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Short communications

Association between intracellular rickettsial-like infections of midgut cells and susceptibility to Trypanosome infection in *Glossina* spp.

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Rickettsia-like organisms (RLOs) have been observed in several species of tsetse both in laboratory colonies and in wild flies. Reinhardt et al. (1972) first reported the presence of RLOs in the mycetome of *Glossina morsitans* and *G. fuscipes* and in the cytoplasm of muscle cells of *G. brevipalpis*. Pinnock and Hess (1974) found intracellular RLOs in midgut, fat body and oocytes of field-caught *G. fuscipes, G. brevipalpis* and *G. pallidipes* and in a laboratory colony of *G. morsitans* ssp. *morsitans*. The aim of the present work was to examine lines of *G. morsitans* ssp. *morsitans*, selected for susceptibility and refractoriness to *Trypanosoma congolense* infection, for the presence of RLOs. A survey for RLOs was also conducted on a sample of flies collected in the field.

Two iso-female lines of *G. morsitans morsitans* were examined by electron microscopy. The 'susceptible' line (family 1/6) has been shown experimentally to produce a mean midgut infection rate of 77% with *T. congolense*, while the 'refractory' line (family 29/2) had a mean midgut infection rate of 11% with the same trypanosome species (Maudlin and Dukes, 1985). Flies from each line were collected on the day of emergence from the puparium and the tissues fixed for electron microscopy.

Wild flies were trapped at the Rekomitjie Research Station in the Zambezi Valley, Zimbabwe. *Glossina pallidipes* and *G. morsitans morsitans* fales were dissected, examined for the presence of *T. congolense* and *T. brucei* infections, and their ovaries and midguts were fixed for electron microscopy as in Curtis et al. (1983).

Rickettsia were found in 17 of the 20 ovaries from susceptible *G. morsitans morsitans* (85%), the heaviest infections being found in the nurse cells (Fig. 1). All of the 20 female midguts and 16 of the 20 male midguts examined in the susceptible group were found to have rickettsia within the epithe-

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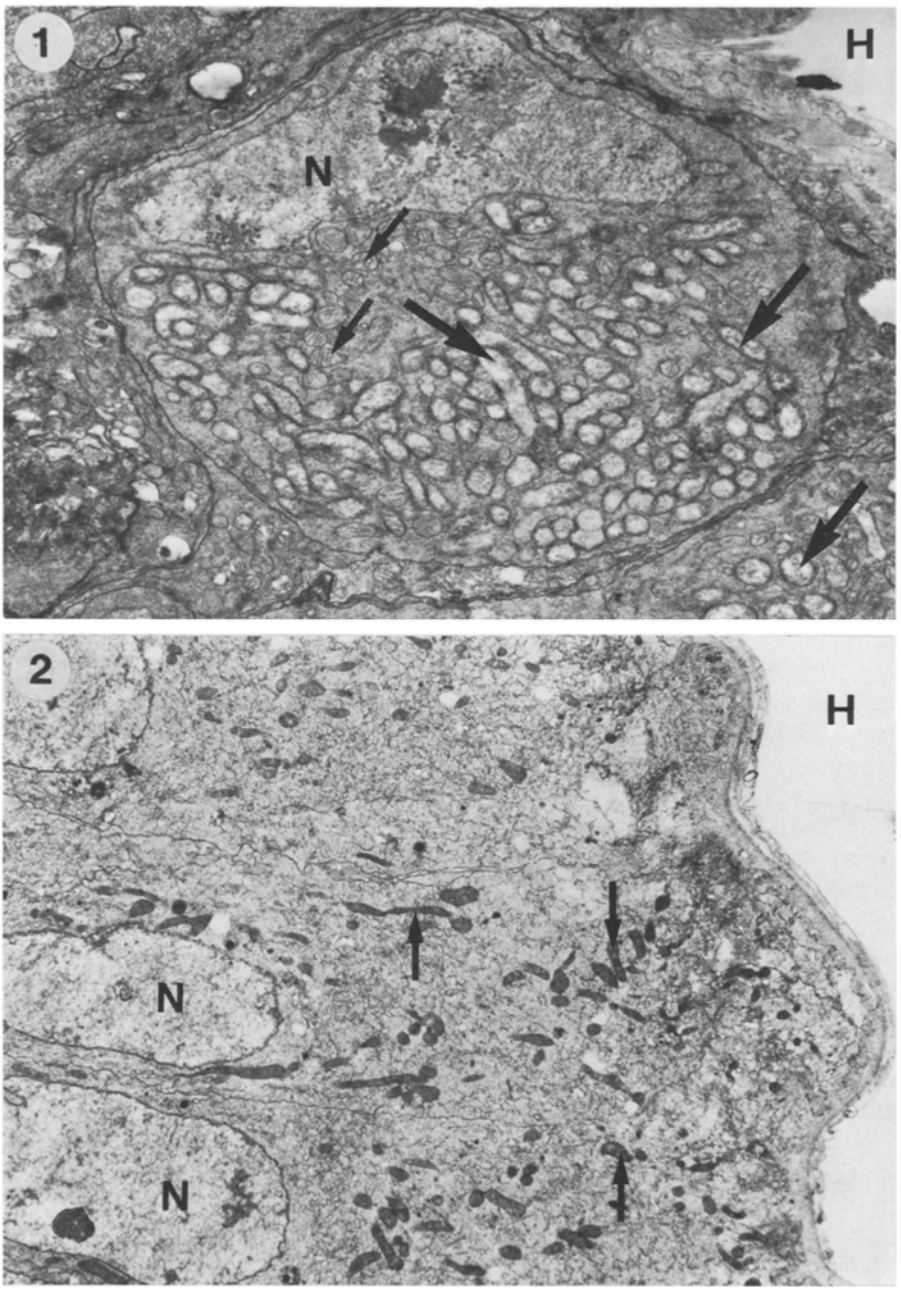


Fig. 1. Rickettsia-like organisms (RLOs), some marked by thick arrows, within nurse cells of the ovary of a tsetse fly from a *G. morsitans morsitans* iso-female line selected for susceptibility to trypanosome infection. Some mitochondria are marked with thin arrows. *N*, nucleus; *H*, haemocoel. ×8,750

Fig. 2. A section across three nurse cells from the ovary of a *G. morsitans morsitans* female from an iso-female line selected for refractoriness to trypanosome infection. Some mitochondria marked with thin arrows. *N*, nucleus; *H*, haemocoel. ×4,550

lium (90% overall), high concentrations being found in the region of the mycetome (Fig. 3).

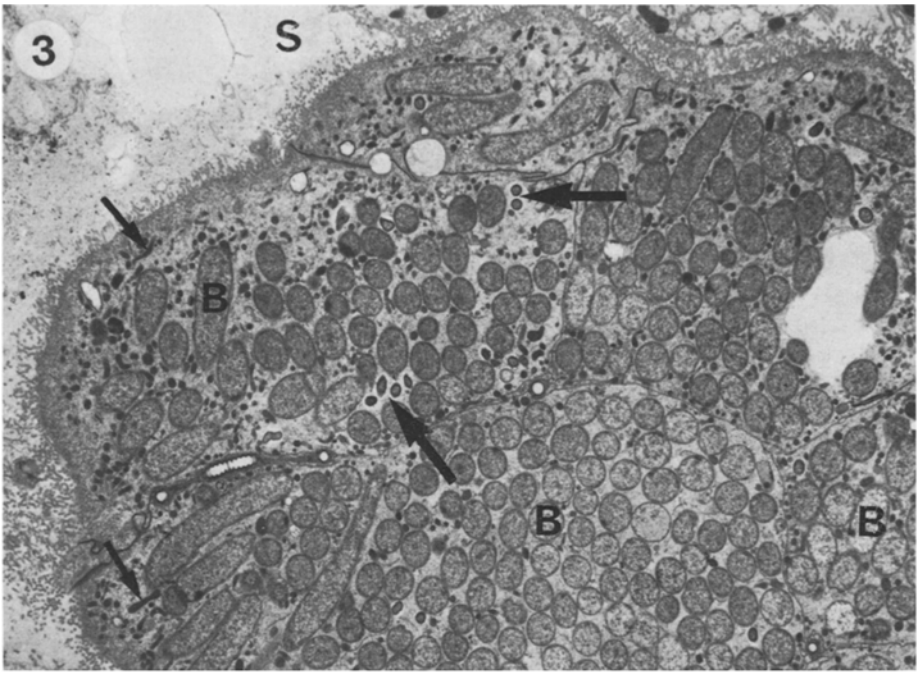
Rickettsia were detected in only three of the 20 ovaries from refractory *G. morsitans morsitans* (15%). Figure 2 shows a section of ovary from one of these specimens with normal uninfected cells. Rickettsia were detected in the midgut cells of only two of the 20 refractory females and five of the 20 refractory males examined (18% overall).

Forty-seven female tsetse from Zimbabwe were examined by electron microscopy, 34 of which were *G. pallidipes* and 13 *G. morsitans morsitans*. Thirteen of these flies (ten *G. pallidipes* and three *G. morsitans morsitans*) were infected with trypanosomes, eight of which were mature *T. congolense* infections and five, which had only immature midgut infections, were presumed to be either *T. congolense, T. brucei* or mixed infections. In only two of the ovaries examined were RLOs found, one of which was from a *G. pallidipes* with a mature *T. congolense* infection, the other an uninfected *G. morsitans morsitans*. Only two of the 24 *G. pallidipes* uninfected by trypanosomes had RLOs in midgut cells whereas four of the ten flies with trypanosomes also had midgut rickettsia (Fig. 4 shows a section of midgut from a *G. pallidipes* female with a rickettsial infection). All three of the wild *G. morsitans morsitans* infected with trypanosomes had RLOs in their midgut cells compared with two of the ten *G. morsitans morsitans* uninfected with trypanosomes.

While RLOs have been found in several species of tsetse (Pinnock and Hess 1974), their role in the biology of the fly has never been clear, although Pell and Southern (1976) suggested that RLOs produced essential metabolites in the ovaries of *G. morsitans morsitans*. In certain mosquitoes, maternally inherited differences in filaria susceptibility are thought to be due to the presence of rickettsia-like symbionts (Trpis et al. 1981; Duhrkopf and Trpis 1981) but work on other mosquito species has produced conflicting evidence (Meek and Macdonald 1982; Curtis et al. 1983).

The present results from both field and laboratory-reared tsetse show a strong association between susceptibility to trypanosome infection and presence of RLOs. The fact that there are exceptions to this association is not surprising; susceptibility to trypanosome infection in the laboratory is not an all-or-nothing phenomenon (Maudlin 1982). In the wild, even more variation in this association would be expected since the likelihood of a fly being infected with trypanosomes is largely related to the species of hosts fed upon and the probability of these animals being infected rather than intrinsic factors (Jordan 1965). That RLOs may be involved in susceptibility to trypanosome infection in tsetse is supported by the fact that RLOs (Pell and Southern 1976) share the same mode of extrachromosomal inheritance as susceptibility (Maudlin and Dukes 1985).

If a causal relationship between rickettsia and susceptiblity to trypanosome infection were to be demonstrated than a novel method of trypanosomiasis control could be proposed. Nogge (1978) has shown that antisera can eliminate the giant bacteroids of the tsetse mycetome and the rickettsial load of wild fly populations might be similarly reduced by raising antisera in preferred domestic hosts of tsetse. While not eliminating trypanosomes



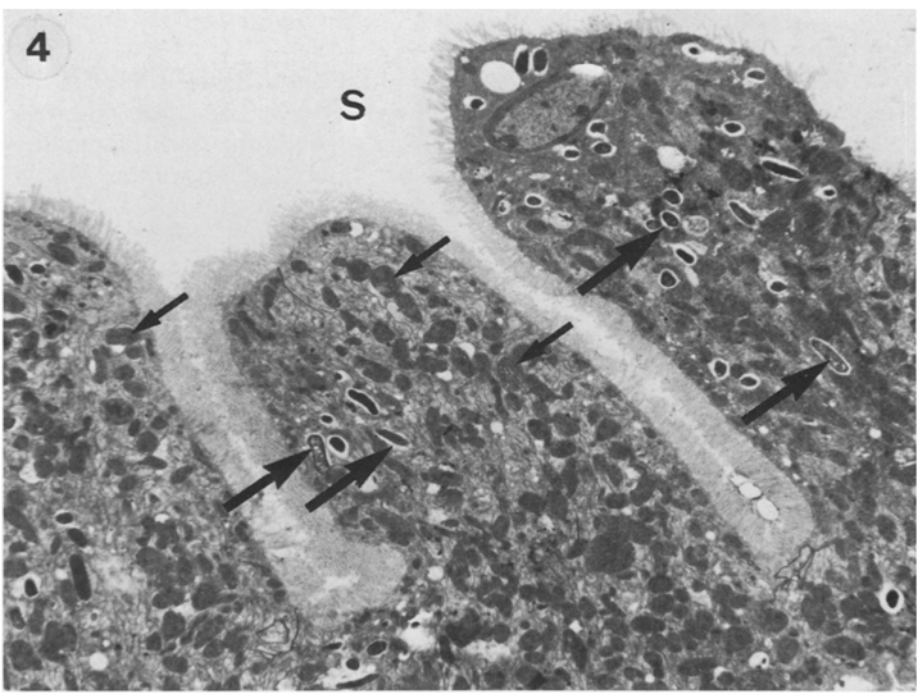


Fig. 3. The mycetome region of the midgut of a *G. morsitans morsitans* female from the susceptible family (1/6). Some of the bacteroids are identified (*B*) and two groups of RLOs are marked with thick arrows. Some mitochondria are shown with thin arrows near the microvilli of this gut cell. *S*, ecto-peritrophic space within the gut lumen. × 3,200

Fig. 4. A section of the midgut of a wild-caught female *G. pallidipes* from Zimbabwe. Some of the RLOs are marked with thick arrows. Note the contrast in appearance with the mitochondria, some of which are marked with thin arrows. × 5,200

from the fly population, such an approach could possibly reduce the 'challenge' of a tsetse population to domestic livestock.

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References

Curtis CF, Ellis DS, Doyle PE, Hill N, Ramji BD, Irungu LW, Townson H (1983) Susceptibility of aposymbiotic *Culex quinquefasciatus* to *Wuchereria bancrofti*. J Invert Path 41:214–223

Duhrkopf RE, Trpis M (1981) The effect of tetracycline treatment on filarial susceptibility in members of the *Aedes scutellaris* complex. Mosquito News 41:729–732

Jordan AM (1965) The hosts of *Glossina* as the main factor affecting trypanosome infection rates of tsetse flies in Nigeria. Trans R Soc trop Med Hyg 59:423–431

Maudlin I (1982) Inheritance of susceptibility to *Trypanosoma congolense* infection in *Glossina morsitans*. An Trop Med Parasitol 76:225–227

Maudlin I, Dukes P (1985) Extrachromosomal inheritance of susceptibility to trypanosome infection in tsetse flies. 1. Selection of susceptible and refractory lines of *Glossina morsitans morsitans*. Ann Trop Med Parasitol (in press)

Meek SR, Macdonald WW (1982) Studies on the inheritance of susceptibility to infection with *Brugia pahangi* and *Wuchereria bancrofti* in the *Aedes scutellaris* group of mosquitoes. Ann Trop Med Parasitol 76:347–354

Nogge G (1978) Aposymbiotic tsetse flies, *Glossina morsitans morsitans* obtained by feeding on rabbits immunized specifically with symbionts. J Insect Physiol 24:299–304

Pell PE, Southern DI (1976) Effect of the coccidiostat, sulphaquinoxaline, on symbiosis in the tsetse fly, *Glossina* species. Microbios Letters 2:203–211

Pinnock DE, Hess RT (1974) The occurrence of intracellular rickettsia-like organisms in the tsetse flies, *Glossina morsitans, G. fuscipes, G. brevipalpis* and *G. pallidipes*. Acta Trop 31:70–79

Reinhardt C, Steiger R, Hecker H (1972) Ultrastructural study of the midgut mycetome-bacteroids of the tsetse flies *Glossina morsitans, G. fuscipes* and *G. brevipalpis* (Diptera, Brachycera). Acta Trop 29:280–288

Trpis M, Duhrkopf RE, Parker KL (1981) Non-Mendelian inheritance of mosquito susceptibility to infection with *Brugia malayi* and *Brugia pahangi*. Science 211:1435–1437

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