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Ecological studies on the mosquito vectors of Japanese encephalitis

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These studies were conducted in China (Province of Taiwan) to assess the populations of known and potential mosquito vectors of Japanese encephalitis in a typical endemic area, and to evaluate a variety of sampling techniques, some of which were new. Culex annulus *was found to be the predominant vector species in the study area during the epidemic season;* C. tritaeniorhynchus *was never abundant, and* C. fuscocephalus *was rare.* C. annulus *and* C. tritaeniorhynchus *were active throughout the year, although populations were at a low level during the cool season. The results show that attention must be given to* C. annulus *as a possible vector where it is present in JE foci. The collection of mosquitos during the early evening hours from buffalo bait tethered outdoors was found to be the most efficient and sensitive means of monitoring vector populations throughout the year. During the JE epidemic season remarkable results were obtained with a vacuum sweep-net.*

In China (Province of Taiwan), the mosquito vectors of Japanese encephalitis, a disease affecting pigs and man, breed mainly in rice fields. Since *Culex tritaeniorhynchus*, *C. annulus*, and *C. fuscocephalus* have been incriminated as JE vectors (Wang et al., 1962a, 1962b; Detels et al., 1970) it was necessary to assess their relative abundance throughout the year, and particularly during the JE epidemic season. Earlier studies had been compromised by the fact that mosquito collections identified as *C. tritaeniorhynchus* may have also contained the very similar *C. annulus* (Cates & Detels, 1969). Although Reuben (1969) considered *C. annulus* to be a synonym of *C. vishnui*, we concur with other investigators conducting related studies on JE in treating *C. annulus* as a distinct species.

STUDY AREA

These studies were conducted in 4 hamlets in Taoyuan Township situated about 23 km WSW of Taipei City, where 2 crops of rice are harvested per annum. Since hamlet number 2 was treated with a residual spray on 17 June 1971 only certain data from this site are considered here. Pigsties are located close to or adjoining human habitations, thus allowing mosquitos to pass from one to the other. The inhabitants of the hamlets sleep in small rooms, on mats placed on elevated platforms or on the floor, and commonly use mosquito nets. During August 1970 the 4 hamlets contained from 5 to 15 families comprising between 47 and 119 individuals. Swine were the most abundant among domestic mammals, and chicken and duck the most common domestic bird species.

METHODS

The 4 study sites were sampled for mosquito larvae and pupae by weekly collections made within a radius of 100 m of each hamlet from October 1970 to the end of the first week of November 1971 and during February and March 1972. Larvae and pupae were assessed by sampling all their potential breeding habitats with an aluminium dipper.

Adult populations were assessed from September 1970 to March 1972 by regular outdoor collections from buffalo bait, made by means of suction-tubes with the aid of flashlights, during periods of either 1 or 2 hours starting about 30 minutes before sundown. The numbers of females of the 3 vector species of *Culex* were tabulated.

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Lard-can mosquito-traps (Bellamy & Reeves, 1952) were also used periodically from June to September 1971. Approximately 1 kg of frozen CO_2 wrapped in aluminium foil was placed in each trap a few minutes before sundown, and the entry portals were closed the following morning shortly after sunrise. The traps were suspended from trees or from bamboo or building structures at a height of 2 m, the premises being sampled in each case by a central and a peripheral trap.

Two Magoon traps with " Egyptian " type ingress baffles (Bates, 1944) were operated weekly from the end of June 1971, one to late September, the other to November. A 20–30-kg pig was placed in each trap shortly before sundown and removed the following morning after the mosquitos had been collected by suction-tube.

Weekly collections of mosquitos resting inside human and animal habitations were made between 08 h 00 and 12 h 00 at site 1 from October 1970 to the end of November 1971 and during March 1972. Habitats sampled included bedrooms, storerooms, sitting-rooms, kitchens, toilets, cowsheds, pigsties, and hen-houses.

Outdoor collections were made with a D-Vac vacuum sweep-net weekly at the 4 study sites from September 1970 to March 1972. Habitats sampled included straw piles, bamboo fences, ditch sides, pathways, field grasses, sweet-potato fields, vegetable fields, and wood piles. Each habitat was sampled usually for at least 10 minutes between 10 h 00 and 15 h 00.

During 1970, New Jersey light traps were operated at the 4 study sites from August to mid-October. Collections were resumed during March 1971 and continued at 2 of the study sites to the end of November. The traps were usually operated 4 nights per week.

RESULTS

The assessments of larvae and pupae (Table 1) show that *C. annulus* constituted 95.3% and *C. tritaeniorhynchus* 4.7% of the 40 698 specimens collected during the entire study period. *C. fuscocephalus*

Table 1. Relative abundance of *C. annulus* and *C. tritaeniorhynchus* larvae and pupae in Taoyuan Township

Months	No. of man-hours expended	No. of larvae and pupae collected		% of total		No. per man-hour	
		C. a.	*C. t.*	*C. a.*	*C. t.*	*C. a.*	*C. t.*
Oct. 1970	10.0	185	2	99	1	19	1
Nov.	7.0	225	0	100	0	32	0
Dec.	36.5	359	47	88	12	10	1
Jan. 1971	39.2	1	17	6	94	1	1
Feb.	53.2	0	2	0	100	0	1
Mar.	99.3	0	0	0	0	0	0
Apr.	50.5	320	0	100	0	6	0
May	34.0	1 553	52	94	6	46	2
June	35.0	2 807	143	95	5	80	4
July	33.0	5 524	1 074	84	16	167	32
Aug.	43.0	12 561	61	99	1	292	1
Sept.	32.0	8 572	213	98	2	268	7
Oct.	26.5	6 351	271	96	4	240	10
Nov.[a]	6.0	342	0	100	0	57	0
Feb. 1972	27.0	2	7	22	78	1	1
Mar.	17.7	0	7	0	100	0	1

[a] Collections on a man-hour basis were discontinued from the second week in November 1971 to January 1972.

was not found. Larvae of *C. annulus* were conspicuous from April to December, being most abundant in August. Larvae of *C. tritaeniorhynchus* were found in every month of the year except April, with peak abundance in early July. Approximately 85% of the *C. annulus* came from paddy fields, 13% from cesspools, and the remaining 2% from a variety of temporary and permanent pools; the pattern was similar for *C. tritaeniorhynchus*.

In the adult assessments on buffalo bait located outdoors (Table 2) both *C. annulus* and *C. tritaeniorhynchus* were present in every month of the year, although the former species was absent in January 1972. *C. annulus* was reasonably abundant from April through September, reaching a peak during August. *C. tritaeniorhynchus* reached an adult population peak in September and this species was predominant over *C. annulus* from December to March. *C. fuscocephalus*, which is strongly attracted to buffalo bait, was never a prominent component of the Taoyuan collections.

During 39 trap nights a total of 9 193 mosquitos was collected in traps baited with frozen CO_2, comprising 95.1% *C. annulus*, 3.5% *C. tritaeniorhynchus*, and a few specimens each of *C. pipiens fatigans*, *Anopheles sinensis*, *Armigeres subalbatus*, and *C. fuscocephalus*. *C. annulus* was attracted to the traps in the greatest number during August and September, 1 687 being collected in a single trap during the night. Traps located on the peripheries of the hamlets were the most productive.

A total of 7 770 female mosquitos was collected from swine-baited Magoon traps during 39 trap nights. This total comprised 81.0% *C. annulus*, 8.3% *C. p. fatigans*, 7.5% *A. sinensis*, 2.3% *A. subalbatus*, and 0.9% *C. tritaeniorhynchus*. *C. annulus* was most abundant in the traps during August, with averages of 478 and 280 per trap night at sites 1 and 2.

Table 2. Relative abundance of females of 3 *Culex* species attracted to buffalo bait located outdoors during early evening hours [a] in Taoyuan Township

Months	No. of man-hours expended	Average no. of females collected per man-hour (% of total for all species)					
		C. annulus		*C. tritaeniorhynchus*		*C. fuscocephalus*	
Sept. 1970	46	92.6	(71)	27.4	(27)	2.6	(2)
Oct.	38	22.4	(85)	3.7	(14)	0.3	(1)
Nov.	27.5	25.4	(36)	7.8	(11)	37.4	(53)
Dec.	20.6	2.1	(32)	2.6	(40)	1.8	(28)
Jan. 1971	15.6	0.1	(5)	2.2	(92)	0.1	(3)
Feb.	21.2	0.1	(11)	0.3	(78)	0.1	(11)
Mar.	26.8	0.2	(35)	0.4	(65)	0	(0)
Apr.	45.5	30.4	(97)	1.0	(3)	0	(0)
May	17.4	28.1	(79)	7.6	(21)	0.1	(1)
June	11.0	169.5	(69)	76.5	(31)	0	(0)
July	4.0	263.3	(82)	57.7	(18)	0	(0)
Aug.	7.7	1 067.8	(98)	22.6	(2)	0	(0)
Sept.	23.0	224.9	(57)	169.1	(43)	1.8	(1)
Oct.	17.0	10.9	(14)	66.7	(85)	0.6	(1)
Nov.	48.7	3.0	(56)	2.4	(44)	0	(0)
Dec.	51.0	0.3	(44)	0.4	(56)	0	(0)
Jan. 1972	45.0	0	(0)	0.2	(100)	0	(0)
Feb.	30.0	0.1	(9)	0.7	(91)	0	(0)
Mar.	42.0	0.2	(40)	0.4	(60)	0	(0)

[a] Collections were begun shortly before sunset and continued for 1–2 hours thereafter.

Among the female mosquitos collected from indoor resting sites (Table 3), *C. p. fatigans* was the predominant species during the late autumn, winter, and spring, while *C. annulus* was predominant in July, August, and September. *A. sinensis* was collected in every month and was most abundant in April and May, while *A. subalbatus* was collected each month except February 1971 and was most abundant during October and November. Other species, including *C. tritaeniorhynchus*, make up fewer than 1% of the specimens collected.

Among the female mosquitos collected from outdoor resting sites (Table 4), *C. p. fatigans* was the predominant species from January to March and in May, October, and December; it was as prevalent as *A. subalbatus* during November. *A. sinensis* was predominant during April, and *C. annulus* was predominant from June to September. *C. tritaeniorhynchus* and *C. fuscocephalus* were never abundant in the outdoor collections.

The light-trap collections showed that the peaks of *C. annulus* occurred during late August or early September at both the sites sampled, an average of 288 females per trap night being collected at site 2 during the last week of August 1970. *C. tritaeniorhynchus* was never abundant, the number collected in 1970 never exceeding a weekly average of 5 females per trap night, even in the period from August to October. In 1971 the weekly average was usually less than 10 per trap night, the highest figure being 32 females at site 1 during the week ending 23 October.

DISCUSSION AND CONCLUSIONS

The fact that no less than 85% of the larvae and pupae of *C. annulus* and *C. tritaeniorhynchus* collected came from paddy fields was due to the relative scarcity of other stable habitats such as temporary pools in fallow fields during the dry summer of 1971.

While neither the pre-harvest drying of the paddy fields during late June and early July nor aerial applications of insecticide for agricultural purposes during 22–25 July and 14–18 August persistently dampened the predominant *C. annulus* populations,

Table 3. Relative abundance of females of 4 mosquito species in collections from human and domestic animal habitations at study site no. 1 in Taoyuan Township

Months	No. of man-hours expended	Average no. of females collected per man-hour (% of total for all species)				
		C. annulus	*C. p. fatigans*	*Armigeres subalbatus*	*Anopheles sinensis*	Other species [b]
Oct. 1970	13.0	0.5 (1)	32.9 (59)	1.7 (3)	20.9 (37)	0 (0)
Nov.	13.5	0.2 (1)	69.3 (71)	5.8 (6)	22.4 (23)	0 (0)
Dec.	18.4	0.1 (1)	81.7 (93)	2.2 (2)	4.2 (5)	0 (0)
Jan. 1971	10.0	(0)	94.6 (89)	2.6 (3)	8.9 (8)	0 (0)
Feb.	9.8	0 (0)	97.7 (97)	0 (0)	3.0 (3)	0 (0)
Mar.	11.0	0 (0)	44.3 (90)	0.6 (1)	4.5 (9)	0 (0)
Apr.	14.3	0 (0)	81.3 (57)	7.8 (6)	52.9 (37)	0.1 (1)
May	7.8	0.1 (1)	86.1 (63)	5.0 (4)	44.4 (33)	0 (0)
June	20.0	6.5 (5)	98.1 (77)	11.3 (9)	12.1 (9)	0 (0)
July	21.0	43.6 (48)	20.2 (22)	11.3 (12)	16.1 (18)	0.1 (1)
Aug.	15.0	237.7 (92)	2.9 (1)	5.1 (2)	13.3 (5)	0.4 (1)
Sept.	18.0	74.4 (57)	24.0 (19)	1.9 (1)	29.0 (22)	0.1 (1)
Oct.	12.0	0.9 (1)	31.7 (45)	21.7 (31)	16.4 (23)	0.1 (1)
Nov. [a]	11.0	0 (0)	43.8 (49)	35.4 (39)	11.2 (12)	0 (0)
Mar. 1972	8.0	0 (0)	61.1 (69)	1.6 (2)	25.5 (29)	0 (0)

[a] Collections were discontinued from December 1971 to February 1972.

[b] Other species included 11 *C. tritaeniorhynchus*, 2 *C. kangi*, 1 *C. fuscanus*, and 1 *Anopheles tessellatus*.

Table 4. Relative abundance of females of 4 mosquito species in outdoor D-Vac collections in Taoyuan Township

Months	No. of minutes	Average no. of females collected per 10 minutes (% of total)				
		C. annulus	*C. p. fatigans*	*Armigeres subalbatus*	*Anopheles sinensis*	Other species [a]
Sept. 1970	320	5.6 (88)	0.2 (3)	0.3 (5)	0.1 (0.1)	0.2 (3)
Oct.	760	0.4 (18)	1.1 (47)	0.2 (10)	0.6 (26)	0 (0)
Nov.	679	0.2 (1)	11.5 (88)	0.8 (6)	0.1 (3)	0.1 (1)
Dec.	379	0.1 (1)	14.6 (85)	1.3 (7)	1.1 (6)	0.2 (1)
Jan. 1971	667	0 (0)	10.6 (95)	0.1 (1)	0.3 (2)	0.1 (1)
Feb.	485	0 (0)	19.6 (97)	0.1 (1)	0.5 (3)	0.1 (1)
Mar.	468	0.1 (1)	30.2 (89)	0.9 (3)	2.6 (8)	0.1 (1)
Apr.	406	4.6 (13)	15.0 (41)	0.7 (2)	15.3 (42)	0.7 (2)
May	495	12.9 (36)	16.8 (47)	0.8 (2)	3.9 (11)	1.6 (4)
June	658	24.4 (68)	7.0 (20)	1.6 (5)	2.1 (6)	0.5 (1)
July	584	131.6 (91)	0.6 (1)	2.9 (2)	7.2 (5)	2.3 (2)
Aug.	235	1 209.5 (97)	0.9 (1)	11.9 (1)	18.5 (1)	2.9 (1)
Sept.	303	105.6 (94)	0.6 (1)	1.7 (2)	2.4 (2)	1.7 (2)
Oct.	470	0.9 (25)	1.0 (29)	0.5 (14)	0.9 (25)	0.2 (7)
Nov.	560	0.1 (6)	1.0 (40)	1.0 (40)	0.1 (6)	0.2 (8)
Dec.	620	0.1 (1)	20.8 (90)	1.3 (6)	0.7 (3)	0.2 (1)
Jan. 1972	480	0.1 (1)	68.7 (97)	0.1 (1)	2.0 (2)	0.1 (1)
Feb.	370	0.1 (1)	8.8 (79)	0 (0)	2.4 (21)	0 (0)
Mar.	780	0 (0)	7.7 (73)	0.1 (1)	2.7 (25)	0.1 (1)

[a] Other species included 221 *C. tritaeniorhynchus*, 109 *C. bitaeniorhynchus*, 86 *C. fuscanus*, 20 *C. kangi*, 17 *C. rubrithoracis*, 13 *C. fuscocephalus*, 10 *Aedes albopictus*, and 1 *Anopheles tesselatus*.

the larval population of *C. tritaeniorhynchus* did not recover when the fields were reflooded during mid-July for the second crop, it being perhaps significant that these larvae are somewhat more susceptible than those of *C. annulus* to certain agricultural insecticides under field conditions. We agree with the conclusion of Cates (1968) that flood-drying schedules and insecticide applications are the most important factors regulating mosquito production in paddy fields in China (Province of Taiwan). It is noteworthy that in an area where untreated swamps are present, namely Peitou District within Taipei Municipality, *C. tritaeniorhynchus* was found to be the predominant vector species during the JE epidemic season (unpublished data, 1971).

Collections from buffalo tethered outdoors during the early evening hours are the most efficient and sensitive means of monitoring adult populations of *C. annulus*, *C. tritaeniorhynchus*, and *C. fuscocephalus* throughout the year. However, more than 1 000 female *C. annulus* could be collected in 10 minutes by sweeping outdoor mosquito resting sites with the D-Vac vacuum sweep-net, which thus demonstrated its usefulness in assessing JE vector mosquito populations during the epidemic season.

The lard-can mosquito-trap baited with frozen CO_2 is costly to operate; however, the expense would seem to be justified if the objective is to sample *C. annulus* populations. On the other hand, our results with swine-baited Magoon traps were not outstanding, despite the use of " Egyptian " type ingress baffles (Bates, 1944). It might be profitable, however, to assess the use of this type of baffle on Magoon traps large enough to accommodate bovine bait.

Collections of mosquitos resting indoors during the daytime yielded reasonable numbers of *C. annulus* during the JE epidemic season, particularly during August, thus providing valuable information on points of epidemiological importance. The resting and feeding habits of the vector mosquitos are discussed in detail by Mitchell et al. (1973).

It has been stated that the most important vector of JE, wherever JE occurs, is *C. t. summorosus* (Ree et al., 1969). Recent reports, however, suggest that *C. annulus* may be equally, if not more, important in certain areas of China (Province of Taiwan) (Detels et al., 1970; Okuno et al., 1971). In our assessments at Taoyuan, *C. annulus* was by far the more abundant species. This observation, together with the considerable virus isolation rates in this species and its strong propensity to seek swine blood (Mitchell, C. J. et al., 1973), reinforces the thesis that *C. annulus* is an important vector of JE in China (Province of Taiwan).

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RÉSUMÉ

ÉTUDES ÉCOLOGIQUES SUR LES MOUSTIQUES VECTEURS DE L'ENCÉPHALITE JAPONAISE

Des études ont été menées pendant 18 mois dans une région de rizières du nord de la Chine (province de Taïwan) où l'encéphalite japonaise est endémique. Leur objectif était d'évaluer les populations de vecteurs connus ou potentiels et de tester un certain nombre de techniques d'échantillonnage.

Les collectes de moustiques ont eu lieu d'octobre 1970 à novembre 1971 et en février et mars 1972, généralement à intervalles d'une semaine. Les larves et les nymphes présentes dans les gîtes ont été prélevées et identifiées. Les adultes ont été capturés au repos à l'intérieur des locaux occupés par l'homme ou les animaux domestiques à l'aide d'un tube à aspiration, et à l'extérieur à l'aide d'un filet spécial (D-Vac). On a aussi eu recours aux pièges lumineux, aux pièges à neige carbonique et aux captures sur appât (porc, buffle).

Le moustique le plus souvent rencontré au cours de la saison épidémique a été *Culex annulus*; *C. tritaeniorhynchus* n'a jamais été abondant et *C. fuscocephalus* a été rarement observé. *C. annulus* et *C. tritaeniorhynchus* étaient actifs pendant toute l'année en dépit de la faible densité de leurs populations durant la saison froide. Ces faits plaident en faveur de l'hypothèse attribuant à *C. annulus* un rôle de transmission important dans certaines régions où l'encéphalite japonaise est endémique.

L'évaluation comparée des techniques d'échantillonnage a montré que la capture sur buffle exposé à l'extérieur des habitations pendant les premières heures de la soirée est la méthode la plus efficace et la plus sensible pour la surveillance des populations de vecteurs durant toute l'année. En saison épidémique, on a obtenu des résultats spectaculaires avec le filet D-Vac qui a permis de récolter plus de 1000 femelles de *C. annulus* en 10 minutes. Cette technique, ainsi que les pièges à neige carbonique, est utile pour l'étude des populations de vecteurs partout où sévit l'encéphalite japonaise.

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