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## Influence of the *salmon* mutant of *Glossina morsitans morsitans* on the susceptibility to infection with *Trypanosoma congolense*

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### Summary

Four phenotypes of a sex-linked, maternally influenced semi-lethal eye color mutant of *Glossina morsitans morsitans* Westwood were fed on *Trypanosoma congolense* Broden infected guinea pigs. Infection rates were evaluated 25 days later by means of dissection. Procyclic as well as mature infections were significantly more common among females with salmon-colored eyes (*sal/sal*) than among heterozygous (+/*sal*, phenotypically wild-type) females. A tendency was found for more mature infections among *sal/Y* males than among wild-type males. Similarly, females tended to be more infected than males with both procyclic and mature infections. These results indicate that the genotype of the fly, exemplified by the allele *salmon*, might influence the development of *T. congolense* in *G. m. morsitans*. A possible explanation for this phenomenon is discussed.

**Key words:** *Glossina*; *Trypanosoma*; vectoring; genetics.

### Introduction

Although several factors influence vectorial capacity of tsetse flies (Jordan, 1974; Maudlin, 1980; Molyneux, 1980; Maudlin et al., 1984), there is little

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direct evidence that vectorial capacity is influenced by genetics of the flies. There is a maternally influenced inheritance pattern in *Glossina morsitans morsitans* Westwood fed upon procyclic forms of *Trypanosoma congolense* Broden; parental genotype had little effect upon development of mature infections (Maudlin, 1982).

A sex-linked maternally influenced semi-lethal eye color mutant, designated *salmon* (= *sal*), was discovered in a self-supporting colony of *G. m. morsitans* (Gooding, 1979). This mutant is unable to metabolize tryptophan normally (Davis and Gooding, 1983; Gooding and Rolseth, 1984) and was chosen for study because tryptophan is an essential amino acid metabolized by several species of trypanosomes (Hall et al., 1981). The present study was undertaken to determine the susceptibility of salmon *G. m. morsitans* to infection with *T. congolense*.

## Methods and Material

### Host animal

The host animals were guinea pigs infected with *T. congolense* TORORO/69/EATRO/1157 by cyclic transmission using *Glossina palpalis palpalis* (Robineau-Desvoidy) (Distelmans et al., 1982). Peripheral blood was examined three times a week by means of the microhaematocrit method of Woo (1969). Peak parasitaemia (at least  $10^8$  tryp/ml) was reached 20 days after the guinea pigs were found positive, and about 15 days later the animals died.

### Tsetse fly

Origin and maintenance of the *G. m. morsitans* colony were previously described (Gooding and Rolseth, 1976; Rolseth and Gooding, 1978). The *salmon* allele is maintained by mating heterozygous (+/*sal*) females with hemizygous (*sal*/Y) males (Gooding, 1979). This cross produces the following types of offspring which were transported as puparia, by air and train, from Edmonton to

Table 1. Infection of *Glossina morsitans morsitans* with *Trypanosoma congolense*

Genotype	Fly sex	Eye color <sup>1</sup>	Number of flies				
			examined	infected	P <sup>2</sup>	G <sup>3</sup>	M <sup>4</sup>
<i>sal/sal</i> . . . . .	♀	sal	53	27 (51) <sup>6</sup>	10 (19)	17 (32)	0.630
+/ <i>sal</i> . . . . .	♀	wt	134	46 (34)	24 (18)	22 (16)	0.478
<i>sal/Y</i> . . . . .	♂	sal	25	8 (32)	3 (12)	5 (20)	0.625
+/ <i>Y</i> . . . . .	♂	wt	91	22 (24)	15 (16)	7 (8)	0.318

<sup>1</sup> Eye color: sal = salmon; wt = wild-type (dark brown)

<sup>2</sup> P = flies with procyclic trypanosomes in the gut

<sup>3</sup> G = flies with gut infection only

<sup>4</sup> M = flies with mature infection, i.e. trypanosomes in the hypopharynx

<sup>5</sup> R = ratio, M/P

<sup>6</sup> Numbers in parentheses are percentages.

Antwerp by one of us (R.H.G.): salmon females (*sal/sal*), wild-type females (+/*sal*), salmon males (*sal/Y*) and wild-types males (+/*Y*). All four phenotypes were fed, within 32 h of emergence, on an infected guinea pig at peak parasitaemia. Fully engorged flies were retained and, except for the single infective meal, they were fed on uninfected guinea pigs seven days a week. These animals were examined for trypanosomes three times a week and were replaced every week in order to eliminate the possibility of secondary cyclical transmission. Flies were maintained at approximately 23 °C and 65% R.H. in Weiss climatic chambers.

#### *Evaluation of the susceptibility to trypanosome infection*

Twenty-three days after the infective meal, flies were given their last meal and then starved for two days to reduce or eliminate partially digested blood and thus facilitate dissection of the gut and observation of trypanosomes. Flies surviving to day 25 were dissected and gut, proventriculus, and mouth parts (labrum and hypopharynx of the proboscis) were examined. All flies dying before the end of this period were dissected.

A  $2 \times 2$  G-test (with Williams' correction; Sokol and Rohlf, 1981) was used to determine the statistical significance of sex and phenotype upon midgut (procyclic) and hypopharyngeal or mature infection of flies by trypanosomes.

## **Results**

A group of teneral flies, emerging on seven consecutive days, was fed within 32 h of emergence on the same guinea pig at peak parasitaemia (at least  $10^8$  tryp/ml blood). Another group of ternals, emerging on nine consecutive days was fed on a second guinea pig. Results showed that, within each sex and phenotype, the two groups did not differ. Results obtained with the two groups have therefore been pooled.

Results of dissections on day 25 post-infection are given in Table 1 and statistical analyses are presented in Table 2. None of the flies dying before the end of the test period were included in these results. However, a mature infection was found in a salmon female as early as seven days post-infection.

#### *Influence of the salmon phenotype*

Procyclic as well as mature infections were significantly more common among salmon females (*sal/sal*) than among the heterozygous (+/*sal*, phenotypically wild-type) females (Tables 1 and 2). There was also a tendency for both more procyclic and mature infections among *salmon* males than among wild-type males. However, the low sample size of *salmon* males would be a possible explanation that these differences have not been found statistically significant.

#### *Influence of sex*

Within each phenotype there was a tendency for a greater percentage of infected females than of infected males (Table 1). This was found with both procyclic and mature infections. However here again none of these differences were statistically significant (Table 2). Our findings differ from previous reports

Table 2. Statistical comparison of infections of *Glossina morsitans morsitans* with *Trypanosoma congolense*

	<i>sal/sal</i>	<i>+/sal</i>	<i>sal/Y</i>	<i>+/Y</i>
<i>sal/sal</i> . . . . .	0.000	5.230	1.236	13.575
<i>+/sal</i> . . . . .	4.297	0.000	0.178	3.823
<i>sal/Y</i> . . . . .	2.454	0.017	0.000	2.605
<i>+/Y</i> . . . . .	10.415	2.670	0.589	0.000

Numbers given in the body of the table are G-stats (with Williams' correction; Sokal and Rohlf, 1981). Values above the diagonal line of zeros refer to mature infections; values below the line refer to procyclic infections. Critical values, for 1 d.f. are: 2.706,  $p = 0.10$ ; 3.841,  $p = 0.05$ ; 5.024,  $p = 0.025$ ; 6.635,  $p = 0.01$ ; 7.879,  $p = 0.005$  (Rohlf and Sokal, 1981).

of slightly higher prevalence of *T. congolense* among males than among females (Clarke, 1969; Distelmans et al., 1982).

#### *Influence of the salmon allele upon the gut barrier*

The ratio (R) of the number of flies having mature infection (M) divided by the total number of flies having a procyclic infection (P) may be considered to be the probability that a gut infection will proceed to a mature infection. The R values obtained in the present study (Table 1) were highest for salmon males and females (0.625 and 0.630, respectively), lowest for flies completely lacking the *salmon* allele (0.318), and intermediate for the heterozygous females (0.478). Although low sample sizes (particularly of salmon males) necessitate caution in interpreting this finding, the results indicate that the *salmon* allele may favour development of a mature infection.

#### Discussion

Though certain findings have to be interpreted with caution, due to low sample sizes of *salmon* males, the *salmon* allele significantly influences the prevalence of both procyclic and mature infections of *T. congolense* in *G. m. morsitans*. Moreover, this positive influence of the *salmon* allele on the infection rate of *G. m. morsitans* corroborates the studies of Makumyaviri et al. (in press), using the *T. b. brucei/G. m. morsitans* model.

We can conclude that these results indicate that the genome of the tsetse fly plays a role in the cyclic development of trypanosomes.

The pleiotropic allele *salmon* affects a number of morphological and physiological traits in *G. m. morsitans* (Gooding, 1979, 1982; Davis and Gooding, 1983), but the only known biochemical lesions are a marked depression of tryptophan oxygenase activity in *salmon* flies, and excretion of large quantities of tryptophan by these flies (Gooding and Rolseth, 1984). Results of the present

experiments suggest a link between infection, of *G. m. morsitans* with *T. congolense*, and tryptophan, an essential amino acid which is extensively metabolized by several species of trypanosomes (Hall et al., 1981). It is hoped that further comparative studies using a variety of strains and species of trypanosomes and an appropriate number of various genetically marked flies will further elucidate the relationships between the trypanosomes and their tsetse vectors.

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