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Host-feeding patterns and behaviour of 4 *Culex* species in an endemic area of Japanese encephalitis

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Studies were made on the host-feeding patterns and behaviour of 4 Culex *species in China (Province of Taiwan) to further assess their importance as vectors of Japanese encephalitis. A relatively unbiased, non-attractant technique (vacuum sweep-net) was used for collecting mosquitos resting outdoors on rural premises; blood meals from engorged specimens were identified by precipitin tests. Additional information was obtained by analysing data from related studies. Precipitin-test results indicated that* C. annulus, C. tritaeniorhynchus, *and* C. fuscocephalus *feed principally on mammals, the first species mainly on swine and the latter two largely on bovine hosts. Mosquito forage ratios suggested that the apparent preference of* C. annulus *for swine over bovine hosts might be a function of host density.* C. annulus *was found to be zoophilic, exophilic, and exophagic; however, in view of its abundance during the JE epidemic season, contact with human hosts may reach a significant level, even indoors. The other 3 species of* Culex *were considered to be of lesser importance as JE vectors during the 1971 epidemic season because of their host-feeding patterns and/or population densities.*

An understanding of the variations in the host-feeding pattern and the behaviour of a mosquito species is necessary to assess its importance as a disease vector, and to delimit the types of control procedures that may be used effectively against it. The present study was undertaken in order to clarify these factors for *Culex annulus*, *C. tritaeniorhynchus*, *C. fuscocephalus*, and *C. pipiens fatigans*, all known or potential vectors of Japanese encephalitis in China (Province of Taiwan). The methods hitherto employed in this study area have included the use of bait animals and precipitin testing of blood meals from mosquitos collected by a variety of methods. It is recognized that blood-meal identification as carried out by precipitin tests may not indicate true host preferences, owing to variables such as host availability and host behaviour; none the less, when used wisely, the precipitin test is valuable in host selection studies. A great problem in interpreting precipitin-test results is the doubtful comparability between the sample results and the population factor being estimated. In an attempt to minimize sampling bias a non-attractant collecting device (vacuum sweep-net) was used to obtain most of the samples, all of which were collected outdoors.

METHODS

Collections were made between September 1970 and May 1972 in typical rural hamlets in Taoyuan, Hsinchu, Miaoli, and Pingtung Counties, situated in the north-western, west-central, and southern parts of the island. The sites in Taoyuan, Hsinchu, and Miaoli Counties are in predominantly rice-growing areas, while at the Pingtung County site bananas and sugar cane predominate over rice. A census of humans and domestic animals was made at each site once or twice during the study.

Collections of engorged mosquitos for precipitin tests were made exclusively with a vacuum sweep-net (D-Vac) in Miaoli and Pingtung Counties, and were supplemented with a few specimens from light-trap and CO_2-trap collections in Taoyuan and Hsinchu Counties. Potential outdoor resting sites were sam-

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pled weekly or bi-monthly with a vacuum sweep-net, usually between 10 h 00 and 15 h 00. The sites in Taoyuan County were sampled without interruption over a period of several months.

Blood-engorged mosquitos were processed for precipitin tests (Weitz, 1956) by smearing them individually on labelled sectors of filter-paper disks, which were sent periodically to the Imperial College Field Station for testing. Normally, each blood-meal smear of sufficient size was first screened by testing with non-specific anti-mammal sera. If positive, the meals were tested with specific antisera for man, bovine species, swine, dog, cat, and horse. In practice, the first screening could often be omitted. Non-mammalian feeds were tested with avian and reptilian antisera. Mosquito forage ratios (Hess et al., 1968) for man and other animals were computed from the results when 12 or more engorged mosquitos of a single species were collected at a single site.

Ancillary information on the feeding habits and behaviour of certain species was gleaned from related studies conducted in Taoyuan County during the 1971 JE epidemic season (Mitchell & Chen, 1973).

RESULTS

Of the total of 2 839 mosquito blood meals tested, 2 734 (96.3%) reacted, the remainder consisting generally of weak feeds. Almost all (96.6%) of the specimens were collected in a vacuum sweep-net. The remainder came from light traps (3.2%) and CO_2 traps (0.2%). The results (Table 1) show that the majority of *C. annulus* feeds came from swine in every area except site 1 in Miaoli County; altogether 53.7% of its feeds came from swine. Bovine feeds ranged from 2.1% to 77.1% in different areas, accounting for 24.7% of the total. Other hosts fed upon included dogs (15.0%), birds (2.7%), and man (0.8%). By contrast, *C. tritaeniorhynchus* had fed at each main site predominantly on bovine hosts, which accounted for 72.3% of its total feeds. Other feeds came from swine (22.8%) and from avian hosts (1.6%). *C. fuscocephalus* had fed on bovine hosts (88.6%), swine (10.0%), birds (0.9%), and dogs (0.1%). *C. p. fatigans* had fed principally on birds at each site, and in lesser numbers on dogs, cats, swine, and bovine and human hosts.

Only 6 multiple feeds (0.2%) were detected, although the testing procedure did not specifically look for them. One *C. annulus* had fed on swine and on bovine hosts, 3 had fed on swine and dogs, and 1 had fed on a bird and an unidentified mammal; 1 *C. fuscocephalus* had fed on both swine and bovine hosts.

The census data for humans and domestic animals at the principal sites (Table 2) show that man was the most abundant mammalian species and swine the next most abundant. Water buffalo (*Bubalus bubalus*) was the predominant bovine species, being present at every site except Taoyuan sites 3 and 5. Cattle (*Bos taurus*) were present only at 2 sites—1 each in Miaoli and Pingtung Counties. Dogs and cats were always present. There was a variety of domestic avian species at each site, with chicken and duck predominating. The total number of potential avian hosts was greater than that of potential mammalian hosts at each of the principal sites.

Mosquito forage ratios, i.e., the percentage of mosquito blood meals from a given host divided by the percentage that host represents of the total host population, were computed for each species at each of the principal sites (Table 3). *C. annulus*, *C. tritaeniorhynchus*, and *C. fuscocephalus* each showed forage ratios of 2 : 1 or greater for mammals, and 0.1 : 1 or less for birds. These 3 mosquito species had the highest forage ratios for bovine hosts. *C. p. fatigans* had forage ratios of 1.2 : 1 to 1.4 : 1 for avian hosts and 0.6 : 1 or less for mammalian hosts.

The relative abundance of female *C. annulus*, including unfed as well as engorged specimens, collected in Taoyuan County during the 1971 JE epidemic season (Table 4), shows that grasses in fallow fields were preferred resting sites during August and September. Straw piles yielded the majority of engorged *C. annulus* (55%), *C. tritaeniorhynchus* (41%), and *C. p. fatigans* (95%) collected with the vacuum sweep-net during the entire study in Taoyuan. In Miaoli County most of the engorged specimens of *C. tritaeniorhynchus* came from sweet-potato fields (30%), vegetation in a guava orchard (27%), and peanut fields (23%). Straw piles in Miaoli County yielded only a few specimens of *C. annulus* since most collections were made from that area during the cool season. In Pingtung County sweet-potato fields yielded the majority of the engorged *C. fuscocephalus* (68%) and *C. annulus* (40%) collected, and straw piles yielded a high proportion (34%) of the engorged *C. annulus*.

Indoor collections from human bait made at Taoyuan sites 1 and 2 from May through September during evenings between 18 h 00 and 22 h 00 revealed that *C. annulus* was most active between 18 h 30 and 19 h 30, when 39% of the 1 882 specimens were collected. A total of 5 160 mosquitos was collected from human bait; these consisted of 36% *C. annulus*, 45% *C. p. fatigans*, and 18% *Anopheles sinensis*, with

Table 1. Host-feeding patterns of 4 *Culex* species in China (Province of Taiwan)

Locality	Site no.	No. of mosquitos reacted	Proportion (%) reactive according to species or group						
			swine	bovine	dog	cat	human	mammalian [a]	avian
						C. annulus			
Taoyuan	(1)	325	63.4	24.6	7.1	0.0	0.3	2.8	1.9
	(2)	447 [b]	55.5	17.5	20.8	0.0	0.7	2.5	3.6
	(3)	141 [b]	64.5	2.1	22.7	0.0	3.5	4.3	4.3
	(4)	101	62.4	10.9	18.8	0.0	0.0	5.9	2.0
	(6)	35	68.6	8.6	14.3	0.0	0.0	8.6	0.0
Miaoli	(1)	157	12.7	77.1	8.9	0.0	1.3	0.0	0.0
	(3)	44	54.5	13.6	20.5	0.0	0.0	6.8	4.5
Pingtung	(2)	39	46.1	35.9	12.8	0.0	0.0	5.1	0.0
	(3)	111	61.3	26.1	6.3	0.0	0.0	1.8	4.5
other areas		37 [b]	27.0	27.0	21.6	0.0	0.0	21.6	5.4
Totals		1 437 [b]	53.7	24.7	15.0	0.0	0.8	3.5	2.7
						C. tritaeniorhynchus			
Taoyuan	(1)	16	12.5	62.5	0.0	0.0	0.0	18.7	6.3
Miaoli	(1)	74	17.6	81.1	0.0	0.0	0.0	1.4	0.0
	(3)	65	24.6	73.9	0.0	0.0	0.0	0.0	1.5
other areas		29	37.9	51.7	0.0	0.0	0.0	6.9	3.5
Totals		184	22.8	72.3	0.0	0.0	0.0	3.3	1.6
						C. fuscocephalus			
Pingtung	(2)	621 [b]	5.3	93.9	0.2	0.0	0.0	0.6	0.2
	(3)	246	21.9	75.2	0.0	0.0	0.0	0.0	2.9
Totals		867 [b]	10.0	88.6	0.1	0.0	0.0	0.5	0.9
						C. p. fatigans			
Taoyuan	(1)	88	0.0	1.1	13.6	2.3	2.3	3.4	77.3
	(2)	93	1.1	7.5	12.9	0.0	0.0	0.0	78.5
	(4)	44	0.0	0.0	11.4	0.0	0.0	0.0	88.6
other areas		21	4.8	0.0	23.8	0.0	0.0	4.8	66.7
Totals		246	0.8	3.3	13.8	0.8	0.8	1.6	78.9

[a] Mammalian feeds further unidentifiable by specific antisera available and weak feeds which gave equivocal results in specific tests below the class Mammalia.

[b] Includes one or more multiple feeds.

Table 2. Humans and domestic animals residing within 100 m of principal mosquito collection sites

Collection site	No.[a] of individuals according to species or group					
	human	bovine	swine	dog	cat	avian
Taoyuan (1)	77	4	37	3	7	152
(2)	109	3	41	7	10	328
(3)	45	1	5	1	3	129
(4)	49	2	15	7	5	161
(6)	131	10	85	15	12	241
Miaoli (1)[b]	143	5	26	25	22	399
(3)[b]	192	3	27	20	7	203
Pingtung (2)	146	8	84	13	11	360
(3)	155	4	74	13	7	391

[a] Averages of two separate censuses were rounded to the nearest whole number.

[b] Only one census made at Miaoli sites 1 and 3.

the remaining 1% made up of *Armigeres subalbatus*, *C. tritaeniorhynchus*, and *Mansonia uniformis*.

Collections of female mosquitos resting indoors at Taoyuan site 1 from May through October 1971 yielded 5 961 *C. annulus*, 3 915 *C. p. fatigans*, and 11 *C. tritaeniorhynchus*. Whereas approximately one-third of the *C. p. fatigans* were found in pig-sheds, fully two-thirds of the *C. annulus* were found in the shelters of this important amplifying host of JE virus. The greater abundance of *C. annulus* in the indoor collections raised the question whether this species was equally or more endophilic than *C. p. fatigans*. The ratios of *C. annulus* to *C. p. fatigans* in indoor collections during June, July, and August were 0.07:1, 1.2:1, and 82:1, respectively. Equivalent ratios in outdoor vacuum-sweep-net collections during the same months were 3:1, 219:1, and 1 344:1. The proportions of *C. annulus* collected outdoors were therefore 43, 110, and 16 times those of *C. p. fatigans* collected outdoors during the peak JE transmission season.

Comparative indoor and outdoor attack rates of *C. annulus* and *C. tritaeniorhynchus* on buffalo during the period of peak activity in the early evening were determined at Taoyuan site 1 from June to September 1971. *C. annulus* was more than 10 times as abundant in outdoor collections (412 per man-hour in 36.4 hours of collection) as in indoor collections (39 per man-hour in 92 hours of collection). *C. tritaeniorhynchus* was about 39 times more abundant in outdoor collections (59 per man-hour) than in indoor collections (1.5 per man-hour). The ratio of *C. annulus* to *C. tritaeniorhynchus* was 7 : 1 in outdoor collections and 26 : 1 in indoor collections.

Comparative attack ratios of *C. annulus* and *C. tritaeniorhynchus* on buffalo, swine, and human bait located indoors were computed for collections made during the period of peak biting activity in the early evening hours in Taoyuan from June to September 1971. The collection at site 1 yielded 4 456 *C. annulus* and 160 *C. tritaeniorhynchus* from buffalo (28 : 1), 13 325 *C. annulus* and 486 *C. tritaeniorhynchus* from swine (27 : 1), and 1 490 *C. annulus* and 10 *C. tritaeniorhynchus* from man (149 : 1). At site 2, which was treated with an interior residual spray on 17 June 1971, the ratios were 103 : 1 (2 686 : 26) on buffalo, and 123 : 1 (4 906 : 40) on swine. Here 376 specimens of *C. annulus*, but no *C. tritaeniorhynchus*, were collected from human bait.

DISCUSSION AND CONCLUSION

At every site sampled, *C. annulus*, *C. tritaeniorhynchus*, and *C. fuscocephalus* showed a strong preference for mammalian hosts, while *C. p. fatigans* had fed predominantly on avian hosts. The samples of *C. tritaeniorhynchus* were admittedly small owing to its relative scarcity, and *C. p. fatigans* was often neglected in favour of the above-mentioned vector species. Our findings strongly suggest that *C. annulus* has greater avidity than *C. tritaeniorhynchus* and *C. fuscocephalus* for swine, a conclusion of epidemiological importance, since swine are the principal vertebrate reservoir of JE virus in the study area during the epidemic season. Other factors being equal, it follows that the species which feeds most on swine has a greater chance of becoming infected. Similarly, *C. p. fatigans* may be concluded to be of negligible importance since its feeds during the epidemic season come mainly from avian hosts and seldom from swine.

None of the *C. tritaeniorhynchus* and *C. fuscocephalus* blood meals identified was of human origin, and fewer than 1% of those from *C. annulus* and *C. p. fatigans* came from man. Although these species undoubtedly are zoophilic, the authors believe that significant contact may occur between man and *C. annulus* during the JE epidemic season. Each of the species tested is known to bite man in China (Province of Taiwan), although *C. fuscocephalus* may do so rarely.

Table 3. Mosquito forage ratios for man and domestic animals at principal mosquito collection sites[a]

Collection site	Mosquito forage ratios according to species or group						
	swine	bovine	dog	cat	human	All mammalian species	avian
			C. annulus				
Taoyuan (1)	4.8	17.6	6.5	0.0	0.1	2.1	0.1
(2)	6.8	21.9	14.9	0.0	0.1	2.8	0.1
(3)	2.4	4.2	45.4	0.0	0.1	3.2	0.1
(4)	9.9	13.6	6.5	0.0	0.0	3.0	0.1
(6)	5.9	6.1	7.1	0.0	0.0	2.9	0.0
Miaoli (1)	3.1	48.2	2.2	0.0	0.1	2.7	0.0
(3)	13.3	17.0	6.6	0.0	0.0	2.5	0.1
Pingtung (2)	3.4	29.9	6.1	0.0	0.0	2.4	0.0
(3)	5.1	37.3	3.0	0.0	0.0	2.3	0.1
			C. tritaeniorhynchus				
Taoyuan (1)	0.9	44.6	0.0	0.0	0.0	2.1	0.1
Miaoli (1)	4.3	50.7	0.0	0.0	0.0	2.7	0.0
(3)	6.0	92.4	0.0	0.0	0.0	2.6	0.1
			C. fuscocephalus				
Pingtung (2)	0.4	78.3	0.1	0.0	0.0	2.4	0.1
(3)	1.9	107.5	0.0	0.0	0.0	2.3	0.1
			C. p. fatigans				
Taoyuan (1)	0.0	0.8	12.4	0.9	0.9	0.5	1.4
(2)	0.1	9.3	9.2	0.0	0.0	0.6	1.2
(4)	0.0	0.0	3.9	0.0	0.0	0.3	1.3

[a] Ratios computed using census averages to the first decimal place.

Table 4. Relative abundance of female *C. annulus* in principal outdoor resting sites in Taoyuan County, June–September 1971 [a]

Habitat	Average no. of female *C. annulus* per 10 minutes of collection (% of total)			
	June	July	August	September
straw	15 (9)	218 (26)	2 779 (47)	170 (34)
fallow fields	107 (64)	395 (46)	975 (17)	99 (20)
ditch sides	27 (16)	37 (4)	1 074 (18)	94 (19)
bamboo fences	11 (6)	78 (9)	358 (6)	100 (20)
firewood piles	—	105 (12)	672 (12)	1 (1)
other [b]	8 (5)	19 (2)	—	37 (7)

[a] Collections were made by sweeping the habitats with a vacuum sweep-net usually between 10 h 00 and 15 h 00.

[b] Other habitats sampled included pathways, sweet-potato fields, and vegetable fields.

The sampling bias discussed by Barnett (1962) was largely avoided in this study by using a non-attractant type of collecting device, and observations were further quantified by applying the forage-ratio technique. Edman (1971) discussed some of the factors that may make it difficult or impossible to correlate host availability with host density, and that may lead to erroneous conclusions if fixed numerical weights such as forage ratios are attached to feeding data. None the less, we believe that the mosquito forage ratios are useful in interpreting our results. For example, the *C. annulus* forage ratio is higher for bovine hosts than for swine despite the fact that more than twice as many of the *C. annulus* blood meals came from swine, suggesting that the apparent preference of *C. annulus* for swine may be a function of host density. Significantly, the forage ratios also confirm the conclusion that *C. annulus* feeds more avidly on swine than do *C. tritaeniorhynchus* and *C. fuscocephalus*. An outstanding case of host preference confirmed by applying the forage ratio is that of *C. annulus* for dog over cat, even though cats outnumbered dogs in Taoyuan.

The collections of mosquitos attracted to human bait located indoors during the early evening hours in Taoyuan indicated that *C. annulus* was most active during the 1-hour period following sunset. These results are similar to those obtained by Hu & Grayston (1962) in their studies on *C. tritaeniorhynchus*. It appears, therefore, that these two important JE vectors may have similar activity patterns.

C. annulus was shown to be essentially exophilic in spite of the fact that it may outnumber the endophilic *C. p. fatigans* in indoor collections during the JE epidemic season, an observation that simply reflects the greater relative abundance of *C. annulus* during this period.

The attack rates of *C. annulus* and *C. tritaeniorhynchus* on buffalo bait were so much greater outdoors than indoors that possible sampling bias cannot change the conclusion that both species are principally exophagic. The comparative ratios of *C. annulus* to *C. tritaeniorhynchus* coming to buffalo indoors and outdoors suggest that *C. annulus* entered the stable almost 4 times as readily as *C. tritaeniorhynchus*. Similar ratios between the 2 species collected from swine and human bait located indoors during the early evening hours indicates that *C. annulus* was the principal vector species coming to these hosts during the 1971 JE epidemic season, whereas the scarcity of *C. tritaeniorhynchus* in the indoor human-bait collections, and its exophagic habits in general, suggest that this species was not a serious indoor threat to man at the Taoyuan sites.

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RÉSUMÉ

HABITUDES TROPHIQUES ET COMPORTEMENT DE 4 ESPÈCES DE *CULEX* DANS UNE RÉGION OÚ L'ENCÉPHALITE JAPONAISE EST ENDÉMIQUE

Au cours d'une enquête de 21 mois sur les habitudes trophiques et le comportement de 4 vecteurs, connus ou potentiels, de l'encéphalite japonaise en Chine (province de Taïwan), on a examiné par l'épreuve des précipitines 2839 repas de sang dont 2734 (96,3%) ont donné une réaction positive.

Culex annulus, *C. tritaeniorhynchus* et *C. fuscocephalus* se nourrissent principalement sur des mammifères, les premiers préférant les porcs (53,7%) et les deux autres les bovins (72,3 et 88,6% respectivement). Cependant si l'on tient compte de la proportion des hôtes disponibles, il semble que *C. annulus* prélève aussi nombre de ses repas de sang sur les bovins. *C. pipiens fatigans* se nourrit surtout sur les oiseaux (78,9%).

Aucun des repas de sang de *C. tritaeniorhynchus* et *C. fuscocephalus* n'avait été prélevé sur l'homme, et moins de 1% de ceux de *C. annulus* et de *C. p. fatigans* avaient cette origine. En dépit du comportement essentiellement zoophile dans cette région de chacune des espèces examinées, on a pu démontrer la fréquence des contacts entre l'homme et *C. annulus* durant la saison épidémique de l'encéphalite japonaise. On comptait 36%

de *C. annulus* parmi les 5160 moustiques capturés sur pièges humains, à l'intérieur des habitations, à Taoyuan, de mai à septembre. Cette espèce, surtout active pendant l'heure suivant le coucher du soleil, s'est néanmoins révélée principalement exophile et exophage.

Il semble, d'après ces observations sur les préférences trophiques et le comportement des diverses espèces, que *C. annulus* soit le principal vecteur de l'encéphalite japonaise dans la région de Taoyuan pendant la saison épidémique. Ce moustique est le plus abondant, il se nourrit surtout sur les porcs qui sont d'importants disséminateurs du virus et on en capture de nombreux spécimens sur pièges humains à l'intérieur des habitations. *C. tritaeniorhynchus*, peu abondant et plus exophage, ne représente apparemment pas une menace. De même, *C. p. fatigans*, qui se nourrit principalement sur les oiseaux, n'apparaît pas comme un vecteur éventuel. Quant à *C. fuscocephalus*, il n'a jamais été trouvé en grand nombre dans la région.

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