

Full Length Research Paper

Comparative association of species of Culicinae (Diptera) at various altitudes in Republic of Korea

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Korean peninsula and south western region (Jeju Island) are heavily covered with natural vegetation and represented by mountains, valleys and streams. This study aims to detect mosquito species from subfamily Culicinae and their breeding places at altitudes 26 to 1345 m. The larvae and adult samples were collected during six months period from June to November 2009 by using plastic pipette and BG Sentinel. 6 genera and 13 species have been recorded from 210 spots in eight major sites. These include *Aedes* represented by five species [*A. albopictus* (Skuse), *A. galloisi* Yamada, *A. japonicus* (Theobald), *A. koreicus* Edwards, and an unidentified form] followed by genus *Culex* represented by six species (*C. sasai* Kano, *C. pipiens* f., *C. vagans* Wiedemann., *C. halifaxii* Theobald and two unidentified forms) and genus *Ochlerotatus* represented by two species [*O. hatorii* (Taylor) and *O. togoi* (Theobald)]. While, three genera *Armigeres*, *Tripteroides* and *Manisonia* each represented by one species [*A. subalbatus* (Coquillett), *T. bambusa* (Yamada), *M. uniformis* (Theobald) respectively]. Our study also revealed 40 different types of breeding places, some of them from nature such as water catchment areas formed as a result of rain, overflow of streams, rock pools, partly dried streams, tree holes and tops of stones as the most potential breeding places, and other formed as a result of human involvement (artificial) such as flowering pots, sewerage lines, disposal areas, pickle pots, construction material, plastic sheets used to cover the equipments, bottles, stone pots, red buckets and flowering stone pots as the most favorable breeding sites.

Key words: Climate change, *Aedes* mosquitoes, Korea, altitude, dengue, culicinae.

INTRODUCTION

Living standards are the most reasonable explanation for preventing the spread of any epidemic. Such standards promote better sanitation facilities, public education, that is, the knowledge and willingness to manage their surroundings like standing water around households, implementing programs to control mosquitoes, and employing screens and air-conditioning. All these quality parameters ultimately reduce the overall burden of mosquito-borne diseases (Moore, 1998). During the recent years, much is being focused on climate change that is likely to increase the dengue fever transmission and may risk large proportion of humans (Hales et al., 2002; Watson 2002). As mosquito-borne disease

transmission is climate sensitive for several reasons; mosquitoes require standing water to breed, and a warm temperature is critical to adult feeding behavior, mortality, rate of larval development, and rate of virus replication (Patz et al., 1998; Focks et al., 1993). Areas that have received particular attention are between climate variation and vector-borne diseases (IPCC, 2001; Anonymous, 2001). It has been found that *A. albopictus* is the most abundant *Aedes* species and main dengue vector originating from Asia as the forest-dwelling mosquito species transmitting dengue virus among wild animals. The forest cycle, all have been recorded in Asia, whereas only one has been detected in Africa thus giving evidence that the viruses are of Asian origin (Gubler, 1997). With public participation, breeding sites can be minimized by eliminations of containers, bush clearing around houses, tree hole filling and drainage clearing. By

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knowing the mosquito fauna available at a given area, one may understand more about the diseases associated with vector mosquitoes which may be helpful for a successful control program (Rozilawati et al., 2007). From the dengue vector surveillance in urban and settlement areas in Selangor, even in clean residential environment with no water storage, the area still had high *A. aegypti* and *A. albopictus* populations and the only possible breeding habitat was the concrete drainage system outside the houses. The drains had clear stagnant water with fallen tree leaves and other debris (Chen et al., 2005). Therefore, these served as good artificial breeding habitats for *A. aegypti* and *A. albopictus*. In another study, it was found that when the water temperature rises, the larvae take shorter time to mature (Rueda et al., 1990) and have greater capacity to produce more offspring during this season because the adult female mosquitoes digest blood faster and feed more frequently in warmer climates, thus increasing the oviposition activity (Gillies, 1953). On the other hand, the abundance of vectors is associated with the environmental factors such as the rainfall, temperature and relative humidity (Okogun et al., 2003). The onset of rainfall supports the development of additional mosquito breeding sites, hatching of eggs following oviposition, high relative humidity (Igbinosa, 1989) in addition to growth of vegetation cover and cool shaded environment for the development of aquatic stages, and the recruitment of young adults and their survivors (Evan, 1938). A growing number of investigators propose that vector-borne diseases (VBDs), (e.g., involving insects and snails as carriers), could shift their range in response to climate change (Leaf, 1989; Shope, 1991; Patz et al., 1996; McMichael et al., 1996; Carcavallo and de Casas, 1996). It has been found that *Anopheles* spp. and *A. aegypti* mosquitoes have established temperature thresholds for survival, and there are temperature-dependent incubation periods for the parasites and viruses within them (the extrinsic incubation period or EIP). Provided sufficient moisture, warmer temperatures within the survivable ranges increase mosquito populations, biting rates (blood meals), mosquito activity, and abundance decreases the EIP or duration of sporogony (Macdonald, 1957; Patz et al., 1996; Martens et al., 1997). A rise from 20 to 25°C, for example, reduces EIP for *P. falciparum* parasites from 26 to 13 days (Macdonald, 1957). Mosquito survival drops as ambient temperatures approach 40°C. Bioclimatographs of temperature and humidity levels, and of the geographic distribution of areas permitting mosquito and pathogen development, can be constructed (Dobson and Carper, 1993). Dengue fever previously limited to about 1000 m in elevation in the Tropics by the 10°C winter isotherm has appeared at 1700 m (Koopman et al., 1991); *A. aegypti*, the mosquito that can carry dengue and yellow fever viruses, has been reported at an elevation of 2200 m (Suarez and Nelson, 1981). Mosquitoes, in particular,

are highly sensitive to climatic factors (Gill, 1920a, b; Macdonald, 1957; MacArthur, 1972; Billett, 1974; Burgos, 1990; Burgos et al., 1994; Carcavallo and de Casas, 1994). Environmental and geographical factors, particularly temperature, rainfall, humidity, land use and land cover, each play a role in determining the habitat as well as the distribution of *Anopheles* and *Aedes* mosquitoes. Contribution of these factors as well as the role of climate change in outbreaks of disease and their spatial distribution has been poorly understood. Current evidence suggests that climate variability has a direct influence on the epidemiology of vector-borne diseases (Githeko et al., 2000; Haines et al., 2000). In permanent ground water habitats, generalist predators appear to limit mosquito populations and so render mortality additive (Juliano, 2007). Industrialized countries face the effects of increasingly frequent climatic events, resulting in a diverse array of conditions including temperature-related illness and the spread of tropical pathogens (Tsail and Liu, 2005). It has been observed that the distribution of many vector-borne diseases, such as malaria and yellow fever, are greatly restricted to regions in "climate envelopes", where local climate supports disease transmission. However, as a result of climate change, the intensity of disease transmission has varied in the region in the past few decades (Thomson and Connor, 2004). The finding that the baseline distribution of dengue is well predicted on the basis of reported vapor pressure is biologically credible. With a suitable climate, other factors, including a source of infection, a vector, and susceptible human populations would also need to be present for an epidemic to happen. Where and when dengue occurs in the future will therefore depend on diverse economic, social, and environmental factors (Hales et al., 2002).

Mosquito borne disease have been on the high and with an alarming climate change the distributional patterns of the mosquitoes have also changed along with the geographical limits of the disease, vector and its occurrence (Reiter, 2001). The global increase in the temperatures by many degrees by 2100 would increase the vectoring capacity of the mosquitoes by 100 folds in temperate countries (Marten et al., 1995). However, this catastrophic situation may ease down if there is a permanent surveillance and source reduction program in operation in the temperate countries. Moreover, the high living standards would be another limiting factor in the spread of the mosquitoes particularly the dengue (Trent et al., 1983).

Analysis of increase in temperature from 2 to 4°C shows that areas adjacent in altitude or elevation to current endemic zones may become more receptive to viral introduction and enhance transmission (Jetten and Focks, 1997). The aforementioned studies depicted that the mosquito species and their behavior have been effected with climate factors and environment surrounding. Therefore, the present study was conducted

for the detection of mosquito species that coexist in different environments.

MATERIALS AND METHODS

The present study was carried out from June to November 2009 to explore the mosquito fauna of the Korean peninsula at altitudes from 26 to 1308 m. All stages of the mosquitoes were collected from forest, high mountains, streams, sewerage line, tree trunks, stones, roofs, plastic covering sheets, Korean pots, Korean temples, lids of Korean pots, trays and Jeju Island etc.

The larvae were collected by using plastic pipette, to draw water and larvae and put in corked bottles with small hole for keeping the air. These bottles were filled with the water from larval collecting sites; while, BG-Sentinel Traps were placed side by side to collect adults. These traps visited after 12 h, had no adult captures, although the area had plenty of mosquito breeding sites from larvae to adults.

Collection glasses were covered by muslin cloth and small hole in the bottom was made to fit well on the top of the bottle lid. These glasses were fixed on the bottles so that the emerging adults could fly up and enter these glasses and consequently these adults could be collected. Small cotton wicks wetted with a sugar solution (20%) were placed on the top of muslin to feed the adult. The newly emerged adults after 3 to 4 h of emergence were found feeding on these cotton wicks.

The populations collected from 210 spots belonging to eight major sites were brought to the NIH, Entomology laboratory, Seoul. The larval populations were maintained under laboratory conditions of 30°C and RH 70 to 75% with a photoperiod from 14 to 12 h. The emerged were transferred to cold storage at temperature -24°C. They were kept for 24 h and then delicately pinned and placed in the collection boxes. Everyday population from the emerging larvae was counted and the data was recorded for each site and sub-site subsequently, the total diversity in a particular area was measured.

The pinned adults were identified under the stereoscope using Entomological keys (Lee and Egan, 1985; Lee and Zorka, 1991). The data so collected was subject to co-relation matrix and standard statistical techniques (Howell, 2007).

RESULTS AND DISCUSSION

6 genera with 13 species have been recorded from 210 spots visited in eight major sites. *Aedes* was the most commonly recorded genera with *A. koreicus*, *A. albopictus*, *A. japonicus*, *A. galloisi*, and an unidentified form. *Culex* has been represented with *C. sasai*, *C. pipiens*, *C. vagans*, *C. halifaxii* and two unidentified forms. *Armigeres* was represented by a single species *A. subalbatus*; *Tripteroides* by *T. bambusa* and *Manisonia* by *M. uniformis*. *Ochlerotatus* has been represented by *O. hatorii* and *O. togoi* during the six month survey in the summer, rainy and autumn of 2009 from altitudes between 26 to 1346 m. The species have been found to associate with one another at different altitudes, habitat conditions and combinations of water temperature, RH% along with a number of parameters that were observed during the study (Table 1). The association of *A. albopictus* was recorded with other genera and species. *A. albopictus* was collected from 49 spots out of the total 210 visited. In 14 situations, its larvae were found in

waters that did not harbor any other mosquito species, while in 35 cases it was associated with other species with different population levels. On overall basis one is to one type of association in maximum (12) spots for *A. albopictus* was recorded with *A. koreicus* as against *A. albopictus* to *A. japonicus* in 3 spots; *A. albopictus* to *A. sineroides*, *A. albopictus* to *C. halifaxii* one spot each. While one is to two level of association of *A. albopictus* was recorded with *A. koreicus* and *A. japonicus* at 9 spots; followed by *A. albopictus* to *A. koreicus* and *O. hatorii* at 4 spots. The association of *A. albopictus* to *A. koreicus* and *A. subalbatus*; *A. albopictus* to *O. togoi* and *C. halifaxii*; *A. albopictus* to *O. hatorii* and *T. bambusa*; *A. albopictus* to *C. sasai* and *O. togoi*; *A. albopictus* to *A. subalbatus* and *C. sasai* was recorded from one spot for each of the combinations respectively during the collection (Table 2).

A. albopictus was found to have strong association with other species as well as genera. However, the population level of *albopictus* was usually less than the main dominating form *A. koreicus* and sometimes *A. japonicus*. There have been a few cases where *A. albopictus* was found to dominate the entire species combination irrespective of the fact that it was associated with *A. koreicus* or the *A. japonicas* thus indicating strong evidence that *A. albopictus* are superior in competition among larvae in man-made containers (Braks et al., 2004).

Associating species and the habitat characteristics

Various habitat characters were recorded at each collection site during the study period. The association between different mosquito species and habitat characteristics is presented in Table 4. Relatively small populations of *A. albopictus* were collected from automobile tires placed along length of the road at an altitude of 244m with temperature 37°C and RH 38%. The water in them was foul but clear and there was plant debris at its bottom. Many copepods were also observed in these tires. Other forms like the *A. koreicus*, *O. hatorii*, *A. galloisi*, *A. sineroides* and unknown *Aedes* were generally recorded between altitudes of 244 to 647 m at temperatures of 22.5 to 27°C with RH 35.6 to 75.5%. The sites were under the shade, in thick plantation, and the water in them was clear to brown and foul. The population collected included all samples from receptacles like the plastic containers, a few from stone pots and none from the natural rock pools.

Populations collected from Bopjusa generally included samples from six spots lying between altitudes of 358 to 463 m. The temperatures recorded here were between 26.5 to 29.6°C with RH 40 to 51.6%. The populations included *A. japonicus*, *A. koreicus* and *C. vagans* from habitats that were under exposed to shady conditions, with water that was clear but had plant debris at its bottom. All of these samples were from the artificial

Table 1. Sites with altitude representing the association of *A. albopictus* with other species.

| Site | Number | Altitude | Associating species | Populations size of species |
|----------------|--------|----------|--|-----------------------------|
| Talgoam | 1C7 | 485 | <i>albopictus</i> to <i>koreicus</i> | 3 : 14 |
| Hapcheon | 1E12 | 334 | <i>albopictus</i> to <i>koreicus</i> | 1 : 1 |
| Songsogotaek | 2C6 | 188 | <i>albopictus</i> to <i>koreicus</i> | 1 : 21 |
| Naejangsan | 3B1 | 200 | <i>albopictus</i> to <i>koreicus</i> and <i>japonicus</i> | 3 : 17 : 4 |
| Naejangsan | 3B5 | 210 | <i>albopictus</i> to <i>koreicus</i> and <i>japonicus</i> | 1 : 1 : 10 |
| Naejangsan | 3B7 | 205 | <i>albopictus</i> to <i>japonicus</i> | 33 : 1 |
| Naejangsan | 3B10 | 201 | <i>albopictus</i> to <i>koreicus</i> and <i>japonicus</i> | 12 : 1 : 1 |
| Naejangsan | 3C4 | 191 | <i>albopictus</i> to <i>koreicus</i> and <i>japonicus</i> | 1 : 9 : 6 |
| Naejangsan | 3C5 | 191 | <i>albopictus</i> to <i>A. sineroides</i> | 3 : 3 |
| Samhwasa | 4A2 | 165 | <i>albopictus</i> to <i>koreicus</i> | 2 : 1 |
| Sangwonsan | 4C8 | 735 | <i>albopictus</i> to <i>koreicus</i> and <i>japonicus</i> | 10 : 14 : 12 |
| Mangwolsa | 4D1 | 367 | <i>albopictus</i> to <i>O. hatorii</i> and <i>T. bambusa</i> | 24 : 4 : 3 |
| Jongaksa | 5B2 | 135 | <i>albopictus</i> to <i>koreicus</i> and <i>hatorii</i> | 1 : 1 : 1 |
| Jongaksa | 5B3 | 150 | <i>albopictus</i> to <i>koreicus</i> and <i>hatorii</i> | 25 : 3 : 2 |
| Jongaksa | 5B4 | 115 | <i>albopictus</i> to <i>koreicus</i> | 5 : 6 |
| Chilgapsan | 5C5 | 291 | <i>albopictus</i> to <i>koreicus</i> | 6 : 5 |
| Namhansan | 5E1 | 334 | <i>albopictus</i> to <i>koreicus</i> | 5 : 6 |
| Namhansan | 5F3 | 330 | <i>albopictus</i> to <i>koreicus</i> and <i>A. subalbatus</i> | 1 : 1 : 1 |
| Andong | 6B6 | 636 | <i>albopictus</i> to <i>koreicus</i> | 3 : 1 |
| Andong | 6B10 | 455 | <i>albopictus</i> to <i>japonicus</i> | 1 : 1 |
| Danyang | 6C5 | 370 | <i>albopictus</i> to <i>koreicus</i> | 9 : 89 |
| Danyang | 6D1 | 561 | <i>albopictus</i> to <i>koreicus</i> | 1 : 18 |
| Mungyeong | 6D5 | 534 | <i>albopictus</i> to <i>koreicus</i> | 2 : 25 |
| Namhansanseong | 7-1 | 26 | <i>albopictus</i> to <i>koreicus</i> | 1 : 15 |
| Eorimok | 8A1 | 977 | <i>albopictus</i> to <i>Cx. sasai</i> and <i>A. subalbatus</i> | 2 : 2 : 6 |
| Eorimok | 8A2 | 977 | <i>albopictus</i> to <i>koreicus</i> and <i>japonicus</i> | 2 : 4 : 8 |
| Cheonwhangsa | 8A3 | 707 | <i>albopictus</i> to <i>koreicus</i> and <i>hatorii</i> | 1 : 1 : 8 |
| Cheonwhangsa | 8A7 | 684 | <i>albopictus</i> to <i>O. togoi</i> and <i>C. sasai</i> | 1 : 1 : 1 |
| Cheonwhangsa | 8A8 | 573 | <i>albopictus</i> to <i>koreicus</i> and <i>japonicus</i> | 1 : 2 : 2 |
| Cheonwhangsa | 8A10 | 593 | <i>albopictus</i> to <i>koreicus</i> and <i>japonicus</i> | 3 : 44 : 4 |
| Cheonwhangsa | 8A12 | 591 | <i>albopictus</i> to <i>koreicus</i> and <i>japonicus</i> | 4 : 50 : 7 |
| Bijarim | 8A18 | 121 | <i>albopictus</i> to <i>C. halifaxii</i> | 1 : 4 |
| Yeongsil | 8B1 | 681 | <i>albopictus</i> to <i>japonicus</i> | 1 : 7 |
| Seongup | 8B7 | 180 | <i>albopictus</i> to <i>O. togoi</i> and <i>C. halifaxii</i> | 5 : 15 : 4 |
| Seongup | 8B8 | 198 | <i>albopictus</i> to <i>koreicus</i> & <i>hatorii</i> | 3 : 2 : 1 |

containers that included plastic sheets, pickle pots and plastic buckets. None except the samples at 463 m were found to have copepods in them along with *O. hatorii*. The populations collected from Talogam and the adjoining mountainous areas were between elevations of 427 to 485 m with temperatures 25.2 to 35.8°C and RH 29 to 45%. *A. albopictus*, *A. koreicus* and *A. subalbatus* were commonly recorded species. These were found coexisting at one to one level of association, that is, *A. koreicus* with *A. subalbatus* under exposed conditions in natural habitats, that is, rock pools, while with *A. albopictus* under shady conditions in artificial fiber stones. The water color ranged from brown to grey and ash in these sites. No predators were recorded from these sites. Samples from Chiak Mountain ranged between altitudes of 359 to 365 m with temperatures between 20.4 to

23.6°C and RH 63.2 to 81.3%. *A. koreicus* was the dominating species found in green and foul waters lying in shady to exposed conditions with plenty of tadpoles. All of the samples were from artificial habitats that included plastic sheets. There was only one sample that was collected from natural habitat that included the rock pool and it was without predators, the water color was grey. Samples from different directions of the same sampling site at altitudes of 334 m with temperature between 21.5 to 37.2°C and RH 38 to 78.2% included *A. albopictus*, *A. japonicus*, *A. koreicus*, *O. hatorii* and *T. bambusa*. All of these were from artificial habitats that included tires, plastic sheets, red and white plastic jars and Korean pots, water color in these ranged from colorless to black, brown, green yellow, ash and grey. Samples from tires at one place included *A. japonicus*, *A. koreicus* and *T.*

Table 2. Number of times a species is trapped from natural habitat.

| Species | Bamboo | stream | Rock pool | stone | Tree hole | soil | Spring | Stream stones | Tree branches | Total |
|----------------------|--------|--------|-----------|-------|-----------|------|--------|---------------|---------------|-------|
| <i>A. albopictus</i> | 1 | 0 | 1 | 5 | 1 | 0 | 1 | 1 | 1 | 11 |
| <i>A. japonicus</i> | 1 | 1 | 3 | 6 | 0 | 0 | 0 | 2 | 0 | 13 |
| <i>A. koreicus</i> | 1 | 0 | 6 | 13 | 0 | 1 | 1 | 3 | 0 | 25 |
| <i>A. galloisi</i> | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| <i>O. hatorii</i> | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 1 |
| <i>Aedes</i> | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 1 |
| <i>C. vagans</i> | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 1 |
| <i>A. subalbatus</i> | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 |
| <i>T. bambusa</i> | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 |
| Total | 3 | 3 | 10 | 26 | 1 | 1 | 3 | 6 | 1 | |

Table 3. Breeding sites of the species collected from Korean Peninsula from June – Nov 2009.

| Breeding site | <i>albopictus</i> | <i>japonicus</i> | <i>koreicus</i> | <i>galloisi</i> | <i>Aedes</i> | <i>O. hatorii</i> | <i>Togoi</i> | <i>Och.</i> | <i>pipiens</i> | <i>sasai</i> | <i>halifaxii</i> | <i>A. subalbatus</i> | <i>T. bambusa</i> | <i>A. sineroides</i> | Total |
|---------------------|-------------------|------------------|-----------------|-----------------|--------------|-------------------|--------------|-------------|----------------|--------------|------------------|----------------------|-------------------|----------------------|-------|
| White bucket | | 1 | 1 | | | | | | | | | | | | 2 |
| Red bucket | 3 | 2 | 7 | | | | | | | 2 | | 1 | | 3 | 18 |
| Bucket lid | | | 2 | | | | | | | | | | | | 2 |
| Stone pot | 4 | 4 | 11 | | | 4 | 2 | | | 2 | 1 | | 1 | | 29 |
| Tires | 8 | 1 | 3 | | | | | | | | | | 2 | | 14 |
| Thermopore box | 1 | 2 | 2 | | | | | | | | | | | | 5 |
| Blue plastic cover | 3 | 2 | 7 | | | 1 | | | | | | | | | 13 |
| Man made stream | | 2 | 2 | | | | | | | | | | | | 4 |
| Sewerage tank | | | 1 | 1 | 1 | | | | | | | | | | 3 |
| Kimchi pot | 1 | 1 | 1 | | | | | | | | | | | | 3 |
| Grey plastic cover | | 1 | 1 | | | | | | | | | | | | 2 |
| Fiber stone | 1 | | 1 | | | | | | | | | | | | 2 |
| Sewerage line | | | 1 | | | | | | | | | | | | 1 |
| Yellow iron trolley | | 1 | 1 | | | 1 | | | | | | | | | 3 |
| Korean pot | 6 | 1 | 4 | | | 1 | | | | 1 | | 1 | | 1 | 15 |
| Green plastic cover | 1 | 1 | 5 | | | 2 | | | | | | | | | 9 |
| White paint drum | 1 | | 1 | | | | | | | | | | | | 2 |
| Pond | | | | | | | | | | | | | | | |
| Inverted pot top | 1 | 1 | 3 | | | | | | | 1 | | | | | 6 |

Table 3. Contd.

| | | | | | | | | | | | | | | | | | |
|------------------------|----|----|----|---|---|----|---|---|---|---|---|---|---|---|--|--|----|
| Orange plastic sheet | | | 1 | | | | | | | | | | | | | | 1 |
| White plastic plate | 1 | 1 | 1 | | | | | | | | | | | | | | 3 |
| Flowering stone pot | 1 | 3 | 10 | | | 1 | | | | 1 | 1 | 1 | 1 | | | | 18 |
| Red plastic tub | 1 | 2 | 1 | | 2 | | | | | 1 | | | 1 | | | | 8 |
| Gutter lid | 1 | 1 | 2 | | | | | | | | | | | | | | 4 |
| Black plastic drum | | | 1 | | | | | | | | | | | | | | 2 |
| Flower plant pot | 1 | 1 | 1 | | | | | | 2 | | | | | | | | 5 |
| Roof top | | | 1 | | | | | | | | | | | | | | 2 |
| Iron flower pot | | | 1 | | | | | | | | | | | | | | 2 |
| Stone pot | | | 2 | | | | | | | | | | | | | | 2 |
| Yellow plastic trolley | 1 | 1 | | | | | | | | | | | | | | | 2 |
| Concrete box | | | 1 | | | | | | | | | | | | | | 1 |
| Capillary water | | | 1 | | | | | | | | | | | | | | 1 |
| Total | 36 | 32 | 77 | 1 | 3 | 10 | 2 | 2 | 1 | 7 | 2 | 4 | 3 | 4 | | | |

Table 4. Correlation matrix.

| Variables | Altitude | Temp. (°C) | R.H.% | Water temp (°C) | <i>A. japonicus</i> | <i>A. albopictus</i> | <i>A. koreicus</i> | <i>O. hatorii</i> | <i>A. sineroides</i> | <i>C. vagans</i> | <i>A. togoi</i> | <i>T. bambusa</i> | <i>C. pipiens</i> | <i>C. sasai</i> | <i>C. halifaxii</i> | <i>A. subalbus</i> |
|----------------------|----------|------------|--------|-----------------|---------------------|----------------------|--------------------|-------------------|----------------------|------------------|-----------------|-------------------|-------------------|-----------------|---------------------|--------------------|
| Temp. (°C) | -0.484 | | | | | | | | | | | | | | | |
| R.H.% | -0.202 | -0.527 | | | | | | | | | | | | | | |
| Water temp. (°C) | -0.578 | 0.931* | -0.471 | | | | | | | | | | | | | |
| <i>A. japonicus</i> | -0.393 | 0.439 | 0.235 | 0.314 | | | | | | | | | | | | |
| <i>A. albopictus</i> | -0.805** | 0.358 | 0.474 | 0.495 | 0.597* | | | | | | | | | | | |
| <i>A. koreicus</i> | -0.448 | 0.357 | 0.036 | 0.400 | 0.723** | 0.542 | | | | | | | | | | |
| <i>Och. hatorii</i> | -0.557 | 0.140 | 0.393 | 0.164 | 0.764** | 0.596* | 0.805** | | | | | | | | | |
| <i>A. sineroides</i> | -0.453 | 0.343 | 0.296 | 0.278 | 0.593 | 0.538 | 0.374 | 0.472 | | | | | | | | |
| <i>C. vagans</i> | -0.195 | 0.157 | 0.027 | 0.169 | 0.399 | 0.308 | 0.684* | 0.376 | 0.562 | | | | | | | |
| <i>A. togoi</i> | -0.406 | -0.079 | 0.527 | 0.036 | 0.404 | 0.613* | 0.105 | 0.499 | 0.337 | -0.200 | | | | | | |
| <i>T. bambusa</i> | -0.174 | -0.042 | 0.100 | 0.082 | 0.275 | 0.310 | 0.750** | 0.400 | -0.180 | 0.523 | -0.111 | | | | | |
| <i>C. pipiens</i> | -0.174 | -0.042 | 0.100 | 0.093 | 0.284 | 0.220 | 0.764** | 0.356 | -0.160 | 0.411 | -0.111 | 1.000** | | | | |
| <i>C. sasai</i> | 0.290 | -0.744* | 0.498 | -0.851** | -0.281 | -0.419 | -0.378 | -0.019 | -0.182 | -0.199 | -0.400 | -0.111 | -0.111 | | | |
| <i>C. halifaxii</i> | -0.406 | -0.079 | 0.527 | 0.036 | 0.404 | 0.614* | 0.104 | 0.499 | 0.337 | -0.199 | 1.000** | -0.111 | -0.300 | -0.111 | | |
| <i>A. subalbus</i> | -0.269 | -0.061 | 0.225 | 0.101 | 0.379 | 0.454 | 0.773** | 0.615* | -0.101 | 0.475 | 0.124 | 0.972** | 0.980** | -0.137 | 0.124 | |

Table 4. Correlation matrix.

| | | | | | | | | | | | | | | | | |
|--------------------|-------|--------|--------|--------|--------|--------|--------|--------|--------|--------|-------|--------|--------|--------|--------|--------|
| <i>A. galloisi</i> | 0.174 | -0.216 | -0.034 | -0.110 | -0.315 | -0.161 | -0.053 | -0.171 | -0.180 | -0.200 | 0.411 | -0.122 | -0.111 | -0.111 | -0.121 | -0.137 |
|--------------------|-------|--------|--------|--------|--------|--------|--------|--------|--------|--------|-------|--------|--------|--------|--------|--------|

* Significant at $P < 0.05$; ** Significant at $P < 0.01$.

bambusa, while samples from tires at other places included either *A. albopictus* or *T. bambusa*. There were no predators recorded from these samples that were present in the shady areas. Sampling at Khajesan at 375 m altitude where the temperature was 17 to 22.4°C and RH 81 to 100% yielded *A. albopictus*, *A. japonicus*, *A. koreicus*, *A. subalbatus*, unknown forms and *C. pipiens*. Most of the samples were from flowering pots with water ranging from light green, to light grey or brown and were found in exposed conditions. Few collection sites were with copepods while others were generally free from any invasion by any fauna, although all had plant debris, mud and stones in them. Populations collected from the plastic tub were found associating with one another; however, at other places, single species were collected from each collection site. *A. albopictus* was collected from rock pool, in the shady area with water clear and containing stone debris.

Populations from Juwangsan, Taejonsa and Jusanji Mt located at altitudes of 279 to 326 m with temperature 26 to 32.9°C and RH between 63 to 80% contained *A. koreicus*, *A. japonicus* and *C. vagans* with former dominating in number and spots. Most of the populations were collected from exposed areas with water colorless or ash to grey. All of the collection sites had tadpoles, copepods and water scorpions in them. Out of the eight spots visited in these sites two were artificial habitats, while rest were natural pools on the rocks formed as a result of rain or over flowing of the streams. Except for two rock pool collections which were found associating as

A. koreicus with *A. japonicus* and *A. koreicus* with *C. vagans*, populations at remaining sites were of single species in each spot.

Localities surveyed along Juwangsan at elevation of 188 and 315 m followed by Songsogotaek at 188m with temperature between 23.7 to 25.2°C and RH 83.5 to 94.6% had *A. albopictus* and *A. koreicus*. Except in one natural spot that is stone where both species were found together, all other collection sites including the artificial habitats like the sewerage line, red plastic bucket, plastic mat, stone pot and tires had single species. The water in these sites was colorless to grey or even green. These habitats were generally located in the exposed conditions and had a few copepods together with the *A. albopictus*, while those with tadpoles had no mosquito fauna.

Populations collected from altitude of 61 m around Haenam were from a rural home and the only species recorded was *A. japonicus* from flowering pots kept in exposed–shady place that had naiads of dragon flies and algae. The temperature recorded was 28°C with 100% RH. The populations collected from altitudes between 190 to 310m included 16 spots at Najangsan Mt. and its surrounding. The species recorded were *A. albopictus*, *A. japonicus*, *A. koreicus*, *O. hatorii*, *A. subalbatus* and *A. sineroides*. As most of the populations were from mountainous area therefore the habitats formed as a result of rain were mostly natural with a few artificial. The sites were under shade and contained black, orange, green or colorless water rich in plant debris, grasses, algae and soil particles. Populations collected from the stones, tree holes, branch

junction were all *A. albopictus*, while in most of the artificial containers *A. koreicus* and *A. japonicus* were recorded. The samples from branch junction with water temperature 20.4°C yielded maximum number of *A. albopictus* (33). Samples collected from artificial habitats at same altitude included plastic tubs, plastic buckets, sheets, Korean pots, plastic trolley and the thermo pore box. All of these contained maximum of *A. albopictus* followed by *A. japonicus*, *A. koreicus*, *A. sineroides* and *A. subalbatus*. Out of the total six spots from this site *A. albopictus* larvae were collected from four sites where they were present independently except in thermo pore box near the drain pipe, where it was associated with *A. japonicus* and *A. koreicus*. As far as *A. subalbatus* was concerned it was found together with *A. koreicus* in Korean pot that contained black color water.

In our field collection from Samhwasa three sites located at an altitude of 160 to 165m were surveyed and the temperature recorded were 27.6 to 29.4°C with RH 63.9 to 65.5%. All of the spots were exposed and under direct sunlight, the temperature recorded near them was 26.5 to 34.4°C. The populations collected were *A. albopictus*, *A. koreicus* and *O. hatorii*. Populations of *A. koreicus* were collected from pots around the statue which contained different stages of mosquito larvae and naiads of dragon flies in the green color water. *A. albopictus* larvae were recorded from the Korean pot that had grey color water which was without any other population. Six sites from Wolleongsa were visited at altitudes of 650 to 655 m. The temperature recorded here

was 21.1 to 25.6°C with RH 64.6 to 89.8%. The water temperature recorded was 20.9 to 25.1°C. All of the sites were occupied by *A. koreicus* with two by *A. japonicus* and one by unknown *Aedes*.

Two spots were from stones, while the remaining were either from the sewerage line, gutter lid, back of the Korean pot or the iron flower pot. The water was generally colorless that had grasses, algae, plant debris and stones. All of these sites included collection from exposed to shady sites. Populations from gutter lid contained the dragon flies naiads together with *A. koreicus* (Table 3).

Sangwonsan site had eight collection spots distributed at an altitude of 560 to 870 m. The temperatures ranges were irrespective of the altitude and found to fluctuate between 20.7 to 29.0°C with RH 56.5 to 79.2%. The water temperatures recorded were between 17.8 to 29.8°C being negatively associated with the altitude. Three mosquito species viz., *A. albopictus*, *A. japonicus* and *A. koreicus* were recorded from these sites with *A. japonicus* as the leading followed by *A. koreicus*. All of the samples were from artificial containers that included pots; buckets etc lying in exposed to shady places with colorless to light green water that had dragon fly naiads and plant debris.

Populations collected from Mangwolsa at altitude of 367 m with temperature 28.3°C, RH 73.3% and water temperature 27°C were from ash colored foul water, exposed under the sun during the day. The only species recorded from this habitat was *A. albopictus* with 24 larvae collected from stone pot having algae and many non-predator forms in it. Two collection spots from Osaek at altitudes of 450 m were recorded with temperatures between 25.7 to 28.5°C and RH 68.5 ± 0.1%. The water temperature 22.5°C in ash colored sample from rock pool revealed *A. japonicus* and *A. koreicus* along with many tadpoles from the spot formed as a result of seepage from the drain pipe. Samples from stone pot at the same site yielded *A. koreicus* larvae.

Two sites were surveyed at Hangyreyong 918 m; the temperature recorded was at an average of 24.8°C with RH 62.9 to 64.3%. Mosquitoes from waters with temperature of 26.2 to 27.7°C yielded *A. japonicus*, *A. koreicus* and *C. vagans* from plastic sheet and the large red bucket placed in exposed to shady conditions with clear to grey and foul water. No predator to non-predator was recorded, while some plant debris, grasses and mud at the bottom was present. Vicinity of Naeseorksans, Seorksans and Baekdamsa all located at 468 m only yielded *A. koreicus* at atmospheric and water temperatures which were at par (av. 26.5°C) with RH between 62%. The populations comprised both from the tires, inverted buckets and rock pools in the exposed to shady conditions. All had plant debris in them however no predators were recorded from these collecting sites.

Populations collected from four Sinheungsa at altitudes of 230 to 235 m only yielded *A. koreicus* from all sites

except one that contained *A. japonicus* together with the *A. koreicus*. All of the sites had dragon fly naiads and abundant plant debris, mud, ferns and algae. The water color from blue and green plastic sheets, Korean pots with shallow water ranged from grey to green.

Eleven spot toward Donghaksa temple were surveyed located at altitudes of 152 to 195 m, the temperature in these spots generally ranged from 21.6 to 25.2°C with RH fluctuating from 79.9 to 99.1% probably because of the very cloudy conditions followed by some slight showers. Two spots included collection from rock pools while rest of the sites were all artificial habitats ranging from tires, plastic covers, flower pots, plastic buckets. Most of the sites contained colorless, while some had green color water. Collections from flowering pots contained the tadpoles; while, plant debris, mud and stones were present in all habitats. There was one site where the *A. koreicus* along with *A. japonicus* were recorded from flowing water. While, *A. subalbatus* was for the first time recorded from automobile tires besides other collection sites. All the sites ranged from shady to exposed.

Four sites at Jongaksa were surveyed that were relatively at lower altitudes (113 to 150 m), with temperatures and RH% at two points between 22 to 27.2°C and 71.6 to 88.7, while at other on an average of 40.8°C and 39.9 to 40.3%, respectively. All of the sites were artificial that included the green or blue plastic covers, or the seepage from drain pipes in the natural stream. *A. albopictus*, *O. hatorii* and *A. koreicus* were the three recorded species with *A. albopictus* as the most commonly found. The species were found associating with one another. The collection sites were in exposed to shady habitats with water color varying from green to brown or even grey or colorless probably because of the plant debris, leaves, fungus, algae and stones. Copepods were recorded from colorless to green color water, with the later having maximum *A. albopictus* population. Outer edges of Chilgapsan Mt were surveyed at five different points located at 291 m. All of these sites had *A. koreicus*, except one where *albopictus* was also present, in waters ranging from colorless to green, lying in shady to exposed conditions. The temperatures recorded were in the upper ranges of 30.4 to 44.9°C with RH 29.9 to 60.2%. All collection sites had waters with plant debris, ferns, algae and stones thus giving a foul fragrance. The water color ranged from black, green, grey, brown to ash. Different sites of Namhansanseong were surveyed located at altitudes of 330 to 334 m at temperatures between 25 to 33.6°C with RH between 53.1 to 80%. The major dominating species has been *A. koreicus* followed by *A. albopictus* and *A. subalbatus* from nine spots in this major collection site. As the sites were in the forest area therefore most of the breeding places were either the trash that was left by the visitors, or plastic sheets that were used for covering the construction material which had the tadpoles, copepods, frogs and sometime

dragonfly naiads in them. The water color in these sites depended on the quantity of organic matter, the color generally ranged from green, grey, ash and black to brown.

Seven spot at altitudes between 77 to 85 m were visited at temperatures between 24.7 to 29°C with RH 55.2 to 71.8%. The collection sites mostly included the larval stages. While in two stone pots the larvae were in proportion to the pupa. Two species of *Aedes* viz., *A. albopictus* and *A. koreicus* were recorded from these sites with *A. albopictus* much more common.

Sites visited at the Danyang located at elevation of 455 to 755 m with temperatures between 19.5 to 25.3°C with RH 71.9 to 100% included all artificial sites except one from the rock pool. The sites included plastic buckets, cement tanks, jars, Korean pots, tires, stone pots. The species recorded included *A. albopictus*, *A. koreicus*, *A. japonicus* and *C. sasai* with the later most dominating species in the plastic jars. All collection sites included more proportionate of larval stages in comparison to pupae.

At five more sites of Danyang located at altitude of 370 to 402 m with temperatures 25.1 to 28.5°C with RH 72.7 to 86.7% yielded *A. albopictus*, *A. koreicus* and *O. hatorii*. All of the collection sites included relatively more fourth instar larval populations.

Nineteen sites were visited on first day in Jeju that included elevations from 85 to 977 m (Manjangul a cave to Eorimok). The temperature recorded inside the cave was 16°C while at the highest elevation was 23°C with RH 88 and 58.3% at these points. However, for other sites visited the temperature ranged from 15.5 to 39.3°C, while the RH fluctuated between 39 to 85%. Out of all the 19 collections, two were from natural habitats that included the bamboo and the stream. The larvae recorded from bamboo were *A. albopictus*, *A. japonicus* and *A. koreicus*. The water temperature was 16.1°C with water generally clear, under the shade and with few plant debris. While from stream (707 m) only *A. japonicus* was collected with colorless water that had algae. Other collections sites included buckets of different colors (red and white), stone pots, automobile tires, artificial streams, white thermo pore box, fountain, blue plastic cover and white plastic plate. The water color in the bucket at 977 m was green and the populations recorded were of *A. albopictus*, *A. subalbus* and *C. sasai*, while buckets at 593 m contained colorless water and had *A. japonicus* and *A. koreicus*. Populations collected from four stone pots at altitudes of 707, 707, 573 and 411 m generally contained turbid with green color, colorless, foul with grey and green with foul water, respectively. The species included *A. albopictus* with *O. hatorii*, *A. japonicus* alone, *A. albopictus* with *A. japonicus* and *A. koreicus* and no populations. Automobile tires examined at two different situations at altitude of 680 and 684 m ranged from exposed and exposed to shady conditions with water color light and black. A total of 30 dips were made from

tires in exposed conditions while 10 from those in exposed to shady. Both had populations of *Ae. albopictus* only; while, artificial stream at Jeolmul forest yielded *A. japonicus* and *A. koreicus* at altitudes of 535 m, both sites had colorless water with some plant debris in it. Populations recorded in the white thermo pore box included *A. albopictus*, *A. japonicus* and *A. koreicus*. The populations recorded from the fountain at 411 m yielded *O. hatorii*, *A. japonicus* and *A. koreicus*. The water was flowing, generally clear and without any debris. The larvae collected from white plastic plate at altitude of 121 m from Bijarim forest had water color grey, with algae and grasses in it. The species recorded were *A. albopictus* and *C. sasai*. No population was recorded from the Manjangul cave although it had many water catchment sites with plenty of visitor. The only factor that could be predicted was the cold temperature in the cave and darkness. The water temperatures recorded at a number of places was on an average between 15 to 16°C.

Nine other sites were visited in Jeju between altitudes of 123 and 681 m. The atmospheric and water temperatures were between 23.6 to 28.1°C and 21.5 to 28°C respectively, while the RH recorded was between 65.8 and 83.8%. The populations included *A. albopictus*, *A. japonicus*, *A. koreicus*, *O. hatorii* and *C. halfaxii*. The populations were collected from covered stone pot at a shrine, rock pool, three stone pots placed at different sites and altitudes, Korean pot, horse shed and tires along road side. These sites had waters which were grey, green or colorless. Sampling sites at altitude of 681, 291 and 180 m contained the tadpoles, and frog or the water bugs. All were with algae, spirogyra and fern. Most of the sources were in the shady, exposed to shady and exposed conditions.

Mosquito collection sites were also assessed to find the most potential breeding habitat of the mosquitoes. It was found that the mosquitoes frequently breed in natural to artificial habitats, however the total population that have been trapped are more from the artificial one, thus indicating that human involvement is the main cause of the mosquito multiplication. Generally, out of the total 40 different types of habitats nine were natural while 31 were artificial.

Maximum times a species was trapped from the artificial habitats included the stone pots (29), red buckets and flowering plant pots (18), Korean pots (15), automobile tires (14), blue plastic sheets (13), followed by remaining in single digits. Amongst the natural collection sites stones (25) were the most preferred places for larval development subject to presence of water on them, rock pools (10) and stones lying in the stream (6) were the three most preferred sites for oviposition. These were followed by bamboo (3), stream water (3), springs (3), tree holes (1), tree branches (1) and soil (1) (Table 2).

It is finally concluded that there is range of mosquito species breeding in these altitudes, however there was

no difference found in terms of the climatic factors that were associated with each species. As the weather conditions tend to remain constant throughout the peninsula and the Jeju Island, regardless of the altitude which only shows a few degree difference from lower to higher therefore the distribution of the mosquito species seems to be uniform. The only factor that favors the breeding of the species in the mountain areas has been the presence of water source. Size of the water catchment area in some cases has been found to affect the number of developing larvae, but on overall basis even very shallow waters had plenty of larvae, and pupae. Shady conditions and exposed breeding areas have been found at par in terms of presence of larvae. The presence of predators, like tadpoles, frogs, naiads of dragon flies, copepods, water measurer, scorpions in some cases were found to have strong effect on the mosquito larvae while in other cases it was observed that despite their presence eggs, all stage larvae, pupae and newly emerging adults were seen on the surface of water indicating a more balanced coexistence in terms of net benefit for all the associating fauna. The abundances of mosquito larvae are often limited by biotic factors, such as predators and competitors (Blaustein and Karban, 1990; Blaustein and Margalit, 1996; Blaustein, 1998; Stav et al., 2000; Mokany and Shine, 2003). In addition, the importance of these biotic interactions varies depending on the type of wetland. Wetlands can be divided into three classes: temporary, permanent and semi permanent, based on their probability of retaining standing water throughout the year; this in turn determines the types of species that can live in those habitats and their interspecific interactions (Schneider and Frost, 1996; Wellborn et al., 1996; Williams, 1996). A range of artificial breeding places have been recorded for the Culicinae mosquitoes particularly the *Aedes*, *Ochlerotatus*, *Tripteroides* and *Armigeres*. *Aedes* has shown more diversity in terms of the habitat selection and breeding. The numbers of individuals captured for this genus irrespective of the species have been dependent on the habitat type. Generally the plastic sheets, kimchi (Korean pickle) pots, buckets have contained maximum population, while tree holes have been the most potential natural breeding sites for these mosquitoes. All of the populations have been found breeding in the standing waters with only a few cases where *A. koreicus* was recorded from waters that were continuously changing as a result of the inflow from a pipe and discharge at times of over flowing. Water characters like color were negatively associated with mosquito larvae. We have recorded a range of colors, starting from colorless to black followed by orange to yellow, dull to dark green, brown to ash and grey, that were either foul or otherwise, all were found to contain mosquito larvae, with the only strong factor the presence of temporary breeding sites. Those areas which had received rain had plenty of clear water breeding places so these were preferred over the places that had stained water in them and vice versa.

Maximum breeding activities were recorded near the temples, resting areas, as these places were regularly visited by the people and thus provided a continuous source of blood to the female mosquitoes. Presence of flora, algae, small plants were found associating with the aquatic life. Almost all the breeding sites had the plant debris, algae, ferns, spirogyra, tree barks, grasses and stones in them. In some situations the plant debris was so much that hardly 20 cc of water could be collected from it, however the larval population in this was considerable. At some places more water was added to the spot so as to collect the 4th instar larvae and pupae. Some cut bamboo plants were also observed no populations from these were however recorded.

In conclusion, the statistical analysis recorded in Table 4 showed that *A. albopictus* have highly significant negative correlation with altitude and *C. sasai* have significant negative correlation with temperature and highly significant negative correlation with water temperature while all the other spp. showed no significant correlation with the studied parameters. *A. albopictus* showed significant positive correlation with *A. japonicus* while *A. koreicus* and *O. hatorii* showed highly significant positive correlation with *A. japonicus*. *O. hatorii* also showed significant positive correlation with *A. albopictus* and *A. subalbatus* and highly significant positive correlation with *A. koreicus*. *C. vagans* showed significant positive correlation with only *A. koreicus*. *A. togoi* showed significant correlation with *albopictus* and highly significant perfect correlation with *C. halifaxii*. *T. bambusa* showed highly significant correlation with *A. koreicus* and *A. subalbatus* while highly significant perfect correlation with *C. pipiens*. *C. pipiens* showed highly positive significant correlation with *A. koreicus* and *A. subalbatus*. *A. galloisi*, *A. sineroides* and *C. sasai* showed non significant correlation with all the mosquito spp.

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