Seasonal population density and daily survival of anopheline mosquitoes (Diptera: Culicidae) in a malaria endemic area, Republic of Korea

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ABSTRACT**:** Mosquito surveillance was conducted near the Korean Demilitarized Zone (Paju County, Gyeonggi Province) from April to October, 1999, where malaria cases were reported. Adult mosquito surveillance, using black light and CDC UV light traps, was conducted at five and two sites, respectively. Weekly larval collections were made at five rice paddies located adjacent to the adult collection sites. *Anopheles sinensis* was the most abundant mosquito of 11 species collected throughout the surveillance period in 1999, comprising 47-48% of the total number of mosquitoes collected at cow sheds and residence. At all five sites surveyed by CDC UV light traps, anophelines appeared early in the year (May 3) and were most abundant in the cow sheds followed by the hillside forest, residence, stream/river bank, and were least abundant in rice fields. The population density of the larvae and the adults of *An. sinensis* increased steadily in June and reached their peaks during the second week of July (mean 112 females/trap/night). The parity rates were higher in July and September, when populations were highest. The probabilities of daily survival of *An. sinensis* were 0.804 in June to 0.895 in July. Cross-correlation showed a significant relationship between the number of adult anopheline mosquitoes and the number of larvae collected on the previous day, the same day, and also three and seven days later, which may be useful for determining treatment thresholds.
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Keyword Index: *Anopheles sinensis*, daily survival, parous rate, malaria vector, Korea.

INTRODUCTION

Vivax malaria (*Plasmodium vivax*) re-emerged in the Republic of Korea (ROK, South Korea) in 1993 following its eradication in the late 1970s (Paik et al. 1988) (Chai et al. 1994). While malaria cases peaked in 2000, there continues to be an ongoing epidemic of malaria, primarily in northern Gyeonggi and Gangwon Provinces. Since its re-emergence, the number of reported cases has risen exponentially with a total of 6,249 cases by the end of 1998 (Chai 1999). Before 1999, most cases were reported in military personnel in the northwestern part of the country, northern Gyeonggi Province, and northwestern Gangwon Province (Kho et al. 1999, Strickman et al. 2000). The epidemic began in the Panmunjom Valley where ROK, American, and North Korean military personnel are in close proximity within the Demilitarized Zone (DMZ) (Cho et al. 1994). Although the disease has spread to the civilian population, the high concentration of soldiers along the DMZ causes great concern (Strickman et al. 2001). It has been postulated that most of the initial malaria cases in the ROK resulted from transmission by *Anopheles sinensis* Wiedmann that were infected in North Korea and subsequently dispersed across the DMZ into the ROK (Chai 1999, Kho et al. 1999). However, vivax malaria is now firmly established and endemic within the ROK (Lee et al. 1998). Vector surveillance, resulting from the re-emergence of vivax malaria in the ROK, has been primarily focused on determining the seasonality and distribution of presumptive vectors at military installations and training sites near the DMZ using light traps and/or landing collections (Kim et al. 1997, 1999, Shim et al. 1997, Strickman et al. 1999, 2000), with little effort to determine population densities and parous rates of potential vectors near areas where civilians reside.

The current study was conducted to determine the seasonal periodicity of *An. sinensis*, associated non-vector mosquitoes species, seasonal parous rates of *An. sinensis*, and correlation of larval and light trap collections for *An. sinensis* near a civilian area bordering the DMZ, Paju County.

MATERIALS AND METHODS

Study site

The study was conducted in Jangpa-ri, Papyung-myun, Paju-si (city), a civilian area south of the Imjin River near the DMZ of northern Gyeonggi Province (37°56'N, 126°50'E) where malaria was first reported in a ROK soldier near this area in 1993. The study site, characterized as rural with 1,881 residents, borders low wooded hills interspersed with small narrow valleys. The primary agriculture is wetland rice farming and associated irrigation ditches in the low-lying valley areas. Intensive rice culture has resulted in a drainage pattern dominated by human-managed irrigation, with few natural streams or ponds. Dairy/beef (498 cattle), swine (246 pigs) and domesticated deer (105) were interspersed within

the community on small farms, along with domesticated and free roaming dogs (233)

Sampling

Weekly light trap and larval collections were made at Jangpa-ri from April 5 to October 25, 1999, except for the first week of August and the third week of September when there were heavy rains. The trap sites, within 2 km radius of the community of Jangpa-ri, included: a hillside deciduous/pine forest, the border of a rice paddy, a cow shed, a human dwelling (outside), and along the branch bank of the Imjin River. The light traps (New Standard CDC-style Miniature Fluorescent UV light trap, model No. 1212, John W. Hock Co., Gainesville, FL, or Yoshisawa type, Shinyoung Korea Co.) were hung from trees or fences about 1.5 m above the ground. Both UV and black light traps were operated without additional attractants from 18:00 to 06:00 h. Captured mosquitoes were transferred to paper cups containing chloroform impregnated cotton used as a killing agent. Collected mosquitoes were transported the following morning after each trap night to the National Institute of Health (NIH) where they were identified, separated, and counted. To avoid fungal contamination, all adult mosquitoes were dried and stored in petri dishes separated by layers of tissue paper. The physiological age of *An. sinensis* females was determined by ovarian dissection of females collected at a residence house in Jangpa-ri by CDC UV light traps. Ovaries of *An. sinensis* females were removed and covered with a 21mm cover glass, dried, and parity scored by Detinova's method (Detinova 1962). Additionally, larval collections were made weekly from five rice paddies near the town of Jangpa-ri and adjacent to areas where light trap surveillance was conducted. Larval collections were conducted in accordance with standard techniques, e.g., mosquito larvae were collected with a 350-ml dipper (BioQuip®) by taking 20 dips per rice paddy along each of the rice paddy sides. Adult and larval identification were based on keys and descriptions of Tanaka et al. (1976) and Lee (1998b).

Analyses

The relationship between light trap and larval collections was determined by linear regression of the log of total number of *An. sinensis* females collected daily from five traps and number of larvae captured from the five nearby rice paddies during the same period. We performed similar analysis as Lines et al. (1991) and Strickman et al. (2000) on our data, regressing the ratio of the log of light trap and larval collections on the geometric mean of light trap and larval counts. When the collections had no *An. sinensis*, the number was adjusted by adding 1 (Strickman et al. 2000). All other observations were greater than zero, so a $\log(x + 1)$ adjustment was not necessary.

Excel-97 (Microsoft Corp., Seattle, WA) was used to calculate linear regression and correlation, while SPSS (SPSS for Windows, Release 6.1.2, SPSS Inc., Chicago, IL) was used for analysis of variance (one-way ANOVA with Duncan's multiple range test).

RESULTS

Vector bionomics

Totals of 47,000 and 1,314 female mosquitoes were captured in 28 trap-night collections using Yoshisawa UV light traps in a cow shed and a residence during 1999, respectively (Table 1). Five of the 11 mosquito species comprised 99.9 and 99.3% of the total collected at the cow shed and residence, respectively. Overall, *Anopheles sinensis* Wiedemann was the most abundant mosquito collected at the cow shed (47.0%) and residence (48.1%). *Aedes vexans nipponii* (Theobald) (40.8 and 29.8%), *Anopheles pullus* (6.9 and 7.2%), *Culex pipiens* L. (3.4 and 13.9%), and *Culex tritaeniorhynchus* (0.8 and 1.4%) were the primary other mosquito species collected at the cow shed and residence, respectively. *Anopheles sineroides* Yamada (1), *Culex. vagans* Wiedemann (1), *Culex bitaeniorhynchys* Giles (1), *Culex orientalis* Edwards (5), *Aedes albopictus* (Skuse) (1), and *Armigeres subalbatus* (Coquillett) (4) were infrequently collected from all sites.

The four species of mosquitoes most frequently collected during 1999 followed various seasonal and habitat distributions (Figure 1). Three of the species, *An. sinensis, Cx. pipiens,* and *Ae. vexans*, were collected throughout the "mosquito season," while *Cx. tritaeniorhynchus* did not appear until late in the season

Anopheline distribution

The relative abundance of four predominant mosquito species collected by CDC light traps for five different habitats varied seasonally (Figure 1). The overwhelmingly predominant anopheline species in all habitats was *An. sinensis* (558 of 624 anopheline mosquitoes) from June through September, 1999. Anophelines were collected more frequently from the cow shed followed by the forested hillside, residence, river/stream bank, and were least abundant in the rice paddy. *Anopheles pullus* was collected primarily from the cow shed.

The population density of the larvae and the adults of *An. sinensis* increased steadily in June, and peaked during the second week of July (112 females/trap/night) (Figure 2), following flooding of the paddy and rice planting in June. A second smaller peak occurred during the second week of September that might be attributed to emergence and dispersal from the rice paddy habitat and lower rainfall and/or a result of rice harvesting and reflooding of the paddies.

Cross-correlation showed a significant relationship between the number of collected adult mosquitoes and the number of larvae collected the previous day, the same day, and also three and seven days later (Figure 3). The numbers of all anopheline larvae collected from the rice paddies in Jangpa-ri were correlated to the numbers of *An. sinensis* captured in CDC light trap collections on the same night. A regression of the log of the number of larvae (independent variable) and the log of the number of adults in light trap collections (dependent variable) showed a high correlation ($y = 0.693x + 0.731$, $r^2 = 0.572$, $F = 16.01$, $df = 12$, $n = 14$, $P = 0.002$) for collections made on the same day (Figure 4).

Table 1. Seasonal distribution of adult female mosquitoes collected in Yoshisawa UV (black) light traps from a cow shed and a residence in Jangpa-ri, Paju, 1999.

Species[b]	Total collected (%)[a]		Date of collection					
			First Positive		Last Positive		Highest	
	Cow Shed	Residence	Cow Shed	Residence	Cow Shed	Residence	Cow Shed	Residence
Aedes vexans nipponii	9,176 (40.8)	392 (29.8)	May 3	May 3	Oct 25	Oct. 25	Jul 19	May 31
Anopheles sinensis	22,618 (48.1)	617(47.0)	May 31	May 17	Oct 18	Sep. 27	Jul 19	July 12
Anopheles pullus	3,258 (6.9)	94 (7.2)	May 31	May 17	Sep 27	Sep. 13	Jun 21	June 21
Culex pipiens	1,588 (3.4)	183 (13.9)	Apr 19	Apr 19	Oct 11	Oct. 25	Jul 5	July 12
Culex tritaeniorhynchus	360 (0.8)	19 (1.4)	Aug 6	Aug 16	Sep 27	Sep. 27	Aug 16	Sep. 6
Total	47,000	1,314						

[a] A total of 28 trap-night collections were made at each of the two trap sites.
[b] Insufficient numbers of other mosquitoes were collected for analysis: *Ae. albopictus* (1), *An. sineroides* (1), *Ar. Subalbatus* (4), *Cx. orientalis* (5), *Cx. bitaeniorhynchus* (1), and *Cx. vavans* (1).

Parous rate

Parity rates and the probability of daily survival for *An. sinensis* were determined for adult females captured in CDC UV light traps from June through the first week of October, 1999 (Table 2). The parity rates were lower during June (avg. 52.0%), August (60.0%), and October (63.2%), while they were higher in July and September at 71.6% and 71.4%, respectively. The probabilities of daily survival of *An. sinensis* ranged from 0.804 in June to 0.895 during July. Too few adult *An. sinensis* were collected during October to provide an accurate estimate of anopheline parity.

DISCUSSION

Anopheles sinensis, the principal vector of *P. vivax* malaria (Ree et al. 1967) and *Brugian filariasis* in the south (Ree 1990), was the most frequently collected mosquito in the Yoshisawa UV light trap collections made at a cow shed and a residence in Jangpa-ri in 1999. It is commonly found throughout Korea and is often found associated with *Ae. vexans* and *Cx. tritaeniorhynchus*, breeding in such habitats as ground and stream pools, marshes, rice paddies, and irrigation ditches.

The seasonal distribution of *An. sinensis* larval populations in Jangpa-ri was similar to that observed previously near Seoul (Ree et al. 1981, 1982) in southwestern ROK (Lee 1998a), and near the DMZ (Strickman et al. 2000). In those studies, larvae increased in numbers during July and then decreased markedly in late September. Large variations, often due to heavy rains, flooding, and extended dry periods, in numbers of *An. sinensis* larvae collected in our study corresponded to similar studies during the course of the season. While adult *An. sinensis* were sometimes present as early as late March, they were infrequently collected in May collections (0.2% of the total number collected during the season) in residences. During June, populations rapidly increased in numbers (15.5% of the total number collected during the season) and accounted for 58% of all mosquitoes collected in various types of light traps in 18 cities during a five-year period in southwestern Korea (Lee and Ree 1991). Collection location (habitat) influenced the total number of *An. sinensis* collected when using CDC UV light traps, varying from an average of 0.7 females per light trap/night at rice paddies to a high of 11.1 females at a cow shed. This variation might be explained, in part, by host-seeking zoophagous behavior (Paik et al. 1988). Relatively large numbers of adult females (10.9 per trap/night) were collected at hillside forests, perhaps as a result of resting behavior of newly-emerged and blood fed mosquitoes (Strickman et al. 2000). Human attraction similarly accounted for relatively large numbers of females collected (9.6 per trap/night) at the residence. Rice paddy and stream bank habitats resulted in relatively low adult populations (4.7 per trap/night), indicating that newly-emerged adults and ovipositing females have a low propensity for searching for blood meals and are likely seeking resting/oviposition habitats. Similar results were also shown for mosquitoes collected among rice paddies and ROK Army military bases (unpublished data).

Other anopheline species, *An. pullus* and *An sineroides*, were collected much less frequently than *An. sinensis*.

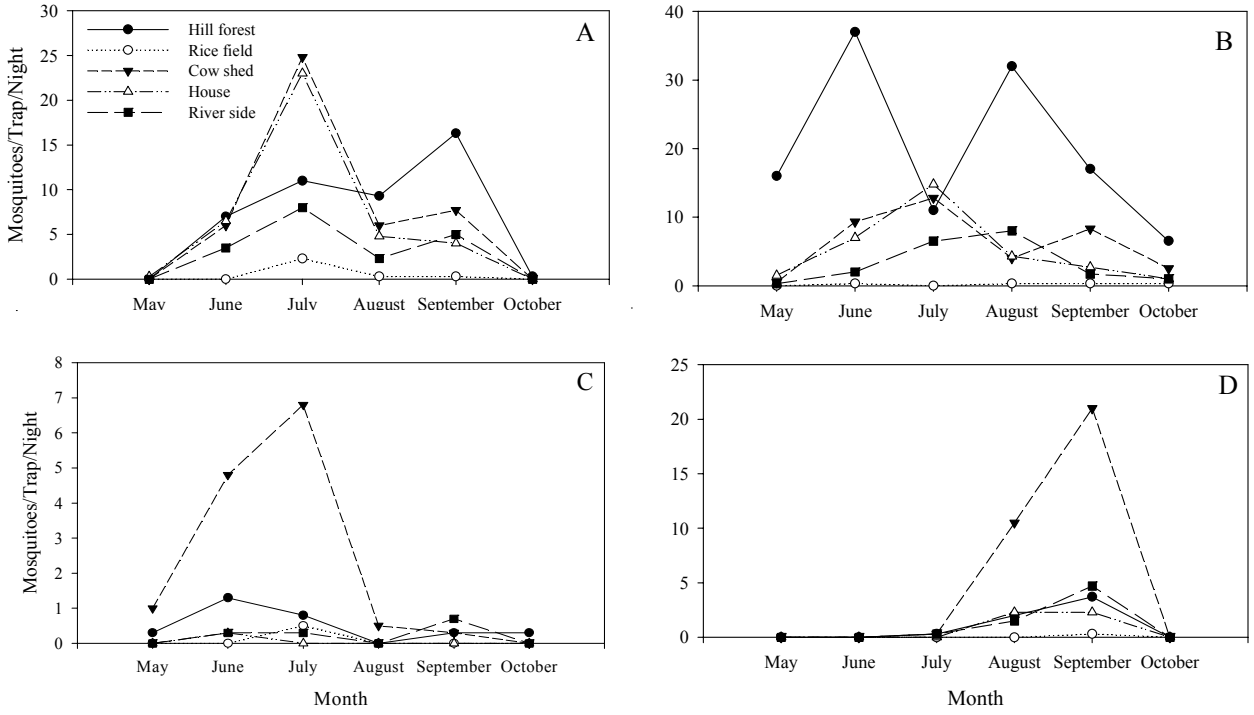

Figure 1. Density of four predominant species of female mosquitoes collected from CDC light traps at five different sites in Jangpa-ri, Paju county, 1999. A, *Anopheles sinensis;* B*, Aedes vexans;* C, *Anopheles yatsushiroensis*, D, *Culex tritaeniorhynchus.*

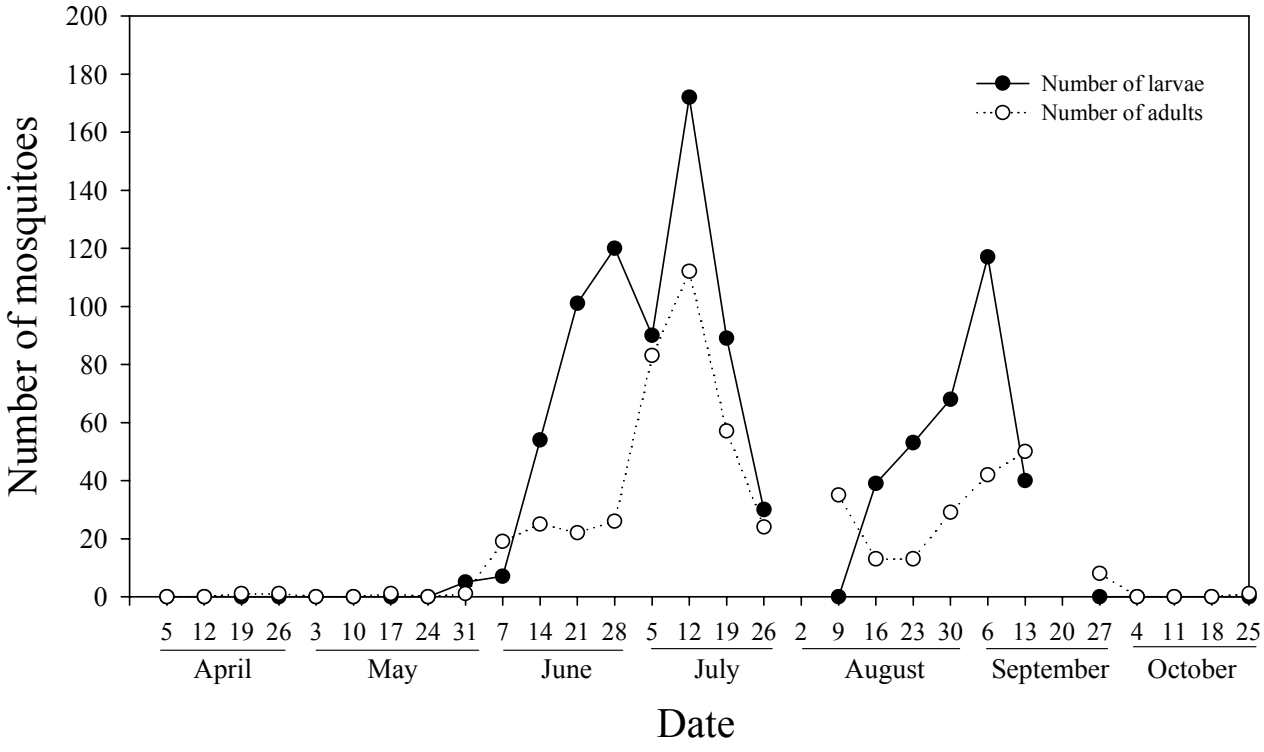

Figure 2. Mean number of larvae per dip and adults per night trap of *Anopheles sinensis* in Jangpa-ri, 1999.

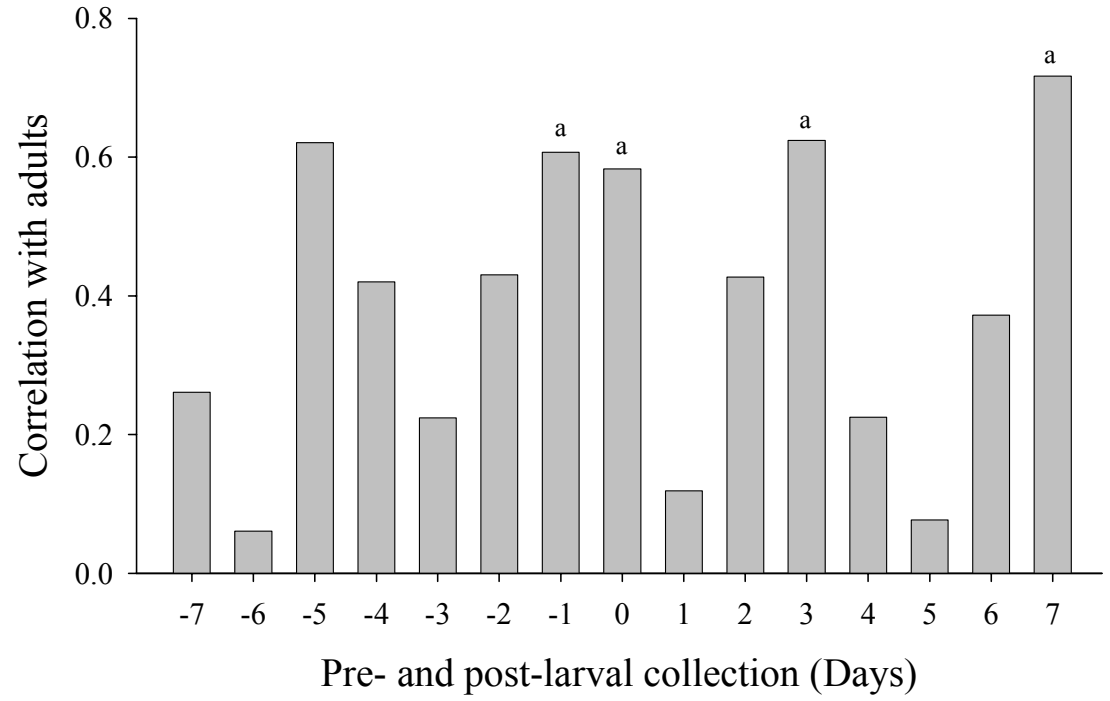

Figure 3. Correlation between the number of adult mosquitoes and the number of larvae on the various days. The letter (a) designates a significant relationship between the numbers of adult and larvae mosquitoes collected on the designated days.

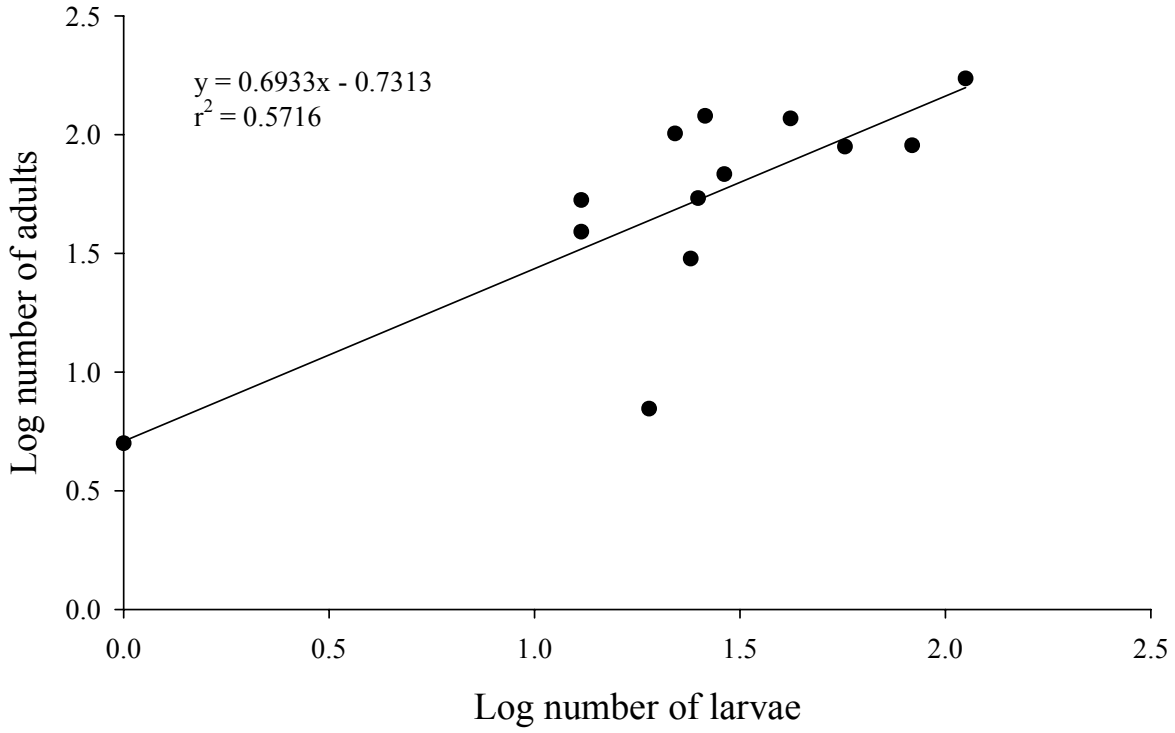

Figure 4. Relation between log numbers of larvae and adults of *Anopheles sinensis* collected.

Table 2. Parous rates and probability of daily survival of *Anopheles sinensis*collected from five different sites in Jangpa-ri, Paju in 1999.

Month	No. of weekly collections	Number dissected	Parity rates (%)	Probability of daily survival
June	4	127	52.0	0.804
July	4	115	71.6	0.895
August	4	109	60.0	0.843
September	3	69	71.4	0.894
October[1]	1	9	63.2	0.858

[1] Insufficient numbers of mosquitoes were dissected for accurate estimates of parity rates and daily survival rates.

Anopheles pullus accounted for only 3.4% of the total number of mosquitoes (0.7 per trap/night) at the five sites in Jangpa-ri during 1999. Only one female *An. sineroides* was collected (hillside forest habitat) and was infrequently collected in other mosquito collections throughout Korea (Kim et al. 1995, 1997, 1999). Strickman et al. (2000) reported that *An. lesteri* was the second most abundant *Anopheles* near DMZ during 1996 and 1997. However, we now know that this species cannot be morphologically distinguished from *An. sinensis* (Wilkerson, R., personal communication), and in collections identified by DNA, accounts for less than 5% of the population (H.C. Kim, unpublished data). The seasonal activity pattern of *An. sinensis* observed in our study corresponded to previous studies. New Jersey light traps operated for seven years in Paju County, near the DMZ and north of Seoul, collected the greatest number of *An. sinensis* in July over 4 years and in August over 3 years, with no or very few mosquitoes collected in May (Kim et al. 1995, 1997, 1999, Strickman et al. 2000).

Seasonal changes in the parous rates result from changes in population age structure due to emergence rates, etc. (Strickman et al. 2000). The overall parous rate (63.6%) in our study was not significantly different than that shown by Ree et al. (2001) (66.1%) in northwest Gyeonggi Province near Seoul in 1999. The proportion of daily survival for *An. sinensis* (0.86) in our study was estimated to be similar, compared to previous reports of *An. sinensis* and other anopheline species from other countries. [0.87 for *An. sinensis* in the northern Gyeonggi Province (Ree et al. 2001), 0.89 for *An. pharoensis* and 0.80 for *An. multicolor* in Egypt (Kenawy 1991), 0.85 for *An. gambiae* in southern Sierra Leone (Bockarie et al. 1995), and 0.80-0.88 for *An. gambiae* in Sudan (Costantini et al. 1996)]. The parous rates were highest in July and September when adult populations also were high, accounting for potential increased malaria transmission during these periods, compared to those in August and October, 1999, when populations were lower. However, overall monthly trends in malaria cases in the ROK do not support this hypothesis, as malaria cases peaked in July/August and declined in September (Lee et al. 2002). Decreases in both parous rates (34.3 - 50.0%) and adult population densities during the third and fourth weeks of August 1999 most likely resulted from heavy rains in the fourth week (329mm) of July and the first week (270mm) of August that led to flooding and fast flowing water in many areas. We assumed that the parous rates would increase late in the season as fewer adults emerged, but too few adults were evaluated for parity during September to provide a good estimate. However, as only unfed adults survive winter, it may be possible that as environmental conditions change and the temperatures get colder, a large proportion of the collected mosquitoes would be unfed adults preparing to overwinter.

During the 1999 survey, only four species (*An. pullus, Ae. vexans, Cx. pipiens*, and *Cx. tritaeniorhynchus*) were commonly associated in CDC light trap collections with *An. sinensis*. Light trap collections showed that *An. sinensis and Ae. vexans* were the most commonly collected mosquitoes throughout the season. In Korea, *Ae. vexans* appears very early in the season and is found to breed in rice paddies, ground and stream pools, and ponds in association with *An. sinensis* and *Cx. tritaeniorhynchus*. Although widely distributed throughout Korea, *Ae. vexans* is most commonly collected in the northern part of Korea, whereas *Cx. tritaeniorhynchus* is more commonly collected in the south (Kim et al. 1997). *Ae. vexans* was also found to be the predominant species in the cow shed from August through October and it has a low propensity to bite men when compared to Mosquito Magnet® (American Biophysics) and human landing collections (unpublished data). Kim et al. (1995, 1997, 1999) reported that *An. pullus*, *Cx. tritaeniorhynchus*, *Cx. pipiens,* and *Ae. vexans* were associated with *An. sinenesis* in New Jersey light trap collections made from 1993 - 1997 at 12 US military installations throughout Korea.

Although landing collections were more efficient at collecting *An. sinensis* than light traps at high mosquito densities (Strickman et al. 2000), larval collections were highly correlated to light trap collections. Strickman et al. (2000) showed that larval sampling provides immediate results, that sampling can be conducted during daylight hours (a major advantage to night-time landing collections), and that the development and application of more accurate sampling methods might be worthwhile for operational purposes. Additionally, factors such as habitat shifts when rice paddies dry up during harvesting and reflooding after the harvest, heavy rains and flooding, etc., must be included into any surveillance and operational treatment analysis. A significant linear relationship was found between the log of the number of larvae to the log of the number of adults at the five light trap collection sites (Figure 4). However, an estimate of adult populations based on larval collection data alone was not sufficient to make an accurate prediction of the ratio of larval

collections to light trap collections. According to our data, it was only possible to make a general estimate of light trap collection rates from larval collection data. Strickman et al. (2000) reported that a less direct approach, comparing landing and CO_2-baited light trap collections, provided more promising results. According to their report, a single threshold number of *An. sinensis* for light traps accurately predicted whether landing rates at Taesong'dong, a small rice-farming town in the DMZ, exceeded a minimum value. Their results showed that light traps augmented with carbon dioxide can be used as a reasonably accurate tool for determining a treatment threshold based on the number of landing *An. sinensis*. Based on our results, we suggest that the number of *An. sinensis* larvae collected may be useful for determining treatment thresholds on the same day, three days, and seven days later.

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