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TRYPTOPHAN METABOLISM IN TSETSE FLIES AND THE CONSEQUENCES OF ITS DERANGEMENT

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Literature comparing salmon and wild type Glossina morsitans morsitans *and that comparing tan and wild type* Glossina palpalis palpalis *is reviewed. New information is presented on behaviour and biochemistry of salmon and wild type* G. m. morsitans. *The eye color mutants result from two lesions in the tryptophan to xanthommatin pathway: lack of tryptophan oxygenase in* G. m morsitans *and failure to produce or retain xanthommatin in eyes (but not in testes) of* G. p. palpalis. *The* salmon *allele in* G. m. morsitans *is pleiotropic and profoundly affects many aspects of fly biology including longevity, reproductive capacity, vision, vectorial capacity and duration of flight, but not circadian rhythms. The* tan *allele in* G. p. palpalis *has little effect upon the biology of flies under laboratory conditions, except that tan flies appear less active than normal. Adult tsetse flies metabolize tryptophan to kynurenine which is excreted; fluctuations in activities of the enzymes producing kynurenine suggest this pathway is under metabolic control.*

Tryptophan is one of the rarer essential amino acids, making up from 0.5 to 1.0% of the amino acid residues in protein. In insects its two principal uses are as components of protein and as a precursor of the eye pigment, xanthommatin. Metabolism of tryptophan by insects has been reviewed by Linzen (1974) and Summers et al., (1982) but the subject has received little attention in tsetse flies. The objectives of this paper are to review what is known about tryptophan metabolism in tsetse, to discuss the consequences of derangement of tryptophan metabolism, to present some new information suggesting possible links between tryptophan metabolism and reproduction in tsetse, and to speculate upon some aspects of tryptophan metabolism in tsetse (and possibly other insects).

Tsetse mutants probably involving tryptophan metabolism

Historically, genetically determined variations in eye color have been the first indicators of derangement of tryptophan metabolism in insects; tsetse flies are no exception. Three eye color mutants have been described in tsetse flies: *salmon* in *Glossina morsitans morsitans* (Gooding, 1979), *pink* in *Glossina morsitans centralis* (Rawlings, 1985), and *tan* in *Glossina palpalis palpalis* (D'Haeseleer et al., 1987). Unfortunately the *pink* mutant in *G. m. centralis*, although described as a non-deleterious allele, had low fecundity (Rawlings, 1985) and the mutant was lost (A.M. Jordan personal communication to R.H.G.).

Biochemical lesions causing salmon and tan-colored eyes

The pathway from tryptophan to xanthommatin is well established in insects; the metabolites are listed here in order of appearance in the pathway (with names of enzymes catalyzing each step given in parentheses): tryptophan (tryptophan oxygenase) formylkynurenine (kynurenine formamidase) kynurenine (kynurenine-3-hydroxylase) 3-hydroxykynurenine (phenoxazinone synthetase) xanthommatin. Identification of the lesion(s) which result in abnormally colored eyes is based upon demonstration of metabolites and enzyme activities in mutant and wild type flies.

Material extracted from heads of wild type *G. m. morsitans* and *G. p. palpalis* has the spectrum and the redox properties typical of xanthommatin (Gooding & Rolseth, 1984; D'Haeseleer et al., 1987). Relatively little of this material is found in heads of *salmon* and *tan* mutants (Table I). Thin layer chromatography, amino acid analyzer and mass spectroscopy techniques indicate that feces of normal *G. m. morsitans* contain kynurenine while the *salmon* mutants excrete tryptophan. These results suggest that the lesion in the *salmon* mutant occurs early in the tryptophan to xanthommatin path-

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TABLE I

Comparison of mutant and wild type tsetse flies with regard to various parameters of tryptophan metabolism*

Parameter	*G. m. morsitans***		*G. p. palpalis****	
	wild type	salmon	wild type	tan
1) xanthommatin in heads	100%	2.7%	100%	0.8%
2) xanthommatin in testes	n.r.+	n.r.	100%	150%
3) metabolites in feces	kynurenine	tryptophan	kynurenine	kynurenine
4) metabolites in pharate adults	n.r.	n.r.	kynurenine 3-OH-kyn++	kynurenine 3-OH-kyn
5) tryptophan oxygenase	100%	0%	100%	75%
6) kynurenine formamidase	100%	63%	100%	79%

*Except for parameters in rows 2 and 4, information pertains to post-teneral females. For ease of comparison quantities are reported as a percentage of what was found in wild type flies.
**Data based on Gooding & Rolseth (1984).
***Data based on D'Haeseleer et al. (1987).
+ n.r. = not reported.
++ 3-OH-kyn = 3-hydroxykynurenine.

way. This conclusion is supported by enzyme assays; tryptophan oxygenase, the first enzyme in the pathway, is absent from (or present at extremely low levels in) salmon flies (Table I). Phenocopies (of the wild type fly) have been produced in *G. m. morsitans,* which were genetically destined to have salmon-colored eyes, by treating pharate adults, in puparia, with formylkynurenine or kynurenine sulfate but not by treating with tryptophan (Gooding & Rolseth, 1984).

In contrast to the situation in *G. m. morsitans,* the testes of mutant *G. p. palpalis* have a near normal color and contain more than the normal amount of xanthommatin (Table I). This, and excretion of kynurenine by both wild type and tan *G. p. palpalis,* suggests that the lesion causing tan-colored eyes occurs late in metabolism of tryptophan. Further evidence supporting this conclusion is the demonstration of kynurenine and 3-hydroxykynurenine in heads of pharate adults removed from puparia, and the nearly normal levels of tryptophan oxygenase and kynurenine formamidase in post-teneral adults (Table I). Unfortunately it has not yet been possible for us to detect the last two enzymes in the tryptophan metabolism pathway in tsetse flies. Nonetheless the weight of the evidence suggests that the lesion producing tan-colored eyes in *G. p. palpalis* occurs late in metabolism of tryptophan, probably at the level of binding of xanthommatin in the eyes but not in the testes (D'Haeseleer et al., 1987).

CONSEQUENCES OF DERANGED METABOLISM OF TRYPTOPHAN

Effects on vision

Effects, upon vision, of deranged tryptophan metabolism have been reported for *G. m. morsitans* but no direct studies have been conducted using *G. p. palpalis.* However, for the latter species subjective observations suggest that tan flies are either partially blind or that their movements are at least strongly inhibited at normal light intensities.

The shape of the spectral sensitivity curves, and the flicker fusion frequencies at threshold light intensities, are similar in wild type and salmon *G. m. morsitans* (Davis & Gooding, 1983). However at most wavelengths salmon flies are about 100x more sensitive to light than are wild type flies and at light intensities above threshold these flies rapidly adapt to the stimulating sourse and the flicker fusion frequency increases by 200 to 300%.

The response, of salmon and wild type *G. m. morsitans,* to a moving target was studied as follows. Puparia were hand carried from Edmonton, Canada to Silwood Park, England, and males emerging within a few days were fed one or more times (on man or rabbit) and were then tested in a flight chamber at least 16 hours after the last meal.

The flight chamber had a cross section of 50 cm x 50 cm and its length was varied by changing the position of one end wall. The end

walls were screens, and onto the fixed end a black rectangular image could be projected so that it passed from left to right at various speeds. The chamber was lit from above by three banks of fluorescent bulbs, the mains current for which was arranged so as to minimize mains 'ripple', which is detectable by *G. morsitans* (Miall, 1978). Light intensity in the chamber was controlled by a system of mechanically controlled baffles. With overhead lights on full, and image screen lit, (but no image projected onto the screen), light intensity at the chamber floor varied from 230 to 320 lux and at the ceiling from 230 to 400 lux. With overhead lights 'half dimmed' and image screen lit, light intensity at the floor ranged from 120 to 270 lux and at the ceiling from 200 to 210 lux. Access to the chamber was through a "sleeve port", at approximately the middle of one wall, 60 cm from the fixed end of the flight space. When the image was centred on the screen it was approximately 17 cm wide by 50 cm high. The flight chamber was in a room at approximately 27°C.

The flight chamber was tested under three conditions using 11 or more flies individually in each test. At the beginning of each test, a fly was placed on the floor of the chamber, approximately half way between the sides, near the moveable end of the chamber.

Test Condition I: The chamber was 125 cm long with 50 cm of floor, walls, and ceiling nearest the image screen covered with black paper. When flies were placed in the chamber, the overhead lights were on 'full', and the image screen was lit but no image was projected on it. After the fly settled down, the image projector was turned on, and one minute later overhead lights were dimmed completely. For three minutes the image had an angular velocity of 5°/sec (when viewed 100 cm from the image screen), but this was increased, during the last two minutes, to 11°/sec.

Test Condition II. As per Condition I, except that the black paper had been removed, and the chamber was 100 cm long. Image velocity was 5°/sec throughout the observation period. Overhead lights were on 'full' for two minutes, dimmed to 'half' for two minutes and were dimmed completely for the final two minutes.

Test Condition III. As per Condition II. except that the chamber had overhead lights 'half dimmed', and the image screen was left dark until the fly had settled. At zero time the image screen was lit and the image turned on, at a velocity of 5°/sec. After two minutes overhead lights were dimmed completely and observations continued for another three minutes.

Flies were observed for 5 or 6 minutes (as indicated above) or until the fly moved to the image screen. Full responses are defined as directed flight to the image. Data were analyzed by contingency chi-square analysis. There was no statistically significant difference in the responses of the two types of males (Table II).

Effects on mating behaviour

Under laboratory conditions, and in relatively small cages, both salmon and tan flies appear to be less competitive (or at least less active) than are their wild type counterparts and in both *G. m. morsitans* an *G. p. palpalis* there is assortative mating (Gooding 1982; D'Haeseleer et al., 1987). For *G. m. morsitans* these conclusions are based upon the numbers of each phenotype produced by salmon and wild type females after they had been caged with salmon and wild type males. The results could, therefore, have been influenced by post-

TABLE II

Responses of male *Glossina morsitans morsitans* to a moving image in a flight chamber

Experimental Conditions*	Full Response WT**	Full Response salmon	Not Full Response WT**	Not Full Response salmon	χ^2 with Yates correction
I	1	5	3	2	0.74
II	2	5	7	9	0.05
III	5	9	13	8	1.38
I-III	8	19	23	19	3.24

*Experimental conditions varied in details of lighting and duration of observation periods as described in Section: Effect on vision.

**WT = wild type.

TABLE III

Some general comparisons of wild type and mutant *Glossina morsitans morsitans* and *Glossina palpalis palpalis**

Parameter	*G. m. morsitans*		*G. p. palpalis*	
	wild type	salmon	wild type	tan
1) puparia/female lifetime**	4.2	2.1	4.4	4.3
2) emergence rate	92%	17%	96%	92%
3) longevity of males	>90 days	ca. 50 days	n.r.***	n.r.
4) longevity of females	>90 days	ca. 80 days	n.r.	n.r.
5) infections with *T. b. brucei*				
5.1) midgut of males	28%	64%	27%	18%
5.2) salivary glands of males	17%	34%	19%	15%
5.3) midgut of females	42%	75%	15%	9%
5.4) salivary glands of females	8%	11%	16%	11%

* Information from Gooding (1982), D'Haeseleer et al. (1987) and Makumyaviri et al. (1984).
** *G. m. morsitans* females kept 90 days, *G. p. palpalis* females kept 80 days.
*** n.r. = not reported.

mating events but there is no evidence for this. Direct mating competitiveness tests comparing tan and wild type *G. p. palpalis* have not been reported. However, observations made during assortative mating tests (D'Haeseleer et al., 1987) showed that wild type males were more likely than tan males to be among the first involved in mating. This is consistant with the observation, mentioned above, that tan flies generally appear less active than wild type *G. p. palpalis*.

Effects on general bionomic and behavioural characters

Table III summarizes some differences reported between mutant and wild type tsetse flies with respect to general bionomic features and vectoring ability of the flies. The *tan* allele has little or no effect upon puparial production by females or upon emergence rates (D'Haeseleer et al., 1987). Since female productivity was calculated over an arbitrarily chosen period of 80 days, the data imply that tan females live about as long as wild type females. In contrast to this, salmon flies do not live as long as wild type flies (Gooding, 1982) and salmon females, mated with salmon males, produce fewer offspring than do wild type *G. m. morsitans* and adults emerge from less than 20% of the puparia (Gooding 1979, 1982; Table III). These adults usually live only a few days.

Circadian rhythms in salmon and wild type *G. m. morsitans* were studied using males which emerged from puparia within a few days of being hand carried from Edmonton, Canada to Silwood Park, England. Two pieces of equipment were available for the study. Apparatus I permitted simultaneous examination of six insects, their movement being detected by a wire, projecting from the cage, closing an electric circuit whenever the cage holding an insect moved. This apparatus was on a 12 hour light: 12 hour dark cycle, with the light phase beginning at approximately 9 a.m. Apparatus II had cages for ten insects and movement was detected by a small projection from the cages cutting an infra-red beam, to activate an event counter. This apparatus had a 12 hour light phase which was preceeded by, and followed by, 30 minute crepuscular periods. Flies which had emerged during the preceeding 24 hours were given a blood meal and then placed in the apparatus, usually between 3 p.m. and 6 p.m.

Actographs obtained were analyzed by dividing each hour into 12 (5-minute) periods and counting the number of periods in which a fly was active. Activity during the first evening that a fly was in the apparatus was ignored; data analyzed are for 84 hours commencing midnight on the day the fly was placed in the apparatus. Overall activity patterns of salmon and wild type males were compared graphically. Statistical analyses of the total activity were done using the nonparametric Wilcoxon 2-sample test (Sokal & Rohlf, 1969). Actographs were also used to determine whether salmon and wild type males differed in how soon they became active after lights came on. These results were analyzed by a contingency chi-square test. Actographs were used to estimate duration

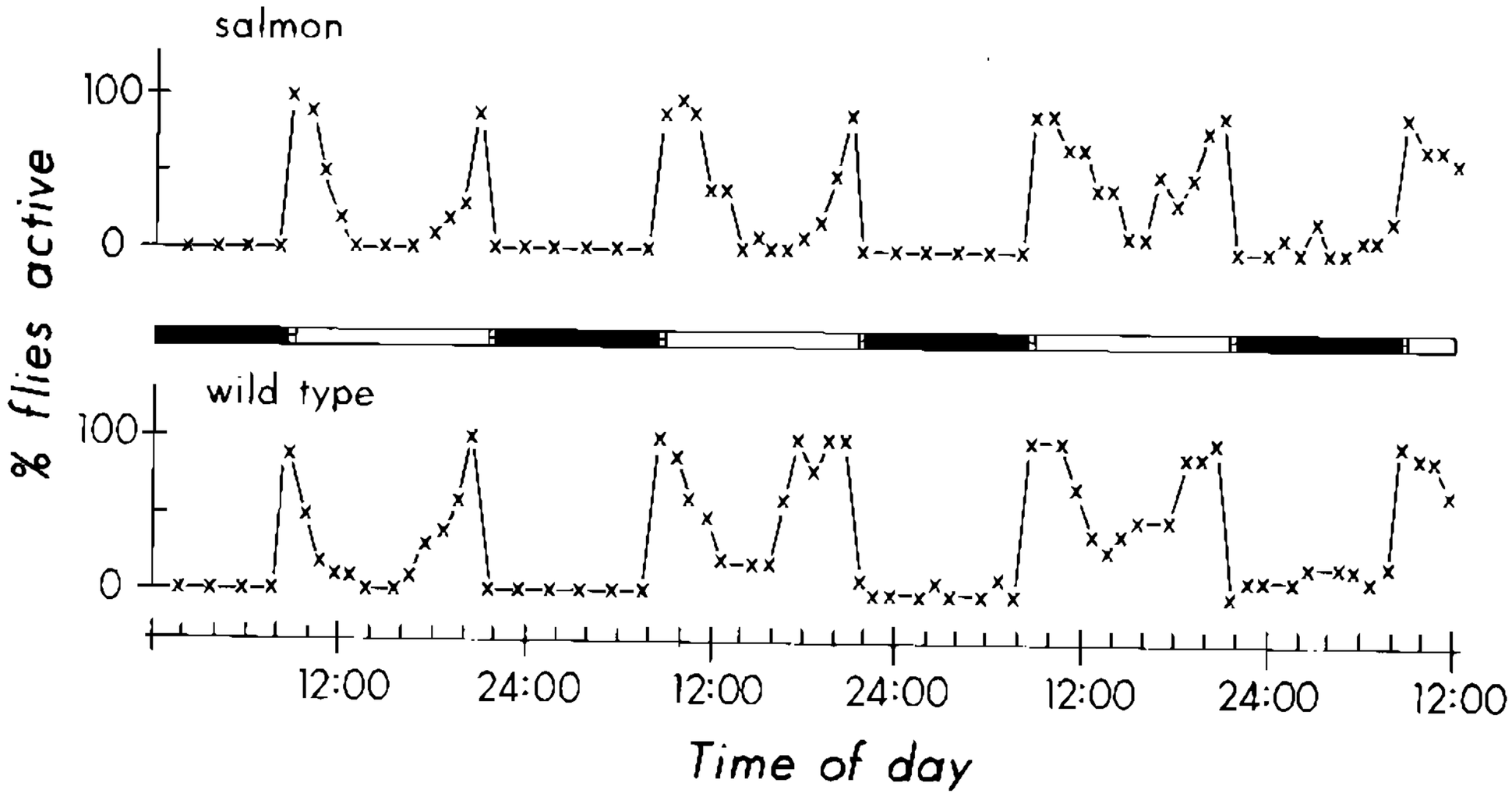

Fig. 1: Percentage of salmon and wild type *Glossina morsitans morsitans* males active at various times of day in actograph apparatus II. Thirty minute crepuscular (=dim light) period began at approximately 8:20 a.m.; 12 hour light phase was followed by a 30 minute crepuscular period.

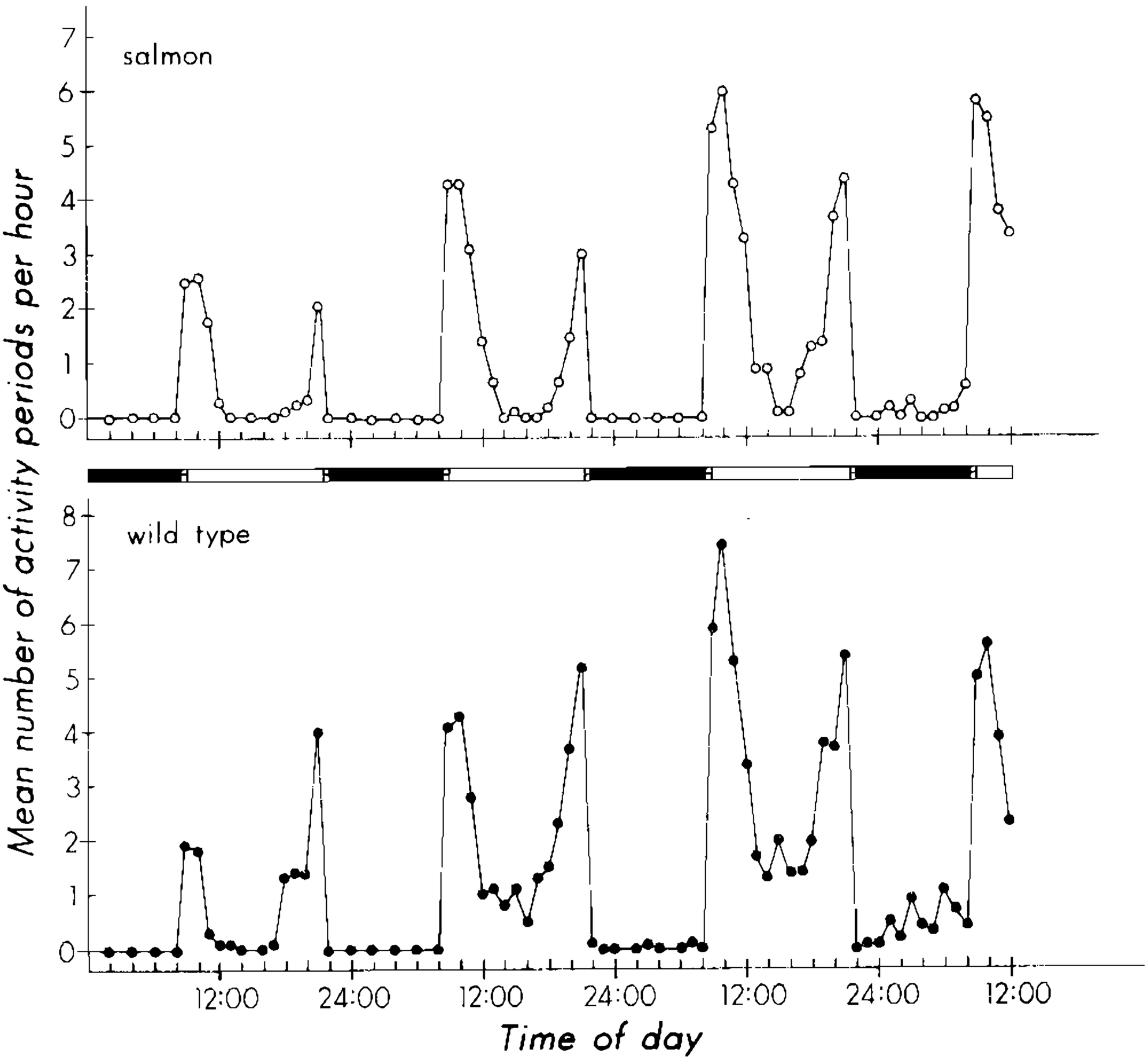

Fig. 2: Mean number of activity periods of salmon and wild type *Glossina morsitans morsitans* males in actograph apparatus II. Thirty minute crepuscular (=dim light) period began at approximately 8:20 a.m.; 12 hour light phase was followed by a 30 minute crepuscular period.

of flights by measuring the length of tracings made during morning and evening activity periods. Mean duration of flight times for salmon and wild type flies were compared using Student's t-test and Wilcoxon 2-sample test.

There is little difference between wild type and salmon males with regard to overall activity pattern (Figs. 1 and 2). Spontaneous activity begins after lights come on and reaches a peak during the second hour of the light phase. The mid-day lull in activity is more pronounced in salmon males than it is in wild type males. The evening peak of activity begins a couple of hours before lights go out and terminates by two hours after the dark phase begins. From the end of evening activity, until lights come on, there is very little activity. These activity patterns are seen in both number of flies active (Fig. 1) and in amount of activity per fly (Fig. 2). Activity per fly during peaks and during lulls, increases each day (Figs. 1 and 2) presumably as a result of the flies becoming hungrier.

There are three sets of data, involving eight flies, in which equal numbers of wild type and salmon males were placed in Apparatus I on the same day and observed for 84 hours. These actographs were examined to see how many flies of each phenotype were among the first half of the flies to become active each morning. There is no tendency for either phenotype to become active first when the data were analyzed one day at a time, or after pooling data from all four mornings. There are five data sets, involving 24 flies in Apparatus II, in which equal numbers of wild type and salmon males were observed each day. In this apparatus, there is a tendency for salmon males to predominate among the first flies which become active each day, and data pooled from all four mornings indicated this tendency is statistically significant (Table IV).

TABLE IV

Number of salmon and wild type *Glossina morsitans morsitans* males among the first flies active each morning in Apparatus II

Day number	Number of each phenotype active among first half of active flies: salmon obs	salmon exp.	wild type obs.	wild type exp.	χ^2 with Yates correction
1	10	6	2	6	5.42
2	9	6	3	6	3.08
3	9	6	3	6	3.08
4	8	6	4	6	1.42
1-4	36	24	12	24	12.02

TABLE V

Average number of five minute periods during which salmon and wild type *Glossina morsitans morsitans* males were active*

Apparatus	phenotype	n**	Day 1	2	3	4***	1-4
I	wild type	5	14	26	31	13	21
	salmon	6	14	18	26	14	18
II	wild type	10	12	30	45	21	27
	salmon	10	10	19	32	20	20

*Numbers in the body of the table are the average of five-minute periods during each day when a fly was active; the last column is the average for the total observation period.

**Number of flies for which complete, uninterupted 84 hour record was available.

***Observations made until noon on day 4.

TABLE VI

Flight duration of *Glossina morsitans morsitans* males in actograph II*

	Day 1		Day 2		Day 3		Over-all
	M**	E**	M	E	M	E	
Wild type							
Mean	57	77	64	82	79	71	72
S.D.	33	25	20	22	28	24	11
Salmon							
Mean	37	41+	41	39+	43+	30+	38
S.D.	12	26	18	28	33	21	18

*Numbers in body of table are means (in seconds) and standard deviations (S.D.) calculated as the unweighted means of average flight duration of 10 males of each phenotype, except where otherwise indicated.
**M = morning activity peak, E = evening activity peak.
+ Means calculated from average flight duration of 9 males.

Wild type males were slightly more active than were salmon males (Table V). However analysis of raw data, using the nonparametric Wilcoxon 2-sample test, showed that, in each apparatus, on each day, as well as during the entire observation period, the differences between wild type and salmon males were never statistically significant (i.e.$p > 0.05$). However, with regard to mean duration of each flight, there were significant differences between wild type and salmon males (Table VI). Flight duration, during morning and evening activity peaks, was not influenced by length of time flies were in Apparatus II. However, flight duration of salmon males, during each activity peak (except the first morning) and over all periods, was significantly less than that of wild type flies. This difference was statistically significant using Student's t-test (Table VI) and the nonparametric Wilcoxon 2-sample test.

The activity of both wild type and salmon *G. m. morsitans*, was typical, in both pattern and magnitude, of what has been previously reported for this species (see for example Brady & Crump, 1978; Crump & Brady, 1979). However, average flight duration of salmon males was significantly less than that of wild type males (Table VI). This result, and their slightly lower total activity, may explain why salmon males were less successful in mating competition experiments (Gooding, 1982).

Mean flight duration of wild type males used here (72 ± 11 sec) is considerably longer than the value (48 ± 3 sec) reported by Brady & Crump (1978). It is not known whether this is due to differences in activity of flies from different colonies, conditions under which they were tested, or the way in which different workers interpret and measure actographs. The difference between flight duration of wild type and salmon discloses yet another parameter which is affected by the pleiotropic allele *salmon*. This difference raises interesting questions about physiological differences between wild type and salmon *G. m. morsitans*. Among the tentative explanations for the difference in flight duration is the possibility that salmon flies, with their greater visual sensitivity (Davis & Gooding, 1983) suffer from a "sensory overload" during flight.

Effects on vectorial capacity

Ability of *G. p. palpalis* to become infected with *Trypanosoma brucei brucei* EATRO 1125 (as measured by either midgut or mature salivary gland infections) is not influenced by the presence of the *tan* allele (D'Haeseleer et al., 1987; Table III). However salmon *G. m. morsitans* are significantly more likely to develop midgut and salivary gland infections of *T. b. brucei* EATRO 1125 than are wild type flies (Makumyaviri et al., 1984; Table III). Similar results have been obtained with *Trypanosoma congolense* (Distelmans et al., 1985).

TRYPTOPHAN OXYGENASE AND KYNURENINE FORMAMIDASE ACTIVITIES

In early experiments on the activities of tryptophan oxygenase and kynurenine formamidase we chose post-teneral flies randomly from our colony and found that levels of these enzymes varied greatly. To see whether activities

TABLE VII

Tryptophan oxygenase and kynurenine formamidase during second larviposition cycle of *Glossina morsitans morsitans*

Day of cycle*	No.	Tryptophan oxygenase** mean ± S.D.	Kynurenine formamidase*** mean ± S.D.
0	8	0.384 ± 0.113	0.078 ± 0.020
1	8	0.323 ± 0.171	0.083 ± 0.021
2	7	0.459 ± 0.251	0.126 ± 0.042
3	8	0.872 ± 0.265	0.206 ± 0.037
4	8+	0.595 ± 0.207	0.178 ± 0.023
5	6	0.388 ± 0.137	0.164 ± 0.041
6	5	0.415 ± 0.118	0.150 ± 0.029
7	9	0.318 ± 0.186	0.118 ± 0.031
8	4++	0.118 ± 0.028	0.078 ± 0.014
9	7	0.384 ± 0.187	0.097 ± 0.020

*Day 0 = 0-24 h after larviposition.
**Tryptophan oxygenase activity = ΔA_{560}/18h/fly at 30°C.
***Kynurenine formamidase activity = ΔA_{365}/min/fly at 30°C.
+ Only 7 assayed for tryptophan oxygenase.
++ Only 3 assayed for kynurenine formamidase.

of these enzymes might be related to the physiological state of the flies we re-examined their activities in flies having more closely defined physiological states.

Virgin heterozygous (+/*sal*) *G. m. morsitans* females were fed daily until 24 hours prior to dissection. After the midgut was removed, each abdomen was homogenized and assayed for kynurenine formamidase using techniques described by Gooding & Rolseth (1984). Five females of each age were assayed. Average activity of kynurenine formamidase (expressed as ΔA_{365}/min/fly) rose from 0.031 in unfed teneral females to 0.089 in three day old females which had fed twice and 0.127 in seven day old females which had fed six times. These results suggest that, although eye pigment formation appears to be complete in teneral flies, the capacity of the tryptophan to kynurenine pathway increases in post-teneral flies.

Heterozygous (+/*sal*) *G. m. morsitans* females were fed 7 days per week. Females, at the black lobe stage, were isolated and those which larviposited each day were pooled in labelled cages and fed daily. Each day several females were assayed for tryptophan oxygenase and for kynurenine formamidase activity using techniques described by Gooding & Rolseth (1984). The results (Table VII) indicate that for each enzyme maximum activity occurs on day 3 (i.e. about the time the egg hatches *in utero*) and that minimal activity occurs at day 8 (i.e. the day before larviposition). The apparently cyclic changes in activity of these enzymes suggests that they are under some sort of control but reasons for the cyclic fluctuations in activity have not yet been elucidated.

SOME TENTATIVE CONCLUSIONS

The *salmon* allele in *G. m. morsitans* and the *tan* allele in *G. p. palpalis* both cause derangement of tryptophan metabolism; salmon flies are profoundly affected by this but tan flies are only slightly affected. The major reason for this difference appears to be the point at which tryptophan metabolism is disrupted. The *salmon* allele results in accumulation and excretion of tryptophan; the *tan* allele merely prevents the production and/or retention of xanthommatin in the eyes while permitting its accumulation in the testes.

The results obtained indicate that tryptophan occurs in excess of what is required, by tsetse flies, for anabolic processes and the excess is metabolized to kynurenine and excreted. Information obtained using salmon flies indicates that tryptophan can be excreted. This raises the question of why adult tsetse flies have a mechanism to produce an excretory product from tryptophan rather than simply excreting any excess tryptophan.

There are two possibilities which we are

investigating. The first is that tryptophan is more toxic or less readily excreted than kynurenine. The second possibility is that it is advantageous to produce kynurenine for this material has a higher nitrogen to carbon ratio than tryptophan and the process liberates a formate which might then be used in synthetic processes such as *de novo* production of uric acid.

Whatever the major function of the tryptophan to kynurenine pathway, it is likely that it would be regulated in such a way as to optimize either removal of an unwanted metabolite or production of a needed one. The results we have obtained with young adult *G. m. morsitans* and with *G. m. morsitans* during the second larviposition cycle indicate that activities of tryptophan oxygenase and kynurenine formamidase may be controlled but we do not yet know how or for what function.

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