POST-HIBERNATING *CULEX TARSALIS* AND *CULISETA INORNATA*: OVIPARITY AND TESTS FOR VIRUS[1]

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ABSTRACT. Mosquito collections made under bridges in Weld County, Colorado, from February to mid-May 1961 indicated that *Culex tarsalis* overwintered as nulliparous females which did not oviposit until April, and that *Culiseta inornata*, first found as gravid and parous females, did not produce nulliparous females until May. None of the *C. tarsalis* but all of the *C. inornata* appeared to have fed on blood before hibernating. No encephalitis virus was recovered from any of the mosquitoes assayed.

The tracheole method of determining oviparity, and hence presumptive feeding on blood, was applied to late summer and fall populations of *Culex tarsalis* Coquillett in and near Weld County, Colorado, in 1960 (Blackmore and Dow 1962). This work was continued in the late winter and spring of 1961 with the addition of tests for arbovirus and the inclusion of *Culiseta inornata* (Williston) as a second subject. The main purpose was to determine, in an area where the mean January temperature was less than 30° F, whether any of the females that survived hibernation were likely to have fed on blood in the previous fall.

PROCEDURES. All the mosquitoes of this study were collected from the under surfaces of bridges in southwestern Weld County, Colorado, in the late winter and spring of 1961. Bridges were inspected near Greeley on warm days in January, but no adult mosquito was seen until February 11 when, during a warm spell, the maximum temperature at Greeley reached 67° F. In the next few weeks the inspection of various bridges was continued, mostly on warm days, until 6 bridges were finally selected for regular inspection. One was the single remaining span of an abandoned concrete bridge, 2 miles north of Johnstown in the drainage of the Big Thompson River. The 5 other bridges (2 of concrete and 3 of wood) were along the course of the St. Vrain River between, a point 5 miles east of Longmont and a point 4 miles WNW of Platteville. Inspection of the less productive wooden bridges was discontinued in mid-April, but collections under the 3 concrete bridges were made for another month.

The live female mosquitoes which externally did not appear to contain host blood were routinely etherized, placed on a sterile slide, identified, and dissected with sterile instruments. The head and thorax were placed in a screw-cap vial, frozen by means of dry ice, and kept chilled for later assay. Except when the abdomen contained a batch of well-developed eggs, the ovaries were removed to a drop of water on a fresh slide. After the slide had dried, the ovarian tracheoles were examined for the unwound condition indicative of parity (Detinova 1962; Kardos and Bellamy 1961). Dead females without host blood were examined similarly but not frozen.

Engorged specimens and females with any external sign of a blood meal were not dissected. Until the blood appeared to have been digested, they were kept outdoors, without food, in netting-topped cartons covered with wet cloths. Next, they were held for at least a week in a warm insectary (72–80° F) where they

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were fed with sugar syrup. Only the survivors were submitted for virus tests.

The pools assayed for virus consisted of 1 to 55 specimens, always grouped by species and by gonotrophic state (blood-fed or as determined at dissection). After trituration in a mortar with 3 ml of Hanks' balanced salt solution containing 20% normal rabbit serum and antibiotics, each pool was centrifuged and the supernatant fluid was inoculated intracerebrally into 2-week-old mice, each receiving 0.03 ml of inoculum. The supernatant of each pool, with the exception of 3 pools that each consisted of a single specimen, was also inoculated into embryonated eggs, with each yolk sac receiving 0.1 ml. When mortality from any cause occurred in mice or eggs, subpassages were made of mouse brain or chick embryo tissue. The supernatant of pools requiring such testing was further assayed for virus in hamster kidney tissue culture.

RESULTS. Of the 363 *Culex tarsalis* collected under bridges, 111 contained host blood, and the gonotrophic state was determined in all but one of the remaining 252. Every female collected through March 25 was nulliparous (Table 1). The first 2 blood-fed specimens were taken on March 26 and 31, the first gravid specimen on April 18, and the first parous specimen on April 26. The number of nulliparous females increased up to the last week of April. The mode of engorged females occurred the same week, and the mode of gravid females the week following. No males of *C. tarsalis* were taken throughout the study. From the sequence in which the 4 gonotrophic states were first obtained, it appears that, in Weld County, *C. tarsalis* normally overwinters as a nulliparous female, then takes blood, develops eggs, and oviposits.

Of the 274 female *Culiseta inornata* collected under bridges, 56 were found to contain host blood and all but 4 of the rest were examined successfully. The first specimens taken (March 22 to April 5) consisted of 9 gravid and 8 parous females. All but one of the gravid females had well-developed eggs. The first blood-fed females were not taken until April 12, and by then might have been parous post-hibernators with a second (or later) blood meal. The first nulliparous female was not obtained until May 5. The only male was found on May 9. Since larvae of *C. inornata* had been collected near the St. Vrain River on April 19 and 27 in the same area as the concrete bridges (personal communication from Fred W. Harmston), it appears that the male and the 9 nullipars taken in May represent a spring generation. From the sequence in which the gonotrophic states were found, it appears unlikely that females of *C. inornata* overwinter in Weld County without having taken a blood meal.

As would be expected from other studies, especially that of Rush et al. (1963a), no virus was recovered from any of the 480 mosquitoes tested. Of most interest were the early nulliparous females of *C. tarsalis,* 29 taken in March and 100 in April. There were also 24 gravid and 2 parous females collected in April, and 10 nulliparous, 41 gravid, and 13 parous females collected in May. (More May specimens were assayed as parous than are reported as such in Table 1 because at least one of 5 females that laid eggs after capture survived until time of dissection and was included with the specimens parous at collection.) *C. inornata* was represented in the pools by 8 gravid and 7 parous females caught in March, 7 gravid and 16 parous females caught in April, and 8 nulliparous, 148 gravid, and 11 parous specimens caught in May. The negative blood-fed females consisted of 45 *C. tarsalis* (1 taken in March, 40 in April, 4 in May) and 11 *C. inornata* (3 taken in April and 8 in May).

Incidental to holding the blood-fed specimens until the blood was digested, observations were made on the time required by 35 females of *C. tarsalis,* caught April 26 and 27, to digest whatever amount of host blood they contained when collected. At least one female finished digesting the blood on each of the first 12 days after

Table 1. Female mosquitoes, by gonotrophic state, collected under concrete and wooden bridges, Weld County, Colorado, 1961. Each mosquito is classified as nulliparous, blood-fed, gravid, or parous.

Dates[1]	Mean air temp.[2]	Mean soil temps.[3]	*Culex tarsalis*						*Culiseta inornata*				
			Under concrete bridges[4]				Under wooden bridges[5]		Under concrete bridges[4]				Under wooden bridges[5]
			Null.	Bl.-fed	Grav.	Par.	Null.	Bl.-fed	Null.	Bl.-fed	Grav.	Par.	Par.
Feb. 11, (12)	34	33/41	1										
Mar. 15, 16	43	36/41	2										
Mar. 22, (23)	39	40/41									1		
Mar. 24, 25	41	40/42	9								2	4	
Mar. 26, 27	44	41/42	13				4	1			4	1	1
Mar. 30, 31	37	42/42	1	1							1	1	
Apr. 1, 5	41	42/43	10				8				1	1	
Apr. 12, 13	38	43/44	9	8			4	3		3		3	
Apr. 18, 19	46	44/44	35	14	4		..	..					..
Apr. 26, 27	48	48/45	40	42	24	3	..	..		9	6	14	..
May 5, 6	50	51/47	5	23	34	6	..	..	4	11	116	5	..
May 15, 16	58	52/48	5	19	28	6	..	..	5	33	38	6	..
Total females			130	107	90	15	16	4	9	56	169	35	1

[1] No mosquitoes were caught on the inspection dates enclosed in parentheses.
[2] Mean (of max. and min.) air temperature (° F) at Greeley in week preceding second date.
[3] Mean 2-ft/mean 6-ft soil temperature (° F at 7 a.m.) at Fort Collins in week preceding second date.
[4] Two concrete bridges inspected each date through Mar. 16; 3 thereafter.
[5] Except 1 omitted and 2 incomplete inspections, 3 wooden bridges inspected each date through Apr. 13.

capture, 2 finished on May 13, and 1 on day 14. For each of the last 3 females the mean of the daily maximum and minimum temperatures on the screened porch where the mosquitoes were kept was 51° F. Rush et al. (1963b) made similar observations on colony females that were blood-fed in the laboratory and kept outdoors at a mean temperature of 50° F (thermograph records read at 2-hour intervals). They found the mean time from feeding to digestion was 10.4±2.4 days (these figures are based on the assumption that a total of 100 mosquitoes was observed). This mean+one standard deviation (12.8 days) is reasonably close to our maximum of 14 days, and suggests that the Colorado specimens, at nearly the same temperature, digested blood at a similar rate. In 10 blood-fed *C. inornata,* again with whatever amount of blood they contained when collected, digestion took a maximum of 11 days in one female held at an estimated mean temperature of 51° F, and 10 days in 2 females held at a mean of 50° F. These lower maxima in *C. inornata* are in accord with its greater abundance in the cooler weather of spring and fall (Harmston and Lawson 1967).

DISCUSSION. In areas where the mean January temperature is less than 30° F, *C. tarsalis* has been found in various types of shelter in the late fall and winter, but the potential hibernators disappear from some retreats before the outside environment seems propitious for survival (Keener 1952; Blackmore and Winn 1956; Dow et al. 1956). Rush et al. (1958) describe natural and man-made rock piles as hibernation sites of *C. tarsalis* in eastern Washington, and establish them as favorable for all-winter shelter by their finding that the population was relatively constant until the end of March when females began to be active outside. Bennington et al. (1958) were the first to report the spring recovery of 2 hibernating *C. tarsalis* from wild animal burrows, but later Shemanchuk (1965) set traps over mammal burrows in southern Alberta in December, and, by recovering *C. tarsalis* in April, May, and June, and *C. inornata* in May and June, proved that both mosquitoes can survive in such shelter all winter. Successful hibernation appears to depend, first, on an adequate reserve of energy, and second, on finding a hibernaculum which remains cool enough so that this energy will not be expended in futile activity before survival is possible outside. If the observations made in a mine tunnel at Farmington, Utah, were correctly interpreted (Dow et al., 1956), hibernating *C. tarsalis* tend to aggregate where the mean air temperature is less than 50° F. In seeking such a temperature (perhaps by avoiding higher ones), *C. tarsalis* might be forced out of many tunnels and burrows prematurely.

Bennington et al. (1958) showed that, in Weld County, there was a general emergence of *C. tarsalis* from hibernation at the time of a spring inversion of soil temperatures. In 4 successive years (1953–56) they observed the first post-hibernators not more than 4 days before, or one day after, the day when the soil temperature at a depth of 2 or 3 feet (not specified) first exceeded that at 6 ft. In the present study, there was a small but general emergence of *C. tarsalis* on March 25, 2 days before the first soil inversion occurred at Fort Collins. This was a temporary inversion, and the 7 a.m. temperature at 2 ft (43° F) was only 1° F higher than that at 6 ft (U. S. Weather Bureau 1961–62). In fact, the soil near the surface continued to warm very gradually (see soil temperatures in Table 1), and the third (and final) inversion did not occur until April 18. The first three *C. tarsalis,* all caught at least 10 days before the first inversion, were taken on 3 different days during warm spells when the air temperature at Greeley rose above 60° F. These early emergents are believed to have been hibernating in shallow burrows or in other shelter which was penetrated by the warm air. They may be considered representatives of those *C. tarsalis* that did not find a sufficiently deep and cool hibernaculum.

The collection of 2 parous *C. tarsalis*

near Jamestown, Colorado, as late as December 2 (Blackmore and Dow 1962) and the recovery of western encephalitis (WE) virus from *C. tarsalis* taken in the same locality on December 30 (Blackmore and Winn 1956) are evidence that blood-fed individuals may survive at least until the season for hibernation. Though Rush (Rush and Tempelis 1967) found 3 parous *C. tarsalis* in the first spring collections that he made in eastern Oregon in 1965 (April 13–17), he also took 21 blood-fed and 29 gravid specimens during the same period, and it therefore seems likely that his parous individuals had not hibernated in that condition. In Weld County, in the very gradual spring of 1961, no parous *C. tarsalis* were collected until 39 days after the first blood-fed specimen and 8 days after the first gravid specimen. Therefore, with no proof that any females that have fed on blood survive hibernation, also with no evidence from the field that *C. tarsalis* metabolizes blood without subsequently developing eggs, *C. tarsalis* remains an unlikely hibernating reservoir of any arbovirus.

Though the first blood-fed *C. tarsalis* of this study was caught on March 26, June is the earliest month in which this mosquito has been reported positive for WE virus in Weld County (Cockburn et al. 1957). Rush et al. (1963b) have shown for *C. tarsalis* that a mean air temperature of 50° F favors neither blood digestion nor extrinsic incubation of WE virus. At Greeley in 1961, the first 5-day period with a max.-min. mean of 50° F or more was April 16–20, and a max.-min. mean of 60° F or more did not occur until May 9–13. Various factors are involved in the spring amplification of virus (Hess et al. 1963; Hess and Hayes 1967), but whenever and however *C. tarsalis* is first infected, temperature is likely to control the rate and perhaps the start of virus multiplication in the mosquito.

In its potential as a hibernating vector, *C. inornata* is quite different from *C. tarsalis*. Though *C. inornata* larvae have been found under ice as late as January in eastern Oregon (Rush 1962), the observations of Thompson (1953) in central Nebraska, and the absence of nulliparous females from our collections until May indicate that only adults survive the winter. Moreover, the fact that Thompson (1953) found fresh (incompletely pigmented) egg rafts as early as March 31 suggests that all of our early parous specimens had overwintered as gravid females and oviposited after emerging from hibernation. Since autogeny has been reported in *C. inornata* reared from wild material (Washino and Shad-Del 1969), the presence of eggs or stretched tracheoles does not prove that a female has fed on blood. However, in our material, except as autogenous egg development might have given a false indication of blood feeding, it appears that all the females caught before April 12 had fed on blood before hibernating. It is concluded that *C. inornata* is a potential winter reservoir of any arbovirus that circulates in its fall hosts.

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DEDICATION OF THE H. H. SCHWARDT LABORATORY AT CORNELL UNIVERSITY, ITHACA, N. Y.

The H. H. Schwardt Laboratory, dealing with the insects of man and animals, will be dedicated on May 10, 1976 in honor of Dr. H. H. Schwardt at a day-long program centered around the theme of Medical-Veterinary Entomology: Perspectives and Prologues to the 21st Century. Tours of the facilities will be followed by speeches by Drs. W. R. Horsfall, W. F. Scherer, E. F. Knipling, Dean Palm and Dr. L. D. Newsom. All are invited.

The late Dr. Schwardt formerly served as head of the Department and was a favorite of students and growers.