A new cell line derived from the tsetse fly *Glossina morsitans morsitans*, vector of trypanosomes of humans and domestic livestock in sub-Saharan Africa

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Insect cell lines play a vital role in many aspects of research on disease vectors [1-3]. Tsetse flies of the genus *Glossina* are important vectors of disease-causing salivarian trypanosomes in sub-Saharan Africa and are major constraints on livestock production, agricultural development and human health in the region [4]. We have recently established a new cell line, GMA/LULS61, derived from tissues of adult female *Glossina morsitans morsitans*.

Methods

- Developing larvae dissected out from gravid female flies (Figure 1)
- Membrane-like tissues surrounding fertilised eggs and larvae cultured in L-15 (Leibovitz) medium supplemented with 10% tryptose phosphate broth, 20% foetal bovine serum, 2 mM L-glutamine, 100 units/ml penicillin, 100 µg/ml streptomycin at 28 °C.
- Medium changed weekly
- Subcultured by scraping or pipetting
- Cells cryopreserved with 10% DMSO [5]
- Species origin confirmed by sequencing fragment of the COI gene [6]
- Absence of contaminating bacteria confirmed by failure to amplify pan-bacterial 16S rRNA gene [7].

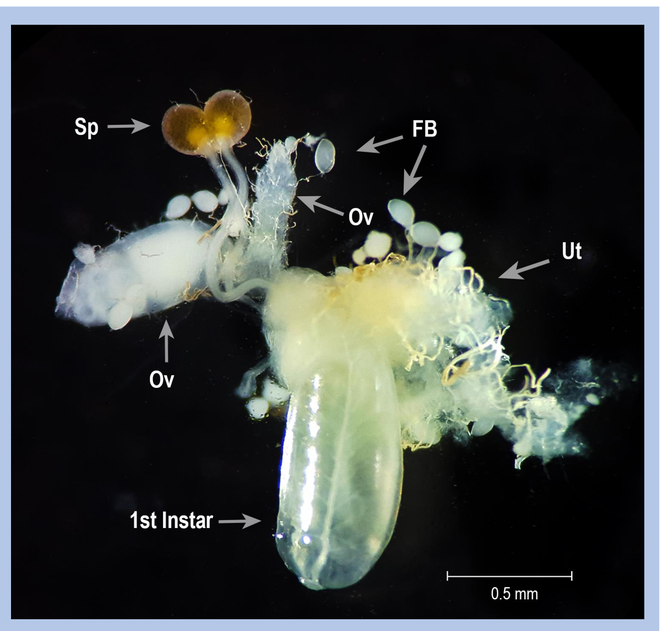

Figure 1. Reproductive tissues dissected out from the body of an adult female *Glossina morsitans morsitans* fly. The uterus was gently retracted to visualise the posterior end of the developing larva. Ov = ovarioles; Sp = spermatheca; FB = fat body; Ut = uterus; 1st instar = L1 larva. Scale bar = 0.5 mm.

- Metaphase chromosome spreads prepared from cells incubated overnight with colcemid, fixed in acetic alcohol and dropped onto ice-cold, wet slides [5]
- GMA/LULS61 cells tested for susceptibility to infection with *Wolbachia* strains *w*Pip and *w*Pap [8], *w*Cfe [9], *w*AlbB [10] and *w*Str1 [11]

Results

- Cell line GMA/LULS61 established from adult *G. m. morsitans* tissues
- First passage carried out on day 437
- Currently at passage 23 (Figure 2)
- Initiated in L-15 medium but also grows well in L-15B medium [12]
- COI sequence analysis grouped GMA/LULS61 cells with other *G. m. morsitans* sequences (Figure 3)
- Karyotyping at passage 17 revealed a predominantly haploid chromosome complement (Figure 4)
- GMA/LULS61 cells supported growth of *Wolbachia* strains *w*Pap, *w*Cfe, *w*AlbB and *w*Str1 (Figure 5) but not *w*Pip

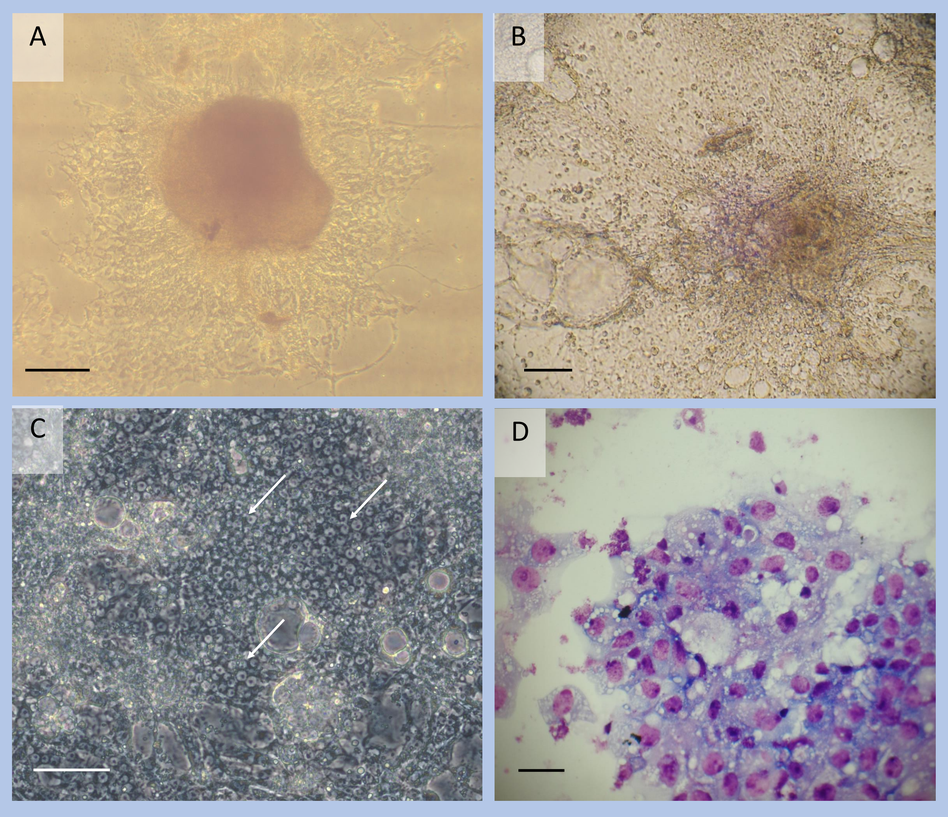

Figure 2. *G. m. morsitans* cell line GMA/LULS61. A. Primary culture at 14 months after initiation. B. GMA/LULS61 cells at passage 4, 18 months after initiation. C. GMA/LULS61 cells at passage 23, 65 months after initiation showing areas of identifiable individual cells (arrows). D. Giemsa-stained cytocentrifuge smear of GMA/LULS61 cells at passage 23. Scale bars = 100 µm (A, B), 50 µm (C), 10 µm (D).

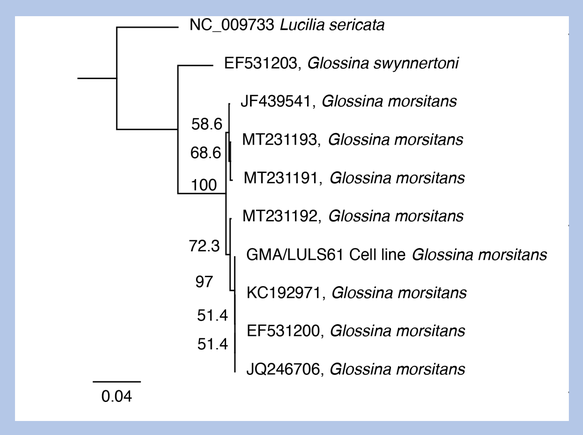

Figure 3. Phylogenetic analysis of the mitochondrially encoded cytochrome c oxidase I (COI) gene sequence amplified from DNA extracted from GMA/LULS61 cells. Tree was generated using the Neighbor-joining method with the Tamura-Nei model and bootstrapped 1000 times. Node labels show bootstrap support as a percentage.

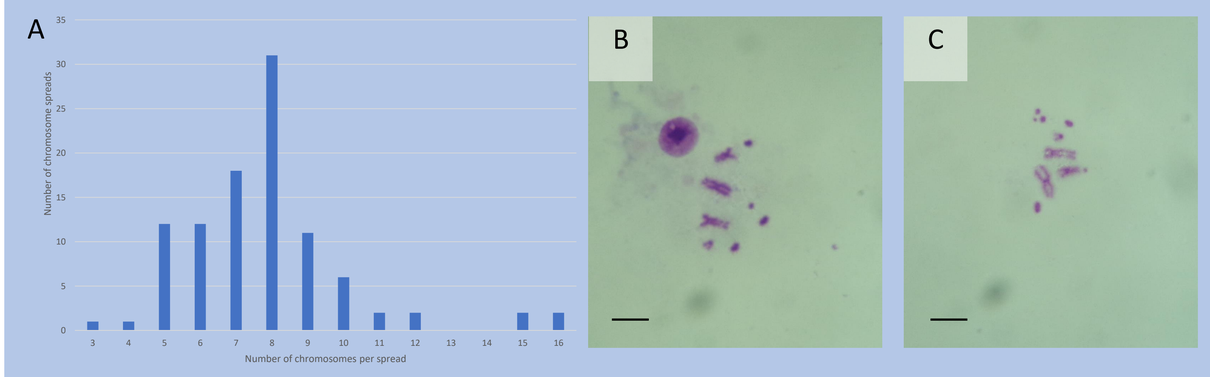

Figure 4. Karyotyping of GMA/LULS61 cells at passage 17. A. Distribution of chromosome numbers in 100 metaphase spreads. B. Typical spread with the modal number of 8 chromosomes. C. Spread with 10 chromosomes. Scale bar = 5 µm.

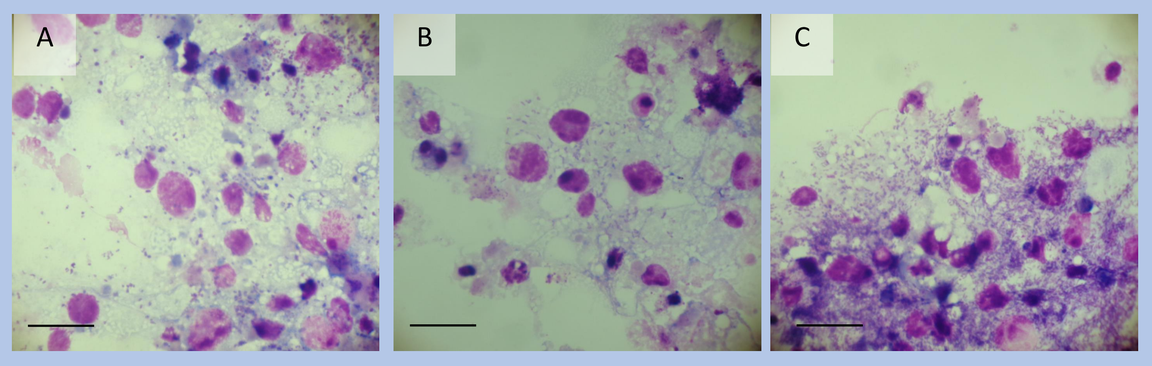

Figure 5. Giemsa-stained cytocentrifuge smears of *Wolbachia*-infected GMA/LULS61 cells prepared at 10 weeks after initial infection. A. Flea-derived *w*Cfe. B. Sand fly-derived *w*Pap. C. Mosquito-derived *w*AlbB passaged onto fresh GMA/LULS61 cells five weeks after initial infection. Scale bars = 10 µm.

Conclusion

The GMA/LULS61 cell line, available from the Tick Cell Biobank at the University of Liverpool, has potential for application in a variety of studies investigating the biology and control of *G. m. morsitans* and its associated pathogenic and symbiotic microorganisms, including salivarian trypanosomes, tsetse viruses and *Wolbachia*.

For further information, contact tickcellbiobankenquiries@liverpool.ac.uk or visit the **TCB website** at https://www.liverpool.ac.uk/research/facilities/tick-cell-biobank/

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