Original Article

Horizontal and elevational distribution of *Culex pipiens* complex mosquitoes compared with *Aedes albopictus* in an inland mountain area, Nagano Prefecture, Japan

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Abstract: Nagano Prefecture, an inland mountain area of Japan, extends widely north and south, with elevation that varies greatly by location. Mosquitoes transmitting infectious disease have a diversity of habitats in Nagano, and many species can be expected there. However, there have been few reports about mosquito fauna in this region; in particular, little information is available on mosquitoes such as *Culex* (*Culex*) *pipiens* complex and *Aedes* (*Stegomyia*) *albopictus*. In this study, we investigated the mosquito fauna in 17 areas to clarify their horizontal and elevational distribution patterns between July and September from 2012 to 2014. We focused especially on *Cx. pipiens* complex compared with *Ae. albopictus*. A total of 1,117 mosquitoes were collected, and identified as belonging to 17 species. The most abundant species was *Cx. pipiens* complex, followed by *Ae. albopictus*. Both species were mainly collected in the southern and eastern parts of Nagano Prefecture, excluding highland areas. *Ae. albopictus* was collected at a maximum elevation of 789 m while *Cx. pipiens* complex was collected at a maximum elevation of 996 m. That means the habitat of *Cx. pipiens* complex was more widely distributed than that of *Ae. albopictus*. The annual mean air temperature of the site at 789 m was estimated to be 10.2°C and that of the site at 996 m was estimated to be 8.5°C. There was a difference of 207 m in elevational distribution between the two species, with a difference in annual mean air temperature of 1.7°C.

Key words: CDC trap, *Culex pipiens* complex, distribution, fauna of mosquitoes, larval collection, temple and shrine

INTRODUCTION

Culex (*Culex*) *pipiens pallens* Coquillett, *Cx. pipiens* form *molestus* Forskal, and *Aedes* (*Stegomyia*) *albopictus* (Skuse) are the most common mosquito species in urban as well as rural areas of Japan (Tsuda et al., 2006; Tsuda, 2011). These *Culex pipiens* mosquitoes are considered to be the most important potential vector of West Nile virus (Molaei et al., 2012; Turell, 2012), while *Ae. albopictus* is a major vector of dengue virus in Japan (Knudsen, 1995; Knudsen et al., 1996; Kobayashi et al., 2014). According to Hotta (1953), Japan experienced dengue outbreaks in several coastal cities with naval ports in 1942–1945. Although dengue outbreaks did not occur in Japan for 50 years after World War II, the possibility of a dengue outbreak exists because of the common distribution of the vector *Ae. albopictus* (Hotta, 1998). In fact, more than 160 people contracted dengue fever around

Yoyogi Park in Tokyo in 2014 (Kutsuna et al., 2015). Vector control is an essential measure for controlling the outbreak of viral disease (Guzman et al., 2010). Thus, collecting and sharing information about the distribution and ecology of vectors might be the most important and effective method of vector control (European Centre for Disease Prevention and Control, 2009). The elevational and horizontal distribution ranges of these mosquitoes are an important factor determining the risk area for transmission of vector-borne diseases. However, there have been no attempts to evaluate the elevational distribution of *Cx. pipiens* mosquitoes in Japan.

Nagano Prefecture in Japan comprises an inland mountain area that extends widely north and south, with an elevation that varies greatly by location. Therefore, the epidemiological structure of mosquito borne infectious diseases in Nagano is diverse, and many mosquito species can be expected there

(Kamimura, 1968; Uchikawa, 1977). However, there have been few reports on mosquito fauna; in particular, little information on mosquitoes such as *Cx. pipiens* complex is available. For *Ae. albopictus*, Kamimura (1968) reported a total of 1,065 (qualitative data) adult or larval mosquitoes collected in Nagano Prefecture in July–August 1964 and in August, September–October 1965. However, he did not describe the collection sites and densities, so we do not know which area *Ae. albopictus* inhabited or how many individual *Ae. albopictus* were collected at each site. From 1972 to 1974, Uchikawa (1977) investigated the mosquito fauna in Matsumoto and its environs using light traps and a survey of collected larvae from June to September, but no *Ae. albopictus* were collected during the collection period. Kurihara et al. (2000) reported many individual *Ae. albopictus* adults were collected from Nagano, Matsumoto and Ueda Cities. They also reported that the density of *Ae. albopictus* might have increased from ca. 1992/1993. On the other hand, Shirai et al. (2001) reported that no *Ae. albopictus* larvae were collected from Matsumoto and its environs in August of 2000 or July of 2001. They doubted the habitation of *Ae. albopictus* around Matsumoto and its environs in Nagano Prefecture.

Recently, however, Hirabayashi et al. (2020) reported that *Ae. albopictus* were widely distributed and collected at elevations <800 m in Nagano and Yamanashi Prefectures.

The distribution of *Ae. albopictus* is centered on the Tropical Zone. However, this species is a southern hemisphere species whose geographical distribution ranges widely from the Tropical Zone to the Temperate Zone, whereas *Cx. pipiens* complex is a northern hemisphere species with a very wide geographical distribution from the Frigid Zone to the Temperate Zone (Hawley, 1988; Mogi, 2012). In addition, it is known for its stronger larval cold tolerance. *Cx. pipiens* complex is better adapted to tolerate low water temperature than *Ae. albopictus* (Tsuda and Takagi, 2001; Mogi, 2012). Our hypothesis was that *Cx. pipiens* complex would be distributed at areas of higher elevation than *Ae. albopictus*. In the present study, we attempted to clarify the horizontal and elevational mosquito distribution patterns in 17 areas by investigating the mosquito fauna there, focusing especially on *Cx. pipiens* complex (*Culex pipiens pallens* and *Cx. pipiens form molestus*) as compared with *Ae. albopictus*, from the end of June to early September for three years from 2012 to 2014.

Fig. 1. Distribution of the 17 studied areas in Nagano Prefecture. Open circles indicate the two highland areas.

MATERIALS AND METHODS

Mosquito surveys were carried out from July to September 2012–2014 in 17 areas, including 11 cities, 3 towns, 5 villages, and 2 highlands, in Nagano Prefecture (Fig. 1). Both adults and larvae were collected at a total of 91 sampling sites, e.g., temples and shrines. The latitude ranged from 35°30′21.24″N to 36°55′28.23″N, longitude ranged from 137°48′46.32″E to 138°38′10.77″E, and elevation was 317–1,534 m, respectively.

For adult collections, Centers for Disease Control and Prevention (CDC) traps were set up at 72 sites (a total of 82 nights; in some cases, we collected mosquitoes at 1 site on 2 nights) in Nagano Prefecture. Table 1 lists the study areas, sampling sites, nights of collection, elevation, and environmental conditions (annual mean air temperature in each area over 10 years from 2005 to 2014) of the mosquito collection sites where CDC traps were used. CDC traps without a light bulb and baited with 1 kg dry ice were used and operated for 24 hrs. In addition, mosquitoes were also collected by sweeping a net around the CDC trap. Mosquitoes in the traps/nets were collected in tubes by aspirator and placed in a cooler and then transported to our laboratory where they were killed by low temperature (−10°C) in a freezer, and then identified morphologically to species or species group (Tanaka et al., 1979). *Cx. pipiens* complex included *Cx. pipiens pallens* and *Cx. pipiens* form *molestus*. Part of the data for *Ae. albopictus* in this paper are quoted from Hirabayashi et al. (2020).

Larval collection sites were selected in each sampling area. Larvae were collected at 54 sites (128 water samples) in Nagano Prefecture. Various kinds of containers, e.g., flower vases in graveyards of Buddhist temples, stone wash basins in shrines, used tires stacked in backyards or along roadsides, used cans or plastic containers, and bamboo stumps, were examined for larvae, which were removed using a glass pipette and then transferred to small polypropylene bottles (ca. 100 mL volume). They were transported to our laboratory and maintained at 25±2°C. Larvae were transferred to a rearing plastic bottle (ca. 9 cm diameter by 10 cm deep) and reared to the adult stage. Emerged adults were killed by low temperature (−10°C) in a freezer and then identified to species as described above.

RESULTS AND DISCUSSION

Table 2 shows the list of mosquito species in Nagano Prefecture during the investigation periods. A total of 1,117 mosquitoes were collected. 685 mosquitoes were collected in CDC traps and 22 mosquitoes were collected in sweeping nets; 410 mosquitoes emerged in the laboratory. They were identified as 17 species belonging to 2 subfamilies, i.e., 2 species of Anopheline

and 15 species of Culicinae. The most abundant genus was *Aedes* (7 species), followed by *Culex* (6 species). The mosquito fauna in this study was dominated by two species, *Cx. pipiens* complex (*pallens* or *molestus*; 419 individuals) and *Ae. albopictus* (345 individuals). Kamimura (1968) reported 19 mosquito species from Nagano Prefecture, and 13 of them were found in this study. Twelve species of mosquitoes were described by Uchikawa (1977) using light traps and larval collection in the central part of Nagano Prefecture (Matsumoto City, Akashina Town, Hotaka Town and Hakuba Village). We were able to capture almost all the species described by Uchikawa (1977). The only one we could not capture in this study was *Lutzia (Metaluzia) vorax*.

Aedes (Stegomyia) galloisi and *Tripteroides (Tripteroides) bambusa* were recorded for the first time in Nagano Prefecture (Table 2). The larvae of the former were collected from a stone water basin at Keinenji Temple in Okaya City in the Suwa and Okaya area, and the latter were collected by CDC trap and larval collection from flower pots etc. in the Suwa and Okaya, Ina and Minamiminowa, and Iida areas.

The data from the CDC traps showed a total of 685 individual adult mosquitoes were collected during the investigation periods, including 398 *Cx. pipiens* complex (58.1%) and 139 *Ae. albopictus* (20.3%, Table 1). Although mosquitoes were not collected in the Sugadaira highland area or the Kamikochi highland area, they were captured in the other 15 areas with an average of 8.4 individuals/night in Nagano Prefecture. A large number of mosquitos were collected from the Ina and Minamiminowa area, with a total of 175 individuals and an average of 43.8 individuals/night/CDC trap.

Although *Cx. pipiens* complex was not collected in the Nozawaonsen area, Omachi area or either of the highland areas. It was captured in the other 13 areas from the northern to the southern part of Nagano Prefecture. A large number of *Cx. pipiens* complex were collected from the Ina and Minamiminowa area, with an average of 40.5 individuals/night/CDC trap and a total of 162 individuals (4 nights). This was followed by the Komagane and Miyata area with an average of 11.8 individuals/night/CDC trap and a total of 47 individuals (4 nights), and the Karuizawa area with an average of 10.6 individuals/night/CDC trap and a total of 85 individuals (8 nights). *Cx. pipiens* complex were mainly collected in the southern and eastern parts of Nagano Prefecture (Fig. 2).

Meanwhile, *Ae. albopictus* was captured in 13 areas from the northern to the southern parts of Nagano Prefecture. The largest number of *Ae. albopictus* (an average) were collected from the Ueda area, with an average of 14.0 individuals/night/CDC trap and a total of 14 individuals (1 night), followed by the Iida area with an average of 7.0 individuals/night/CDC trap and a total of 49 individuals (7 nights), and the Kijimadaira

Table 1. Location and environmental conditions of *Culex pipiens* complex and *Aedes albopictus* collection sites using CDC traps in Nagano Prefecture, Japan.

Study area	Sampling site, collecting nights	Elevation (m)*	10 years annual mean air temperature ($\pm$ S.D.)°C**	Total No.	Average
				of mosquitoes	(Ind./night by CDC)
				(Cx. pipiens com., Ae. albopictus***)	
Nozawaonsen	3 sites (2 shrines, 1 temple), 3 nights	576	10.0 ($\pm$ 0.5)	4 (0, 0)	1.3 (0.0, 0.0)
Iiyama	4 sites (4 temples), 4 nights	313	11.2 ($\pm$ 0.4)	31 (4, 11)	7.8 (1.0, 2.8)
Kijimadaira	2 sites (2 temples), 2 nights	none	none	28 (6, 12)	14.0 (3.0, 6.0)
Shinanomachi	3 sites (2 shrines, 1 temple), 3 nights	685	9.4 ($\pm$ 0.2)	8 (1, 7)	2.7 (0.3, 2.3)
Nagano	2 sites (2 temples), 2 nights	418	12.2 ($\pm$ 0.3)	19 (2, 11)	9.5 (1.0, 5.5)
Ueda	1 site (1 other), 1 night	502	12.0 ($\pm$ 0.3)	16 (1, 14)	16.0 (1.0, 14.0)
Tomi and Sanada	7 sites (2 shrines, 5 temples), 7 nights	958	9.3 ($\pm$ 0.3)	43 (29, 2)	6.1 (4.1, 0.3)
Sugadaira	1 site (1 shrine), 1 night	1,253	6.5 ($\pm$ 0.3)	0 (0, 0)	0.0 (0.0, 0.0)
Karuizawa	2 sites (1 temple, 1 other), 8 nights	999	8.6 ($\pm$ 0.3)	88 (85, 0)	11.0 (10.6, 0.0)
Hakuba	4 sites (2 shrines, 2 temples), 4 nights	703	9.6 ($\pm$ 0.3)	9 (3, 1)	2.3 (0.8, 0.3)
Omachi	8 sites (3 shrines, 5 temples), 8 nights	784	9.5 ($\pm$ 0.3)	16 (0, 2)	2.0 (0.0, 0.3)
Kamikochi	1 site (1 other), 1 night	1,534	5.4 ($\pm$ 0.4)	0 (0, 0)	0.0 (0.0, 0.0)
Matsumoto	7 sites (4 shrines, 3 temples), 7 nights	610	12.1 ($\pm$ 0.2)	32 (10, 8)	4.6 (1.4, 1.1)
Suwa and Okaya	12 sites (5 shrines, 7 temples), 16 nights	760	11.3 ($\pm$ 0.2)	88 (30, 3)	5.5 (1.9, 0.2)
Ina and Minamiminowa	4 sites (2 shrines, 2 temples), 4 nights	633	11.8 ($\pm$ 0.5)	175 (162, 10)	43.8 (40.5, 2.5)
Komagane and Miyata	4 sites (1shrine, 3 temples), 4 nights	none	none	56 (47, 9)	14.0 (11.8, 2.3)
Iida	7 sites (1shrine, 6 temples), 7 nights	516	13.0 ($\pm$ 0.3)	72 (18, 49)	10.3 (2.6, 7.0)
Total	72 sites (25 shrines, 44 temples and 3 others), 82 nights			685 (398, 139)	8.4 (4.9, 1.7)

*Elevation of the meteorological station in the study area, **annual mean air temperature at each area during 10 years (2005–2014), ***data source from Hirabayashi et al. (2020)

Table 2. List of mosquito species collected by larval collections and adult sampling (CDC traps and sweeping net) in Nagano Prefecture.

Scientific name	Capturing number				Kamimura (1968)	Uchikawa (1977)	Hirabayashi (2020)	Comments
	Adult No. by CDC trap	Adult No. by sweeping net	Larval No.	Total No.				
Anophelinae								
<i>Anopheles (Anopheles) hyrcanus</i> complex					○			
<i>Anopheles (Anopheles) lindesayi japonicus</i>	0	0	5	5	○		○	
<i>Anopheles (Anopheles) sinensis</i>	3	0	0	3		○	○	
<i>Anopheles (Anopheles) sineroides</i>					○			
Culicinae								
<i>Aedes (Aedimorphus) vexans nipponii</i>	0	0	15	15	○			
<i>Ae. (Colllessius) hatori</i>					○			
<i>Ae. (Downsiomyia) nipponicus</i>	1	0	0	1	○		○	
<i>Ae. (Hulecoeteomyia) japonicus</i>	0	0	92	92	○		○	
<i>Ae. (Stegomyia) albopictus*</i>	139	21	185	345	○			
<i>Ae. (Stegomyia) flavopictus</i>	24	1	46	71	○			
<i>Ae. (Stegomyia) albopictus</i> or <i>Aedes (Stegomyia) flavopictus</i>	0	0	3	3				First record in Nagano Prefecture
<i>Ae. (Stegomyia) galloisi</i>	0	0	3	3				
<i>Aedes</i> sp.	7	0	8	15	○			
<i>Armigeres (Armigeres) subalbatus</i>	14	0	0	14	○			
<i>Culex (Culex) orientalis</i>	8	0	3	11	○		○	
<i>Cx. (Culex) pipiens</i> complex (<i>pallens</i> or <i>molestus</i>)	398	0	21	419	○		○	
<i>Cx. (Culex) tritaeniorhynchus</i>	61	0	1	62	○		○	
<i>Cx. (Culicomyia) kyotoensis</i>					○			
<i>Cx. (Eumelanomyia) hayashii</i>	2	0	0	2	○			
<i>Cx. (Neoculex) rubens</i>					○			
<i>Cx. (Oculeomyia) bitaeniorhynchus</i>	18	0	0	18	○			
<i>Cx. (Oculeomyia) sinensis</i>	1	0	0	1	○			
<i>Lutzia (Metalutzia) vorax</i>					○			
<i>Tripteroides (Tripteroides) bambusa</i>	5	0	2	7				First record in Nagano Prefecture
unknown	4	0	26	30				
Total capturing number	685	22	410	1,117	19 species	12 species	6 species	

2 subfamilies, 5 genus and 17 species (*Anopheles*: 2 species, *Aedes*: 7 species, *Culex*: 6 species, *Tripteroides*: 1 species, *Armigeres*: 1 species)

*Data source from Hirabayashi et al. (2020)

Fig. 2. Individual number of *Culex pipiens* complex by CDC trap/night in Nagano Prefecture.

Fig. 3. Individual number of *Aedes albopictus* by CDC trap/night in Nagano Prefecture. Data source: Hirabayashi et al. (2020).

area with an average of 6.0 individuals/night/CDC trap and a total of 12 individuals 2 nights). *Ae. albopictus* were also mainly collected in the eastern and southern parts of Nagano Prefecture (Fig. 3).

Figure 4 shows the relationship between the number of individual *Cx. pipiens* complex (symbol X) and *Ae. albopictus* (closed circle) by CDC trap/night and the elevation of the CDC trap site. At investigation sites with elevations of more than 1,000 m (only 2 sites), no adult *Cx. pipiens* complex were captured. Only one individual *Cx. pipiens* complex was collected at a residence (996 m asl.). This was in the Karuizawa area, and was the maximum elevation at which *Cx. pipiens* complex was collected in this study. Eighty-four individual *Cx. pipiens* complex were collected at Seishyouji Temple (964 m asl.) in the Karuizawa area. On the other hand, at investigation sites with elevations of more than 790 m (10 sites), no adult *Ae. albopictus* were captured. One individual adult *Ae. albopictus* was collected at Syoukouji Temple (789 m asl.) in the Suwa and Okaya area, which was the maximum elevation at which the mosquito species was collected in this study. Thus, *Ae. albopictus* was collected at a maximum elevation of 789 m asl. while *Cx. pipiens* complex was collected at a maximum elevation of 996 m asl. According to Hirabayashi et al. (2020), a strong negative correlation was found

Fig. 4. Relationship between numbers of *Culex pipiens* complex and *Aedes albopictus** collected by CDC trap/night and elevation of the trap site. *Data source: Hirabayashi et al. (2020).

between the elevation of the meteorological station in this study area ($y = -123.8x + 2048.1$) and the annual mean air temperatures at each area over 10 years (x) ($r^2 = 0.84$) in this area. The mean air temperature of the site at 789 m was estimated to be 10.2°C and that of the site at 996 m was estimated to be 8.5°C. There was a difference in elevation of 207 m in vertical distribution between the two species, and a difference of 1.7°C in annual mean air temperature. According

to Kobayashi et al. (2002), there is a strong correlation between *Ae. albopictus* mosquito-infested areas and annual mean air temperature above 11°C. In our study, *Ae. albopictus* was not collected in mountainous areas, with an elevation of above 790 m. It was captured at elevations of 789 m and lower where annual mean air temperature was 10.2°C. Thus, our results showed a slightly lower annual mean air temperature than in Kobayashi et al. (2002).

This paper looks at the elevational distribution of *Cx. pipiens* complex with the altitude and annual mean air temperature. However, for the elevational distribution of this species, the distribution of residential areas might greatly influence their distribution. This is because there is human sucking blood directivity in *Cx. pipiens* complex (Mogi, 2012) and their larval habitat is human-made puddles such as catch basins, foam polystyrene boxes and buckets for water reservoirs, washbasins, and so on (Tsuda et al., 2006). Further follow-up field investigations will be necessary to collect more adults using a combination of several sampling methods, and may well further improve our understanding of the mosquito fauna in Nagano Prefecture.

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