OVERWINTERING SITES AND OVARIAN DEVELOPMENT OF SOME MOSQUITOES IN CENTRAL ALBERTA, CANADA

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ABSTRACT. Searches in winter near George Lake (53°57'N, 114°06' W) revealed 108 females of *Anopheles earlei* Vargas in man-made rockpiles, 210 were collected from root cellars, and 127 were trapped emerging from badger burrows in spring. Rockpiles also yielded 102 female *Culex territans* Walker, one *Culiseta alaskaensis* (Ludlow) and one *Culiseta silvestris minnesotae* Barr. Two *Cx. territans* females were found in a logpile and one was trapped emerging from a burrow. All 271 *An. earlei* and 87 *Cx. territans* from overwintering sites were without any trace of blood meals, and all were inseminated and nulliparous, except for one uninseminated and one parous *An. earlei* from the burrows. Follicle development of overwintering females suggested that they were still in diapause, except for some *An. earlei* from the root cellars and burrows.

INTRODUCTION

The mode of overwintering of western equine encephalitis (WEE) virus remains unknown, in spite of many investigations on the subject. Reeves (1974) considered that the most likely method was by latent infections in hibernating vertebrates, but female mosquitoes that overwinter could also be involved if they fed on such vertebrates in fall or spring. The feeding would be most likely where mosquitoes and vertebrates shared hibernacula.

The transmission and persistence of WEE is particularly puzzling in central Alberta, where the primary vector, *Culex tarsalis*, is rare, and winter lasts for 5–6 months of the year. Cases of WEE have been confirmed in horses in Alberta as far as 56°N (Morgante et al. 1968), in the boreal forest zone. WEE virus has been isolated from *Aedes vexans* (Meigen), *Cx. tarsalis* and *Culiseta inornata* females in the irrigated prairie region at about 50°N (Shemanchuk and Morgante 1968), but the vectors in the boreal forest zone are not known. In the only previous study of mosquito overwintering in Alberta, Shemanchuk (1965) trapped *Anopheles earlei*, *Cx. tarsalis* and *Cs. inornata* females entering mammal burrows in fall and leaving them in spring, both in the prairies and in the aspen parkland zone up to 53°N. WEE virus isolations were made from mosquitoes in some of these burrows up to August 19 (*Cs. inornata*) and August 25 (*Cx. tarsalis*), but not in September (Schemanchuk and Morgante 1968). Since mosquitoes continued to enter the burrows until November it is not certain that the infected females were the ones that would have overwintered, and no virus isolation attempts were reported from mosquitoes in winter or spring.

The objectives of the present study were to find overwintering sites of *Anopheles*, *Culex* and *Culiseta* females in central Alberta, and to examine collected mosquitoes for any signs of blood feeding, ovarian development or associations with overwintering vertebrates, that might indicate that the mosquitoes could play a role in the overwintering of WEE.

MATERIALS AND METHODS

Most searches were made in three winters, 1972–75, on a farm and in an aspen-spruce wood near George Lake, Alberta (53°57'N, 114°06'W), which lies in the boreal forest (*taiga*) zone. Mosquito dispersal is unlikely during winter (November-March), when normal mean temperatures at Sion, 3.6 km south of George Lake, are below 0°C (Environment Canada 1973). The normal snowfall is 152 cm each winter (Environment

Canada 1973), and there is usually standing snow from November to April. Mosquitoes described as "overwintering" were collected in these months, and most of the searching was done after January, the coldest month, to lessen the chances of recording mosquitoes from sites where they could not have spent the whole winter. Some collections in fall (September-October) and spring (April-May) suggested other possible overwintering sites.

Searches in the winter of 1972–73 covered the greatest variety of sites. In the winters of 1973–74 and 1974–75 most searching was at sites that had revealed mosquitoes in the first winter, (rockpiles, burrows and root cellars), so results are biased in their favor. Mosquito collections in the study area in summer and fall, 1972–75 (Hudson 1977), included 7 species that overwinter as adults. *An. earlei* Vargas, *Cx. territans* Walker, *Cs. alaskaensis* (Ludlow) and *Cs. inornata* (Williston) adults were common, but *Cx. tarsalis* Coquillett, *Cs. impatiens* (Walker) and *Culiseta silvestris minnesotae* Barr[1] were rarely seen.

Rockpiles created by farmers clearing their fields are common in the George Lake area, and will become more so as new ploughings uncover more rocks. The smallest rocks were the size of an adult's fist and the largest more than 50 cm across. Since the piles sloped gently most had complete snow cover in winter. The rockpiles were searched by removing the snow and turning the stones one by one. Mosquitoes were aspirated into vials and stored on snow until brought to the laboratory. Air and rockpile temperatures were measured with a telethermometer (Yellow Springs Instruments), the probe attached to a piece of stiff wire and pushed in between the rocks.

Root cellars are cool, dark buildings, often sunk into the ground, used for storing potatoes and other vegetables through the winter. Fifteen cellars were examined, ranging in size and complexity from an excavation in a hillside 3 m wide, roofed with boards and earth, to metal hangers more than 50 m long, with machinery to warm, humidify and circulate the air.

Burrows of badger (*Taxidea taxus*) were examined in roadside banks at 3 sites near George Lake, and in a bank at the edge of field near St. Albert, north of Edmonton. Most of the burrows were 15–25 cm in diameter and at least 2 m deep, with nearly horizontal entrances in banks of well-drained, sandy soil. Nearly all the mosquitoes were collected emerging from the burrows in spring, in traps based on the design of Harwood and Halfhill (1960). The traps were made from cans 10 cm in diameter and 15 cm long, from which the bottoms were removed and replaced with a cone of 1 mm mesh black plastic screen pointing inwards with a 1.5 cm hole at the apex. A 2.5 cm hole was cut in the push-in lid and covered with 2 mm mesh metal screen. The can was fixed at its base in a hole in a 20 cm square of plywood, with a skirt of black polyethylene attached to its edges to aid in sealing the trap in the burrow mouth with earth. Entrance cones of the first traps had holes chewed in them probably by small rodents. Further damage was prevented by a protective screen of 6 mm mesh galvanized wire. In fall 1972 and 1973, the traps were pointed inwards to catch mosquitoes entering, but none were caught. In spring 1973, 1974 and 1975 the traps were positioned to catch mosquitoes emerging. Once every 7–10 days the lids were carefully removed and mosquitoes collected by aspirator: 10–20% escaped.

Mosquitoes were held alive at 2°C and dissected a few days after capture. Abdomens were opened in 0.7% NaCl under a stereo microscope at x25. Fatbody development was rated as follows: 0 = no visible fatbody, abdomen flat; 1 = trace of fatbody; 2 = abdomen filled; 3 = abdomen distended with fatbody, (Burdick and Kardos 1963). A few follicles

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[1] The validity of *silvestris* is questionable, but *Mosquito News* follows the Knight and Stone 1977 Catalog (Thomas Say Foundation VI).

were teased out of one ovary, examined at x150, assigned a stage of Christophers (Detinova 1962), and the lengths of a typical follicle and its germarium were measured using an eyepiece micrometer. Spermathecae were crushed and examined under a compound microscope at x200 for sperm. Ovaries were dried on microscope slides and the surface tracheation later examined to see if the mosquito was parous (Detinova 1962).

RESULTS

Four hundred and twenty-five females of 4 species were collected from rockpiles, under logs, and root cellars in winter, and another 128 trapped emerging from badger burrows in spring (Table 1).

ROCKPILES. Twelve searches in 7 rockpiles, costing 116.5 man-hours, revealed 212 mosquitoes, or 1.82 per man-hour. *An. earlei* females were found in 10 of 12 searches, at a maximum rate of 2.25 per man-hour, and *Cx. territans* in 9 of 12 searches, at a maximum rate of 4.0 per man-hour. Vertical distribution of mosquitoes in three of the piles is shown in Fig. 1. Females of both species were distributed from under the first stone (5–10 cm deep) almost to the bottom of the piles, but the *Cx. territans* (mean depth 25 cm) were closer to the surface than the *An. earlei* (mean depth 32 cm). The mean temperature between the rocks (−4°C) was close to the mean temperature in the open air (−3°C), but air temperatures will have been above average because searches were made during warm spells. All but four of the mosquitoes were taken in February and March, so they could probably have survived the whole winter in the rockpiles.

LOGS. Two *Cx. territans* females were found in a narrow space between branches of a fallen aspen tree, but only on the 19 November 1972, thus it was not confirmed that they could have survived the whole winter at this site. Many other fallen trees and logs were searched in winter, but no mosquitoes were found.

ROOT CELLARS. An estimated 415 *An. earlei* females were seen in four root cellars, and 210 of them were collected. Others were left for later visits, to study ovarian development through the winter. One of the cellars was visited in 4 successive winters, and 183 of the mosquitoes were collected from it. This cellar, 20 km south of Edmonton, was 9 m long by 7 m wide, with walls 2 m high sunk 1.75 m into the ground, and a gabled roof raised a further 1.5 m. Two loading hatches at ground level on the west wall remained open during September, providing burrow-like entrances for mosquitoes. The cellar was estimated to contain 330 *An. earlei* females on the 17 September 1974, but only 19 were found on the 14 March 1975, after 165 had been collected

Table 1. Female mosquitoes collected from overwintering sites in central Alberta during winter and spring, 1972–72.

	Anopheles earlei	*Culex territans*	*Culiseta alaskaensis*	*Culiseta silvestris minnesotae*	Total	%
November-March						
Rockpiles	108	102	1	1	212	38.3
Under logs	0	2[b]	0	0	2	0.4
Root cellars	210	1[c]	0	0	211	38.2
March-May						
Badger Burrows[a]	127	1	0	0	128	23.1
Total	445	106	1	1	553	100.0
%	80.5	19.2	0.2	0.2	100.1	

[a] Mosquitoes caught in exit traps.
[b] On 19 November, 1972, winter survival unconfirmed.
[c] On 31 March, 1973, late in snowmelt. May have overwintered elsewhere.

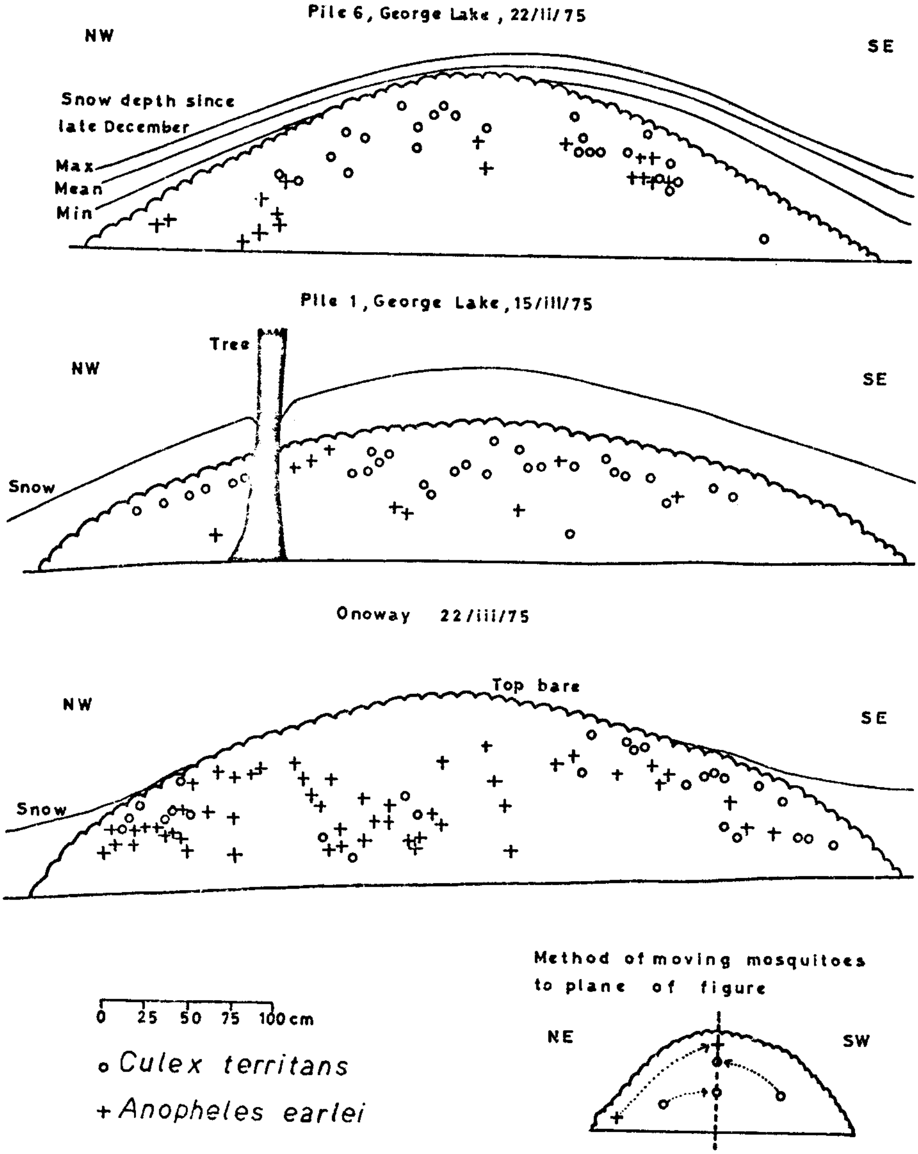

Figure 1. Distribution of female mosquitoes in three rockpiles near George Lake, Alberta. The figure indicates the true depth of the mosquitoes below the surface of the pile, but exaggerates the height above the soil of those that were not in the plane of figure.

at intervals through the winter, making a natural mortality of 88.5%. Most mosquitoes taken after December had little fatbody and the rather high temperatures (mean 6.4°C) may have caused many to die of starvation. This cellar also had many spiders, mostly Linyphiidae. Mosquito remains were seen in webs, and on the 24 February 1976 a Linyphiid was seen feeding on a freshly-killed *An. earlei* female.

Burrows. Numbers of *An. earlei* females trapped emerging from burrows varied considerably from burrow to burrow and year to year. Thirty-five were trapped from 5 burrows in 1973 (7.0 per burrow), 11 from 21 burrows in 1974 (0.5 per burrow) and 81 from 27 burrows in 1975 (3.0 per burrow). Six of the burrows used in 1974 had had entry traps over them in the fall of 1973, but if these are excluded from the calculation the mean number for 1974 was still only 0.6 per burrow. No mosquitoes were taken from 49% of the burrows. 20.8% gave only 1–2 mosquitoes per season, 24.6% gave 3–10, and only 5.7% gave more than 10 per season. At George Lake in 1975, the burrows facing north, which were covered with snow about 2 weeks longer than the others, never yielded any mosquitoes, and those facing south yielded the largest mean number per burrow (Table 2). Burrows with signs of recent mammal activity (paw marks, fresh digging, or trap displacement) more often had mosquitoes than did disused burrows (Table 3). The association between mosquitoes and recent mammal activity was not statistically significant, but escapes from burrows with mammal activity probably were more frequent because traps were displaced.

Some traps were set when the mouths of the burrows were still under snow, and most were set before any *An. earlei* females were seen on the wing. Later in the spring, however, some burrow mouths were left open when traps were displaced by mammals or removed and damaged by vandals. Although some of the mosquitoes trapped from these burrows could have overwintered elsewhere and used them only as a spring resting place, most were taken before the first disturbance of the traps, (Table 4). The single *Cx. territans* was taken quite late in spring, on 15 May 1975, but it was from a trap that had never been disturbed. A gravid *An. earlei* was taken on the 23 May 1975, from a trap that had been displaced some time between the 7 and 15 May 1975, leaving the burrow mouth open. *An. earlei* females had been seen on cattle on the 7 May 1975, so it seems most likely that this gravid female had taken a blood meal earlier the same spring, and used the burrow as a temporary resting site. It is not included in the total of overwintering *An. earlei.*

The emergence of *An. earlei* lasted from snow melt until 3 (1974) to 7 (1973) weeks later, (Fig. 2). The interval between the middle of the snowmelt period and 50% emergence was 21 days in 1973, 5 days in 1974 and 15 days in 1975. In 1973 and 1974, trapping ceased 1 week after the 1st occasion that no mosquitoes were taken in the traps, but in 1975, trapping was continued from June through August at two sites using 18 burrows and 9 exit traps alternating between visits. This was important because Shemanchuk (1965) found that most of his *Cs. inornata* did not emerge from the burrows until June,

Table 2. *An earlei* females trapped emerging from burrows with different aspects, George Lake, Alberta, April-May 1975.

	Aspect				
	North	South	East	West	Total
No. of burrows	4	4	6	8	22
No. with *An. earlei*	0	4	4	6	14
Total *An. earlei*	0	25	17	36	78
Mean *An. earlei*/burrow	0	6.25	2.83	4.50	3.54

Table 3. *An. earlei* females collected from burrows with and without signs of mammal activity, George Lake, Alberta, April-May 1975.

	With mammal activity	Without mammal activity	Total
Numbers of burrows[1]			
with *An. earlei*	6	8	14
without *An. earlei*	2	6	8
Total	8	14	22
Numbers of *An. earlei*			
Total	33	45	78
Numbers per burrow	4.1	3.2	3.5

[1] For upper part of table, $Chi^2 = 0.81$, no significant association between *An. earlei* and mammals.

though his *An. earlei* emerged in April and May. No mosquitoes were taken in the traps in June, but a few unfed, gravid and male *An. earlei* were taken in July and August. The traps did not catch any mosquitoes entering burrows in the fall of 1972 or 1973. A variety of other insects, spiders, and even a few frogs and toads, were caught in the traps. There were many flying insects in spring but few in fall, which suggests the traps deterred insects such as mosquitoes that would have flown into the burrows.

FALL AND SPRING COLLECTIONS. Many other sites (listed by Hudson 1977) were searched fruitlessly in winter, but a few mosquitoes were found during fall and spring in sites where they could conceivably have overwintered. In the fall *An. earlei* females were found in animal sheds and under logs, *Cs. inornata* females in a sedge meadow and in the mouth of a badger burrow. The earliest *Cs. inornata* female collected in spring was under a log on the 9 May 1973. Many *An. earlei* females were found in a beaver lodge on the 9 and 17 May 1976, but this was more than a month after snow melt and *An. earlei* females flew earlier than this in other years. Two other lodges opened in February 1977 had no mosquitoes.

DISSECTIONS. None of the mosquitoes collected had blood in the gut. The 271 *An. earlei* dissected came in about equal numbers from rockpiles, root cellars and burrows, and all but 2 of the 97 *Cx. territans* came from rockpiles (Table 5). All females of both species were inseminated and nulliparous, except for one uninseminated and one parous *An. earlei* from the burrows. The parous *An. earlei* was collected on the 2 May 1974 and would have to have been in the burrow on the 24 April, when the trap was set.

There were differences in fatbody and follicle development between sites,

Table 4. Numbers of unfed *An. earlei* females in disturbed and undisturbed traps over badger burrows, George Lake, Alberta, April and May, 1975.

	Numbers of *An. earlei*			
	in disturbed traps	in undisturbed traps	Total	%
Before first disturbance	34	34[b]	68	87
After first disturbance	10[a]	—	10	13
Total	44	34	78	100

[a] Plus 1 gravid *An. earlei*.

[b] Plus 1 unfed *Culex territans*.

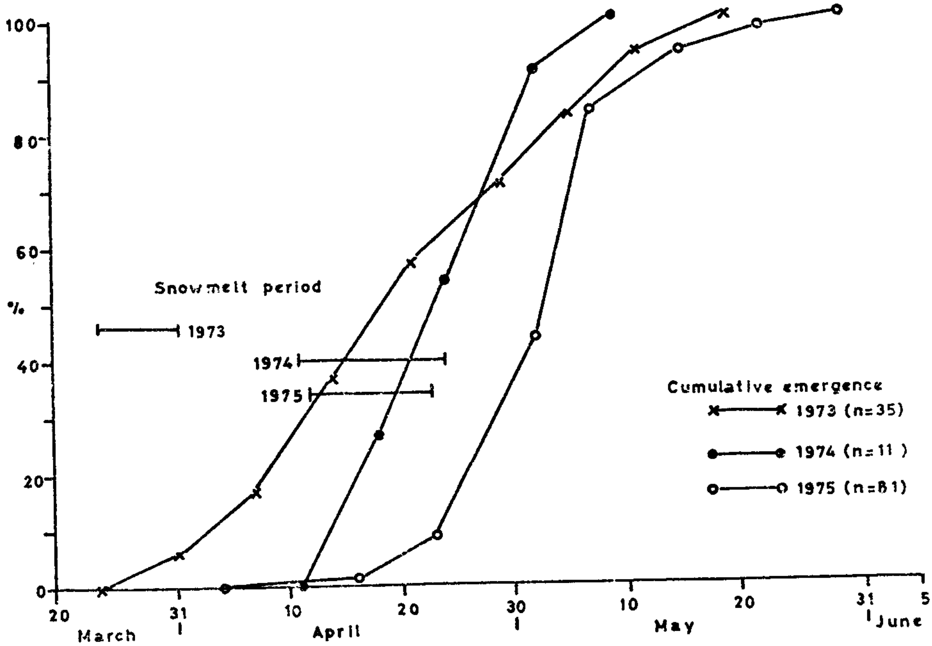

Figure 2. Cumulative emergence of *Anopheles earlei* females from badger burrows near George Lake, Alberta, in relation to time of snowmelt, 1973–75.

Table 5. Follicle and fatbody development of *Anopheles earlei* and *Culex territans* females from rockpiles (January-March), root cellars (November-March) and badger burrows (April-May). All females were inseminated and nulliparous unless otherwise indicated.

Species and Site	Number dissected	Follicle Stage		Fatbody rating[a]				Mean F:G ratio	Numbered measured
		I	II	0	1	2	3		
Anopheles earlei									
Rockpiles	88	77	11	0	10	65	3	2.04	67
Root cellars	90	83	7	0	26	34	30	2.13	64
Burrows	93[b]	47	46[c]	3	43	41	6	2.31	88
Total	271	207	64	3	79	154	39	2.16[d]	19
Culex territans									
Rockpiles	85	85	0	0	0	61	24	1.55	53
Root cellar	1	1	0	0	0	1	0	—	0
Burrow	1	0	1	0	0	1	0	2.5	1
Total	87	86	1	0	0	63	24	2.02[d]	54

[a] Defined in materials and methods section.
[b] One uninseminated and one parous.
[c] One Stage III follicle.
[d] Mean of means for sites.

possibly because the mosquitoes were collected from them during different time periods, (January-March from rockpiles, November-March from root cellars, and April-May from burrows). Most females of both species in the rockpiles and root cellars had fatbody ratings of 2 or 3 (abdomens filled or distended with fatbody), follicles in stage I, and the F:G (follicle:germarium) ratios were less than 2.0 in most *An. earlei* and less than 1.5 in most *Cx. territans*. In these features they resembled diapausing females in the fall. About half the *An. earlei* females from burrows in spring had follicles in stage II, half had no visible fatbody or only a trace of it, and the mean F:G ratio, 2.31, was of a magnitude usually found in gonoactive females in summer.

DISCUSSION

The numbers of females recorded reflect more the ease of collection than the relative importance of different overwintering sites to the mosquito populations. More *An. earlei* were collected from root cellars than from any other site, but root cellars are far less common than rockpiles and burrows, at least in the George area. Only *An. earlei* were taken in the root cellars, plus one doubtful record for *Cx. territans,* while 4 species were recorded from the rockpiles. Moreover, the mortality of *An. earlei* in a root cellar from September to March was 88.5%, at a mean temperature of 6.4°C. This mortality rate falls between Khelevin's (1941) two figures for *An. maculipennis* females in the U. S. .S. R., 96.8% in shelters at 8–10°C and 8% in shelters at 2–6°C.

Females of only 4 of the 8 species known to be present were found during winter, and they were found in fewer sites than expected from records in the literature. All the mosquitoes found were in subterranean or subnivean sites and this is probably a true record of distribution because the more exposed sites were much easier to search. *An. earlei* has been found elsewhere in burrows (Shemanchuk 1965), caves (Owen 1937) and sheds (McLeod and McLintock 1947). *Cx. territans* females were already known to overwinter in rockpiles (Howard et al. quoted by Hearle 1926) and root cellars (Keener 1952), also in caves (Price et al. 1960), a tree squirrel nest (Hopla 1965) and clumps of grass (Hopla 1970). *Cs. alaskaensis* females were known to overwinter in cellars (Minář and Hájková 1966), mammal burrows, clumps of grass (Hopla 1970) and hollow logs (Dyar 1922). There are no previous records for overwintering *Cs. minnesotae* females.

The failure to find *Cs. inornata* females in winter is most surprising because they were the commonest of the species studied in summer and fall, and three kinds of site where females are known to overwinter, mammal burrows (Shemanchuk 1965), cellars (Rees quoted in Horsfall 1955) and rockpiles (Rush et al. 1958), were searched. The late Dr. R. E. Bellamy (personal communication 1975) found that *An. earlei* females overwintered in mammal burrows in southern Saskatchewan, but did not find any *Cx. tarsalis* or *Cs. inornata*, though populations of all 3 species occur in the area in summer. Dr. J. McLintock (personal communication 1977) considers that all 3 species enter the burrows in Saskatchewan but the survival rate of *An. earlei* is higher than that of the other 2 because it is much more cold-hardy. Data on cold-hardiness of mosquitoes in central Alberta (Hudson 1977) support this view. *Cs. inornata* females collected in fall were much less cold-hardy than *Cx. territans* and *An. earlei* females, and would have been unable to overwinter in the rockpiles, though they might survive in some of the burrows. In spring 1973–75 *Cs. inornata* females appeared much later and in smaller numbers than those of other species. This pattern of arrival suggests dispersal from a few widely-scattered sites where the females survived.

An. earlei and *Cx. territans* females are not known to be vectors of WEE, but the virus has so many vertebrate reservoirs in nature (Burton et al. 1966a, b) that any bloodsucking mosquito might be involved in enzootic transmission. The females found in winter could not have been infected, however, because they were all nulliparous. Gonotrophic dissociation (blood feeding without egg development) is one way in which mosquitoes could become infected while remaining nulliparous (Washino 1977), but there is no evidence that females of *An. earlei, Cx. territans, Cs. alaskaensis* or *Cs. inornata* take blood meals in the fall in central Alberta (Hudson 1977). The study of overwintering sites is still of interest, because the mosquitoes might feed on hosts of WEE in winter or spring.

Cx. pipiens females have been classed as diapausing if their F:G ratios do not exceed 1.5 (Spielman and Wong 1973), and it is possible to predict the end of diapause by follicle growth (Oda and Kuhlow 1974). If we consider *An. earlei* and *Cx. territans* females to be in diapause if they have F:G ratios no greater than 2.0 and follicles in stage I or earlier, then nearly all females of both species from the rockpiles were still in diapause. Diapause seemed to have ended in *An. earlei* females from the root cellar in January, because 4 of 12 had follicles in stage II, the mean F:G ratio was 2.2, 17 of 20 took blood in the laboratory and all the blood-feds matured eggs, though very few females survived the winter in the cellar. Diapause had ended in about half the *An. earlei* females from the badger burrows, but this development may have taken place in the traps rather than the burrows. Females in spring must have been gonoactive by the time of their 1st flights, because the 1st blood-feds and gravids were taken only a few days after the 1st unfeds. Thus we have no good evidence that *An. earlei* or *Cx. territans* females in natural hibernacula are ready to take blood before spring, but this could be a critical period in the transmission of WEE if latent infections in hibernating vertebrates are involved, and viremia does not appear until the host's final arousal from diapause. WEE virus has been isolated from Richardson's ground squirrels (which hibernate) in Saskatchewan in mid-May, earlier in the year than from any other host or vector (Leung et al. 1975). If the mosquitoes fed on such hosts as both emerged from hibernation, transmission would get off to a flying start.

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