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## Culicid and Chaoborid flies (Diptera: Culicidae and Chaoboridae) attracted to a CDC miniature frog call trap at Iriomote Island, the Ryukyu Archipelago, Japan

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**Abstract:** Mosquito collections were made at two foothill sites of Iriomote Island, the Ryukyu Archipelago, Japan, by modified CDC miniature light traps with a frog call CD player from June 12 to 18, 2004. A total of 777 and 257 female Diptera was collected at sites 1 and 2, respectively. Among them, 4 haematophagous species were predominant at sites 1 and 2—*Uranotaenia macfarlanei* with 580 (74.6%) and 193 (75.1%) individuals respectively, *Ur. yaeyamana* 19 (2.4%) and 27 (10.5%), and *Corethrella nippon* 106 (13.6%) and 20 (7.8%) which is a new record for Iriomote Island, and *Mimomyia luzonensis* with 39 (5.0%) individuals was predominant at site 1. The females of these species collected by the traps fed easily on a frog, *Rana limnocharis* exposed in the cage. The results demonstrated clearly that frog call is the primary stimulus in guiding these Culicine and Chaoborine flies in their search for host animals in nature.

**Key words:** frog call, Culicidae, Chaoboridae, feeding behavior, Japan

### INTRODUCTION

McKeever (1977) reported for the first time that the females of the chaoborid gnats *Corethrella brakeleyi* and *C. wirthi* were attracted to the frog calls that were broadcasted from a cassette player. He collected successfully a large sample of the female *Corethrella* by a modified CDC miniature light trap placed close to the cassette player in Statesboro, Georgia, USA (McKeever and Hartberg, 1980). The genus *Corethrella* is closely related to the mosquito biologically and morphological-

ly, and had been treated as a subfamily Chaoborinae of the family Culicidae (Edwards, 1932). Stone (1956) elevated the subfamily Chaoborinae to a separate family Chaoboridae. The females of the genus *Corethrella* are known to feed readily on animals, especially frogs in the field (Williams and Edman, 1968; Miyagi, 1974; MacKeever, 1977). MacKeever and Hartberg (1980) reported also 9 female mosquitoes together with 566 *Corethrella* collected by the frog call trap during one 30-min. test period in Statesboro, Georgia, USA, but these mosquitoes were not identified as to species.

To determine whether mosquito and *Corethrella* flies occurring in the Ryukyu Archipelago, Japan, are attracted to frog calls, which were broadcast from a CD player in the foothill areas of Iriomote Island. Diptera were collected by the CDC miniature traps hung close to the player. The feeding behavior of some mosquitoes and *Corethrella* attracted to the frog calls is discussed briefly.

#### MATERIALS AND METHODS

Iriomote Island ( $24^{\circ}23'N$ ,  $123^{\circ}45'E$ ) has an area of 284,44 km<sup>2</sup> and is located approximately 700 km south of Okinawa Islands, with about 90% covered with thick forests (Fig. 1). The climate is subtropical, having an average annual temperature of 23.2°C (the coldest 15.0°C in February, hottest 30.1°C in July) and average annual rainfall of 1,912 mm according to the 1999 census. The collections were made over 5 nights between June 12 and 18, 2004 in Iriomote Island, Ryukyu Archipelago, Japan. Two sites were selected at foothill areas of Komi for observation. Site 1 is a mountainous area with paddy fields, along

the Fukari River, while site 2 is the backyard of the Iriomote Wildlife Center, about 200 m from site 1.

A set of CD (Compact Disc) player and CDC miniature light trap without a bulb (McKeener and Hartberg, 1980) was hung close on a tree, about 1.5 m above the

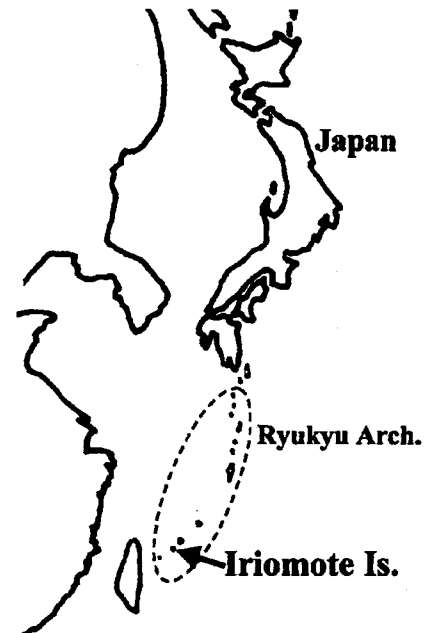


Fig. 1. Map of Japan and the Ryukyu Archipelago showing Iriomote Island.

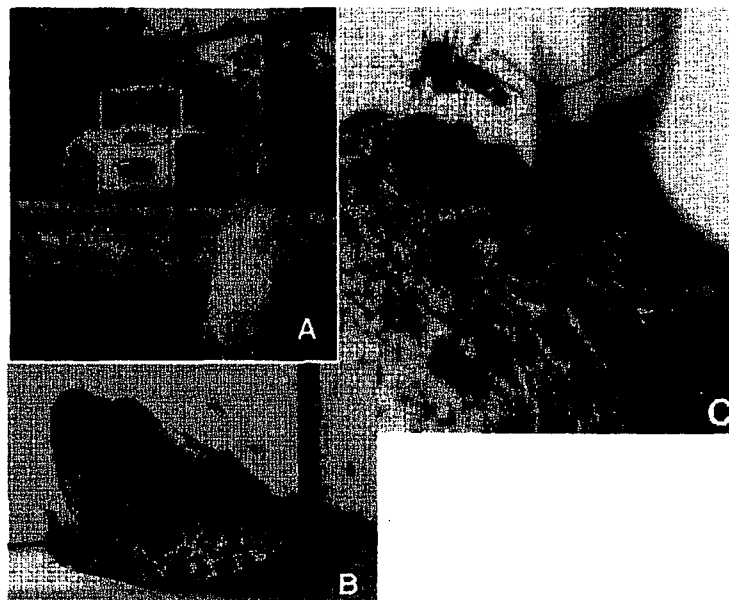


Fig. 2. A, CDC miniature light trap and compact disc player for collecting Diptera; B, A frog, *Rana limnocharis*, exposed to Culicine and Chaoborine flies in a cage; C, *Uranotaenia macfarlanei* feeding on a frog.

ground (Fig. 2A). The calls of 8 common species of frogs (*Hyla hallowellii*, *Rhacophorus owstoni*, *Chirixalus eiffingeri*, *Buergeria japonica*, *Microhyla ornate*, *Rana limnocharis*, *R. narina* and *R. psaltes*) were recorded in a compact disc (Kabaya and Maeda, 2002) and used in the field collection. Broadcasting of the calls took place continuously overnight, from 7:00 p.m. to 7:00 a.m. the next morning. As control, collections by the same type of trap without the broadcasting of frog calls and a bulb, were made at the sites, operated about 10 m apart from the CD player, during the same period. Two day-time collections were also made from 8:00 a.m. to 5:00 p.m. at site 2 where the frog call

traps were carried out. The flies absorbed into the bag attached on the traps were sorted and identified under a binocular microscope. Culicine and Chaoborine flies were identified to species following Miyagi (1975), Miyagi and Toma (1980), Tanaka et al. (1979) and Toma and Miyagi (1986); other Diptera were identified to family and genus. The sex of the Culicidae, Chaoboridae, *Sergentomya* sp. and *Culicoides* sp. were distinguished but other dipterans were not distinguished.

In order to know the host preferences of the Culicid and Chaoborid flies attracted to the frog-call traps, biting tests were undertaken outdoors. A frog, *R. limnocharis*, captured around the study site was

Table 1. Diptera collected by frog call and control traps at site 1, Fukari River, Komi, Iriomote Island, Okinawa, June 12-18, 2004.

Names of flies	June 12-13		June 14-15		June 15-16		June 16-17		June 17-18		Total		
	Frog*	C**	Frog	C	Frog	C	Frog	C	Frog	C	Frog	%	C
<b>Culicidae</b>													
<i>Ur. macfarlanei</i>	108	1	137	0	110	0	93	0	132	0	580	74.6	1
<i>Ur. yaeyamana</i>	4	0	3	0	6	0	3	(1)	3	0	19	2.4	(1)
<i>Ur. ohamai</i>	1	0	0	0	1	0	0	0	0	0	2	0.3	0
<i>Mi. luzonensis</i>	16	0	6	0	6	0	3	0	8	0	39	5.0	0
<i>Ve. iriomotensis</i>	0	0	0	0	0	0	0	1	0	0	0		1
<i>Ve. atriisimilis</i>	0	0	0	0	0	0	0	(1)	0	0	0		(1)
<i>Ae. vexans nipponii</i>	0	0	0	0	0	0	0	0	1	0	1		0
<i>Ae. riversi</i>	0	1	0	0	0	0	0	0	0	0	0		1
<i>Cx. bitaeniorynchus</i>	0	0	1	0	0	1	0	0	0	0	1		1
<i>Cx. fuscocephala</i>	0	0	0	0	0	0	0	0	2	0	2		0
<i>Lu. halifaxii</i>	0	0	0	0	0	1	0	0	0	0	0		1
<i>Ar. subalbatus</i>	0	0	0	0	0	0	0	(1)	0	0	0		(1)
<b>Chaoboridae</b>													
<i>C. nippon</i>	4	0	16	0	12	0	19	0	55	0	106	13.6	0
<i>C. urumense</i>	0	0	0	0	1	0	0	0	4	0	5	0.6	0
<b>Other Diptera</b>													
Mycetophailidae	1	0	3	1	0	1	0	0	0	0	4		2
Tipulidae	0	0	0	2	0	1	0	0	0	0	0		3
Cecidomyiidae	3	0	6	9	0	6	0	5	0	0	9	1.2	20
Psychodidae	1	0	0	5	0	0	0	5	0	0	1		10
<i>Sergentomyia</i> sp.	0	(2)	0	0	0	1	0	0	0	0	0		1 (2)
<i>Culicoides</i> sp.	2	0	0	0	0	0	0	0	0	0	2		0
Sphaeroceridae	1	0	5	5	0	0	0	8	0	0	6	0.8	13
<b>Total</b>											<b>777</b>	<b>100</b>	<b>54 (5)</b>

\* Frog: Trap with frog call. \*\* C: Control trap without frog call.

( ): Number in parentheses shows male.

Collections were made usually from 7:00 p.m. to 7:00 a.m.

Table 2. Diptera collected by frog call and control traps at site 2, Iriomote Wildlife Center of Iriomote Island, Okinawa, June 12-17, 2004.

Names of flies	June 12-13		June 13-14		June 14-15		June 15-16		June 16-17		Total		
	Frog*	C**	Frog	C	Frog	C	Frog	C	Frog	C	Frog	%	C
<b>Culicidae</b>													
<i>Ur. macfarlanei</i>	49	0	23	0	57	0	32	0	32	0	193	75.1	0
<i>Ur. yaeyamana</i>	4	0	1	0	8	1	3	0	11	0	27	10.5	1
<i>Ur. ohamai</i>	2	0	0	0	0	0	0	0	1	0	3	1.2	0
<i>Ae. riversi</i>	1	0	0	0	0	0	0	0	0	0	1	0.4	0
<b>Chaoboridae</b>													
<i>C. nippon</i>	5		0	0	3	0	4	0	8	0	20	7.8	0
<b>Other Diptera</b>													
Mycetophallidae	0	0	0	0	2	0	0	0	0	0	2		0
Cecidomyiade	6	0	1	4	2	3	0	11	0	5	9		23
Psychodidae	0	0	0	0	0	0	0	3	0	1	0		4
<i>Sergentomyia</i> sp.	0	0	0	1	0	(1)	0	0	0	0	0		1 (1)
Sphaeroceridae	2	0	0	0	0	3	0	0	0	1	2		4
<b>Total</b>											<b>257</b>	<b>100</b>	<b>33 (1)</b>

\* Frog: Trap with frog call. \*\* C: Control trap without frog call.

( ): Number in parentheses shows male.

Collections were made usually from 7:00 p.m. to 7:00 a.m.

exposed to the flies collected by the frog call traps in a cage (20×30×20 cm) for several hours from 24:00 to 06:00 (Fig. 2B). This test was repeated three times. One test was carried out in the daytime from 08:00 to 17:00. Feeding activities of the flies in the cage were observed and photographed. The number of engorged females was counted.

## RESULTS

A total of 777 and 257 Diptera was collected by frog call CDC traps at sites 1 and 2, respectively, in Komi, Iriomote Island, over 5 nights from June 12 to 18, 2004 (Tables 1 and 2). They were all females, comprising 12 species of the family Culicidae [*Uranotaenia (Uranotaenia) macfarlanei* Edwards, *Ur. (Pseudoficalbia) yaeyamana* Tanaka et al., *Ur. (Pfc.) ohamai* Tanaka et al., *Mimomyia (Etorleptomysia) luzonensis* (Ludlow), *Verrallina iriomotensis* (Tanaka and Muzusawa), *Ve. atrisimilis* (Tanaka and Mizusawa), *Aedes (Aedimorphus) vexans nipponii* (Theobald), *Ae. (Stegomyia) riversi* Bohart and Ingram,

*Culex (Culex) bitaeniorhynchus* Giles, *Cx. (Cux.) fuscocephala* Theobald, *Lutzia (Metalutzia) halifaxii* (Theobald) and *Armigeres (Armigeres) subalbatus* (Coquillett)], and 2 species of the family Chaoboridae [*C. nippon* Miyagi and *C. urumense* Miyagi]. The predominant culicid species at sites 1 and 2 were *Ur. macfarlanei* with 580 (74.6%) and 193 (75.1%) individuals, respectively, and *Ur. yaeyamana* 19 (2.4%) and 27 (10.5%). *Mimomyia luzonensis* 39 (5.0%) was predominant only at site 1. A few *Ur. ohamai* were collected at both sites and *Cx. fuscocephala* at site 1. Other mosquitoes collected comprised only one female or male. Of the two chaoborid species, *C. nippon* with 106 (13.6%) and 20 (7.8%) individuals, was collected at sites 1 and 2, respectively, and *C. urumense* with 5 (0.6%) individuals at site 1. Other Dipteran comprised Mycetophallidae, Tipulidae, Cecidomyiidae, Psychodidae, Sphaeroceridae, and *Culicoides* sp. which were small in number, and miscellaneous taxa including males.

In the control traps (Tables 1 and 2), a total of 54 and 33 Diptera was collected at

Table 3. Culicine and Chaoborine flies fed on frog *Rana limnocharis* in a cage.

Names of flies	Test 1 (24:00-06:00)		Test 2 (08:00-17:00)		Test 3 (24:00-06:00)		Total	
	No. exam.*	No. fed**	No. exam.	No. fed	No. exam.	No. fed	No. exam.	No. fed
<i>Mi. luzonensis</i>	4	2	0	0	3	2	7	4
<i>Ur. macfarlanei</i>	38	24	55	11	34	6	127	41
<i>Ur. yaeyamana</i>	2	0	1	1	3	0	6	1
<i>Cx. fuscocephala</i>	0	0	0	0	2	0	2	0
<i>C. nippon</i>	13	0	6	0	32	2	51	2

\* Numbers of examined flies collected by the frog call traps and exposed to the frog in a cage (30×20×20 cm).

\*\* Number of flies fed on frog.

( ): Times exposed to a frog.

sites 1 and 2, respectively. Most of them were non-biting flies—Cecidomyiidae (20 at sites 1 and 23 at site 2), and Sphaeroceridae (13 at site 1) were predominant. Only one female or male of *Ur. macfarlanei*, *Ve. iriomotensis*, *Ve. atrisimilis*, *Ae. riversi*, *Cx. bitaeniorynchus*, *Lu. halifaxii* and *Ar. subalbatus* were trapped. One female and 2 males of *Sergentomyia* sp. at site 1, and 1 female and 1 male at site 2 were also collected in the control traps. No Chaoboridae were trapped.

A total of 51 female culicine and chaoborine flies collected by frog call traps was exposed to a frog in a cage for several hours, twice at night and once during the day (Table 3). The cages covered with dark wet paper were placed in a small outdoor shed. As soon as being placed with the frog, *Ur. macfarlanei* showed interest, and some alit on the dorsal part of the frog and began to feed (Fig. 2C). Some alit on the floor behind the hind legs of the frog and walked slowly toward it. Other flies in the cage were inactive and showed no desire to feed under a direct light, and it was difficult to observe their feeding. Several hours after exposure to the frog in the dark, *Ur. macfarlanei* (41/127 females), *Mi. luzonensis* (4/7), *Ur. yaeyamana* (1/6) and *C. nippon* (2/51) fed on the frog, with reddish blood in their stomachs. Two females of *Cx. fuscocephala* did not feed on the frog in the cage. No flies were collected by the frog call traps operated during the day.

## DISCUSSION

It is well known that only the female mosquitoes bite, and body odour and carbon dioxide carried on the wind, stimulate the sense receptors on the antennae and palpus of female mosquitoes, alerting them to the presence of a host (Clements, 1992). Temperature and humidity gradients also may play an important part in the final descent on the host. These stimuli may be similar in all mosquitoes that bite warm-blooded animals, though the relative importance of different classes of stimuli may vary with the species of mosquito, and the threshold of stimulus necessary to elicit a response may also vary, since it is well known that different species of mosquitoes show characteristic host preferences (Bates, 1949). So far as we are aware, there is little information on the importance of calls of host animals as the primary stimulus in guiding a mosquito in its search for blood meal. McKeever (1977) indicated for the first time that *Corethrella* flies were attracted to the calls of their host, tree frogs *Hyla* spp. in Georgia.

The mosquito fauna of the island is well studied and it is very rich; 59 species of 14 genera have been reported (Miyagi and Toma, 1980; Toma et al., 1983; Toma and Higa, 2004). However, our knowledge on the feeding habits of the mosquitoes in the island is limited. Twenty-six (44%) of the

59 species are zoophilic or anthorophilic, collected readily by human-bait net traps, but others are unknown, except *Toxorhynchites yaeyamae* Bohart, *Malaya genurostris* Leicester and *Topomyia yanbarendis* Miyagi which are well-known as non-haematophagous mosquitoes. Some species of *Uranotaenia* and the subgenera *Eumelanomyia* and *Lophoceraomyia* of *Culex* are known to bite amphibians. Miyagi (1972) reported *Cx. (Eum.) hayashii* Yamada and *Ur. (Psf.) novobscura* Barraud collected in Nagasaki, mainland Japan, to feed on frogs, and *Cx. (Lop.) infantulus* Edwards on cold- and warm-blooded animals in the cages. Recently, Okudo et al. (2004), reported that *Ochlerotratus (Geosuksea) baisasi* Knight and Hull fed on a mudskipper (*Periophthalmus argentilineatus* Valenciennes) in the mangrove forests of this island. However, there have been no studies on the feeding habits of the culicid and chaoborid flies attracted to calls of their host animals.

A considerable number of the females of *Ur. macfarlanei*, *Ur. yaeyamana*, *Mi. luzonensis* and *C. nippon* was attracted to frog calls in the present study. Due to the broadcast of 9 species of frog calls continuously throughout the night, it is difficult to know which frog's call acted as the stimulus for each fly. In the control traps with no broadcasting, most of the flies trapped were non-biting, and no females of *Ur. macfarlanei*, *Ur. yaeyamana*, *Mi. luzonensis* and *C. nippon* came to the speaker.

Site 1 in the present study is a mountainous area with paddy fields where 3 species of *Uranotaenia*—*Ur. (Psf.) novobscura novobscura* Tanaka et al., *Ur. (Ura.) annandalei* Barraud and *Ur. (Ura.) lateralis*, other than *Ur. macfarlanei*, *Ur. yaeyamana* and *Ur. ohamai*, and *Culex (Eum.) hayashii*, *Cx. (Eum.) okinawae* Bohart, *Cx. (Lop.) cinctellus* Edwards, *Cx. (Lop.) infantulus*, and *Cx. (Lop.) bicornutus* (Theobald)—were found by Miyagi and Toma (1980). The adults of these species are abundant in the vicinity of the larval habitats, such as

dark recesses in the stream bank, including freshwater crab and tree holes. Although they were not attracted to the broadcasting of frog calls in the study, some of them may feed on amphibians in nature. *Uranotaenia albescens* Taylor and *Culex (Lop.)* sp. fed on a large green frog, *Hyla caerulea* (Shaw) in the Australian region (Marks, 1960) and *Ur. lowii* Theobald fed on a tree frog (*Hyla cinerea*) in Louisiana (Remington, 1945).

The larvae of *Mi. luzonensis* and *Mi. elegans* (Taylor) are found in paddy fields containing decaying vegetable matter in Iriomote Island. The feeding habits of *Mi. elegans* in the island are unknown, but like *Mi. luzonensis* shown by the present study, *Mi. elegans* may be attracted to frog calls and feed on frog in nature. *Mimomyia elegans* was observed to feed on the introduced toad, *Bufo marinus* in Australia (Van Beurden, 1980).

*Corethrella nippon* is known to occur in Nagasaki, Amami Island (Miyagi, 1980) and Taiwan (Lien et al., 1998). The distribution record of this species in the Ryukyu Archipelago is new. The females of *C. nippon* were engorged with reddish or darkened stomach content, the blood presumably of vertebrate animals such as frogs (Miyagi, 1974; Lien et al., 1998). Only 5 females of *C. urumense* Miyagi were collected by the frog-call traps at site 1 which is the type locality of this species (Miyagi, 1975). They may feed on frog like *C. nippon*. McKeever (1977) and McKeever and Hartberg (1980), reported that *C. brakeleyi* and *C. wirthi* were attracted to calls of their host (*Hyla* spp.) in Georgia.

The present study indicates the likelihood of the females of *Ur. macfarlanei*, *Ur. yaeyamana*, *Mi. luzonensis* and *C. nippon* to be attracted to frog calls and feed on frog in nature. The biting activities of these flies may be nocturnal and they came to the speaker only at night. However, these nocturnal mosquitoes attracted to the frog calls may be hungry and often bite at day-time in the cage placed in a dark shed. It also indicates that this biting behavior

may be more widespread in the genus *Corethrella* and some species of mosquito genera *Mimomyia* and *Uranotaenia*.

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