Symbionts in the Female Tsetse Fly *Glossina morsitans morsitans*[1]

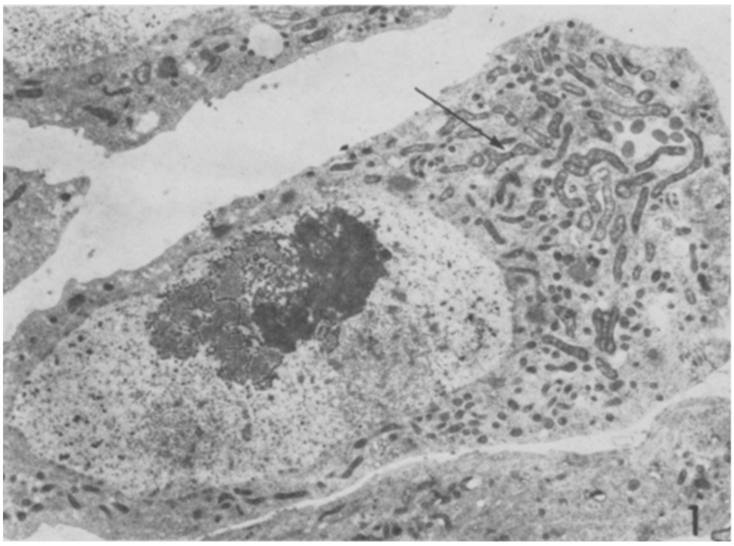
Gram negative bacterial symbionts[2] are known to reside in the giant cell region (mycetome) of the anterior mid-gut of tsetse flies and it has been suggested that they are maternally transmitted to subsequent generations[3,4]. The generally accepted route is via the milk glands which are structures designed to synthesize and release a lipid and protein secretion to the intra-uterine larva. Both light microscope[3,4] and ultrastructural[5] studies have shown that bacteroids tend to concentrate in the lumen of these glands from where they may become incorporated into the milk and thus have direct access to the cells of the larval gut.

However, the presence of bacteroids has recently been reported within all areas of the ovaries of *Glossina austeni*[6]. Although much smaller than the mid-gut symbionts it is proposed that they may be their progenitors and that transovarian transmission is an alternative or additional pathway. Our study has been extended to include the developing embryo and we confirm that symbionts do pass from ovary to progeny in this manner. In *G. m. morsitans*, however, they appear to be of a different type compared with those of the mid-gut cells and the milk gland lumen and more closely resemble the inclusions recently described in certain tissues of other species of *Glossina*[7].

Materials and methods. Embryos were removed from females $7^1/_2$ h after the deposition of the first larva. From work carried out in this laboratory we know that this coincides with early embryogenesis and is at least 48 h before the first instar larva receives secretions from the milk gland. The embryos were fixed in 2.5% glutaraldehyde at 4°C for 58 h followed by 2% osmium tetroxide for 2 h. All fixatives were buffered to pH 7.1 with 0.02 *M* Soerensen's buffer adjusted to 0.25 *M* with sucrose. The tissue was prestained with 1% uranyl acetate in 70% ethanol for 1 h during dehydration, and finally embedded in Spurr resin. The ovaries and anterior mid-gut of teneral females were subjected to a similar fixation. Silver/gold sections, cut with a Reichert OMU3 ultramicrotome, were supported on uncoated copper grids and contrasted with lead citrate prior to examination with an AEI EM6B electron microscope.

Results and discussion. The ovarian symbionts of *G. m. morsitans* occur in their highest concentration within the nurse cell cytoplasm of young egg follicles, and both light and electron microscope studies reveal that they are extremely pleomorphic, occuring as rods and occasionally as branched structures (Figure 1). They have a rather sparse internal ribosomal grana and what appears to be a Gram negative cell wall (Figure 2). In all these respects, including their size, they are identical to those located in the developing embryos (Figure 3). As far as we can ascertain the only difference resides in the fact that the symbionts in nurse cells, follicle cells and ooplasm are enveloped by a host membrane whereas those in the embryo are not. It is worth noting that the distribution of the embryonic forms corresponds to that in the amblyommines[8], aggregating at the periphery of the yolk. It is pertinent to note that both ovarian and embryonic symbionts in *G. m. morsitans* share features ascribed to rickettsia[9] and are similar to those reported in the ovaries of ticks[10]. The mid-gut symbionts lack a host membrane[2] and are at least 4 times larger than the ovarian and embryonic forms. They have a dense internal grana, Gram negative cell wall and more closely resemble true Gram negative bacteria than rickettsia (Figure 4).

It has been suggested that in cockroach spp.[11] the morphological differences displayed by symbionts are simply a reflection of their metabolic activity and the type of host tissue they are living in. But in other in-



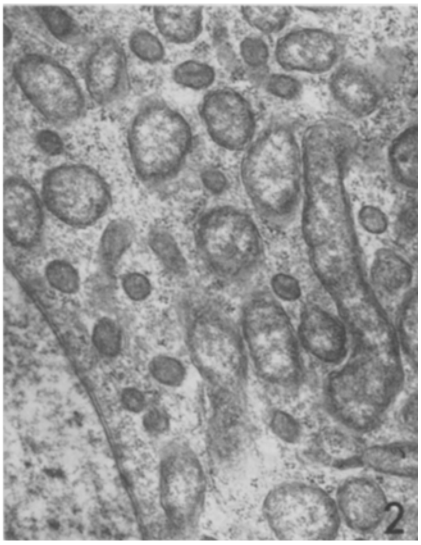


Fig. 1. High concentrations of ovarian symbionts in nurse cell cytoplasm of young egg follicle. Arrow indicates branched form. ×3,750.
Fig. 2. Ovarian symbionts in nurse cell cytoplasm. ×15,000.

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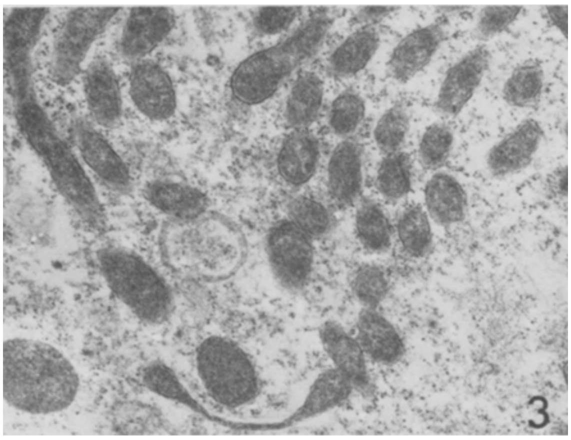

Fig. 3. Symbionts in the yolky region of young embryo. Note the absence of a host membrane. ×14,250.

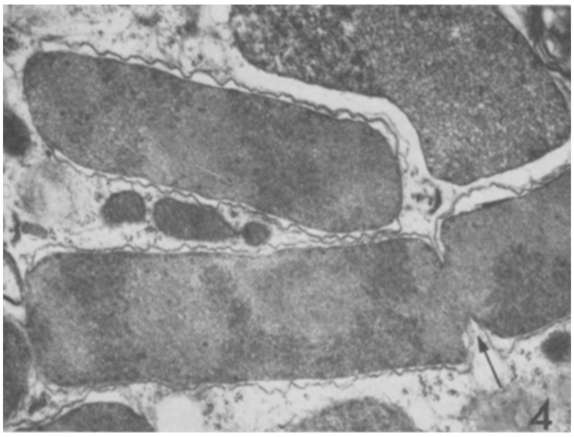

Fig. 4. Bacteroids in mid-gut mycetome. Arrow indicates characteristic division of Gram negative bacteria. ×14,500.

sects[12,13], certain of which have a similar reproductive system to tsetse flies, it has been shown that 2 symbiont types are definitely present, a gut form and a rickettsial ovarian form. Each of these is conveyed to the offspring along an independent pathway, the gut form moving in the milk secretion and the ovarian form via the egg. On present evidence we suggest that the rickettsia-like inclusions in this *Glossina* sp. are also passed from generation to generation in the egg, and that the Gram negative mid-gut symbionts, which from our and other[2] observations closely resemble the milk gland forms, are transmitted in the milk.

The precise role of bacterial symbionts within insect tissues has not been fully elucidated although there is evidence they contribute to the synthesis of factors essential for normal insect development[12]. Indeed, it has been demonstrated in some insect spp. that when the symbiont population is removed or reduced by antibiotics then growth is retarded and egg production is restricted[14]. Some recent experiments involving the antibiotic treatment of tsetse have also provided evidence for a considerable reduction of bacteroids in the mycetome[15]. Although it was not known at this time that ovaries carry symbionts it is interesting to record that the authors report degeneration of egg follicles and germaria. Similarly, female *G. austeni* fed upon rabbits whose diet contained a coccidiostat, sulphaquinoxaline and pyrimethamine, suffered marked interference in their ability to produce offspring, although it failed to increase the mortality rate of the mothers[16]. Although we have not examined females treated in this way one is nevertheless tempted to suggest that the rickettsia-like population could also be significantly reduced by the chemicals, in which case their presence may be essential for normal ovarian development. It should, however, be pointed out that this conflicts with the suggestion[7] that the rickettsia-like inclusions are to some extent parasitic and appear to cause degeneration of the tissues. We could detect no ill-effects in the host tissues and, moreover, the colony from which our material was drawn had normal fecundity.

As *Glossina* spp. are the vectors of the African trypanosomiases, our observations may have some bearing on the problem of insect control. It has been described how under certain conditions tsetses are virtually dependent on one species of domestic animal for their nutrition. The results outlined in this article serve to underline the suggestion[16] that by suitable host treatment a means of localized tsetse eradication may be achieved.

Summary. A rickettsia-like symbiont, located in the ovaries of *G. m. morsitans* is maternally transmitted to the offspring via the egg. It is suggested that they may be essential for normal ovarian development.

Résumé. Un symbionte de type rickettsien localisé dans les ovaires de *G. m. morsitans* est transmis maternellement par l'œuf à la progéniture. Le symbionte est probablement indispensable au développement normal des ovaires.

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Hybridization of Incompatible Poplars Following Solvent Treatment of Stigmas

Interspecific incompatibility barriers, normally preventing hybridization between white poplars and black poplars, are now well known. It was reported recently[1,2] that successful hybridization between *Populus deltoides*, from Section Aigeiros[3], the black poplars, and *P. alba*, from Section Leuce[3], the white poplars, could be accomplished experimentally using recognition pollen, i.e. compatible pollen rendered inviable by various means, which was then mixed with incompatible pollen and dusted on to receptive stigmas. Success rates of 15–30% seed set (5–11 fertile seeds per capsule, as compared with

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