STUDIES ON THE SEASONALITY OF *CULISETA INORNATA* IN KERN COUNTY, CALIFORNIA

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ABSTRACT. The seasonal abundance of adult *Culiseta inornata* was markedly bimodal in the San Joaquin Valley of Kern County, California, with minima observed during both summer and midwinter. Larvae were abundant in most surface water habitats during winter, but could not be found during summer. The occasional collection of females during summer indicated the persistence of an adult population. The midwinter decrease in adult abundance was attributed to the progressive mortality of the autumnal cohort and delayed emergence due to cold water temperature. Reproductive diapause was not induced experimentally when field or laboratory populations were exposed as larvae, pupae or adults to simulated summer or winter photoperiod and temperature regimens. In comparison, *Culex tarsalis* readily entered a winter diapause when concurrently exposed to simulated winter conditions. The aestivation, and perhaps hibernation, of reproductively quiescent females makes *Cs. inornata* theoretically attractive as a maintenance host of encephalitis viruses, while the bimodal seasonality of host-seeking activity defines periods when Jamestown Canyon virus may be transmitted horizontally.

INTRODUCTION

Mechanism(s) for the interepidemic maintenance of temperate encephalitis viruses such as western equine encephalomyelitis (WEE) and St. Louis encephalitis (SLE) viruses are understood incompletely despite in-depth investigation. One hypothesis to explain the seasonality of these viruses is that they use mosquito-vertebrate transmission cycles other than the primary *Culex*-bird cycle recognized during the summer epidemic period (Reeves 1971). A thorough understanding of the population dynamics and ecology of the suspected mosquito vector is necessary in planning research to elucidate possible secondary cycles. The present study was undertaken to more fully understand the potential for *Culiseta inornata* Williston to serve as an alternate or secondary maintenance host for WEE or SLE viruses, as well as define the periods of active horizontal transmission of Jamestown Canyon virus, a recently recognized and widespread infection in man (Grimstad et al. 1986).

In California, WEE and SLE viruses are transmitted most actively during summer by *Culex tarsalis* Coquillett, the primary endemic and epidemic vector (Reeves and Hammon 1962). The infrequent detection of virus infection in the primary vector from November through March has been attributed to decreases in host-seeking activity associated with facultative reproductive diapause (Bellamy and Reeves 1963, Nelson 1964, Reisen et al. 1986a). Although viral persistence through this period has been demonstrated for experimentally infected *Cx. tarsalis* (Bellamy et al. 1967, 1968) and passerine birds (Reeves et al. 1958), evidence to describe viral maintenance in nature is lacking.

The bimodal seasonality of *Cs. inornata* host-seeking activity (Washino et al. 1962, Meyer et al. 1982a, Barnard and Mulla 1978a) has made this species an attractive candidate as a secondary virus vector. Decreases in abundance during summer and winter have been associated with aestivation in southern California (Barnard and Mulla 1977, 1978a, 1978b) and hibernation in Manitoba, Canada (Hudson 1977[1]), respectively. However, in Kern County, California, females have been collected occasionally during summer by CO_2 and New Jersey (NJ) light traps, while in Colorado both gravid and parous females were collected resting during early spring (Dow et al. 1976). These findings led to questions concerning the physiological nature of the aestivation and hibernation states. If *Cs. inornata* females enter a summer or winter quiescence rather than a reproductive diapause, they could serve as a virus reservoir during either summer or winter, providing that they acquire a viral infection before the inhibition of host-seeking activity.

MATERIALS AND METHODS

Field studies: Field studies were designed to confirm the pattern of seasonal abundance by different sampling methods, identify the natural resting sites used by adults during summer and winter, and determine seasonal changes in the reproductive status of resting and host-seeking

[1] I. ..son, J. E. 1977. Seasonal biology of *Anopheles*, *Culex* and *Culiseta* in central Alberta (Diptera: Culicidae). Ph.D. Diss., Univ. Alberta, Alberta.

females. Marked seasonal changes in abundance, ovarian condition and parity may indicate a preparation for aestivation or hibernation.

Adult and immature *Cs. inornata* were sampled from January 1982 through October 1987 at foothill, riparian, marshland and agricultural sites in Kern County, California, using five methods:

1) NJ light trap: Phototactic females and males were sampled from April to October by New Jersey light traps operated for three consecutive days a week at 25 to 28 localities by the Kern Mosquito Abatement District. Additional trap records for the years 1976 to 1986 were examined to identify weeks when the last and first adults were collected each spring and fall, respectively, and when during the summer that occasional adults were collected. Mean air temperature recorded at the Bakersfield Airport by the National Oceanographic and Atmospheric Association for five days prior to each positive collection date of *Cs. inornata* were compared to 30-year normals to determine if the appearance of females during summer could be related to short term changes in temperature.

2) CO_2 traps: Host-seeking females were collected from dusk to dawn weekly at each of three localities from April 1983 through June 1987 by three to six CDC-style traps that were baited with dry ice and had the light bulbs removed. Habitats sampled included 1) a managed alkali marsh (Kern National Wildlife Refuge), 2) irrigated cotton and alfalfa fields bordering a duck hunting club (John Dale Ranch) and 3) riparian vegetation bordering the Kern River 5 km southwest of Bakersfield.

3) Walk-in red boxes: Resting males and females were sampled shortly after sunrise at one to three walk-in red boxes (Meyer 1987) positioned near CO_2 trap sites (see 2 above).

4) Natural and man-made resting sites: Decreases in adult abundance at walk-in red boxes during summer and winter prompted the search for resting adults at man-made and natural refugia. Man-made resting sites such as privies, bridges and deserted houses were inspected using a flashlight and mechanical aspirator (Hauscherr's Machine Works, Freehold, NJ). Vegetation was sampled using a back-pack sweeper (Meyer et al. 1983) or a more powerful gasoline engine powered D-Vac suction device (Dietrick et al. 1959). Ground squirrel burrows were sampled by egress traps (Reisen et al. 1985) placed over the entrances in the afternoon and then examined for mosquitoes the following morning. Pipe traps (Nelson 1980) were used during summer to simulate burrows. Burrows, small caves, brush piles, woodrat (*Neotoma*) nests and erosion furrows were sampled using an aerial net after resting females were stimulated to egress by discharging atomized chloroform into the habitat (Mortenson 1953).

5) Collection of immature stages: Mosquito breeding sites throughout Kern County were sampled sporadically from 1985 through 1986 with emphasis on the late winter-early spring period. Specimens were identified as either third or fourth instar larvae, or after rearing to the adult stage.

The reproductive state of females collected resting and host-seeking were scored as empty, blood fed or gravid by examination of the abdomen. Specimens collected from May 1986 through October 1987 were dissected to determine reproductive age. One ovary was disrupted with a vibrating needle (Hitchcock 1968), follicular maturation classified by the scheme of Christophers (1911) and the number of dilatations on the pedicel counted to determine reproductive age (Detinova 1962). The second intact ovary was allowed to dry, and parity was determined by the degree of coiling of the tracheolar skeins (Detinova 1962).

From December 1986 through May 1987, adults were tested for the presence of fructose, using the cold anthrone reaction (Van Handel 1972).

Laboratory studies: Laboratory studies on *Cs. inornata* were conducted to describe the reproductive state induced by both summer and winter environmental conditions and thus elucidate the seasonal patterns observed during concurrent field studies. Observations on *Cx. tarsalis* were included for comparison to ensure that our experimental procedures would induce a winter diapause response as described previously (Reisen et al. 1986b).

During the fall of 1985 and the spring of 1986, *Cs. inornata* and *Cx. tarsalis* were collected for experimentation from sites in Kern County. Mosquitoes used included: 1) f_1 progeny of host-seeking females which blood fed and oviposited in the insectary, 2) wild-caught (WC) larvae (L) or pupae (P) and 3) immatures from the Kern National Wildlife Refuge colony (KNWR-84) maintained in an insectary at 23–27°C and 16:8 (L:D).

In experiment 1, the f_1 progeny of *Cs. inornata* collected during November 1985 from the KNWR were compared to the f_1 progeny of *Cx. tarsalis* collected from Poso West (PW), a riparian foothill site. The newly eclosed first stadial (L1) f_1 progeny were reared to adult emergence (density = 200 L1/pan, replication = 2 pans, total food = 3 g of finely ground rodent chow per pan) and maintained as adults in bioenvironmental chambers set at four temperature and photoperiod regimens: 1) 26°C, 14:10 (L:D), 2) 26°C, 10:14, 3) 18°C, 14:10 and 4) 18°C, 10:14. For comparison, pupae of both species were col-

lected from the same sites during December just prior to the winter solstice, and then were allowed to emerge and were held for 2 weeks in an unheated building under seminatural conditions (water temperature = 18°C; air temperature range, 16–20°C; day length range, 9:52–9:46 hr:min).

In experiment 2, mosquitoes were allowed to emerge and were maintained as adults in light boxes with photophases of 8, 10, 12, 14 and 16 hours of illumination created by 25 watt bulbs on timers. Light boxes were maintained at 16 and 18 ± 1°C or 25 ± 3°C to simulate winter and summer temperatures, respectively. Mosquitoes used during the spring of 1986 included: A) *Cs. inornata* larvae collected from seepage pools at the KNWR on 5 March 1986 (KNWRWC-L), B) mature *Cs. inornata* larvae collected on 10 May 1986 from seepage pools adjacent to the Kern River (KRWC-L), C) progeny of *Cs. inornata* females collected by CO_2 traps on 27–28 March 1986 at the KNWR ($KNWRf_1$) and D) *Cx. tarsalis* larvae collected on 24 February 1986 from a sump at Poso West (PWWC-L). Groups A and D were reared in an unheated building under natural photoperiod (day length range, 11:14–11:45 hr:min) until first pupation. The first pupae in groups A, B and D were discarded and the remaining mature fourth instar larvae transferred to the experimental regimens for pupation and emergence. Larvae in group C were reared from egg eclosion through adult emergence under the experimental regimens. Comparison groups of *Cs. inornata* included KNWR-84 and $KNWRf_1$ reared in the insectary, KNWRWC-P emerging under natural photoperiod (photophase range, 13:37–14:22 hr:min) and host-seeking females collected by CO_2 traps during 16 April–28 May 1986.

Adults were allowed to emerge and were maintained for 12–16 days on 10% sucrose solution in each experimental regimen, after which females were dissected to determine reproductive status. The maturation of the primary follicles was classified by the scheme of Christophers (1911). The length of the primary and secondary follicles was measured for five ovarioles per female. Females with Stage V follicles were considered to be autogenous and were not measured.

During experiment 1, females not dissected were offered a restrained chick suspended from the lid of a 1 gal (3.8 liters) carton cage for two consecutive nights and then a chick placed on the screened top of a 1 pt carton (0.5 liters) for two consecutive nights. Each morning the numbers of unfed, partially blood fed and replete females were counted. Unfed females were used in subsequent blood feeding attempts, while partially fed and replete females were held under the experimental regimens for more than six days and then dissected to determine ovarian maturation.

Statistics: Abundance estimates were transformed by $\ln(y + 1)$ to control the variance and account for zero values, and seasonal relationships were described by correlation analysis (Sokal and Rohlf 1981). Model I, nested analyses of variance (ANOVA) and multiple range tests (Duncan 1955) were used to test for differences among photoperiod-temperature regimens in laboratory experiments 1 and 2.

RESULTS

Field studies: NJ light traps. A total of 3,635 female and 537 male *Cs. inornata* were collected during 7,914 NJ light trap nights from April 1982 through October 1986. The relative abundance pattern indicated a cessation of flight activity during midsummer with few females and no males collected during July and August (Fig. 1A). Lowest abundance occurred when mean monthly temperature peaked during July–August (Fig. 2).

Female *Cs. inornata* were collected sporadically at NJ light traps during summer from 1976 through 1986 (Fig. 2). The mean temperature at the Bakersfield Airport for 5 days prior to the date of collection of the last female in spring, all females during summer and the first female in fall were plotted comparatively with the average 30-yr temperatures from 1956 to 1985 (Fig. 2). Most collections of *Cs. inornata* during summer (79%) were made during periods which were warmer than the mean 30-yr monthly temperature.

CO_2 traps: A total of 14,104 *Cs. inornata* females were collected during 3,947 CO_2 trap nights at the Kern NWR, the Kern River and John Dale Ranch during 1983–87. The seasonal abundance pattern was bimodal (Fig. 1B), although abundance levels varied markedly among years and sites. Mean monthly female abundance at the Kern NWR was correlated temporally with female abundance at both John Dale Ranch and the Kern River ($r = 0.569$ and 0.696, respectively; $df = 37$; $P < 0.01$). Maximal numbers at the Kern NWR and the John Dale Ranch were collected during November after the f_1 progeny of the aestival cohort emerged from ponds flooded for duck hunting (Fig. 1B). Conversely, the vernal pattern of abundance at the Kern River was associated with the late winter inundation of riparian sources and was not correlated significantly with abundance at John Dale Ranch ($r = 0.354$; $df = 37$; $P > 0.05$). The magnitude of the vernal maxima at the Kern River study area was related to river discharge and equaled 30.3 and 47.2 females/trap night during 1983 and 1986 when 285,531 and 69,582

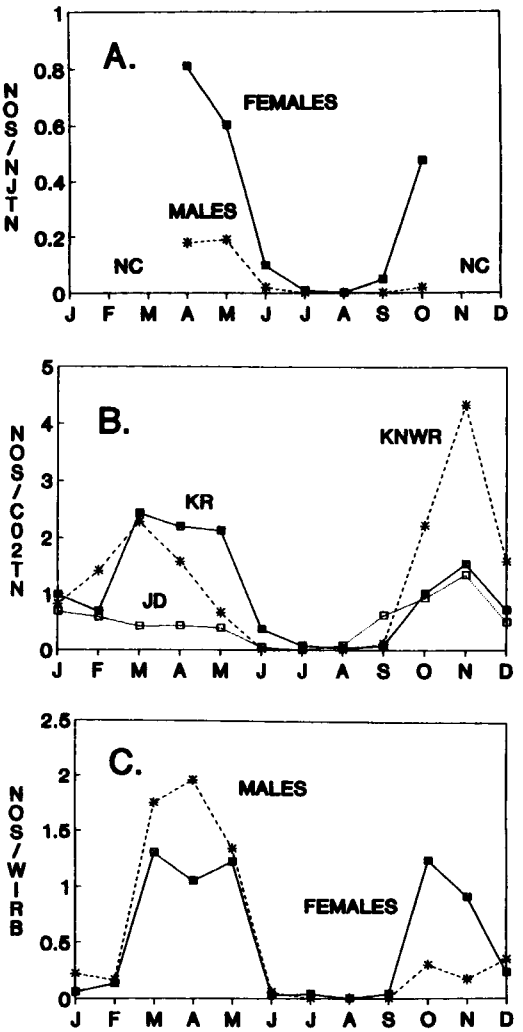

Fig. 1. Average monthly abundance of *Culiseta inornata* in Kern County [transformed to ln(y + 1)] estimated by A) NJ light traps operated throughout Kern County (n = 26–28 traps, NC = no collections made); B) CO_2 traps (n = 4–6) operated at the Kern National Wildlife Refuge (KNWR), the Kern River (KR) and John Dale Ranch (JD); and C) walk-in red boxes (WIRB) positioned at the Kern National Wildlife Refuge (n = 2–3 WIRBs sampled weekly), 1983–87.

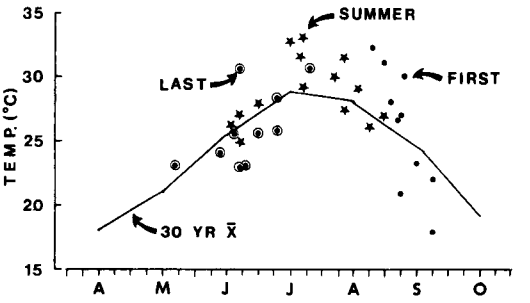

Fig. 2. Mean air temperature recorded at Bakersfield Airport for five days before the date that the last spring (encircled point), all summer (star) and the first fall (point) female *Culiseta inornata* were collected by NJ light trap in Kern County during 1976–86. Mean monthly air temperature for 1956–85 is included for comparison.

acre/feet of water were released into the river bed, respectively, but decreased to 1.0 and 2.0 females/trap night during 1984 and 1985 when only 3,217 and 71 acre/feet were discharged, respectively.

Walk-in red box: A total of 443 females and 584 males were collected in 459 walk-in red box samples taken at the KNWR from November 1983 to June 1987. The seasonal pattern of resting female and male abundance was bimodal (Fig. 1C) and did not agree well with the autumnal-dominated pattern of female abundance monitored concurrently by CO_2 traps (Fig. 1B). Although resting female abundance was comparable during the vernal and autumnal increases, a significantly greater percentage ($\chi^2 = 38.69$; $df = 4$; $P < 0.001$) of the females collected during October–November were blood fed which agreed with increases in the number of host-seeking females collected at this time (Fig. 1B, Table 1). Male abundance was greatest during spring (Fig. 1C).

Natural and man-made resting sites: A total of 1,480 females and 14,563 males were collected using eight different methods to sample resting adults (Table 1). Small 1-ft^3 red boxes placed under vegetation at the Kern River and John Dale Ranch, and along the banks of a foothill rivulet at Poso West collected only four females and seven males from May through August (remaining specimens in Table 1 were taken earlier or later than this period). In addition, few adults were collected from man-made shelters such as bridges, culverts, privies and farm buildings during summer.

Since adults were collected infrequently from resting sites above ground, we also sampled simulated burrows (pipe traps) and natural rodent burrows, woodrat nests, small caves, erosion furrows or brush piles (cone traps or burrow collections) at foothill, riparian or agricultural habitats with minimal success (Table 1). Sweeper collections from vegetation during summer were unrewarding; however, collections from vegetation bordering breeding sites from November through May produced large numbers of males and newly emerged females (Table 1).

Reproductive status: From May 1986 through October 1987, a total of 131 resting and 533 host-seeking *Cs. inornata* females were dissected to determine reproductive status (Table 2). Females which had vitellogenesis progressed to ≥

Table 1. Sampling effort and the total number of *Culiseta inornata* females in each metabolic state (empty, blood fed and gravid) and males collected by 8 methods in Kern County, 1983–87.

Season (month)	Effort[2] Sex-State	Sampling methods[1] WIRBs KNWR	WIRBs Rest	Small RB	Man-made shelters	Pipe trap	Cone trap	Burrows	Vegetation Sweeper	Vegetation D-vac	Total
Winter (D-F)	N	122	293	154	0	0	5	605[3]	17	2	1,198
	Gravid	35	9	28			0	0	1	0	73
	Blooded	1	6	9			0	2	0	0	18
	Empty	2	23	54			0	2	212	113	406
	Male	17	7	19			0	2	11,752	978	12,775
Spring (M-M)	N	120	319	54	4	0	0	0	4	3	504
	Gravid	9	25	12	0				0	0	46
	Blooded	24	31	4	0				1	0	60
	Empty	242	202	7	0				3	76	530
	Male	527	259	3	2				12	549	1,352
Summer (J-S)	N	145	503	53	195	160	95	15[4]	5	0	1,171
	Gravid	1	15	4	1	0	0	0	0		21
	Blooded	0	12	4	0	0	0	0	0		16
	Empty	2	19	18	3	1	1	0	0		44
	Male	2	9	7	5	1	0	0	0		24
Fall (O-N)	N	72	210	53	3	0	0	0	5	0	343
	Gravid	6	20	12	0				0		38
	Blooded	42	22	5	0				0		69
	Empty	97	22	33	0				7		159
	Male	20	5	1	0				386		412

[1] Sampling methods: WIRB = walk-in red boxes positioned at the Kern National Wildlife Refuge (KNWR) and Rest = 5 additional sites (Breckenridge Rd, Tracy Ranch, Hart Park, Poso West and John Dale Ranch); Small RB = 1-ft^3 red boxes; Man made shelters = bridges, farm buildings, privies, culverts, etc.; Burrows = ground squirrel burrows, *Neotoma* nests, brush piles, caves, erosion furrows, etc.; Vegetation sampled by AFS sweeper or gasoline driven D-Vac at margin of breeding sites.

[2] Effort, N = number of collections made; total gravid, blooded and empty females and males collected.

[3] 605 burrows examined on 30 occasions.

[4] 15 burrows examined on three occasions.

Stage II and/or were parous were collected throughout the year, indicating that a reproductive diapause similar to that described for *Cx. tarsalis* (Reisen et al. 1986b) may not have occurred. Of special interest was the recovery of gonotrophically active and host-seeking females during the June-August aestival period. Most females collected host-seeking during this period were nulliparous (96%), whereas most of the females collected during September were parous (89%). These data indicated that females may imbibe a blood meal prior to or during summer and then oviposit in September–October shortly after the termination of aestivation. In agreement, the first two resting females collected in September were gravid and nulliparous, and 50% of the parous host-seeking females collected during September had sacculate dilatations, indicating recent oviposition.

Many females collected during early fall had well-formed dilatations, while the skeins on the ovarian tracheoles were coiled tightly, indicating that follicular maturation and oviposition most likely had not occurred (Table 2). These females possibly could have imbibed a partial blood meal or oviposited a small autogenous egg raft. However, few host-seeking females had follicles at ≥ Stage III or contained blood in the gut from an interrupted or partial blood meal (Table 2). In addition, the probability of autogenous oogenesis during summer was slight, since autogeny rates in Kern County were typically low (<13%); and previously Meyer et al. (1982b) had demonstrated that the rate of autogeny varied as an inverse function of temperature. Most likely, these dilatations were formed when hormonally active females with ovarioles at Stage I–II or IIa failed to imbibe a blood meal during summer and then degenerated the primary follicle. By the end of summer, the primary follicle had again matured to Stage I–II or IIa when host-seeking activity was renewed. Females collected from December through August exhibited concordance between skein uncoiling and dilatation formation, indicating that follicular degeneration apparently was restricted to late summer and early fall.

Females collected in CO_2 traps imbibed plant fluids significantly less frequently ($\chi^2 = 16.8$; $df = 1$; $P < 0.001$) during winter (December 1986–February 1987, 35% fructose positive, n = 89) than during spring (March–May 1987; 67% fructose positive, n = 85). Increased fructose positivity occurred coincidentally with increased NJ light and CO_2 trap counts and changes in plant growth and flowering.

Collection of immature stages: During 1984–86, 80 surface water habitats sampled in Kern

Table 2. Reproductive status in percent of female *Culiseta inornata* collected resting in walk-in red boxes or host-seeking at CO_2 traps, Kern County, California, 1986–87.

	Months				
	Dec–Feb	Mar–May	Jun–Aug	Sep	Oct–Nov
	Resting in red boxes				
No. dissected	11	13	7	2	98
Foll. stage[1]					
N	36[2]	31	29	0	6
I	45	38	0	0	23
I–II	9	0	29	0	17
II	0	0	29	0	21
III	0	15	0	0	18
IV	9	8	14	0	5
V	0	8	0	100	10
Dilatations					
0	91	77	71	100	49
1	9	23	29	0	42 (17)[3]
2	0	0	0	0	5 (25)[3]
3	0	0	0	0	2
4	0	0	0	0	1
Sac.[4]	0	33	50	0	28
	Host-seeking at CO_2 traps				
No. dissected	78 (2)[5]	99 (1)[5]	25	18	313 (13)[5]
Foll. stages[1]					
N	0	0	0	0	0
I	17	24	16	6	20
I–II	55	38	44	67	55
II	26	37	40	28	25
III	3	0	0	0	<1
Dilatations					
0	62	51	96	11	32
1	32	44	4	61 (18)[3]	49 (40)[3]
2	5	5	0	28	16 (4)[3]
3	2	0	0	0	3
Sac.[4]	52	62	0	50	24

[1] Follicular developmental stages of Christophers (1911).
[2] Percentage of total dissected.
[3] Percentage of females with dilatations that had tracheolar skeins that were not uncoiled.
[4] Percentage of parous females with sacculate dilatations indicating recent oviposition.
[5] Number of females partially blood fed in parentheses.

County were found positive for mosquito larvae, of which 61 (76%) contained *Cs. inornata* immatures. Collection effort emphasized the winter period (December–March) when 40 of 44 (91%) positive sites contained *Cs. inornata.* The percentage positive for *Cs. inornata* decreased to 73% (n = 15 sites) during April and then to 40% (n = 10) during May. None of five sites sampled from June to September contained *Cs. inornata* larvae. The 61 collections positive for *Cs. inornata* also contained larvae of *Cx. tarsalis* (57% of 61 sites), *Aedes melanimon* Dyar (26%), *Cx. peus* Speiser (previously *Cx. thriambus* Dyar, Strickman 1988) (10%), *Cs. incidens* (Thomson) (5%), *Cs. erythrothorax* Dyar (3%) and *Cx. stigmatosoma* Dyar (previously *Cx. peus* Speiser, Strickman 1988) (2%). Collections negative for *Cs. inornata* during late winter and early spring frequently contained *Ae. melanimon* larvae, indicating recent inundation. Collections negative for *Cs. inornata* during summer contained *Cx. tarsalis* larvae, which indicated that the habitats probably were suited for *Cs. inornata* but that oviposition had ceased. In agreement, sources sequentially sampled along a foothill rivulet at Poso West were positive for *Cx. tarsalis* from 14 March to 5 November, but were only positive for *Cs. inornata* from 14 March through 5 April 1985 (Reisen et al. 1989).

Laboratory studies: In experiment 1 (Table 3), follicular maturation was not arrested uniformly when KNWRf_1 *Cs. inornata* females were reared under either simulated summer (regimen 1) or winter (regimen 4) conditions. Females in regimen 2 under the aberrant conditions of 26°C and 10:14 L:D exhibited the strongest reproduc-

Table 3. Reproductive status of *Culiseta inornata* and *Culex tarsalis* exposed to four temperature/photoperiod regimens in a bioenvironmental chamber, natural photoperiod in an unheated building and "summer" conditions in an insectary.

Regimen	No. diss.	Aut. (%)[1]	No. measured	$\bar{x}$[2] stage (1–5)	$\bar{x}$[3] 1° Foll (μm)	$\bar{x}$[4] 1°/2° ratio
			Cs. inornata KNWRf$_1$			
Bioenvironmental chamber						
1. 26C, 14:10 L:D	16	6	15	2.60	93.0a	2.06a
2. 26C, 10:14 L:D	33	0	33	2.27	73.6b	1.74b
3. 18C, 14:10 L:D	23	0	23	2.91	91.9a	1.99ab
4. 18C, 10:14 L:D	25	4	24	3.38	107.4a	2.26a
Unheated building (temp = 16–18°C, photophase = 9:52–9:46 hr)						
5. KNWRWC-L,P	15	13	13	3.54	113.4a	2.18a
6. PWWC-L,P	15	0	15	3.13	106.1a	2.07a
Insectary (temp = 16–25°C, 16:8 L:D)						
7. KNWRf$_1$	6	0	6	1.50	47.7b	1.30b
8. KNWR-lab colony	20	0	20	2.20	62.5b	1.41b
			Cx. tarsalis PWf$_1$			
Bioenvironmental chamber						
1. 26C, 14:10 L:D	25	68	8	3.13	95.0a	2.29a
2. 26C, 10:14 L:D	25	76	6	3.33	94.5a	2.27a
3. 18C, 14:10 L:D	25	28	18	3.61	96.9a	2.34a
4. 18C, 10:14 L:D	25	8	23	2.39	65.0b	1.50b
Unheated building (temp 16–18°C, photophase = 9:52–9:46 hr)						
5. PWWC-P	14	0	14	2.57	72.5b	1.63b
6. PWWC-L,P	17	6	15	2.47	67.3b	1.47b

[1] Autogeny, percent of females dissected that contained Stage V eggs.

[2] Mean ovariole stage of measured females where N = 1, I = 2, I–II = 3, IIa = 4, IIb = 5.

[3] Length in microns of primary follicle (5 measured/female). Means in the same column for the same species followed by the same letter were not significantly different ($P > 0.05$) when tested by a Duncan's (1955) multiple range test.

[4] Mean ratio of the primary/secondary follicles. Means tested as in footnote 3.

tive arrest. However, in all regimens vitellogenesis progressed to ≥Stage I–II in some females (mean >2), the mean primary to secondary (1°/2°) follicular ratio was greater than the 1.5 considered indicative of reproductive diapause (Hudson 1977), and a high percentage of females blood fed when offered a restrained chick (Table 4). Females reared under "summer" insectary conditions (regimens 7 and 8) exhibited a stronger aestival response than did those reared under regimen 1 (Table 3), and few of these females imbibed a blood meal (Table 4). Of the 50 engorged females that were held for >6 days under cool temperature (regimens 3, 4, 5 and 6, Table 4), 14% had primary follicles still at ≤ Stage IIb upon dissection; i.e., exhibited gonotrophic dissociation. In contrast, all of 45 engorged females that were held under warm temperatures (regimens 1, 2 and 8) matured their follicles to Stage V; i.e., exhibited gonotrophic concordance.

In contrast to the *Cs. inornata*, PWf$_1$ *Cx. tarsalis* females entered reproductive diapause in response to simulated (regimen 4) or semi-natural (regimens 5, 6) winter conditions in experiment 1 (Table 3). Females in regimens 4, 5 and 6 reared under cool temperature and short day length had a significantly ($P < 0.05$) lower rate of autogeny, shorter mean primary follicular length, lower mean 1°/2° follicular length ratio, and a more inhibited blood feeding drive than females reared in regimens 1–3 (Tables 3 and 4).

Results of experiment 2 (Table 5) were comparable to experiment 1 (Table 3). *Culiseta inornata* developing under natural conditions at the KNWR (group A) and the Kern River (group B) and then transferred to experimental conditions for pupation and emergence were similar to KNWRf$_1$ developing under experimental conditions from eclosion to emergence (group C) and failed to uniformly enter reproductive diapause under simulated summer or winter conditions (Table 5). Mean 1° follicular developmental stage, length or 1°/2° follicular ratio were variable among and within the temperature/photoperiod regimens and did not exhibit a pattern indicative of either aestivation or hiberna-

Table 4. Blood feeding avidity of *Culex tarsalis* and *Culiseta inornata* females offered restrained chickens suspended from the lid of bucket cages or taped to the lid of pint cartons.

Regimen[1]	Bucket cage		Pint carton	
	Day 1 % fed (n)	Day 2 % fed (n)	Day 1 % fed (n)	Day 2 % fed (n)
	Cs. inornata			
Bioenvironmental chamber				
1. 26C, 14:10 L:D	18 (22)	6 (17)	82 (17)	100 (1)
2. 26C, 10:14 L:D	29 (49)	6 (35)	13 (30)	11 (9)
3. 18C, 14:10 L:D	0 (68)	0 (62)	6 (56)	24 (45)
4. 18C, 10:14 L:D	14 (83)	0 (67)	20 (56)	52 (38)
Unheated building				
5. KNWRWC-L,P—duck pond	0 (15)	7 (14)	9 (11)	40 (10)
6. PWWC-P—warm pond	0 (22)	0 (13)	8 (13)	36 (11)
Insectary				
8. KNWR lab colony	0 (22)	0 (22)	40 (20)	27 (11)
	Cx. tarsalis			
Bioenvironmental chamber				
1. 26C, 14:10 L:D	64 (101)	20 (35)	18 (22)	0 (11)
2. 26C, 10:14 L:D	61 (72)	21 (28)	15 (20)	20 (5)
3. 18C, 14:10 L:D	94 (53)	n.d.[2]	n.d.	n.d.
4. 18C, 10:14 L:D	4 (23)	0 (22)	10 (20)	0 (16)
Unheated building				
5. PWWC-P—rivulet	28 (14)	10 (10)	11 (9)	17 (6)
6. PWWC-P—warm pond	22 (9)	0 (6)	0 (6)	0 (6)

[1] Regimens defined in Table 3.
[2] n.d. = not done.

tion. Results were hampered by poor immature survival (group A) and a high percentage of females with degenerated primary follicles at the time of dissection (group B = 58%, n = 79; group C = 24%, n = 147). Follicular degeneration indicated continued endocrine activity and thus a failure to enter reproductive diapause.

In contrast to *Cs. inornata*, *Cx. tarsalis* PWWC-L pupating under experimental photoperiod/temperature regimens readily entered reproductive diapause under simulated winter conditions (photophase ≤12 hr, temp = 16°C, Table 5, group D). Results with *Cx. tarsalis* indicated that light box conditions were suitable for diapause induction and that the failure of *Cs. inornata* to enter a reproductive arrest most likely was due to an intrinsic response to experimental conditions.

To put the reproductive data measured in experiments 1 and 2 (Tables 3 and 5) into a natural context, comparative estimates were made for four additional groups of *Cs. inornata* females (Table 6). Females collected host-seeking at CO_2 traps during April and May that had a significantly greater mean 1° follicular length and 1°/2° follicular length ratio than did females that emerged from pupae reared under semi-natural conditions were reared under insectary conditions or emerged under our experimental regimens (Tables 3 and 5).

DISCUSSION

The pattern of *Cs. inornata* relative abundance in Kern County was markedly bimodal. Decreases in the adult population were observed during both winter and summer, while decreases in larval abundance occurred during summer, agreeing with previous studies in the Central Valley (Washino et al. 1962, Meyer et al. 1982a) and southern California (Barnard and Mulla 1978a). Attempts to sample adults during winter and summer periods of reduced flight activity were markedly unsuccessful. Decreases in adult abundance during midwinter may have reflected the progressive mortality of the autumnal cohort without sufficient replacement by emergence because cool water temperature delayed immature development. In contrast, large numbers of host-seeking females were collected during September–October before larvae were found in suitable breeding sites. These data indicated that large numbers of females successfully aestivate each summer, but that our collection methods were ineffective or were directed at the wrong habitat. The sampling methods used during summer in the present study were similar to those used successfully for the collection of hibernating *Cx. tarsalis* during winter (e.g., Reisen et al. 1986a, 1986c).

Culiseta inornata did not seem to enter a reproductive diapause in the southern San Joa-

Table 5. Reproductive status of *Culiseta inornata* and *Culex tarsalis* exposed to five photoperiods in light boxes maintained at two temperature regimens during experiment 2.

Temp (C)	25					16				
Light (h)	8	10	12	14	16	8	10	12	14	16
A. *Cs. inornata* KNWRWC-L										
No. diss.:	14	15	13	11	1	15	7	4	8	15
Foll. stage[1]:	2.8a	2.8a	2.4a	2.4a	2.0a	2.8a	2.3a	2.3a	2.5a	2.3a
Size (μm):	86a	91a	74b	69bc	54c	86a	68bc	65bc	66bc	71b
1°/2° ratio:	2.00a	2.07a	1.70b	1.64b	1.55b	1.66b	1.48b	1.37b	1.40b	1.44b
B. *Cs. inornata* KRWC-L3,4										
No. diss.:	15		13		13	7		2		12
Foll. stage:	2.5a		2.4a		2.5a	2.9a		2.0a		2.3a
Size (μm):	76a		76a		72a	81a		62a		72a
1°/2° ratio:	1.74a		1.91a		1.71a	1.69a		1.44a		1.60a
C. *Cs. inornata* KNWRf$_1$										
No. diss.:	15	15	13	15	13	14	12	10	13	11
Foll. stage:	2.5a	2.6a	2.5a	2.5a	2.4a	2.2a	2.5a	2.3a	2.5a	2.8a
Size (μm):	81abc	78abc	68c	72bc	68c	77abc	78abc	71bc	84ab	88a
1°/2° ratio:	1.92a	1.95a	1.73a	1.75a	1.68a	1.64a	1.69a	1.64a	1.84a	1.89a
D. *Cx. tarsalis* PWWC-L										
No. diss.:	17	19	17	14	16	15	15	15	15	14
Foll. stage:	2.7d	2.7d	3.0bcd	3.5a	2.9cd	2.0e	2.0e	2.2e	3.1abc	3.4ab
Size (μm):	60cd	61cd	68c	80b	66c	48e	45e	57d	83ab	97a
1°/2° ratio:	1.64cd	1.55d	1.86bc	2.05ab	1.8bcd	1.14e	1.10e	1.28e	2.07ab	2.29a
Autogeny (%):	15	5	15	30	20	0	0	0	6	6

[1] Foll. stage, primary follicles classified according to the scheme of Christophers (1911) with Stage N = 1, I = 2, I–II = 3, IIa = 4. Size, length of the primary follicle. 1°/2° ratio, ratio of the length of the primary/secondary follicle. Values only for measured females. Values within the same row followed by the same letter were not significantly different when tested by a Duncan's (1955) multiple range test ($P > 0.05$).

Table 6. Mean primary follicular stage,[1] size and the 1°/2° follicular ratio of *Culiseta inornata* females reared in the insectary (25°C, 16:8 L:D) or under natural photoperiod and temperature in May and collected host-seeking at CO_2 baited traps.[2]

Mosquito source	No. diss.	Foll. stage	Foll. size (μm)	1°/2° ratio
1. KNWR-84 colony	14	3.0a	95b	2.14b
2. KNWRf_1 insectary reared	15	2.5b	80c	1.86b
3. WCP—natural photoperiod	29	3.0a	94b	2.04b
4. WC CO_2—Apr/May	40	3.2a	116a	2.64a

[1] Primary follicles classified according to the scheme of Christophers (1911) with Stage N = 1, I = 2, I–II = 3, II = 4. Values only for measured females.

[2] Values within the same column followed by the same letter were not significantly different when tested by a Duncan's (1955) multiple range test ($P > 0.05$).

quin Valley of California during summer. Females arrested flight and host-seeking activity during the hot, dry June–September period and entered an aestival quiescence as parous, gravid or nulliparous females. Nullipars remained relatively sedentary, but occasionally egressed to blood feed prior to becoming fully active during September–October. Similarly, Meyer et al. (1982a) reported that ca. 75% of females collected by CO_2 baited traps in central California were nulliparous in May just prior to aestivation. In contrast, all females collected in the Coachella Valley during the pre- and postaestival periods were either gravid or parous (Barnard and Mulla 1978a). In Kern County, nullipars that were not blood fed remained hormonally active, as indicated by the collection of host-seeking individuals during midsummer, the failure to uniformly arrest follicular development experimentally and the presence of degenerative dilatations in postaestival nullipars collected during fall. Thus, the few females collected during summer in NJ light or CO_2 traps may have been individuals with depleted energy reserves or those that failed to blood feed during late spring.

The stimulus for midsummer flight activity and the termination of aestivation may be the depletion of energy reserves. Barnard and Mulla (1978b) showed a marked decrease in the lipid content between females collected in April–June and September–October. Upon terminating aestivation, females immediately sought a blood meal and/or oviposited, since the first females collected at CO_2 traps were either nulliparous with degenerative dilatations or parous with a high percentage with sacculate dilatations.

The f_1 progeny of aestival females emerged during late October and November when large numbers of males were collected resting in vegetation adjacent to breeding sites such as flooded duck ponds at the KNWR. Mating occurred immediately after the females emerged with the males presumably locating the newly emerged females by a volatile pheromone (Kliewer et al. 1966). Females subsequently dispersed from breeding sites as indicated by the predominance of males in sweeper collections at breeding sites and the predominance of females in collections from walk-in red boxes. However, during midwinter, female abundance at red boxes seemed disproportionately low, indicating that alternative sites were being used for diurnal resting. Cool winter temperatures delayed immature development, as indicated by the predominance of older larvae during winter, and may have precluded flight and host-seeking activity, as indicated by the decrease in CO_2 trap counts. Meyer et al. (1982a, 1982b) previously reported similar winter observations in central California. In Kern County, females of the aestival cohort presumably were long-lived and overlapped with the newly emerged f_1 cohort, which resulted in an age structure during November–December with proportionately more multiparous females. With the onset of warmer weather during late winter and the completion of emergence by the progeny of autumnal females, both males and females became abundant at walk-in red boxes. Increased dispersal and flight activity during late winter was accompanied by increased fructose ingestion.

Females emerging under winter conditions of cool temperature and short daylength did not undergo reproductive diapause under either natural or experimental conditions. In addition, females collected as larvae during May (>13 hr daylength) and allowed to pupate and emerge under conditions of cool temperature and short daylength (experiment 2) did not enter reproductive diapause. These results differed markedly from summer-active Canadian populations which initiated a reproductive diapause naturally in late summer[1] and experimentally when transferred from long day to short day photoperiods (Hudson 1977).

In Kern County, females emerging from April to June presumably comprised the aestival cohort; however, in the present or previous studies (Barnard and Mulla 1978a, Meyer et al. 1982a) the population did not bifurcate into parous, host-seeking and nulliparous, non-host-seeking components, similar to that observed for dia-

pausing and nondiapausing *Cx. tarsalis* populations in autumn (Nelson 1964, Reisen et al. 1986a, 1986c). In the present study, only 29% of resting and 4% of host-seeking females collected during June–August were parous, which sharply contrasted results from the Coachella Valley where Barnard and Mulla (1978a) reported that all aestivating females were either gravid or parous.

The deposition of hypertrophic fat through nectar feeding was critical to successful aestivation (Barnard and Mulla 1978b). In the present study, females collected host-seeking or resting during March–May frequently were fructose positive, indicating recent plant sugar ingestion. However, hypertrophic fat accumulation seemed independent of gonotrophic activity, since Barnard and Mulla (1977) found that when both nonblood fed and blood fed females were offered sugar and exposed to long photoperiods, they deposited comparable quantities of lipids and, thus, were capable of aestivation.

The bimodality of seasonal abundance and the lack of a reproductive diapause make *Cs. inornata* an attractive candidate for virus interepidemic maintenance during both summer and winter. In southern California, WEE virus activity decreases after the midsummer decline in *Cx. tarsalis* abundance and often does not reappear until the following spring or early summer (Work et al. 1974, Workman et al. 1976, Reisen et al. 1988). Thus, virus must be maintained during both late summer and winter in these areas. The summer spread of WEE virus into the rabbit (*Lepus*) population by *Cx. tarsalis* (Hardy 1987) could provide a source of infection for host-seeking *Cs. inornata* during the preaestival period, which may be carried over the summer into the fall by aestivating gravid females. *Cs. inornata* could then seed virus into autumnal populations of either *Culiseta inornata* or winter-active *Cx. tarsalis*. However, the failure to isolate WEE virus from over 10,000 adult *Cs. inornata* tested in California over the past 40 years would seem to refute this speculative scenario. Most likely the bovine/equine dominated host selection pattern of this species (Bohart and Washino 1978) precludes frequent contact with viremic hosts.

In more northern latitudes where *Cs. inornata* hibernate (Hudson 1977[1]), the lack of reproductive diapause, low rate of gonotrophic disassociation and overwintering by gravid females (Dow et al. 1976) also may be important in virus maintenance. Although few isolations of WEE virus have been made from *Cs. inornata* in the United States, WEE virus has been isolated frequently from large Canadian *Cs. inornata* populations, leading McLintock et al. (1970) to postulate that in northern latitudes spring populations of *Cs. inornata* and several *Aedes* species may introduce virus into the *Cx. tarsalis*-bird cycle.

The Jerry Slough variant of Jamestown Canyon virus has been isolated from *Cs. inornata* during winter in Kern County (Reeves 1985) and in the Imperial Valley,[2] where it is maintained presumably by transovarial transmission similar to other closely related Bunyaviridae (Turell and LeDuc 1983). Thus, the seasonality of *Cs. inornata* host-seeking activity also indicates time periods in California when Jamestown Canyon virus may be transmitted horizontally to susceptible vertebrate hosts including man.

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