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□ Mini-Review □

Studies on *Anopheles sinensis*, the vector species of vivax malaria in Korea

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Abstract: Extensive previous studies on taxonomy, behavior/bionomics and control of *Anopheles sinensis* are reviewed and summarized. Recent molecular identification revealed that the population of *An. sinensis* complex includes *An. sinensis*, *An. pullus*, *An. lesteri* and at least two new species, and *An. yatsushiroensis* is synonmy of *An. pullus*. *An. sinensis* is the main vector specie of vivax malaria in Korea. Larvae of *An. sinensis* breed in wide range of habitats which are naturally-made clean water, stagnant or flowing; main habitats include rice fields, ditches, streams, irrigation cannals, marshes, ponds, ground pools, etc. Their host preferences are highly zoophilic. Human blood rate is very low (0.7-1.7%); nevertheless *An. sinensis* readily feeds on man when domestic animals are not found near by. They feed on hosts throughout the night from dusk to dawn with a peak period of 02:00-04:00 hours; they are slightly more exophagic (biting outdoors); much larger numbers come into the room when light is on. Main resting places are outdoors such as grasses, vegetable fields and rice fields. A mark-release-recapture study resulted that 37.1% was recaptured within 1 km, 29.4% at 1-3 km, 21.1% at 3-6 km, 10.3% at 6-9 km and 2.1% at 9-12 km distance. *An. sinensis* hibernate outdoors (mostly under part of dense grasses) during October-March. At the end of the hibernation period (March-April) they feed on cows at daytime. Until today any single measure to effectively control *An. sinensis* population has not been found. Indoor residual spray with a long-lasting insecticide can not reduce vector population densities, but shorten their life spans in some degree, so contributes to malaria control.

Key words: *Anopheles sinensis*, malaria, Korea

INTRODUCTION

In Korea, vivax malaria which had been prevalent throughout the country for many years was eradicated in 1979. Fifteen years later, malaria re-emerged in South Korea in 1993 and an outbreak has been occurring, with thousands of cases every year (Chai, 1999; Ree, 2000). Moreover, in North Korea hundred thousands of malaria cases have been found recently, being reported 204,428 cases and 300,000 cases in 2000 and 2001, respectively. Infection rate of the inhabitants was 32.9% in Kaepung-gun, Hwhanghaenam-do in 2001 (unpublished report).

The degree of epidemicity of malaria is decided by many factors, of which vector efficiency is one of the most important ones. The main vector species of vivax malaria in Korea is *Anopheles sinensis* (Ree et al., 1967; Hong, 1977; Lee et al., 2000; Strickman et al., 2001). The knowledge of behavior and bionomics of the vector species is of vital importance to understand the epidemiological features, to find effective control measures, and to interrupt vector-human contact. Studies of *An. sinensis* in Korea initiated by several

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Japanese workers in 1910s-1930s, providing very primitive informations. Thereafter, comprehensive studies were implemented by the entomological team of National Malaria Eradication Service (NMES) in 1960s and by various workers in 1990s. The author reviewed all the available study results of *An. sinensis*, which would contribute for the control / eradication of recent outbreak vivax malaria not only in South Korea but also in North Korea.

Anopheles sinensis Wiedmann 1828 is a member of the *Anopheles hyrcanus* group belonging to the Myzorhynchus series of the subgenus *Anopheles*, which are distinguished from other series by the presence of pale bands (usually four) on the palpi and by the presence of a tuft of dark scales on the clypeus on each side in the female adult. *An. sinensis* is distributed in Assam, Burma, Thailand, Malaysia, Indonesia, Kampuchea, Vietnam, Nam, China, Taiwan, Japan and Korea. Among 28 related species of the Hyrcanus group, five species was found in Korea. *An. sinensis* was reported in Korea as the new species, named *An. yesoensis* by Hasegawa (1913), and it was revealed that it was synonym of *An. sinensis* by Yamada (1924). In 1936 *An. sineroides* Yamada, 1924 was found near Seoul, and *An. pullus*, a new species was collected in Taejeon, Chungchongnam-do (Yamada, 1937). *An. lesetri* Baisa et Hu, 1936 was reported for the first time in Korea by Whang (1964). *An. yatsushiroensis* Miyazaki, 1951 was found by Hong and Ree (1968). Besides five related species of the Hyrcanus group, *An. koreicus* Yamada and Watanabe, 1918 and *An. lindesai japonicus* Yamada, 1958 were listed in Korean mosquito fauna by Yamada (1937) and Hong and Ree (1968), respectively. Recently *An. yatsushiroensis* was synonimized of *An. pullus* by morphological observation (Shin and Hong, 2001) and by molecular evidences (Hwang et al., 2004). Recent molecular studies revealed that *An. lesteri* from Korea, *An. anthropophagus* from China and *An. lesteri* from the Philippines were all same species (Wilkerson et al., 2003), and *An. lesteri* from Japan and *An. anthropophagus* from China were same species (Hwang et al., 2005). Ree et al. (2005) reported an unknown *Anopheles* species which was morphologically identical to *An. sinensis*, and Li et al. (2005) also found two unknown species. Rueda (2005) designated these two species as new species, *An. belenrae* sp. nov. and *An. kleini* sp. nov. They were morphologically identical with *An. pullus* and *An. sinensis*, respectively. Morphological identification of *sinensis* complex is extremely difficult, so that some numbers of *An. lesteri*, *An. pullus* (including the form *yatsushiroensis*) and two species (at least) are mixed in the population of *An. sinensis* in Korea.

TAXONOMY OF *ANOPHELES HYRCANUS* COMPLEX

Malaria Infection of *An. sinensis*

Within the range of distribution of *An. sinensis* its importance as a malaria vector would appear to be confined to China, Taiwan, Japan including the Ryuku Islands, and Korea. *An. sinensis* is not probably considered to be a malaria vector in Indonesia and Malaysia, and of little significance to human health in Thailand. In Japan, an infection rate of 0.9% was reported in Kyoto prefecture in 1903. In China various workers reported 0.006-2.58% of infection rates of *An. sinensis* to *P. vivax*. The infection rate of *An. sinensis* in Taiwan was 0.02% in 1947-1949.

In Korea, salivary gland dissections of *Anopheles* mosquitoes for natural infection rate of vivax malaria were implemented in three different locations in 1960-1962 (Table 1). Total 7,517 females of *An. sinensis* were dissected and one positive was found, showing the infection rate of 0.01% in total of three locations (Ree et al., 1967). In 1966-1967, 4,225 *An. sinensis* and 1,469, *An. yatsushiroensis* were dissected for sporozoites and oocysts, and two and one positive cases were found from the former (0.05%) and the latter species (0.07%), respectively, as shown in Table 2 (Hong, 1977).

At 14 locations of malaria endemic areas, 4,866 *An. sinensis*, 673 *An. pullus*, 58 *An. sineroides* and 12 *An. lesteri* were collected in August - September 1996. They were pooled (50 ± 5) and nested-PCR were implemented for application of *P. vivax* specific gene, and two positives from *An. sinensis* pools were found showing at minimum 0.04% of infection rate (Lee et al., 2000). *Anopheles* mosquitoes which were collected

Table 1. Salivary gland dissections of *Anopheles* mosquitoes for natural infection of vivax malaria in Korea in 1960-1962 (Ree et al., 1967)

Locality	*A. sinensis*	*A. sineroides*	*A. koreicus*
Yeongju	1,589	6	287
Andong	842	339	80
Yangpyeong	4,906 (1)[a)]	2	5
Total	7,337 (1)[b)]	347	372

[a)]positive (0.04% of infection rate).
[b)]positive (0.01% of infection rate).

during landing or biting humans in Paju city, Kyonggi-do in July 1996 were studied for vivax infections by ELISA assay of circumsporozoite antigen and 0.28% (1 positive / 361 females) and 0.06% (1 / 1,559) of the infection rate were found at Taesong-dong and Camp Bonifas, respectively (Strickman et al., 2001). None of 2,005 *An. sinensis* from other collecting sites were infected. None of 82 *An. lesteri* or 29 *An. yatsushiroensis* was positive for vivax malaria.

Breeding Habitat

Broadly speaking, the breeding habitat of *An. sinensis* is everywhere much the same. The larvae are found in rice fields, open grassy ponds, ground pools, swamps, marshes, shores of lakes, stream margins, ditches, and seepages. All these are normally fresh, shallow water habitats, either stagnant or flowing, usually with emergent vegetation and exposed to sunlight. Results of larval collections of *An. sinensis* in May-September by dipping are given in Table 3 (NMES, 1968) and Table 4 (Self et al., 1971). Though numbers of the larvae collected in rice fields are smaller than those collected in parceley fields and marshes, the main breeding place of *An. sinensis* is undoubtedly the rice field, taking into account the total area size of each breeding place throughout the whole country. Comparative studies between adult population densities and size of the rice field were carried out in 13 counties of Chollabuk-do in 1985-1990 (Ree and Lee, 1993). Population densities of *An. sinensis* had no correlation with sizes of the rice fields (r = 0.12), whereas those of *Cx. tritaeniorhynchus* had a positive correlation (r = 0.62). These findings indicate that *An. sinensis* had many other breeding habitats together with rice fields so that density of *An. sinensis* population was not much influenced by the size of rice fields. Large or small temporary ground pools, particularly in rainy season, provide ideal breeding places for *An. sinensis*. Streams are also one of the main breeding sources. The author collected 347 larvae of *An. sinensis* in 30 minutes at water weed of a rapidly flowing stream in

Table 2. Salivary gland dissections of *Anopheles* mosquitoes for natural infection of vivax malaria in 1966-1967 (Hong, 1977)

Area (Year)	*An. sinensis*		*An. yatsushiroensis*	
	No. dissected	No. positives	No. dissected	No. positives
Andong (1966)	207	0	89	1 (1.12)[a)]
Cheongsong (1967)	4,018	2 (0.05)	1,380	0
Total	4,225	2 (0.05)	1,469	1 (0.07)

[a)]Infection rate in parenthesis.

Table 3. Collections of *An. sinensis* larvae by dipping in May-September, 167 (NMES, 1968)

Location	Number of larvae per man hour				
	Rice field	Ditch	Parsley field	Pond	Ground Pool
Jeongup-gun	33.1 (24)[a)]	41.0 (2)	-	26 (1)	5 (1)
Cheongsong-gun	77.0 (21)	4.4 (11)	74.8 (4)	11.1 (13)	14.6 (14)
Average	55.1	22.7	74.8	18.6	9.8

[a)]Number of weekly collections in May-September are given in parenthesis.

Table 4. Number of 3rd-4th instar larvae of *An. sinensis* collected by dipping in 1971 (Self et al., 1971)

Locality	Habitat	Hr.[a)]	No. of larvae per man per hour					
			May	June	July	Aug.	Sept.	Ave.
Busan	Rice field	40	0.3	2	9	4	-	3.8
	Marsh	19	0	45	1	3	-	12.3
	Parsley field	59	0.7	1	16	8	-	6.4
	Others[b)]	52	0.6	0.2	0.5	4	-	1.3
Sintaein	Rice field	163	3	8	13	3	0.3	5.5
	Parsley field	66	4	8	15	6	0	6.6
	Others	40	2	2	65	1	0	14.0
Seoul	Rice field	48	0.3	1	3	18	-	5.6
	Swamp	156	0.1	1	8	2	17	5.6
	Others[b)]	53	0	0.8	4	0	-	1.2
Average	Rice field	103	1.2	3.7	8.3	8.3	0.3	4.4
	Marsh/swamp	115	0.1	23	4.5	2.5	17	9.4
	Parsley field	77	2.4	4.5	15.5	7.0	0	5.9
	Others[b)]	53	0.9	1.0	23.2	1.7	0	5.4

[a)]Total hours of the collections.
[b)]ground pools, ditches, streams, etc.

Table 5. Cow biting collectionsa) of *An. sinensis* at Yangpyeong and Yeoju, Gyeonggi-do and Okgu, Jeollabuk-do in 1964 (Paik et al., 1965)

Area	Month	Time					Total (5 hrs)	Average (per hr)
		20:00-21:00	22:00-23:00	24:00-01:00	02:00-03:00	04:00-05:00		
Yangpyeong	May	2	4	2	2	1	11	2.2
	Jun.	125	83	79	50	25	310	62.0
	Jul.	97	85	134	135	93	544	108.8
	Aug.	91	144	144	138	117	633	126.6
	Sep.	71	26	25	44	41	207	41.1
Yeoju	Jun.	73	52	28	36	24	213	42.6
	Jul.	134	123	124	139	76	592	118.4
	Aug.	113	171	156	127	71	637	127.4
	Sep.	54	43	45	32	35	208	41.6
Okgu	May	1	1	0	0	0	2	0.4
	Jun.	38	64	47	50	21	220	44.0
	Jul.	236	125	145	124	103	567	113.4
	Aug.	75	106	51	153	143	493	98.6
	Sep.	28	40	22	21	5	103	20.6

[a)]Average of 4 night collections each month.

August 1992 (unpublished data).

Recently, Claborn et al. (2002) reported that six primary habitats of *An. sinensis* were rice fields, irrigation ditches, drainage ditches, stream pools, irrigation pools and marshes, most of which harbored similar densities of larvae. They also reported that environmental factors, such as pH, total dissolved solids, percent of surface covered with floating vegetation, and nitrate and phosphate concentrations were not correlated with larval densities.

Seasonal Prevalence

The seasonal prevalence of *An. sinensis* population has been studied by many workers in Korea, mainly applying two monitoring methods, either cow biting collections throughout the night during the period of

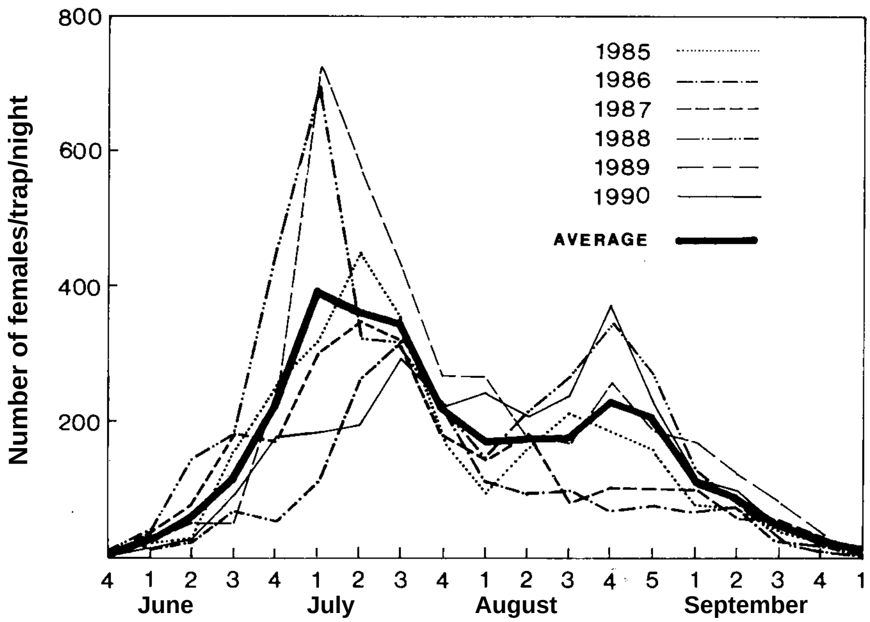

Fig. 1. Seasonal prevalence of *Anopheles sinensis* adult populations in Jeollabuk-do in 1985-1990 (Average of 18 Si/Gun collections) (Lee and Ree, 1991).

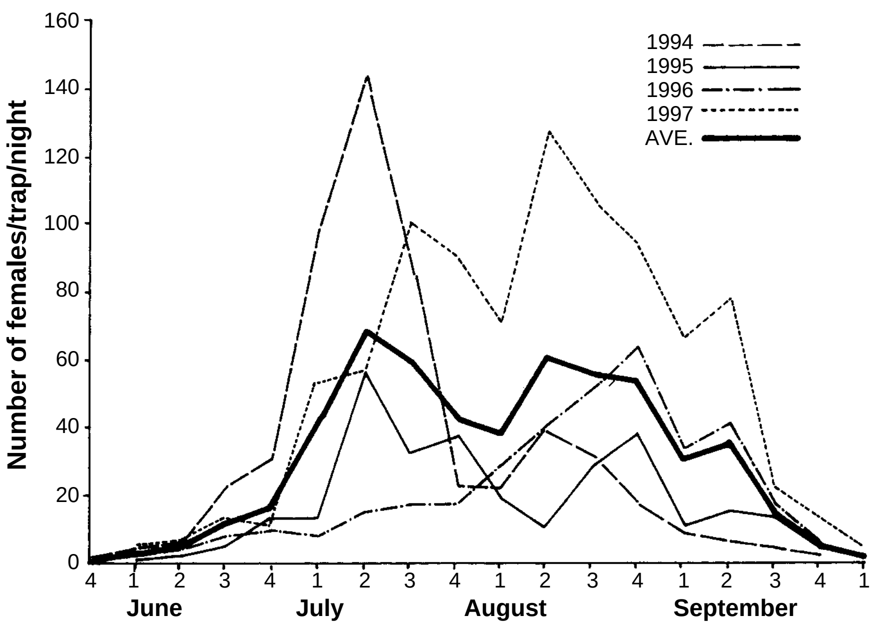

Fig. 2. Seasonal prevalence of *Anopheles sinensis* collected by New Jersey light traps at Munsan area, Gyeonggi-do in 1994-1997 (Kim et al., 2000).

1960s - early 1970s (Whang, 1964; Paik et al., 1965; NMES, 1966, 1968; Hong, 1967; Kim et al., 1978), or light trap collections in 1970s-1990s (Kim et al., 1978; Hong, 1983; Sohn, 1984; Lee and Ree, 1991; Yoon et al., 1994; Kim et al., 1995, 1997, 1999, 2000, 2001, 2003a, 2003b, 2004; Shim et al., 1997; Lee et al., 1999; Strickman et al., 2000; Ree et al., 2001, Lee and Kim, 2001).

Cow biting collections with two hour intervals throughout the night were carried out at three different areas in 1964, and the peak appeared in August at Yangpyeong and Yeoju, Gyeonggi-do and in July at

Okgu, Jeollabuk-do, as shown in Table 5 (Paik et al., 1965). Seasonal prevalence of this species was also studied in 1967 by means of cow biting collections with weekly intervals in Jeongup-gun, Jeollabuk-do and Cheongsong-gun, Gyeongsangbuk-do, showing the peak period at late July in both areas as shown in Table 6 (NMES, 1968). In Daegu, Gyeongsangbuk-do, the peak of *An. sinensis* population were appeared at the 5th week of July in 1981 and at the 3rd week of July in 1982; population density was markedly different, being 5,556 mosquitoes collected in 1981 whereas 29,755 mosquitoes in 1982 (Sohn, 1984). Most extensive studies on seasonal prevalence were done by Lee and Ree (1991), who monitored seasonal trend of mosquitoes with weekly operation of light traps at 18 cities/guns of Jeollabuk-do in 1985-1990 (Fig. 1), and also by Kim et al. (2000) who weekly operated light traps at four different locations in the same area (Munsan, Gyeonggi-do) in 1994-1997 (Fig. 2). It is noteworthy that the peak of the population density has appeared one to two weeks earlier since late 1980s, compared to the peak period of 1960s-1970s.

Summarizing all the study results of various workers mentioned above, seasonal fluctuations and population sizes of *An. sinensis* are markedly different from location to location and from year to year. In general, females of the new generation start appearing in late April - early May. Population sizes steadily increase from early - mid June, reach the peak during the perod of late June - mid July, follow by a significant decline in August, appear a small secondary peak in early September, and disappear in mid-late October.

Only a few studies on weather factors influencing population dynamics of *An. sinensis* were carried out in Korea. Ree and Lee (1993) reported that the summer air temperature was correlated in some degree with the adult population size, but other factors such as precipitation, sunshine hours, relative humidity and winter temperature were not correlated. Lee and Kim (2001) reported that temperature and precipitation were the major factors influencing *An. sinensis* populations.

Table 6. Seasonal prevalence of *An. sinensis* by means of cow biting collections at Jeongup, Jeollabuk-do and Cheongsong, Gyeongsangbuk-do in 1967 (cow/man/8 hours) (NMES, 1968)

Month	No. of week	Number/cow/man/night (8hr)	
		Jeongup	Cheongsong
May	1	4	1
	2	20	3
	3	10	8
	4	170	4
June	1	94	126
	2	234	46
	3	254	220
	4	1,066	58
July	1	1,239	70
	2	628	220
	3	1,028	824
	4	1,394	848
August	1	952	236
	2	112	352
	3	185	84
	4	76	244
	5	82	136
September	1	540	640
	2	88	24

Resting Habit

After blood feeding, female mosquitoes rest in shaded and humid places, waiting for development of follicles during the period of the gonotrophic cycle. Gonotrophic period of *An. sinensis* was 2.7 days in average (2-3 days) in July-August in north Gyeonggi-do (Ree et al., 2001).

Indoor resting place collections of *An. sinensis* were weekly done in 10 houses and 10 cowsheds in 5 different localities in June-September 1964-1967, and the results are summarized in Table 7 (Paik et al., 1965; Won and Hong, 1968). Very small numbers were found in houses, being collected 4.0 females/room, 2.3 females/verandah, 2.4 females/kitchen and 42.3 females/stock place, whereas large numbers were collected in animal sheds, being collected 370.3 females/pigsty and 1,077.9 females/cowshed.

On the other hand, 8.3 females of *An. sinensis* resting on walls of a bedroom (living room) were collected at night (21:30-22:30 hours), as shown in Table 8

(NMES, 1966). Majority (86.4%) of total mosquitoes were unfed, and only 4.1% was blood fed, which means that most of the females entered rooms rest on walls for a while before feeding, whereas 93.4% of *An. sinensis* females resting on the wall of a cowshed during the night was fed ones (Hong and Kim, 1990), which means that they rest after fed for a while, before flying outside. It is not clear why a majority of females resting in living rooms were unfed whereas a majority of females resting in cowsheds were fed; it would be resulted from their animal host preference. When they rest on the wall of cowsheds, they prefer low side of the wall (65.3%) rather than upper part (34.7%).

As shown in Table 9, resting place collections in cowsheds in the morning revealed that most of *An. sinensis* females after feeding flew out of sheds before sunlight, particularly in August, taking into account that numbers of resting mosquitoes were much smaller compared to numbers of biting mosquitoes on cows (Refer Tables 5 and 6). Experimental hut collections with exit window traps showed that only 17.2% of the total *An. sinensis* rested on walls of the hut, and the others (82.8%) were trapped into the exit window traps in June-July (Table 10), which means that *An. sinensis* is highly exophilic.

Outdoor resting mosquitoes were collected by sweeping an insect net at a wide range of habitats, such as grasses, rice fields, parsley fields, potato fields, bean fields and other vegetable fields, as shown in Table 11 (NMES, 1968). Though the highest number of the mosquitoes were collected in parsley fields, it is not a main resting place, as the size of parsley fields was very small, compared to the areas of grasses and rice fields that are the main resting places.

Host Preferences

It was known that *An. sinensis* was almost entirely zoophilic in Thailand; in comparative biting tests involving man and cow, almost none of them were attracted to man (Harrison and Scanlon, 1975). In Japan, Sasa (1951) showed that *An. sinensis* was

Table 7. Indoor resting place collectionsa) of *An. sinensis* at 5 locations in June-September in 1964-1967 (Paik et al., 1965; Won and Hong, 1968)

Resting place	Okgu (1964)	Yangpyeong (1964)	Asan (1965)	Andong (1966)	Cheongsong (1967)	Average
Room	1.5	0.3	5.3	2	11	4.0
Verandah	0.9	1.2	8.3	0.4	0.6	2.3
Kitchen	0	0.5	8.5	0.8	2	2.4
Stock place	48	5.5	42	65	51	42.3
Toilet	3.7	0.0	7.1	6	4.2	4.2
Under eaves	1.2	1.0	6.1	2	2.2	2.5
Hen	7.5	-	3.5	22	2.4	8.9
Pigsty	734	-	6.5	-	-	370.3
Cowshed	1,333	162	1,610	964	1,320	1,077.8

[a)]Ten randomly selected houses and cowsheds were checked every week.

Table 8. Resting place collections in bedrooms at night (21:30-22:30) at Yeongju, Gyeongsangbuk-do in June-July 1963, and Jeongup, Jeollabuk-do in July 1967 (NMES, 1968)

Locality	No. of rooms collected	Unfed	Fed	Gravid	Total	No./room
Yeongju	417	2,999	139	337	3,475	8.3
Jeongup	27	194	14	11	219	8.1
Total/Ave.	444	3,193	153	348	3,694	8.3
		(86.4)	(4.1)	(9.4)	(100)[a)]	

[a)]Per cent in parenthesis.

Table 9. Resting place collections at *An. sinensis* in cowsheds at daytime (09:00-10:00) by month in Jeongup, Jeollabuk-do and Cheongsong, Gyeongsangbuk-do in 1967 (NMES, 1968)

Month	Number/cowshed		
	Jeongup	Cheongsong	Average
April	0.9	0.2	0.6
May	44.4	18.8	31.6
June	267.5	124	195.8
July	153.4	270	211.7
August	9.0	17.1	13.1
September	34.5	6.0	47.3
Total	509.7	490.1	500.1

Table 10. *An. sinensis* collections in an experimental hut attached with exit window traps at Yeongju, Gyeongsangbuk-do in June-July, 1963 (unpublished)

Time	Number	%
No. in exit window traps at:		
20:00-22:00	79	5.2
22:00-24:00	142	9.4
24:00-02:00	251	16.7
02:00-04:00	356	23.3
04:00-06:00	419	27.0
No. remained in the hut	259	17.2
Total	1,506	100

strongly attracted by big animals and usually very few fed on man.

In Korea precipitin tests of the blood fed females of *An. sinensis* collected at outdoor resting places in Yangpyeong, Gyeonggi-do in 1962 showed that this mosquito fed exclusively bovines (54.8%) and pigs (42.5%); human blood rate was 1.7% (Table 12; Ree et al., 1967). In north Kyonggi-do in 1999 host blood analysis of *An. sinensis* collected at outdoor resting places by ELISA also showed similar results, giving 0.7% for human, 89.8% for bovine, 3.3% for pig (Ree et al., 2001). A significant difference between two localities was pig blood meals (42.5% vs 3.3%), which resulted from the availability of pigs for blood feeding. Lee et al. (2001) also studied host preference of *An. sinensis* in paju, Kyonggi-do in 2000, showing 0.8% of human blood rate (Table 12). Though human blood rates of these host blood tests were very low, they readily fed on man in great numbers where domestic animals (cows and pigs) were not near by for feeding. Number of human biting mosquitoes were 30/man/night at Taesong-dong and 130/man/night at Camp Bonifas located in DMZ where domestic animals were not found (Strickman et al., 2001). *An. sinensis* fed also dogs, chicken and cats in small numbers.

Feeding Time and Place

Cow biting collections of *An. sinensis* were hourly carried out at three different locations in 1965-1967 (Table 13; Hong, 1977). Human biting collections throughout the night were also carried out at Okgu, Jeollabuk-do and Yangpyeong, Gyeonggi-do in July 1965 (Table 15; NMES, 1966) and at several different

Table 11. Outdoor resting place collections[a)] of *An. sinensis* by sweeping at Cheongsong, Gyeongsangbuk-do and Jeongup, Jeollabuk-do in 1967 (NMES, 1968)

Area	Month	Grasses	Parsley field	Bamboo bush	Rice field	Potato field	Bean field	Vegetable fields	Total
Cheongsong	May	0	7	-	0	-	-	-	7
	Jun.	4	265	-	124	18	-	-	411
	Jul.	128	434	-	-	4	15	-	581
	Aug.	120	132	-	97	8	8	-	365
	Total	252	831	-	221	30	23	-	1,357
Jeongup	Jun.	3	-	-	39	18	-	3	63
	Jul.	14	-	60	10	12	-	193	289
	Aug.	3	-	12	49	-	11	-	75
	Total	20	-	72	98	30	11	196	427

[a)]Number/2 man/4 hours.

Table 12. Host blood analysis of *An. sinensis* collected at outdoor resting places in Yeoju, Gyeonggi-do in July 1962 (Ree et al., 1967), Goyang, Gyeonggi-do in 1999 (Ree et al., 2001), and Paju, Gyeonggi-do in 2000 (Lee et al., 2001)

Locality	No.	No. of host blood							
	tested	Human	Cow	Pig	Dog	Chicken	Cat	Cow / Pig	Unknown
Yeoju (1962)	301	5	165	128	0	0	-	1	2
		(1.7)[a]	(54.8)	(42.5)				(0.3)	(0.7)
Goyang (1999)	305	2	274	10	2	5	-	2	11
		(0.7)	(89.8)	(3.3)	(0.7)	(1.6)		(0.7)	(3.6)
Paju (2000)	241	2	155	55	33	2	4	0	35
		(0.8)	(61.8)	(21.9)	(13.1)	(0.8)	(1.6)		(13.9)

[a]Per cent in parenthesis.

Table 13. Cow biting collections[a] of *An. sinensis* at Asan, Chungcheongnam-do in 1965, Andong, Gyeongsangbuk-do in 1966 and Cheongsong, Gyeongsangbuk-do in 1967 (Hong, 1977)

Area	Month	20:00-21:00	21:00-22:00	22:00-23:00	23:00-24:00	24:00-01:00	01:00-02:00	02:00-03:00	03:00-04:00	04:00-05:00	05:00-06:00	Total	No. / Cow / hour
Asan	May	1	1	1	0	0	0	0	0	0	0	3	0.3
	Jun.	38	160	65	28	39	26	50	20	21	0	477	47.7
	Jul.	65	125	90	202	152	160	124	110	103	2	1,133	113.3
	Aug.	40	120	106	29	51	68	153	263	133	10	973	97.3
	Sep.	22	26	40	23	22	25	21	12	5	3	199	19.9
Andong	May	5	1	4	4	-	3	1	-	1	-	19	2.7
	Jun.	16	33	31	14	13	13	7	13	10	-	150	16.7
	Jul.	48	66	42	103	109	82	230	137	103	1	920	92.0
	Aug.	24	34	52	74	103	84	87	121	85	10	674	67.4
	Sep.	20	28	31	35	20	40	43	53	31	21	322	32.2
Cheongsong	May	1	2	1	2	1	1	0	0	0	-	8	0.9
	Jun.	39	50	41	33	28	20	15	17	10	-	253	28.1
	Jul.	32	52	89	81	120	106	73	85	78	13	729	72.9
	Aug.	29	53	50	46	41	56	44	50	46	18	433	43.3
	Sep.	66	30	53	30	16	15	17	24	21	11	283	28.3

[a]Average of 3-4 collections each month at each locality.

locations of northern Gyeonggi-do in July-August 1995-1996 (Tables 14; Shim et al., 1997; Strickman et al., 2000). All of the study results showed that *An. sinensis* fed throughout night from dusk to dawn, apparently feeding more actively from 24:00 to 04:00. Exceptionally, the peak of feeding time appeared at 05:00 in the study result of Strickman et al. (2000). Figure 3 shows that feeding time on animal bait and human bait is not different. Feeding activity at outdoors and indoors was not much different when the room was dark as shown in Table 15. However, when the light was on, much higher numbers of *An. sinensis* came into the room and fed on man (212.0 / man / night with light vs 86.7 without light in Okgu; 17.5 with light vs 5.0 without light at Yangpyeong).

Feeding activity of *An. sinensis* were significantly different by month, showing that the peak time appeared in early night (2000-2300 hr) in May, June and September, whereas it appeared after midnight in July and August (Table 13). These results strongly support that feeding activity of *An. sinensis* largely relies upon meteorological factors, particularly air temperature, with the majority of individuals biting late at night during warm weather (>20°C) and early at night during cool weather.

It was observed that hibernated *An. sinensis* fed on cow during daytime (12:00-17:00) when temperature was above 12°C in March-April (NMES, 1968). The

Table 14. Human biting collections of *An. sinensis* at northern part of Gyeonggi-do in July-August in 1995 and 1996

Time	1995[a)]		1996[b)]	
	No.	%	No.	%
18:00-19:00	-		6	0.1
19:00-20:00	2.9	0.3	12	0.3
20:00-21:00	17.4	2.1	108	2.7
21:00-22:00	23.8	2.8	222	5.5
22:00-23:00	51.6	6.2	285	7.1
23:00-24:00	80.8	9.7	391	9.7
24:00-01:00	83.0	9.9	379	9.4
01:00-02:00	124.9	14.9	358	8.9
02:00-03:00	169.3	20.2	410	10.1
03:00-04:00	153.6	18.4	785	19.5
04:00-05:00	115.9	13.9	850	21.1
05:00-06:00	12.9	1.5	229	5.7
Total	836.1	100	4,029	100

[a)]Average of the collections at 4 locations; 2 men collections in a tent with light (Shim et al., 1997).
[b)]Total of 19 night collections at 5 different sites near Pammunjeom; 2 men collections outdoors (Strickman et al., 2000).

biting activities of *An. sinensis* were sensitibly influenced by wind speed and direction (Whang, 1964), and negatively correlated with size of the moon (Strickman et al., 2000).

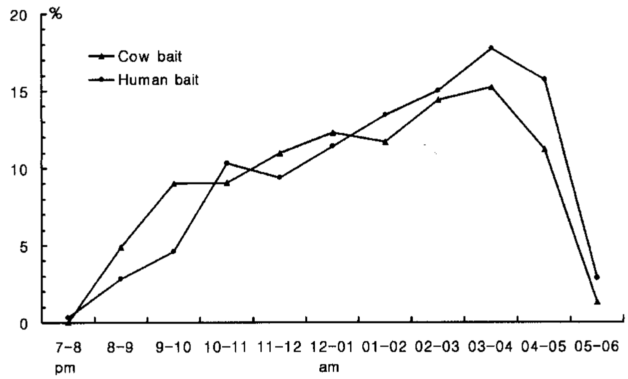

Fig. 3. Comparison of feeding time of *An. sinensis* between cow biting and human biting (Hong, 1977; Shim et al., 1997; Strickman et al., 2000).

Physiological Age

For physiological age determination, ovaries were dissected for parous rate at three topographically different areas in 1964 and 1967 (Table 16) (Paik et al., 1965; Hong, 1977). Parous rates were significantly different at three study areas. It was 50.6% (0.711 of proportion of daily survival) in Okgu, Jeollabuk-do, located in plain, malaria-free area, whereas it was 70.4% (0.890 of proportion of daily survival) in Yangpyeong,

Table 15. Human biting collections at Okgu, Jeollabuk-do in 1964 and Yangpyeong, Gyeonggi-do in July 1965 (NMES, 1966)

man / hour

Time	No. of mosquitoes / man / hour							
	Okgu[a)]				Yangpyeong[b)]			
	Room		Out-door	Ave-rage	Room		Out-door	Ave-rage
	Light	No light			Light	No light		
20:00-21:00	7.3	3	5	5.1	0.6	0.5	0.5	0.5
21:00-22:00	9.7	4	9	7.6	1.6	0.3	0.8	0.9
22:00-23:00	14.3	6	10	10.1	1.6	0.5	0.3	0.8
23:00-24:00	17.7	7	12	12.2	2.5	0.9	1	1.5
24:00-01:00	36.3	12.7	12.7	20.6	2.4	0.9	0.6	1.3
01:00-02:00	37.3	15.7	15.3	22.8	2.5	0.5	0.7	1.2
02:00-03:00	30.7	11.7	18.3	20.2	2.8	0.5	0.4	1.2
03:00-04:00	29.3	14.7	19	21.0	2.8	0.8	0.5	1.4
04:00-05:00	25.7	9.7	14.3	16.6	0.8	0.3	0.3	0.5
05:00-06:00	3.7	2.3	0.7	2.2	-	-	-	-
Total	212.0	86.7	116.3	138.4	17.5	5.0	5.1	9.2
No. per hr	21.2	8.7	11.6	13.8	1.8	0.5	0.5	0.9

[a)]Average of 3 nights. [b)]Average of 8 nights indoors and 11 nights outdoors.

Table 16. Parous rate of *An. sinensis* at Okgu, Jeollabuk-do and Yangpyeong, Gyeonggi-do in 1964 (Paik et al., 1965) and Cheongsong, Gyeongsangbuk-do in 1967 (Hong, 1977)

Area	Month	No. dissected	Parous rate (%)	Daily survival ratio
Okgu, Jeollabuk-do	June	103	27.2	
(Malaria-free area)	July		773	53.8
	Aug.	142	50.0	
	Total	1,018	50.6	0.711
Yangpyeong, Gyeonggi-do	June		173	66.5
(Endemic area)	July		474	68.4
	Aug.	104	86.5	
	Total	751	70.4	0.890
Cheongsong	June	677	71.9	
(High-endemic area)	July		218	74.3
	Aug.		124	87.9
	Total	1,019	75.3	0.911

Gyeonggi-do, located in hilly, moderately endemic area of malaria, and 75.3% (0.911 of proportion of daily survival) in Cheongsong, Gyeongsangbuk-do, located in mountainous area where malaria was highly endemic. These results showed positive correlation between longevity of the vector species and degree of malaria endemicity, i.e. the longer life span of the vector mosquitoes, the more malaria cases. Recently in northern part of Gyeonggi-do, where outbreaks of malaria cases have occurred, parous rate of *An. sinensis* was 70.5% (0.890 of daily survival ratio) at Gusan-dong, Goyang-si, Gyeonggi-do in 1999 (Ree et al., 2001), which was very similar to 70.4% at Gaegun-myeon, Yongpyeong-gun, Gyeonggi-do in 1964. Contrary to the above result, Lee et al. (2001) reported that parous rate was 48.8% (0.787 of daily survival ratio) in Paju-si in 2000 (Table 17). Ree et al. (2001) observed that adult population density of *An. sinensis* and their parous rate (longevity) were positively correlated, showing that sharp decrease of the population density during late July-August resulted from the short life span of the vector mosquitoes (Fig. 4).

Table 17. Parous rate of *An. sinensis* at two localities in Gyeonggi-do in 1999-2000

Area	Month	No. dissected	Parous rate (%)	Daily survival ratio
Goyang[a]	July	825	75.2	
	Aug.	639	56.5	
	Sep.	801	78.5	
	Oct.	115	60.0	
	Total	2,380	70.5	0.890
Paju[b]	June	145	35.2	
	July	111	55.0	
	Aug.	74	66.2	
	Total	330	48.8	0.787

[a]Collected by light traps in 1999 (Ree et al., 2001).
[b]Collected by human bait in 2000 (Lee et al., 2001).

Flight Range and Dispersal

Geographical expansion of malaria transmission is largely determined by flight / dispersal activities of the vector mosquitoes, when vivid human movement is not observed. Therefore, flight / dispersal range of *An. sinensis*, the vector species of vivax malaria, is a vital important factor for understanding recent malaria epidemics in border areas between North and South Korea.

A mark-release recapture experiment of *An. sinensis* was carried out in Paju city, Gyeonggi-do, Korea during the period of 7-28 September 1998 (Cho et al., 2002). Total 12,772 unfed females were marked with fluorescent dye and released. Recaptures of marked females were made from following days of the release, by operating 13 light traps, each of which was set up in a cowshed at various distance from the release point. Light trap collections continued every night for 21 days. Among total 194 marked females recaptured (1.52% recapture rate), 37.1% were recap-

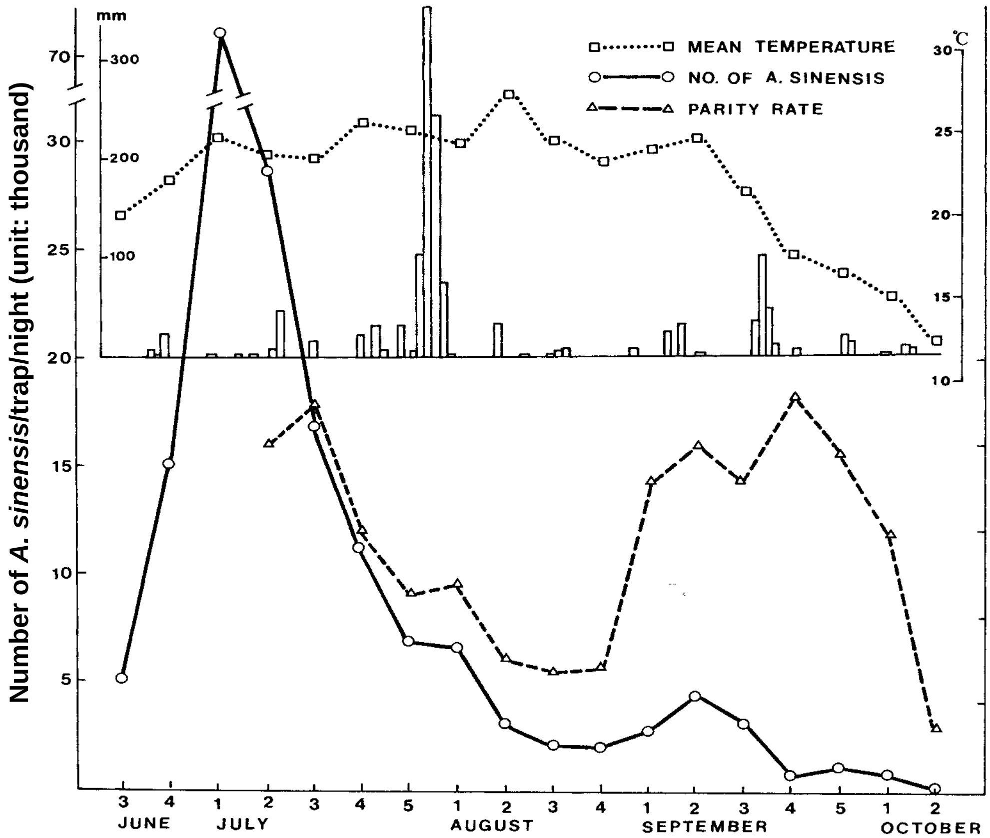

Fig. 4. Weekly occurrence of population density and parous rate of *Anopheles sinensis* at Gusan-dong, Goyang-si, Gyeonggi-do, in 1999. Mean temperature by week and daily rainfall are also shown (Ree et al., 2001).

tured within 1 km from the release point, 29.4% at 1-3 km distance, 21.1% at 3-6 km, 10.3% at 6-9 km, and 2.1% (4 females) at 9-12 km. Correlation between flight distance and number of the recaptured *An. sinensis* was observed, shown as Fig. 5.

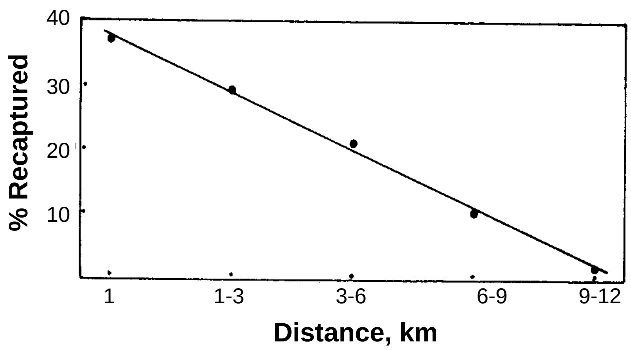

Fig. 5. Correlation between flight distance (km) and per cent of *An. sinensis* recaptured (Cho et al., 2002; the figure drawn by Ree).

Hibernation Habit

Studies on finding hibernation places of *An. sinensis* have been rather extensively carried out by various workers (Whang, 1961, 1964; NMES, 1968; Hong, 1977; Ree et al., 1976; Shim et al., 1987c, 1989). Whang (1964) reported that among total 4,402 hibernating females 22 *An. sinensis* were found in culverts and 6 *An. sinensis* under bridges in November-December in 1959-1960. Two straw piles (one 2.5 × 3 × 3 m, and the other 2.7 × 3.5 × 1.4 m) were covered with specially-made extra-large mosquito nets in Jeongup, Jeollabuk-do from 11 March to 30 April 1966. A collector searched mosquitoes resting in the mosquito net every day (twice a day) and 6 females of *An. sinensis* and one *Culex pipiens* were collected (NMES, 1968). An exit window trap was covered over an abandoned well (stone-piled with 10 m deep), and 108 rat holes at banks of rice fields were covered with specially designed cone-shape trap from February to May. The

Table 18. Dry ice / mosquito net and cow biting collections of hibernating *An. sinensis* at daytime in Jeongup, Jeollabuk-do in March - April 1967 (NMES, 1968)

Date	Time	Temp. (°C)	Dry ice	Cow biting
12 March	12:00-13:00	13.5	3	-
	13:00-14:00	14.0	1	-
	15:00-16:00	15.0	1	-
13 March	14:00-15:00	14.0	3	0
	15:00-16:00	15.5	1	7
14 March	14:00-15:00	17.0	2	-
	15:00-16:00	17.0	3	-
	16:00-17:00	16.0	4	-
15 March	14:00-15:00	13.0	1	-
	16:00-17:00	14.5	1	-
18 March	11:00-13:00	12.0	1	-
20 March	10:00-11:00	12.0	1	0
	11:00-12:00	12.0	1	1
	14:00-15:00	16.5	2	2
	15:00-16:00	16.0	1	2
	16:00-17:00	14.5	1	11
6 April	17:00-18:00	15.0	3	-
7 April[a)]	15:00-17:00	14-16	-	6[a)]
9 April[a)]	-	14-17.5	-	10[a)]
13 April[a)]	-	12-16	-	5[a)]
18 April[a)]	-	13-16	-	1[a)]
22 April	18:30-19:00	13.0	4	-
27 April	19:00-19:30	12.0	2	-
Total			36	45

[a)]Collections in Cheongsong, Gyeongsangbuk-do.

results of both trials were negative (NMES, 1968). A vinyl tent was covered over grasses in December in Buan-gun, Jeollabuk-do, and collected 6 females of *An. sinensis* (Shim et al., 1987c). The same collection method was applied at Gosan, Cheju-do and 3 females of *An. sinensis* were collected (Shim et al., 1989). Stone piles were covered with a plastic tent and stones were removed one by one in December 1972 in Busan, and 15 females of *An. sinensis* were found together with 1 *An. pullus*, 31 *Cx. pipiens pallens* and 4 *Cx. orientalis* (Ree et al., 1976). A mobile vinyl tent was covered over heavy grasses of rice paddy banks at Samha-ri, Yangju-gun, Gyeonggi-do in January-March 1999. When temperature inside the tent raised high (>15°C) by sun light, hibernating mosquitoes flew out from grasses in a few minutes. At total 46 sites, 145 females of *An. sinensis* complex were collected. Most

Table 19. Comparison of population densities of *An. sinensis* between DDT-sprayed (Daesin-myeon) and control (Gaegun-myeon) areas in Gyeonggi-do, by means of cow biting collection at 2 hour intervals in June-September in 1964-1965. Total coverage of DDT indoor residual spray was completed in April-May each year (NMES, 1966)

Area	No. of *An. sinensis*[a)]				
	June	July	Aug.	Sep.	Total
Sprayed (Daesin)	42.6	118.4	127.4	41.6	330
Unsprayed (Gaegun)	62	108.8	126.6	41.4	340.8

[a)]Number / cow / hour, in average of 1964-1965.

Table 20. Comparison of parous rate of *An. sinensis* between DDT-sprayed and control aeras in June-September 1965 (NMES, 1966)

Area	No. dissected	No. of parous	Parous rate (%)
Sprayed (Yeoju)	1,041	711	68.3
Control (Yangpyeong)	947	703	74.2

of them were *An. pullus* (Hwang et al., 2004).

The first collection of *An. sinensis* larvae of the first new generation of the season was on April 14 (one larva from ditch) in Cheongsong, Gyeongsangbuk-do and on April 15 (two 3rd instar larvae from rice fields) in Jeongup, Jeollabuk-do in 1967 (NMES, 1968). These larval findings in mid April indicate that hibernated females fed on hosts in March and laid eggs in late March - early April. Because temperature at night was too low to feed, cow biting collections at daytime were carried out in Jeongup, Jeollabuk-do from early March to late April 1967. *An. sinensis* started collecting from mid March. The results are shown in Table 18 (NMES, 1968). It is interesting to note that their feeding activity was observed during daytime even when temperature was 12-13°C.

Indoor resting place collections in cowsheds at daytime and cow biting collections at night showed that hibernating females appeared for feeding at night in small numbers in April - early May; 0.3 and 4.0 females per cow per night were collected in April and

Table 21. Comparison of malaria cases between DDT sprayed and unsprayed areas in 1963-1965

Area	Population	No. of houses	Pre-spray(1963)		Post-spray			
					1st spray (1964)		2nd spray (1965)	
			Slides[a)]	SPR[b)]	Slides[a)]	SPR[b)]	Slides[a)]	SPR[b)]
Sprayed area (Yeoju)	22,215	3,588	518	12.9	1,313	6.8	3,243	1.1
Unsprayed area (Yangpyeong)	15,433	2,504	553	30.4	4,364	3.1	1,474	4.5

[a)]Number of blood slides of the suspected fever cases by PCD.
[b)]Slide positive rate.

May respectively in Cheongsong, Gyeongsanbuk-do in 1967, and 3.3 females per cow per night were collected on cow bait in May in Asan, Chungcheongnam-do in 1965 (Hong, 1977).

In conclusion, females of *An. sinensis* hibernate outdoors at various concealed places such as grasses, straw piles, stone piles and others. Successfully survived females during several months of overwintering period appear for feeding on cows in daytime in mid March-April. They start feeding on cows when temperature reaches above 12°C at night.

CONTROL

Two different control measures are applied for malaria vector control, one larval control and the other adult control. Principal objective of the former is to reduce absolute population density and that of the latter is to cut off vector-human contact by selective killing of old-age mosquitoes and/or reducing longevity of the vector mosquitoes. Indoor residual spray with a long-lasting insecticide is the most classical methodology for the latter purpose.

In 1964 and 1965, Daesin-myeon with a population of 22,215 in 3,586 houses were selected for the treated area in Yeoju-gun, Gyeonggi-do and the neighbouring Gaegun-myeon, Yangpyeong-gun was selected for untreated (control) area. All surfaces of the houses and animal sheds in Daesin-myeon were sprayed with 2 g/m^2 of DDT in April-May each year of 1964 and 1965. Passive case detection for malaria cases was carried out in both areas and suspected fever cases were dosed with chloroquine (NMES, 1966). Relative population densities of *An. sinensis* were compared by cow biting collections throughout night with a week interval. The result is summarized in Table 19, showing no significant difference of densities and seasonal trends of the vector mosquitoes between in the DDT-sprayed and control areas. In other words, total coverage of the houses and animal sheds with DDT could not suppress density of *An. sinensis* population. Parous rate of *An. sinensis* was 68.3% in the treated area and 74.2% in the control area, which was slightly lower in the treated area (Table 20). Malaria cases were decreased in both the sprayed and unsprayed areas in the first year of the treatment, and then significantly decreased in the sprayed area in the second year of the treatment (Table 21). The slide positive rate (SPR) of malaria before treatment (1963) were 12.9% and 30.4% in the treated and untreated areas, respectively, and SPR in 1964 (after 1st treatment) was 6.8% and 3.1%, respectively, which would probably be resulted from the passive case detection and drug administration in both areas. SPR in 1965 (after the 2nd treatment) was further decreased (1.1%) in the sprayed area, whereas increased (4.5%) in the unsprayed area. It can be explained that PCD (case finding and treatment) could significantly reduce the cases in a short-term period only where incidence rate was high, and when the incidence rate decreased to very low level PCD alone could not reduce cases further, but DDT spray, as an additional supplemental control measure, reduced the cases further.

Thermal foggings which have been widely and extensively applied in villages in Korea, and permethrin-treated mosquito nets which are successfully applied in tropical countries have not been evaluated for the effectiveness of *An. sinensis* control. Therefore such studies are required with high priority.

For larval control of *An. sinensis*, susceptibility of *An. sinensis* larvae to various insecticides including *Bacillus thuringiensis israelensis* (B.t.i.) and *Bacillus sphaericus* was tested by many researchers (Self et al., 1974; Shim and Kim, 1980, 1981; Ree et al., 1981b; Shim et al., 1987 a, b, 1995; Lee et al., 1996; Lee and Yu, 1999; Shin et al., 2003). These studies revealed that *An. sinensis* has developed high level of resistance since 1980s to most of the organophosphorous and carbamate insecticides which had been extensively applied on rice fields for the control of agricultural pests. Ree et al. (1981 a, b; 1982) reported that the seasonal prevalence of *An. sinensis* larvae in the rice field was rather stable, not being affected by insecticide pressure at all, whereas natural predators such as larvae of *Odonata* and *Coleoptera* drastically decreased by agricultural insecticide applications. Field trials in rice fields also showed that 14 insecticides tested were ineffective to control of *An. sinensis* larval population except cartap and B.t.i. (Ree et al., 1981a; Yu et al., 1982a; Shim et al., 1987a, b, d). Entomological studies were performed on the community structures of aquatic animals including mosquito larvae in organically-farmed rice fields (insecticides not applied) and conventionally-farmed rice fields (insecticides applied) in Boseong, Jeollanam-do in 1995-1996 (Lee et al., 1997; Lee, 1998). The abundance of *An. sinensis* larvae was lower in the organically-farmed rice fields in which number of the Chinese muddy loaches (*Misgurnus mizolepis*), predator of mosquito larvae was high, as compared to the conventionally-farmed rice fields.

For the development of biological control measures against larval population of *An. sinensis*, laboratory and field tests were carried out to find effective biological control agents by Yu et al. (1981, 1982a,b, 1993), Ree and Lee (1983), Yu (1986), Shim et al. (1987a,d), Yu and Kim (1993), and Kim et al. (1994). Potential biological control agents as effective predators of *An. sinensis* larvae were *Aphyocypris chinensis*, *Aplochilus latipes*, *Moroco oxycephalus* and *Misgurnus anguillicaudatus* among native fishes, and nymphs of *Orthetrum triagulare melania* and *Sympetrum* sp. in *Odonata*, and larva of *Cybister japonicus*, *lacophilus* sp. and *Hydrophilus affinis* in Coleoptera.

Analyzing all available data on biological control against *An. sinensis* larvae, it would not practically applicable in the rice field, not only in technical but in economical ponts of view, except in a very limited area or isolated habitats.

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