Host-feeding patterns of Japanese mosquitoes II A host-blood identification study of daytime-resting *Culex pipiens pallens* in Kyoto, Japan

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Abstract: Blood meal sources of *Culex pipiens pallens* collected in the field during the daytime were serologically identified by the Ouchterlony method. Mosquito collections were made at two stations in two areas 4 km apart from each other in the suburbs of Kyoto in 1973 and 1974 by using a drop-net cage and sweeping. Blood removed from the engorged mosquito was tested against ten kinds of antisera. Eight different hosts identified were mammals to reptiles, *i. e.*, bird, dog, cat, cow, pig, mouse, snake and human, showing that the blood meal sources of this mosquito were not restricted to a particular host, but covered a variety of animals. Feedings on birds and dogs made up the greater part of the total identified at the two stations, but the ratio of bird-feeding: dog-feeding differed between the stations suggesting that the host selectivity by this mosquito might vary depending on the availability of host animals.

INTRODUCTION

Host-blood identification studies of *Culex pipiens pallens* are valuable in examining the natural cycles of Japanese encephalitis (JE) virus, since several isolations of JE virus have been made from this mosquito in some localities of Japan (Buescher *et al.*, 1959; Matsuyama *et al.*, 1960; Hayashi *et al.*, 1966).

Field studies of the host-feeding patterns of this mosquito showed two different results; in Japan this mosquito was observed to have fed mainly on birds (Toshioka, 1960; Takahashi *et al.*, 1971), while in China it fed chiefly on humans (Liu *et al.*, 1959; Lu *et al.*, 1959).

Laboratory experiments demonstrated that this mosquito could feed on a variety of hosts though there appeared to be a feeding preference among the host animals (Miyagi,

1972). Also in the field study, it was shown that the host range of this mosquito was comparatively wide (Karoji *et al.*, 1980).

Host-blood identification studies of field-caught engorged mosquitoes have revealed some differences in host preferences among mosquitoes. It has been suggested that the host-feeding pattern of a mosquito species is not always fixed as has previously been believed, but it is changeable due to the availability of hosts (Tempelis, 1975).

Accordingly, the present study was made using a gel diffusion method in order to clarify if host availability influences on the host-feeding patterns of *Cx. pipiens pallens*.

MATERIALS AND METHODS

Collection of mosquitoes

Collections of blood-fed mosquitoes were carried out at two stations (A and B), 4 km apart from each other, in the paddy areas of Kyoto City, Japan.

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Station A was on the edge of a swamp covered with weeds and shrubs, chiefly *Zizania latifolia*, *Polygonum Thunbergii*, *Salix* sp., and surrounded by paddy fields. Station B was on the edge of irrigation ditches densely covered with vegetation similar to that at Station A and surrounded by paddy fields.

Mosquito collections were made at Station A from May to September 1973, and at Stations A and B from April to October 1974. Samplings were performed once a week at each station. The daytime-resting mosquitoes in the vegetation at Station A were captured by using a drop-net cage (0.5 × 0.5 m at the base, 1.0 m high, the four sides covered with nylon gauze) during the two years. We could enclose the mosquitoes with the drop-net cage by placing the cage on the vegetation, and all the mosquitoes within the cage were captured by the battery-operated suction catcher. A sweep-net (42 cm in diameter) was also used at the two stations in 1974.

Blood-meal identification of engorged mosquitoes

Captured mosquitoes were anesthetized, stored in a chilled ice-box and brought to the laboratory. The blood-fed specimens were separated, counted and then dissected under a binocular microscope to remove the blood contained in the gut. The undigested blood was dissolved in 0.1 ml of 0.01 M phosphate-buffered saline pH 7.2. After frozen storage and subsequent thawing these samples were used as antigens in the Ouchterlony method (Ouchterlony, 1958).

The antisera used in the Ouchterlony method were prepared by immunizing rabbits against the sera or blood of ten kinds of animals, human, horse, cow, pig, dog, cat, mouse, chicken, snake (*Elaphe quadri-virgata*) and frog (*Rana breviped*). The immunization schedule was the same as that mentioned in the previous study (Karoji *et al.*, 1980). Titers and cross-reactivity were checked by the Ouchterlony method using the sera of homologous and heterologous species as antigens. The antisera titers were 1:2,000 or more except for Lot 1 of the pig antiserum (1:500). Though cross-reactions occurred between the dog antiserum and cat

serum, and *vice versa*, these could be eliminated by dilution (dog antiserum 5 times and cat antiserum 7 times). Antichicken serum was used as antibird serum since it was known to react broadly with the sera of various species of birds (Tempelis and Reeves, 1962).

The procedure of the Ouchterlony method used for the host-blood identification was the same as in a previous study (Karoji *et al.*, 1980).

Estimation of host selectivity in Cx. p. pallens

First, a comparison was made by the χ^2 -test between the feeding patterns of *Cx. p. pallens* and the frequency of domestic animals present in each area. The numbers of domestic animals distributed within 0.5, 1.0 and 1.5 km radii from each station were counted.

Next, the degree of host selectivity was estimated by calculating Ivlev's index of electivity (Ivlev, 1961) which was proposed as a measurement of the predator-prey relationships and is formulated as follows:

$$E_i = (r_i - p_i) / (r_i + p_i),$$

where r_i is the percentage of a certain kind of food in the digestive organ of a predator and p_i is that of the same kind of food present in the environment. In the present study, r_i is the percentage of a given host-blood in the guts of the mosquitoes collected and p_i is that of the same host present in the study area.

The value E_i can range from +1 to -1; zero signifies that the mosquitoes utilize a certain kind of host without particular preference. The closer to +1 the value, the higher the degree of preference for a particular host and the closer to -1, the higher the degree of rejection for that host.

In this procedure, the wildlife in each area was not taken into consideration as very few was present due to the heavy urbanization of the outer margins of the city. For instance, black-crowned night heron was absent from the study areas and only during the daytime could eastern carrion crows and plumed egrets be seen.

Seasonal change of Cx. p. pallens

Seasonal changes of *Cx. p. pallens* were observed during the two years at Station A.

The population density was estimated based on the daytime-resting females captured with the drop-net cage. Because the daytime-resting mosquitoes showed the contagious distribution at their resting sites (Karoji, 1980), the magnitude of error relative to mean density was estimated by the I_d -index method (Ono and Morisita, 1969), and it (ϵ) is expressed as follows:

First, I_d is calculated by the formula:

$$I_d = q \frac{\sum x_i (x_i - 1)}{T (T - 1)},$$

where q is the number of samples taken, x_i is the number of individuals occurred in the i -th unit ($i=1, 2, \dots, q$), and T is the total number of individuals ($T = \sum x_i$).

Then,

$$\epsilon = t \sqrt{\frac{1}{q-1} (I_d - 1) + \frac{1}{T}},$$

where t is "Student's t ."

RESULTS

Host range of *Cx. p. pallens* at Stations A and B

Yearly totals of the identified blood at the two stations are summarized in Table 1.

In total, 728 blood meals were examined at Station A in 1973, of which 725 were identified. These blood meals were from six hosts, bird, dog, cow, cat, mouse and snake; bird- and dog-feedings made up 97% of the total identified. Only one case of heterologous double feeding (dog+bird) was observed. Three samples did not react against

any of the antisera prepared.

In 1974, 539 blood meals were identified at Station A. The host range of this mosquito in 1974 was similar to that observed in the preceding year except that human blood was identified. Two cases of heterologous double feedings were observed, both of which were of dog+bird.

At Station B, 1,001 blood meals were tested, of which 984 were identified. Seven different host bloods, bird, dog, pig, cat, human, snake and cow were identified. The former two were most frequently identified as was observed at Station A. Feedings on a poikilothermic host (snake) were observed at this station, too. Four cases of heterologous double feedings were observed; one was from cat+bird and the others were all from dog+bird. Seventeen samples were unidentified.

In total at Stations A and B, it was shown that *Cx. p. pallens* fed on eight different hosts: bird, dog, cat, cow, pig, mouse, snake and human.

Host-feeding patterns of *Cx. p. pallens*

It appeared that the host-feeding pattern of *Cx. p. pallens* was related to the availability of domestic animals present in the study area, especially when the 0.5 km radius was considered (cf. Table 2). It was concluded from the χ^2 -test that the bird-feeding: dog-feeding ratio was not significantly different from the chicken: dog ratio in the study area. For example, the χ^2 -value calculated between the bird-feeding: dog-feeding ratio and the chicken: dog ratio within

Table 1 Host-feeding patterns of field-caught *Cx. p. pallens* at Stations A and B in 1973 and 1974

Station (Year)	No. and the percentage (in parentheses) of positives against									Unidenti- fied	Total
	Human	Cow	Pig	Dog	Cat	Mouse	Bird	Snake	Double hosts		
A (1973)	0 (0.0)	8 (1.1)	0 (0.0)	168 (23.2)	8 (1.1)	3 (0.4)	536 (73.9)	1 (0.1)	1* (0.1)	3	728
A (1974)	1 (0.2)	3 (0.6)	0 (0.0)	110 (20.4)	3 (0.6)	1 (0.2)	418 (77.6)	1 (0.2)	2* (0.4)	0	539
B (1974)	2 (0.2)	1 (0.1)	9 (0.9)	406 (41.3)	9 (0.9)	0 (0.0)	551 (56.0)	2 (0.2)	4** (0.4)	17	1,001

* Positive against dog and chicken antisera.

** One sample was positive against cat and chicken antisera, and the others were positive against dog and chicken antisera.

Table 2 Indices of electivity in *Cx. p. pallens* calculated for the radii of different distances from each station

Distance (Radius in km)	Ivlev's index of electivity				
	Chicken	Dog	Cat	Cow	Pig
Station A in 1973					
0.5	0.08(60)	0.13(17)	-0.87(15)	—	—
1.0	0.04(157)	0.18(37)	-0.85(31)	—	—
1.5	0.05(264)	0.23(57)	-0.78(36)	-0.77(34)	—
Station A in 1974					
0.5	0.09(60)	0.06(17)	-0.93(15)	—	—
1.0	0.06(157)	0.12(37)	-0.92(31)	—	—
1.5	0.05(304)	0.22(57)	-0.87(36)	-0.87(34)	—
Station B in 1974					
0.5	-0.01(52)	0.08(32)	-0.70(5)	—	—
1.0	-0.08(8,000)	0.95(130)	0.71(20)	—	-0.95(4,000)
1.5	-0.07(8,000)	0.90(280)	0.45(45)	-0.60(50)	-0.95(4,000)

Actual number of individuals are shown in parentheses.

—: absent.

Fig. 1 The seasonal changes in the population density of the daytime-resting females of *Cx. p. pallens* at Station A in 1973 and 1974

Mean density (open circle) and the magnitude of error relative to mean density calculated by the I_{δ} -index method (vertical thin line) are shown.

0.5 km radius from Station A in 1973 was 0.122 ($p > 0.70$), and the comparable χ^2 -values calculated at Station A and at Station B in 1974 were 0.063 ($p > 0.80$) and 0.594 ($p > 0.30$), respectively.

It was observed that the feeding pattern of this mosquito at Station A differed from that at Station B in 1974 ($\chi^2 = 69.959$, $p < 0.001$).

Secondly, Ivlev's indices of electivity were calculated and tabulated for 0.5, 1.0 and 1.5 km ranges from each station, respectively (Table 2).

The indices were comparatively close to zero in the cases of chicken and dog, except for those of dog within 1.0 and 1.5 km radii from Station B. In contrast, indices were generally close to -1 in the cases of cat, cow and pig.

Seasonal changes in Cx. p. pallens population

Seasonal changes in the female *Cx. p. pallens* population at Station A are shown for the two years (Fig. 1). It is apparent that a similar fluctuation pattern prevailed both in 1973 and 1974 though the population density was higher in 1973 than in 1974. Active populations of *Cx. p. pallens* were already present as early as May and attained the highest density in late-June.

DISCUSSION

In the past, *Cx. p. pallens* was believed to feed exclusively on birds in Japan (Sasa, 1955). When observed in the field, however, it was clear that this mosquito fed on several kinds of animals as well as birds (Liu *et al.*, 1959; Takahashi *et al.*, 1971; Karoji *et al.*, 1980). Toshioka (1960) stated that this mosquito preferred chickens but it also fed on other animals when chickens were not readily available.

The present study showed that the host range of this mosquito in the field was not restricted to birds but that it included eight different hosts ranging from mammals to reptiles. It was also demonstrated that the host-feeding patterns differed from those of preceding works. Our result might be interpreted as indicating that this mosquito fed on chickens and dogs, not because it had a particular preference for either of these

animals, but because they were readily available in the study areas. It was shown that the feeding patterns were different between the two areas and paralleled with the ratio of chickens to dogs in the areas. Therefore, the result could be interpreted as showing that *Cx. p. pallens* has flexible preferences and these are determined by the available hosts.

The problem how large a study area should be carried out for collecting unbiased samples of engorged mosquitoes for the host-blood identification study has already been discussed. The flight range of mosquitoes after taking blood-meals generally has been thought to be short (Nakao, 1959; Shemanchuk *et al.*, 1963), but others observed that it was surprisingly wide (Edman and Bidlingmayer, 1969). *Cx. p. pallens* appears to be able to move over 1 km after engorgement, as exemplified in the present study, since Station A where the cow-feedings were detected was about 1.3 km away from the hosts and similarly Station B about 1.2 km away. A more accurate survey should be made on the flight dispersal of mosquitoes after blood-feeding.

It is noteworthy that *Cx. p. pallens* is already active, despite low density, in May in Japan, and attains a high level of population density in late-June. The population trend in the fall was not followed in the present study, but *Cx. p. pallens* was observed to feed on its hosts and rest in the ripened rice fields even in mid-October (Karoji, unpublished data). Thus, the active period of this mosquito lasts from spring to fall as has been observed by other workers (Sasa *et al.*, 1951).

It could be considered that *Cx. p. pallens* does not play an important role in the pig-mosquito cycle of JE virus during the epidemic season since laboratory experiments have revealed that the susceptibility of this mosquito to JE virus was lower than that of *Cx. tritaeniorhynchus* (Hammon *et al.*, 1949; Doi *et al.*, 1977; Karoji *et al.*, 1977), and only occasionally has JE virus been isolated from field-caught *Cx. p. pallens* (Buescher *et al.*, 1959; Matsuyama *et al.*, 1960; Hayashi *et al.*, 1966).

Despite the facts mentioned above, the

role of *Cx. p. pallens* in the natural cycles of JE virus should not be ignored because the unknown pathways of this virus may involve this species which has a wide range of hosts in the field.

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摘 要

日本産蚊類の宿主吸血パターン II 京都市の水田地域におけるアカイエカの 吸血宿主

日中、野外で休止している吸血アカイエカを採集し、その吸血源をゲル内沈降反応の1種 Ouchterlony 法により判定した。吸血蚊の採集は、1973年と1974年に京都市南部の水田地域の、東西にほぼ4 km 離れた2カ所のステーションで、ドロップネット・ケージとスーピングにより行った。吸血蚊の吸血血液を、哺乳類から爬虫類にわたる10種の動物の抗血清を用いてテストした結果、トリ、イヌ、ネコ、ウシ、ブタ、ネズミ、ヘビ、ヒトの8種の吸血源が判定され、この蚊の吸血は特定の宿主に限定されているのではなく、広い宿主幅を持っていることが示された。いずれのステーションにおいても、トリ吸血とイヌ吸血が吸血例の大部分を占めていたが、両者の比率はステーション間で異なっており、この蚊の宿主選択が、その地域に分布している宿主動物の比率に依存している可能性を示していた。