

Difference in Characteristics of *Culex tritaeniorhynchus* Originated from Thailand and Japan

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Abstract: The morphology of *Culex tritaeniorhynchus* of Chantaburi, Thailand, Amami-Oshima and Nagasaki in Japan were compared. Adult females collected at Nagasaki were the largest and those at Chantaburi were the smallest in any season. The results of laboratory experiments with two colonies originated from Bangkok and Nagasaki indicated that larval plus pupal period was shorter and body sizes of resulting adults were smaller in Bangkok colony than in Nagasaki colony. At low temperature and short photoperiod, all females of Nagasaki colony entered into diapause and did not feed on blood meals, while a part of Bangkok colony did not show any sign of diapause and fed on blood meals 7 to 14 days after emergence. These results clearly showed the geographical difference in morphology and physiology between populations of Thailand and Japan.

Key words: *Culex tritaeniorhynchus*, geographical variation, seasonal variation

Culex tritaeniorhynchus Giles, a vector of Japanese encephalitis (JE), is widely distributed in Asia (Stone *et al.*, 1959). Adult females of this species feed actively on blood meals at any seasons of the year in the tropics such as Thailand and Malaysia, and JE virus is isolated from them throughout the year in those countries (Gould *et al.*, 1974; Simasathien *et al.*, 1972; Simpson *et al.*, 1970). In the temperate zones such as Japan, however, only unfed adult females in diapause can overwinter. Therefore, in Japan, JE

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virus is considered to be unable to overwinter in the body of *Cx. tritaeniorhynchus* (Oda *et al.*, 1978). The supposition that the migratory bird carries JE virus from outside of Japan has not been proved yet; antibody to JE virus was found from migratory birds but JE virus itself was not isolated from them (Takahashi, Personal communication). Hayashi *et al.* (1978) inferred that *Cx. tritaeniorhynchus* bring JE virus into Japan from the tropics or subtropics, as suggested by the facts that brown plant hoppers fly into Japan from overseas every early summer (Kishimoto, 1971), and some *Cx. tritaeniorhynchus* were caught in the Pacific Ocean and the East China Sea far from the land (Asanina, 1970; Asahina and Tsuruoka, 1969, 1970). In order to give the proof of their hypotheses, Hayashi and his co-workers attempted to catch *Cx. tritaeniorhynchus* in the East China Sea and caught some females of *Cx. tritaeniorhynchus*, but JE virus was not isolated (Hayashi *et al.*, 1978; Suzuki *et al.*, 1977). If we can prove that those *Cx. tritaeniorhynchus* came from tropics or subtropics, the hypotheses of Hayashi *et al.* (1978) will confirm its authenticity. According to Reisen *et al.* (1979), several attributes are different clearly between *Cx. tritaeniorhynchus* from Pakistan and Japan. Nakamura (1982) also showed a cline of morphological and ecological characteristics among the laboratory colonies from Japan and Taiwan. These results suggested the possibility to guess the native place of *Cx. tritaeniorhynchus*, which were caught, for example, in the ocean from the inherent characteristics of this species in each region. However, much more data are needed to do so on the morphological and ecological differences in *Cx. tritaeniorhynchus* populations from south to north, particularly in relation to seasonal changes of these characteristics. Therefore, comparisons were made with *Cx. tritaeniorhynchus* of Thailand in the tropics, Amami-Oshima in the subtropics and Nagasaki in the temperate zones.

MATERIALS AND METHODS

For the observations on the seasonal changes of the length of wing and hind tibia of *Cx. tritaeniorhynchus* in the field, collections of mosquitoes were carried out at Chantaburi (12°36'N), Thailand, Amami-Oshima (28°10'N) and Nagasaki (32°44'N), Japan, using Nozawa type light trap. In Chantaburi, the light trap was operated in the front of a house once a month from July, 1981 to June, 1982. In Amami-Oshima, the light trap was set at pigsty from January to December in 1973, and in Nagasaki from March to October in 1973. Collected *Cx. tritaeniorhynchus* females were dried and then measured the lengths of their wings and hind tibiae by micrometer.

The histories of laboratory colonies of *Cx. tritaeniorhynchus* used in this experiment are described as follows. The Bangkok colony of *Cx. tritaeniorhynchus* was obtained originally from females collected by human bait at Bangkok, Thailand in October, 1980, and the 3rd to 5th generations of this colony were used. The Nagasaki colony was originated from fed females collected at a pigsty in Nagasaki, Japan in July, 1965, and

the 156th generation was used. Colonies of both strains have been maintained in the insectarium at 27°C, 80% RH, and 16 hr daylength. One hundred first instar larvae of each colony, within 12 hr after hatch were put in the tray (22 × 28 cm) and transferred to the insectarium at 21°C under 10 hr daylength, 25°C under 16 hr daylength or 30°C under 16 hr daylength. The larvae in each tray were given 0.1 g equally mixed powder of Brewer's yeast and mouse pellets as food every day, and the developmental periods were recorded. The resulting adults were provided with a maintenance diet of 2% sugar solution. Females from 25°C and 30°C were killed one week after emergence, and the size of wing and hind tibia were measured and the developmental stages of follicles (Kawai, 1969) were examined. Females from 21°C were killed one to two weeks after emergence and treated in the same way as stated above. As for the females from 25°C, the number of ovarioles were counted.

RESULTS

The results of the measurement of wing and hind tibia of *Cx. tritaeniorhynchus* females caught at three places are given in Fig. 1 and 2. As it is well known that the

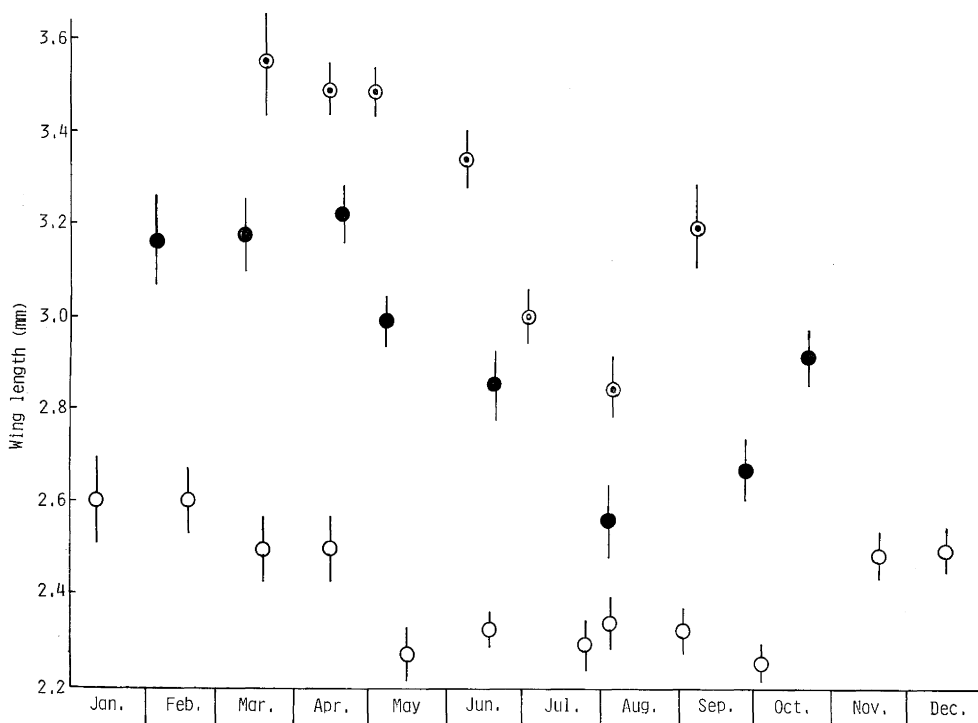


Fig. 1. Seasonal changes of wing length of female *Culex tritaeniorhynchus* caught at Chantaburi, Thailand, from July, 1981 to June, 1982 and at Amami-Oshima and Nagasaki, Japan from January to December, 1973. ○; Chantaburi, ●; Amami-Oshima, ◐; Nagasaki. Vertical line shows 95% confidence limit.

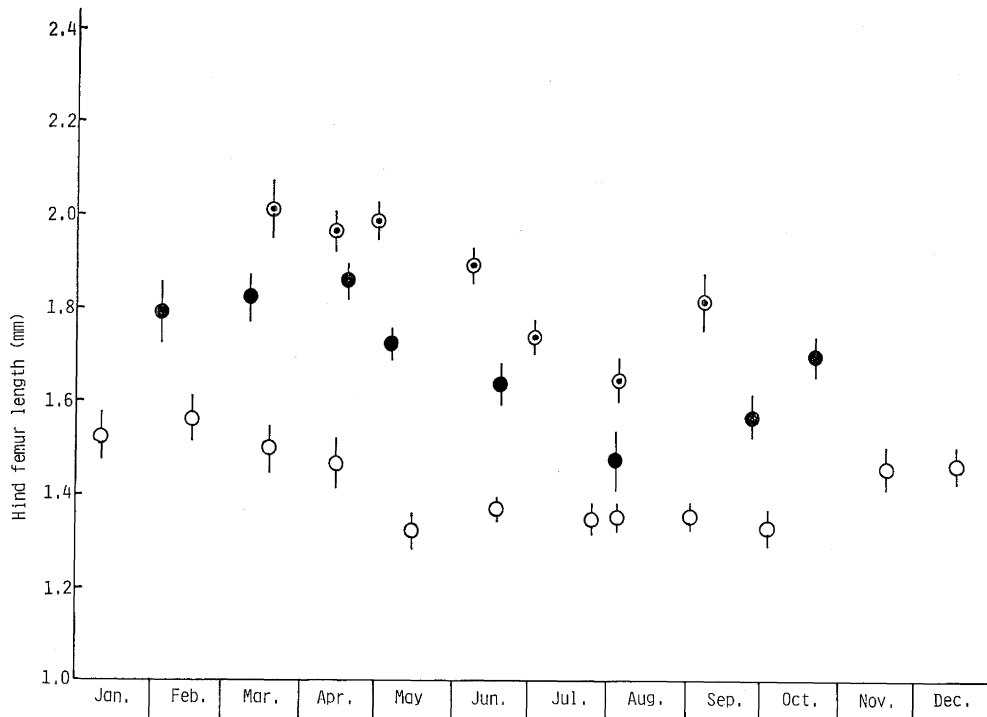


Fig. 2. Seasonal changes of hind tibia length of female *Culex tritaeniorhynchus* caught at Chantaburi, Thailand from July, 1981 to June, 1982 and at Amami-Oshima and Nagasaki, Japan from January to December, 1973. ○; Chantaburi, ●; Amami-Oshima, ⊙; Nagasaki. Vertical line shows 95% confidence limit.

wing length of mosquitoes is correlated with rearing temperature, monthly averages of mean air temperature at Chantaburi from July, 1981 to June, 1982 and Amami-Oshima and Nagasaki from January to December, 1973 are given in Fig. 3.

In Nagasaki, the wing length of overwintered females caught in March, April and early May were the largest in the year. These females had emerged in the previous autumn before and overwintered. The wing became smaller from early to mid summer and it became larger again in September, that is wing length tended to change with the fluctuation of mean air temperature, which increased from May toward August and decreased from September.

In Amami-Oshima, the adults were caught almost all through the year. Seasonal changes of their wing length showed a similar tendency to those in Nagasaki and were correlated with the fluctuation of mean air temperature. However, they were smaller than those in Nagasaki throughout the year.

As for *Cx. tritaeniorhynchus* from Chantaburi, the wing length in rainy season from May to October were smaller than in dry season from November to April. Mean air temperature in Chantaburi, which is almost stable throughout the year, did not seem

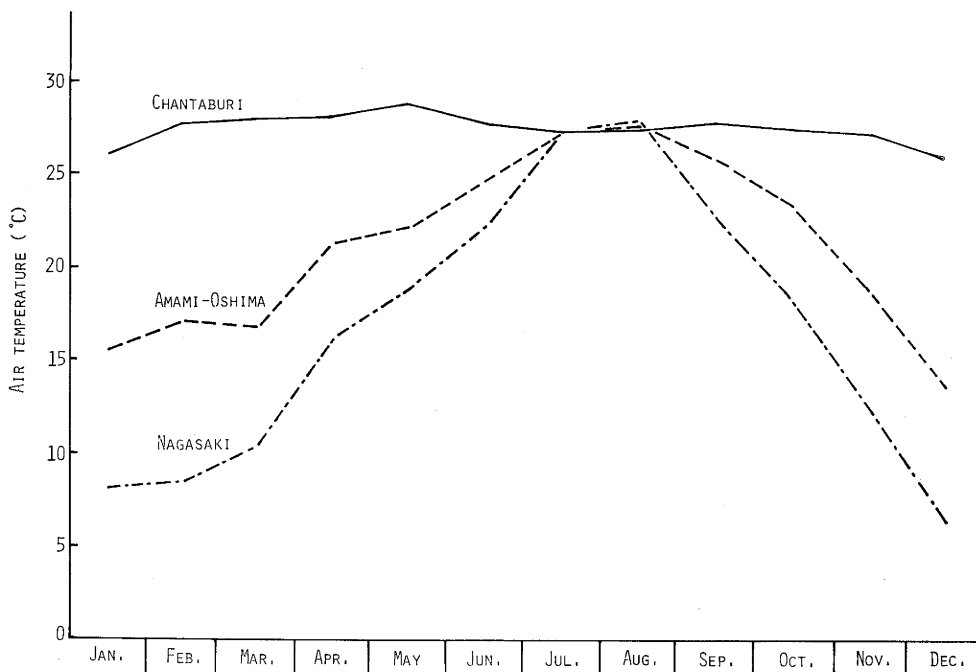


Fig. 3. Monthly averages of mean air temperature at Chantaburi from July, 1981 to June, 1982 and at Amami-Oshima and Nagasaki from January to December, 1973. Obtained from the Chantaburi Observatory, the Naze observatory and the Nagasaki Marine Observatory.

to correlate with the wing length of *Cx. tritaeniorhynchus*. As humidity in dry season is lower than that in rainy season, the water temperature in breeding site of this mosquito may be lower in dry season than in rainy season. It is also interesting in the fact that *Cx. tritaeniorhynchus* females in Chantaburi had by far smaller wing than those in Japan throughout the year, in spite of the fact that the temperature of Nagasaki or Amami-Oshima increased to the level of the temperature of Chantaburi in mid summer.

The seasonal changes of the length of hind tibia were parallel with those of the wing length in all regions. This indicates that not only wing length but also hind tibia length, and whole body size also, changes seasonally.

Median periods from hatch to adult emergence of Bangkok and Nagasaki colony at 21, 25 and 30°C are given in Table 1. The development period of Bangkok colony tended to be shorter than that of Nagasaki colony at every temperature.

Table 2 shows the wing length of females reared at various temperatures. Though the wing length shortened in both colonies with increasing temperature, Nagasaki colony had larger wing than Bangkok colony at any temperatures. The largest difference of wing length in Nagasaki colony was 0.62 mm between females resulting from 21°C and 30°C, while the largest difference in Bangkok colony was only 0.19 mm between those from 21°C and 25°C. The difference of wing length between these two colonies was

Table 1. Median periods in days from hatch to emergence of females of *Culex tritaeniorhynchus* originated from Bangkok, Thailand and from Nagasaki, Japan at various temperatures

| Temperature \ Location | 30°C | 25°C | 21°C |
|------------------------|------|------|------|
| Bangkok | 5.5 | 8.1 | 15.8 |
| Nagasaki | 6.4 | 8.4 | 16.9 |

Table 2. Wing length (mean [mm] \pm 95% confidence limit) of *Culex tritaeniorhynchus* females originated from Bangkok, Thailand and from Nagasaki, Japan at various temperatures

| Temperature \ Location | 30°C | 25°C | 21°C |
|------------------------|-----------------|-----------------|-----------------|
| Bangkok | 2.55 \pm 0.02 | 2.51 \pm 0.04 | 2.70 \pm 0.02 |
| Nagasaki | 3.03 \pm 0.03 | 3.25 \pm 0.03 | 3.65 \pm 0.03 |

Table 3. Hind tibia length (mean [mm] \pm 95% confidence limit) of *Culex tritaeniorhynchus* females originated from Bangkok, Thailand and from Nagasaki, Japan at various temperatures

| Temperature \ Location | 30°C | 25°C | 21°C |
|------------------------|-----------------|-----------------|-----------------|
| Bangkok | 1.74 \pm 0.03 | 1.71 \pm 0.03 | 1.79 \pm 0.02 |
| Nagasaki | 2.03 \pm 0.04 | 2.13 \pm 0.03 | 2.09 \pm 0.04 |

significant at relatively low temperature as 21°C.

The developmental stages and size of follicles in the ovary of females are given in Fig. 4 and Table 4. At the condition of high temperature and long photoperiod, such as 30°C under 16 hr daylength or 25°C under 16 hr daylength, most of females in both colonies had stage I follicles. The feeding activity of these females were very high. At 21°C under 10 hr daylength, follicles in all the females of Nagasaki colony were at follicular stage N. On the other hand, 33 % of females of Bangkok colony had stage I or more developed follicles under the same condition. In addition, about a half of females fed on blood meals when they were permitted to feed on a mouse overnight 7 to 14 days after emergence, and most of fed females made mature eggs (Table 5), without showing gonotrophic dissociation. Therefore, the females of Bangkok colony do not go into diapause.

Table 6 gives the number of ovarioles in one of the two ovaries in resulting females at 25°C under 16 hr daylength. Nagasaki colony had about 30 more eggs than Bangkok colony in all conditions. This result indicated that a female of Nagasaki colony may lay a larger number of eggs than Bangkok colony.

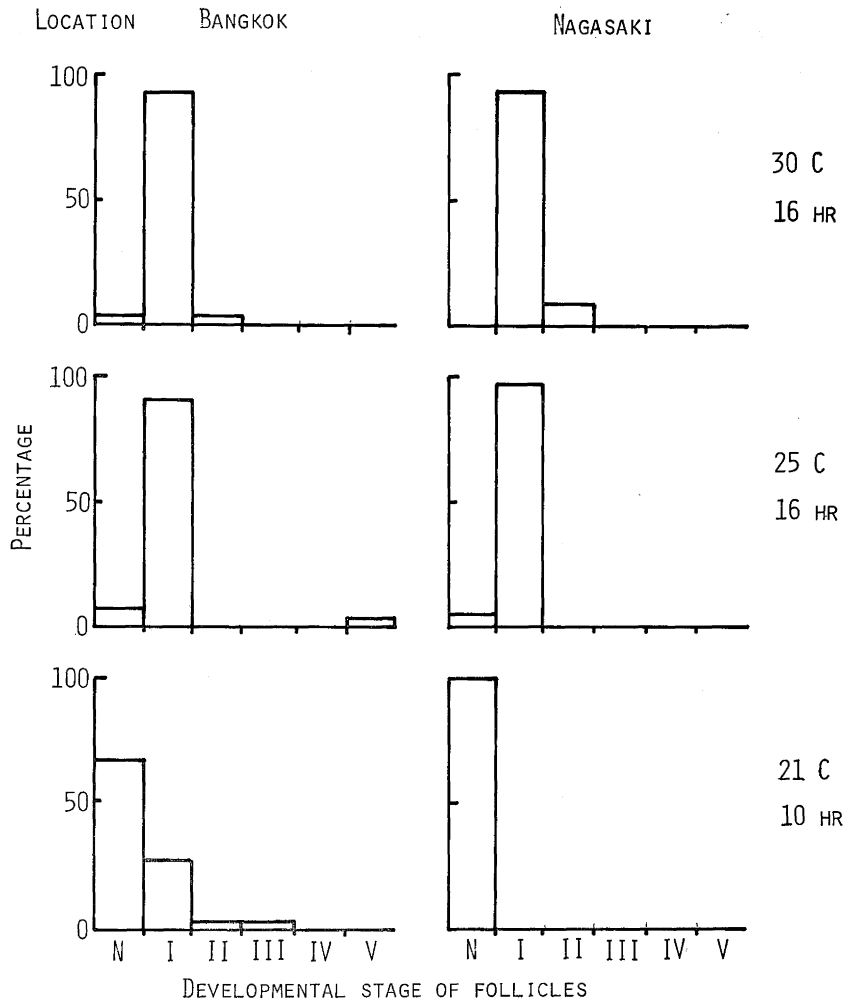


Fig. 4. Developmental stages of follicles 7 days after emergence in an ovary of *Culex tritaeniorhynchus* originated from Thailand and Japan reared at various temperatures and daylengths. The definition of the developmental stages of follicles is given in Kawai (1969).

Table 4. Size of follicles 7 days after emergence in an ovary of *Culex tritaeniorhynchus* originated from Bangkok, Thailand and from Nagasaki, Japan reared at various temperatures and photoperiods

| Location | Temperature (°C) | Photo- period (hr) | No. ♀ dissected | No. (%) ♀ with 1st follicles of the indicated size* | | | | | | | | | | | No. ♀ allowed to feed on a mouse | No. fed (%) |
|----------|---------------------|--------------------------|--------------------|---|-------------|--------------|--------------|--------------|-------------|------------|------------|-----|-----|------------|---|-------------------|
| | | | | 4- | 5- | 6- | 7- | 8- | 9- | 10- | 11- | 12- | 13- | 14- | | |
| Bangkok | 21 | 10 | 30 | 11 (36.7) | 9 (30.0) | 4 (13.3) | 3 (10.0) | 1 (3.3) | | | | | | 1 (3.3) | 60 | 31 (51.7) |
| Nagasaki | 21 | 10 | 26 | 17 (65.4) | 8 (30.8) | 1 (3.8) | | | | | | | | | 100 | 0 (0.0) |
| Bangkok | 25 | 16 | 30 | 1 (3.3) | 1 (3.3) | 11 (36.7) | 13 (43.3) | 3 (10.0) | | | | | | 1 (3.3) | | |
| Nagasaki | 25 | 16 | 29 | | | | 6 (20.7) | 12 (41.4) | 4 (13.8) | 1 (3.4) | | | | | | |
| Bangkok | 30 | 16 | 30 | 1 (3.3) | | | 9 (30.0) | 16 (53.3) | 2 (6.7) | 1 (3.3) | 1 (3.3) | | | | | |
| Nagasaki | 30 | 16 | 15 | | | | 3 (20.0) | 8 (53.3) | 3 (20.0) | 1 (6.7) | | | | | | |

* 1 unit: 10 μ

Table 5. Number of mature eggs 7 days after feeding on a mouse in gravid females of *Culex tritaeniorhynchus* of Bangkok colony which were reared at 21°C under 10 hr daylength

| No. egg* | No. | (%) | |
|-----------|-----|-------|--------|
| 0 | 4 | 13.8 | |
| 1 - 50 | 3 | 10.3 | } 86.2 |
| 51 - 100 | 9 | 31.0 | |
| 101 - 150 | 10 | 34.5 | |
| 151 - | 2 | 6.9 | |
| uncounted | 1 | 3.5 | |
| Total | 29 | 100.0 | |

Table 6. Number of ovarioles (mean \pm 95% confidence limit) in an ovary of *Culex tritaeniorhynchus* originated from Bangkok, Thailand and from Nagasaki, Japan reared at 25°C

| Location | Number of ovarioles |
|----------|---------------------|
| Bangkok | 76.8 \pm 4.6 |
| Nagasaki | 105.7 \pm 5.3 |

DISCUSSION

According to Wada *et al.* (1969), in Nagasaki *Cx. tritaeniorhynchus* moved about 1 km in one night on an average in the dispersal experiment, while Baily and Gould (1975) caught marked *Cx. tritaeniorhynchus* at 1800 m distance up from the releasing point in the same experiment in Chiang Mai, Thailand. These results do not seem to indicate a great difference in the flight range of *Cx. tritaeniorhynchus* between two regions, though the wing length and the whole body size of adults of Thailand are smaller than those of Nagasaki.

At low temperature under short photoperiod, most of *Cx. tritaeniorhynchus* females of Nagasaki colony stop their follicular development at stage N and the size of their follicles remained small. It is said that these mosquitoes are in diapause without developing their follicles to stage I, and do not feed on blood meal, unless they encounter the condition of high temperature and long photoperiod (Kawai, 1969). Females of Bangkok colony did not show diapause even when reared at low temperature under short photoperiod (21°C under 10 hr daylength); a half of females fed on blood meals 7 to 14 days after emergence. Mogi *et al.* (1971) showed that *Cx. tritaeniorhynchus* in Japan has the geographical variation in the condition to induce diapause. It may be natural that the mechanism for entering diapause is lacking in the females of Bangkok where there is no winter.

It is recognized that the geographical variation in some physiological and morphological characteristics exists in some mosquito species that are widely distributed from south to north. Examples are the depth of diapause of *Cx. pipiens* complex (Oda *et al.*, 1974), the condition inducing or awakening diapause of *Aedes triseriatus* (Kappus and Venard, 1967), *Ae. sierrensis* (Jordan and Bardshaw, 1978), *Cx. pipiens pipiens* (Vinogradova, 1960) and *Wyeomyia smithii* (Bradshaw, 1967; Bradshaw and Lounibos, 1977) and the ratio of DV/D in male genitalia of *Cx. pipiens pallens* (Bekku, 1956). In

Cx. tritaeniorhynchus, variations are found between females from Bangkok and Nagasaki not only in the physiology for the induction of diapause, but also in some characteristics of morphology and biology.

It is proved that the possibility of overwintering of JE virus in mosquitoes in Japan, if ever, extremely small (Oda *et al.*, 1978), and one of the remaining possibility is the introduction of JE virus from outside of Japan by mosquitoes (Hayashi *et al.*, 1978). If the laboratory colony is established from *Cx. tritaeniorhynchus* caught, for example, in the ocean and some morphological and physiological characteristics of the colony are observed, it might be possible to assume the original place from which they came, based on the results of the present experiment.

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タイ国と日本のコガタアカイエカの形質の変異

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現在、日本内地で日本脳炎ウイルスが蚊の体内で越冬する可能性はたいへん小さいことが証明されている。陸から遠く離れた洋上で蚊が採集されることから、コガタアカイエカが外国から日脳ウイルスを持ち込んでいるのではないかとの疑いがでている。しかし、これらのコガタアカイエカがどこから飛んでくるかを明らかにしなければ蚊によるウイルス持ち込みを立証することはできない。コガタアカイエカの産地による変異をそれぞれ明らかにしておけば洋上の蚊の産地の推定に役に立つ。そこで長崎、奄美大島、タイのチャンタブリで捕えたコガタアカイエカの形態の比較を行った。その結果、野外で採れた成虫は長崎のものが一年中最も大きく、ついで奄美大島が大きく、チャンタブリのものは気温が同じでも長崎のものより小さかった。次いで長崎とバンコクで得られたコガタアカイエカを同一条件で飼育すると、バンコクのものの方が幼虫・蛹期間は短かく、羽化成虫は小さかった。低温短日で飼育すると長崎産はすべて休眠し、濾胞は小さく吸血しないのに、バンコク産の一部のものは濾胞が大きくなり吸血し成熟卵を形成した。これらの結果は洋上で捕えた蚊から系統を作れば産地を推定することが可能であることを示している。

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