FLIGHT AND DISPERSAL OF JAPANESE ENCEPHALITIS VECTORS IN NORTHERN THAILAND

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ABSTRACT. Large numbers of engorged *Culex fuscocephala* Theobald, *C. gelidus* Theobald, and *C. tritaeniorhynchus* Giles were collected in CDC traps near buffalo or cattle pens, marked with fluorescent zinc-sulphide dusts, and released. CDC-trap collections at over 300 recapture stations were made. Most recaptured specimens were taken within 48 hours of their release. In many instances the recapture rate approximated 0.3%. *C. tritaeniorhynchus* were generally recaptured at greater distances (up to 1800 meters) from the release site than were the other 2 species. Dispersal was in a random fashion.

INTRODUCTION

In Thailand, the mosquitoes *Culex fuscocephala* Theobald, *C. gelidus* Theobald and *C. tritaeniorhynchus* Giles have been incriminated in the transmission of Japanese encephalitis (JE) virus (Simasathien et al., 1972; Gould et al., 1974). Large domestic animals are the principal hosts of these mosquitoes, and pigs are apparently the most important vertebrate reservoirs of JE virus in Thailand (Johnsen et al., 1974; Grossman et al., 1974). During 1972, we undertook a study of the flight range of the vector species, through the release and recapture of marked females, in the Chiangmai Valley of Northern Thailand. The main objective of this study was to measure the ability of these mosquitoes to disseminate JE virus by determining the time, distance and direction of their dispersal following engorgement upon domestic animal hosts.

MATERIALS AND METHODS

DESCRIPTION OF AREA. The Chiangmai Valley is located at 18° 45′ N latitude and 99° E longitude. The valley has an area of approximately 1500 km^2 and is surrounded by mountains rising to elevations of more than 2000 meters. Except for the two urban centers of Chiangmai and Lamphun, the population of the valley, estimated at 680,000 in 1969, is fairly evenly distributed in villages of 400–1000 people. Rice is the principal crop during the rainy season (April–November), and during that period paddy-fields occupy more than 70% of the valley floor. The three *Culex* species breed in enormous numbers in the flooded rice fields and irrigation ditches, and the females are attracted to feed on the numerous domestic animals present in the nearby villages. During the rainy seasons of 1969, 1970 and 1971 epidemics of Japanese encephalitis occurred in the Chiangmai Valley (Grossman et al., 1973). The area selected for these flight range studies is located in Saraphi district near the center of the valley and was the source of numerous encephalitis cases during those outbreaks. The study villages and intervening ricefields cover an area approximately 3.5 km in diameter (Fig. 1).

COLLECTION OF MOSQUITOES. Freshly engorged females of the three vector species were collected in large numbers in CDC light-traps set near animal pens during earlier studies in this area (Gould et al., 1974), so this technique was used to provide the mosquitoes for these mark-release studies. The CDC traps were operated between 1800 and 0600 hours in close proximity to buffalo or cattle pens within the study area. Engorged females were aspirated from the CDC collection bags and transferred to 45 x 45 x 45 cm holding cages, the bottoms of which were hinged to allow for release of the mosquitoes after marking. We estimated the number of mosquitoes per cage by counting those resting on each of 2 sides on which grids of 64, 5 cm squares were demarcated by string. Ten percent of these estimated totals

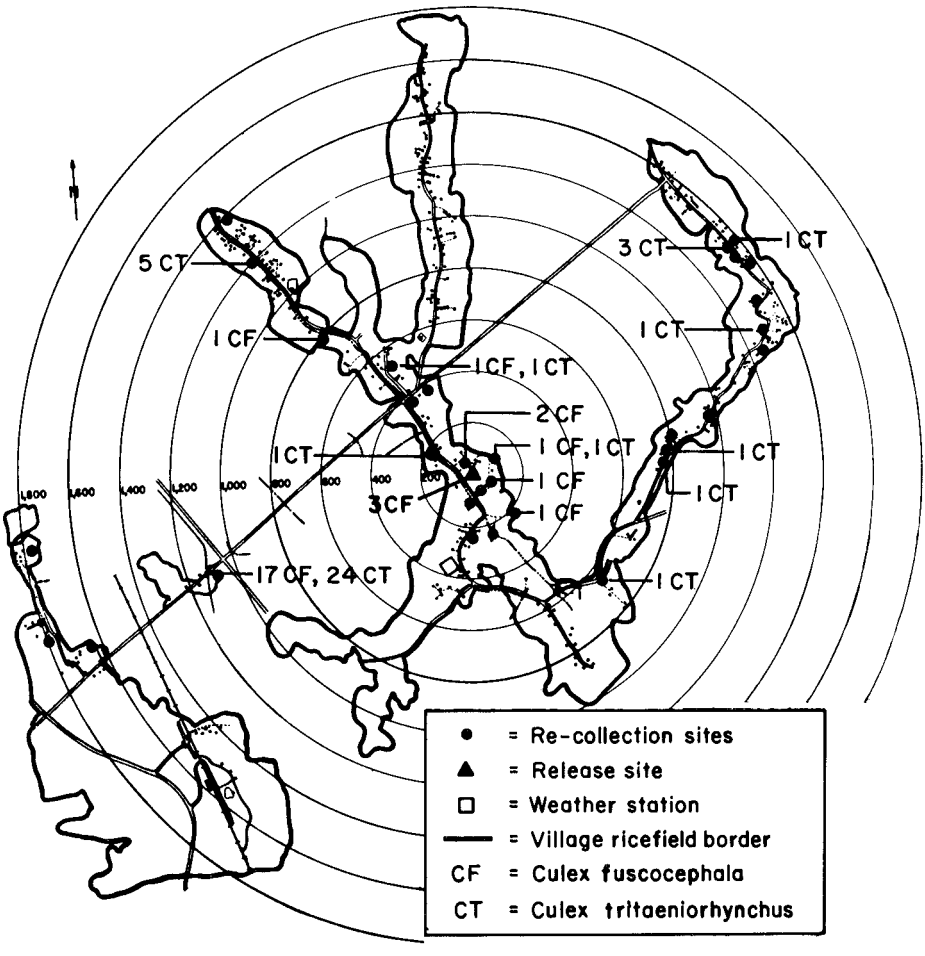

Fig. 1. Map showing location of sites where marked *C. fuscocephala* and *C. tritaeniorhynchus* were recaptured in the first experiment—July 1972. Circles are separated by 400 meters.

were removed from each cage and examined microscopically to determine the proportions of the three vector species present. Although we tried to select only freshly engorged females from the CDC traps, 11 to 20% of the mosquitoes in the samples were unfed. No sugar was given the caged mosquitoes; instead, rain-water soaked cotton pads were placed in the cages as a source of moisture during the holding period.

MARKING. Using techniques similar to those described by Bailey and his associates (1965), fluorescent zinc-sulfide dusts[1] were used to mark the mosquitoes just prior to release. Holding cages were inverted, placed inside close-fitting cardboard boxes, and marking dust was blown into the cages through a hole in the hinged floor. The amount of dust required to mark mosquitoes adequately varied according to the color used, and we found it

[1] Helecon Fluorescent Pigments #1757 (red), #1953 (green), #2200 (blue) and #2266 (yellow), United States Radium Corp., Hackettston, N.J.

necessary to examine them periodically under black light[2] until sufficient dust had been applied. A different color of dust was applied to mosquitoes according to the date of their collection.

RELEASE. Two releases of marked mosquitoes were made during this study. To obtain sufficient numbers of engorged females for the 1st release, mosquitoes were collected for 3 consecutive nights (19–20, 20–21 and 21–22 July); all were marked and released at 0730 hours on 22 July. Mosquitoes for the 2nd release were collected on the nights of 6–7, 7–8 and 8–9 August, but in this case each night's collection was held for 48 hours before it was marked and released at 0600 hours on 9, 10 and 11 August, respectively. The release point was the same in both experiments and was located near the center of the study area (Figs. 1, 2). Marked mosquitoes were released by opening the hinged bottoms of the inverted holding cages. All dead or immobile mosquitoes remaining in the holding cages after release were removed and counted; the majority (92%) of these were unfed females. Only seven males, all *Mansonia uniformis,* were found among these mosquitoes.

RECAPTURE. To recapture marked mosquitoes, CDC light-traps were placed near buffalo or cattle pens within the village of release and across rice fields in nearby villages, at distances up to 1800 meters from the release point. Thirty-six hours after the first release, CDC-trap collections were begun at the 32 sites shown in figure 1 and continued for the next 7 nights. In the 2nd experiment CDC traps were operated at 38 recapture stations (Fig. 2). Collections were begun 12 hours after the 1st batch of marked mosquitoes was released and continued for 9 nights.

Mosquitoes from recapture station collections were frozen until examined for marked specimens. Each trap collection was spread in a thin layer on the bottom of an enameled pan (37.5 cm x 32.5 cm) and quickly scanned under long wave radiation (3650°A) in an ultraviolet light cabinet.[3] Marked specimens were removed with forceps and confirmed under the 3x power of a stereoscopic microscope with a handheld ultraviolet lamp. All marked specimens were identified and their color marking and recapture sites recorded.

A recording anemometer,[4] similar to the one used by Bailey et al. (1965), was used throughout the course of this study to measure both the direction and velocity of winds (Fig. 1).

RESULTS AND DISCUSSION

An estimated 12,723 *C. fuscocephala,* 444 *C. gelidus,* and 21,380 *C. tritaeniorhynchus* were marked and released on 22 July; and, of these, 27 *C. fuscocephala* and 40 *C. tritaeniorhynchus* were recaptured (Table 1). Dispersal of both species appeared to be in all directions, and mosquitoes were recaptured in traps located as far distant as 1400 meters from the release point (Fig. 1). A majority (61%) of the recaptured specimens were collected in one trap, located at an isolated point surrounded by rice fields. Nighttime wind velocities during this period were usually less than 2 km per hour and were predominantly from the southwest. It is possible that the marked mosquitoes tended to concentrate at that point because it was the only place within hundreds of meters where livestock were sheltered during the night and was located upwind from the release point.

There were major differences in the stages of blood-meal digestion and ovarian development reached by the 3 groups of mosquitoes released together on 22 July. Thus, most of the females from the 19–20 July collection were gravid at the time of release, while blood-meals in the mosquitoes collected on 20–21 and 21–22 July

[2] SL 3600 Longwave, Hand model, 115V, 50–60 hertz, Blacklight Eastern Corp., Westbury, Long Island, N.Y.

[3] Chromato-vue Cabinet, Model C-5, Arthur H. Thomas Co., Philadelphia, Pa.

[4] California Spot Climate Station. The Foxboro Co., Foxboro, Mass.

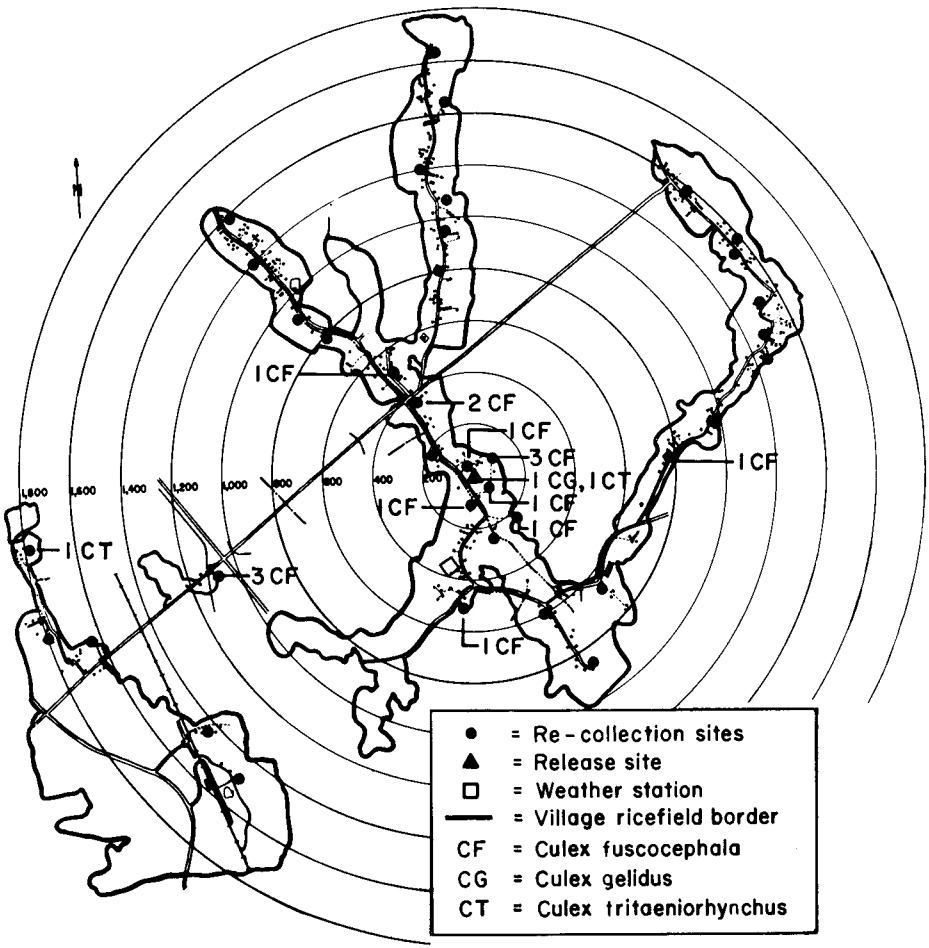

Fig. 2. Map showing location of sites where marked *C. fuscocephala, C. gelidus* and *C. tritaeniorhynchus* were recaptured in the second experiment—August 1972. Circles are separated by 400 meters.

were still only partially digested and ovarian development was incomplete. While the 19–20 July mosquitoes represented only 46% of the total number released, they constituted 88% of marked mosquitoes recaptured in this experiment. All of the 19–20 July mosquitoes which were recaptured were taken within 3 days of their release, while those mosquitoes released at earlier stages of blood-meal digestion were recaptured as late as 5–6 days after release. Freshly engorged specimens appeared to be less active and not as prone to fly as gravid mosquitoes at the time of release, and the above recapture results suggest that the newly blooded females probably rested nearby the release point for 24–48 hours or longer before beginning their dispersal flights.

Because 88% of the mosquitoes recaptured in the 1st experiment came from the group which were gravid at the time of release, we decided, in the 2nd experiment, to hold all mosquitoes for at least 48 hours prior to marking and release. An estimated total of 6,096 *C. fuscocephala,*

Table 1. First release-recapture experiment—July 1972.

Species released	Date and time collected 1800–0600 hrs.	Est'd no. released	% of total released	No. re-captured	% re-captured
C. fuscocephala	19–20 July	6,185	33.7	24	0.39
C. gelidus	"	238	01.3	0	0.0
C. tritaeniorhynchus	"	9,507	51.8	35	0.37
C. fuscocephala	20–21 July	4,637	34.1	1	0.02
C. gelidus	"	70	00.5	0	0.0
C. tritaeniorhynchus	"	4,962	36.5	4	0.08
C. fuscocephala	21–22 July	1,901	15.2	2	0.11
C. gelidus	"	136	01.1	0	0.0
C. tritaeniorhynchus	"	6,911	55.1	1	0.01

481 *C. gelidus* and 10,718 *C. tritaeniorhynchus* were released in this experiment; 15 *C. fuscocephala,* 1 *C. gelidus* and 2 *C. tritaeniorhynchus* were recaptured at various distances up to 1800 meters from the release point—the maximum distance at which recaptures were atempted (Fig. 2). One of the 2 *C. tritaeniorhynchus* recaptured was taken at this distance. The lone *C. gelidus* was recaptured in the vicinity of the release point. Although wind velocity and direction during this experiment were similar to those prevailing during the first release experiment, there was no similar concentration of marked specimens in traps located upwind from the release point.

The intervals that elapsed between the time of original collection and recapture of marked *C. fuscocephala* and *C. tritaeniorhynchus* are summarized for both experiments in Table 3. Eighty-six percent (36/42) and 78% (33/42) of the recaptured *C. fuscocephala* and *C. tritaeniorhynchus,* respectively, were freshly engorged at the time of recapture. The single *C. gelidus* recaptured, although collected nearby the release point, was also freshly fed. Forty-eight percent (20/42) and 76% (32/42) of the recaptured *C. fuscocephala* and *C. tritaeniorhynchus,* respectively, were collected at distances of 1000 meters or greater from the release point. We estimate that the mosquitoes recaptured at these distances had taken their pre-release blood meals from 96 to 132 hours earlier.

Very few studies have been made of the dispersal of blood-engorged mosquitoes. In Florida, Edman and Bidlingmayer (1969) collected freshly engorged mosquitoes of a variety of species at a central collection point surrounded by marshes and at least 1 mile distant from the nearest host. Wada et al. (1969) in Japan recap-

Table 2. Second release-recapture experiment—August 1972.

Species released	Date and time collected 1800–0600 hrs.	Est'd no. released	% of total released	No. re-captured	% re-captured
C. fuscocephala	6–7 Aug	1,708	27.4	5	0.29
C. gelidus	"	181	02.9	0	0.0
C. tritaeniorhynchus	"	3,039	48.8	0	0.0
C. fuscocephala	7–8 Aug	3,049	24.8	10	0.33
C. gelidus	"	267	02.2	1	0.37
C. tritaeniorhynchus	"	5,184	42.1	2	0.04
C. tritaeniorhynchus	8–9 Aug	1,339	19.8	0	0.0
C. gelidus	"	33	00.5	0	0.0
C. fuscocephala	"	2,495	36.8	0	0.0

Table 3. Summary of *Culex fuscocephala* and *C. tritaenirohynchus* re-captures at various time intervals after original collection, Chiangmai, Thailand July–August 1972.

Hours from original collection to recapture	Experiment #1				Experiment #2			
	C. fuscocephala		*C. tritaeniorhynchus*		*C. fuscocephala*		*C. tritaeniorhynchus*	
	No.	%	No.	%	No.	%	No.	%
74–86	0	...	1	2.5	0	30.0	0	0.0
96–108	23	85.1	30	75.0	6	40.0	2	100.0
120–132	4	14.9	7	17.5	7	46.6	0	0.0
144–156	0	0.0	0	0.0	1	6.7	0	0.0
168–180	0	0.0	2	5.0	1	6.7	0	0.0

tured marked *C. tritaeniorhynchus* at distances up to 8.4 km from the release point, although the mean dispersal distance recorded after 7 days was 1.0 km. These authors collected their mosquitoes for mark and release in "dry ice traps and at a horse shed," but they did not describe their condition (i.e., unfed, engorged or gravid) at the time of release. In our study, *C. tritaeniorhynchus* were generally recaptured at greater distances from the release site than were *C. fuscocephala.* In view of the flight ranges recorded for *C. tritaeniorhynchus* in Japan, it is probable that in Thailand this species is capable of making flights of greater lengths than the 1800 meter maximum observed in our Chiangmai Valley study. Because only 1 *C. gelidus* was recaptured, and because that specimen was taken nearby the release point, we were unable to draw any conclusions regarding this species' dispersal capabilities.

During the course of these studies, mosquitoes other than the JE vector species were recaptured. These species, belonging to the genera *Aedes, Anopheles, Culex* and *Mansonia,* are listed in Table 4. Included were some specimens which could not be identified beyond genus, possibly because of the damage sustained by 2 passages through light traps. Probably a large proportion of those identified as *Culex (Culex)* spp. were, in fact, *C. tritaeniorhynchus.* The maximum distance at which both *Aedes* and *Culex* species were recaptured was similar, but none of the *Anopheles* or *Mansonia* species was recaptured at a distance greater than 400 meters from the release site, even though many were recaptured 4 to 7 days following release.

Table 4. List of other mosquito species recaptured in release-recapture experiments, July–August 1972.

Species	Estimated number released	Number re-captured	% Re-captured	No. days release to re-capture	Greatest distance meters
Aedes lineatopennis	367	3	0.08	1	1,100
Aedes vexans	477	6	0.13	1–3	1,100
Aedes spp.*	...	2	...	1	1,100
Anopheles barbirostris group*	...	1	...	3	50
Anopheles nivipes	1098	5	0.05	1–7	400
*Anopheles peditaeniatus**	...	1	...	1	Release site
Anopheles vagus	587	5	0.09	1–4	400
Anopheles spp.*	...	1	...	1	100
Culex bitaeniorhynchus	202	2	0.10	1	1,100
Culex vishnui group	238	2	0.08	1	1,400
*Culex whitemorei**	...	1	...	2	1,100
Culex (Culex) spp.*	...	15	...	1	1,100
Mansonia uniformis	403	3	0.07	1–5	375

* No specimens in sample.

CONCLUSIONS

Recently engorged *Culex fuscocephala* and *C. tritaeniorhynchus* were recaptured at points located in all directions from the release point, across rice fields to other villages. The observed pattern of dispersal allows for rapid dissemination of JE virus from village to village. Thus, the feasibility of halting an outbreak of Japanese encephalitis in the Chiangmai Valley by control of vectors within villages where the disease first appears does not appear to be great.

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