Journal of Insect Science OPEN ACCESS

RESEARCH

Measure Post-Bloodmeal Dispersal of Mosquitoes and Duration of Radioactivity by Using the Isotope ^{32}P

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Subject Editor: Thomas Scott

J. Insect Sci. 14(196): 2014; DOI: 10.1093/jisesa/ieu058

ABSTRACT. The radioactive isotope ^{32}P-labeled disodium phosphate ($Na_2H^{32}PO_4$) was injected via the jugular vein into a cow kept in a shed in Maozhuang Village, Cao Township of Shanxian County, China. Over the following 5 d, mosquitoes feeding on the cow were captured at distances up to 400 m to determine dispersal distance. The duration of radioactivity in the cow and marked mosquitoes was 10 d. The results showed that after blood feeding, *Anopheles sinensis* and *Culex tritaeniorhynchus* temporarily rested in the cattle shed and then flew outdoors. In contrast, *Culex pipiens pallens* remained in the cattle shed after feeding. These findings confirmed that local *An. sinensis* and *Cx. tritaeniorhynchus* were partially endophilic and tended to rest out of doors, whereas *Cx. pipiens pallens* was endophilic. For marked *An. sinensis* and *Cx. tritaeniorhynchus*, there was a significant tendency for dispersal to be in a northeast and east direction, probably because of the presence of heavy shading by an agricultural field, a small river for mosquito oviposition sites, and locations downwind from the blood source. The furthest flight distances for *An. sinensis* and *Cx. tritaeniorhynchus* were 210 and 240 m; therefore, control of these mosquitoes should include resting places indoors and outdoors within a radius of 250 m from confirmed cases.

Key Words: mosquito dispersal, isotope, *Anopheles sinensis*, *Culex tritaeniorhynchus*, *Culex pipiens pallens*

The dispersal of mosquitoes is related to seeking blood hosts, nectar sources, mates, oviposition sites, and resting sites (Killeen et al. 2003, Chaves et al. 2010). Knowledge of the dispersal of mosquito species in rural villages can provide key data for the determination of the vector control range for prevention of secondary cases, and allows vector control authorities to design and implement efficient strategies for vector control (Tempelis 1975, Vinogradova 2000). Therefore, the study of dispersal ranges of mosquitoes is an important factor to detect potential sources of infection and prevent transmission (Liu et al. 2012).

Mark-release-recapture techniques have provided useful insights into the adult mosquito gonotrophic cycle, survival, feeding behavior, population sizes, and dispersal range (Silver 2008). The mark-release-recapture technique has been widely used to investigate the behavior of mosquitoes, such as *Anopheles* species (Toure et al. 1998; Tsuda et al. 1999, 2000; Achee et al. 2005, 2007; Fabian et al. 2005; Midega et al. 2007; Hii et al. 2008; Liu et al. 2012) and *Culex* species (Lindquist et al. 1967, Wada et al. 1969, Dow 1971, Reisen et al. 1978, Tsuda et al. 2008, Estep et al. 2010, Ciota et al. 2012, Greenberg et al. 2012). Service (1997) reviewed all aspects of adult mosquito dispersal. However, so far, only Greenberg et al. (2012) and Russell et al. (2005) have analyzed the post-bloodmeal flight distances in mosquitoes.

Dispersal and survival are of considerable importance in studying the ecology of mosquitoes (Baber et al. 2010). Measuring the dispersal range is critically important for vector control and the prevention of vector-borne disease. *Anopheles sinensis* Wiedemann plays a major role in the maintenance of *Plasmodium vivax* malaria transmission in China (Liu et al. 2011). *Culex tritaeniorhynchus* Giles is the primary vector of the Japanese encephalitis virus, and *Culex pipiens pallens* Coquillett is the primary vector of the Japanese encephalitis virus and filariasis in China (Rosen et al. 1980, Masuoka et al. 2010). However, little information is available regarding the dispersal range of mosquitoes in the Heze District of Shandong Province, China. In this study, we investigated the dispersal distances of these three prevalent mosquitoes in Shandong Province, using the isotope ^{32}P-labeled disodium phosphate as the marker.

Materials and Methods

Study Area. This study was conducted in Maozhuang Village, Cao Township of Shanxian County, Heze District (34° 46′41″ N, 116° 04′39″ E). This isolated village is ~2 km from other villages and located in the southwest of Shandong Province in the delta between the ancient and modern Yellow River. The immediate area surrounding the village consisted of heterogeneous farmland ~250 m away with soybean, sorghum, corn, etc. The study within an area delimited to the northeast by a small river. There was one country road from north to the south. In this village, there were eight houses, one cattle shed in the center of the village, one cattle shed south of the village, and two cattle sheds located west of the village.

The cattle shed in the center of this village was selected as the test shed, and a study area of 400-m radius from the cattle shed boundaries was defined (Fig. 1), the area was subdivided into northwest, northeast, southeast, and southwest quadrants. Based on radii at 1–50, 51–100, 100–150, 151–200, 201–250, 251–300, 301–350, and 351–400 m from the boundary of the release cattle shed, 120 sticky ovitraps were established. During the study period (10–14 August 2011), the wind velocity was low (~1.6–5.3 m/s), and the main wind direction was southwest. The relative humidity (RH) was 70–76%, the temperature was 27–36°C, and the environmental conditions were all favorable.

Injection of Cow With $Na_2H^{32}PO_4$. Before the experiment, one healthy cow (355 kg) was selected and physically examined by a veterinarian. In addition to this cow, there were two cows and two horses in the same cattle shed. At 4:30 p.m. 9 August 2011, $Na_2H^{32}PO_4$ was injected via the jugular vein of the cow; 43 mCi of $Na_2H^{32}PO_4$ was injected (0.1211 mCi/kg) (Zhang et al. 2014). After injection, the cow was kept in the original livestock shed.

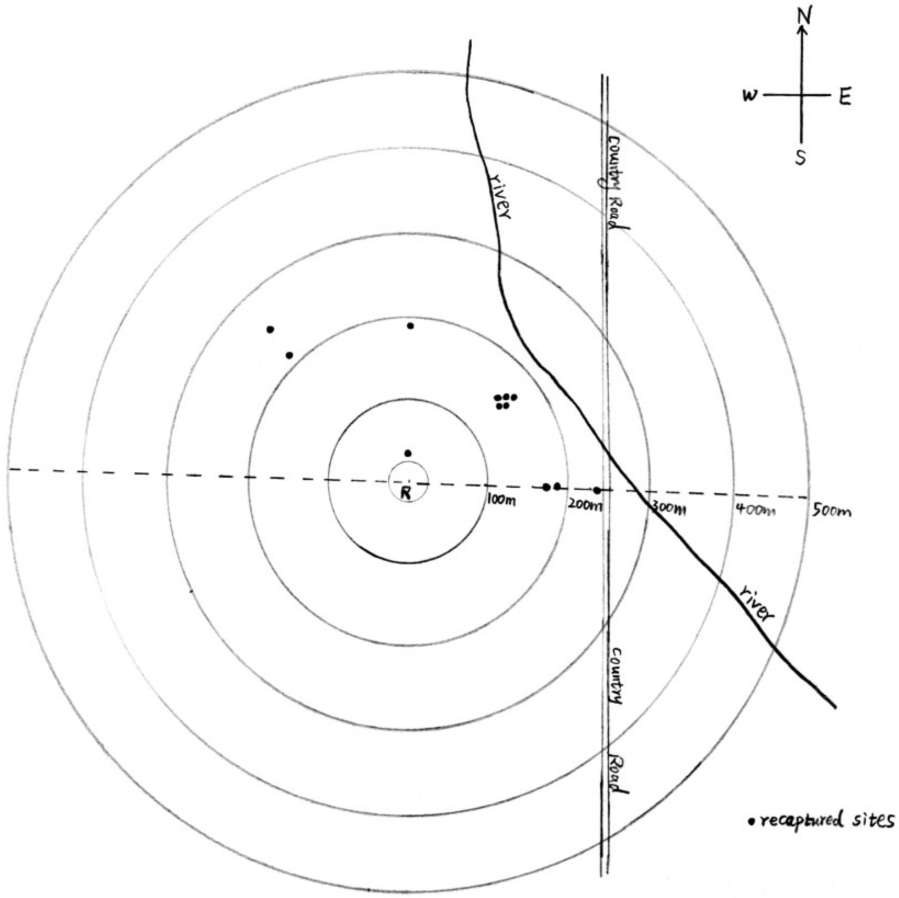

Fig. 1. Map of the recapture sites; each annulus is 100 m consecutively farther from the boundary of the release point (R).

Capturing Mosquitoes. After $Na_2H^{32}PO_4$ was injected into the cow, mosquitoes were captured with handheld battery-operated aspirators during the following 5 d in human houses and livestock sheds. Sticky ovitraps were established to capture mosquitoes outdoors in the village within 400 m (seven zones were created: 0–50, 51–100, 101–150, 151–200, 201–250, 251–300, and 301–400 m). Sticky ovitraps were prepared as described by Ritchie et al. (2003, 2004) and Russell and Ritchie (2004). Captured mosquitoes were brought to the laboratory and species identified using a dissecting microscope. Labeling condition and duration of radioactivity of mosquitoes were also measured; after the injection of the cow, every evening several mosquitoes were captured blood feeding on the cow and tested for ^{32}P as described below.

Measuring Methods. The measurement of radioactivity was conducted using a liquid scintillation counter (Model YSJ-76). One day before injection of ^{32}P, some adult mosquitoes were captured from the local area to measure normal background radiation intensity, which was determined as ~19–20 counts per minute. For assay, mosquitoes were anesthetized with ether and placed in a β-bell counter tube of the vitriol chambers for 1 min. Counts that exceeded 50% of background (>30 counts per minute) were considered positive.

Ethics Clearance and Informed Consent. The experimental project was reviewed and approved by the Ethical Committee of Shandong Academy of Medical Sciences (Jinan, Shandong). Also, permission was obtained from the Municipal Health Bureau and Center for Disease Control and Prevention in Heze District. The experiment posed very low risk of public health because the radioactivity intensity was determined to be within the safety dosage (Zhang et al. 2014). Urine and feces of the cattle were collected and sent to the Institute of Radiation Medicine, Shandong Academy of Medical Sciences for appropriate processing to prevent spread of the isotope; the half-life of ^{32}P was 14.3 d. In addition, written consent was obtained from the households to permit mosquito collection from their houses and livestock sheds. Individuals who conducted the catches during the study were trained.

Results

Mosquito Numbers Captured and Radioactively Labeled. From 10 to 14 August (within 5 d after injection), a total of 1,540 female mosquitoes were captured: 341 from human houses, 253 in the test cattle shed, 303 in other cattle sheds, and 643 outdoors. Of all female mosquitoes captured, 106 were marked; the marked *An. sinensis*, *Cx. pipiens pallens*, *Cx. tritaeniorhynchus*, and other mosquito species were 30, 7, 59, and 10, respectively. ^{32}P-labeled mosquitoes were found only in the cattle shed and outdoors. The ^{32}P-labeled *An. sinensis* and *Cx. tritaeniorhynchus* were found only in the test cattle shed and outdoors, whereas *Cx. pipiens pallens* and other ^{32}P-labeled mosquitoes were found only in the test cattle shed (Table 1).

Mosquito Numbers Captured and Radioactively Labeled at Various Distances. Of 143 *An. sinensis* and 345 *Cx. tritaeniorhynchus* captured outdoors, each of the 6 were labeled and distributed in the north, northeast, east, and northwest from the test shed. The number captured and ^{32}P-labeled *An. sinensis* were distributed 101–150, 151–200, and 201–250 m from the test shed. The number captured and ^{32}P-labeled *Cx. tritaeniorhynchus* were distributed 0–50, 101–150, 151–200, and 201–250 m from the test shed (Table 2). After bloodmeal, the dispersal distance of *An. sinensis* and *Cx. tritaeniorhynchus* was 210 and 240 m, respectively.

Conditions of Mosquitoes Radioactively Labeled and Duration of ^{32}P. Within 5 d, a total of 253 female mosquitoes were captured in the test cattle shed; among them, 94 were marked (37%). Otherwise, 51

Table 1. Number of mosquito species captured and labeled from different places

Species	Houses		Shed with injected cow			Other cattle sheds		Outdoors			Total		
	No. collected	No. labeled	No. collected	No. labeled	Percentage	No. collected	No. labeled	No. collected	No. labeled	Percentage	No. collected	No. labeled	Percentage
An. sinensis	72	0	57	24	42.1	82	0	143	6	4.19	354	30	8.47
Cx. tritaeniorhynchus	28	0	139	53	38.1	186	0	345	6	1.74	698	59	8.45
Cx. pipiens pallens	240	0	21	7	33.3	8	0	41	0	0	310	7	2.26
Other mosquitoes	1	0	36	10	27.8	27	0	114	0	0	178	10	5.61
Total	341	0	253	94	37.1	303	0	643	12	1.87	1,540	106	6.88

Table 2. Number and percentage of mosquitoes captured and labeled at various distance

Species	Distance (m)													
	0–50		51–100		101–150		151–200		201–250		251–300		301–400	
	No. collected	No. labeled	No. collected	No. labeled	No. collected	No. labeled	No. collected	No. labeled	No. collected	No. labeled	No. collected	No. labeled	No. collected	No. labeled
An. sinensis			2	0	70	4	18	1	36	1	13	0	4	0
Cx. tritaeniorhynchus	3	1	5	0	78	1	82	2	157	2	11	0	9	0
Cx. pipiens pallens					23	0	5	0	10	0	3	0		
Other mosquitoes			2	0	17	0	46	0	37	0	2	0	10	0

Table 3. Mosquitoes captured from the cow's body and in the cattle shed

Collect place		Cow's body				Shed with the injected cow	
Collect date		9 Aug.	9–13 Aug.	19 Aug.	24 Aug.	10–14 Aug.	19 Aug.
Species	Time after injection of ^{32}P into cow	20 min	20 min to 4 d	10 d	15 d	1–5 d	10 d
An. sinensis	No. mosquito	2	3	20	17	57	24
	No. labeled	1	2	1	0	24	3
Cx. tritaeniorhynchus	No. mosquito	3	47	10	38	139	34
	No. labeled	3	29	2	0	53	1
Cx. pipiens pallens	No. Mosquito		1			21	
	No. labeled		1			7	
Other mosquitoes	No. mosquito					36	
	No. labeled					10	
Total	No. mosquito	5	51	30	55	253	58
	No. labeled	4	32	3	0	94	4

female mosquitoes were captured from the cow's body, and 32 were marked (62%). A greater proportion of ^{32}P-labeled mosquitoes was collected from the cattle body (62%) compared with the cattle shed (37%) (Tables 1 and 3). After injection, most of the female mosquitoes captured in the cow's body and test shed were labeled within 10 d (Table 3). After $Na_2H^{32}PO_4$ was injected into the cow, for up to 1 month, no adverse reaction was observed.

Table 4 shows that, after blood feeding on the cow, for female mosquitoes captured outdoors, on the cow's body, or in the test shed, the radioactivity intensity in the blood of the cow gradually decreased. After 15 d, the mean radioactive intensity of mosquitoes fed on the cow was close to the background (Table 4).

Discussion

The total number of female mosquitoes captured was 1,540 during the study period, with *Cx. tritaeniorhynchus* being the most prevalent (698), versus *An. sinensis* (354) and *Cx. pipiens pallens* (310), and other mosquitoes (178). According to the results, we can determine that, in this region, these three species dominate. Within 5 d, all the ^{32}P-labeled *An. sinensis* and *Cx. tritaeniorhynchus* mosquitoes were captured only in the test cattle shed containing the injected cow, and outdoors. However, all the ^{32}P-labeled *Cx. pipiens pallens* mosquitoes remained in the test shed with the cow, and were not found outdoors. After blood feeding, most of the *An. sinensis* and *Cx. tritaeniorhynchus* mosquitoes stayed at the blood source temporarily; others flew outdoors rather than resting in the human houses or other cattle sheds, whereas *Cx. pipiens pallens* preferred to stay in the room that contained the cow to feed on, rather than traveling to other places. These findings confirmed that the local *An. sinensis* and *Cx. tritaeniorhynchus* mosquitoes were half residential and tended to be outdoors, whereas *Cx. pipiens pallens* was a residential mosquito species (Tables 1, 2, and 5).

In our study, within 5 d after blood feeding on the cow, the furthest dispersal distance for *An. sinensis* and *Cx. tritaeniorhynchus* was 210 and 240 m, respectively; otherwise, *Cx. pipiens pallens* remained in test shed. For *An. sinensis*, the dispersal distance in this study was shorter than that of the studies in Yongcheng, China (Liu et al. 2011) and Gyeonggi-do, Korea (Cho et al. 2002). The results of *An. sinensis* were also consistent with the experiment in Yongcheng, as in their study, 80 and 90% of the marked *An. sinensis* were recaptured within a radius of 100 m from the release point, and the furthest recapture ranges were

Table 4. Levels of radioactivity in the mosquitoes collected at different time and places after start of experiment

Collection places			Outdoors	Cow's body				Shed with injected cow						
Collection date			11–14 Aug.	9 Aug.	13 Aug.	19 Aug.	24 Aug.	10 Aug.	11 Aug.	12 Aug.	13 Aug.	14 Aug.	Total 10–14 Aug.	19 Aug.
Days after injection of ^{32}P			2–5 d	20 min	4 d	10 d	15 d	1 d	2 d	3 d	4 d	5 d	1–5 d	10 d
An. sinensis	No. Mosquito		6	1	2	1	5	1	2	3	6	12	24	3
	CPM	Total	263	99	147	34	115	37	153	142	240	612	1,184	148
		Mean	43.8	99	73.5	34	23	37	76.5	47.3	40	51	49.3	49.3
Cx. tritaeniorhynchus	No. Mosquito		6	3	29	2	8	2	11	4	3	33	53	1
	CPM	Total	305	165	1,373	64	186	158	533	176	137	1,293	2,297	42
		Mean	50.8	55	47.3	32	23	79	48.4	44	45.6	39.1	43.3	42
Cx. pipiens pallens	No. mosquito				1				1		2	4	7	
	CPM	Total			45				41		90	161	292	
		Mean			45				41		45	40.3	41.7	
Other mosquitoes	No. mosquito								1	5		4	10	
	CPM	Total							46	234		139	419	
		Mean							46	46.8		34.7	41.9	
Total	No. mosquito		12	4	32	3	13	3	15	12	11	53	94	4
	CPM	Total	568	264	1,565	98	301	195	733	552	467	2,205	4,192	190
		Mean	47.3	66	48.9	32.7	23.1	65	51.7	46	42.4	41.7	44.5	47.5

CPM, counts per minute.

Table 5. Dispersal range and resting places of An. sinensis and Cx. tritaeniorhynchus

Mosquito marked		Date captured	Days after blood fed	Captured place		Distance from blood source (m)
Species	Mosquito			Direction from village	Crop type	
An. sinensis	A	11 Aug.	2 d	Northeast	Soybean, sorghum	150
	B	11 Aug.	2 d	East	Soybean, corn	193
	C	13 Aug.	1 d	Northeast	Soybean, sorghum	150
	D	13 Aug.	2 d	Northwest	Soybean, corn	210
	E	14 Aug.	1 d	Northeast	Soybean, sorghum	150
	F	14 Aug.	2 d	Northeast	Soybean, sorghum	150
Cx. tritaeniorhynchus	G	11 Aug.		North	*Helianthus tuberosus*	30
	H	11 Aug.		East	Soybean, corn	193
	I	11 Aug.		Northwest	Soybean	240
	J	14 Aug.		East	Roadside shrub	230
	K	14 Aug.		East	Tussock, grassplot	180
	L	14 Aug.		Northeast	Soybean, sorghum	150

300–400 m (Liu et al. 2011). In addition, the dispersal distance of *Cx. tritaeniorhynchus* was shorter than that of the studies by Reisen et al. (1978) in Pakistan and Wada et al. (1969) in Japan.The dispersal distance of *Cx. pipiens pallens* was shorter than sibling species *Culex quinquefasciatus* as described by Reisen et al. (1992) in rural areas in the United States and by Lindquist et al. (1967) in Rangoon. In their study, they analyzed only unfed females, and for the fed female mosquitoes, they did not measure their distances. Greenberg et al. (2012) found that mosquitoes captured at the zoo flew no more than 170 m, with an average distance of 106.7 m, after taking a bloodmeal. Russell et al. (2005) found, within 8 d, the furthest flight distances for *Aedes aegypti* was 200 m from the release point, and the mean distance traveled was 78 m. So, in this study, results were consistent with them.

In our study, although there were cattle sheds to the south and west of the village, no marked female mosquitoes were captured. The marked mosquitoes most often dispersed to the north, northeast, and east of the village. This finding suggests that the dispersal pattern of *An. sinensis* and *Cx. tritaeniorhynchus* may correlate with wind direction after blood feeding because, from 9 to 13 August, although the wind velocity was low (1.6–5.3 m/s), the main wind direction was southwest; thus, additional ecological and meteorological influences, likely related, drive dispersal in the area. It seems that after blood feeding, mosquitoes were not attracted by other cows.

From our study, after blood feeding, we can see that these three species of mosquitoes have different dispersal distances. Previous research showed that the dispersal distance of mosquitoes is mainly influenced by local environmental characteristics rather than mosquito species. The cattle shed with the labeled cow was located in an isolated village far from other villages. Other factors, such as riverside, which was the major habitat, did not apply, as no mosquitoes flew more than 240 m from the cattle shed with the injected cow. So, during the critical period, when a confirmed or suspected mosquito case is reported, the emergency mosquito control and prevention measures for *An. sinensis* should include various places indoors and outdoors, larvae, and adults within a 210-m radius of confirmed or suspected cases; 100–150 m is the key radius of the vector control activities. For *Cx. tritaeniorhynchus*, the focus should be within 240 m, whereas for *Cx. pipiens pallens*, the focus should be on the resident houses.

After injection, within 10 d, most of the female ^{32}P-labeled mosquitoes were captured on the cow's body and in the test shed (Table 3). A greater percentage of the blood-fed mosquitoes was found on the cow's body than in the cattle shed. There are two possible reasons for this: 1) in addition to the injected cow, two other cows and two horses were in the same cattle shed and 2) among the female mosquitoes in the cattle shed, excluding those on the cow's body, some mosquitoes did not take blood.

In conclusion, we have estimated the dispersal distances of *Cx. pipiens pallens*, *An. sinensis*, and *Cx. tritaeniorhynchus* by mark capture experiments using ^{32}P as the marker. From our results, we suggest that there is a need to conduct more mark capture experiments using this method. Information on the dispersal of mosquitoes is important especially where mosquito control by reducing human–vector contact is a priority. This information is also necessary for mosquito vector control programs focusing on integrated vector management methods where dispersal data are important for determining the range of barrier zones around management areas.

Acknowledgments

This study was supported by the National Natural Science Foundation of China (NSFC) (grant 81271877) and the development of medical science and technology project of Shandong Province (grant 2013WS0353).

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Received 4 August 2013; accepted 1 March 2014.