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MOSQUITO SURVEILLANCE IN THE DEMILITARIZED ZONE, REPUBLIC OF KOREA, DURING AN OUTBREAK OF *PLASMODIUM VIVAX* MALARIA IN 1996 AND 1997[1]

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ABSTRACT. Since 1993, more than 2,000 cases of vivax malaria have occurred in the Republic of Korea in an epidemic that ended nearly 20 malaria-free years. Most malaria has occurred in the northwestern part of the country, mainly affecting Korean military personnel. As a part of an operational surveillance effort, we sampled mosquitoes in and near the Demilitarized Zone (Paju County, Kyonggi Province) during the last 2 wk of July in 1996 and from May 15 to September 10 in 1997. The 1st year, landing collections were done at 5 different sites; the 2nd year, carbon-dioxide–baited light traps at 5 sites, larval collections in 10 adjacent fields, and landing collections at 1 site in the Demilitarized Zone were performed weekly. Of 17 species collected, *Anopheles sinensis* was consistently the most abundant mosquito, comprising 79–96% of mosquitoes. The diel pattern of biting by *An. sinensis* varied by location and season, with the majority of individuals biting late at night during warm weather (>20°C) and early at night during cool weather. In contrast, *Aedes vexans nipponii* (the 2nd most abundant species) bit in the greatest numbers at the same time all season, from 2000 to 2300 h. Among the correlates with abundance of *An. sinensis* were average nighttime temperature 2 wk previous to the night in question, wind late at night (negatively correlated), and apparent size of the moon (negatively correlated). The data showed that the exact number of *An. sinensis* biting could not be estimated from numbers collected in carbon-dioxide–baited light traps. On the other hand, a threshold of 15 *An. sinensis* per trap night corresponded (88% accuracy) to a threshold of 12 mosquitoes biting 2 adjacent collectors per night. Larval collections were also significantly correlated with landing collections, despite inexact sampling methods and separation of the larval habitat from the site where landing collections were performed. Operational entomology assets using nighttime temperature records, carbon-dioxide–baited light traps, and larval collections should be able to target their efforts in Korea more efficiently.

KEY WORDS *Anopheles sinensis, Plasmodium vivax,* malaria, surveillance, Korea

INTRODUCTION

The Republic of Korea (ROK) currently is experiencing an expanding epidemic of *Plasmodium vivax* malaria (Feighner et al. 1998, Lee et al. 1998). The 1st case in the last 20 years occurred in 1993 (Chai et al. 1994), followed by 2 more cases in 1994 (Cho et al. 1994). Since then, the number of new malaria infections has risen exponentially to the unofficial total of more than 1,600 cases in ROK military personnel in 1998, despite application of chemoprophylaxis to 35,000 ROK troops (Seminar for Malaria Control, Korean National Institute of Health, November 25, 1998). The 1st 3 cases included 2 Korean soldiers stationed near Panmunjom, Paju County, Kyonggi Province. The other case was a civilian woman in the nearby town of Munsan, only 4 km from the edge of the Demilitarized Zone (DMZ) separating the ROK from the People's Democratic Republic of Korea (PDRK). During the next 4 years, the outbreak spread to many points near the border from Chorwon west to Kangwha Island and south to Inchon and Seoul (Lee et al. 1998). More infected soldiers (total of 1,596 cases from 1993 through 1997, including 40 cases in U.S. Army personnel; Feighner et al. 1998) than infected civilians (total of 650 cases, Lee et al. 1998) have been detected.

Malaria is not new to Korea (Paik et al. 1988). In 1910, the 1st documented cases were described from occupying Japanese forces and Korean civilians distributed throughout the peninsula. The number of cases declined steadily during the 1930s for unknown reasons. Malaria again became a major problem during the Korean War (1950–53), when U.S. forces suffered a 6.6% attack rate. In 1960, the ROK and the World Health Organization initiated the National Malaria Eradication Service (NMES). The NMES performed active and passive case detection documenting widespread distribution of vivax malaria in 1960, with the greatest number of cases in the eastern mountains (Kyungsang Pukdo Province). Treatment of cases, vector control, and a general improvement in living conditions reduced malaria transmission so that by 1965 the only areas with malaria were northwest of Seoul (including the area where the current epidemic started), east of Seoul, and the eastern mountainous area. The NMES was disbanded in 1970 when malaria was very much reduced. A thorough search in

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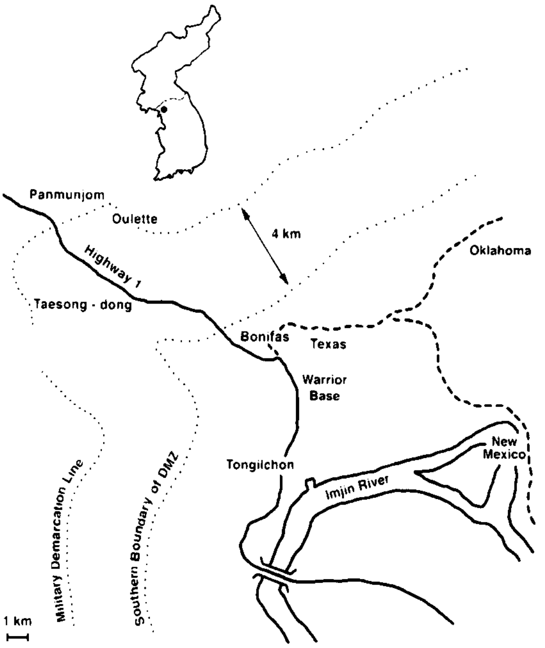

Fig. 1. Map of study sites in military-controlled area of Paju County.

1986 failed to find a single case in the area where malaria had once been most prevalent.

The principal purpose of the current study was surveillance of malaria vectors as part of a successful effort to protect U.S. forces serving near the DMZ in Paju County. In 1996, only a 2-wk period was sampled at the time when the vector was thought to be most abundant. We correctly assumed that the epidemic would increase in 1997, so we planned more extensive vector surveillance in order to make the following determinations: what nonvector species of mosquitoes are likely to generate complaints about biting in the DMZ; whether diel periodicity of *Anopheles sinensis* Wiedemann varied seasonally; whether carbon-dioxide–baited light trap and larval collection data could be correlated to biting collections of *An. sinensis*; and whether temperature, wind, and moon could be used to predict the biting rate of *An. sinensis.*

MATERIALS AND METHODS

Study site: This study was conducted in a military-controlled area north of the Imjin River near Panmunjom (37°55′N, 126°38′E; Fig. 1). The area consisted of low wooded hills interspersed with valleys usually planted in rice. Intensive rice culture had resulted in a drainage pattern dominated by human-managed irrigation, with few natural streams or ponds. Other crops in the area included peppers, corn, and ginseng. Large domestic animals were located at only a few sites, probably because of a curfew on civilian nighttime activity. Dairy cattle were present in Tongil'chon and a pig farm and some domesticated deer were present in Taesong'dong. The area included numerous ROK and American military activities, including garrisons, training areas, and weapon ranges. A broad valley at least 2 km wide was located on the other side of the border in the PDRK. This valley was almost completely cultivated in rice, which was observed on several occasions to be worked with oxen by residents of nearby villages. Higher ground to the north of the valley rose into a range of rocky mountains that lacked wooded cover. The population on the PDRK side of the border also included considerable numbers of soldiers, who were sometimes visible from the DMZ.

Sampling in 1996: Landing collections were made from 1800 to 0600 h, with 1 pair of soldiers collecting for 2 h and then switching with another pair of soldiers. All landing mosquitoes were captured with an aspirator and then blown into a paper carton cage, separating each hour's collection. Collectors wore the standard U.S. Army field uniform (Battle Dress Uniform, not treated with permethrin) and sat with the legs of the trousers rolled up to the knee. Most mosquitoes were collected from exposed skin on the collector's own legs; however, mosquitoes anywhere on exposed skin were captured. Occasions occurred when it was more convenient for a collector to capture a mosquito from his partner. Temperature and wind speed (as maximum value during 90 sec) at the site of the collection were both recorded at the beginning of each hour.

Five locations within 10 km of each other were sampled in 1996. The placement of collectors at each location was chosen based on practicality and proximity to the representative population. The decision was made to place collectors at a small distance (10–50 m) from military or civilian residents for 2 reasons. First, collectors were active all night so that their presence would have been disruptive for local residents and, 2nd, the number of mosquitoes attracted to collectors was less likely to be influenced by movement of residents. Tongil'chon was a village with approximately 1,000 inhabitants, modern masonry housing, paved streets, running water, and electricity. Collections were made at the edge of the village on a small dairy farm overlooking a valley with rice fields. Camp Bonifas was a military post adjacent to the DMZ bordered on 1 side by rice fields. The West Bonifas site was less than 50 m from the rice fields, whereas the East Bonifas site was in the angle of low hills and at least 300 m from the nearest rice field. Observation Point Oullette was on a steep hill (100 m high) inside the DMZ and directly adjacent to the Military Line of Demarcation between the 2 Koreas. Collections were made just south of the crest of the hill. Taesong'dong was the only ROK civilian village in the DMZ, with approximately 750 residents. Collections were made near the church, within site of both giant flags raised on towers by the ROK and the DPRK.

Sampling in 1997: Following the results of 1996, we were particularly concerned about the possibility of seasonal variation in diel periodicity that would have affected the efficacy of insecticidal fogging. In addition, the work was designed to find a correlation between light trap, larval, and landing collections, so that light trap and larval survey results could be interpreted according to real risk to soldiers. These efforts also provided data that could be used to test the correlation between weather factors and landing collections. By recording the occurrence of nonanopheline mosquitoes, we developed information on the biting pressure to be expected from nonvector species.

Weekly light trap (Solid State Army Miniature trap, Driggers et al. 1980) and landing collections were made at Taesong'dong from May 15 to September 10, 1997. Only the week of July 9 was missed, because of a military alert. Traps were hung from a tree or fence about 1.5 m above the ground. Each trap was operated with the light on and was baited with carbon dioxide provided by dry ice in an insulated, 1-liter jug with an open pour spout. Using this arrangement, the dry ice always lasted the entire night. The locations of traps included Taesong'dong (approximately 300 m from the landing collection site) and 4 weapons training ranges (designated Texas, Kansas, New Mexico, and Oklahoma) located within 15 km of Taesong'dong. Larvae were collected from 10 rice fields on the morning after each landing collection. The fields were across the road (Highway 1) from Warrior Base, located 1 km from Camp Bonifas and 4 km from Taesong'dong. In an effort to perform larval collection in a standard manner, 4 collectors spread out among the fields and captured as many anopheline larvae as possible during 30 min of dipping with a shallow pan (20 × 36 cm). Each week, a subsample of the larvae was reared to the adult stage for identification.

Analyses: Morphologic characters of adults (K. W. Lee 1998) were used to identify mosquitoes. Although the characters used to distinguish between *An. sinensis* and *An. lesteri* Baisas and Hu were sometimes obscured in light trap samples, landing collections produced specimens in much better condition. Representative specimens were deposited in the collection of the 5th Medical Detachment, 18th Medical Command, Yongsan Garrison, Seoul, ROK.

Excel97 (Microsoft Corp., Seattle, WA) or SPSS (SPSS for Windows, Release 6.1.2, SPSS Inc., Chicago, IL) software were used to perform statistical analyses. Excel97 calculated linear regression and SPSS performed chi-square tests of 2 × 2 contingency tables (Crosstabs procedure with Pearson's estimate of chi-square), analysis of variance (one-way ANOVA with Duncan's multiple range test), cross correlation (Graphs, Time Series, Cross Correlations), and 2-tailed, linear partial correlation (Correlation, Partial). Phases of the moon and times of sunrise and sunset were calculated using the Interactive Computer Ephemeris (Version 0.51, U.S. Naval Observatory Nautical Almanac Office, Washington, DC).

The relationship between light trap and landing collections was studied by performing linear regression on the log of total female *An. sinensis* in the Taesong'dong trap, the mean of females per trap for all 5 light traps, and females captured by 2 collectors per night. Only 3 observations from the Taesong'dong light trap had no *An. sinensis,* which then required adjustment by adding 1. All other observations were greater than zero, so that a $\log(x + 1)$ adjustment was not necessary.[4] The analysis involved a test of the possibility that the ratio of light trap to landing collections was related to density of mosquitoes. We performed the same analysis as Lines et al. (1991) on our data, regressing the ratio of the log of light trap and landing collections on the geometric mean of light trap and landing counts.

In a separate analysis, we examined the significance of a threshold number of female *An. sinensis* in light traps that might correspond to a minimum number of biting mosquitoes. This analysis was accomplished by comparing a series of threshold values of the mean of all 5 light traps to a minimum biting rate ($\geq$12 bites per night for 2 collectors). The correspondence of each threshold value was evaluated for standard epidemiologic measurements (sensitivity, specificity, and accuracy) and tested for significance by chi-square (Griner et al. 1981). The threshold with the greatest accuracy and sensitivity was considered the most appropriate.

RESULTS

Although the data from 1997 were more extensive, the 1996 data had the advantage of greater replication over a short period of time and sampling from several different sites. *Anopheles sinensis* was consistently the most abundant biting mosquito in 1996, comprising 79.3–95.8% of the mosquitoes

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[4] Avoiding this adjustment overcame the objection of Smith (1995) to the analysis of Lines et al. (1991). Smith (1995) was concerned (for landing collections represented by x and light trap collections represented by y), that whereas for constant x/y and changing mosquito density values the ratio of $\log(x)$ to $\log(y)$ would be constant, the ratio of $\log(x + 1)$ to $\log(y + 1)$ would not be constant. Because log conversion was considered necessary to achieve linearity, the inequivalence of $\log(x)$ and $\log(x + 1)$ would make it difficult analyze constancy of the ratio of landing to light trap collections with respect to mosquito density. Smith (1995) suggested that the problem could be avoided if collections were sufficiently large to avoid the $\log(x + 1)$ conversion or by using Poisson regression techniques. Our data allowed us to use the former solution.

Table 1. Mosquitoes captured ≥5 times in landing collections at 5 locations in and near the Demilitarized Zone, Republic of Korea, between July 23 and August 2, 1996.

	Location (No. of nights of collection)									
	E Bonifas (4)		Oullette (2)		Taesong (5)		Tongil (2)		W Bonifas (6)	
Species	No.[1]	%[2]	No.	%	No.	%	No.	%	No.	%
Anopheles sinensis	376.2	95.8	163.0	86.5	72.2	79.3	87.0	87.4	278.2	88.2
An. lesteri	6.0	1.5	5.5	2.9	1.6	1.8	5.0	5.0	5.7	1.8
An. yatsushiroensis	0.8	0.2	3.5	1.9	2.8	3.1	0.5	0.5	1.2	0.4
Culex pipiens	6.2	1.6	1.0	0.5	10.2	11.2	3.5	3.5	8.3	2.6
Aedes vexans	1.5	0.4	8.5	4.5	2.0	2.2	2.0	2.0	21.7	6.9
Ae. alboscutellatus (Theobald)	1.0	0.3	0	0	0.4	0.4	0.5	0.5	0	0

[1] Per night per 2 collectors.
[2] Of total at that location.

collected at the 5 sites (Table 1). The next most abundant biting species, *Aedes vexans nipponii* (Theobald), exceeded 0.5 bites/person/h at only 1 site, West Bonifas. *Anopheles sinensis, An. lesteri, An. yatsushiroensis* Miyazaki, *Culex pipiens pallens* Coquillett, and *Ae. vexans nipponii* were the only species present at all 5 sites. Species collected landing on humans less than 5 times were *An. sineroides* Yamada, *Cx. bitaeniorhynchus* Giles, *Cx. orientalis* Edwards, *Cx. mimeticus* Noe, *Cx. tritaeniorhynchus* Giles, *Ae. koreicus* (Edwards), *Ae. albopictus* (Skuse), *Ae. esoensis* Yamada, *Ae. nipponicus* LaCasse and Yamaguti, and *Armigeres subalbatus* (Coquillett).

The species of mosquitoes collected in landing collections in 1997 followed several different patterns of seasonality (Table 2). All abundant species persisted until late in the season (September), but some appeared earlier (in May, *Ae. vexans, An. sinensis, Cx. pipiens*) than others (in June, *Ae. koreicus, Ae. albopictus, An. lesteri, Cx. bitaeniorhynchus*). The earlier group gradually increased to maximum numbers later in the year (August 6–27). In contrast, the later group of species reached maximum numbers relatively soon after their 1st appearance (July 16–August 6). Examining the 2 most abundant species more closely (Fig. 2), *Ae. vexans* 1st appeared early in the season (May) and gradually increased in numbers through a series of probably 3 broods. *Anopheles sinensis* increased suddenly in late June and peaked late in the season.

The diel pattern of landing by *An. sinensis* was not the same at each location in 1996 (Fig. 3). The pattern of landing at East Bonifas (n = 4 nights) and Tongil'chon (n = 2 nights) was biphasic. In contrast, the pattern of landing at Taesong'dong (n = 3 nights) and West Bonifas (n = 6 nights) was a single, late-night peak. Despite this variation in diel pattern at different locations, it was still useful to examine the pattern of activity of landing mosquitoes for all locations during a short sampling period (2 wk), which represented a single point in the seasonal cycle of abundance. The average pattern for the entire area during late July (determined by summing hourly landing collections from all locations for a total of 19 nights) was a peak in landing late at night. Analysis of variance of all locations showed significant differences in number of landing *An. sinensis* between hours of collection (F = 3.05, df = 11,216, P = 0.0008), with statistically

Table 2. Seasonality of mosquitoes collected more than once[1] in landing collections during 1997 at Taesong'dong.

		Date of		
Species	Total collected	First collection	Last collection	Highest collection
Aedes vexans	527	May 15	Sept. 10	Aug. 13
Anopheles sinensis	1,164	May 15	Sept. 10	Aug. 27
Culex pipiens	70	May 15	Sept. 10	Aug. 6
Ae. koreicus	66	June 4	Sept. 3	July 16
Ae. albopictus	47	June 11	Sept. 10	July 23
Ae. esoensis	9	June 11	Sept. 3	July 23
An. lesteri	105	June 11	Sept. 10	Aug. 6
An. yatsushiriroensis	5	June 24	July 23	July 3–23
Cx. bitaeniorhynchus	150	June 24	Sept. 3	July 28
Cx. orientalis	10	July 16	Sept. 3	Aug. 27
Armigeres subalbatus	16	July 23	Sept. 10	Aug. 27
Ae. nipponicus	4	July 23	July 28	July 28
Ae. alboscutellatus	4	Aug. 13	Aug. 13	Aug. 13
Cx. tritaeniorhynchus	16	Aug. 13	Sept. 10	Aug. 18

[1] Species collected only once: *Cx. vagans* Wiedemann, May 15; *Ae. bekkui* Mogi, June 18; *Mansonia uniformis* (Theobald), Aug. 18.

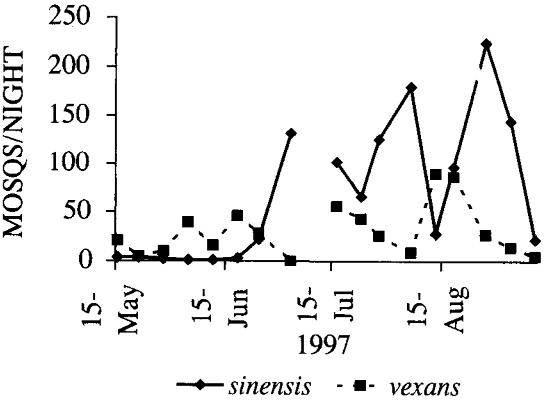

Fig. 2. Seasonal occurrence of *Anopheles sinensis* and *Aedes vexans* in landing collections (per 2 collectors) in 1997 at Taesong'dong. Note that no sampling took place on July 9.

homogeneous groups of 1800–0200 h and 0500 h, 2300–0300 h, and 0200–0400 h. The diel landing pattern of *An. sinensis* was unique among the 16 species collected in 1996 (Table 3). Many of these species were collected rarely, but the data are included because studies concentrating on these species are unlikely to occur in the near future. The 2 species with diel patterns of landing most similar to *An. sinensis* were *An. lesteri* and ***Cx. pipiens pallens. Culex bitaeniorhynchus*** was collected only 4 times and *Ae. esoensis* only once, but these included late-night collections. All other species were most frequently collected within 2 h after sunset (time of sunset between 1942 h and 1951 h during this period). The greatest variety of mosquitoes (10 species) was collected between 2100 h and 2300 h and the least variety (1 species) between 1800 h and 1900 h.

Seasonal analysis of *An. sinensis* and *Ae. vexans*

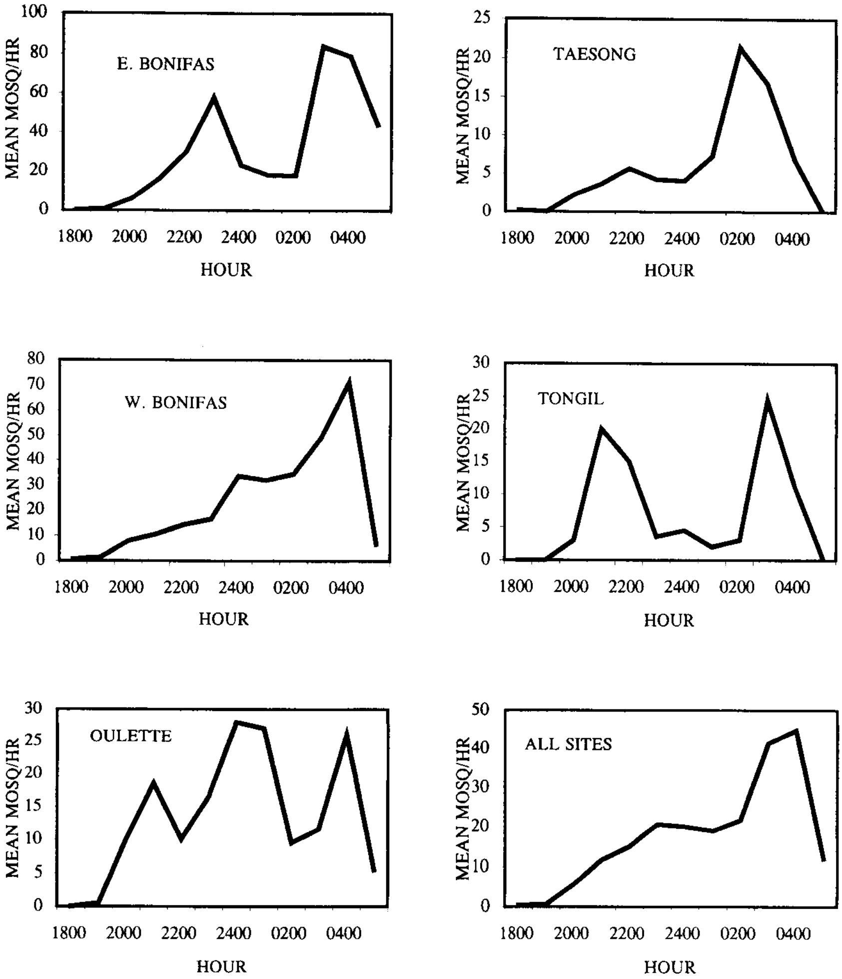

Fig. 3. Hourly distribution of *Anopheles sinensis* captured in landing collections (mean per night for that hour for 2 collectors) during July 23–August 2, 1996, at 5 locations near Panmunjom. Graph for all sites is mean for each hour per night for all locations sampled.

Table 3. Total hourly collections of all species of mosquitoes captured in landing collections (19 all-night collections) at 5 different sites near Panmunjom during July 23–August 2, 1996.

Species	Hour when collection began											
	1800	1900	2000	2100	2200	2300	2400	0100	0200	0300	0400	0500
Anopheles sinensis	6	12	108	222	285	391	379	358	410	785	850	229
An. lesteri	0	0	8	8	8	10	14	10	8	10	9	2
An. yatsushiroensis	0	0	11	6	3	2	2	2	3	2	0	1
An. sineroides	0	0	0	1	0	0	0	0	0	0	0	0
Culex pipiens	0	0	9	14	23	25	15	9	18	15	7	1
Cx. bitaeniorhynchus	0	0	0	1	1	0	0	0	1	1	0	0
Cx. orientalis	0	0	0	2	0	1	1	0	0	0	0	0
Cx. mimeticus	0	0	0	1	1	0	0	0	0	0	0	0
Cx. tritaeniorhynchus	0	0	0	0	1	0	0	0	0	0	0	0
Aedes vexans	0	0	114	17	9	7	8	6	1	1	3	0
Ae. koreicus	0	0	11	0	0	0	1	0	0	0	1	0
Ae. alboscutellatus	0	0	2	2	1	0	1	0	1	0	0	0
Ae. albopictus	0	0	2	0	1	0	0	0	0	0	0	0
Ae. esoensis	0	0	0	0	0	0	0	0	0	0	1	0
Armigeres subalbatus	0	0	2	0	0	0	0	0	0	0	0	0

in 1997 showed that there were 4 statistically distinct parts of the season based on the ANOVA and Duncan's multiple range test. These 4 time periods were May 15–June 18 (6 wk), June 24–July 23 (4 wk), July 28–August 18 (4 wk), and August 27–September 10 (3 wk). The pattern of diel periodicity and the total number of mosquitoes both varied seasonally (Fig. 4). Most *An. sinensis* bit early at night (before 2300 h) in the 1st part of the season, throughout the night during June 24–July 23 (peak at 2200–0500 h), very late at night during July 28–August 18 (peak at 0200–0500 h), and earlier at night from August 27 to September 10 (peak at 2000–2300 h). Changes in time of sunset (1937 h on May 15, 1959 h on the summer solstice, and 1850 h on September 10) and sunrise (0524 h on May 15, 0511 h on the summer solstice, and 0611 h on September 10) were not great enough to be the cause of these seasonal changes in diel landing pattern. Although difficult to correlate with diel landing pattern or total number of *An. sinensis,* wind speeds decreased in the latter half of the season (after July 23), particularly in the early evening. Nightly variation in temperature documented during 11 consecutive nights in 1996 provided some replication of the temperature profile during the peak of the season for *An. sinensis* (Table 4). Seasonal changes in the hourly pattern of temperature during 1997 were related to changes in the diel landing collection pattern of *An. sinensis* (Fig. 4). In the early part of the season, temperature quickly descended below 20°C, corresponding to a decrease

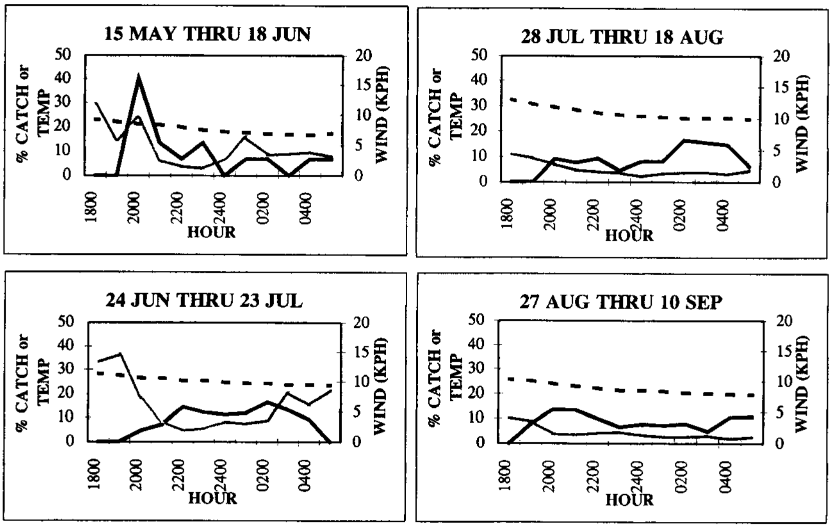

Fig. 4. Seasonal diel distribution of *Anopheles sinensis* in landing collections (solid line), hourly temperature in °C (dashed line), and hourly wind speed (gray line) during 1997, Taesong'dong.

Table 4. Hourly temperature during 11 nights (July 22–August 1) in 1996 near Panmunjom.

Hour	Temperature (°C)		
	Mean[1]	Standard deviation	Range
1800	28.0^{a}	3.41	22.5–33.5
1900	27.0^{ab}	2.70	22.5–31.5
2000	25.7^{bc}	2.20	22.0–29.0
2100	24.8^{cd}	1.99	21.0–27.0
2200	24.7^{cd}	1.56	22.0–26.5
2300	24.5^{cd}	1.54	22.0–26.5
2400	24.3^{cd}	1.86	21.0–27.0
0100	23.9^{cd}	1.66	21.0–27.0
0200	24.0^{cd}	1.51	21.0–26.0
0300	24.4^{d}	2.21	21.0–29.0
0400	23.6^{d}	1.38	21.0–25.0
0500	23.4^{d}	1.39	21.0–25.0

[1] Means not followed by the same superscripted letter were in separate homogeneous groups based on analysis of variance followed by Duncan's multiple range test (F = 5.162, df = 11,120, $P \leq 0.0001$).

in landing. From July 23 to August 27, the temperature remained above 25°C all night and *An. sinensis* continued landing all night. In the last part of the season (August 27–September 10), temperature descended below 25°C (although above 20°C) late at night and the landing rate was higher in the early part of the evening. In contrast to *An. sinensis*, landing collections of *Ae. vexans* were greatest at the same time of night during the entire season, peaking between 2000 and 2300 h (Fig. 5).

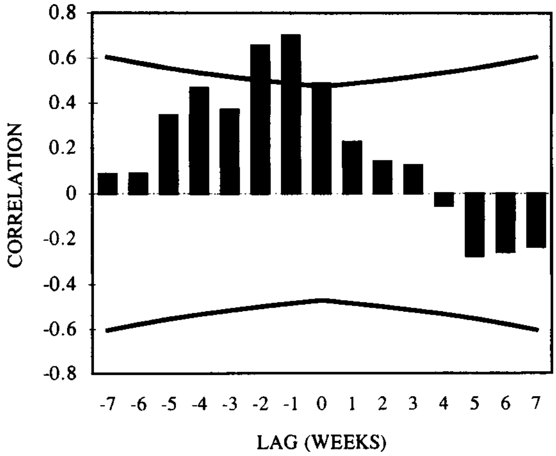

Fig. 6. Cross-correlation between mean nightly temperature (lagged factor) and number of *Anopheles sinensis* captured in landing collections at Taesong'dong, May 15–September 10, 1997. Bars are correlation and lines are twice standard deviation.

Seasonal variation in the mean nighttime temperature was related to the total number of *An. sinensis* captured per night in landing collections during 1997. Mean nightly temperatures on the night of, 1 wk before, and 2 wk before a landing collection were significantly related to the number of landing mosquitoes (Fig. 6).

Mean nightly wind speed was not cross-correlated to landing or light trap collections of female *An.*

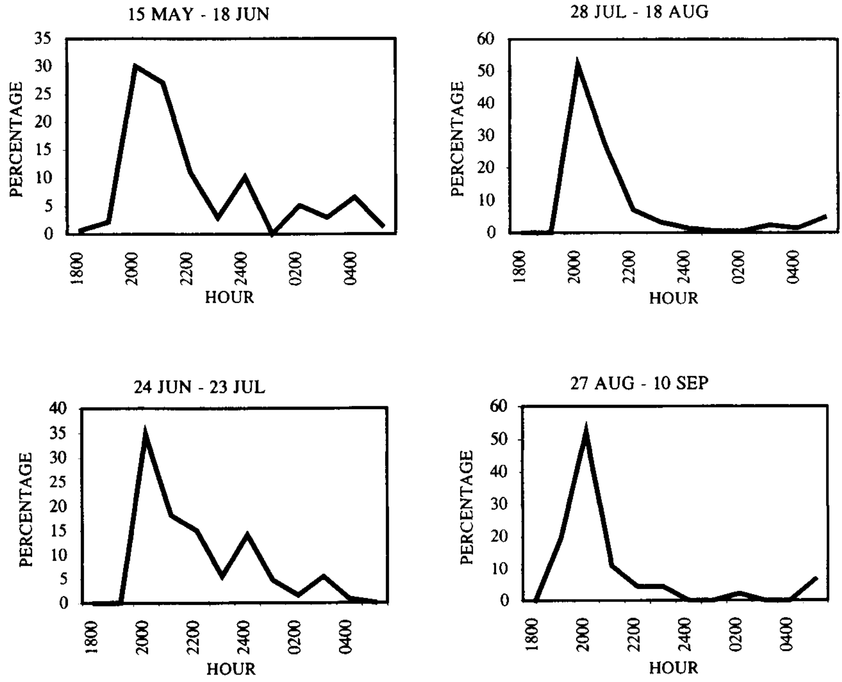

Fig. 5. Seasonal diel distribution of *Aedes vexans* in landing collections during 1997, Taesong'dong.

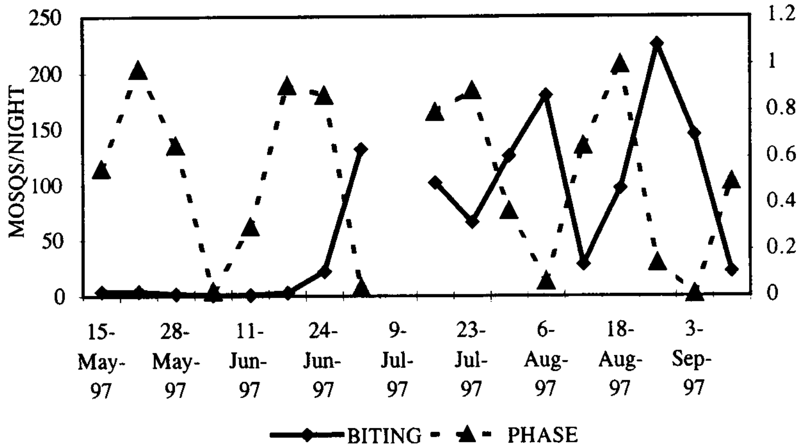

Fig. 7. Moon phase (expressed as proportion of full disk) compared to landing collections of *Anopheles sinensis* at Taesong'dong, May 15–September 10, 1997. Note that no sampling took place on July 9.

sinensis through 1997. Any further analysis of the influence of wind on landing in 1997 was unlikely to produce significant results, because replication occurred over a long period with seasonal effects. Data from 1996 were collected during a single 2-wk interval so that replication was presumably free of seasonal variation. For this analysis, we used all locations in a single analysis for a total of 19 collection nights during 1996. The linear regression of wind in kilometers per hour (independent variable) on number of landing *An. sinensis* for each hour of the night (dependent variable) was weak but significant ($y = -0.82(x) + 21.43$, $r^2 = 0.043$, $P = 0.0177$, df = 1,129). Dividing the night into statistically grouped intervals (based on abundance of mosquitoes) and performing linear regression of wind speed on landing showed an interesting trend. Regression was not significant during the period from 1800 to 2200 h ($y = 0.0088(x) + 7.02$, $r^2 = 0.00003$, $P = 0.9683$, df = 1,53). Only a weak indication of a relationship was found between wind and landing in the middle of the night from 2300 to 0100 h ($y = -0.97(x) + 26.91$, $r^2 = 0.0995$, $P = 0.0738$, df = 1,31). However, the regression was stronger during the early morning hours of 0200–0400 h ($y = -2.93(x) + 49.75$, $r^2 = 0.2535$, $P = 0.0028$, df = 1,31).

Hourly landing collections of *An. sinensis* in 1997 were negatively correlated to the phase of the moon present during the hour of collection. Controlling for effects of wind (zero order partial correlation = −0.1133, df = 202, $P = 0.107$) and temperature (zero order partial correlation = 0.1161, df = 202, $P = 0.001$), the correlation coefficient between phase of the moon (or absence if it had not yet risen or if it had already set) and hourly landing collections was −0.2245 (df = 200, $P = 0.001$). This statistical result supported the apparent negative correlation between moon phase and total nightly landing collections of *An. sinensis* (Fig. 7).

The numbers of mosquitoes in light traps during 1997 were compared to landing collections (for pair of collectors) at Taesong'dong in several ways: examination of relative ranking of species collected, percentage of each species compared to the total, and number of females of each species (Table 5). Ranking of the 3 most abundant species was similar for light trap and landing collections. Absolute numbers of *An. sinensis* in the Taesong'dong light trap (1,284) and landing collections (1,164) were also similar. Regression of the log of the number of *An. sinensis* in landing collections at Taesong'dong (dependent variable) and the log of the number in the light trap at Taesong'dong (independent variable) did not result in a significant relation ($r^2 = 0.051$, F = 0.75, df = 1,14, $P = 0.400$). However, regression between the log of landing collections and the log of the mean of all 5 light traps was significant ($y = 1.14(x) - 0.297$, $r^2 = 0.386$, F = 10.05, df = 1,16, $P = 0.0059$; Fig. 8). The ratio of the log of light trap to log of landing collections (dependent variable) was not constant. This proportion decreased significantly ($y = -0.72(x) + 1.065$, $r^2 = 0.407$, F = 10.98, df = 1,16, $P = 0.0044$; Fig. 8) as the number of mosquitoes in light trap and landing collections (independent variable, expressed as the mean of the logs of light trap and landing collections) increased. The relationship was not significant ($r^2 = 0.010$, F = 0.16, df = 1,16, $P = 0.695$) when the log of the mean of all light traps was used as a measure of mosquito density. Other differences were found between light trap and landing collections. *Aedes vexans* and *Cx. tritaeniorhynchus* were more abundant in light traps than in landing collections. In contrast, *An. sinensis* (by percentage of collection), *Ae. koreicus*, and *Ae. albopictus* were more abundant in landing collections than in light traps.

Establishing statistically significant thresholds was possible for light trap collections of *An. sinen-*

Table 5. Comparison of landing collections and carbon-dioxide–baited light trap collections during May 15–September 10, 1997. Landing collections were at Taesong'dong; light traps were at Taesong'dong, Texas Range, Kansas Range, New Mexico Range, and Oklahoma Range, Paju County. Only those species ranked among the 10 most common by 1 of the collection methods are included.[1]

	Landing collection			Taesong'dong trap			All light traps		
Species	Rank	Number	%	Rank	Number	%	Rank	Number	%
Anopheles sinensis	1	1,164	53.0	2	1,284	6.8	3	3,826	5.5
Aedes vexans	2	527	24.0	1	15,894	83.6	1	56,150	81.4
Culex bitaeniorhynchus	3	150	6.8	3	806	4.2	5	1,391	2.0
An. lesteri	4	105	4.8	9	26	0.1	11	71	0.1
Cx. pipiens	5	70	3.2	5	154	0.8	4	1,599	2.3
Ae. koreicus	6	66	3.0	13	2	<0.1	14	11	<0.1
Ae. albopictus	7	47	2.1	11	10	0.1	8	250	0.4
Ar. subalbatus	8	16	0.7		NC[2]		15	6	<0.1
Cx. tritaeniorhynchus	9	16	0.7	4	546	2.9	2	3,936	5.7
Cx. orientalis	10	10	0.5	8	58	0.3	6	684	1.0
Ae. esoensis	11	9	0.4	10	20	0.1	9	190	0.3
An. yatsushiroensis	12	5	0.2	7	59	0.3	10	165	0.2
Cx. vagans	16	1	<0.1	6	146	0.8	7	659	1.0

[1] Species not among the 10 most common by any of the collecting techniques (positive collection methods indicated in parentheses: L, landing collection; T, Taesong'dong trap; A, all light traps): *Ae. alboscutellatus* (L, A), *Ae. nipponicus* (L, A), *Mansonia uniformis* (L, T, A), *Ae. bekkui* (L), *An. sineroides* Yamada (T, A), *Cx. mimeticus* (T, A), *Tripteroides bambusa bambusa* (Yamada) (A), *Ae. chemulpoensis* Yamada (A), *Cx. jacksoni* Edwards (A), *Ae. flavopictus flavopictus* Yamada (A), *Ae. lineatopennis* (Ludlow) (A).

[2] NC, not collected.

sis based on landing collections at Taesong'dong in 1997. The most accurate threshold was based on the mean catch for all 5 light traps compared to a landing collection considered to be the minimum significant potential biting rate. Therefore, the threshold of landing rate was either >12 *An. sinensis* per night (i.e., above significant landing rate chosen based on an average of 1 potential bite per collector each 2 h) or ≤12 *An. sinensis* per night (i.e., below significant landing rate). Contingency tables constructed with light trap thresholds ranging from 10 to 40 female *An. sinensis* per night in intervals of 5 were all significant at $P \leq 0.05$ ($n = 17$). The greatest accuracy (i.e., percentage of times that the light trap threshold corresponded to the landing threshold) of 88.2% was achieved at light trap thresholds of 10, 15, or 20 *An. sinensis* per night per trap (Fig. 9). As expected, the highest sensitivity (100%) was observed at the lowest threshold of 10 *An. sinensis* per night and the highest specificity (100%) at the highest thresholds of 20 or more *An. sinensis* per night. Using only the Taesong'dong light trap, maximum accuracy of 80.0% was achieved at a threshold of 30 *An. sinensis* per night in the trap with a sensitivity of 66.7% and specificity of 100% ($P = 0.0098$, $n = 15$).

The number of anopheline larvae captured by 4 people during 30 min of collection at Warrior Base was related to the number of *An. sinensis* captured in landing collections at Taesong'dong on the previous night (Fig. 10). Regression of the log of the number of larvae (independent variable) and the log of the number of adults in landing collections (dependent variable) was significant ($y = 0.796(x) + 0.709$, $r^2 = 0.465$, $F = 13.046$, df = 1,15, $n = 17$, $P = 0.00256$). Cross-correlation showed a signifi-

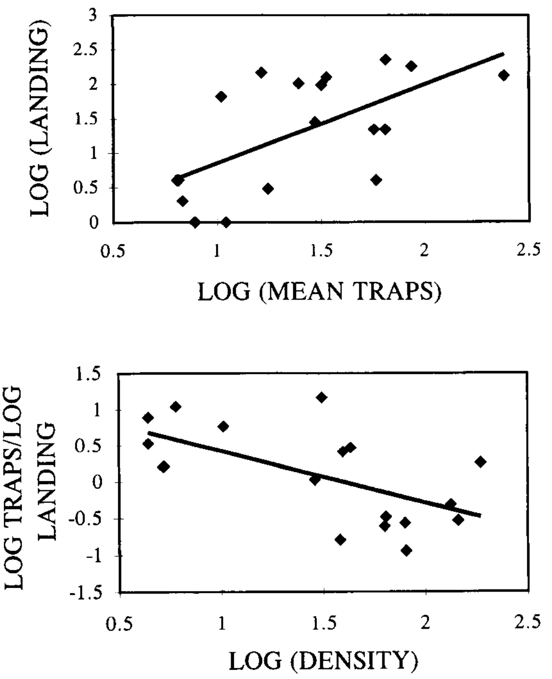

Fig. 8. Comparison of the logs of nightly mean of collections of *Anopheles sinensis* in 5 carbon-dioxide–baited light traps and landing collections at Taesong'dong with regression line indicated (top graph), and relationship between ratio of the 2 kinds of collections to combined density of trap and landing collections with regression line indicated (bottom graph), May 15–September 10, 1997.

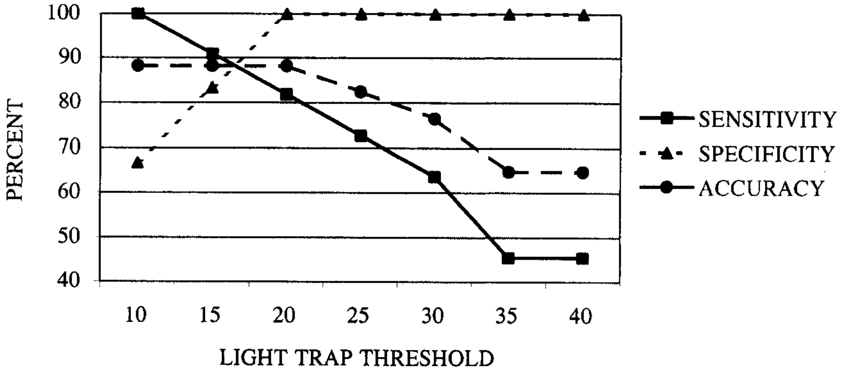

Fig. 9. Accuracy, sensitivity, and specificity of carbon-dioxide–baited light trap catches (mean per night for 5 traps) for prediction of whether or not landing collections at Taesong'dong exceeded 12 *Anopheles sinensis* per night. Light trap collections expressed as thresholds from 10 to 40 in intervals of 5 female *An. sinensis* mosquitoes. Data from 17 nights during weekly collections from May 15 to September 10, 1997, Paju County.

cant relationship between the number of landing mosquitoes and the number of larvae collected on the next morning and also 7 days later (Fig. 11).

DISCUSSION

Anopheles sinensis was the mosquito most often collected in landing collections at 5 different collection sites during 2 wk in 1996 and throughout the season in 1997. In addition to being a vector of *P. vivax* (Ree et al. 1967) and *Brugia malayi* (Ree 1990), *An. sinensis* is a very abundant mosquito throughout the ROK. This species accounted for 81% of mosquitoes in landing collections in northwestern ROK during July and August (Shim et al. 1997), 58% of mosquitoes in light traps in 18 cities during 5 years in southwestern Korea (Lee and Ree 1991), and 37, 43, and 25% of mosquitoes in light traps at 13 U.S. military installations throughout the ROK during 1991–92, 1993–94, and 1995–96 respectively (Kim et al. 1995, 1997, 1999).

The location of a collection influenced the total number of *An. sinensis* landing on humans, varying from a mean of 72 females per night (for 2 collectors) at Taesong'dong to 376 at East Bonifas. This variation might be explained in part by larval abundance of the species near the collection sites, because *An. sinensis* apparently does not fly very far from larval sites when a source of blood meals is nearby (Strickman et al. 1999). In addition, particular aspects of each collection site could have influenced the number of *An. sinensis* landing. At Tongil'chon, nearby cattle may have attracted mosquitoes away from human collectors, because *An. sinensis* is zoophagic (Paik et al. 1988). Although no significant concentrations of large animals were present at Taesong'dong, human residents of the village may have attracted mosquitoes away from

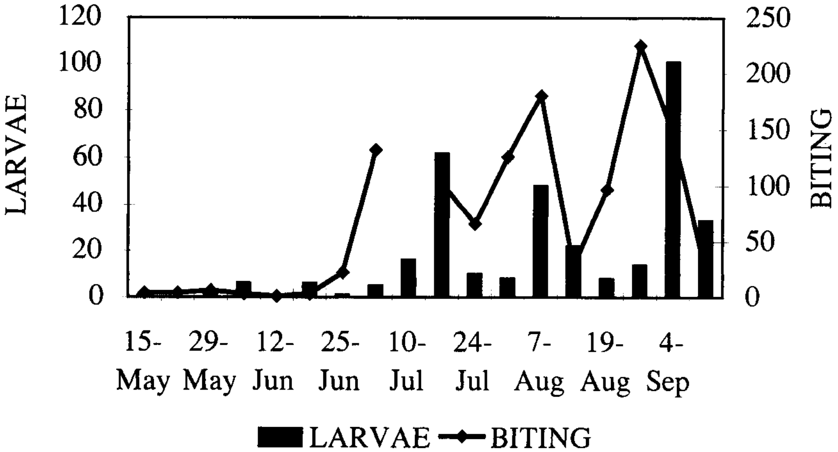

Fig. 10. Number of larvae of *Anopheles sinensis* captured by 4 collectors during 30 min per week at fields near Warrior Base compared to number of mosquitoes captured in landing collections the previous night at Taesong'dong, 1997.

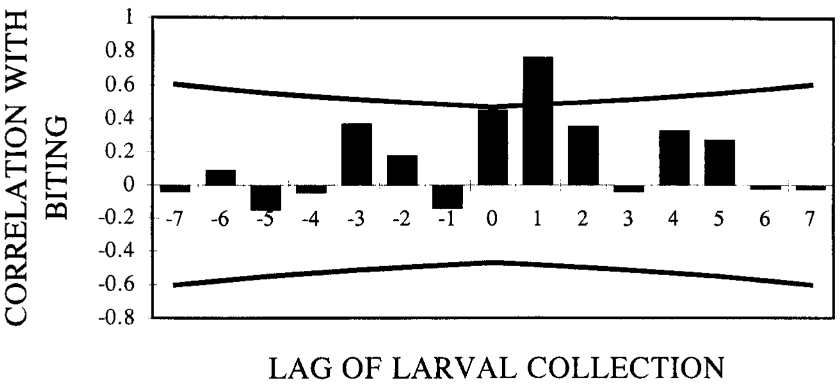

Fig. 11. Cross-correlation between number of *Anopheles sinensis* larvae captured by 4 collectors during 30 min per week (lag) and number of mosquitoes captured in landing collections at Taesong'dong on previous night (May 15–September 10, 1997). Bars are correlation and lines are twice standard deviation. Landing collection for July 9–10 (which was missing because of military alert) was set at the average of number of mosquitoes collected on the previous and following weeks.

collectors. The relatively low landing rate at Observation Point Oullette may indicate that females of *An. sinensis* were reluctant to fly to a higher elevation to seek hosts. The greater number of mosquitoes collected at East Bonifas compared to West Bonifas could have been associated with the topography of these 2 nearby sites. Hills on either side of East Bonifas would have tended to concentrate mosquitoes flying from rice fields.

Other anophelines landing on humans were much less numerous than *An. sinensis*. *Anopheles lesteri* was the next most abundant *Anopheles*, accounting for only 1.5–5.0% of mosquitoes (1.6–6.0 females per 2 collectors per night) at the 5 locations in 1996 and only 4.8% of the mosquitoes landing during 1997 at Taesong'dong. The vector status of *An. lesteri* in Korea is uncertain. This species was 1st described from the Philippines (Baisas and Hu 1936), although the distribution of the species as originally described extended throughout temperate and tropical eastern Asia. Since the original description, *An. paraliae* Sandosham has been raised to a separate species from *An. lesteri* in southeastern Asia (Harrison et al. 1991). Similarly, *An. anthropophagus* Xu and Feng has been described as a separate species in China (Xu and Feng 1975, Ma 1981). No information has been published on the actual taxonomic relationship of Korean *An. lesteri* and Chinese *An. anthropophagus*, although they might be expected to be related or identical. A close relationship between the 2 species would be significant because *An. anthropophagus* is the major vector of *P. vivax* in China (Chi'i et al. 1962). The difficulty and uncertainty of distinguishing adults of *An. lesteri* from *An. sinensis* (K. W. Lee 1998) further complicates the situation. We collected *An. yatsushiroensis* (a suspected malaria vector, Paik et al. 1988) and *An. sineroides* in very low numbers.

In 1996, only 3 species (*An. sinensis*, *Ae. vexans*, and *Cx. pipiens*) were common in landing collections. A greater diversity of mosquitoes was collected in larger numbers in 1997 than in 1996. Seven species in 1997 each accounted for ≥2.0% of the collections (*An. sinensis*, *Ae. vexans*, *Cx. bitaeniorhynchus*, *An. lesteri*, *Cx. pipiens*, *Ae. koreicus*, and *Ae. albopictus*). Seventeen other species were collected in 1997, for a total of 24 species that came to bite humans at a single location in Taesong'dong.

Anopheles sinensis and *Ae. vexans* were present during the entire season. Their numbers were very low until late June, when rice was well established (approximately 33 cm tall) and the average nighttime temperature exceeded 22°C. Both species reached their greatest numbers in August, apparently having increased their populations through the season. Such a steady increase in the populations was a possibility because rice fields in this area retained standing water throughout the growing season almost until the time of harvest. Because *Ae. vexans* deposits its eggs on moist soil likely to flood, it tends to occur in distinct broods caused by simultaneous hatching of eggs over a large area. The number of landing *An. sinensis* also varied during the season, but in a less regular pattern. Temperature during 2 wk before the landing collection accounted for more than 50% of variation in abundance. Possibly, higher temperatures supported faster larval development resulting in production of more adult mosquitoes after 1 or 2 wk.

The seasonal pattern of activity of *An. sinensis* observed in our study corresponded to previous studies. New Jersey light traps operated for 6 years in Paju County collected the greatest number of *An. sinensis* in July during 4 years and in August during 2 years, with no or very few mosquitoes collected in May (Kim et al. 1995, 1997, 1999). Black-light traps collected the greatest number of *An. sinensis* in July at 3 of 4 sites in the northwestern part of the ROK, including Paju County (Shim et al. 1997).

This seasonal pattern corresponds to the incidence of malaria cases, which peaks in August (Yim et al. 1996, Feighner et al. 1998, Lee et al. 1998).

During the season of its peak abundance, *An. sinensis* bit most frequently between 0200 and 0500 h, although departures from this average pattern occurred on some nights at some locations. Shim et al. (1997) also observed highest landing rates in Paju County late at night, between 0100 and 0400 h during July and August 1995. Early and late in the season, the times of peak landing shifted to an earlier hour, apparently corresponding to times of night when temperatures were ≥23°C. Late night landing and seasonal shifts in diel activity by *An. sinensis* were in marked contrast to *Ae. vexans,* which bit in greatest numbers between 2000 and 2300 h throughout the season. Unlike *An. dirus* Peyton and Harrison (Colless 1956, Rosenberg and Maheswary 1982) and *An. farauti* Laveran (Charlwood et al. 1986), landing by *An. sinensis* was negatively correlated to the apparent size of the moon during the hour of collection.

Mean wind speed during an entire night was not strongly correlated with the number of *An. sinensis* captured in landing collections. Closer examination of the data showed that wind had a great influence (accounting for 25% of variation) only during the late-night peak in landing (0200–0400 h). This may be an indication that mosquitoes landing late at night traveled a greater distance to find a host, and therefore were influenced more by wind before reaching the host.

Landing collections of *Anopheles* are presumably the most accurate way of evaluating the biting rate. Although landing collections are practical in the sense that they require a minimum amount of equipment, the technique has numerous disadvantages. Landing collections are tedious and uncomfortable, pose a small disease risk to the collectors (who can take chemoprophylaxis), and include inherent biases from collectors' individual attractiveness to host-seeking mosquitoes. From an operational standpoint, performing all-night landing collections at all sites where soldiers are scheduled to train or deploy usually is not possible. For these reasons, we defined the relationship between light trap and landing collections of *An. sinensis.*

On a seasonal basis the light trap catches and landing collections showed that the same 2 species, *Ae. vexans* and *An. sinensis,* were the most abundant. Perhaps more significantly, the total number of *An. sinensis* captured in a nearby light trap was close to the total number in landing collections in 1997. Comparison of weekly landing collections to carbon-dioxide–baited light traps did not produce a simple relationship. A significant linear relationship was found between the log of the mean of 5 light traps to the log of the landing collection, but this relationship accounted for only about 39% of the observed variation. What is more, the ratio of light trap to landing collections varied systematically, decreasing from a positive to a negative proportion as the mosquito population increased (i.e., landing collections were more efficient than light traps at high mosquito densities). The seasonal change in ratio may have resulted from changes in the population age structure of the vector; however, we did not measure gonotrophic age in order to test this possibility. Unfortunately, an estimate of population from light trap data alone was not sufficient to make an accurate prediction of the ratio of light trap to landing collections. Therefore, using our data, it would only be possible to make a general estimate of landing rate from light trap data. Another study failed to find a useful index of landing based on unbaited light traps for a malaria vector in India, *An. fluviatilis* James (Gunasekaran et al. 1994). In contrast, Lines et al. (1991) showed that they could achieve a constant ratio of about 1.5 between a light trap (placed adjacent to humans sleeping under mosquito nets) and landing collections of *An. gambiae* Giles and *An. funestus* Giles in Africa.

A less direct approach comparing landing and carbon-dioxide–baited light trap collections provided more promising results in our study. A single threshold number of *An. sinensis* in the light traps accurately predicted whether landing rates at Taesong'dong exceeded a minimum value. Using the average from all light traps, 15 or more *An. sinensis* per trap night indicated with 91% sensitivity, 83% specificity, and 88% accuracy that more than 12 *An. sinensis* would bite 2 collectors. A single light trap, although located near the landing collections, was much less predictive. These results show that a light trap augmented with carbon dioxide can be used as a reasonably accurate tool for determining a treatment threshold based on number of landing *An. sinensis.* The results also highlight the importance of using replicate traps.

Despite the inherent inaccuracies of our method for sampling, larval collections were correlated closely to landing collections. Considering that larval sampling provides immediate results and that it can be carried out during daylight hours, application of more accurate sampling methods (Ree 1982) might be worthwhile for operational purposes. The seasonal distribution of larvae at Warrior Base was similar to that observed previously near Seoul (Ree et al. 1981, 1982) and in southwestern ROK (D.-K. Lee 1998). In those studies, larvae 1st increased in numbers during July and then decreased markedly in late September. These studies also documented some sharp fluctuations in number of larvae during the course of the season.

Probably the most practical result from this study was establishment of a threshold for light trap collections of *An. sinensis* based on landing collections. Using this threshold, it would be possible to decide when and where to concentrate vector control efforts based on an implied landing rate threshold. The relationship of landing rates to wind, moon phase, and temperature could also be useful for predicting likely landing rate when trapping data are not available.

Close examination of temperature in Korea could have value in predicting landing populations up to 2 wk later, if finding or estimating an initial landing rate was possible. Temperature might be especially useful for determining the 1st seasonal appearance of large numbers of landing *An. sinensis*. Using the mathematical relationship suggested by our data, an average nighttime temperature over 20°C corresponds to a landing rate of 36 mosquitoes per night 2 wk later. Larval counts of *An. sinensis* might provide another measure of probable landing rates, although important questions remain about the best method for sampling.

The time of night of landing is sometimes portrayed as a constant feature of a species, but the data from this study clearly show that the pattern for *An. sinensis* in Korea can vary from place to place and during a season. This variation complicates the optimum time for application of insecticidal fogs, assuming that the times of greatest landing activity correspond to the times of greatest potential exposure to aerial toxicants. Analysis of our data indicates that the maximum number of mosquitoes would be exposed late at night unless temperatures descend below 23°C. The effect of fogging might also be improved by avoiding times when the moon is in a large phase. Study of diel periodicity throughout the season at several sites would probably provide adequate data to establish a useful model to maximize the effectiveness of insecticidal fogging.

Vector control could be a particularly practical component of attempts to eliminate vivax malaria from the Korean peninsula, because vector activity is limited to a short season. Although the bionomic information from this and previous studies is adequate for rational design of insect pest management plans, a number of questions should be answered in order to improve vector control. First, the systematics of *An. sinensis* and the related members of the Hyrcanus Group are uncertain. Clarifying the taxonomic status of *An. sinensis, An. lesteri,* and *An. yatsushiroensis* in Korea would make comparisons with these species in other regions much clearer. Second, the flight range of *An. sinensis* is virtually unknown except for a single study showing indirect evidence that most individuals do not fly as far as 1 km (Strickman et al. 1999). Better knowledge of flight range is essential to decide the extent of larval and adult treatment for protection of human populations from malaria. Third, the possibility that *P. vivax* overwinters in adult mosquitoes as well as human hosts (Korean strains of this parasite are particularly prone to prolonged incubation times before the 1st symptomatic parasitemia; Brunetti et al. 1954, Feighner et al. 1998, Park et al. 1999) has not been adequately tested. If the parasite can overwinter in the mosquito, limited winter or early spring transmission is possible. And finally, very little information exists on larval habitats other than rice fields, particularly early in the season before planting. Although the current malaria epidemic in Korea is an unfortunate event, it may stimulate research on the vectors that will enable public health authorities to respond more decisively. The malaria situation in Korea could also warrant action from an American perspective, considering that vivax malaria was formerly an overwhelming problem in the United States (Anonymous 1920, Faust 1949) and that it has been locally transmitted in the USA in recent years (Zucker 1996). American soldiers returning from Korea with active infections (Guzenhauser et al. 1997) could become the source for introduction of *P. vivax* parasites that are preadapted to temperate conditions.

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