Trop. Med., 30 (2), 155−161, June, 1988 

# Studies on the Developing Period of Larval Stage of the *Culex pipiens* Complex in Japan

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**Abstract**: The developing period and the developmental zero were compared among the members of the *Culex pipiens* complex in Japan. The larval period of all members became shorter with raised water temperature in the range from 14 to 29℃. This period in northern strains of *Cx. p. pallens* tended to be longer than in southern ones, and *Cx. p. quinquefasciatus* needed the same developing period as *Cx. p. pallens* in southern Kyushu. However, the developing period and the developmental zero of *Cx. p. molestus* were apparently different from those of *Cx. p. pallens* or *Cx. p. quinquefasciatus*.

*Key words*: Developing period, Developmental zero, *Culex pipiens pallens, Culex pipiens quinquefasciatus, Culex pipiens molestus*

## INTRODUCTION

The mosquito of *Culex pipiens* complex in Japan consists of three members, *Culex pipiens quinquefasciatus, Culex pipiens pallens* and *Culex pipiens molestus. Cx. p. quinquefasciatus* is distributed in Ryukyu Islands and Ogasawara Islands and *Cx. p. pallens* and *Cx. p. molestus* in Kyushu main island and northward. It is hard to differentiate the members by external structures of larvae and adults alone. But some physiological and ecological characteristics are remarkably different among these members. The females of *Cx. p. quinquefasciatus* and *Cx. p. pallens* lay eggs only after taking blood meal, and the larvae are foundg in a very wide variety of artificial containers and other types of stagnant water such as ditches, gutters, ground pools, *etc.* On the other hand, *Cx. p. molestus* breeds most frequently in underground waterpools and occasionally in open water, and the female exhibits autogeny, the phenomenon of laying the first egg batch without taking blood meal (Sasa *et al.*, 1966; Tanaka *et al.*, 1979). *Cx. p. pallens* females exhibit the imaginal diapause, but *Cx. p. quinquefasciatus* and *Cx. p. molestus* females lack the mechanism of inducing diapause (Oda, 1977). At a high temperature of 30℃ the oviposition rate of

Received for Publication, March 29, 1988.
Contribution No. 316 from the Department of Medical Zoology, Nagasaki University School of Medicine.

autogenous females and the hatching rate of eggs of *Cx. p. molestus* were low, but in *Cx. p. quinquefasciatus* the rates of oviposition and egg-hatching were high at 30℃ (Oda *et al.*, 1980). In the present study, the developmental zero and the developing period in immature stages, which are known to be the limiting factors in the distribution of some insects, were compared among the members of the *Culex pipiens* complex in Japan. The results obtained in the present study are to provide with very useful information in discussing and understanding the distribution of the members of *Culex pipiens* complex in Japan.

## MATERIALS AND METHODS

One strain of *Cx p. quinquefasciatus*, four strains of *Cx. p. pallens* and one *Cx. p. molestus* strain were used in the present study. The histories of these strains are given in Table 1. Fig. 1 shows the originating sites of the strains in a map. All the strains had been kept in an insectary at temperature of 25℃ under daylength of 16 hr. The strains of *Cx. p. quinquefasciatus* and *Cx. p. pallens* had been maintained by feeding on blood meals from mice and the strain of *Cx. p. molestus* without blood.

Immature stages of each strain were reared from first instar larvae at water temperatures of 14, 18, 23 and 29℃ to determine the developing period. One hundred newly hatched larvae were put in an enamel tray (22×28×4㎝) with *ca.* 1,500 ml water. Equally mixed powder of Brewer's yeast and finely ground mouse pellet was served as the larval food. A water suspension of 0.2 g of this mixture was added to the rearing tray every day at 18℃ and higher temperatures until all larvae pupated. At 14℃, the same amount of the mixture was given every two days. To prevent the formation of sucm on the water surface, there was used an aeration system that flowed out small bubbles from the open end of polyvinyl tubule in the water. Pupae were transferred to a laboratory dish after recording their number each day.

Table 1. Strains of *Culex pipiens* complex used in the present study

| Strain | Collection site (latitude) | | Data of collection | Generation used in this study |
|---|---|---|---|---|
| *Cx. p. quinquefasciatus* | | | | |
| Okinawa | Naha City, Okinawa | (26°14′ N) | March 1980 | F2−F6 |
| *Cx. p. pallens* | | | | |
| Kagoshima | Kagoshima City, Kagoshima | (31°34′ N) | November 1977 | F30−F35 |
| Nagasaki | Aino, Nagasaki | (32°48′ N) | December 1979 | F4−F6 |
| Tokushima | Tokushima City, Tokushima | (34°04′ N) | March 1986 | F2 |
| Abashiri | Abashiri City, Hokkaido | (44°01′ N) | May 1976 | F50+$\alpha$ |
| *Cx. p. molestus* | | | | |
| Nagasaki | Nagasaki City, Nagasaki | (32°47′ N) | November 1979 | F6−F10 |

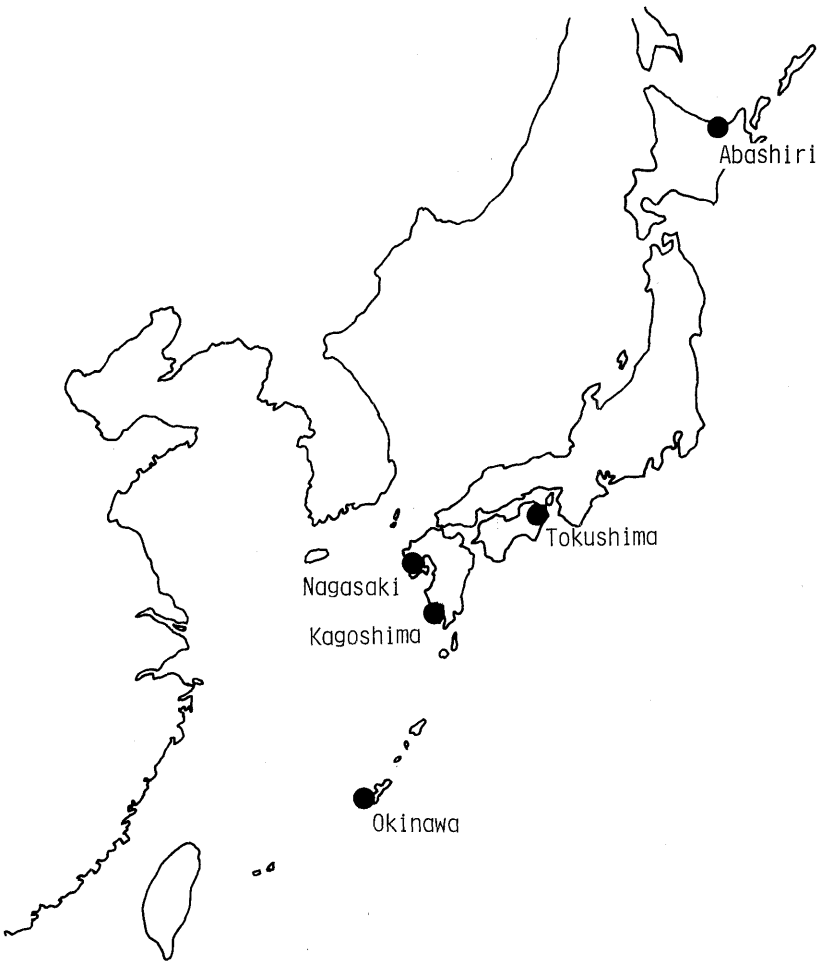


Fig.1. The map of sites where the strains of *Culex pipiens* complex were collected.

## RESULTS

Median larval periods of females and males in each strain are given in Table 2. In all strains, this periods became shorter with rising temperature in the range from 14 to 29℃. The developing period of *Cx. p. molestus* larvae originated from Nagasaki was longer at any temperature than that of *Cx. p. pallens* from any regions or *Cx. p. quinquefasciatus* from Okinawa. There was a tendency that larval period in *Cx. p. pallens* became longer in northern strains than in southern ones.

Figs. 2 and 3 show the relation between the rearing temperature and the reciprocal of median larval period. As the relation was clearly linear in these three members of the *Cx. pipiens* complex, the developmental zeros and thermal constants were calculated for laval periods (Table 3). The developmental zero of *Cx. p. molestus* was the highest among the members. The thermal constant in *Cx. p. molestus* was the largest, and the smallest in

*Cx. p. quinquefasciatus*. *Cx. p. pallens* in northern areas tended to have larger thermal constant than in southern areas.

Table 2. Median larval periods of *Culex pipiens quinquefasciatus*, *Culex pipiens pallens* and *Culex pipiens molestus* at various temperatures

| Temperature | Larval period (Days)*, ** | | | | | |
|---|---|---|---|---|---|---|
| | *Cx. p. quinquefasciatus* | *Cx. p. pallens* | | | | *Cx. p. molestus* |
| | Okinawa | Kagoshima | Nagasaki | Tokushima | Abashiri | Nagasaki |
| 14℃ | 18.3±0.8[a] | 17.8±0.5[ab] | 19.6±0.6[c] | 19.3±0.9[bc] | 20.4±0.4[c] | 24.9±0.8 |
| 18℃ | 9.6±0.2[a] | 10.7±0.1[b] | 10.9±0.2[b] | 9.8±0.2[a] | 12.2±0.2 | 15.6±0.5 |
| 23℃ | 7.3±0.3[a] | 6.4±0.2 | 7.2±0.2[a] | 7.2±0.3[a] | 7.4±0.2[a] | 9.5±0.1 |
| 29℃ | 4.8±0.3 | 5.2±0.1[a] | 5.2±0.2[ab] | 5.2±0.1[a] | 5.5±0.2[b] | 6.5±0.1 |

* Mean±S.D.

** Medians followded by the same letter within a holizontal line were not significantly different (P=0.05) according to Duncan's New Multiple Range Test.

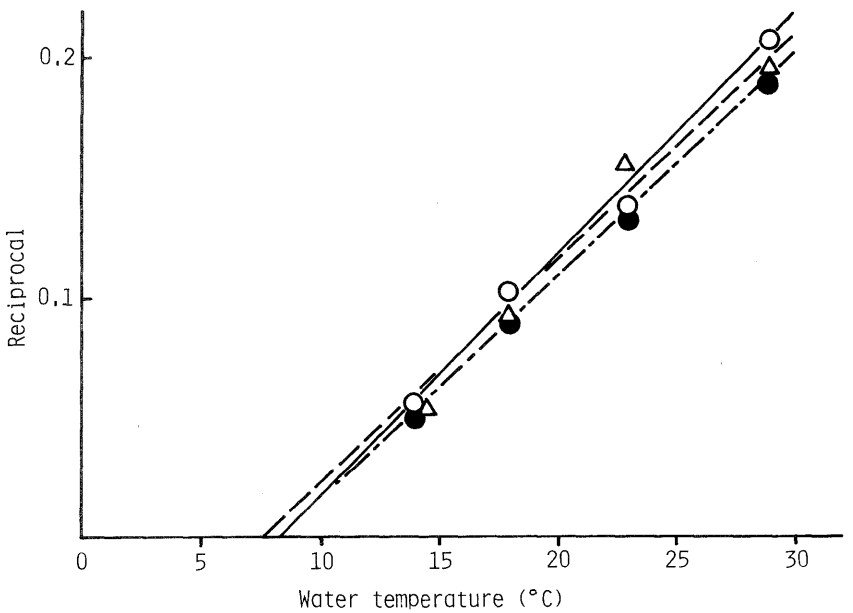


Fig.2. Relation between temperatures and reciprocals of larval periods in days of *Culex pipiens quinquefasciatus* from Okinawa ———○, and *Culex pipiens pallens* from Kagoshima – – – –△, and from Nagasaki ——·——●.

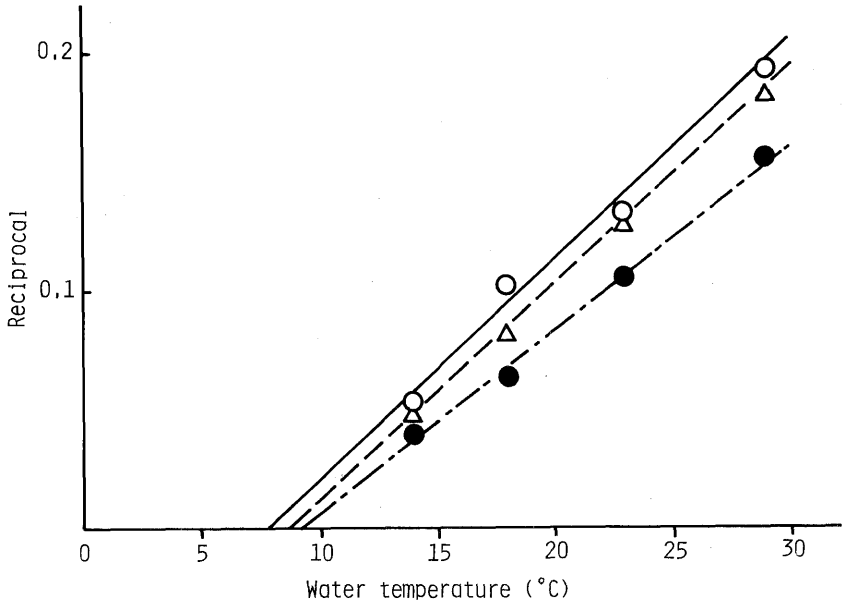


Fig.3. Relation between temperatures and reciprocals of larval periods in days of *Culex pipiens pallens* from Tokushima ———○, and from Abashiri – – – –△, and *Culex pipiens molestus* from Nagasaki —— · ——●.

Table 3. Theoretical calculations of developmental zero and thermal constant (K) of larval period of *Culex pipiens quinquefasciatus, Culex pipiens pallens* and *Culex pipiens molestus*

| Strain | Regreesion | ($r^2$) | Developmental zero (℃) | K (day-degrees) |
|---|---|---|---|---|
| *Cx. p. quinquefasciatus* | | | | |
| Okinawa | Y=0.00999X−0.08294 | (0.99384) | 8.3 | 100.8 |
| *Cx. p. pallens* | | | | |
| Kagoshima | Y=0.00938X−0.07212 | (0.99002) | 7.7 | 106.6 |
| Nagaski | Y=0.00929X−0.07698 | (0.99933) | 8.3 | 107.6 |
| Tokushima | Y=0.00920X−0.07107 | (0.99522) | 7.7 | 109.1 |
| Abashiri | Y=0.00898X−0.07643 | (0.99741) | 8.5 | 111.4 |
| *Cx. p. molestus* | | | | |
| Nagasaki | Y=0.00762X−0.06943 | (0.99841) | 9.1 | 131.2 |

## DISCUSSION

Hosoi (1945) observed the influence of temperatures ranging from 14 to 37 ℃ on the development of *Cx. p. pallens* in Shanghai, China, and stated that the surviving rate in immature stages became low with rising temperature. He also showed that the larvae of *Cx. p. pallens* could not develop at 5℃, and many larvae and pupae died during their development at 37℃. Kamura (1959) reported that the temperature between 16 and 24℃ was suitable for the development of immature *Cx. p. molestus*, but 27℃ was not so. Within a range of suitable temperature, the larval period was lengthened with falling temperature. The results of Hosoi (1945) and Kamura (1959) approximately conform to those of the present study.

The developmental zero is higher and the thermal constant is larger in *Cx. p. molestus* than in all the strains of *Cx. p. pallens* from any places. This implies that the duration of immature stages of *Cx. p. molestus* is genetically longer than that of *Cx. p. pallens.* It seems probable, as indicated by Spielman (1967), that *Cx. p. molestus* females need longer larval period to take the nutrition for egg-maturation without taking blood meals.

Kurihara (1963) reported that the larval period was influenced greatly with water temperature in *Cx. pipiens* s.l. in Amamioshima island, which was identified as *Cx. p. quinquefasciatus* by Tanaka *et al.* (1979). From the result in Kurihara (1963), the developmental zero of *Cx. p. quinquefasciatus* in Amamioshima island, which is the northern extremity of distribution of this mosquito, is calculated as 9.0℃. This is slightly higher than the relult in the present study. The discrepancy between these two experiments might be due to different quality of larval food.

The present study showed that the cline from north to south is present in thermal constant for the larval development of *Cx. p. pallens* in Japan. The larval period also varies geographically with cline north to south. Similar clines are found in morphological characters in this mosquito, such as the DV/D ratio of male genitalia, the cell-stem ratio at R−2 vein in female wing, the size of dark patches on abdominal sternites, and the shape of white basal bands on abdominal tergites (Hori, 1960; Yamaguchi, 1965). Furthermore, individuals of *Cx. p. pallens* and *Cx. p. quinquefasciatus* in southern Kyushu are difficult to be distinguished, because these biometrical values almost completely overlap in this area. It seems that these characteristics gradually change from *Cx. p. quinquefasciatus* to *Cx. p. pallens*. This is an interesting fact in studying the speciation of *Cx. p. quinquefasciatus* and *Cx. p. pallens*.

## ACKNOWLEDGMENTS

The authors wish to thank Professor Y. Wada, Department of Medical Entomology, Insitute of Tropical Medicine, Nagasaki University for his critical reading of the manuscript.

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日本産アカイエカ群の幼虫期間について

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アカイエカ群を構成している蚊の中で，日本に分布するアカイエカを網走，徳島，長崎，鹿児島から，ネッタイイエカを沖縄から，チカイエカを長崎から採集して実験室系統を作り，温度条件を変えて1令より飼育して発育期間を比較した．すべての系統において水温14～29℃の範囲では温度が高くなるに従って発育期間は短くなった．網走系のアカイエカは四国，九州産のものより発育期間が長かった．ネッタイイエカは四国、九州産のアカイエカと発育期間はほぼ同じであった．しかし，チカイエカの発育期間はアカイエカのいずれの系統よりもあきらかに長いことがわかった．推定された幼虫の発育零点はチカイエカがアカイエカやネッタイイエカに比べわずかに高かった．

熱帯医学　第30巻　第2号　155−161頁，1988年6月