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The Vegetable Diet Theory of *Glossina Pallidipes.*

By

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Introduction.

In a paper recently read before a meeting of Durban Members of the Royal Society of Tropical Medicine and Hygiene by Mr. A. Davidson, it was stated that the tsetse fly, *G. pallidipes*, can live on the latex of plants. This worker specially mentions *Euphorbia tirucalli* and *Sarcostemnus viminale* as being two of the latex bearing plants upon which the fly feeds. He declares that it will take up sufficient nutritive fluid therefrom to support life and even to reproduce.

No experimental proof is given, though it appears that this worker attempts to correlate the flagellate infection found in the latex of Euphorbia species and other latex bearing plants (described by Lafont in 1909), with the trypanosome infection normally found in Glossinae, and their vertebrate hosts, the wild animals.

The present investigation was conducted at the Nagana Research Station in Zululand.

G. pallidipes was the insect material employed. The latex was derived from the plants *E. tirucalli* and *Sarcostemnus viminale*, the species referred to by Mr. Davidson. Flagellate infection was found in *E. tirucalli* but none in *S. viminale*. These two species occur in abundance throughout the habitat of *G. pallidipes* in Zululand. During the experiments the tsetses were given every opportunity under both normal and artificial conditions to ingest latex from both of these plants. It will be shown that the insects do not in nature attempt to feed upon this substance, and therefore any theory in regard to the trypanosome infection of tsetses being derived from plant flagellates is entirely without foundation.

Early Investigations.

The history of Glossinology is presented in an instructive manner by Hegh (1930). Entomologists were at first content to describe the few tsetses brought home by travellers. Wiedman (1830) established his genus Glossina without comment. Robineau-Desvoidy boldly added to his description of *G. palpalis* the remark that "the proboscis was innocuous" by which he perhaps meant that the fly did not suck blood.

Macquart embroidered this opinion. "It is probable", he says, "that this fly does not live on animal blood like stable flies, but on the nectar of flowers. The two setae contained in the proboscis, and forming a sucking apparatus are so fine that one can hardly conceive how they might be able to pierce the skin; the weakness of this organ seems to be further shown by the modifcation of the palpi, which are lengthened and hollowed out into a sheath for the proboscis." Indeed, the proboscis of the tsetse fly does seem eminently adapted to the sucking of nectar, yet none of the species have been seen visiting flowers. The males of the Tabanidae naturally suck the nectar from flowers, and the females of mosquitoes will suck both blood and nectar so that by analogy it is perhaps not difficult to imagine that some of the Glossina species might likewise visit flowers.

David Livingstone (1857) focussed the attention of the scientific world upon the ravages of the tsetse, and since that time entomologists, veterinarians, medical men, protozoologists, botanists and many others, even colonial politicians have studied the behaviour of this insect.

Ecological Factors.

Doubtless every species of *Glossina* is associated with a particular type of country on account of the fly's special requirements in regard to floral conditions, shade, etc., either as an adult or as a pupa.

The species *G. pallidipes* with which we now deal belongs to that group of tsetse which can live through prolonged dry seasons without apparent suffering, and in consequence is found in regions clothed with one of the many types of open vegetation included under the general term "Savannah".

Ecological conditions alone, however, are inadequate to account for the local abundance or scarcity of tsetses. Their feeding habits are of more importance, and because they thrive best and most numerously where animal hosts are most abundant, there appears to be a very close association between the tsetses and the wild animals. There can be no doubt that the insects are strictly haematophagous.

The Tsetses Reaction to Visual Impressions.

In Nature, Glossinae while beset with many hazards and contingencies manage to thrive, notwithstanding the apparent handicap of extremely slow reproduction. An observer cannot help being impressed by the apparently unobtrusive, yet deliberate manner in which a tsetse when attacking, darts towards a victim, and it has been established beyond doubt that the fly hunts by sight, reacting in a peculiar manner to visual impressions.

The most convincing evidence of this is supplied by the operations carried out by Harris and his staff at the Nagana Research Station where millions of tsetse (*G. pallidipes*) have been destroyed by the "Harris" fly trap. This trap is a visual impression one, and has no olfactory stimulus as bait. The flies are attracted to it by its conspicuousness and its resemblance to the bulk of an animal, and, darting towards the object they dive into the shadow of the ventral surface. They are then attracted to the light showing through the opening in the platform on which the cage rests; making their way through this into the cage above, and are caught.

Digestive Anatomy of the Fly.

There are many features in the external and internal anatomy of the tsetse which show a high degree of specialization. The mouth parts and the digestive tract offer, perhaps the most striking peculiarities, and are of importance in connection with the rôle of the flies as vectors of the disease.

(*a*) In the proboscis the mandibles and maxillae, found in some species of haematophagous diptera, are absent. The labrum and labium in apposition form together a needle-like tube enclosing the long slender hypopharynx. The saliva from the salivary glands passes into the wound when the proboscis punctures the skin of the vertebrate, and prevents coagulation of the blood.

At rest, the proboscis is carried horizontally between the palpi which ensheathe it. When the insect is about to feed the proboscis is lowered vertically, the palpi retaining the horizontal position. The skin is pierced by the rapid movement of the labella situated at the tip of the labium, and the proboscis is thrust into the wound as far as its bulbous base will permit. When a suitable well of blood has been found, the proboscis is partially withdrawn, and the blood may be seen passing up the tube, the fly becoming engorged in a few seconds.

(*b*) The digestive tract and the complicated process of digestion have recently been studied in great detail by Wigglesworth (1930).

Mr. Davidson passes over this creditable work with the following remarks: " Some observers go so far as to assert that the digestive organs of tsetse are only capable of dealing with blood; but this is a rash statement, since a gut that can digest blood would almost certainly be able to digest any fluid which contains suitable proteins, such as milk and the latex of plants ".

Mr. Davidson gives no points of similarity between milk and the latex of plants, and the reader is left to surmise that the substances are similar. Whereas milk is essentially a blood product of mammals, latex is a storage product of the green cells of plants.

Wigglesworth discusses the physiology of digestion in the cockroach, and shows that the properties of the digestive enzymes are similar to those of vertebrates. Comparing the digestive system of the tsetse fly (Glossina) with that of the blue-bottle (Calliphora), he states that the adaptation to a diet of blood in the former is the loss of enzymes acting upon carbohydrates, while he notes a great increase in the activity of the proteolytic enzymes and the acquisition of factors affecting blood coagulation.

Wigglesworth also suggests that the so-called " symbionts " found in the mid gut might be contributing some accessory food factor which is lacking in the sterile diet of their host.

In this connection it may be mentioned that if the region of the gut in which these symbionts occur is cut the symbionts float out, and, to an inexperienced worker they may readily appear similar to starch grains seen in the latex of euphorbias. Mr. Davidson has referred freely to starch grains in the gut of tsetses.

Temperature in Regard to Feeding.

Harris (1930) has shown that the tsetse *G. pallidipes* is highly sensitive to warmth, and responds to this stimulus by probing even when the warmth is in the form of hot air. He has pointed out that those flies, which on alighting do not explore a cool object, whether that object resembles a quadruped or not, appear to be those which because not acutely hungry require some accessory stimulus such as warmth to cause them to probe. Flies which immediately probe appear to be those in which hunger has reached an acute stage.

From these facts it would seem that there should be some contrast in temperature between an object and the surrounding media to induce most flies to probe.

The following temperatures of objects represent the averages of five readings taken over half an hour. The temperature of the air and that of the surface mentioned were read at the same time.

Temperature of air.....	24·5° C.	Temperature of bark of Euphorbia....	25·5° C.
Temperature of air.....	25·6° C.	Temperature of green Asclepiad.......	24·5° C.
Temperature of air.....	25·6° C.	Temperature of man's arm...........	34·9° C.
Temperature of air.....	29·2° C.	Temperature of donkey's skin........	34·8° C.

The Table shows that the temperature of the surface of trees may be lower or slightly higher than that of the surrounding atmosphere. On the other hand the temperature of the surface of a mammal is generally much higher than that of the surrounding atmosphere.

For this reason in the following experiments, when attempting to induce flies to feed on latex bearing plants, it was found necessary to warm their naturally cool surfaces in order to induce the tsetse to probe gainst them.

Latex in Nature.

Latex occurs in certain plants of the families *Euphorbiaceae*, *Asclepiadaceae* and others. It is a milky fluid, a storage product of the green cells and is contained in lactiferous tubes running longitudinally in the xylem. When the stem of a latex bearing plant is punctured the latex exudes. The only manner in which the tsetse could feed upon the latex therefore, is by puncturing a containing vessel with its proboscis.

Under the microscope, dumb-bell shaped starch grains are seen in plenty in the latex of euphorbias, but similar starch grains have not been found in the asclepiad.

Any fly which ingested latex should upon dissection and examination reveal either traces of latex, or the peculiar shaped starch grains, in the gut.

Material and Method.

The tsetses for the experiments were captured flies taken from the " Harris " traps operating in the Umfolozi game reserve.

Groups of ten flies each were placed in glass battery jars covered with mosquito netting. All flies were given a preliminary feed of blood from a donkey to ensure that none were hungry and that all might start the experiments on a common hunger basis.

Subsequently each group of flies was given the opportunity to feed daily on the respective foods, blood or latex, while some were starved to act as a control.

In that the flies used in the experiments were captured flies, no definite age could be assigned to them, though none were young.

When giving the flies the opportunity to feed on the latex bearing plants, twigs were placed side by side and clamped at the ends to prevent movement, and to form a mat presenting a more or less even surface. The mat was placed over a basin of warm water (37° C. to 40° C.) and left for a few minutes in order to warm the twigs to give inducement for the flies to probe. Both plants, *S. viminale* and *E. tirucalli* were employed.

The jar containing the flies was then inverted on the mat so that the mosquito net covering the jar came into contact with it. By this method it was possible for the proboscides of the flies to reach the twigs without difficulty. Similarly when feeding the flies on the donkey, the jar was inverted on the shaven skin of the animal.

When the flies died they were dissected, and the gut contents specially examined for traces of latex. The surface of the plants was also examined for signs of exudation.

The experiments were twice repeated, and the results are given in the following Tables.

Flies Fed on Blood of Donkey.

Table I shows that of the ten flies in the jar eight lived fifteen days and two fourteen days. Their deaths were due to a succession of unusually hot days when the highest temperature recorded was 105° F., and the relative humidity dropped to 15 per cent. Seven of them deposited larvae of which six successfully emerged.

The experiment was repeated, the results being given in Table II. It is here seen that four flies died after seventeen and eighteen days, one after forty-two and another after forty-three days. The four remaining flies lived fifty-seven days, when the experiment was stopped. Eleven larvae were deposited, and there was one abortion. Flies emerged from all the puparia.

TABLE I.

Flies Given Opportunity to Feed on Donkey.

Date.	No. of Flies.	Died.	Days Lived.	Larvae Deposited.	Emerged.
1/10/32	10	—	—	—	—
—	—	—	—	1, 6/10/32	12/11/32, ♀
—	—	—	—	1, 8/10/32	15/11/32, ♀
—	—	—	—	2, 9/10/32	16, 18/11/32, ♀, ♂
—	—	—	—	2, 11/10/32	18, 21/11/32, ♀, ♂
14/10/32	10	—	—	1, 15/10/32	—
15/10/32	8	2♀	14	—	—
16/10/32	—	3♂, 5♀	15	—	—

TABLE II.

Flies Given Opportunity to Feed on Donkey.

Date.	No. of Flies.	Died	Days Lived.	Larvae Deposited.	Emerged.
21/10/32	10	—	—	—	—
—	—	—	—	1. 25/10/32	27/11/32, ♀
—	—	—	—	1, 26/10/32	1/12/32, ♂
—	—	—	—	1, 27/10/32	1/12/32, ♂
—	—	—	—	1, 1/11/32	3/12/32, ♀
—	—	—	—	1, 2/11/32	7/12/32, ♂
6/11/32	10	—	—	1, 4/11/32	11/12/32, ♂
7/11/32	7	1♂, 2 ♀	17	—	—
8/11/32	6	1♂	18	—	—
—	—	—	—	1, 17/11/32	21/12/32, ♂
2/12/32	5	1♂	42	1, 21/11/32	♂
3/12/32	4	1♂	43	1 Abortion,	—
—	—	—	—	27/11/32	—
—	—	—	—	1, 29/11/32	♀
—	—	—	—	1, 1/12/32	♂
16/12/32	—	1♂, 3♀	57	—	—

TABLE III.

Flies Given Opportunity to Feed on Latex-bearing Plant.

SARCOSTEMNUS VIMINALE.

Date.	No. of Flies.	Fed.	Died.	Days Lived.	Larvae.	Remarks.
1/10/32	10	8	—	—	—	Fed on blood.
2/10/32	—	—	—	—	—	Stimulated to probe by warmth.
4/10/32	9	—	1♀	3	—	Thin.
5/10/32	9	—	—	—	1 Abortion	—
6/10/32	7	—	1♂, 1♀	5	—	—
7/10/32	5	—	1♂, 1♀	6	—	Starving.
8/10/32	0	—	1♂, 4♀	7	—	—

TABLE IV.

Flies Given Opportunity to Feed on Latex-bearing Plant.

SARCOSTEMNUS VIMINALE.

Date.	No. of Flies.	Fed.	Died.	Days Lived.	Larvae.	Remarks.
21/10/32	10	10	—	—	—	Fed on blood.
22/10/32	—	—	—	—	—	Stimulated to probe by warmth.
24/10/32	10	—	—	—	1 Abortion	—
25/10/32	10	—	—	—	1 Abortion	—
26/10/32	8	—	2♀	5	—	—
28/10/32	7	—	1♀	7	—	Flies weak.
29/10/32	3	—	3♂, 1♀	8	—	—
30/10/32	3	—	—	—	—	—
31/10/32	2	—	1♀	10	—	—
1/11/32	0	—	1♂, 1♀	11	—	—

TABLE V.

Flies Given Opportunity to Feed on Latex-bearing Plant.

EUPHORBIA TIRUCALLI.

Date.	No. of Flies.	Fed.	Died.	Days Lived.	Larvae.	Remarks.
21/10/32	10	10	—	—	—	Fed on blood.
22/10/32	10	—	—	—	—	Stimulated to probe by warmth.
25/10/32	9	—	1♀	4	1 Abortion	
26/10/32	6	—	2♂, 1♀	5	—	Thin. —
28/10/32	4	—	1♂, 1♀	7	—	—
29/10/32	2	—	1♂, 1♀	8	—	—
31/10/32	1	—	1♀	10	—	—
1/11/32	0	—	1♂	11	—	—

Flies Given the Opportunity to Feed on Latex-bearing Plants.

As will be seen in Table III eight flies had a perliminary feed of blood. On the following day the jar of flies was inverted on the mat of *S. viminale*, previously described. None attempted to feed. Next day the mat was again warmed over a basin of hot water and the flies given further opportunity to pierce the twigs. This procedure was repeated daily. On each occasion the flies reacted to the warmth of the mat, but were never at any time able to pierce the cuticle and epidermis of the twigs, and thus did not feed. As the days passed, the flies became more and more hungry, and the probing reaction to warmth became more pronounced. On the third and forth day they were definitely thin and hungry, and eventually became too weak to attempt to probe. The last flies died on the seventh day. One female aborted on the fifth day, but there were no births.

This experiment was repeated with another batch of twenty flies, when both of the latex bearing plants *S. viminale* and *E. tirucalli* were used.

Methods of feeding similar to the foregoing were employed. As seen in Table IV and V the longest lives recorded were eleven days. There were three abortions. When the flies reacted to the stimulus of warmth, none were able to pierce the twigs. The action of the proboscides against the twigs was carefully watched, and though numerous attempts were made to pierce them none succeeded. Microscopic examination of the surface of the mat revealed no signs of exuding latex, such as would occur had the cuticle been punctured.

All dead flies from the " latex jars " were dissected. The abdomens were totally collapsed, and the guts contained only greenish traces of digested blood at the posterior end. No trace of latex was found, and the iodine test for starch gave no reaction. In all cases the proboscides contained no trace of latex.

Flies Given the Opportunity to Feed on Latex Through a Membrane.

As the flies were unable to pierce the epidermis of the latex-bearing plants, it was decided, to tap the latex into tubes, to cover them with a membrane and endeavour to induce the flies to feed through it.

Two types of membrane were used. The skin of a freshly killed bird, and the caecum of an antelope. In the former case the skin, devoid of feathers, was stretched tightly over the mouth of the tube filled with latex. In the latter case the blind caecum was filled and tied at the open end.

The latex of both *Euphorbiaceae* and *Asclepiadaceae* tend to coagulate when drawn from the plant forming a viscous fluid. For this reason it was necessary to dilute the latex with physiological saline for the flies to ingest. A mixture of citrated blood and latex was also tried as a diet.

As in the case of the plant experiment it was necessary to warm the mixture slightly (37° C. to 39° C.) in order to stimulate the flies to probe.

Only by presenting the latex in this extremely artificial manner was it found possible to induce the flies to feed.

Results.

Two jars of ten flies each were employed. They were first given a feed of blood to ensure working from a common hunger basis. They were then starved for three days so that all were sufficiently hungry to respond readily to the stimulus of warmth.

The jars containing the flies were inverted, and the membrane covering the tubes of warm latex was brought into contact with the flies. Stimulated by the warmth the tsetses probed and pierced the membrane, but rapidly withdrew the proboscis and commenced cleaning it with their front legs. In several cases the flies sucked up some of the latex and fell on their backs, dying in a few minutes, while in other cases they survived from one to twelve hours, according to the amount of material ingested. Similar findings were noticed in the case of the latex of the Asclepiad and in the mixture of blood and latex.

On dissection of the dead flies the dumb-bell shaped starch grains of the Euphorbia were found in their guts, and the proboscides were clogged with latex.

Flies Given the Opportunity to Feed on Citrated Blood Through a Membrane.

To ensure that the above method of feeding flies through a membrane could have no adverse effect upon the length of life of the fly, a jar of twelve flies was fed experimentally upon citrated blood alone.

The blood was drawn from a donkey into tubes containing a 2 per cent. solution of potassium citrate, the mixture comprising 12 c.c. of blood and 2 c.c. of citrate.

As previously the skin of a bird was stretched tightly over the mouth of the tube and it was then placed in a water bath and kept at a temperature between 37° C. and 39° C.

The tube containing the warm citrated blood was then brought into contact with the tsetses.

Stimulated by the warmth the flies pierced the membrane and their abdomens swelled in a few seconds, as though engorging on an animal host.

Table VI shows that the flies were given the opportunity to feed in this manner on nine occasions. The opportunities to feed varied from one to five days interval.

TABLE VI.

Flies Given Opportunity to Feed Through a Membrane on Citrated Blood.

Date.	No. of Flies.	Fed.	Died.	Days Lived.	Larvae. Deposited.	Remarks.
15/6/33	12	12	—	—	—	Fed through a membrane.
16/6/33	12	—	—	—	—	—
17/6/33	11	6	1♀	2	3 Abortions..	Fed through a membrane.
19/6/33	11	—	—	—	1 Pupa......	—
20/6/33	11	—	—	—	2 Abortions..	—
21/6/33	9	9	2 [illegible]	6	1 Pupa......	Fed through a membrane.
24/6/33	9	7	—	—	—	Fed through a membrane.
27/6/33	9	9	—	—	1 Pupa......	Fed through a membrane.
1/7/33	9	7	—	—	—	Fed through a membrane.
3/7/33	9	—	—	—	1 Pupa......	—
5/7/33	8	8	1♂	20	—	Fed through a membrane.
11/7/33	8	5	—	—	—	Fed through a membrane.
12/7/33	6	—	2♀♀	27	—	—
15/7/33	5	5	1♀	30	—	Fed through a membrane.
16/7/33	4	—	1♂	31	—	—
17/7/33	4	—	—	—	—	—
18/7/33	0	—	1♂, 3 [illegible]	34	—	Experiment stopped.

TABLE VII.

STARVATION CONTROL

Flies Allowed to Feed Once on Blood and then Starved.

Date.	No. of Flies.	Fed.	Died.	Days. Lived.	Larvae Deposited.	Remarks.
1/10/32	10	8	—	—	—	Fed on blood.
4/10/32	10	—	—	—	1 Abortion...	Flies thin.
5/10/32	8	—	1♂, 1♀	4	—	—
7/10/32	5	—	1♂, 2♀	6	—	Starving.
8/10/32	3	—	2 ♀	7	—	—
9/10/32	3	—	—	—	—	Abdomens wafer-like.
10/10/32	0	—	1♂, 2♀	9	—	—

TABLE VIII.

STARVATION CONTROL.

Flies Allowed to Feed Once on Blood and then Starved.

Date.	No. of Flies.	Fed.	Died.	Days Lived.	Larvae Deposited.	Remarks.
21/10/32	10	10	—	—	—	Fed on blood.
24/10/32	9	—	1 ♀	3	2 Abortions..	—
26/10/32	7	—	1♂, 1 ♀	5	1 Larva......	Starving and weak.
28/10/32	6	—	1♀	7	—	—
29/10/32	4	—	1♂, 1♀	8	—	—
30/10/32	3	—	1♀	9	—	—
31/10/32	2	—	1♀	10	—	—
1/11/32	1	—	1♀	11	—	—
2/11/32	1	—	—	—	—	—
3/11/32	0	—	1♀	13	—	—

One fly died after two days, two after six days, one after twenty days, two after twenty-seven days, one after thirty days, and one after thirty-one days. The remaining four lived thirty-four days. The experiment was then discontinued. Four fully developed larvae were deposited, and five abortions occurred.

From the foregoing it is clear that flies fed through a membrane on blood while not actually thriving, nevertheless can be kept alive for a considerable time, and will even reproduce.

STARVATION CONTROL FLIES.

Two jars of ten flies each were kept as controls. These flies were given one feed of blood and then starved.

Tables VII and VIII show that the longest lived of these starved flies was nine and thirteen days; this is longer than the longest life of any of the flies which had the opportunity to feed on the latex bearing plants. Three abortions occurred and one fully developed larva was deposited. Flies do not readily reproduce when starved, and the one larva was from a female well advanced in pregnancy.

SUMMARY AND CONCLUSIONS.

(1) This paper gives a brief account of the history of Glossinology. Some habits of Glossina with special reference to *G. pallidipes* have been discussed, together with points of interest in the tsetse's anatomy.

(2) There can be no doubt that the tsetse is strictly haematophagous, and when allowed to feed on blood it will thrive and reproduce. It reacts in a peculiar manner to visual impressions, seeking its food by sight.

(3) The proboscis though a delicate organ, is yet capable of being rapidly thrust deep into the tissues of the insect's animal host.

(4) The complicated process of digestion as studied by Wigglesworth has been briefly discussed, and he has shown that the tsetse's adaptation to a diet of blood has resulted in the great increase of factors affecting blood coagulation.

(5) Further, the tsetse will generally probe against any inert object, provided that object is warm.

(6) Latex, a storage product of certain plants, is contained in lactiferous tubes in the xylem, and the only manner in which the tsetse could feed upon it would be by thrusting its proboscis into a containing vessel.

(7) In the experiments here set forth it is clear that the flies are totally incapable of piercing the plant tissues, and if given opportunity to feed on latex as it occurs in Nature, they soon die and no reproduction takes place.

(8) Further, the latex has an undoubted toxic effect upon those flies which have been artificially induced to partake of it through a membrane.