Discovery, Distribution, and Abundance of the Newly Introduced Mosquito *Ochlerotatus japonicus* (Diptera: Culicidae) in Connecticut, USA

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ABSTRACT The earliest documented specimen of an exotic east Asian mosquito *Ochlerotatus* (*Finlaya*) *japonicus japonicus* (Theobald) in the Western Hemisphere is reported along with the results of a state wide survey to determine the distribution and abundance of this mosquito in Connecticut. *Ochlerotatus japonicus* was collected from 87 locations in eight counties. It is established throughout the state and occurs in a variety of natural and artificial container habitats including discarded tire casings, bird baths, wooden barrels, porcelain bath tubs (used for watering animals), plastic milk cartons, toys, vinyl tarpaulins (covering wood piles and swimming pools), exposed rock holes in stream beds, tree holes, subterranean catch basins, surface water rain pools, and spring-fed depressions. Larvae were particularly common in containers with water, decaying leaves, and algae, in shaded and sunlit areas and, in rock-pool habitats along streambeds, in association with *Ochlerotatus atropalpus* (Coquillett). Adult females were collected in sod grass-infused gravid and CO_2-baited light traps, from early June through October, with peak collections in September. Biting females were collected by human bait method augmented with CO_2, verifying its capacity to feed on humans. The ovitraps used in this study were not effective for recovering this species. Our results suggest that *Oc. japonicus* was introduced into Connecticut between 1992 and 1998. Because of the ability of *Oc. japonicus* to transmit West Nile virus, and because of the recent detection of this virus in field-collected specimens, the introduction of *Oc. japonicus* is considered a significant public health development.

KEY WORDS *Ochlerotatus japonicus*, mosquito, first record, distribution, abundance, Connecticut

IN AUGUST AND September of 1998, four adult females of the exotic East Asian mosquito *Oclerotatus* (formally genus *Aedes*) (*Finlaya*) *japonicus japonicus* (Theobald) were captured in light traps from two locations on eastern Long Island (Suffolk County), NY, and one locale in the Pine Barrens (Ocean County) of northern New Jersey (Peyton et al. 1999). This was the first reported occurrence of *Oc. japonicus* in the United States. Following this discovery, Peyton et al. (1999) conducted a survey of container habitats in the New Jersey collection site, but was unable to find *Oc. japonicus* larvae.

Ocherotatus japonicus is common throughout Japan (Hokkaido, Honshu, Shikkoku and Kyushu) and is also known from Korea (Korean Peninsula and Cheju Do) (Tanaka et al. 1979). Larvae occur in a variety of natural and artificial containers, including used tire casings, concrete barrels, stone vessels, bamboo stumps and tree holes (Sato et al. 1980, Sota et al. 1994, Tsuda et al. 1994, Sunahara and Mogi 1997), but rock holes are the favored habitat (Tanaka et al. 1979). Adults principally inhabit forested areas, are day biters, and are reluctant to feed on man (Tanaka et al. 1979), although Iriarte et al. (1991) reported *Oc. japonicus* to be abundant near human dwellings in urban areas. Females readily feed on chickens and mice in the laboratory (Miyagi 1972) but host feeding preferences in the wild are unknown.

Ochlerotatus japonicus was intercepted in New Zealand along with the Asian tiger mosquito, *Aedes* (*Stegomyia*) *albopictus* (Skuse) in used tire shipments from Japan in the early 1990s (Laird et al. 1994). Peyton et al. (1999) suggested the mode of introduction of *Oc. japonicus* into the northeastern United States was in used tires. The abundance, distribution, and actual time of establishment of this mosquito is unknown, however.

In the summer of 1999, we initiated a survey of artificial and natural container habitats in Connecticut for *Oc. japonicus* to ascertain its establishment in the state. We also inspected archival mosquito collections for evidence of the earlier occurrence of this species.

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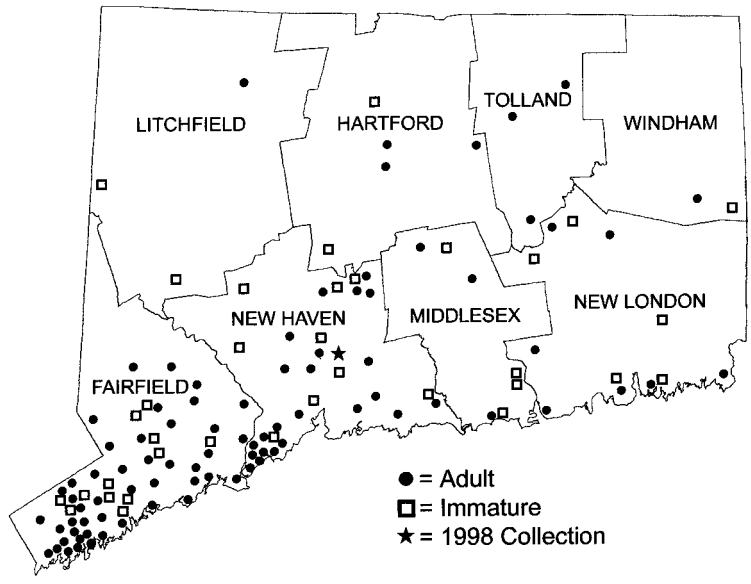

Fig. 1. Collection records for *Ochlerotatus japonicus* in Connecticut 1999–2000.

Statewide surveillance was expanded in the following year using oviposition traps, human bait collections and gravid and CO_2-baited light traps. Season long studies compared the relative abundance of immature *Oc. japonicus* with resident species at one rock hole and one tire dump site. The results of these investigations and earlier records of *Oc. japonicus* retrieved from archival mosquito collections are reported.

Materials and Methods

Larval Surveys. Surveys for immature *Oc. japonicus* were conducted statewide in eight counties. The search focused on used tire disposal sites and natural rock hole habitats in stream beds in 1999, but was expanded in 2000 to include catch basins, tree holes and artificial containers found in urban, suburban, rural and agricultural settings. Surveys were made from June to October with some sites sampled once, but other sites sampled repeatedly throughout the season. Mosquitoes were collected by removing the water from containers with a plastic cup or, for catch basins, using a long-handled 300-ml dipper. Mosquito larvae were sorted, counted and identified in the laboratory, using the descriptions/keys of Darsie and Ward (1981), Means (1979, 1987) and Peyton et al. (1999). Early instar larvae that could not be identified were reared to the fourth instar or allowed to emerge and identified as adults. All mosquitoes were identified to the species level. Larval specimens were deposited in the collections of T.G.A. and the Division of Entomology, Yale Peabody Museum of Natural History, New Haven, CT.

Larval Population Studies. Studies of seasonal abundance of immature *Oc. japonicus* were conducted at one tire dump in North Haven (New Haven County) and one natural rock hole site on the Housatonic River in Kent (Litchfield County) from July to November 1999. Every 1–2 wk, at each site, an average of 50 tire casings scattered throughout the dump, and 15 rock holes along the streambed were sampled. Collections were made from locations in direct sunlight and shaded by trees and vegetation. Mosquitoes were sorted, counted and identified to species as described above.

Adult Surveys. Surveys for adult *Oc. japonicus* were carried out in conjunction with a state wide arbovirus surveillance program for eastern equine encephalitis and West Nile (WN) virus (Anderson et al. 1999, Andreadis et al. 1999) from June through October in 1999 and 2000. In 1999, trapping was conducted using CO_2 (dry ice)-baited CDC light traps and was expanded in 2000 to include sod grass-infused CDC gravid mosquito traps (Reiter, 1983, Lampman and Novak 1996). Traps were placed at 37 permanent and 80 supplemental locations in 1999, and 73 permanent (same 37 plus 35 new) and 75 supplemental locations in 2000. At the permanent trap sites, collections were made once every 10 d for the entire season; trapping frequency at the supplemental sites was irregular. Traps were placed in or near permanent wetlands, freshwater swamps, waterways, parks, golf courses and undeveloped wood lots. Traps were set in each of the eight counties but were concentrated in the southwestern sector of the state (Fairfield and New Haven counties) (Fig. 1). Typically, traps were placed in the field during the late afternoon and the contents were retrieved the following morning; the average length of operation was 18 h. Adult mosquitoes were transported to the laboratory, sorted on chill tables, and identified using the descriptions/keys of Darsie and Ward (1981), Means (1979, 1987) and Peyton et al.

(1999), and by comparison with Theobald's (1901) original description and figure of *Oc. japonicus.* The numbers and species of female mosquitoes were recorded for each trap.

Human bait collections were undertaken in mid-July to assess biting activity of female *Oc. japonicus* at two locations; a forest in a sparsely populated rural setting (Winsted, Litchfield County) and a wood lot in a densely populated suburban setting (Stamford, Fairfield County). Two-person teams were stationed in the field between 1000 and 1400 hours. Carbon dioxide was used as additional attractant. Female mosquitoes that landed on the skin were captured with a hand held battery-powered aspirator, transported to the laboratory, and identified to species as described above.

Ovitrap Study. Collections of *Oc. japonicus* eggs in ovitraps were made between June and October 1999. Collection sites included the 37 permanent light-trap surveillance sites noted above and one used tire recycling plant. Each ovitrap comprised a 900 ml black plastic cup filled with 500 ml of tap water to which 10 ml of an aqueous suspension containing 1.5% of a 3:2 mixture of liver powder and brewer's yeast was added. Cups were lined with brown seed germination paper as an oviposition substrate. Ovitraps were attached to trees at 2 m above ground level and inspected every 2 wk at which time the contents of the cup were removed and brought to the laboratory for examination. At the same time, traps were replenished with fresh water, infusion and oviposition paper. Larvae in ovitrap samples were identified to species. In addition, each oviposition paper was inspected for eggs and then inundated with 2-d-old (to dissipate Chlorine) tap water to stimulate egg hatch. Resulting larvae were reared in 100 by 80-ml glass culture dishes and identified to species as fourth instars.

Results

Archival Search. Two adult female *Oc. japonicus* were identified in an archival search of specimens collected by LEM from suburban Hamden, CT (41° 23' 31" N, 71° 53' 49" W), in 1998. The first specimen was collected 17 July 1998, by UV light attraction at dusk, approximately 2130 hours, and aspirated from a white reflecting linen sheet. The second was attracted to human bait in late afternoon at 1730 hours, on 25 October 1998. The 17 July mosquito represents the earliest documented specimen of *Oc. japonicus* in the Western Hemisphere. Both were deposited in the collections of the Division of Entomology, Yale Peabody Museum of Natural History, New Haven, CT.

Larval Surveys. *Ochlerotatus japonicus* larvae were found in 35 locations in seven counties during the 2-yr survey (Fig. 1). Collection records are summarized in Table 1. Larvae were found in eight of nine waste tire disposal sites in Hartford, Middlesex, New Haven, New London, and Windham counties and were common in tires with decaying leaves and algae.

Sampling at the tire disposal site in North Haven revealed the presence of *Oc. japonicus* larvae between late July and mid-October (Fig. 2). *Ochlerotatus japonicus* ranked fourth in overall abundance (9.4%). Similar results were obtained at the tire disposal site in Essex where *Oc. japonicus* larvae were found between early June and August.

Ochlerotatus japonicus larvae were detected from other artificial containers sampled in urban, suburban and rural habitats (Table 1). Other mosquitoes collected concurrently included *Ochlerotatus triseriatus*

Table 1. Occurrence of immature *Ochlerotatus japonicus* in container and groundwater habitats in Connecticut

Habitat	No. sites	Dates collected	Associated species
Artificial containers[a]	10	3/24–9/22	*An. punctipennis*, *Cx. pipiens*, *Cx. restuans*, *Oc. triseriatus*
Used tire casings	8	6/21–10/22	*An. punctipennis*, *Cx. pipiens*, *Cx. restuans*, *Cx. salinarius*, *Cs. melanura*, *Oc. atropalpus*, *Oc. triseriatus*, *Tx. rutilus*
Catch basins	8	6/26–10/6	*Cx. pipiens*, *Cx. restuans*
Surface water pools	2	8/16–9/19	*An. punctipennis*, *Cx. pipiens*, *Cx. restuans*
Rock hole	1	5/24–11/10	*Ae. vexans*, *An. punctipennis*, *Cx. restuans*, *Cx. territans*, *Oc. atropalpus*
Tree hole	1	10/2	None

[a] Bird bath (cast iron and concrete), porcelain bath tub, vinyl tarpaulin, wooden tub, and plastic and metal containers.

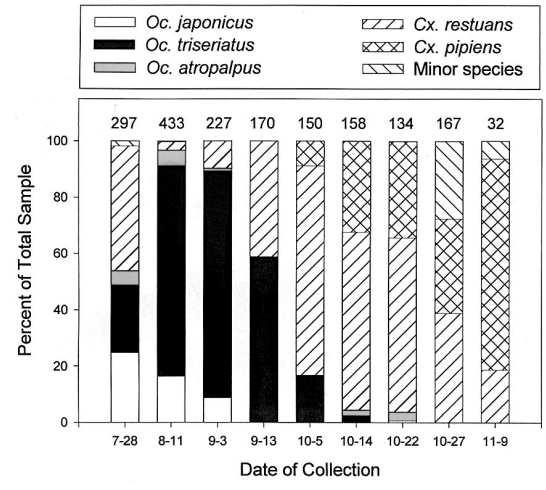

Fig. 2. Comparative abundance of *Ochlerotatus japonicus* and other immature mosquito species collected from used tire casings in a tire dump along the Quinnipiac River in North Haven, CT, 1999. Numbers on top of bars indicate sample size.

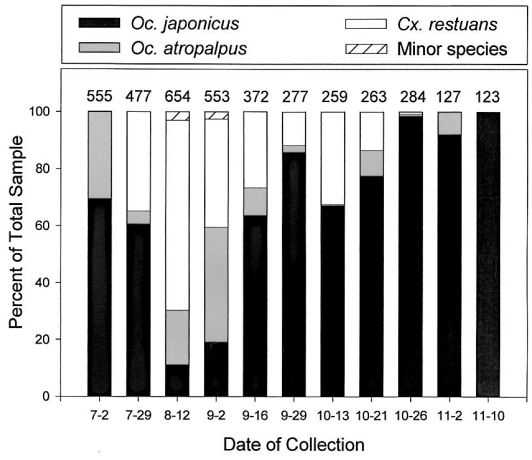

Fig. 3. Comparative abundance of *Ochlerotatus japonicus* and other immature mosquito species collected from water-holding rock holes along the Housatonic River in Kent, CT, 1999. Numbers on top of bars indicate sample size.

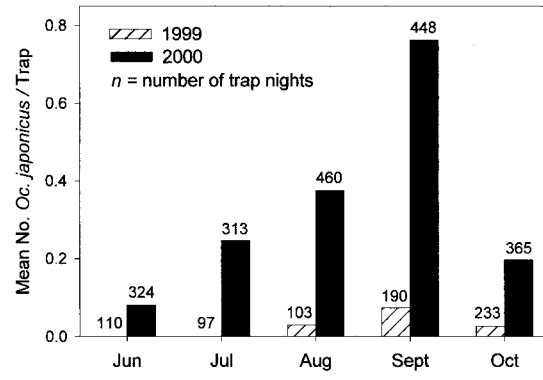

Fig. 4. Monthly CO_2-baited light and gravid trap collections of adult female *Ochlerotatus. japonicus* in Connecticut in 1999 and 2000.

(Say), *Anopheles punctipennis* (Say), *Culex pipiens* L., and *Culex restuans* Theobald. The earliest collection of *Oc. japonicus* larvae was of third instars from a decorative wooden barrel on the porch of a residence on 24 March 2000.

Ochlerotatus japonicus larvae were recovered from catch basins in eight of 40 locations in Fairfield, New Haven, and New London counties between late June and early October. Monthly sampling in catch basins at Oxford and Weston resulted in *Oc. japonicus* larvae on five occasions during the same period. Cohabiting mosquitoes included *Cx. restuans* and *Cx. pipiens.*

Natural habitats yielding *Oc. japonicus* larvae included rock holes along a stream bed, a tree hole, two surface water pools, small impoundments (temporary and permanent), street gutters (with *An. punctipennis*), and a spring-fed depression in a wooded park (with *Cx. pipiens* and *Cx. restuans*). Sampling of rock holes for mosquito larvae along the Housatonic River in Kent, CT, showed *Oc. japonicus* to be common comprising 56.4% of all larvae collected between early July and mid-November 1999 (Fig. 3). *Ochlerotatus japonicus* was most abundant in July and between mid-September and November. Larvae were common in shaded and sunlit pools and pools with algae. In May of the following year, fourth instars were collected from the same rock pools.

Adult Surveys. Adult *Oc. japonicus* were collected from 85 locations in Connecticut (Fig. 1). Fifty (68%) of the 73 permanent trapping sites established in 2000 yielded *Oc. japonicus* females in season-long sampling (Table 2). The largest number of repetitive collections (n = 10) was at a zoo in an urban community in Fairfield county.

In 1999 and 2000, peak collections of adult female *Oc. japonicus* were in September (Fig. 4). Overall, the number of adult *Oc. japonicus* collected was low relative to other species representing 0.05% (23 of 45,391; 30 species) and 0.5% (690 of 137,199; 32 species) of all mosquitoes collected in 1999 and 2000, respectively. The mean number of adult females recovered in light traps (n = 78) positive for *Oc. japonicus* was 2.5 ± 5.2 SD (range, 1–41), and 1.3 ± 0.5 SD (range, 1–2) in gravid traps (n = 29).

Biting adult female *Oc. japonicus* were collected in mid-July using CO_2 and human bait between 1000 and 1400 hours. Five females of 76 total mosquitoes collected on six July were in a wooded habitat in Litchfield County. Other species collected in order of abundance included: *Ochlerotatus canadensis* (Theobald)

Table 2. Adult female collections of *Ochlerotatus japonicus* with CO_2-baited CDC light traps and CDC gravid traps in Connecticut 1999–2000

County	1999		2000	
	No. trap sites	No. sites *Oc. japonicus* collected (%)	No. trap sites	No. sites *Oc. japonicus* collected (%)
Fairfield	72	11 (15.2)	59	46 (78.0)
New Haven	5	2 (40.0)	33	22 (66.7)
New London	15	2 (13.3)	19	7 (36.8)
Hartford	1	0	13	3 (23.0)
Middlesex	4	1 (25.0)	10	3 (30.0)
Litchfield	4	0	6	0
Tolland	3	0	5	3 (60.0)
Windham	13	0	3	1 (33.3)
Totals	117	16 (13.7)	148	85 (57.4)

(70.8%), *Coquillettidia perturbans* (Walker) (35.1%), *Ochlerotatus excrucians* (Walker) (20.3%), *Oc. triseriatus* (4.1%), *Anopheles quadrimaculatus* Say (4.1%), *Ochlerotatus trivittatus* (Coquillett) (2.7%), *Aedes cinereus* Meigen (2.7%), and *Aedes vexans* (Meigen) (1.4%). Fifteen females of 57 mosquitoes total collected on 19 July were in a wooded area in Fairfield County. Other species collected at this site were as follows: *Oc. triseriatus* (40.4%), *Oc. canadensis* (24.6%), *An. punctipennis* (7.0%), and *Ae. vexans* (1.8%).

Ovitrap Study. *Ochlerotatus japonicus* was recovered from two of 38 ovitrap locations between June and October 1999. Positive sites included a woodland swamp in Fairfield County where adult females were collected in light and gravid traps on five different occasions, and a tire recycling plant in Windham County. Ovitraps collected large numbers of *Oc. triseriatus.* Other species recovered in ovitraps included: *Cx. restuans* (three sites), *Anopheles barberi* Coquillett, and *Tx. r. rutilus* (two sites each).

Discussion

Ochlerotatus japonicus is established in Connecticut where it has invaded a variety of natural and artificial container habitats in urban, suburban, rural and agricultural settings. Common habitats for larvae were used tire casings, cast iron and concrete bird baths, wooden barrels, porcelain bath tubs, plastic milk cartons, metal toys, vinyl tarpaulins, rock holes in stream beds, and tree holes. Larvae were particularly common in algal- and leaf-filled containers, and were observed in both shaded and sunlit areas. Our findings are consistent with observed habitat preferences of *Oc. japonicus* in Japan and Korea (Tanaka et al. 1979, Sato et al. 1980, Sota et al. 1994, Tsuda et al. 1994). Tanaka et al. (1979) indicated a preference by larvae for shaded locations; however, we observed larvae in containers in full sun as did Sota et al. (1994) in cemetery stone vessels in Kyushu Province, Japan. Tsuda et al. (1994) collected *Oc. japonicus* larvae from tree holes in Nagasaki, Japan.

The occurrence of *Oc. japonicus* in rock pools on stream beds concurs with the use of this habitat by larvae in Japan (Tanaka et al. 1979). Its abundance relative to other species, especially *Oc. atropalpus*, provides a natural setting for the study of competition between these two rock pool specialists (Zavortink 1972). In earlier collections in 1989 and 1992 from the Housatonic River rock pools, *Oc. atropalpus* was the predominant mosquito and *Oc. japonicus* was not detected. Although our observations did not directly measure competition, clearly *Oc. japonicus* and *Oc. atropalpus* can co-exist in this highly specialized habitat.

Collections of *Oc. japonicus* larvae from catch basins indicates its occurrence in this habitat is not accidental and represents a previously unreported larval habitat for the species. Because catch basins have been recognized as a major urban source for *Cx. pipiens* and *Cx. restuans* (Munstermann and Craig 1977), the importance of catch basins as a development site for *Oc. japonicus* warrants further study.

In Connecticut *Oc. japonicus* is multivoltine. Iriarte et al. (1991) also describe *Oc. japonicus* as multivoltine in southern Japan, with immatures and biting adults collected between May and August. Its presence in Connecticut from late May to early November further indicates a cold tolerance. The overwintering stage is as freeze- and desiccation-resistant eggs in both tree holes and artificial containers (unpublished data). This parallels the descriptions provided for northeastern Japanese populations (Tanaka et al. 1979).

The attraction of female *Oc. japonicus* to human bait indicates that this species will feed on humans. Caged adult *Oc. japonicus* in the laboratory readily feed on human hosts and guinea pigs. We have collected females attempting to bite indoors during September, as well as from midsummer through autumn. Iriarte et al. (1991) has reported *Oc. japonicus* to be the most dominant adult mosquito obtained in human bait catches at multiple sites in forests near human dwellings in Nagasaki City, Japan from May through August.

Our results demonstrate that sod grass-infused gravid and CO_2-baited light traps are effective devices for detecting female *Oc. japonicus.* However, ovitraps were not effective. In many sites where adult females were collected in light and gravid traps, no eggs or larvae were recovered in ovitraps. Ovitraps have been reported to be successful in forested areas of southern Japan (Iriarte et al. 1991). More studies are needed to evaluate the efficacy of these traps.

Although *Oc. japonicus* was first discovered in used tire dumps, both larvae and adults were collected in a variety of natural and artificial containers, over a wide geographic region. We concur with Peyton et al. (1999), who suggested the most likely mode of introduction of *Oc. japonicus* into the northeast United States was in used tires. Moreover, our observations suggest *Oc. japonicus* has been resident in Connecticut for some time. In surveys in the Kent rock pools in 1989 and 1992, and in several of the tire dumps surveyed in the current study in 1987 (Andreadis 1989), no unusual species were found. On this basis, *Oc. japonicus* appears to have become established in Connecticut in the last 6 yr. Recently completed genetic analysis of *Oc. japonicus* populations show at least two genetically independent foci of expansion in the United States (New York/New Jersey/Connecticut and Pennsylvania/Maryland) (Fonseca et al. 2001), however, it is not clear if these populations originated from separate introductions.

The public health significance of the introduction of *Oc. japonicus* into the United States is great. *Ochlerotatus japonicus* transmits Japanese B encephalitis (JE) virus (Mitamura et al. 1947; as referenced by Hammon et al. 1949). More recently studies by Takashima and Rosen (1989) indicate high JE infection rates in this mosquito and efficient vertical and horizontal transmission (to suckling mice) after feeding on viremic chicks. Of greatest significance is the detection of WN virus in multiple pools of *Oc. japonicus* collected in the northeast during the summer of 2000 (CDC 2000)

combined with a high level of susceptibility to infection, and transmission to chickens, under laboratory conditions (Turell et al. 2001). The ability of *Oc. japonicus* to feed on birds in the laboratory and on humans makes it a candidate bridge vector for WN virus.

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