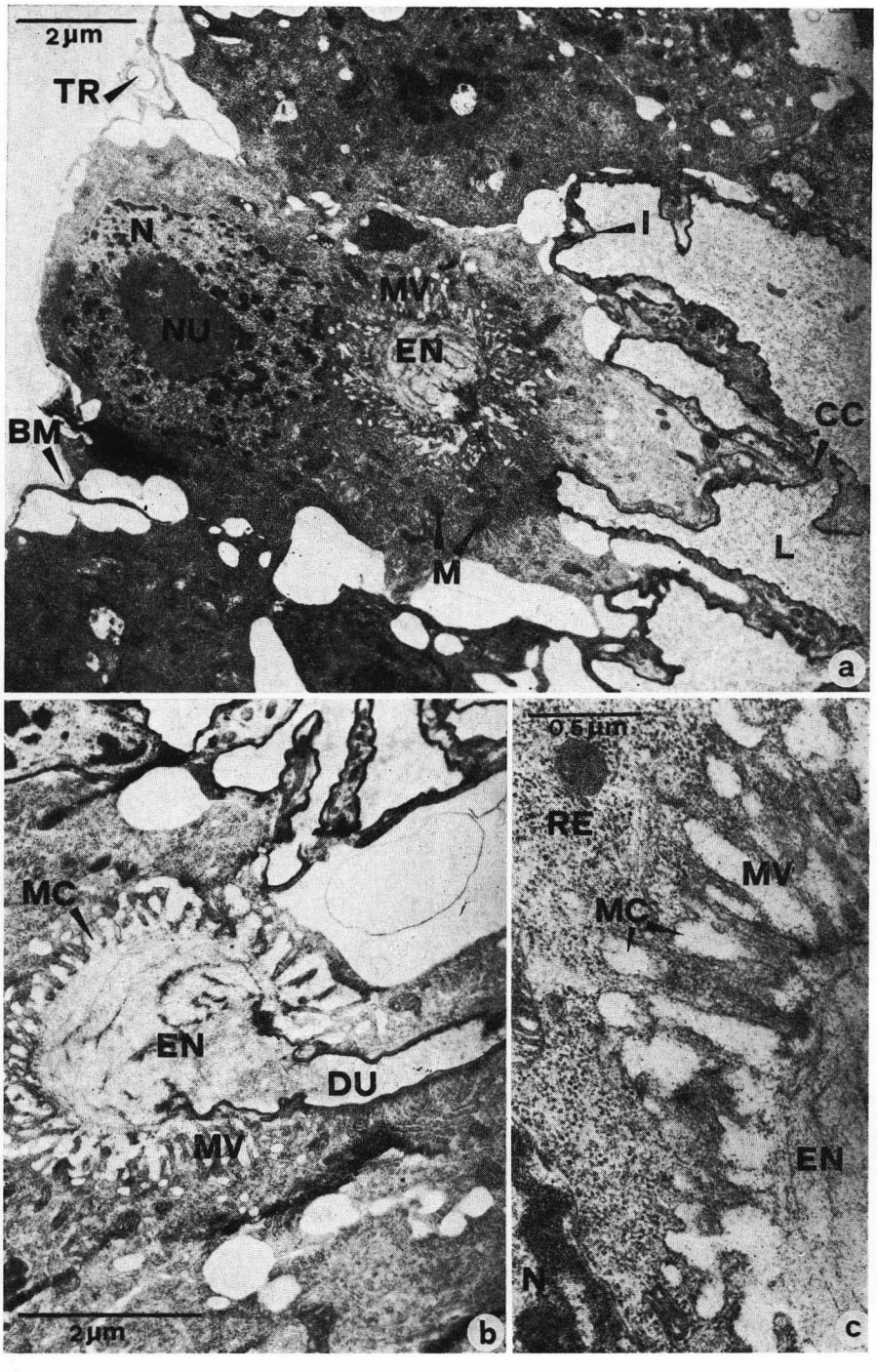

Fig. 14 - Reproductive accessory glands of newly emerged female. Transmission electron micrographs showing details of a cross section. (For explanation, see text).

Ghiandole genitali accessorie di femmina neosfarfallata. Micrografie elettroniche a trasmissione di particolari di sezione trasversale. Spiegazione nel testo.

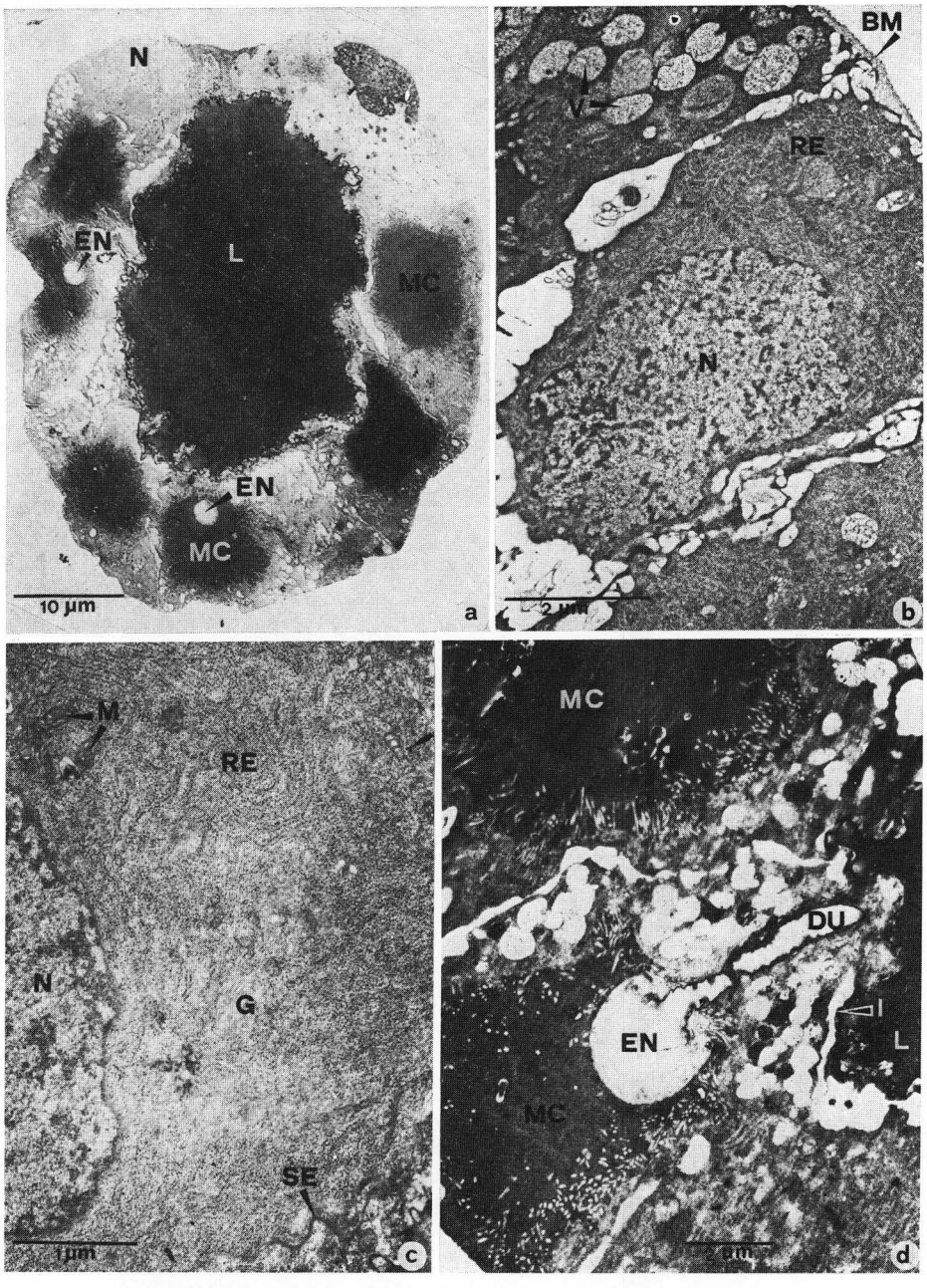

Fig. 15 - Reproductive accessory glands of a female dissected after copulation. Transmission electron micrographs of: a) cross section of a gland; b), c), d) details of the same. (For explanation, see text).

Ghiandole genitali accessorie di femmina sacrificata dopo la copula. Micrografie elettroniche a trasmissione di: a) sezione trasversale di una ghiandola; b), c), d) particolari della medesima. Spiegazione nel testo.

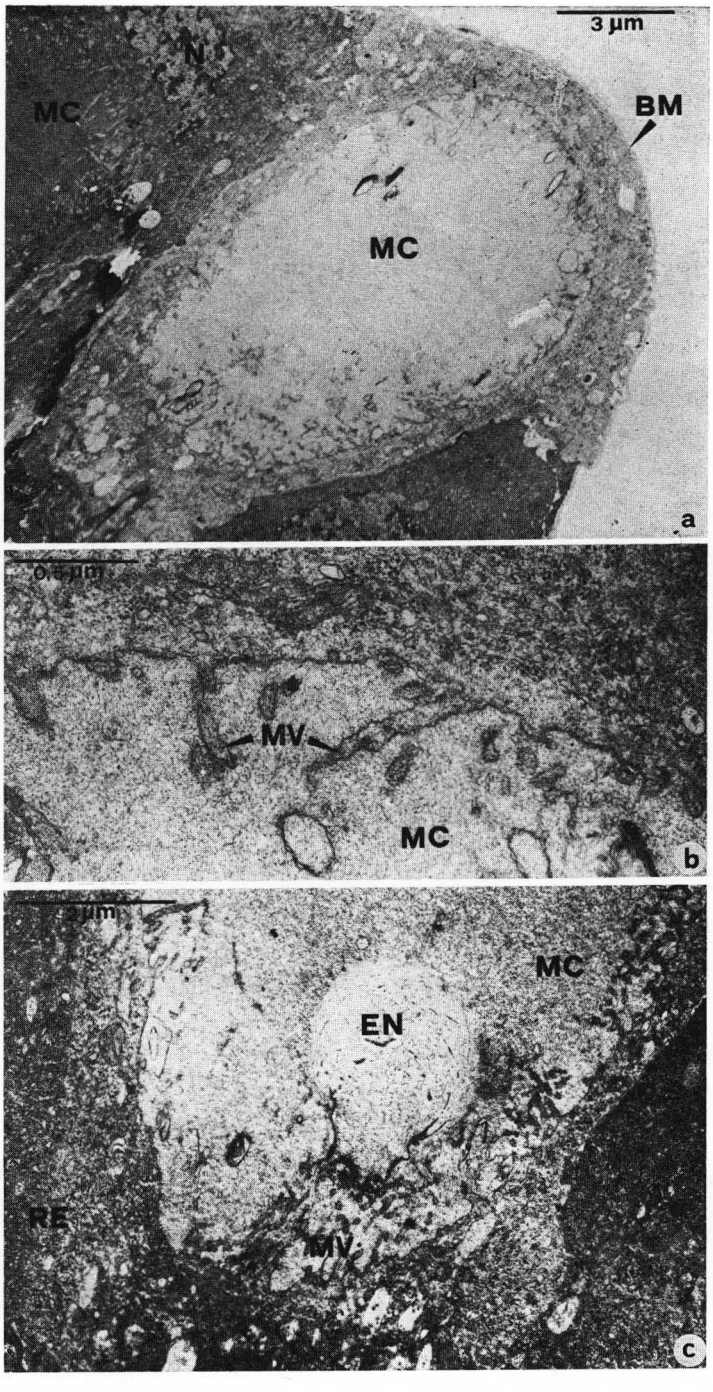

Fig. 16 - Reproductive accessory glands of a female dissected after copulation. Transmission electron micrographs of: a) a secretory cell with enormously developed microvillar cavity; b), c) details of the same cell.

Ghiandole genitali accessorie di femmina sacrificata dopo la copula. Micrografie elettroniche a trasmissione di: a) cellula secretrice con cavità microvillare enormemente sviluppata; b), c) particolari della stessa cellula.

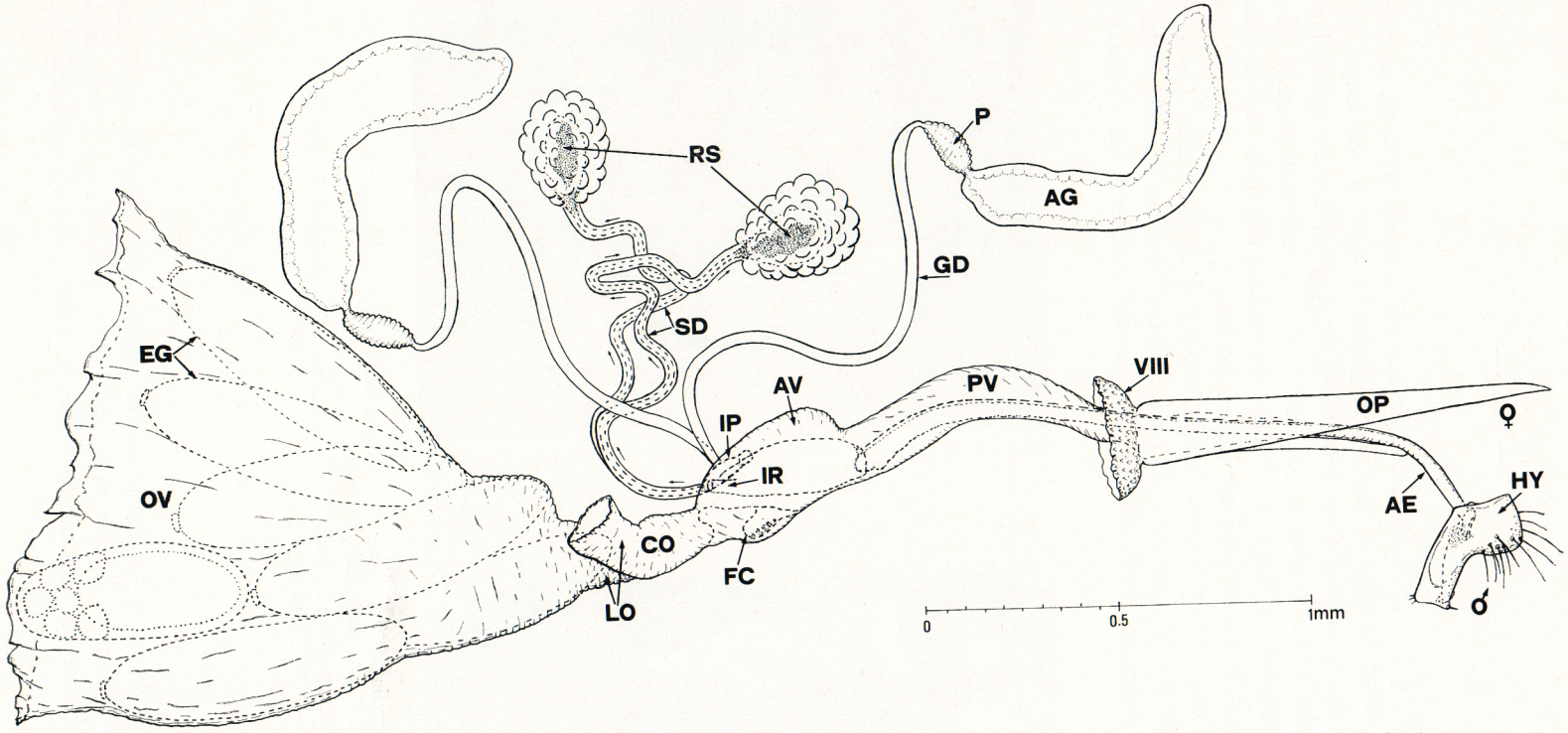

Fig. 17 - Genitalia of a sexually mature female with inserted male copulatory apparatus during insemination. (Semi-schematic lateral view).
Apparato genitale di femmina sessualmente matura con apparato copulatore maschile in atto d'inseminare. (Disegno semischematico).

secretory cells (Fig. 12, b, c, d). Certainly, the said passive transport may better explane the filling rapidity of the spermathecae in spite of the length of the spermathecal ducts and the slow proceeding of spermatozoa by swimming. In fact, about ten minutes after copulation onset, spermatozoa already fill the receptaculum seminis, while few of them are still in the ducts and almost no one may be still found in the insemination pocket.

During storage, spermatozoa remain active and motile within the receptaculum seminis, where they must find all they need to live on. Therefore the spermathecal secretions must contain the essential nutrients for spermatozoa.

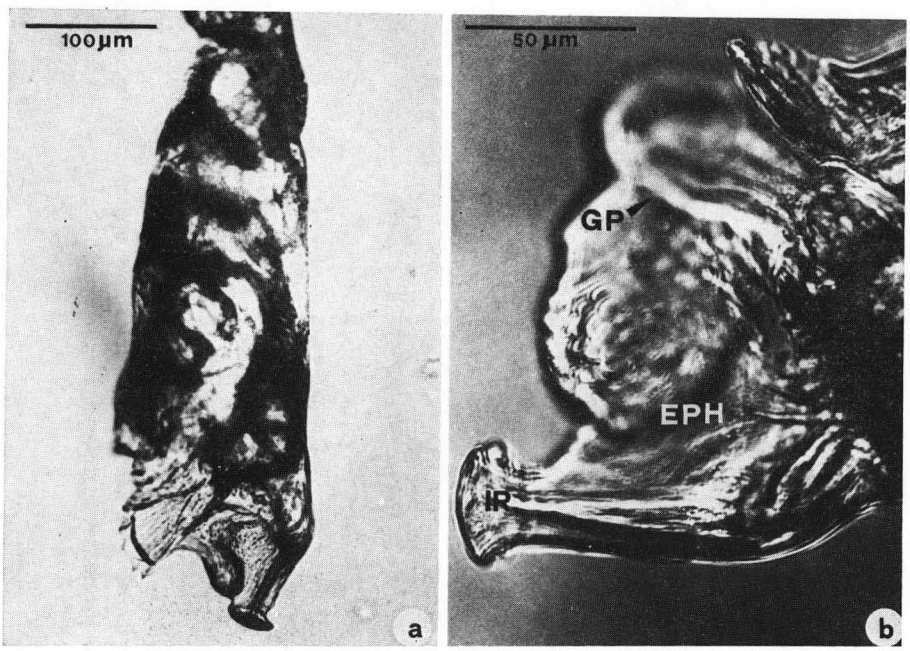

Fig. 18 - Male copulatory apparatus: a) aedeagus distal piece (left side); b) apical detail of the same showing endophallus and gonopore.

Apparato copulatore maschile: a) pezzo distale dell'edeago (lato sinistro); b) particolare apicale mostrante endofallo e gonoporo.

3.5 - Sperm-egg encounter.

How sperm do leave the receptaculum seminis and reach the fertilization chamber was not resolved in this study. But we may conceive a sperm displacement from the receptaculum and their transport down the spermathecal ducts

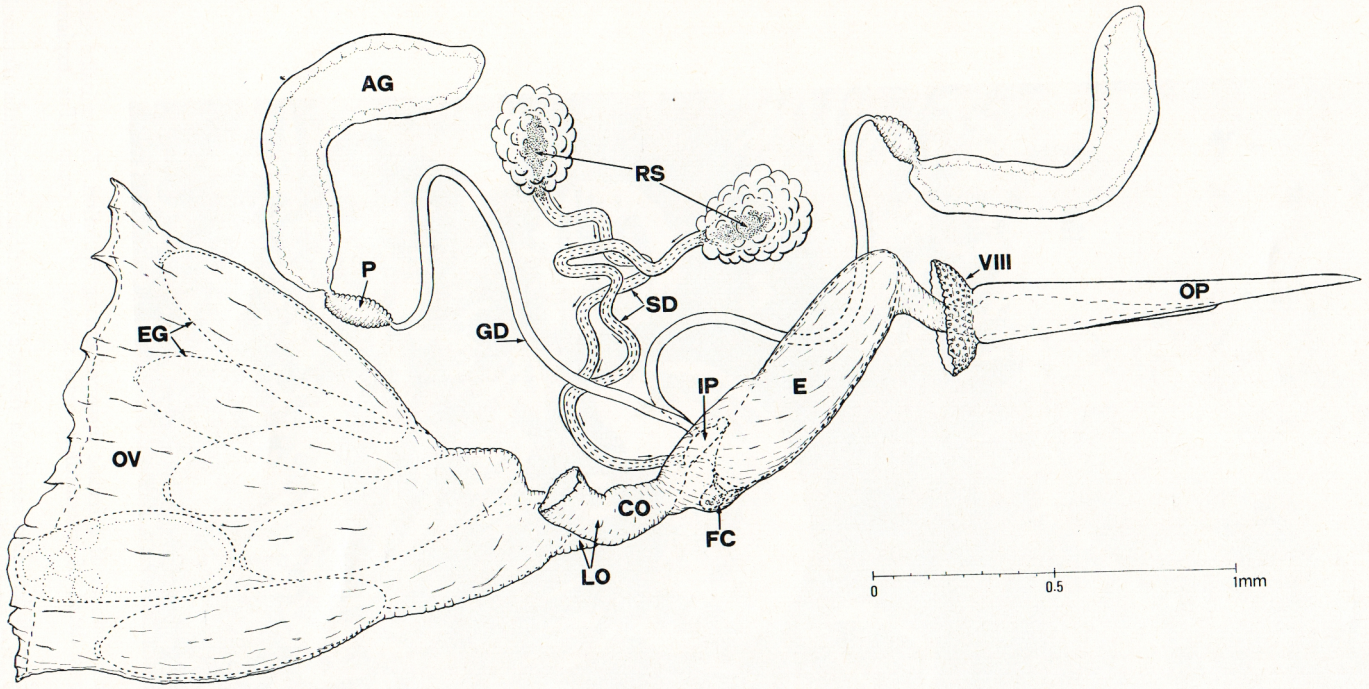

Fig. 19 - Female genitalia during ovulation-fertilization. (Semi-schematic lateral view).
Apparato genitale femminile durante il processo di ovulazione-fecondazione. (Disegno semi-schematico).

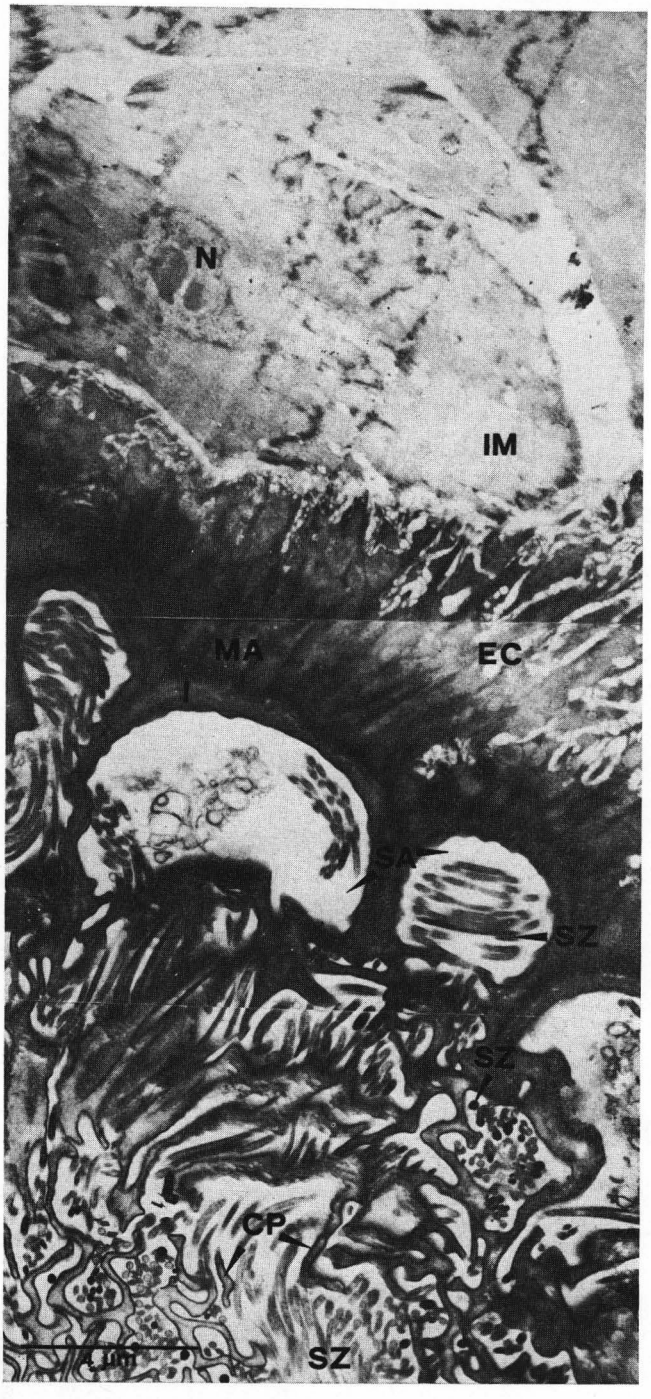

Fig. 20 - Fertilization chamber of a female dissected during ovulation. Transmission electron micrograph showing a detail of intermediate cross section of the organ. (For explanation, see text).

Camera di fecondazione di femmina sacrificata durante l'ovulazione. Micrografia elettronica a trasmissione di un particolare di sezione trasversale intermedia dell'organo.

to the insemination pocket by antiperistaltic movements of the duct muscular sheath; while sperm transfer from insemination pocket to fertilization chamber, which in a rest position are in close contact with one another (Figs 1; 5a, b), may be achieved by a sucking action of the chamber through contraction of its extrinsic muscles (Fig. 5, d: EM). Moreover, an important role seems to be played in this process by the accessory gland secretions. As above reported, these glands are narrow and immature in newly emerged females, distended and full of very electrondense secretion in ovigerous females; and during oviposition, said glands look (sometimes only one and sometimes both) more or less reduced in size (i.e. deflated through the release of secretion). That strongly suggest that, at ovulation (between the deposition of successive eggs), a relatively abundant portion of the secretion must be employed. Presumably, it could be forced down the gland ducts (by the pumping device) and, through the insemination pocket, into the spermathecal ducts, in order to serve principally as a fluid carrier for the sperm to reach the fertilization chamber.

Just after ovulation the egg passes rapidly and continuously down the lateral and common oviducts and enters the vagina, where it stops for a while,

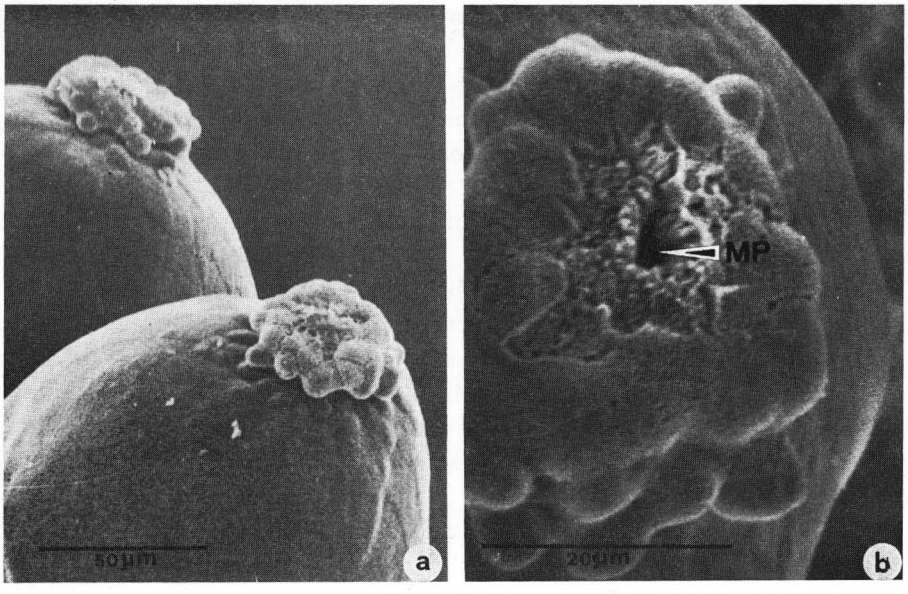

Fig. 21 - Ovarian eggs. Scanning electron micrographs showing the micropyle region: a) whole view; b) detail with micropyle.

Uova ovariche. Micrografie elettroniche a scansione mostranti la regione micropilare: a) visione d'insieme; b) particolare con micropilo.

having the micropylar region anteriorly directed (Fig. 19) and partially plugged in the fertilization chamber. At this time, a limited number of spermatozoa are in the chamber, almost all singularly housed, coiled up, within the « spermiophore alveoli » (Figs 2, d; 5; 20: SA). The egg bears a single micropyle free from any occluding substance (not like in *Musca domestica*: LEOPOLD, MEOLA, DEGRUGILLIER, 1977) also in the ovary (Fig. 21).

Within the fertilization chamber the micropyle presumably (given the dimensions and position of the involved structures) comes into tight contact with a spermiophore alveolus and receives the spermatozoon lodged in it as ready to spring. Sperm wastage is kept to a minimum also because of the cuticular microprocesses which fill like a packing the chamber entrance (Figs 2, b, d; 3; 20: CP) preventing spermatozoa from being waudering away when the end of the egg is withdrawn after fertilization.

4. CONCLUSIONS

To our knowledge, our observations provide new findings on the functional anatomy of the *Dacus oleae* female reproductive system. Furthermore we think they contribute some confirmation and some new information about:

a) the mechanisms (chemotactism and pumping up) which effect sperm transfer from the vagina (or bursa copulatrix) to the receptaculum seminis;

b) the maintenance of spermatozoa by means of the secretions from the spermathecal secretory cells;

c) the mechanisms which accomplish sperm removal from the spermatheca and trasfer to the fertilization site;

d) the mechanisms which bring about fertilization, strictly speaking.

In fact we have observed an insect, i.e. *Dacus oleae*, where:

a) sperm transfer from the bursa copulatrix (more precisely, from the « insemination pocket ») to the spermatheca, i.e. receptaculum seminis, is effected (1) mainly by peristaltic contractions of the muscular sheath of the spermathecal ducts, and (2) by a chemotactic attraction of the spermatozoa towards the receptaculum;

b) sperm maintenance is ensured in the spermatheca by the secretions from the spermathecal secretory cells;

c) sperm displacement from the receptaculum seminis to the « fertilization chamber » is presumably (1) effected by antiperistaltic contractions of the muscular sheath of the spermathecal ducts, (2) aided by the secretions from the

reproductive accessory glands, acting principally as a fluid carrier for the spermatozoa, and (3) completed by sucking of the sperm into the fertilization chamber by some vacuum produced in it by the contractions of the extrinsic muscles of the chamber;

d) fertilization takes place just after ovulation; as the egg enters the vagina and its micropylar end is thrust into the fertilization chamber; then, presumably, as a conseguence of the combined contraction of the muscular sheath of the vagina and chamber, the micropyle comes into tight contact with a « spermiophore alveolus » and receives a spermatozoon;

e) sperm utilization appears remarcably efficient both in insemination and fertilization processes; particularly in the latter where there seems to be a virtually 100 percent efficiency.

RIASSUNTO

ANATOMIA FUNZIONALE DELL'APPARATO GENITALE FEMMINILE DI *Dacus oleae* GMEL. IN RELAZIONE AI PROCESSI D'INSEMINAZIONE E FECONDAZIONE

Nel *Dacus oleae*, come in molti altri insetti, l'incontro tra i pronuclei maschile e femminile richiede il superamento preliminare di vari problemi legati al trasferimento dello sperma dal maschio alla femmina (accoppiamento e inseminazione), alla conservazione in vita degli spermatozoi per un periodo di attesa più o meno lungo, di solito in una spermateca, al prelevamento degli spermatozoi dalla spermateca al momento dell'uso e alla penetrazione dello spermio nell'uovo attraverso il micropilo (fecondazione p.d.).

Gli spermatozoi vengono trasmessi dal maschio alla femmina del *Dacus* per inoculazione diretta del seme in una « tasca d'inseminazione », situata come un atrio all'ingresso dei dotti delle spermateche, sulla parete interna dorsale della vagina, dove sboccano anche i dotti delle due ghiandole accessorie. Il trasferimento degli spermatozoi dalla tasca d'inseminazione al receptaculum seminis avviene principalmente per azione peristaltica della potente tunica muscolare dei dotti delle spermateche; ma una azione chemiotattica importante nei confronti degli spermatozoi sembra da attribuirsi ai secreti dell'epitelio ghiandolare del receptaculum seminis. Prima che l'uovo scenda nella vagina, un numero relativamente modesto di spermatozoi raggiunge la « camera di fecondazione », situata di fronte alla tasca d'inseminazione, sulla parete interna ventrale della vagina. Detti spermatozoi vanno ad alloggiarsi singolarmente negli « alveoli spermiofori », nei quali si ritrovano avvolti a spirale e come pronti a scattare verso il micropilo appena questo verrà a trovarsi giusto allineato di fronte. L'anatomia funzionale, gli spostamenti degli spermatozoi (inseminazione e fecondazione) e la loro conservazione nella spermateca, il presumibile ruolo svolto dai secreti dell'epitelio spermatecale e delle ghiandole accessorie sono stati studiati attraverso osservazioni dirette di organi in vivo in soluzione fisiologica al microscopio ottico e attraverso sezioni in serie esaminate e fotografate in microscopia ottica ed elettronica a trasmissione ed a scansione.

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