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PHYSIOLOGICAL AGE DETERMINATION IN FEMALE *STOMOXYS CALCITRANS* LINNAEUS (DIPTERA: MUSCIDAE)*

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ABSTRACT

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The primary sex organs of female *Stomoxys calcitrans* consist of a single pair of ovaries, each containing 80–100 polytrophic ovarioles. A natural population of these females can be grouped according to age into different reproductive categories. The technique described below defines these categories and enables one to distinguish between newly-emerged, nulliparous and uniparous females and females that have reproduced twice (biparous) or more (pauciparous).

Résumé

DÉTERMINATION DE L'ÂGE PHYSIOLOGIQUE DES FEMELLES STOMOXYS CALCITRANS *LINNAEUS (DIPTERA: MUSCIDAE)*

Les organes sexuels primaires de la femelle Stomoxys calcitrans *consistent d'une seule paire d'ovaires, chacune contenant* 80–100 *ovarioles polytrophiques. Une population naturelle de ces femelles peut être groupée suivant l'âge en différentes catégorie reproductives. La technique décrite ci-après définit ces catégories et permet de distinguer entre les femelles nullipares et unipares nouvellement émergées et les femelles qui ont reproduit deux fois (bipares) ou plus (paucipares).*

INTRODUCTION

It is possible to determine the physiological age of female *Stomoxys calcitrans,* as with most female blood-feeding Diptera, with a technique which was proposed by Kuzina (1942) and Detinova (1962).

This technique involves the determination of the number of follicular relics (yellow bodies) in the ovaries of parous females, and the maturation phase of the follicles in nulliparous females where no follicular relics are present. According to Detinova (1962), these follicular relics are similar to corpora lutea in mammals and are formed by the aggregation of trophocyte remnants and degenerating epithelial cells of the follicular egg chamber.

Detinova (1962) grouped the parous females of *S. calcitrans* by taking into account both the follicular stages of the ovaries and the number of follicular relics present in each ovary. This author, unfortunately, did not describe the various stages of follicular development in this species, but presented only a generalized diagram based on that given by Kuzina (1942). The descriptions of the follicular stages given by Gillies, Hamon, Davidson & De Meillon (1961) (who cited Christophers 1911) make it clear that Kuzina (1942) and Detinova (1962) confused the follicular stages described in mosquitoes by Christophers (1911) with those in *S. calcitrans.*

Since comparatively little is known about the primary sex organs of female *S. calcitrans,* a detailed study of these organs was undertaken in order to describe the follicular stages in this species. These characteristics are essential for the physiological age grouping of female stable flies.

MATERIALS AND METHODS

Nomenclature

In this study 'anterior' refers to any part of the primary sex organs situated towards the head of the fly, while 'posterior' refers to structures situated towards the anal split. 'Dorsal' refers to those structures nearest to the terga, while those closest to the sterna will be referred to as 'ventral'. In the mature oocyte (Stage V of follicular development), the surface bearing the hatching split will be 'dorsal', while the opposite side will be 'ventral'. The 'anterior' surface of the oocyte and the fertilized egg after deposition bear the microyple, with the 'posterior' surface opposite.

Stereomicroscopic studies

Teneral flies originating from the stock colony were maintained under conditions identical with those in the main colony (Sutherland, 1978). A few flies were killed twice daily in diethyl ether vapour, but only females were dissected.

Small Petri dishes, partially filled with beeswax, were used for dissecting. The flies were pinned in these dishes with their dorsal side uppermost and were completely covered with Pampel's fluid (formalin 6 parts, glacial acetic acid 4 parts, 95% ethanol 15 parts and distilled water 30 parts). They were examined under a Wild M5 stereoscopic microscope after their dorsa had been removed.

Both ovaries of each fly were examined *in situ* for the presence of tracheal skeins and follicular relics, after which one ovary was loosened and carefully transferred to a drop of Pampel's fluid on a glass microscope slide. Subsequently, the ovarioles were carefully separated under 25 × magnification, spread in the drop of Pampel's fluid, covered with a coverslip and examined under 100 × magnification with a Wild compound microscope.

Scanning electron microscopic studies

Old females from the stock colony were dissected in distilled water under a Wild M5 stereoscopic microscope. Ovaries containing mature oocytes were removed and transferred to Petri dishes filled with distilled water. The oocytes were removed from the ovarioles, thoroughly washed in distilled water and fixed with filtered 25% gluteraldehyde 1,0 mℓ + buffer 9,0 mℓ for 1 hour. The buffer consisted of a 19: 81 mixture of solutions of monobasic sodium phosphate ($NaH_2PO_4[2H_2]$) 3,1202 g + 100 mℓ of distilled water and dibasic sodium phosphate ($Na_2HPO_4[12H_2O]$) 7,1628 g + 100 mℓ of distilled water.

After fixation, the oocytes were dehydrated in 30, 50, 65, 75, 85, 90, 95 and 100% methanol and kept for 25 min in each concentration. The specimens were then left for 30 min in a 1: 1 solution of absolute methanol plus amyl acetate, and finally transferred to 100% amyl acetate for 1 hour. Immediately after

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removal from the amyl acetate transitional fluid, the oocytes were critical point dried using CO_2, mounted on specimen stubs with conductive silver paint and subsequently coated with gold, using an ion sputtering device. Finally, they were examined with a Hitachi scanning electron microscope equipped with a photographic camera.

Results

The primary sex organs of a *S. calcitrans* female consist of a pair of ovaries (Fig. 1), each situated dorso-laterally on the side of the fly's abdomen. The anterior extremity of each ovary is attached to the body wall by means of a suspensory ligament, and each opens into a calyx formed by the anterior portion of the lateral oviduct. Posteriorly, the lateral oviducts unite to form a short common oviduct, which in turn opens into the anterior portion of a bell-shaped vagina. Antero-dorsally, the vagina also receives the short ducts from the 2 conspicuous, dark-coloured spermathecae as well as ducts from the 2 club-shaped accessory glands.

Each ovary consists of 80–100 polytrophic ovarioles (Fig. 2), each of which has a terminal filament at its anterior extremity and opens posteriorly, via a short pedicel, into the calyx of the lateral oviduct. The entire ovariole is enclosed in a membranous covering, the peritoneal sheath, and the terminal filaments of the ovarioles unite to form the suspensory ligaments. A germarium, consisting of oogonia, is situated posterior to the terminal filament of each ovariole and is followed posteriorly by follicles at different stages of development. In newly-emerged females only one such follicle is present, but these increase in number with the ages of the flies, and 4–5 follicles in different stages of development may be present in each ovariole. Each follicle consists of a single oocyte and 7 trophocytes which are surrounded by a thin layer of epithelium, and the follicles are separated from one another by an epithelial plug.

The various developmental stages of the follicles of *S. calcitrans* can be described as follows:

Stage I (Fig. 2 & 3)

Follicles spherical in shape without being clearly separated from the germaria, as the follicular epithelia are not fully differentiated.

Early Stage IIa (Fig. 2)

Although still spherical in shape, the follicles are larger, almost completely separated from their germaria, and their epithelia are clearly differentiated. The contents of the follicles appear uniformly granulated and no cells are visible.

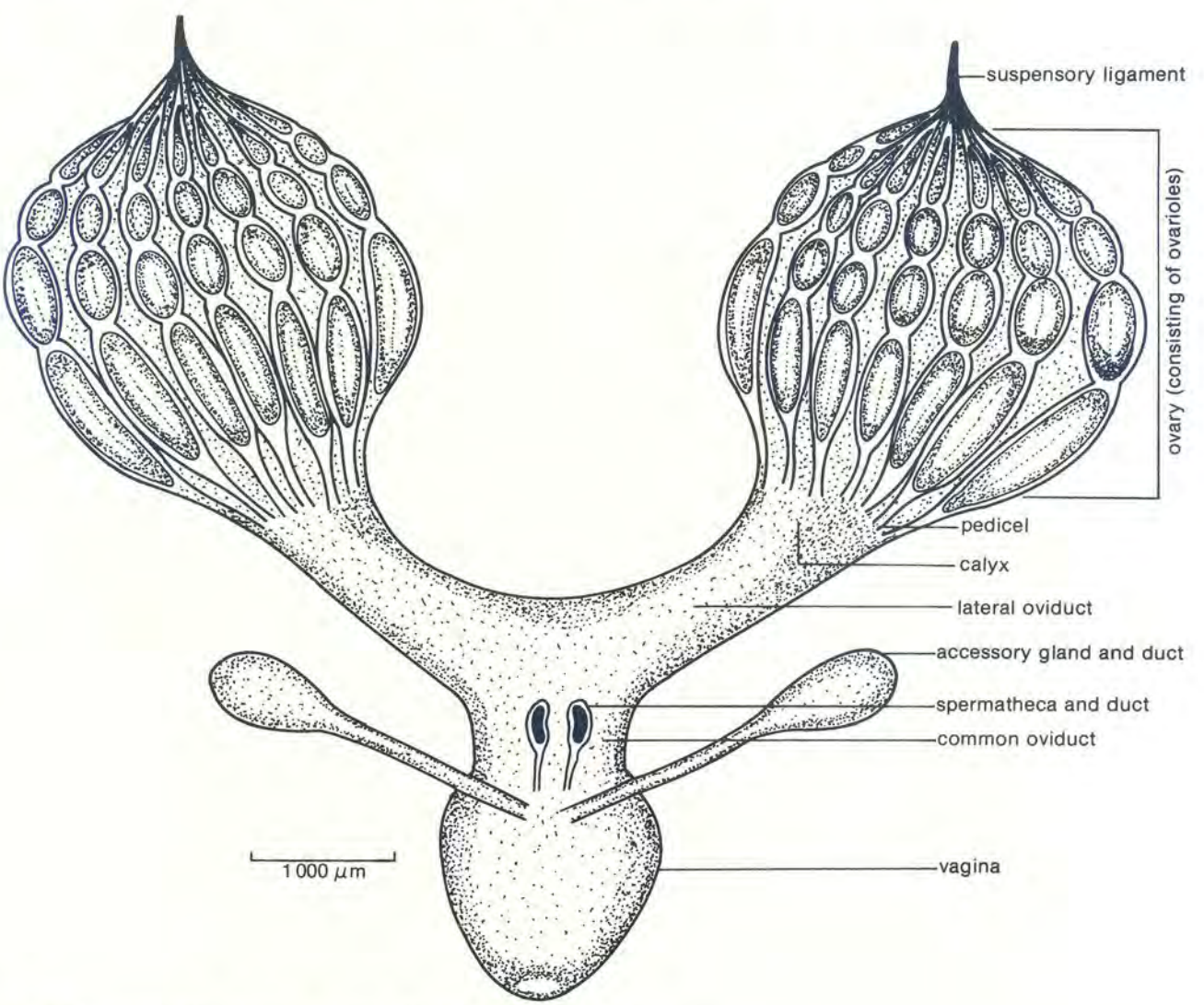

FIG. 1 Diagram of the primary sex organs of a female *Stomoxys calcitrans*

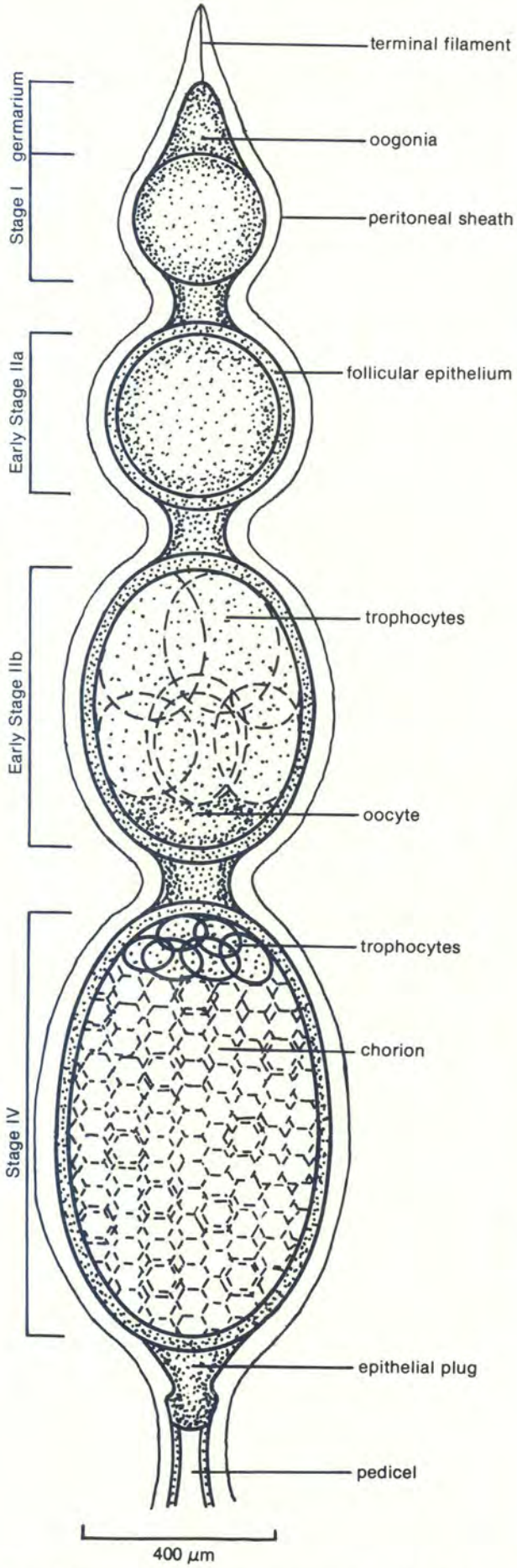

FIG. 2 Ovariole of *Stomoxys calcitrans*, showing the germarium and follicles in Stages I, early IIa, early IIb and IV of development

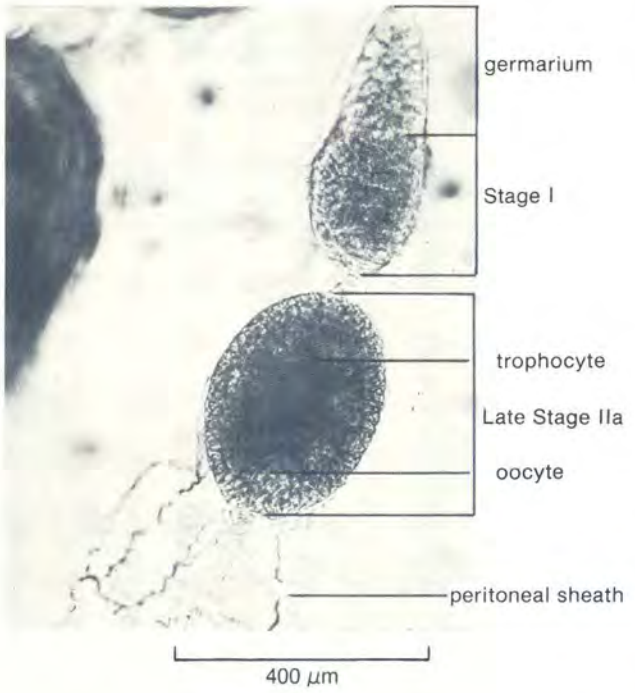

FIG. 3 Ovariole of *Stomocys calcitrans*, showing the germarium and follicles in Stages I and late IIa of development

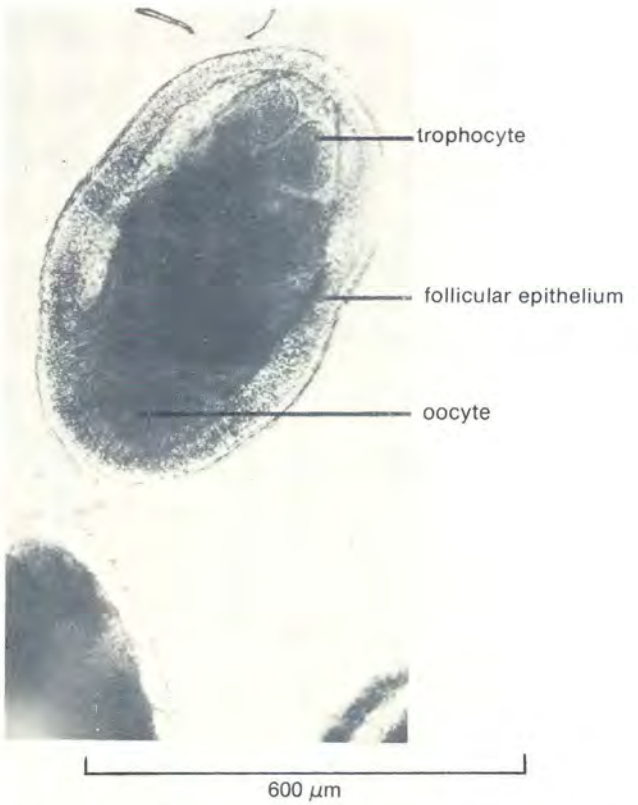

FIG. 4 The follicle of *Stomoxys calcitrans* in late Stage IIb of development

Late State IIa (Fig. 3)

Follicles resemble the earlier ones, except that their granulated contents are less dense and the oocyte and 7 trophocytes are just visible.

Early Stage IIb (Fig. 2)

The follicles are larger, distinctly separated from their germaria, and oval in shape, while the oocytes and trophocytes are more conspicuous.

Late Stage IIb (Fig. 4)

The follicles resemble those in the previous stage, and the oocytes and trophocytes are clearly visible, with the 7 trophocytes filling the larger part of the anterior portion of each follicle.

Stage III (Fig. 5)

The follicles are elongate and considerably larger than those in the previous stage. The oocytes, which are still developing, increase in size and occupy between 30% and 50% of the volume of their respective follicles. Anteriorly, the remainder of each follicle is filled with the conspicuous trophocytes. The chorion, with its characteristic honeycomb pattern, starts to develop on the outer surface of each oocyte during this stage.

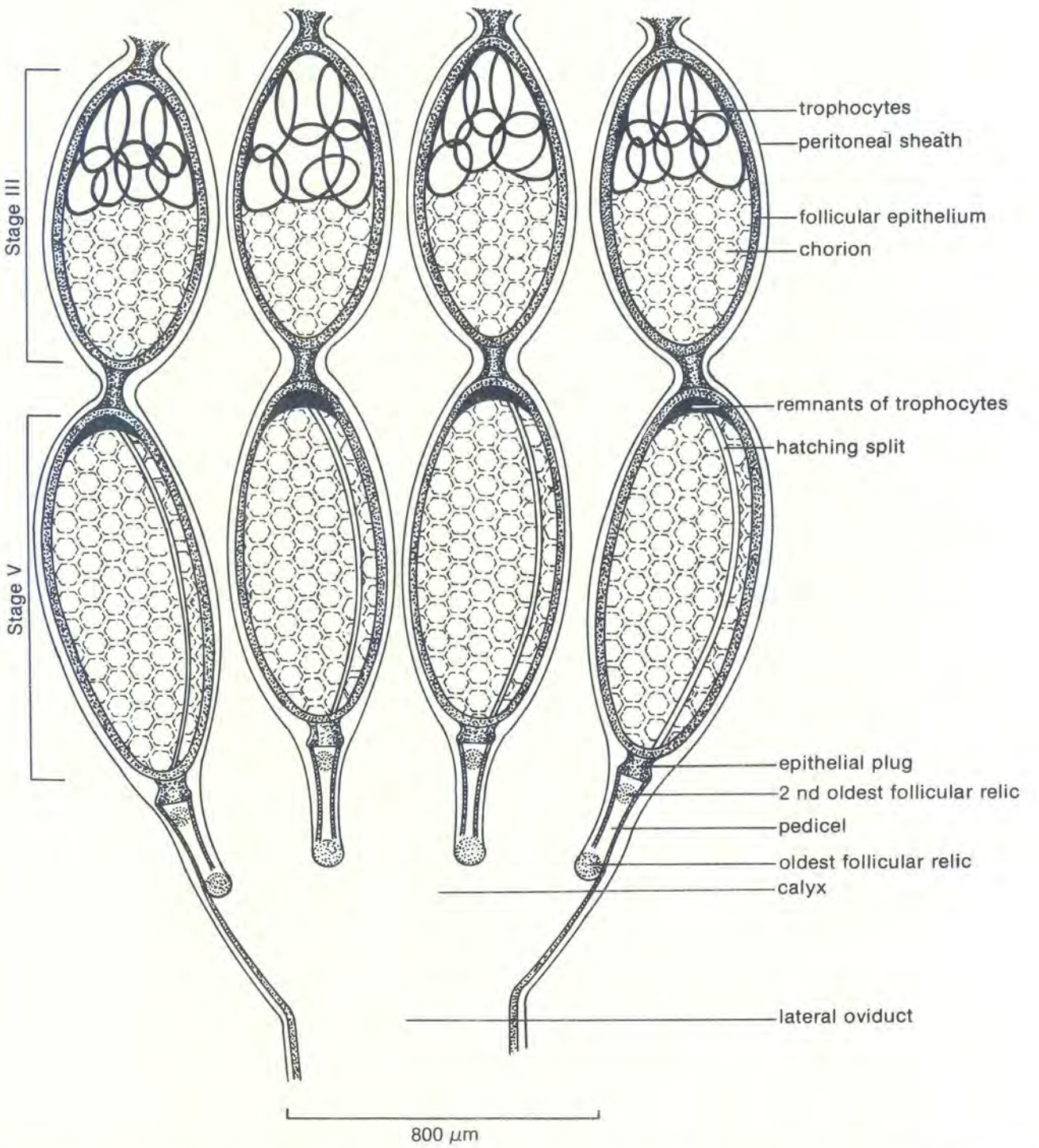

FIG. 5 Diagram of a portion of the primary sex organs of a female *Stomoxys calcitrans*, showing the follicular relics (corpora lutea) and follicles in Stages III and V of development

Stage IV (Fig. 2)

Follicles in this stage of development are larger than those in Stage III, greater areas are covered oy the developing chorions, and the oocytes fill approximately 90% of the follicular volume.

Stage V (Fig. 5)

The oocytes complete their development during this stage. They are characterized by the presence of hatching splits and by chorions with polygonal reticulations covering their surfaces. The oocytes fill the follicles and the remnants of the trophocytes are present anteriorly as rather inconspicuous, yellow, dome-shaped structures.

Scanning electron micrographs reveal the strong morphological resemblance of the chorion (Fig. 6), hatching split (Fig. 7) and micropyle (Fig. 8) of mature oocytes to those of oviposited eggs.

FIG. 6 Chorionic surface of a mature oocyte (Stage V) of *Stomoxys calcitrans* ×400

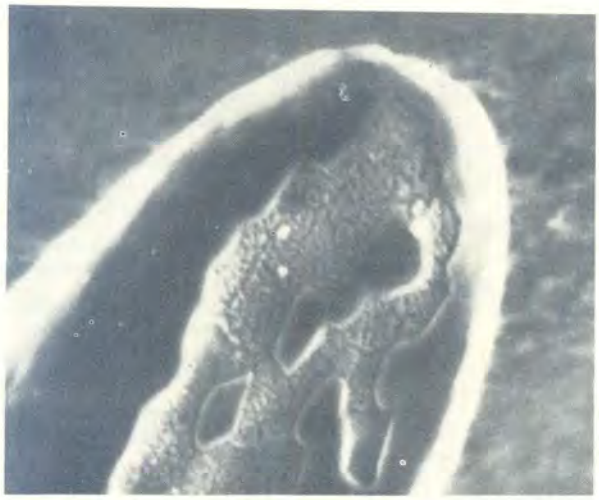

FIG. 7 The hatching split in a mature oocyte (Stage V) of *Stomoxys calcitrans* ×400

The minute, underdeveloped ovaries of newly-emerged females are indicative of their physiological age. They contain no follicular relics and are easily detectable in the abdomens of the flies, as they are surrounded by dark brown tracheae from which they receive their oxygen supply (Fig. 9).

Microscopic examination of the ovaries of newly-emerged *S. calcitrans* females revealed that the oldest follicles were always in Stage I of development (Fig. 10). Immediately after the mature oocytes had passed into the lateral oviducts, the epithelial cells of the recently evacuated follicle in parous females remained as large, nucleated, sac-like structures posterior to the oldest follicles. Within 24–48 hours, however, these structures had contracted and formed follicular relics in the terminal pedicels of the ovarioles. On subsequent oviposition, these relics were ejected into the calyces of the lateral oviducts and replaced by new relics which had been formed in the same sites as the old ones (Fig. 5). The presence of numerous follicular relics in the calyces of the lateral oviducts resulted in the formation of yellow deposits, thus making it impossible to identify individual relics.

In old pauciparous females, the follicles tended to be irregularly developed, and residual mature oocytes were frequently present in some of the ovarioles.

The females of *S. calcitrans* did not exhibit gonotrophic concordance because ovary development is not directly related to the number of blood meals ingested.

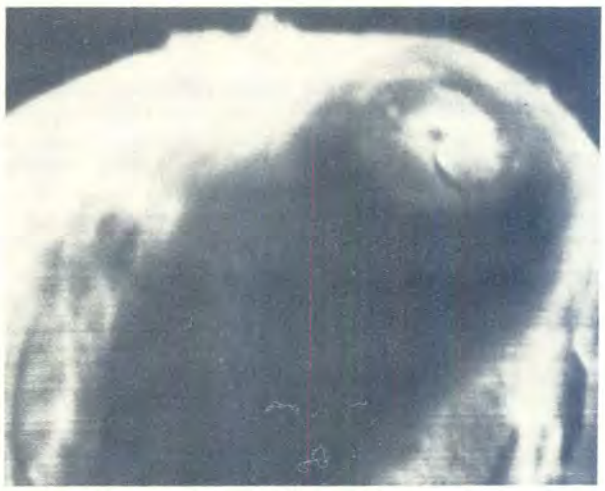

FIG. 8 Micropyle in a mature oocyte (Stage V) of *Stomoxys calcitrans* ×700

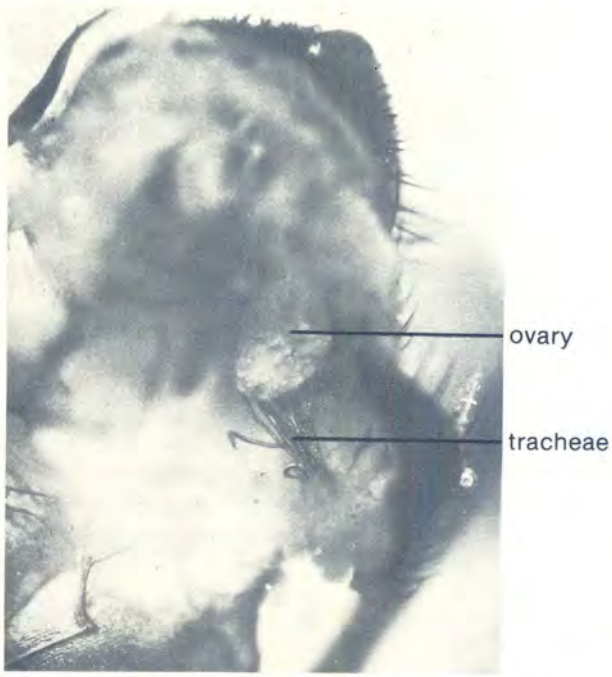

FIG. 9 Ovary of newly-emerged *Stomoxys calcitrans* showing tracheae ×10

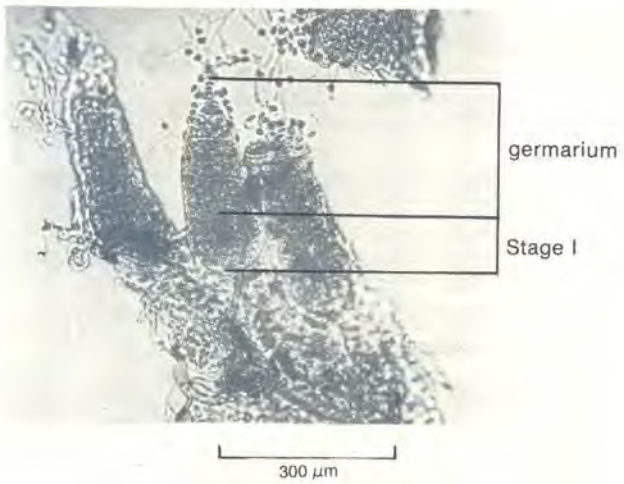

FIG. 10 Ovariole of a newly-emerged female *Stomoxys calcitrans*, showing the germarium and the oldest follicle in Stage I of development

Discussion

Pampel's fluid clears the specimens more effectively than saline (Sutherland, 1973), and was therefore used as the dissecting medium in the present study. In Diptera, the number of trophocytes and oocytes present per ovarian follicle apparently varies from species to species. There are 7 trophocytes and 1 oocyte present per follicle in mosquitoes (Detinova, 1962), while in both *Musca* and *Drosophila* each follicle contains 15 trophocytes and 1 oocyte (De Wilde, 1964). Moobola & Cupp (1978), who studied the ovarian development in *S. calcitrans* in relation to diet and juvenile hormone control, did not state the number of trophocytes present per follicle in this species. This study revealed that each ovarian follicle in *S. calcitrans* contains 1 oocyte and 7 trophocytes, a relationship similar to that in *Hippobosca capensis* (Detinova, 1962).

The absence of gonotrophic concordance in the females of *S. calcitrans* was also found by Detinova (1962) and Anderson & Tempelis (1970). Since trophocyte secretions and massive yolk formation in the oocyte totally mask the contents of the follicles from an early stage onwards, the sketches of Kuzina (1942) and Detinova (1962) do not accurately represent the various stages of follicular development. It is therefore suggested that the classification of follicular stages presented here should be used to group *S. calcitrans* females into 4 different reproductive categories, as follows:

Newly-emerged females—the ovaries ensheathed in tracheal skeins with the oldest follicles in Stage I.

Nulliparous females—the most mature follicles at any stage from IIa–V, with no follicular relics present in the ovaries.

Uniparous females—a single follicular relic present in each terminal pedicle that has passed a mature oocyte.

Biparous or pauciparous females—a single follicular relic present in the distal pedicel and one or more in the calyces of the lateral oviducts. In very old females the ovarioles tend to be irregularly developed and mature oocytes might be retained in some.

This system has the disadvantage that it can only differentiate between 4 categories of females: newly-emerged, nulliparous and uniparous females and females that have reproduced twice or more. These should, however, enable one to structure a natural population of *S. calcitrans* females according to age.

By using this method of age grouping, economic entomologists should be able to determine the efficacy of control measures against this species. In cases where control is aimed at the immature stages of the stable fly, the appearance of young nulliparous females in the natural population might indicate that the control measure is becoming less efficient. Where control is aimed at adult fly populations in the field, the presence of old females in these populations would indicate that the control measures were either ineffective or only partially so. Apart from its practical application in control programmes, this method of physiological age determination can also serve as a very handy meause for monitoring the overwintering populations of this non-diapausing insect. Sutherland (1978) also used it to assess the effects of different blood diets on adult *S. calcitrans* in the laboratory.

Acknowledgements

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