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Evaluation of Fecundity for Variants of Laboratory Bred Glossina Morsitan Submorsitan and Glossina Palpalis Palpalis Exposed to Ethanol at Larval Stage

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Abstract

22 nos Larvae of *Glossina morsitan submorsitan* and *Glossina palpalis palpalis* collected from the insectary after larviposition was immediately exposed to 50% ethanol and observed for puparium time; *Glossina Palpalis palpalis* indicated an average of 13.22min while *Glossina morsitan submorsitan* indicated an average of 10.72mins. The average emergence period of the F^1 generation of young tsetse flies for both species were 30 days. After mating, 16 nos exposed variant of all *Glossina morsitan submorsitan* did not produce any offspring while 2 nos of *Glossina palpalis palpalis* larviposited. The study reveals that *Glossina palpalis palpalis* maintained a normal fecundity irrespective of larvae exposure to alcohol while *Glossina morsitan submorsitan* maintained a null fecundity thus showing remarkable result for possible derivative insecticidal control of the *Glossina morsitan submorsitan*.

Keywords: fecundity, Glossina morsitan submorsitan, Glossina palpalia palpalis, trypanosomiasis

INTRODUCTION

Tsetse flies are the sole vectors of Human African Trypanosomiasis (HAT) in sub-Saharan Africa and are efficient vectors of African trypanosomes, causative agents of sleeping sickness in humans and nagana in domesticated animals [Geiger A, 2007, Gooding RH, 2005; Steelman CD 1976].

.*Glossina morsitan submorsitan* and *Glossina palpalis palpalis* are dipteral of the family Glossinidae and responsible as a vector for the transmission of trypanosomes, the parasite responsible for the disease trypanosomiasis. Reduction of tsetse fly populations in affected areas is suggest to be very effective due to tsetse's low reproductive rate (Joja and Okoli 2001; Jordan 1986), also amongst dipterans which are usually oviparous (depositing eggs), tsetse are the only ones known to be viviparous (intrauterine development and birth of live offspring), a closer understanding of this may help in augmenting vector control techniques based on traps and targets to make them more effective. The disease trypanosomiasis was an epidemic involving several hundred thousand people that spread through Sudan, the Central African Republic, DRC and Angola in the 1990's, and has shown how socially and economically devastating these diseases are [Pepin J 2001]. Trypanosomes kill more than 3 million cattle annually and those animals that survive display low productivity due to the wasting effects of the disease [Hursley BS, 2001]. The annual losses from trypanosomiasis in cattle amount to more than US $4.5 billion [Budd]. The identified factors that influence vector competence (the ability to transmit parasites) include the age of the fly, the number of bloodmeals taken and the activation of fly immune processes, with both antimicrobial (host defense) peptides [Hao Z, 2001], and lectins [Welburn SC 1989; Maudlin SC 1987; Abubakar L 2006]. The disease is debilitating and responsible for loss of livestock of over 30 billion dollars value in Africa alone and also causes sleeping sickness in human. Drug approach to this disease has been hindered by resistance by the parasite, hence the need to look at other approach to control of the vector, in this case tsetse fly control.

Between days 4 and 5 trypanosome infections are eliminated from most flies [Gibson W 2003], There are no effective vaccines against trypanosomosis and the disease is mainly managed by the prophylactic and curative treatment with trypanocidal drugs. However, there have been reports of development of resistance to the available trypanocidal drugs (Aksoy & Rio, 2005).

The SIT (sterile infectivity technique) has been adduced as a good approach with regards to area-wide intergrated insect management (AW-IPM) with promising success (Hendrich J 2007; Msiangi AR 2000). Other novel approach is the incorporation of molecular biology like nanobody development or genetic distortion of enzymes necessary for transialidation to occur invariably leading to no cyclic transmission and no transmittable parasite by vector, amongst others. This novel approaches have been met with skepticism over its long term effect to the ecosystem. However in this case, control methods have to be abducted as less than 15% cases of human trypanosomiasis is diagnosed and treated (Kristjanson et al, 1999; Kamuanga, 2003; Cecchi and Mattoli, 2009)

MATERIALS AND METHOD

Analytical grade absolute (100%) ethanol was used and diluted with distilled water at equal proportion to obtain (50%) ethanol. Standard Geiger cages, mini-pipette and disposable cups were obtained as required.

Tsetse Larvae

Tsetse larva of both *Glossina morsitan submorsitan* and *Glossina palpalis palpalis* were collected from the breeding room of the Insectary at the Vector and Parasitology Studies Department of NITR, Kaduna immediately after they are larviposited.

Larva exposure

G. morsitan submorsitan and *G. palpalis palpalis* larva were collected after larviposition from the Breeding room of the NITR insectary in Kaduna daily with Specie, dates and time of collections recorded. The larvae were placed in separate small cups immediately and subjected to a drop of 50% alcohol and allowed to pupate at suitable environment devoid of sunlight then time of every pupation was recorded against time of exposure to alcohol.

Pupae

The pupae in cups were covered with nets then transferred into the insectary for suitable environmental required condition and monitored for emergence of flies while daily temperature and relative humidity were recorded. Dates of emerged flies were also recorded.

Emergents

Emerged flies were collected from cups and placed into Geiger cages and matched with different sex then allowed to mate for two (2) days after which they are separated.

Separation

The female flies after separation were kept on trays and monitored for larviposition. The date of larviposition and further pupating were recorded.

Mortality

Mortality was monitored and checks were also carried out and the dates recorded.

TABLE 1
CHART SHOWING TSETSE LARVAE SPECIES COLLECTED AND DATA OF DATES AND TIMES OF VARIOUS EXPERIMENTS

SAMPLE	TSETSE SPECIE	SEX	DATE COLLECTED	TIME			DATE EMERGED	DATE MATED	DATE SEPERATED	DATE PUPATED	DATE EXPIRES
				COLLECTED	EXPOSED	PUPATED					
A	GMM	-	15/07/13	08.30	09.49	-	-				-
B	GMM	-	15/07/13	08.28	09.49	10.13	-				-
C	GMM	M	15/07/13	08.50	09.49	-	14/08/13				01/09/13
D	GMM	M	15/07/13	08.35	09.49	-	06/08/13				02/09/13
E	GMM	F	15/07/13	10.06	10.10	12.24	04/08.13	(D + E)			30/08/13
F	GMM	M	16/07/13	10.06	10.10	12.22	14/08/13	08/08/13			01/09/13
G	GMM	M	16/07/13	08.50	08.53	10.35	15/08/13		10/08/13		02/09/13
H	GPP	F	16/07/13	09.00	09.02	13.20	15/08/13	17/08/13		08/09/13	02/09/13
I	GMM	F	18/07/13	09.25	10.02	11.03	17/08/13	G + I	19/08/13		15/09/13
J	GMM	F	18/07/13	10.26	10.31	11.35	18/08/13	19/08/13			16/09/13
K	GMM	M	18/07/13	10.25	10.30	02.00	18/08/13	J + K	21/08/13		16/09/13
L	GMM	F	23/07/13	09.45	10.00	10.28	22/08/13	20/08/13			18/09/13
M	GMM	M	24/07/13	10.55	11.04	12.22	26/08/13		22/08/13		20/09/13
A1	GMM	F	19/07/13	10.42	10.50	12.30	15/08/13	D + A1			02/09/13
A2	GPP	-	19/07/13	10.42	10.50	13.25	-	17/08/13			-
A3	GMM	M	29/07/13	09.58	10.04	10.29	30/08/13		19/08/13		12/09/13
A4	GMM	M	29/07/13	10.23	10.27	12.42	30/08/13				10/09/13
A5	GMM	M	30/07/13	09.15	09.19	10.25	30/08/13				28/09/13
A6	GPP	F	31/07/13	11.20	11.22	12.32	01/09/13	A4 + A8			28/09/13
A7	GMM	-	01/08/13	10.03	10.14	11.24	-	02/09/13			-
A8	GMM	F	01/08/13	10.05	10.19	11.25	30/08/13	A9 + A5	04/09/13		09/09/13
A9	GMM	F	05/06/13	09.20	09.30	10.40	02/09/13	06/09/13			16/09/13
									09/09/13		

TABLE 2
INSECTARY TEMPERATURE & RELATIVE HUMIDITY

DATE	PERIOD	TEMP (%)	RELATIVE HUMIDITY (%)
16/07/2013	**Morning / Afternoon**	**25 / 23**	**84**
17/07/2013	**Morning / Afternoon**	**24 / 22**	**84**
18/07/2013	**Morning / Afternoon**	**25 / 23**	**85**
19/07/3013	**Morning / Afternoon**	**24 / 23**	**75**
20/07/2013	**Morning / Afternoon**	**25 / 23**	**83**
22/07/2013	**Morning / Afternoon**	**23 / 18**	**62**
23/07/2013	**Morning / Afternoon**	**23 / 23**	**83**
24/07/2013	**Morning / Afternoon**	**25 / 21**	**68**
25/07/2013	**Morning / Afternoon**	**20 / 19**	**91**
26/07/2013	**Morning / Afternoon**	**24 / 20**	**70**
27/07/2013	**Morning / Afternoon**	**24 / 20**	**70**
29/07/2013	**Morning / Afternoon**	**25 / 23**	**53**
30/07/2013	**Morning / Afternoon**	**25 / 20**	**64**
31/07/2013	**Morning / Afternoon**	**24 / 21**	**76**
01/08/2013	**Morning / Afternoon**	**25 / 22**	**76**
02/08/2013	**Morning / Afternoon**	**24 / 19**	**64**
03/08/2013	**Morning / Afternoon**	**24 / 21**	**76**
05/08/2013	**Morning / Afternoon**	**24 / 19**	**62**
06/08/2013	**Morning / Afternoon**	**25 / 21**	**70**
07/08/2013	**Morning / Afternoon**	**25 / 23**	**70**
08/08/2013	**Morning / Afternoon**	**25 / 21**	**70**
12/08/2013	**Morning / Afternoon**	**25 / 21**	**70**
13/08/2013	**Morning / Afternoon**	**25 / 20**	**63**
14/08/2013	**Morning / Afternoon**	**25 / 19**	**55**
15/08/2013	**Morning / Afternoon**	**25 / 23**	**83**
16/08/2013	**Morning / Afternoon**	**25 / 20**	**64**
17/08/2013	**Morning / Afternoon**	**25 / 23**	**84**
19/08/2013	**Morning / Afternoon**	**25 / 20**	**63**
20/08/2013	**Morning / Afternoon**	**25 / 22**	**77**
21/08/2013	**Morning / Afternoon**	**24 / 22**	**78**
22/08/2013	**Morning / Afternoon**	**25 / 23**	**70**
23/08/2013	**Morning / Afternoon**	**25 / 24**	**92**
24/08/2013	**Morning / Afternoon**	**25 / 24**	**92**
26/08/2013	**Morning / Afternoon**	**25 / 20**	**64**
27/08/2013	**Morning / Afternoon**	**24 / 22**	**84**
28/08/2013	**Morning / Afternoon**	**24 / 19**	**84**
29/08/2013	**Morning / Afternoon**	**25 / 20**	**64**
30/08/2013	**Morning / Afternoon**	**25 / 21**	**70**
31/08/2013	**Morning / Afternoon**	**25 / 21**	**70**
02/09/2013	**Morning / Afternoon**	**24 / 20**	**70**
03/09/2013	**Morning / Afternoon**	**25 / 23**	**84**
04/09/2013	**Morning / Afternoon**	**25 / 23**	**84**
05/09/2013	**Morning / Afternoon**	**24 / 19**	**62**
06/09/2013	**Morning / Afternoon**	**24 / 22**	**85**
07/09/2013	**Morning / Afternoon**	**25 / 23**	**84**
09/09/2013	**Morning / Afternoon**	**25 / 23**	**84**
10/09/2013	**Morning / Afternoon**	**25 / 22**	**78**
11/09/2013	**Morning / Afternoon**	**25 / 20**	**64**
12/09/2013	**Morning / Afternoon**	**25 / 21**	**70**
13/09/2013	**Morning / Afternoon**	**25 / 20**	**64**
14/09/2013	**Morning / Afternoon**	**25 / 20**	**64**
16/09/2013	**Morning / Afternoon**	**24 / 22**	**85**
17/09/2013	**Morning / Afternoon**	**25 / 22**	**78**
18/09/2013	**Morning / Afternoon**	**26 / 22**	**70**
19/09/2013	**Morning / Afternoon**	**25 / 22**	**78**
20/09/2013	**Morning / Afternoon**	**25 / 21**	**70**

21/09/2013	**Morning / Afternoon**	**25 / 21**	**70**
23/09/2013	**Morning / Afternoon**	**25 / 20**	**63**
24/09/2013	**Morning / Afternoon**	**25 / 21**	**70**
25/09/2013	**Morning / Afternoon**	**24 / 22**	**84**
26/09/2013	**Morning / Afternoon**	**25 / 22**	**75**
27/09/2013	**Morning / Afternoon**	**22 / 21**	**92**
28/09/2013	**Morning / Afternoon**	**25 / 20**	**64**
30/09/2013	**Morning / Afternoon**	**23 / 19**	**70**
02/10/2013	**Morning / Afternoon**	**25 / 22**	**78**
03/10/2013	**Morning / Afternoon**	**24 / 19**	**63**
04/10/2013	**Morning / Afternoon**	**25 / 23**	**78**
05/10/2013	**Morning / Afternoon**	**25 / 22**	**78**
07/10/2013	**Morning / Afternoon**	**25 / 20**	**62**
08/10/2013	**Morning / Afternoon**	**25 / 21**	**70**
09/10/2013	**Morning / Afternoon**	**25 / 20**	**62**
10/10/2013	**Morning / Afternoon**	**25 / 20**	**62**
11/10/2013	**Morning / Afternoon**	**23 / 21**	**84**
12/10/2013	**Morning / Afternoon**	**24 / 20**	**70**

RESULTS

Pupation time for larva of *Glossina morsitan submorsitan* was discovered to be within the range of 70 seconds averagely while *Glossina palpalis palpalis* was discovered to be 188 seconds averagely. Average period for the *Glossina morsitan submorsitan* to emerge is 29 days while the *Glossina palpalis palpalis* is 30 days. All challenged *Glossina morsitan submorsitan* exposed to ethanol were paired and mated then separated according to standard insectary procedure but non larviposited, while the *Glossina palpalis palpalis* were mated with clean male flies from the insectary did larviposite.

DISCUSSION

Exposure of larvae to ethanol for both species was within the time range of 2 to 30 seconds and all larvae of more than a minute before exposure was observed to have pupated immediately on exposure showing that the ethanol acted as a catalyst to the pupating stage as the larvae failed to exhibit the colour change associated with pupation.

All larvae exposed as observed from the result emerged after the standard documented period of 20 to 27 days for *Glossina morsitan submorsitan* and *Glossina palpalis palpalis* (Davies H 1977). The result obtained can be attributed to the exposure to ethanol at larve stage or fluctuation to temperature and relative humidity (RH) change in the insectary.

The standard Relative humidity (RH) of an insectary is 75% while the temp Is 25^{o}C. The temperature as indicated from the result was relatively constant while the relative humidity was fluctuating from the standard level of an insectary with less high relative humidity and lower relative humidity recorded on the average.

The high emergence period may not be attributory to the change in standard documented emerging periods of the flies, but exposure of larvae to ethanol and loss of fecundity of the *Glossina morsitan submorsitan* as the *Glossina palpalis palpalis* did not experience any null fecundity. The fluctuation in temperature and relative humidity of insectary can be attributory to the observed life span of the flies as female tsetse flies live on average for about 90 days and are capable of generating an average of about 8 offspring during her lifetime (Langley and Clutton-Brock 1998). Hence, exposure of larve to coarse chemical environment with high polarity like ethanol can affect the fecundity of some species of tsetse flies like the *Glossina morsitan submorsitan*, as a prelude to determination of incorporation as a insecticidal addictive for control of *Glossina morsitan submorsitan*, however, further research with a higher sample size for *Glossina palpalis palpalis* needs to be conducted with also a closer look at the parturition of the tsetse as the first gonotrophic cycle requires about 21 days from eclosion to parturition of a fully developed third instar larva. The following gonotrophic cycles average about 9 days per offspring (Attardo et al. 2006).

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