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BIOLOGY OF *CULEX TARSALIS* DURING THE SPRING SEASON IN OREGON IN RELATION TO WESTERN ENCEPHALITIS VIRUS¹

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The western encephalitis (WE) and St. Louis encephalitis (SLE) viruses are maintained by cyclic transfer of virus between *Culex tarsalis* and avian hosts during the summer months in the western United States, but it is not known how the viruses survive the winter period when mosquitoes are inactive. The vector overwinters as the hibernating adult female and can carry WE virus for several months during the winter (Bellamy, Reeves, and Scrivani, 1958). The virus has been isolated from *C. tarsalis* in California during all months except Decem-

ber (Reeves, Bellamy, and Scrivani, 1958), and *C. tarsalis* has, therefore, been suspected of maintaining WE virus through the winter. However, most evidence indicates that *C. tarsalis* or other mosquitoes are not the winter reservoir for WE virus in northern areas (Bennington, Sooter, and Baer, 1958; Rush, Brennan, and Eklund, 1958; Rush, Kennedy, and Eklund, 1963a, 1963b) and that mosquitoes become infected after they emerge from hibernation. Rush *et al.* (1963a) reported evidence that *C. tarsalis* did not acquire virus immediately after it left its winter habitat and postulated that it acquired virus later in the spring.

The virus transmission cycles that occur each summer would be impossible if the vector did not feed regularly on infected and susceptible hosts at some time earlier in the year. In the spring and early summer the number of mosquitoes and rate of blood feeding constantly increase and mosquitoes are increasingly exposed to potential sources of infection. As a result, conditions become more and more suitable for virus amplification in a mosquito-bird cycle. Isolations of virus from mosquitoes or birds during this

¹ This investigation was supported in part by the National Institute of Allergy and Infectious Diseases, Research Grant AI 03028, and General Research Support Grant I-SO1-FR-05441-01, from the National Institutes of Health, U. S. Department of Health, Education, and Welfare.

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⁴ Supported by U. S. Public Health Service Research Career Development Award 1-K3-AJ-25, 427-01.

early season offer the greatest opportunity to trace infection back to a basic winter reservoir. However, interpretation of such isolations requires detailed studies of the relationships of the vector to vertebrate hosts, and knowledge as to the season when conditions first become favorable for maintenance of mosquito-bird virus cycles.

This is a report of the latest studies in eastern Oregon on the local distribution of *C. tarsalis*, its food sources, and its frequency of feeding from the time of its emergence from hibernation until the appearance of the first generation of adults in the spring.

METHODS. The study area, a dry part of eastern Oregon, has been described previously (Rush, 1962; Rush *et al.*, 1963a).

In the study of overwintered mosquitoes, *C. tarsalis* were collected from resting sites during two periods: April 13 to 28 and May 7 to 22, 1965. For convenience, these periods have been subdivided (see tables). Collections were made from abandoned buildings, under bridges, unbaited walk-in chicken coop traps, and red wooden boxes. The principal collecting site was selected on April 18, and was designated Site 7. It was at the juncture of irrigated and dry land, and contained an abandoned one-story house with an attic, two sheds, an outside well, an outside cellar, 2 small rabbit hutches, 30 large trees, 8 fruit trees, 3 lilac bushes, and some miscellaneous shrubs. The nearest other trees were about one-half mile away. An adjacent large irrigation ditch contained water throughout the study period and water flowed intermittently in a small ditch that bordered the premises. This site was about 300 yards from a rocky dam that impounded a large reservoir. The face of the dam provided a suitable habitat for mosquito hibernation. Numerous birds and small mammals were observed at the site, both within and outside the buildings.

Light traps were operated at several

sites during suitable weather to detect the first appearance of a new generation of adults in the spring. The mosquitoes so captured were subjected to viral isolation attempts but were not used further.

Each female mosquito from shelter collections was classified as engorged, gravid, or neither (flat). Most of the specimens in the flat category were further characterized as to the presence of appreciable amounts of fluid in the major alimentary diverticulum (crop) or presence of fat bodies in the abdomen. The ovarian tracheation classification technique of Detinova (1945) was applied to most of the flat and freshly engorged females to obtain a history of prior oviposition. This method has been used extensively in similar studies on *C. tarsalis* (Kardos and Bellamy, 1961; Blackmore and Dow, 1962; Burdick and Kardos, 1963).

Data on blood feeding were obtained by precipitin tests performed on engorged *C. tarsalis*. The test system has been described by Tempelis and Lofy (1963) and its use in field studies has been reported by Tempelis *et al.* (1965, 1966) and Reeves *et al.* (1963). The abdomens were removed from all specimens in which any blood was visible and these were submitted for test. Blood meals could usually be identified to family if the mosquitoes fed on mammals, and to order if they fed on nongallinaceous birds. If the mosquitoes had fed on gallinaceous birds, identification was to species.

The head and thorax of each mosquito were stored on dry ice and subsequently tested for virus by intraperitoneal inoculation of suckling mice. This method of preparing specimens may miss virus in previously non-infected specimens which have recently fed on infective blood, but is satisfactory in all other cases. One blind passage of mouse brain was made to detect low-titered or attenuated virus.

In June, July, and August, *C. tarsalis* were collected from the routine resting sites and tested for virus in pools of 50 mosquitoes or less. Fifteen sentinel

chickens that had been placed in the study area (not Site 7) in April were bled every two weeks during the summer and their sera were tested for hemagglutination-inhibition antibodies to WE and SLE viruses.

RESULTS. Site 7 contained abundant food sources, oviposition sites, and resting sites and was topographically delimited. Its mosquito population was, therefore, regarded as a semi-distinct element of the general population, and observations from that site are tabulated separately from all others. Although data from all sites are included in the tables, the results to be given below are, unless otherwise specified, from Site 7 alone.

C. tarsalis had already left their hibernation sites when observations were begun on April 13 and specimens were found at varying distances from known winter habitats. Sizeable collections were made under two bridges that were part of a road that separated cultivated land from surrounding semi-desert area. The greatest number of *C. tarsalis* was collected from Site 7 where they were found resting in the house and outbuildings and in nearby accumulations of dry leaves. Nearly two-thirds of the mosquitoes were collected there, and the remainder were mostly from the two bridges and an artificial shelter (unoccupied chicken coop) placed in a grove of trees. Of the 596 *C. tarsalis* collected from resting sites (Table 1), only 10 were from sagebrush areas away from trees, bridges, or water.

The overwintered population had declined greatly by May 7 and continued to drop throughout the study, as indicated by the daily collection rates shown in Table 1. The new generation was first in evidence on April 27, when a brightly-marked male was collected at a hot spring. The drainage from the spring has a temperature of 90° F. and *C. tarsalis* larvae and pupae have been abundant at this location during the spring, summer, and fall. Single brightly-marked adult females were found on May 11, 14, 19, and

21 at Site 7, which was 6 miles from known hot springs; and on May 20, 10 female specimens, also probably of the new brood, were taken in a light trap far from warm water.

When collecting was initiated in April, many specimens still contained winter fat (Table 1). As this source of energy was depleted, feeding on non-blood sources increased: the proportion of the flat population containing fat (Site 7) dropped from 73 percent (April 18-22) to 23 percent in late April and 0 percent in May, while the corresponding values for fluid content were 57 percent, 61 percent and 75 percent (Table 1). The relationship between fat content and blood feeding is less simple. During the very earliest period, April 13-17, when all the flat population contained fat, the rate of blood engorgement was only 21 percent (Table 1, all sites). Subsequently blood feeding increased rapidly, reaching 68 percent during April 18-22 at Site 7. From then on, as fat incidence continued to decline, blood engorgement also dropped, from 68 percent (April 18-22, Site 7) to 54 percent to 45 percent.

A different pattern is seen, however, if past as well as current blood feeding is taken into account. On this basis, an initial level of 56 percent (all sites) was followed by the consistently high rates of 82 percent, 84 percent, and 85 percent (Site 7). These values were obtained by adding the numbers of engorged and gravid specimens (Table 1) and of the unengorged specimens found to be parous (Table 2) and computing the sum as a proportion of the total number of mosquitoes collected during a given period (Table 1). Unreadable specimens and the occasional individual not checked for parity were considered nulliparous. (In presenting these results it has been necessary to use data from "all sites" for the earliest period, April 13-17, since Site 7 had not been discovered at that time.)

Trends of fat and fluid content and of blood feeding are shown in Figure 1. That

TABLE I.—Composition of the female *Culex tarsalis* population.

Period	Engaged		Gravid		Total		No. Checked, Fat & Fluid		Containing Fat		Containing Fluid		Total		
	Avg. No./Coll.	No.	% †	Avg. No./Coll.	No.	% †	Avg. No./Coll.	No.	% †	No.	% †	No.	% †	Avg. No./Coll.	No.
Site 7															
April 13-17	No collections														
April 18-22	28.0	140	68	3.6	18	9	9.4	47	23	44	32	73	25	57	41.0
April 23-28	16.0	48	54	3.7	11	12	10.0	31	35	31	7	23	19	61	27.7
May 7-22	3.2	45	45	1.0	14	14	3.0	42	42	24	0	0	18	75	7.2
Total	10.6	233	59	2.0	43	11	7.2	120	30	99	39	33	62	63	18.0
Total, All Sites															
April 13-17	2.0	10	21	2.8	14	29	4.8	24	50	18	18	100	Not done	9.6	48
April 18-22	37.0	171	72	8.0	40	15	11.0	55	21	49	25	51	26	53	53.2
April 23-28	15.0	88	60	4.3	17	12	10.3	41	28	40	10	25	25	63	36.5
May 7-22	3.4	48	35	1.6	22	16	4.7	66	49	41	0	0	30	73	9.7
Total	11.3	317	53	3.3	93	16	6.6	186	31	148	53	36	81	55	21.3

* Neither engorged nor gravid.

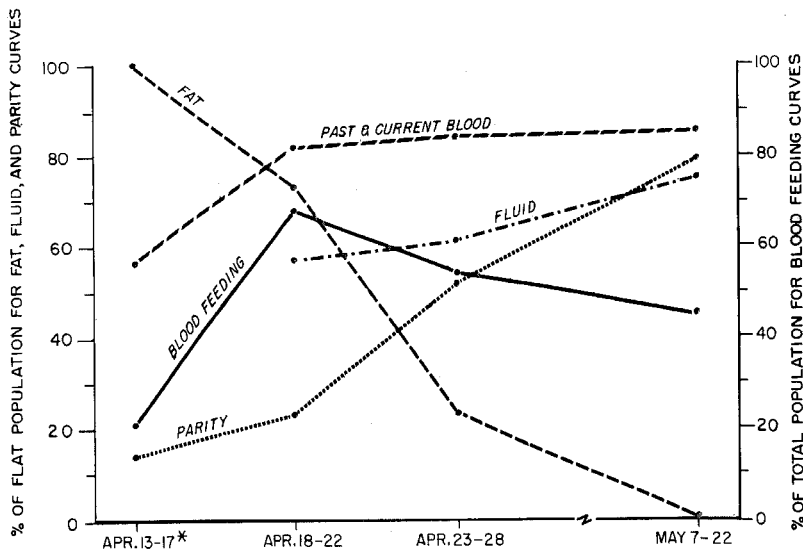
† of total, last column.

‡ of number checked for this quality.

TABLE 2.—Ovarian condition of *Culex tarsalis*.

Period	Unengorged			Engorged		
	No. Readable Specimens	No. Parous	%	No. Readable Specimens	No. Parous	%
	Site 7					
April 13-17		No collections		None done*		
April 18-22	43	10	23			
April 23-28	31	16	52	22	11	50
May 7-22	33	26	79	3	3	100
Total	107	52	49	25	14	56
	Total, All Sites					
April 13-17	21	3	14	None done		
April 18-22	51	13	25	None done*		
April 23-28	41	23	56	26	13	50
May 7-22	52	37	71	4	4	100
Total	168	76	45	30	17	57

* Except for a few specimens collected April 22, which are included in the entry for April 23-28.



* ALL SITES EXCEPT SITE 7. DATA FROM SITE 7 NOT AVAILABLE FOR THIS PERIOD.

FIG. 1.—Trends of feeding, fat content, and parity in *Culex tarsalis*.

total blood feeding remained high in spite of reduced current engorgement reflects the rise in parity shown in Figure 1 and Table 2. Only 23 percent of flat specimens of April 18-22 were classed as par-

ous, compared to 52 percent and 79 percent for the later periods. It was also shown that individuals can feed more than once, since engorged mosquitoes (all the May specimens examined, and half of

TABLE 3.—Precipitin tests on engorged *Culex tarsalis*.*

Period	BIRDS												MAMMALS				TOTALS		
	Passeriform		Columbiform		Strigiform		Unidentified		Miscellaneous		Total		Bovine		Miscellaneous		Total		
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	
April 13-17	No collections																		
April 18-22	45	34	17	13	39	27	27	19	1**	1	129	96	3	2	2†	2	5	4	143
April 23-28	15	20	19	25	12	14	25	29	0	0	71	93	5	7	0	0	5	7	84
May 7-22	20	59	0	0	0	0	9	26	1**	1	30	88	3	9	1++	3	4	12	44
Total	80	33	36	15	51	19	61	23	2	1	230	94	11	5	3	1	14	6	271
Site 7																			
April 13-17	7	100	0	0	0	0	0	0	0	0	7	100	0	0	0	0	0	0	9
April 18-22	68	42	18	11	39	22	30	17	1	0+	156	96	4	2+	2	1+	6	4	177
April 23-28	18	22	19	23	12	13	25	27	0	0	74	90	8	10	0	0	8	10	93
May 7-22	20	57	0	0	0	0	10	29	1	3	31	89	3	9	1	3	4	11+	47
Total	113	40	37	13	51	19	65	24	2	1	268	94	15	5	3	1	18	6	326
Total, All Sites																			

* There were no positives for hosts other than mammals and birds. Each component of a dual feeding was tabulated separately; such feedings all at Site 7, were as follows:

- April 18, canine+bovine
- April 18, canine+passeriform
- April 26, bovine+strigiform
- April 28, passeriform+columbiform

† Canines

** Quail

++ Hawk

*** Unidentified mammal

those of April) were parous (Table 2). This finding in the Northwest corroborates that of Nelson (1964) in California, who found many blood-engorged parous specimens in spring, before appearance of the new generation.

Results of the precipitin tests, which indicate the source of blood meals, are given in Table 3. Ninety-four percent of the mosquitoes fed on birds, and 6 percent on mammals. Four specimens (1.3 percent) contained blood of more than one species. Most of the feedings on birds were on passeriform, columbiform, or strigiform birds. There was one feeding each on hawk and quail and 26 percent of the avian blood meals could not be identified further. Some of the unidentified blood meals were probably of strigiform (owl) origin. In the first tests 36 samples gave a reaction that suggested the mosquitoes had fed on owls, but the samples were used up in the initial tests or were lost in storage, therefore they were not available for confirmatory testing. A confirmatory test was necessary for interpretation because our original strigiform antiserum had a marginal titer and cross-reaction pattern. All the confirmed feedings on owls were at Site 7 and the specimens were collected near nesting owls. Most of the feedings on mammals were on cattle. Except for an apparent increase in mammal-feeding from 4 percent during April 18-22 to 12 percent during May, there was no obvious pattern of host selection. The absence of columbiform feeding during May after a level of 25 percent in late April, is striking, and suggests casualties or migratory wave of hosts at Site 7. Fourteen percent of the specimens did not react with any antisera, primarily because they contained insufficient blood.

The liquid which many mosquitoes contained was not identified, but presumably was either water or a fluid of plant origin. Its consistency varied from that of water to almost that of corn syrup, and in all cases residue was left after it was dried

TABLE 4.—Results of virus isolation attempts* from *Culex tarsalis*.

Period	Number Mosquitoes	Results
April 13-28	482	Neg
May 7-22	145	Neg
June	177	Neg
July	691	Neg
August 3-8	1070	4 WE†
Remainder of August	199	Neg
Total	2764	4 WE†

* Intraperitoneal inoculation of 4-day-old mice, blind passage of brain.

† All isolations were on initial inoculation. WE=western encephalitis.

on glass. The fluid was present in both parous and nulliparous specimens.

During the later parts of the study there was much activity of *C. tarsalis* at dusk at Site 7, both near avian hosts and among blooming shrubs. Because of light conditions, it was never possible to determine whether mosquitoes actually fed on the plants.

Virus was not isolated from mosquitoes collected in the spring, but four isolations of WE virus (all on initial inoculation) were made from 1,070 *C. tarsalis* collected between Aug. 3 and Aug. 8. There were no isolations of other viruses. Results of isolation tests are shown in Table 4.

One of 15 sentinel chickens developed hemagglutination inhibiting antibody to WE virus and none to SLE virus. The WE conversion appeared in blood collected July 9.

DISCUSSION. Site 7 may typify a habitat that *C. tarsalis* will occupy after emergence from hibernation. Although man-made, it is a counterpart of small groves found naturally along streams in dry, un-forested regions of the Northwest. We have previously found that large populations of *C. tarsalis* in such environments in summer are consistent sources of infected mosquitoes. These observations and the infrequency with which *C. tarsalis* can be collected away from trees in Oregon (present study) suggest isolated

groves as loci of mosquito-virus activity. The overwintering generation of mosquitoes goes to such places soon after it becomes active in the spring while the population is changing from a non-feeding to feeding one. They are later joined by progeny of their own generation. The first new brood was observed at Site 7 three weeks after the overwintering population was discovered, but the proportion of mosquitoes of the new generation was never great during the May observation period. In other circumstances, the overlap of generations may be greater, since we removed parental specimens repeatedly and this may have reduced the opportunity for maximum production of a new generation at this location.

There was an opportunity for virus transmission to be established at Site 7 in April and May. Blood feeding by the overwintering generation had reached a high level at the time Site 7 was found; and two major host groups, passeriform and columbiform birds, that are known to circulate virus (Hammon, Reeves, and Sather, 1951; Kaplan, Winn, and Palmer, 1955; Kissling *et al.*, 1957) were present and fed on by the vector. The failure to isolate virus in the spring suggests that virus was not readily accessible to *C. tarsalis* in the area during the observation period.

For cyclic virus maintenance by *C. tarsalis* and birds, multiple feedings by the vector are necessary. The parity studies show that repeat feedings were occurring at Site 7. The flat, parous specimens were collected near hosts, presumably ready to feed again, while the engorged, parous specimens had fed at least twice. It is clear that in northern regions many *C. tarsalis* feed more than once during the spring after emergence from hibernation and potentially they could acquire and transmit virus before the spring generation of mosquitoes appears. A sequential link is provided when the next generation of *C. tarsalis* enters into the feeding complex. Conditions at Site

7, even during April and May, seemed suitable for cyclic maintenance of virus; biologic studies must begin at this time or earlier to relate virus findings to environmental events.

Our failure to isolate virus from *C. tarsalis* during April or May in northern areas may reflect infrequency of infection in the hosts that *C. tarsalis* attacks at this time rather than absolute absence of virus from the biota. It is also conceivable that the number of hosts with viremia varies greatly from one spring to the next. A continuation of this type of study may answer these questions.

SUMMARY. An overwintered population of female *Culex tarsalis* was studied during April and May in eastern Oregon before the new generation of adults appeared. Collections from resting sites consistently included freshly blood-engorged mosquitoes. As the spring progressed, a decreasing proportion of the population contained visible fat, and an increased proportion contained fluid in their alimentary tract. Blood feeding was at a high level. Parity increased throughout the spring and by mid-May reached a level of 79 percent in the non-engorged population. The finding of some parous, engorged specimens, indicated multiple feeding. A new brood of adult mosquitoes was present at a low level by late May. Precipitin tests on mosquito blood meals indicated that most of the mosquitoes had fed on birds. There was some feeding on mammals and none on other vertebrates. Virus was not isolated from *C. tarsalis* collected in the spring, but four isolations of WE virus were made from *C. tarsalis* collected in early August. The presence of *C. tarsalis* in relatively large numbers in the spring and their frequency of attack on avian hosts for WE virus offer potential for virus transmission as early as mid-April in eastern Oregon.

ACKNOWLEDGMENTS. The authors are grateful to the Oregon State Board of Health and the Malheur County Health Department for their cooperation; to Dr.

Leo A. Thomas of the Rocky Mountain Laboratory for the serologic tests on arboviruses; to Miss Mary Lofy of the University of California for professional assistance; and to Dr. Carl M. Eklund of the Rocky Mountain Laboratory and Dr. William C. Reeves of the University of California for consultation and review of the manuscript.

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REQUEST FOR ASSISTANCE

Information is being requested on mosquito rearing techniques to be included in the new AMCA Bulletin 3, Manual for mosquito rearing and experimental techniques. Reprints on rearing or experimental techniques or personal communications on this subject will be appreciated. Appropriate acknowledgment will be made for information included in the manual. Data on the rearing of exotic species, or reprints of articles in not readily available journals are particularly desired.

Please send all information to: Dr. Eugene J. Gerberg, Insect Control & Research, Inc., 1111 N. Rolling Road, Baltimore, Maryland 21228