The Effects of a DNA Virus Infection on the Reproductive Potential of Female Tsetse Flies, *Glossina morsitans centralis* and *Glossina morsitans morsitans* (Diptera: Glossinidae)

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Reproductive anomalies associated with the tsetse DNA virus infection in the female tsetse hosts, Glossina morsitans centralis *Machado and* Glossina morsitans morsitans Westwood, *inoculated with the virus during the 3rd instar larval stage were studied and the data compared to those obtained from the control females injected with sterile physiological saline.*

Virus infected flies had significantly longer first and second pregnancy cycles (P<0.0001) and produced pupae that were of significantly less weight in milligrams (P<0.0001) compared to controls. Transmission of the virus to progeny was not absolute and only 21% of G. m. centralis *and 48% of* G. m. morsitans *first progeny flies from infected females developed salivary gland hypertrophy as a result of transmission from mother to progeny.*

The virus infected females produced significantly fewere pupae compared to the controls during the experimental period (P<0.00001).

Key words: DNA virus - tsetse fly - *Glossina morsitans centralis* - *Glossina morsitans morsitans* - reproductive potential - pregnancy cycle - pupal weights - transovarial transmission

The tsetse virus causing salivary gland hypertrophy and male sterility has been shown to infect some tsetse fly species naturally in the wild including *Glossina pallidipes* and *Glossina morsitans morsitans* (Whitnal 1934, Jaenson 1978, Otieno et al. 1980, Ellis & Maudlin 1987). Subsequently it has been partially characterized as a double stranded DNA virus (Odindo et al. 1986). A method of infecting tsetse flies in the laboratory was developed by Jura et al. (1993), thus facilitating laboratory experimentation on this virus to determine how it affects its hosts.

Tsetse flies are reproductively peculiar because the females give birth to larvae, nourished within the mother by a secretion from the highly modified uterine milk gland (Tobe & Langley 1978). The first oocyte is ovulated about nine days after emergence of the female (Saunders 1970) and is fertilised as it enters the uterus. Embryogenesis takes place for three days after which the larva hatches and goes through three larval instars in the uterus. It is born as a full term 3rd instar larva at the end of a first pregnancy cycle which takes 18 days in the laboratory conditions while subsequent cycles take 9 to 10 days (Langley 1977). The larva pupates in 15 to 30 min and the adult fly emerges from the pupa 30 days later. Tsetse thus have a low reproductive potential in comparison to other insects as only a small number of developed larvae can be produced in the life of a female. The fecundity of tsetse is thus highly dependent on the survival rate and the duration of interlarval period.

Jura et al. (1988) examined the ovarioles of virus infected *G. pallidipes* and observed that the majority of the germaria were affected by degeneration and severe necrosis and virus particles were seen within the germarial cells suggesting that this virus may be transmitted transovarially to the progeny by an infected female. The degenerative changes may affect the development of the larva resulting from such an egg. Sang et al. (1997) demonstrated that flies infected by this virus had difficulty in feeding and this affected their survival significantly. These factors would probably influence the reproductive performance of the infected female fly. Evaluation of the potential of this virus as a biocontrol agent in the control of tsetse flies includes obtaining information on the effects of the virus on the female reproductive potential as well. The specific objectives of this study were to investigate the

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effects of this virus on the duration of the pregnancy cycles, pupal productivity and their weights.

MATERIALS AND METHODS

Virus material - *G. pallidipes* were trapped at the Ruma National Park in the Lambwe Valley Game Reserve in Western Kenya. Flies were dissected in the laboratory and any hypertrophied salivary glands retrieved and frozen in sterile physiological saline. Fifteen pairs of these were later homogenised in 0.5 ml of sterile saline centrifuged at 3,000 rpm for 15 min at 4ºC. The supernatant was used to inoculate experimental flies.

Production of virus infected and control flies - Freshly larviposited larvae of *G. m. centralis* or *G.m. morsitans* were obtained from the insectary where tsetse are reared at 25ºC and 75-80% relative humidity. The larvae were inoculated with 1 ml of virus suspension prepared as above using the method developed by Jura et al. (1993). This technique confers 100% infection in treated flies. Control flies were inoculated with 1 ml of sterile saline. After the larvae pupated they were incubated under the insectary conditions for approximately 30 days to emerge. At emergence the sexes were caged separately in PVC fly holding cages. Flies were always fed on a live rabbit.

Effects of virus infection on pregnancy cycle, pupal production - Virus-infected 2-day old *G.m. centralis* females were fed on rabbit blood and after few hours mated to 8 day-old males obtained from the insectary, where they are fed every other day also on rabbit blood. The males were removed after 24 hr of mating and destroyed. The females were tranferred to single fly holding plastic vials measuring 5 x 2.5 cm with a nylon netting on one end to allow feeding, kept under insectary conditions and fed every other day. A group of control flies were treated similarly. Flies were observed for two pregnancy cycles and the duration of the cycles, pupal production, weights of pupae and their emergence were recorded.

Transmission rate of the virus from the mother to progeny - Both *G. m. morsitans* and *G. m. centralis* were used in these experiments. Females of these species emerging from both control and virus infected pupae were fed on rabbit blood on day 2 and then mated to 8 day-old males as in the previous experiment. The flies were caged singly in single-fly holding plastic vials, fed regularly and checked daily for pupal production for three pregnancy cycles. The pupae from each fly were caged separately in single fly holding tubes and upon emergence they were dissected and examined for salivary gland hypertrophy. At the end of the three pregnancy cycles, the experimental females were dissected to check for insemination and to confirm salivary gland hypertrophy in the virus infected flies.

RESULTS

Effects of virus infection on pregnancy cycle and pupal weights - It was observed that the durations of the first and the second pregnancy cycles in virus infected flies were significantly longer than in the controls and the pupae produced by virus infected females were of significantly lower weights compared to those produced by control females ($P<0.0001$, Table I).

TABLE I

Mean duration of 1st and 2nd pregnancy cycles (in days) and weights (in mg) of pupae produced in 1st and 2nd cycle by virus infected and control female *Glossina morsitans centralis*

Treatment	Cyle	No. larvae	Mean duration	Mean weight
Control	C1	59	17.84±0.54	27.46±0.50
	C2	58	9.09±0.20	29.38±0.35
Infected	C1	50	22.38±0.76	22.38±0.75
	C2	40	12.80±1.17	21.55±0.74

C1: first pregnancy cycle; C2: second pregnancy cycle; $F = 25.115$, $P < 0.001$ for duration of cycle 1 and $F = 13.90$, $P < 0.001$ for duration of cycle cycle 2. $F = 97.671$, $P < 0.001$ for weight of pupa 1 and $F = 112.387$, $P < 0.001$ for weight of pupa 2.

Virus transmission rates to progeny flies by virus infected females - It was observed that an infected female did not always transmit the virus to all her progeny. Only 21% of the progeny from *G. m. centralis* and 48% of the progeny from *G. m. morsitans* developed the syndrome (Table II). Where an infected fly was able to produce more than one puparium, in most cases it either produced all infected progeny or all normal progeny except two flies (nos. 14 and 18, see Table III) which produced mixed progeny (one virus infected and one uninfected). The rest of the flies in the experimental group produced only one or no larvae at all and in few cases the larvae produced failed to pupate.

TABLE II

Transmission rates of hypertrophied salivary gland syndrome by virus infected *Glossina morsitans morsitans* (*Gmm*) and *Glossina morsitans centralis* (*Gmc*)

Fly species	Total pupae	EM pupae	NSG	HSG	%HSG
Gmm	39	29	15	14	48.28
Gmc	32	18	14	4	22.22

EM: emerged; NSG: normal salivary gland; HSG: hypertrophied salivary gland.

Pupal productivity - Table IV demonstrates that 45% of the female *G. m. morsitans* and 47% of *G. m. centralis* did not produce any larvae at all through the experimental period although they were inseminated. Some of the females either died before they larviposited or failed to larviposit over the experimental period. Only 10% the virus infected *G. m. morsitans* and *G. m. centralis* females produced three larvae during the experiment. Among the controls of both species, over 90% of the females produced three larvae during the experimental period and as demonstrated in Table V, infected females produced significantly more pupae per female compared to the controls.

TABLE III

Virus infection rates in 1st progeny of virus infected female *Glossina morsitans centralis* (*Gmc*) producing more than one viable pupa

Fly no.	Species	Pupae produced	1st pupa	2nd pupa	3rd pupa
2	*Gmc*	3	NSG	NSG	NSG
4	*Gmc*	3	HSG	HSG	
15	*Gmc*	2	NSG	NSG	
8	*Gmc*	3	NSG	NSG	NSG
7	*Gmc*	3	NSG	NSG	
5	*Gmc*	2	NSG	NSG	
9	*Gmm*	3	HSG	HSG	HSG
10	*Gmm*	3	HSG	HSG	HSG
5	*Gmm*	2	HSG	HSG	
18	*Gmm*	2	HSG	NSG	
39	*Gmm*	3	NSG	NSG	NSG
14	*Gmm*	3	NSG	NSG	HSG

HSG: hypertrophied salivary gland; NSG: normal salivary gland.

DISCUSSION

These results reveal that the tsetse virus infection has detrimental effects on the female reproductive performance, lengthening the interlarval periods and reducing the pupal production and their weights. The reduction in the weights of pupae of infected flies can be attributed partly to the presence of necrotic lesions in the germaria and viral particles detected in the germarial cell nuclei (Jura et al. 1988). The presence of virogenic stromata in these cells is an indication of replicating sites of the virus. Replication and assembly of virions in cells provides a stress on the affected organ as the cell macromolecular synthetic machinery is switched over to the synthesis of viral protein. The replication of the virus in the ovarioles could have an impact on the nutritive requirements and other developmental factors which could lead to delays in larval development or lack of it. This could also partly explain the increased interlarval periods. The replication of the virus and the pathological changes observed in milk glands of virus infected females (Sang et al. 1996) may also lead to the poor nutritional provision to the larva *in utero* and could also explain the reduced pupal weights and

TABLE IV

Pupal production rates in three pregnancy cycles by virus infected and control *Glossina morsitans morsitans* and *Glossina morsitans centralis* females

	G. m. morsitans				*G. m. centralis*			
	Infec.		Cont.		Infec.		Cont.	
Pupae produced	N	%	N	%	N	%	N	%
0 pupae	18	45	0	0	18	47.4	0	0
1 pupa	9	22.5	0	0	12	31.6	0	0
2 pupae	9	22.5	2	4	4	10.5	3	5.6
3 pupae	4	10	48	96	4	10.5	50	94.3

N: the number of females producing the pupae in the experiment; %: proportion of females producing 0, 1, 2 or 3 pupae during the three cycle period.

TABLE V

Mean number of pupae per female produced in three pregnancy cycles by infected and control *Glossina morsitans morsitans* (*Gmm*) and *Glossina morsitans centralis* (*Gmc*)

	Gmm		*Gmc*	
	Infected	Control	Infected	Control
Females	40	48	38	53
Mean # pupae/female	0.95±0.16	2.96±0.03	0.84±0.16	2.94±0.03

$X^2 = 68.57$; $P<0.0001$ for *Gmm;* $X^2 = 68.72$; $P<0.0001$ for *Gmc.*

lengthened interlarval periods. Other observations (Sang et al. 1997) have shown that infected flies experienced difficulty in feeding and in some cases the digestion was greatly impaired due to lesions occurring in sections of the midgut. This could also affect the development of the larvae *in utero* as the larva depends on the nutritive provision of the mother for its development. Lesions occurring in the midgut would result in impaired digestion and absorption and this would lead to undernourishment of the infected female and hence the larva *in utero*. It has been reported in some laboratory studies that the amount of blood embibed during interlarval period is a major factor determining the reproductive performance of tsetse (Langley & Stafford 1990, Gaston & Randolph 1993). Saunders (1970) showed that factors other than the temperature can alter the interlarval period and also suggested that the milk gland secretion is perhaps initiated by correctly timed blood meals and in the absence of such blood meals the secretion of the milk is delayed and the larva becomes undernourished with consequent retardation in its growth rate.

Results of this study further demonstrate that although a female may show salivary gland hypertrophy, it may fail to transmit the infection to the progeny. Jaenson (1978, 1986) working on field-collected and laboratory reared *G. pallidipes* recorded that infected females, in general, produced infected progeny. However, Jura et al. (1988) demonstrated that only occasional virus particles were found within the ooplasm and the affected oocytes appeared intact without pathological changes. Sang et al. (1996) demonstrated the presence of the virus in milk gland cells with virogenic stroma in the cell nuclei and suggested that the virus could be transmitted through milk secretion to the larva *in utero*. This mode of transmission probably occurs when sufficient virus particles are ingested with the milk.

These effects of the virus on the female reproductive potential partly explain the reported low incidence of the virus infection in nature which ranges between 0.5% to 5%, (Otieno et al. 1980, Ellis et al. 1987, Gouteaux 1987). It is possible that the establishment of this virus in a tsetse infested area could help to regulate the tsetse population by acting as a control agent. Extensive field studies are required to determine the role of the DNA virus in the regulation of tsetse populations in nature.

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REFERENCES

Challier A, Laveissiere C 1973. Un nouveau piege pour la capture des Glossines (*Glossina*; Diptera, Muscidae). Discription et essais sur le terrain. *Cah ORSTOM Ser Entomol Med Parasitol 11*: 251-262.

Ellis DS, Maudlin I 1987. Salivary gland hyperplasia in wild caught tsetse from Zimbabwe. *Entomol Exp Appl 43*: 167-173.

Gaston KA, Randolph SE 1993. Reproductive under performance of tsetse flies in the laboratory, related to feeding frequency. *Physiol Entomol 18*: 130-136.

Gouteaux JP 1987. Prevalence of enlarged salivary glands in *Glossina palpalis*, *G. pallicera* and *G. nigrofusca* (Diptera: Glossinidae) from the Vavoua area, Ivory Coast. *J Med Entomol 24:* 268

Jaenson TGT 1978. Virus like rods associated with salivary gland hyperplasia in tsetse, *Glossina pallidipes. Trans R Soc Trop Med Hyg 72*: 234-238.

Jaenson TGT 1986. Sex ratio distortion and reduced lifespan of *Glossina pallidipes* infected with the virus causing salivary gland hyperplasia. *Entomol Exp App 41*: 265-271.

Jura WGZO, Zdarek J, Otieno LH 1993 A simple method for artificial infection of tsetse, *Glossina m. morsitans* larvae with the DNA virus of *G. pallidipes*. *Insect Sci Applic 14*: 383-387.

Jura WGZO, Odhiambo TR, Otieno LH, Tabu NO 1988. Gonadal lesions in virus-infected male and female tsetse *Glossina pallidipes* (Diptera; Glossinidae). *J Invertebr Pathol 52*: 1-8.

Langley PA 1977. Physiology of tsetse flies (*Glossina* Spp) (Diptera;Glossinidae): a review. *Bull Enomolt Res 67*: 523-574.

Langley PA, Stafford K 1990. Feeding frequency in relation to reproduction in *Glossina m. morsitans* and *G. pallidipes*. *Physiol Entomol 15*: 415-421.

Odindo MO, Payne CC, Crook NE, Jarret P 1986. Properties of a novel DNA virus from a tsetse fly *Glossina pallidipes*. *J Gen Virol 67*: 527-536.

Otieno LH, Kokwaro ED, Chimtawi M, Onyango P 1980. Prevalence of enlarged salivary glands in wild populations of *Glossina pallidipes* in Kenya, with a note on the ultrastructure of the affected organ. *J Invertebr Pathol 36*: 113-118.

Sang RC, Jura WGZO, Otieno LH, Mwangi RW 1997. Effects of a DNA virus infection on the survival of a host fly, *Glossina morsitans centralis* (Diptera; Glossinidae). *J Invertebr Pathol 69*: 253-260.

Sang RC, Jura WGZO, Otieno LH, Ogaja P 1996. Ultrastructural changes in the milk gland of Tsetse *Glossina morsitans centralis* (Diptera; Glossinidae) female infected by a DNA virus. *J Invertebr Pathol 68*: 253-259.

Sounders DE 1970. Mating ovulation and oocyte development in *Glossina morsitans*. *Trans R Soc Trop Med Hyg 64*: 184-185.

Tobe SS, Langley PA 1978. Reproductive physiology of *Glossina*. *Ann Rev Entomol 23*: 283-307.

Whitnall AMB 1934. The trypanosome infections of *Glossina pallidipes* in Umfolosi game reserve, Zululand. *Onderstepoot J Anim Ind 11*: 7-21.