

DESTINATION OF INGESTED BLOOD AND SUGAR SOLUTION ALONG THE ALIMENTARY CANAL OF *TOXORHYNCHITES RUTILUS SEPTENTRIONALIS* MOSQUITOES¹

R. M. K. W. LEE², R. D. MILLER³ AND D. M. DAVIES⁴

Department of Biology, McMaster University, Hamilton, Ontario, Canada. L8S 4K1

ABSTRACT. Adult *Toxorhynchites rutilus septentrionalis* mosquitoes, which are non-bloodsucking, were induced to take blood, and the destination of blood or sugar solution along the alimentary canal of the mosquitoes was studied with or without a 24-hr starvation period prior to the experiments. The effects of removal of the labella, and the presence or absence of the cibarial ventral papillae on distribution pattern of food, were also studied. Unlike most blood-sucking dipterans the blood meal did not go to the midgut alone but to the midgut and crop or, in a few males and females, to the crop alone. The sugar meal frequently went only to the crop, as in most blood-sucking Diptera, but it also went to the crop

and midgut. The removal of labellar lobes had no effect on the distribution of the blood meal in either sex of starved and nonstarved mosquitoes. Almost one third of unstarved females stored blood in the crop alone and two thirds in both crop and midgut whereas all starved females stored blood in both places. With no starvation period removal of the labella affected the distribution of sugar solution only in the females. Furthermore, 24-hr starvation period affected the distribution of sugar solution in both sexes of mosquitoes. The presence or absence of cibarial ventral papillae had no effect on the distribution of blood or sugar meals in either sex. The results are discussed in relation to other bloodsucking insects.

INTRODUCTION

Among bloodsucking Diptera that feed on both sugar solution and vertebrate blood, sugar solution generally goes to the crop and blood to the midgut, although there are exceptions (Lee and Davies 1979). In mosquitoes, the destination of a sugar or blood meal is believed to be dictated by sensilla found in the cibarial pump (Day 1954, Lee 1975) and probably by labellar sensilla as well (Hosoi 1959). Adults of *Toxorhynchites* mosquitoes are non-bloodsucking (Knab 1911) and

have reduced mouthparts (Edwards 1932). Lee and Davies (1978) reported that the cibarial sensilla of the 3 species of *Toxorhynchites* examined by them are similar to those of bloodsucking mosquitoes reported by Lee (1975), except that there was a reduction in number of the ventral group of sensilla in some specimens of *Tx. brevipalpis* Theobald and a complete loss of them in most *Tx. rutilus septentrionalis* (Dyar and Knab). They suggested, therefore, that such reduction is probably related to the non-bloodsucking habits of these mosquitoes. Although blood is not included in the normal diet of *Toxorhynchites* mosquitoes, we were able to induce adults of *Tx. rutilus septentrionalis* to feed on blood in the laboratory, thus enabling us to study the following:

- (1) The destination of blood and sugar solutions in both sexes of these mosquitoes.
- (2) The effect of starvation on the destination of ingested blood and sugar solutions.
- (3) The effect of removing the labellar

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² Present address: Department of Anaesthesia (4V 33), McMaster University, Hamilton, Ontario, Canada. L8N 3Z5.

³ Present address: Department of Radiation Biology and Biophysics, School of Medicine and Dentistry, University of Rochester Medical Center, 601 Elmwood Avenue, Rochester, NY 14642, U.S.A.

⁴ Contact for reprint requests.

sensilla in the proboscis tip on the destination of blood and sugar solutions.

- (4) The relationship between the destination of blood and sugar solutions and the presence or absence of ventral cibarial sensilla.

MATERIALS AND METHODS

Adults, 1–2 months old, and eggs of *Tox. rutilus septentrionalis* were obtained from Dr. Brian Watts of the Department of Biology, University of Waterloo, Waterloo, Ontario, Canada. The eggs were hatched and reared to adults following the methods of Trimble and Corbet (1975). For feeding experiments, adults, temporarily immobilized by chilling, were individually fixed to one end of applicator sticks by the scutum with Paraplast® (Canlab) (Fig. 1). The free end of the applicator stick was attached to one

clamp of a double clamp holder. The other clamp was used to fasten the clamp holder to the rod of a retort stand, so that the mosquito could be moved up and down by adjusting the clamp. Test solutions were contained in a capillary tube sealed at one end with Paraplast. The capillary tube was inserted into the end of a rectangular block of styrofoam so that the open end of the tube was 2–3 mm above the surface of the block. By gently lowering the clamp holder containing a mosquito at the other end of the applicator stick, and adjusting the styrofoam piece, the tip of the proboscis of a mosquito was dipped into the capillary tube (Fig. 1). The legs were allowed to touch the styrofoam pad, thereby eliminating tarsal flight response. Because the slender proboscis of *Toxorhynchites* mosquitoes is usually J-shaped with the tip bent downwards, it was generally easy to position the tip of the proboscis in the capillary tube. Feeding usually began as soon as the proboscis tip was inserted into the capillary tube and lasted for 10–15 min. For “slower feeders,” up to 30 min was allowed for feeding. The fluid level in the capillary tube indicated if a mosquito had taken any solution.

Some mosquitoes inadvertently moved the proboscis out of the capillary tube without taking any test solution. In such cases, the proboscis was reinserted into the test solution until the mosquito had taken some solution. In certain experiments, the proboscis tips of some mosquitoes were excised just before the feeding experiments, to determine whether labellar sensilla in the proboscis tip affected the destination of blood and sugar solution. Such treatment seemed not to affect the mosquitoes in their feeding, as those without labella fed as efficiently as those with labella.

Mosquitoes were killed by freezing immediately after feeding, and later dissected to determine the destination of ingested food. Expired citrated human blood was obtained from the blood bank of the McMaster University Medical Centre in Hamilton, Ontario, Canada.

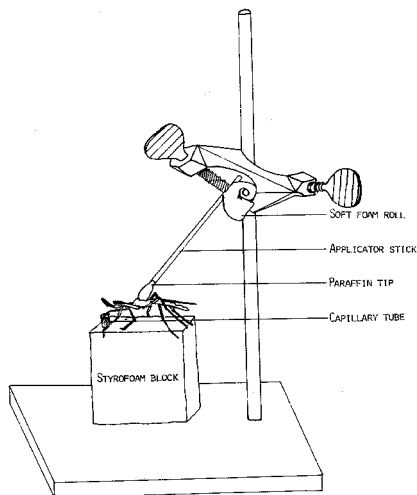


Fig. 1. Diagram to show the feeding apparatus used in the experiments with *Toxorhynchites* mosquitoes. Paraffin tip is affixed to the dorsum of the mosquito's thorax. The capillary tube contains the liquid food.

Sucrose solution (10%) was stained with methylene blue to aid in identifying its location during dissection. The experiments were conducted at room temperature (21°C).

To determine if the presence or absence of ventral cibarial sensilla affected the destination of blood or sugar solution the cibaria of the mosquitoes were dissected out after feeding, cleared in hot 10% KOH, washed several times in distilled water, and then prepared for light microscopy. The presence or absence of these sensilla was noted along with the destination of the test solutions.

Five factors were investigated in the experimental design: (1) sex, (2) blood or sugar feeding, (3) labella present or removed, (4) ventral group of cibarial sensilla present or absent and (5) 24-hr starvation period or no starvation period. After treatment, the location of the meal was scored as either in the midgut or crop, or in both.

RESULTS

Table 1 gives the results of dissecting 259 force-fed adult *Tx. r. septentrionalis*. Each individual was scored as having the meal in one of 3 storage arrangements: the crop alone, the midgut alone, or the crop and midgut. Effectively, the storage arrangements are reduced to two, since none of the individuals in Table 1 had the meal in the midgut alone. The results show similarities in food storage with most bloodsucking Diptera in that the sugar meal frequently went to the crop alone; however, the sugar meal was also found in both the crop and midgut. In contrast with most bloodsucking Diptera, a blood meal never (with 1 exception in Table 2) went to the midgut alone, but only to the crop or to the crop and midgut.

The first of 4 groups in Table 1 is females with no starvation period prior to the forced meal. Some 69% of those with labella and fed sugar, stored the meal in the crop alone. But only 31% of those with labella and fed blood, stored the

meal in the crop alone. These values are statistically different (probability of being the same less than .01) as shown in Table 1 by the results of a 2×2 contingency test using the G statistic with Yates correction for continuity (Sokal and Rohlf 1969). However, those females with their labella removed show no statistical difference in storage of a forced sugar or blood meal. When those with and without labella are compared for storage in the crop or the crop and midgut, the results are statistically significant. Therefore, removal of the labella affects food storage location in this 1st group. This result is based on sugar storage as there is no difference in blood storage. The G values without Yates correction are also given in Table 1. The sum of the 3 comparisons is the same as the 4×2 contingency test value of the 4 categories of non-starved females versus food storage location, since the comparisons are orthogonal contrasts of a larger two-way design. The design is two-way, rather than a higher multiple since the row totals are fixed by the number of mosquitoes examined for a particular class. Statistical subgroups (Sokal and Rohlf 1969) are also shown in Table 1. These represent combinations which no longer are statistically significant when tested with a G test. For non-starved females, there are 2 subgroups: those with labella that were fed sugar, and the remainder.

The 2nd group in Table 1 is males with no starvation period. Considering those with labella, as with females, a higher proportion stored sugar in the crop alone than stored blood in the crop alone. However, the number of individuals examined is less than for females, and the difference is not statistically significant. The G test with Yates correction shows no statistical difference in storage patterns between non-starved males and females with labella for those fed sugar and for those fed blood. Respective G values with Yates correction are 2.3 and 0.1, not shown in Table 1.

The third group is females that were starved for 24 hr prior to the forced meal.

Table 1. Food storage in *Toxorhynchites rutilus septentrionalis* with labella present (+) or removed (-), and given 10% sucrose (S) or citrated human blood (B).

Group	Labella and food	Meal storage frequency ^v		Total examined	Statistical subgroup ^{vv}	Hypothesis	df	G value		
		Crop only	Crop and midgut					With Yates	Regular	
										Rxc analysis and 2 x 2 partition
<i>No starvation</i>										
Females	+	S	.69	.31	45	a	+ : S vs B	1	8.3**	9.8**
	-	B	.31	.69	26	b	- : S vs B	1	1.3	2.2
	+	S	.15	.85	27	b	+ vs -	1	11.6**	13.0**
	-	B	.32	.68	25	b		3		25.0**
Males	+	S	.48	.52	27	ab	+ : S vs B	1	2.5	3.6
	-	B	.21	.79	19	b	- : S vs B	1	2.9	4.8*
	+	S	.61	.39	18	a	+ vs -	1	0.4	0.8
	-	B	.14	.86	7	ab		3		9.3*
<i>Starved 24 hrs.</i>										
Females	+	S	0	1.00	7	c	Females vs Males	1	0.0	0.0
	-	B	0	1.00	6	c	Total non-starved	7		34.9**
	+	S	0	1.00	7	c				
	-	B	0	1.00	6	c		3		0.0
Males	+	S	.89	.11	9	c	+ : S vs B	1	6.5*	9.9**
	-	B	.14	.86	7	de	- : S vs B	1	6.1*	10.2**
	+	S	.50	.50	10	cd	+ vs -	1	3.5	4.9*
	-	B	0	1.00	13	e		3		25.0**
					259		Females vs Males total starved	1	12.5**	16.8**
							Non-starved vs starved	7	7.7**	41.8**
							Total	15		84.8**

^v No individuals had the forced meal stored in the midgut only.

^{vv} From a *posteriori* test of all subsets in the 4 x 2 contingency table of each group (Sokal & Rohlf, p. 703-4).

* Probability less than .05.

** Probability less than .01.

The pattern of storage is quite different from non-starved females in that all individuals examined had food stored in both the crop and midgut for either a blood or sugar meal.

The last group is males that were starved for 24 hr before the forced meal. Storage is similar to that in non-starved males, but accentuated. Averaging both those with and without labella, sugar is stored predominantly in the crop alone, and blood in the crop and midgut. Food storage in starved males is statistically different from that in starved females, especially with regard to sugar. Food storage in non-starved versus starved mosquitoes is also statistically different as shown by the G tests.

In summary, starved females stored their food in both the crop and the midgut, regardless of the type of food, but non-starved females stored food in either the crop or both the crop and midgut depending on the kind of food (Table 1), but only rarely in the midgut alone (Table 2). In non-starved females, presence or absence of labella affected the storage location if a sugar solution was imbibed, but not if blood was imbibed. However, in starved females, labellar presence or absence had no effect on the distribution of sugar solution or blood. The situation in males was the reverse, in that presence or absence of labella affected food distribution only in starved mosquitoes. With their labella intact, non-starved females

and starved males usually stored sugar solution in the crop only.

Table 2 shows the results of investigating 67 *Tx. r. septentrionalis* for the natural presence or absence of the ventral group of cibarial sensilla (ventral papillae). Only 11 of the 67 had these structures. Those having the structures exhibited no obvious differences in food storage patterns from those lacking them.

DISCUSSION

Ingestion of blood by otherwise non-bloodsucking Diptera under laboratory conditions is not uncommon (MacGregor and Lee 1929, MacGregor 1931, Russell 1931, Day 1954, Chao and Wistreich 1959, Salama 1966, and Jones and Pilitt 1973 with male mosquitoes; Trembley 1952 with autogenous *Aedes atropalpus* (Coquillett) female mosquitoes; Yang and Davies 1968 and Wenk 1965 with male black flies; Davies and Anderson 1967 with the house fly). Adler and Theodor (1926) observed 2 male *Phlebotomus papatasii* Scopoli, sand flies which are usually non-blood feeding, gorged with blood, and they reported seeing 1 of the flies leaving a human being after the "meal!" Male mosquitoes have been reported to bite man, but such behavior is abnormal and may involve gynandromorphs (Bates 1949). Reports on the destination of ingested blood in male mosquitoes are confusing. MacGregor

Table 2. Presence or absence (-) of cibarial ventral papillae versus food storage in *T. r. septentrionalis* given 10% sucrose (S) or citrated human blood (B) with no starvation period.

Sex	Papillae	Food	Meal storage frequency		Total examined
			Crop only	Midgut only	
Females	+	S	.25	0	8
		B	.67	0	3
	-	S	.35	.06	17
		B	.28	.03	32
Males	-	S	-	-	0
		B	.14	0	7
					67

* No males with cibarial ventral papillae were found.

and Lee (1929) gave male mosquitoes citrated human blood mixed with honey and found the ingested blood usually in the crop and when the mosquito was fully fed in the other diverticula as well, whereas Russell (1931) found that blood ingested by male mosquitoes went to the midgut. This difference may have resulted from the addition of carbohydrate in the blood meal of the former, as was found in simuliids (Yang and Davies 1968). In autogenous *Ae. atropalpus* females, blood was found in the midgut and sometimes also in the diverticula (Trembley 1952).

In the present study ingested blood was generally found in both the crop and the midgut of both sexes of *Toxorhynchites* mosquitoes with no starvation period. However, females starved for 24 hr prior to the experiments stored blood differently from those without a starvation period, whereas males, whether starved or not, showed little change in the location of blood storage. In starved females, the locations of blood and sugar solution are the same, suggesting that after a starvation period, the type of food ingested by the females may not be discriminated, and all is dispatched to the same locations in the alimentary canal. The presence or absence of the labella did not affect the destination of blood in either starved and non-starved mosquitoes, indicating that the labellar sensilla were not sensitive to blood.

Whether or not non-blood-feeding mosquitoes are capable of digesting blood remains to be investigated. MacGregor (1931) stated that male mosquitoes are capable of digesting blood within the same period as required by the females, and that they would feed on blood many times in succession. However, Chao and Wistreich (1959) referring to their unpublished data, stated that male *Culex pipiens* L. when force-fed blood, were unable to digest it and died shortly after the meal. Davies and Anderson (1967) found in the house fly, *Musca domestica* L., that when human blood was given to the flies with or without various supplements, the

flies were able to lay some eggs, although fewer in number than milk-fed females. Only female black flies feed on blood, but Yang and Davies (1968) detected trypsin activity from the midgut of males, and found that such activity was identical to that of female black flies before their blood meal. They also found that trypsin activity in females increased after a blood meal or a sucrose-blood mixture, whereas in males, ingestion of a sucrose-blood mixture elicited no increase in trypsin activity. Trypsin activity was also noted by them in both sexes of non-blood-feeding black fly, *Cnephia dacotensis* (Dyar and Shannon). It would be interesting to conduct similar studies in male and autogenous female mosquitoes.

When *Toxorhynchites* mosquitoes were given sugar solution with no starvation period, the presence or absence of labellar lobes affected the distribution of food only in the females. In the females with their labella intact, sugar solution was found mostly in the crop alone, whereas removal of the labella abolished the selective dispatching of the sugar solution to the crop. These results suggest that in the females, the labellar sensilla are probably sensitive to the sugar solution, and are responsible in detecting and influencing the destination of sugar solution, whereas in the males without a starvation period, the labellar sensilla are probably less sensitive than those of the females. The dispatch of sugar solution to the crop in the females is similar to that found in most bloodsucking dipterans (Lee and Davies 1979). Such similarity probably indicates the close affinity of *Toxorhynchites* mosquitoes with bloodsucking mosquitoes.

When *Toxorhynchites* mosquitoes were starved for 24 hr before the experiments, the distribution of sugar solution in both sexes with their labella intact was different from the distribution in those without a starvation period. In the starved males, sugar was found mostly in the crop alone, as compared to those without a starvation period, where the number of mosquitoes having sugar in crop, and in both crop and midgut, are

almost the same. Removal of the labella in males abolished such difference due to starvation pattern (cf. Table 1). In the females, after 24-hr starvation period, sugar solution was found both in the crop and midgut, and the presence or absence of the labella had no influence on the food distribution pattern.

The data discussed above regarding the distribution of sugar solution, the pattern of food distribution and utilization, and the role of labellar sensilla before and after starvation periods can be summarized as follows: In the females without a starvation period, as food was always accessible and the mosquito probably made frequent visits to the food source, ingested sugar solution was generally dispatched to the crop for storage, and the stored food was probably released at intervals into midgut for digestion whenever required, as in the case of bloodsucking mosquitoes. Such a selective dispatch mechanism to the crop is probably controlled through the labellar sensilla, and dictated by the central nervous system. When the females were starved for 24 hr, the mosquitoes were in need of food, therefore an initial portion of ingested food probably went into the midgut first, with the remainder going into the crop. The sensitivity threshold of the labellar sensilla probably was changed due to starvation, thereby resulting in a different food distribution pattern. In the males with no starvation period, the sensitivity threshold of the labellar sensilla may have been the same as those of females after the starvation period, a supposition based on the similar food distribution pattern in the 2 groups of mosquitoes. Male mosquitoes were seldom seen close to the food source compared to the females, and the abdomen of the males was generally flattened, in contrast to the full, distended abdomen of the females (unpublished data). It seems that a sugar meal was rarely taken by the males, and whenever it was taken, it was passed to the midgut for immediate utilization as well as to the crop for storage. However, when the males were starved for 24 hr,

the sensitivity threshold of the labellar sensilla was changed, and then in most males sugar was found only in the crop.

With few exceptions, most bloodsucking dipterans take a sugar meal, and the sugar meal provides an energy source for flight, enables them to live longer, and it also influences the fly's blood-feeding and oviposition activities, as well as the development of eggs (Lee and Davies 1979). Bloodsucking female mosquitoes of different age groups have been shown by Magnarelli (1978) to acquire fructose in all phases of the gonotrophic cycle, and the author asserted that: ". . . female mosquitoes obtain nectar whenever possible, utilize some of the carbohydrates for immediate energy in flight and for survival, incorporate some carbohydrates into yolk, and store the remainder as glycogen and tricycleride for later use." It is obvious that nectar feeding in females is more essential and the meal is utilized for various purposes including egg production and oviposition, whereas in the males, the sugar meal probably is an energy source for general activity, and also enables them to live longer. Such differences in utilization probably explain the differences in food distribution patterns observed in the present study.

Our finding that the presence or absence of cibarial ventral papillae had no effect on the food distribution pattern of blood and sugar solution in both sexes of mosquitoes suggested that these sensilla, even when present, are vestigial or non-functional. Since these sensilla are found in the cibarium of bloodsucking mosquitoes (Lee 1975), it is possible that with the loss of blood-feeding habits in *Toxorhynchites* mosquitoes (as bloodsucking in Diptera is considered a primitive trait by Downes 1958), the cibarial ventral papillae have become reduced. These sensilla have been suggested by Lee (1975) to be responsible for the destination of the blood meal in bloodsucking mosquitoes. It is possible that blood is not recognized by any of the chemoreceptors along the alimentary canal of *Toxorhynchites* mosquitoes.

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