MARK-RELEASE-RECAPTURE EXPERIMENTS WITH *ANOPHELES SAPEROI* (DIPTERA: CULICIDAE) IN THE YONA FOREST, NORTHERN OKINAWA, JAPAN

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Abstract. Six mark-release-recapture experiments with *Anopheles saperoi* Bohart and Ingram were performed in the Yona Forest, northern Okinawa, Japan from June 1998 ~ November 1999, in order to estimate the gonotrophic cycle, survival, spatial distribution, flight range, and population size of *An. saperoi*. Adults and immature *An. saperoi* were collected from the Yona Forest area, taken to the laboratory and maintained under a controlled temperature and humidity in order to get a lager number of mosquitos for the mark-release-recapture experiments. Cohorts of *An. saperoi* females and males, numbering 3,016, 4,728, 327, and 2,603 for experiments I, II, III, and IV, respectively, were released. Cohorts of *An. saperoi* females only, numbering 709 and 586 for experiments V and VI, respectively, were also released. At the release site, the *An. saperoi* were marked with 0.5% fluorescent dye Rhodamine B and released. The recapture rates were 14 (0.93%), 33 (1.40%), 3 (1.83%), 32 (2.46%), 14 (1.97%), and 22 (3.75%) for experiments I, II, III, IV, V, and VI, respectively. The length of the gonotrophic cycle of the recaptured *An. saperoi* females was estimated to be about 4 days, through the dissection method. The daily survival rate was estimated to be 0.73 by regression coefficient. The spatial distribution of marked, recaptured *An. saperoi* was similar to that of unmarked captured *An. saperoi*. The spatial distribution of the marked, recaptured *An. saperoi* among the collection site categories was significantly different. The observed differences in the frequency distribution of marked recaptured *An. saperoi* were considered to be due to the spatial variation of the habitats between the collection sites. The maximum flight range of the recaptured *An. saperoi* recorded in this study was 0.93 km. The population size of *An. saperoi* females in the study area was estimated by the Seber method to be 23,841, 1,182, 3,514, 5,679, and 9,238 for experiments I, II, IV, V, and VI, respectively. The estimated population size has a low standard of error using the Seber method, therefore we estimated our population size reasonably well. The population attributes and ecology of *An. saperoi* in the Yona Forest, in northern Okinawa are discussed.

INTRODUCTION

Mark-release-recapture experiments provide the best method for estimating cohort specific dispersal, survival, flight range, and the gonotrophic cycle of mosquitos. These population attributes are important precursors to planning control programs for mosquito-borne diseases. In addition, information on survival, longevity and dispersal are of considerable interest when studying the ecology of vectors mosquito-borne diseases, such as malaria. These important population attributes and other time related biological phenomena can best be studied under field conditions, using mark-release-recapture techniques. Service (1976) has reviewed the literature applying to mosquito population parameters. Extensive mark-release-recapture experiments have been conducted on anopheline mosquitos to study spatial distribution, survivorship, population sizes, and the gonotrophic cycles of malaria mosquito vectors, such as *Anopheles balabacensis* (Hii and Vun, 1985), *An. maculatus* (Loong *et al*, 1990; Chiang *et al*, 1991), *An. stephensi* (Quraishi *et al*, 1966; Reisen *et al*, 1979), *An. gambiae* (Mnzava *et al*, 1995; Thomson *et al*, 1995; Costantini *et al*, 1996; Toure *et al*, 1998), *An. farauti* (Charlwood and Graves, 1987), *An. mini-*

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mus (Nutsathapana *et al*, 1986; Tsuda *et al*, 1999), and *An. vestitipennis* (Arredondo-Jimerez *et al*, 1998). However, no comparable mark-release-recapture data are available for *Anopheles saperoi* Bohart and Ingram, to evaluate population dynamics, such as survival, spatial distribution, and flight range.

In 1946, Bohart and Ingram first described *An. saperoi* on Okinawa Island. *An. saperoi* has a relative limited geographical distribution. It is exclusively found on the northern part of Okinawa Island and always occurs in forest areas, where they breed in the clean water of mountain streams. *An. saperoi* is a daytime biting mosquitos, which is unique from other anopheline mosquitos (Toma and Miyagi, 1981). The ecology of *An. saperoi* had not been studied in nature.

Mark-release-recapture experiments with *An. saperoi* were performed in the Yona Forest area, northern Okinawa, Japan from June 1998 to November 1999. During the first four experiments, cohorts of male and female *An. saperoi* mosquitos were marked and released. During the last two experiments, only cohorts of female *An. saperoi* mosquitos were marked and released. The population parameters, such as population size, survival, spatial distribution, gonotrophic cycle, and flight range of *An. saperoi* are discussed.

MATERIALS AND METHODS

The study area

The Yona Forest area is located about 120 km north of the University of the Ryukyus, at 26º 45′ 30′′ N and 128º 05′ 00′′ E (Fig 1). The climate of Okinawa Island is subtropical. The temperature in the Yona Forest area rises gradually from April to its highest in August, then gradually falls to its lowest in February. Experiment I was carried out during the summer, in June 1998, where the mean temperature recorded was 27.3ºC. Experiment II was performed during the winter, November-December 1998, when the temperature ranged from 18.3º-23ºC, with a mean temperature of 22.3ºC. Experiment III was carried out in the spring, April 1999, when the mean temperature was 19.2ºC. Experiment IV was carried out in the summer, June 1999, when the temperatures ranged from 25º-29.8ºC, with a mean temperature of 27.3ºC. Experiment V was carried out in the summer, August 1999, when the mean temperature was 28.2ºC. Experiment VI was carried out in the winter, November 1999, when the mean temperature was 21.8ºC (Table 4). Rainfall in the Yona Forest area fluctuates throughout the year, with peaks in May, June, and September. The climax vegetation of the Yona Forest area is subtropical evergreen broad-leaf forest.

Mosquito rearing

Adult and immature *An. saperoi* were collected from the study area. Specimens were taken to the laboratory of Medical Zoology, University of the Ryukyus for breeding. *An. saperoi* were maintained in the insectary with a controlled temperature of 25ºC, 15 hours of light per day, and a relative humidity of 80%. Larvae were reared in plastic containers (41 x 29 x 6.5 cm) containing seasoned water. Larvae were fed daily pulverized food consisting of a 1:1 ratio of brewer's yeast and ground mice pellets. The wild caught *An. saperoi* females were allowed to feed on the blood of mice. Engorged females were separated from un-engorged females and held in a medium sized cage (20x30x20 cm) for egg maturation. Four days

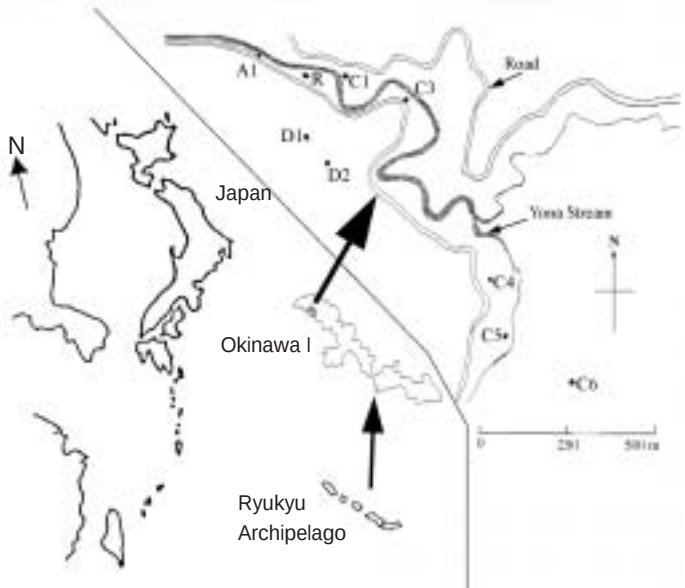

Fig 1–Location of nine collection sites in the mark-release-recapture study of *An. saperoi* in the Yona Forest, Okinawa Island, Japan.

later, gravid females were transferred into small plastic vials (5 x 3 cm) lined with wet tissue paper. The *An. saperoi* oviposited their eggs in the vials. On hatching, the larvae were reared as described above, in order to have a large number of adult *An. saperoi* of the same brood for mark-release-recapture experiments. These mosquitos, aged 1-4 days old, were specifically counted and morphologically identified as to sex before being released into a large cage (56 x 32 x 56 cm). For the cohorts consisting of females and males, the sex ratio of females to males was 1:1. Emerged *An. saperoi* were offered 2% sucrose solution with a cotton pad and transported to the study area for mark-release-recapture experiments.

Mosquito marking and releasing

Laboratory reared *An. saperoi* 1-4 days old were used for the mark-release-recapture experiments. For the first four experiments, cohorts of *An. saperoi* females and males were marked and released. While for the last two experiments, cohorts of *An. saperoi* females only were marked and released. At the release site, cages were put under the shade of bushes. The *An. saperoi* were marked with a 0.5% solution of fluorescent dye Rhodamine B (Wako Pure Chemical Industries Ltd, Japan). Marked *An. saperoi* were left in cages for one hour in order to dry. At 13 00 hours, when the activities of wild *An. saperoi* were observed to be at their peak, the sleeve of each cage was carefully opened to allow the *An. saperoi* to escape and mix with the wild population. The cages were left under the shade for 30 minutes. Marked *An. saperoi* remaining in the cage after 30 minutes were considered dead and their number was subtracted in subsequent calculations.

Mosquito collection

The collection method for mosquitos was the same throughout all the mark-release-recapture experiments. Collection of mosquitos was begun on the day following release. In experiment I, collection was carried out for eight subsequent days, whereas for experiments II and III, collections were carried out for seven subsequent days, and for experiments IV and V collections were carried out for ten subsequent days. In the previous five experiments, *An. saperoi* females were recaptured up to the tenth day post-release and also at the farthest collection site C5, which was located 930 m from the release site. Therefore, in experiment VI collection days were increased to 12 days and an extra collection site, C6, located 1,165 m from the release site was added (Fig 1). Collections of mosquitos were made at nine fixed collection sites that had different environmental conditions. Different environmental conditions were chosen in order to observe the differences in the number of marked and unmarked specimens captured in different environmental conditions. The collection sites were grouped into four environmental categories, according to their respective habitats. The environmental conditions were as follows. The first category site was release site R, which was located near Yona stream, surrounded by forest and green vegetation. The second category sites were streamside collection sites C1 and A1, located 110 m and 150 m from the release site, respectively. The third category sites were foothill collection sites D1 and D2, located in the foothills about 350 m above sea level; their distances from release site were 175 m and 250 m, respectively. The fourth category sites were forest collection sites C3, C4, C5, and C6, These collection sites were located in the middle of the forest; their distances from the release site were 445, 790, 930 and 1,165 m, respectively. The mosquitos were collected from 09 00-16 30 hours. At each collection site, host seeking mosquitos were captured by one person with a hand net for 20 minute/day. The captured mosquitos were held in a small cage (12x16x12 cm) labeled specifically for the site of collection, for species identification and counting.

On the same day, all the captured mosquitos were killed and morphologically identified by species and sex (Toma and Miyagi, 1986). The *An. saperoi* were examined for the presence or absence of dye. Male *An. saperoi* were not collected at any of the sites in any of the experiments. To check for the presence of dye, the *An. saperoi* females were placed on filter paper and rinsed with a few drops of 80% alcohol and examined for the dye under ultraviolet light. All marked and unmarked caught *An. saperoi* females were dissected under the dissecting microscope to determine parous and insemination rates. In the first two experiments,

wild captured *An. saperoi* females were not dissected. The parity was determined by dissecting the ovaries of *An. saperoi* females and examining the degree of tracheole coiling as an index of parity (Detinova, 1962). Insemination of the *An. saperoi* females was determined by dissecting the spermatheca and examining for the presence or absence of sperm under the microscope.

Population estimates

The Seber (1982) method was used to estimate the population size of *An. saperoi* in the study area. Important assumptions of this measure are the following, for estimating N*, the number of animals is a closed population. A sample n_1 of animals was taken from the population; the animals were marked for future identification and then returned to the population. After allowing time for marked and unmarked specimens to mix, a second sample n_2 of animals was then taken, where it was found that m_2 were marked. Then, the population size was estimated by $N^* = (n_1 + 1)(n_2+1)/(m_2 + 1) - 1$ (Seber, 1982).

An estimate of the variance v* of N* was calculated by the following formula: $v^* = (n_1+1)(n_2+1)(n_1-m_2)(n_2-m_2)/(m_2+1)^2(m_2+2)$.

Survival rate estimation

A regression coefficient model was used to estimate survival of recaptured *An. saperoi*. The underlying assumption of this model was that, the daily probability of survival was constant throughout the year. This was done by regressing the number of recaptured *An. saperoi* transformed into ln (y+1) as a function of time in days post-release (Gillies, 1961; Reisen and Aslamkhan, 1979). Then, the daily survival rate was calculated as the antilogarithm of the regression coefficient.

RESULTS

Recapture rates

The recapture rates of *An. saperoi* females in six mark-release-recapture experiments were 14 (0.93%), 33 (1.40%), 3 (1.83%), 32 (2.46%), 14 (1.97%), and 22 (3.75%) for experiments I, II, III, IV, V, and VI respectively (Table 4). For experiments I-IV, cohorts of *An. saperoi* females and males were released. While for experiments V and VI, cohorts of *An. saperoi* females only were released. No valid recapture rate observations were drawn between these two cohorts of *An. saperoi*.

Dispersal

The spatial distribution of marked recaptured *An. saperoi* females was compared with that of unmarked captured *An. saperoi*. Because of the small number of mosquitos captured each day at each collection site, marked and unmarked captured *An. saperoi* from each collection site were pooled together according to their environmental conditions. Pooled data were used for statistical analysis. The number of marked recaptured *An. saperoi* at each collection site category was multiplied by the recapture rates for each experiment to get the expected number of recaptures in each collection site category. The difference between the expected and observed number was tested by the chi-square test. The spatial distribution of marked recaptured *An. saperoi* females was similar with that of unmarked captured *An. saperoi*. However, the spatial distribution of marked recaptured *An. saperoi* among collection site categories was significantly different (Tables 5, 6).

Flight range

The maximum flight distance recorded in the present study was 930 m (Table 4). Seven *An. saperoi* females were recaptured at this distance. Two out of the seven recaptured *An. saperoi* females were parous and the other five were nulliparous. In experiment VI, where an extra collection site C6 was added, located at 1,165 m from the release site, marked *An. saperoi* females were not recaptured at this extra collection site during twelve days of subsequent collection.

Survival

The number of recaptured *An. saperoi* transformed to ln (y+1) decreased significantly as a liner function of time in days post-release during the n^{th} days recapture sequence; *n* was the last day a marked mosquito was recaptured. Initially, survival was estimated for each experiment separately, but the regression coefficients in experiments I, III, and V, where a small number of *An. saperoi* females were recaptured, were

Table 1
Total number of marked and unmarked *An. saperoi* females collected at each collection site in six mark-release-recapture experiments in the Yona Forest, Okinawa Japan.

Collection category	Collection site	Distance (m)	Mark-release-recapture experiment						Total
			I	II	III	IV	V	VI	
Release	R	0	2 (7)	3 (1)	0 (1)	22 (2)	5 (22)	12 (12)	44 (45)
Stream-side	C1	110	1 (36)	8 (2)	1 (1)	2 (5)	0 (6)	2 (13)	14 (63)
	A1	150	3 (24)	0 (0)	0 (1)	0 (0)	0 (0)	0 (1)	3 (26)
Foothill	D1	175	3 (13)	4 (2)	0 (2)	1 (2)	0 (5)	0 (36)	8 (60)
	D2	250	0 (3)	9 (4)	1 (4)	1 (3)	0 (3)	2 (19)	13 (36)
	C3	445	3 (65)	6 (4)	1 (1)	3 (35)	0 (20)	3 (63)	16 (188)
Forest	C4	790	2 (88)	3 (3)	0 (4)	3 (41)	3 (41)	2 (164)	13 (341)
	C5	930					6 (22)	1 (33)	7 (55)
	C6	1,165						0 (20)	0 (20)
	Total		14 (236)	33 (16)	3 (14)	32 (88)	14 (119)	22 (361)	118 (834)

Number of unmarked *An. saperoi* in parenthesis.

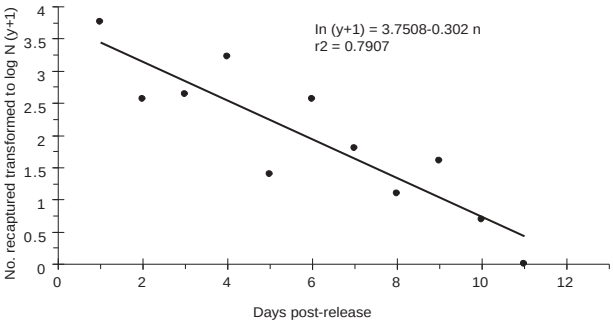

Fig 2–The number of recaptured *An. saperoi* transformed to ln (y+1) and plotted against days post-release.

not significantly different from zero ($p>0.05$). The data from all six experiments were, therefore, pooled to plot the regression line. Fig 2 shows the regression lines fitted to the data. The regression slope was significantly different from zero ($p<0.05$) and had a high coefficient of determination ($r^2 = 0.7907$). The regression equation was ln (y+1) = 3.7508-0.302*n*. The daily survival rate, the antilogarithm of the regression coefficient (-0.302), was calculated to be 0.73.

Gonotrophic cycle

The gonotrophic cycle in the present context refers to the interval between blood feeding and oviposition of eggs. The physiological status of the recaptured *An. saperoi* females in the six mark-release-recapture experiments is shown in Table 2. In experiments I, II, III, and IV, where cohorts of *An. saperoi* females and males were released, the gonotrophic cycles estimated by the dissection method were 3, 4, 4, and 6 days, respectively. For experiments V and VI, where cohorts of *An. saperoi* females only were released, the gonotrophic cycles were found to be 9 and 8 days, respectively. The parous rates of the wild caught *An. saperoi* females were 50, 42.9, 42, and 44.9% for experiments III, IV, V, and VI, respectively (Tables 2). The parous rates for marked, recaptured *An. saperoi* females released with males were 4 (28.6%), 9 (27.3%), 4 (66.7%), and 2 (6.3%) for experiments I, II, III, and IV respectively. Nevertheless, when cohorts of *An. saperoi* females only were released, the parous rates were 1 (7.1%) and 1 (4.45%) for experiments V and VI, respectively (Table 2). The insemination rate for the cohorts of *An. saperoi* females released with the males was 100%, while the insemination rates for the cohorts of *An. saperoi* virgin females released were 64.28 and 86.36% for experiments V and VI, respectively.

Population size

The Seber (1982) method was used to estimate population size of *An. saperoi* females in the Yona Forest area. Given that the number of marked recaptured *An. saperoi* females on day one post-release was <10 in five out of six experiments, the Seber method for the estimate of the population size seems to fit our data. However, in experiment III, the population size was not estimated because the total number

Table 2
Parous rates of marked and unmarked captured *An. saperoi* females estimated by the dissection method.

Days post release	Marked recaptured *An. saperoi*					
	Experiment I No. (parous rate %)	Experiment II No. (parous rate %)	Experiment III No. (parous rate %)	Experiment IV No. (parous rate %)	Experiment V No. (parous rate %)	Experiment VI No. (parous rate %)
1	1 (0)	9 (0)	0 (0)	26 (0)	3 (0)	4 (0)
2	0 (0)	5 (0)	0 (0)	1 (0)	4 (0)	1 (0)
3	5 (20) [1]	5 (0)	0 (0)	1 (0)	0 (0)	2 (0)
4	3 (33.33) [1]	7 (57.1) [4]	1 (100) [1]	2 (0)	4 (0)	7 (0)
5	0 (0)	0 (0)	2 (50) [1]	0 (0)	1 (0)	0 (0)
6	3 (33.33) [1]	5 (60) [3]	3 (66.7) [2]	1 (100) [1]	0 (0)	3 (0)
7	1 (0)	2 (100) [2]	0 (0)	0 (0)	0 (0)	2 (0)
8	1 (100) [1]			0 (0)	0 (0)	1 (100) [1]
9				0 (0)	2 (50) [1]	2 (0)
10				1 (100) [1]	0 (0)	0 (0)
11						0 (0)
12						0 (0)
Total	14 (28.6) [4]	33 (27.3) [9]	6 (66.7) [4]	32 (6.3) [2]	14 (7.1) [1]	22 (4.45) [1]
	Unmarked captured *An. saperoi*					
Total	236[a]	16[a]	88 (50)	14 (42.9)	119 (42)	361 (44.9)

[a]None dissected; [ ] = No. parous

Table 3
Estimates of *An. saperoi* female population size in the Yona Forest by the Seber method. The standard error is calculated as the square root of the variance.

Experiment	No. released (n_1)	No. captured (n_2)	No. recaptured (m_2)	Population size N*	Variance v*	Standard error
I	1,508	236	14	23,841.20	1.90	1.38
II	2,364	16	33	1,181.50	5.74	2.39
IV	1,302	88	32	3,514.15	2.80	1.67
V	709	119	14	5,679.00	9.95	3.15
VI	586	361	22	9,237.86	9.75	3.12

Females comprise half of the population, therefore total population size should be twice these figures.

of recaptured *An. saperoi* females was <10. Thus, the populations of *An. saperoi* females in the Yona Forest area were estimated to be 23,841, 1,182, 3,514, 5,679, and 9,238 for experiments I, II, IV, V, and VI, respectively (Table 3). Females comprised half the population, therefore the total population size in our study area should be twice these figures.

DISCUSSION

This paper describes the first mark-release-recapture experiments with *An. saperoi* in Okinawa, Japan. The principle paradigm for measuring dispersal, survival and population distribution in insects has been mark-release-recapture experiments (Costantini *et al*, 1996). Mark-release-recapture experiments have been used to demonstrate mosquito dispersal and survival, with varying success. Jaal and MacDonald (1992) reported the recapture rates for three-anopheline species *An. lesteri paralie, An. subpictus* and *An. vagus* were 3.34, 1.19, and 0.97%, respectively. The daily survival rate

Table 4
Recapture rates and maximum distance flown by *An. saperoi* females in six mark-release-recapture experiments in the Yona Forest.

Experiment	Mean temp (ºC)	Number released	Recapture rates (%)	Max flight distance (m)
I[a]	27.3	3,016	0.93	790
II[a]	22.3	4,728	1.4	790
III[a]	19.2	327	1.83	445
IV[a]	27.3	2,603	2.46	790
V[b]	28.2	709	1.97	930
VI[b]	21.8	586	3.75	930

[a]Females and males were released
[b]Females only were released

of these species was estimated to be 0.68 by the regression coefficient. In our present study, the numbers of *An. saperoi* females released in the six experiments were 1,508, 2,364, 164, 1,302, 709 and 586, which corresponds to the recapture rates of 0.93, 1.40, 1.83, 2.46, 1.97, and 3.75% (Table 4). Weather conditions, such as heavy rains during experiments I and III may have played an important role in the small recapture rates for *An. saperoi* by restricting the movements of most of the released mosquitos. The recapture rates for *An. saperoi* observed in this study are much lower than the recapture rates reported for some other anopheline mosquitos. In Pakistan, for example, Reisen and Aslamkhan (1979) reported a recapture rate for

Table 5
Observed and expected numbers of marked recaptured *An. saperoi* females in four different environmental conditions.

Experiment	Release (R)		Foothill (D1, D2)		Stream-side (C1, A1)		Forest (C3, C4, C5, C6)	
	Expected	Observed	Expected	Observed	Expected	Observed	Expected	Observed
I	6.51	7	14.88	16	55.8	60	142.29	153
II	1.4	1	8.4	6	2.8	2	9.8	7
III	1.83	1	10.98	6	3.66	2	9.15	5
IV	4.92	2	12.3	5	12.3	5	186.96	76
V	43.34	2	15.76	8	11.82	6	163.51	83
VI	45	12	206.25	55	52.5	14	1,050	280
Total	58	45	268.57	96	138.88	89	1,561.71	604
χ^2	37		122		36.7		674	
p	< 0.001		< 0.001		< 0.001		< 0.001	

Table 6
Observed and expected number of unmarked captured *An. saperoi* females in four different environmental conditions.

Experiment	Release (R)		Foothill (D1, D2)		Stream-side (C1, A1)		Forest (C3, C4, C5, C6)	
	Expected	Observed	Expected	Observed	Expected	Observed	Expected	Observed
I	1.86	2	2.79	3	3.72	4	4.65	5
II	4.2	3	18.2	13	11.2	8	12.6	9
III	0	0	1.83	1	1.83	1	1.83	1
IV	54.12	22	4.92	2	4.92	2	14.76	6
V	9.85	5	0	0	0	0	17.73	9
VI	45	12	7.5	2	7.5	2	22.5	6
Total	115.03	44	35.24	21	29.17	17	74.07	36
χ^2	46		7.64		7.08		23	
p	< 0.001		0.106		0.132		< 0.001	

An. stephensi of 7.13% and Reisen *et al*, (1980) a recapture rate of 8.0% for *An. culicifacies*.

In a smaller sized experiment in Sri Lanka, in which marked *An. culicifacies* were released two times, Sloof and Herath (1980) recaptured only 2.0% of the mosquitos released. However, recapture rates in mark-release-recapture experiments involving anopheline or culicine mosquitos are often less than 1% (Jaal and MacDonald, 1992). Clearly, spatial distribution will be influenced by the different dispersal characteristics of the individual species, resting sites, oviposition sites, host seeking and availability, age of individuals marked and released, local geography and topography of the area (Bidlingmayer, 1971).

The spatial distribution of marked recaptured *An. saperoi* was compared with that of unmarked captured *An. saperoi* to explain the behavioral responses of released *An. saperoi* to heterogeneous environments. In these experiments, the spatial distribution of marked recaptured *An. saperoi* was similar with that of unmarked captured *An. saperoi*. Nevertheless, the spatial distribution of the marked recaptured *An. saperoi* females was observed to be different for the different collection sites (Table 5). For example, 30.1% (36/118) of marked recaptured *An. saperoi* and 72.42% (604/834) of unmarked captured *An. saperoi* were collected at forest collection sites C3, C4, C5, and C6 (Table 1). The differences in the spatial distribution of marked recaptured *An. saperoi* resulted in significant differences in the recaptured *An. saperoi* between the collection sites (Table 5). Since the spatial distribution of marked and unmarked captured *An. saperoi* females was similar, the environmental conditions were a major factor in the spatial distribution of *An. saperoi* in our study area. The spatial distribution of released *An. saperoi* in this study was non-random, related closely to the forest habitats. An abundance of wild captured *An. saperoi* females were also found concentrated in the forest collection sites (Table 1).

The total number of recaptured *An. saperoi* decreased as a function of time post-release. The last day *An. saperoi* were recaptured was ten days post-release. This mosquito had dispersed to collection site C4, located 0.79 km from the release site. Although *An. saperoi* may have the potential to fly further than what is documented by this study, in our study released *An. saperoi* do not appear to disperse widely. A possible explanation for this is that since *An. saperoi* has a short gonotrophic cycle, estimated in our study to be 4 days, the shorter flight distance in the gravid females was associated with searching of oviposition sites, which were plentiful in the study area.

The maximum flight distance of *An. saperoi* recorded in our present study was 0.93 km from the release site (Table 4). Seven *An. saperoi* females flew this distance. Two out of seven *An. saperoi* females were recaptured at this distance two days post-release, while four *An. saperoi* females four days post-release and a single *An. saperoi* eight days post-release. The maximum flight distance recorded in our present study is much less than the 4.5 km flown by unfed *An. stephensi* females in Iran (Quraishi *et al*, 1966), or by other marked and released anopheline mosquitos, for examples *An. culicifacies*, 2.4-2.8 km (Russell *et al*, 1944), *An. gambiae* 3.62 km (Gillies, 1961). The maximum flight distance recorded in this study was greater than that observed for *An. quadrimaculatus*, 0.82 km (Smith *et al*, 1941).

The number of recaptured *An. saperoi* transformed into ln (y+1) decreased significantly as a linear function of time in days post-release during the 12-day recapture sequence (Fig 2). The regression coefficient was calculated to be -0.302 ($p<0.05$) and the daily survival rate, which is the antilogarithm of the regression coefficient, was 0.73. These findings are consistent with other anopheline studies. The survival estimates of *An. saperoi* in our present study can be compared to similarly derived estimates of anopheline mosquitos based on regression analysis. For example, in Pakistan the survival estimate for *An. stephensi* was 0.81-0.86 (Reisen and Aslamkhanl, 1979) and for *An. culicifacies* was 0.66-0.78 (Reisen *et al*, 1980). The survival rate of the forest mosquito *An. balabacensis* in Sabah was estimated by the regression coefficient to be 0.72-0.79 (Hii and Vun, 1985). Tsuda *et al* (1999) reported the daily probabilities of fed and unfed *Anopheles minimus* in Ishigaki Island, Japan were estimated to be 0.747 and 0.756, respectively. The higher survival for *An. saperoi*

of 0.73 suggests a longer life span for this species.

The gonotrophic cycle in our present study was determined by dissecting recaptured *An. saperoi* females. Recaptured parous *An. saperoi* in each experiment were <5; gonotrophic estimates in this study were therefore considered as a day when recaptured parous *An. saperoi* were first collected. The gonotrophic cycle for cohorts of *An. saperoi* females released with males were 3, 4, 4, and 6 days post-release for experiments I, II, III, and IV, respectively (Table 2). Taking the average number of these days, the gonotrophic cycle was estimated to be about 4 days. These observations agree with Quraishi *et al* (1966) who also collected a parous *An. stephensi* females three days post-release. Nevertheless, when cohorts of *An. saperoi* females only were released, parous *An. saperoi* were first recaptured 9 and 8 days post-release for experiments V and VI, respectively (Table 2). Presumably, the reason for the delayed parity in the cohorts of *An. saperoi* virgin females was mating with wild males in nature. The striking observation made in the laboratory of *An. saperoi* virgin females was they mated well with wild *An. saperoi* males. The insemination rates for the released virgin female *An. saperoi* were 64.28% and 86.36% for experiments V and VI, respectively. Thus, the laboratory conditions in which the *An. saperoi* females were reared did not impede them from mating with wild *An. saperoi* males in nature. The insemination rate for the host seeking wild caught *An. saperoi* was 100%. The parous rates for the wild captured *An. saperoi* were 50% (44/88), 42.9% (6/14), 42.0% (50/119), and 44.9% (162/361) for experiments III, IV, V, and VI, respectively (Table 2). The parous rates for the wild caught *An. saperoi* did not significantly differ between seasons. Of epidemiological interest were the relatively high parous rates of wild *An. saperoi* throughout the different seasons. Despite the fact that malaria on Okinawa Island was completely eradicated in the 1970s (Toma and Miyagi, 1981), regular surveys of the *An. saperoi* population in the Yona Forest area are of major public health importance, especially in this new era where the earth is threatened with global warming and re-emergence of infectious diseases.

Estimates of population size assume that: (1) the population is closed; (2) all animals have the same probability of being caught; (3) marking does not affect the catchability of an animal; (4) animals do not lose their marks during sampling; and (5) all the released and wild mosquitos were equally likely to be captured in subsequent efforts (Seber, 1982). These assumptions seemed to be more or less realized with *An. saperoi* in the Yona Forest area. Population size was estimated in five out of six mark-release-recapture experiments. The population size was not estimated for experiment III because the total recaptured *A. saperoi* were <10. Estimates of the population size by Seber's method (1982) seem to be more realistic in this study because it corresponds well to the proportional number of wild captured *An. saperoi*. For example, the wild captured *An. saperoi* females were 236, 16, 88, 119, and 361 for experiments I, II, IV, V, and VI, respectively (Table 3). Estimates of the population size indicate that 1% (236/23,841), 1.35% (16/1,181), 2.5% (88/3,514), 2.1% (119/5,679), and 3.91% (361/9,237) of the wild females in the population were sampled during experiments I, II, IV, V, and VI respectively (*cf* recapture rates). These population estimates seem to be reasonable, taking into consideration the sampling effort of 20 minutes/day expended at each collection site. The low standard errors of our population estimates suggest that Seber's method (1982) estimated the population size reasonably well in the Yona Forest area (Table 3).

This study of the critical population attributes of *An. saperoi* in the Yona Forest, northern Okinawa, Japan and other vectorial factors, such as high longevity, high parous rates, and short gonotrophic cycles, discloses that *An. saperoi* is well suited and can be a potential vector for malaria. In order to be prepared for the immense challenges of re-emerging infectious diseases, such as malaria, the importance of constant surveilance of *An. saperoi* populations in Yona Forest cannot be overemphasized.

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