Distribution and Ecology of Potential Vector Mosquitoes of West Nile Fever in Japan

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Abstract

West Nile virus (WNV) became established in New York in 1999, in the first outbreak in the Western Hemisphere. WNV is propagated in many species of birds and has been detected in more than 300 bird species in the United States, although there are great differences in the viremia level among the various bird species. *Culex* mosquitoes, including *Cx. restuans,* the *Cx. pipiens* complex and *Cx. tarsalis* are important vectors of WNV in the USA. Laboratory transmission of WNV by *Cx. pipiens* form *molestus* showed that they were highly competent vectors of WNV, with infection and dissemination rates of 96% and 81%, respectively. Three species, including two of the *Culex pipiens* group (*Cx. pipiens pallens* (as *pallens* in this article) and *Cx. pipiens* form *molestus* (as *molestus* in this article)) and *Aedes albopictus,* account for more than 99% of collected mosquitoes surrounding Tokyo. The *Cx. pipiens* complex (*pallens*, *molestus* and *Cx. quinquefasciatus*) prefer both human and avian hosts, and there is no clear differences in avian blood-feeding patterns between *pallens* and *molestus*, but there are significant differences among mammalian species, with *molestus* preferring human blood compared with *pallens*. Ducks (mallards & spotbill ducks) and tree sparrows are major blood sources for the *Cx. pipiens* group in Japan, which are moderate or high amplifiers of WNV. From a larval bioassay with several kinds of insecticides it was demonstrated that etofenprox, a synthetic pyrethroid, exhibited relatively low effectiveness against several mosquito colonies of the *Cx. pipiens* group in an urban environment of Japan. The most important larval habitats of the *Cx. pipiens* group and *Ae. albopictus* are catch basins along public roads and this kind of structure should be targeted to control mosquito larvae.

Key words: blood-feeding habit, distribution, insecticide resistance, larval habitat, MIROC K1 model, WNV-vector mosquito

1. Introduction

The term “arthropod-borne virus” (arbovirus) has no taxonomic significance, but is rather an ecological term used to define viruses that require a blood-sucking arthropod for transmission between hosts. There are currently more than 530 viruses registered in the International Catalogue of Arboviruses, and most of the viruses listed in this catalogue are zoonoses or viruses infecting vertebrate animals. WNV was isolated in 1937 in a Ugandan woman who survived the infection (Smithburn *et al*., 1940; Hayes, 2001), it has recently emerged as a major public concern in Europe with the occurrence of an epidemic, with reports of meningoencephalitis and encephalitis cases in urban areas since 1996 (Platonov, 2001; Campbell *et al*., 2001). WNV became established in New York in 1999, in the first outbreak in the Western Hemisphere (Anderson *et al*., 1999). WNV is maintained in nature by many bird species, and the virus has been detected in more than 300 bird species in the United States, although there are great differences in the viremia level among bird species. WNV has also been detected in 62 mosquito species, including genera of *Aedes, Anopheles, Coquillettidai, Culex, Culiseta, Denocerites, Mansonia, Orthopodomyia, Psorophora* and *Uranotaenia* in the USA. During the transmission season, 63 avian species, representing 30 families and 14 orders, have tested positive for WNV in New York State. The highest infection rate of dead birds for WNV was 67% (n=907) observed in American crows at the epicenter (Bernard *et al*., 2001). Detection of WNV from 9,954 mosquito pools was conducted and the minimum infection rate per 1,000 mosquitoes (MIR) was found to be highest at 3.53 for *Cx. pipiens* group at the epicenter for the entire season and 7.49 for the peak week in August. On Staten Island an MIR of 11.42 was recorded in the *Cx. pipiens* group (Bernard *et al*., 2001). *Culex* mosquitoes, including *Cx. restuans, Cx. salinarius,* *Cx. tarsalis* are also important vectors of WNV in the northwestern and northeastern areas of the USA (Bernard *et al*, 2001). In the USA there are three members of the *Cx. pipiens* complex, specifically *Cx. pipiens pipiens*, *Cx. quinquefasciatus* and *Cx. pipiens* form *molestus* (*molestus* in the USA). Experimental transmission of WNV showed that they were highly competent laboratory vectors of WNV, with infection and dissemination rates of 96% and 81%, respectively (Turell *et al*., 2006). In field surveys for WNV detection and/or isolation, correct morphological identification is difficult among the *Cx. pipiens* complex. In Japan about 110 mosquito species have been recorded and some species of the *Cx. pipiens* complex are common, such as, *pallens*, along with *molestus* and *Cx. quinquefasciatus* (Tanaka *et al*., 1979). It is crucial to understand which mosquito species are potential vectors for WNV and are common in urban environments of Japan, which are densely populated. In this review we describe the present status of the mosquito fauna, their population densities in urban areas, the host feeding patterns of mosquitoes which seem to be potential WNV vectors and occurrence of insecticide resistance in Japan.

2. Mosquito Fauna in Urban Areas Surrounding Tokyo and Rural Areas Surrounding Narita International Airport in Chiba Prefecture

Mosquitoes were collected using dry-ice traps (CDC type) in urban areas surrounding Tokyo, Japan in 2003 and 2004. The collection sites beside houses were located in areas with high population densities. A battery-operated trap with 1 kg of dry-ice was operated weekly for 24 hrs. A total of six mosquito species were collected from 18 collection sites, and three species, including the *Cx. pipiens* group (*pallens* and *molestus*, Fig. 1A) and *Ae. albopictus* (Fig. 1B), accounted for more than 99% of the collected mosquitoes (Table 1). The average number of female mosquitoes /trap/day for the *Cx. pipiens* group and *Ae. albopictus* was 7.4 and 1.8 in 2003 and 9.4 and 2.0 in 2004 (Tsuda *et al.*, 2006a), respectively. There were large variations in the number of collected mosquitoes among the collection sites. This variation may be related to the environmental conditions at collection sites, particularly to the number of larval habitats around collection sites and the presence of adequate resting places for adult mosquitoes around houses. Adult mosquitoes of *Ae. albopictus* were collected from May to October, while those of the *Cx. pipiens* group were found from April to December. There are two members of the *Cx. pipiens* group (*pallens* and *molestus*) in the urban areas surrounding Tokyo, but identification of each form is complicated and time-consuming. To distinguish the females of two forms there is a morphological method involving comparison of the number of ommatidia of some parts of the compound eyes of the mosquitoes. These countings clearly showed that the *Cx. pipiens* group mosquitoes contained two forms consisting of *pallens* and *molestus* (Tsuda *et al*., 2006 a).

There have been frequent instances of insects of public health importance being introduced from one country to another. In 1930 *Anopheles gambiae*, a major vector of malaria in Africa, was probably introduced into Brazil from Senegal by a French naval vessel (Gratz *et al*., 2000). Several reports regarding insects carried by aircraft have been published in Japan (Ogata *et al*., 1974; Takahashi, 1984; Haseyama *et al*., 2007). The survey by Ogata *et al*. (1974) was conducted by checking inside international aircraft just after their arrival at Tokyo International Airport (Haneda) from 1972 to 1973. Of 42 aircraft surveyed, ten species of pest insects were captured in 24 aircraft. House flies (*Musca domestica*) and *Cx. quinquefasciatus* were collected most abundantly, 59 and 24 individuals, respectively. Engorged female *Ae. aegypti* and *An. subpictus* were also caught. The important mosquito species were carried mainly by flights via Bangkok, Hong Kong or Manila (Ogata *et al*., 1974). There are big differences in the structures of airport terminals and passenger boarding bridges among reconstructed international airports, but accidental introduction of mosquitoes, flies, cockroaches and other medically important insects from international aircraft is not very rare in international airports.

Adult mosquitoes were collected in adjacent and surrounding areas of Narita International Airport, Chiba Prefecture in 2003 and 2004 (Fig. 2) and the samples were tested for WNV by RT-PCR to check for accidental introduction of WNV in areas near the international air-

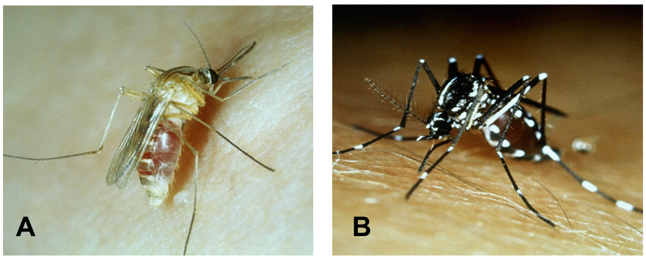

Fig. 1 Typical urban mosquito species in Japan.
A. *Culex pipiens pallens* B. *Aedes albopictus*

Table 1 Mosquito species collected in urban areas surrounding Tokyo, Japan in 2003 and 2004. (from Tsuda *et al*. 2006a)

Species	Female		Male	
	2003	2004	2003	2004
Aedes albopictus	614	908	182	355
Armigeres subalbatus	0	1	0	0
Culex bitaeniorhynchus	2	1	0	0
Cx. orientalis	1	0	0	0
Cx. pipiens group	2,582	4,225	21	35
Cx. sasai	0	0	0	1
Cx. tritaeniorhynchus	2	0	0	0
Cx. (*Culiciomyia*) sp.	1	0	1	0
Ochlerotatus japonicus	1	0	0	0
Total	3,203	5,135	204	391

port. Collection sites of mosquitoes adjacent to Narita International Airport and 8 or 14 km from the airport, were selected for the possibility of release of hidden mosquitoes from the wheel bays of airplanes. Russell (1987) reported that *Cx. quinquefasciatus* had a survival rate averaging 84% in the wheel bays of a Boeing 747B aircraft on several commercial airways including Melbourne-Singapore and Bangkok-Singapore. The minimum external temperature during the flights was −54ºC at 11,890 m above sea level, but the minimum temperature within the wheel bays did not fall below +8ºC and remained as high as +25ºC. These results indicated that disinsection of aircraft wheel bays should be considered to control hidden mosquitoes and other medically important insects (Russell, 1987). During the two-year survey of the area around Narita International Airport a total of 5,882 females of 16 mosquito species in seven genera were collected and all pools were negative for WNV (Tsuda *et al*, 2006b). There are significant differences in species composition of mosquitoes between rural areas of Chiba Prefecture and urban areas surrounding Tokyo. Major species of mosquitoes collected by dry-ice traps at three survey sites in Chiba Prefecture consisted of the *Cx. pipiens* complex, *Ae. albopictus, Ae. flavopictus, Cx. tritaeniorhynchus* and *Uranotaenia novobscura* (Table 2). Our data clearly showed that there were significant differences in the numbers of mosquitoes collected at different survey sites. It is important to know that the species composition of mosquitos in urban environments was extremely simple. There are big differences in the natural environment, climate and socio-economical situation between cities in urban areas and villages or towns in rural areas. If WNV is introduced into Japan, mosquito control, particularly larval control, will have to start quickly. Fundamental data related to species composition, population density of larval and adult mosquitoes and breeding sites will be essential to the controlling of mosquitoes in a variety of areas where the activity of WNV is confirmed.

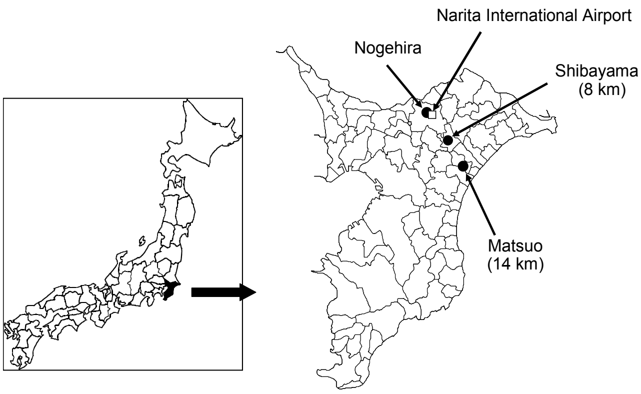

Fig. 2 Collection sites around Narita International Airport in Chiba prefecture. (redrawn from Tsuda *et al*., 2006b)

Table 2 Results of mosquito collection by dry-ice trap conducted in three survey areas around Narita International Airport, Chiba Prefecture, Japan, from July to September 2003. (from Tsuda *et al*., 2006 b)

	Species	Study area			Total
		Nogehira	Matsuo	Shibayama	
Females	*Culex pipiens* group*	178	130	18	326
	Aedes albopictus	139	96	0	235
	Ae. flavopictus	46	34	0	80
	Uranotaenia novobscura	11	18	0	29
	Armigeres subalbatus	6	17	2	25
	Anopheles sp.	8	5	3	16
	Cx. tritaeniorhynchus	12	2	1	15
	Tripteroides bambusa	3	3	0	6
	Ochlerotatus japonicus	2	3	0	5
	Ae. nipponicus	0	4	0	4
	Cx. bitaeniorhynchus	3	0	0	3
	Orthopodomyia anopheloides	0	2	0	2
	Ae. vexans nipponii	1	0	0	1
	Female total	409	314	24	747
Males	*Ae. albopictus*	4	1	0	5
	Ae. flavopictus	0	5	0	5
	Tp. bambusa	2	1	0	3
	Male total	6	7	0	13
	Total	415	321	24	760

* Includes *Cx. pipiens pallens* and form *molestus*.

3. Host Feeding Patterns of Several Mosquito Species Collected in Japan

The WNV virus has become firmly established in the Western Hemisphere since it was discovered in the New York City area in 1999 (Anderson *et al*., 1999). The virus's activity has spread at an unprecedented rate throughout the continental United States and to Canada and Mexico, where it is maintained in an enzootic cycle that involves wild birds and ornithophilic mosquitoes (Komar *et al*., 2003). More than 60 mosquito species have been found to be infected with WNV in North America, and certain *Culex* mosquitoes appear to be the primary vectors (White *et al*., 2001; Nasci *et al*., 2001). To evaluate the role of *Culex* mosquitoes, the hosts of blood-meals were identified by PCR amplification of a portion of the cytochrome b gene of the mitochondrial DNA and sequencing of the PCR products (Molaei *et al*., 2006). All *Cx. restuans* and 93% of *Cx. pipiens* acquired blood from avian hosts; *Cx. salinarius* fed frequently on both mammals (53%) and birds (36%). In this analysis mixed-blood meals were also detected in 11% and 4% of *Cx. salinarius* and *Cx. pipiens,* respectively. Human-derived blood meals were identified from two *Cx. salinarius* and one *Cx. pipiens*. From these results, *Cx. salinarius* was found to be an important bridge vector to humans, while *Cx. pipiens* and *Cx. restuans* were found to be more efficient enzootic vectors in the northeastern United States (Molaei *et al*., 2006). Recent analysis of host-feeding patterns of *Culex* mosquitoes shows that a shift in host choice from birds to mammals, including humans, as robins begin fall migratory movements, seems to be responsible for the seasonal rise in human cases of WNV (Kilpatrick *et al*., 2006). Host-feeding patterns of 21 mosquito species from New York and New Jersey have been checked by DNA sequencing of the cytochrome b gene. *Ae. vexans, Ochlerotatus japonicus* and *Oc. trivitattus* fed exclusively or almost exclusively on mammals, but five species, two of the *Cx. pipiens* complex (*Culex pipiens pipiens* and *molestus* in the USA), *Cx. restuans, Cx. salinarius* and

Culiseta melanura took a blood meal from both birds and mammals. A total of 24 different avian host species were detected among the avian-derived blood meals, with blood meals from the American crow relatively rare (Apperson *et al.*, 2004). Although the activity of WNV was not detected in field-collected mosquitoes in our survey during 2003-2006 in Japan, it is also important to know which species of mosquitoes may become bridge vectors in the urban environment of Japan. Blood-feeding patterns of *Cx. pipiens* group (*pallens* and *molestus*) were separately analyzed by DNA sequencing of the cytochrome b gene. *Ae. albopictus*, the common *Aedes* mosquito in urban environments of Japan was also analyzed. *Cx. pipiens* group preferred both human and avian hosts, and no clear differences between the two members were found in avian species composition, but there were significant differences in mammalian species composition (Fig. 3). *Molestus* preferred human blood compared with *pallens*. Among avian species, ducks (mallards and spotbill ducks) and tree sparrows were major blood sources for the *Cx. pipiens* complex in Japan (Table 3). More than 60% of *Ae. albopictus*, a common daytime biter, took a blood meal from humans and 11% from wild birds (Sawabe & Kobayashi, 2004; Sawabe *et al.*, 2008) (Fig. 3). From these results it is concluded that two members of the *Cx. pipiens* group and *Ae. albopictus* may be important bridge vectors for WNV in urban areas of Japan.

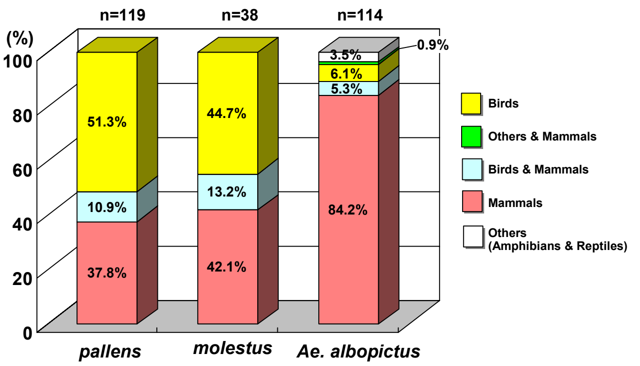

Fig. 3 Blood-feeding habits of three urban mosquitoes, *Culex pipiens pallens*, *Culex pipiens* form *molestus* and *Aedes albopictus.* (drawn from data in Sawabe *et al.*, 2008)

Table 3 Identification of blood sources of three urban mosquitoes in Japan. (from Sawabe *et al.*, 2008)

Species of Blood sources	*pallens* (n=119)	*molestus* (n=38)	*Ae. albopictus* (n=114)
Birds	75	22	13
Ducks	35 (29.4%)	12 (31.6%)	10 (8.8%)
Tree sparrows	30 (25.2%)	7 (18.4%)	
Crows	2 (1.7%)		
Others	6 (5.0%)	1 (2.6%)	2 (1.8%)
Unidentified	2 (1.7%)	2 (5.3%)	1 (0.9%)
Mammals	59	21	104
Humans	24 (20.2%)	17 (44.7%)	75 (65.8%)
Dogs	11 (9.2%)	1 (2.6%)	
Cats	10 (8.4%)	1 (2.6%)	9 (7.9%)
Others	7 (5.9%)		25 (21.9%)
Unidentified	7 (5.9%)	2 (5.3%)	
Others (Amphibians & Reptiles)			5 (4.3%)

4. Insecticide Resistance of *Culex pipiens pallens* and *Culex pipiens* form *molestus* in Japan

If WNV is introduced to a country, control of the vector mosquitoes will be one of the most important countermeasures for preventing transmission of the virus from birds to humans, and successful control of mosquitoes at present heavily relies on the application of insecticides. As mentioned above, the *Cx. pipiens* group is the most important of the potential vector mosquitoes for WNV in Japan. Despite almost identical morphology, these two forms (*pallens* and *molestus*) have some differences in physiological and ecological characteristics. *Pallens* inhabits areas above ground while *molestus* inhabits mostly septic tanks below ground in or around buildings. Therefore, *molestus* tends to be the target of indoor control by insecticides. Since the temperatures of these habitats are suitable in all seasons, *molestus* does not diapause in winter. Sources of blood are probably sparse in such closed dark habitats; however, *molestus* can lay its first egg raft without a blood meal. It has been clarified that *molestus* willingly goes up above ground after its first oviposition (Oda & Fujita, 1986). Using a PCR-based method to identify the *Cx. pipiens* group (Kasai *et al.*, 2008), we also confirmed that this mosquito species breeds in the stagnant water of catch basins above ground in summer. It was clarified that WNV inoculated into the thorax of *molestus* propagated to ca 10^6 PFU / mosquito 14 days after inoculation (unpublished data). Therefore, at present we cannot exclude this species from the list of potential vector mosquitoes of WN fever in Japan.

In order to confirm whether mosquitocides registered in Japan are still effective against these mosquitoes, we investigated the insecticide susceptibility of the *Cx. pipiens* group (Kasai *et al.*, 2007). Overall 37 colonies of *pallens* and 17 colonies of *molestus* were collected in urban environments in 2003 and 2004, and larval bioassays were carried out for five insecticides including a pyrethroid-like insecticide (etofenprox), two organophosphates (fenitrothion and temefos), a chitin synthesis inhibitor (diflubenzuron) and a juvenile hormone mimic (pyriproxyfen). As a result, almost all insecticides demonstrated high effectiveness against both mosquito species except etofenprox, which exhibited relatively low effectiveness against several mosquito colonies (Fig. 4). Twenty-two colonies exhibited survival rates of >10% at an etofenprox concentration of 5.7 ppm which was 100 times higher than the LC_{99} concentration, concentration required to kill 99% of the test population in a susceptible strain. Given that the total number of colonies was 54, overall 41% of the colonies contained resistant larvae. It is noteworthy that three colonies of *molestus* exhibited a 100% survival rate at 5.7 ppm. Low susceptibility of *molestus* to etofenprox may be due

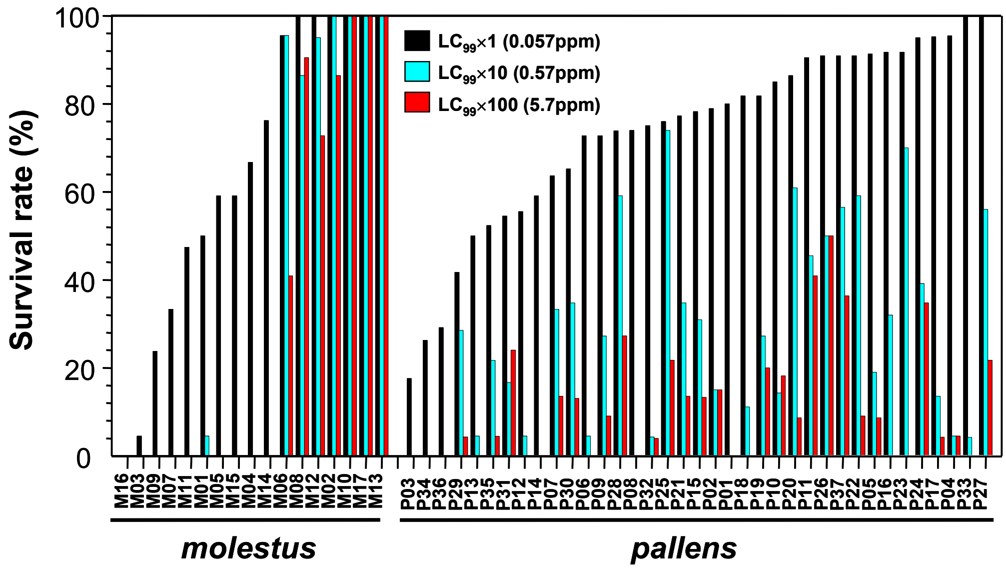

Fig. 4 Percentage of survival of *Culex pipiens* form *molestus* and *Culex pipiens pallens* collected from Japan exposed to pyriproxyfen at three diagnostic concentrations that were calculated according to their toxicities against a susceptible strain (LC_{99}, $LC_{99}\times10$, $LC_{99}\times100$).
(redrawn from Kasai *et al*., 2007)

to the high likelihood of this species to have contact with pyrethroids in underground habitats of buildings, like commercial facilities. Pyrethroid insecticides are popular as adulticides in Japan and it is suspected that frequent and long-term usage of these insecticides results in the development of insecticide resistance of this mosquito species to pyrethroids. Pyriproxyfen, a promising larvicide which is becoming popular in Japan as an IGR (insect growth regulator), exhibited enough effectiveness against most of the colonies of *Cx. pipiens* group at present; however, some colonies already exhibited reduced susceptibilities compared to the standard susceptible strain (Kasai *et al*., 2007). The long history of insecticide usage teaches us that, due to continued use, no insecticide can escape the issue of resistance. The number of registered mosquitocides is limited in Japan. Development of new mosquitocides or diversion of agricultural insecticides to mosquitocides, which are registered as insecticides for controlling medically important insects, would be strongly desirable in the case of an outbreak of WN fever in Japan.

5. Larval Habitats of the *Culex pipiens* group and *Aedes albopictus* and Increasing Size of the Areas of *Ae. albopictus* Distribution in Japan

Mosquito species exploit a wide variety of aquatic habitats for larval development. *Molestus* and *pallens*, which are common *Culex* mosquitoes in urban environments of Japan, can tolerate highly polluted aquatic environments, such as septic tanks and catch basins containing high concentrations of organic matter. Recently, water supply and drainage systems of urban areas in Japan have been widely re-constructed, polluted water from residential houses does not drain into the gutters of public roads. From our preliminary surveillance of larval mosquito habitats in urban environments, *Cx. pipiens* group mosquitoes, were collected mainly from catch basins along public roads (unpublished data). Larval habitats of *Ae. albopictus* in forested areas of the countryside are fundamentally rotted holes in trees, but many kinds of man-made containers such as cans, bottles, used tires, plastic containers, vases on graves and special types of basins for incense sticks at Japanese temples are exploited. Because catch basins contain mainly rainwater, it has been clearly shown that the larvae of *Ae. albopictus* breed in catch basins along public roads and by public facilities and detached houses (unpublished data). From these results, it is clear that catch basins are the main larval habitat in urban environments of Japan. It has also been shown that the target of larval control should be addressed to catch basins in the case of an accidental outbreak of mosquito-borne diseases, such as West Nile, dengue and chikungunya fever, and even in times of ordinary conditions without an outbreak.

Since insects are cold-blooded animals, they are influenced by environmental changes. Recently the IPCC (Intergovernmental Panel on Climate Change) reported that green house gas emissions have already changed our climate. High temperatures may increase the survival rates of vector mosquitoes during winter in temperate zones and change their behavior and ecology. Recent increases in mean annual temperature related to global warming in Japan have accelerated the expansion of the areas of mosquito distribution, such as *Ae. albopictus* in the Tohoku District (Kobayashi *et al*., 2002). Using the MIROC K1 model (Model for Interdisciplinary Research On

Climate K-1), model developers clearly showed that the northern limit of *Ae. albopictus* will move north, particularly into the northern Honshu District of Japan by 2035 and into southern and middle Hokkaido Island by 2100 (Kobayashi *et al.*, 2008). The spreading of vector mosquitoes by world trade in used tires has occurred in recent decades into Africa, the Mideast, Europe and North and South America causing an outbreak of chikungunya fever in northern Italy in 2007 (Rezza *et al.*, 2007). These results alert us to the importance of continuous surveillance of vector mosquitoes, particularly in the temperate zone countries.

6. Conclusion

There are significant differences in species composition of mosquitoes between rural areas of Chiba Prefecture and urban areas surrounding Tokyo. During the two-year survey surrounding Narita International Airport 16 mosquito species were collected, while three species, the *Culex pipiens* group and *Ae. albopictus*, accounted for more than 99% of collected mosquitoes in areas surrounding Tokyo.

Some mosquito species in urban environments of Japan are potential vectors of WNV, dengue and chikungunya fever. The present status of mosquito fauna, population density in urban areas and blood-feeding habits of female *Culex* mosquitoes which are competent vectors of WNV are essential data for construction of a mosquito control strategy. Development of insecticide resistance in the *Cx. pipiens* group was also recognized in urban environments of Japan. The most important larval habitats of these urban mosquitoes were catch basins along public roads so this kind of structure should be modified to control mosquito larvae.

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