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Feeding Patterns of Mosquitoes (Diptera: Culicidae) in the Atlantic Forest, Rio de Janeiro, Brazil

JERONIMO ALENCAR,[1,2] CECÍLIA FERREIRA DE MELLO,[1] HÉLCIO R. GIL-SANTANA,[1] ALESSANDRO PONCE DE LEÃO GIUPPONI,[3] ANDRESSA NUNES ARAÚJO,[1,4] ELIAS SEIXAS LOROSA,[5] ANTHONY ÉRICO GUIMARÃES,[1] AND JÚLIA DOS SANTOS SILVA[6]

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ABSTRACT The stomach contents of culicids from the Atlantic Forest in Rio de Janeiro state, Brazil, were analyzed using the precipitin technique to evaluate the feeding patterns of the species. Sampling was performed from February 2012 to December 2013, using CO_2-baited Centers for Disease Control and Prevention traps to catch mosquitoes from 15 00 to 07 00 hours. The following antisera were used: bird, rodent, opossum, human, horse, capybara, lizard, and frog. Of the 325 adult bloodfed females caught and analyzed, 273 (84.0%) reacted in the precipitin test. The percentage of specimens with a positive reaction to a single antiserum included bird (39.2%), rodent (22.5%), opossum (13.2%), capybara (6.6%), horse (5.7%), frog (6.2%), human (4.0%), and lizard (2.6%). The specimens that reacted positively against more than one blood source (46) most frequently presented the following combinations: bird + rodent and bird + frog (17.4%), followed by bird + human (13.0%). The predominance of positive results for birds suggested that the avian-rich environment might have influenced the feeding behavior of the culicids.

KEY WORDS feeding habit, precipitin test, behavior, mosquito

Knowledge of the feeding sources of culicids is of vital importance to evaluate their potential for pathogen transmission. Contact between a mosquito and its possible host is influenced by multiple factors, including innate mosquito behavior, host availability and abundance, attempts to feed and reefed, and host behavioral reactions (Defoliart et. al. 1987, Clements 1999).

Studying the feeding habits of mosquitoes in areas that are remnants of the typical Brazilian primary Atlantic forest is of great importance for implementing eco-epidemiological analyses of the vector insect fauna. This biome has a high diversity of flora, vertebrates, and invertebrates, thus providing multiple niche options for mosquitoes.

The precipitin technique has aided in detecting the feeding patterns of mosquitoes and has contributed toward evaluating the vector potential of numerous important mosquito species that transmit pathogens to humans (Lorosa et al. 1998). Even with the development of other methods for bloodmeal identification, the precipitin test remains the basic serological tool (Tempelis 1975). This test was used because minimal equipment is needed, it is relatively simple to perform, and it is faster than other techniques (Washino and Tempelis 1983). Moreover, Chamberlain and Sudia (1967) reported that the precipitin test, developed in capillary tubes (Tempelis and Lofy 1963), showed higher sensitivity than immunodiffusion techniques.

The present study examined the feeding habits of mosquitoes collected within the ecological reserve of Guapiaçu, located in the municipality of Cachoeiras de Macacu, Rio de Janeiro, Brazil.

Material and Methods

Study Area. The capture of culicids throughout Brazil was performed in accordance with scientific license number 28733-4 provided by SISBIO/IBAMA and Comissão de Ética no uso de animais (CEUA – LW – 38/14).

Mosquitoes were caught in the Ecological Reserve of Guapiaçu (REGUA), consisting of remnants of the Atlantic forest. In REGUA, there is a flooded, marshy area that was restored in 2005. According to Köppen (1936), the climate in this region is tropical, with a rainy summer and dry winter, classified as type Aw. In the study by Couto (2010) in the same reserve, the

[1] Laboratório de Diptera, Instituto Oswaldo Cruz (FIOCRUZ), Av. Brasil 4365, zip code 21040-360, Manguinhos, Rio de Janeiro, Brasil.
[2] Corresponding author, e-mail: jalencar@ioc.fiocruz.br
[3] Laboratório de Referência Nacional em Vetores das Riquetsioses, Instituto Oswaldo Cruz (FIOCRUZ), Av., Brasil 4365, zip code 21040-360, Manguinhos, Rio de Janeiro, Brasil.
[4] Programa de Pós-graduação em Biologia Animal, Instituto de Biologia, Universidade Federal Rural do Rio de Janeiro, BR-465, Km 7, zip code: 23890-000, Seropédica, Rio de Janeiro, Brasil.
[5] Laboratório Nacional e Internacional de Referência em Taxonomia de Triatomíneos, Instituto Oswaldo Cruz (FIOCRUZ), Av., Brasil 4365, zip code 21040-360, Manguinhos, Rio de Janeiro, Brasil.
[6] Laboratório Interdisciplinar de Vigilância Entomológica em Diptera e Hemiptera, Instituto Oswaldo Cruz (FIOCRUZ), Av., Brasil 4365, zip code 21040-360, Manguinhos, Rio de Janeiro, Brasil.

mean annual temperature was 22.4°C, with the maximum in January and February and minimum in June. The mean annual precipitation was 2,095 mm: December and January had the highest rainfall and June and July had the lowest rainfall. Part of the REGUA area is located in the State Park of Três Picos, while the remainder is in its buffer zone. The vegetation is divided into three altitudinal zones: lowland (0–500 m), submontane (500–1,500 m), and montane (1,500 m) (Veloso et al. 1991).

Mosquito Collection. Culicid sampling took place once every 2 mo (at approximately the same day of the month) for 2 yr, from February 2012 to January 2013. Mosquitoes were caught in two Shannon traps, with the aid of an oral suction tube (Forattini 1962) and in two Centers for Disease Control and Prevention traps baited with 0.5 kg of dry ice, from 1500 to 0700 hours. These CO_2 traps were set in the tree canopy at heights between 2 and 4 m, in two different vegetation environments. The collection points were located in areas of preserved vegetation and close to the reconstructed marsh. Two points were established: A (22° 25′23.9″ S and 42° 44′30.0″ W), characterized by secondary Atlantic forest vegetation, with forest recomposition similar to the original biocenotic community structure; and B (22° 26′37.6″ S and 42° 54′30.4″ W), characterized by a large flooded area with a rich aquatic plant composition.

The mosquitoes were placed in labeled polyethylene cages that were packed in thermal boxes and transported live to the laboratory, which was located near the ecological reserve. Subsequently, the mosquitoes were anesthetized by exposure to chloroform fumes and stored in a freezer (4°C) to halt bloodmeal digestion.

Mosquito Identification. The Culicidae species were identified with dichotomous keys developed by Faran and Linthicum (1981) and Forattini (2002). The names and abbreviations for genera and subgenera followed those proposed by Reinert (2001). For species classification of the Aedini tribe, the Journal of Medical Entomology (Editorial 2005) recommendations were followed. These recommendations suggested that *Ochlerotatus* should be treated as a subgenus within the genus *Aedes*, as opposed to the work by Reinert (2000), which raised *Ochlerotatus* to genus category.

Precipitin Test. To analyze the blood ingested, the capillary precipitin technique was used as described by Siqueira (1960), with some modifications (Lorosa et al. 1998). The precipitin technique was adapted from Bull and King (1923) to determine bloodsucking insect diets. The method is characterized by the formation of a white ring (antigen + antibody) for positive reactions.

Antisera production. Blood for antisera production was taken from field-collected animals by cardiac puncture; the amount of blood collected was 1% of the body weight of the animal. The blood was centrifuged and the sera were labelled and frozen to −20°C. After recovering from anesthesia, the animals were identified and released in their original habitat. To precipitate the serum protein, 10 ml of specific vertebrate serum was used, together with 32 ml of distilled water and 35 ml of 10% potassium alum solution. The pH was adjusted to between 6.5 and 7.0 using sodium hydroxide solution, as serum protein precipitation occurs within this pH range. After precipitation, the material was centrifuged at 1,800 rpm for 5 min, the supernatant was discarded, and the precipitate homogenized with saline solution (pH 7.0) in a volume equal to the initial volume. For washing, this procedure was repeated twice, the supernatant was discarded, and the precipitate was resuspended in 40 ml of merthiolated saline solution (1:10,000; pH 7.0). This precipitate was inoculated once a week into two young rabbits weighing ±1,500 g for 1 mo. Each animal received an intramuscular dose of 5 ml (2.5 ml into each thigh). Three days after all inoculations, blood was collected from one ear to test for the desired antiserum titer of 1:5,000 to 1:15,000. Use of wild animals and rabbits for antisera production was approved by the Ethics Committee on the Use of Animals (CEUA-LW-38/14).

To test antiserum sensitivity, the homologous serum was diluted to 0.85% in dilutions of 1/10, 1/100, 1/1,000, 1/5,000, and 1/15,000 in saline. Antisera were prepared against bird (*Gallus gallus domesticus*), horse (*Equus ferus caballus*), lizard (*Tropidurus* sp.), opossum (*Didelphis marsupialis*), capybara (*Hydrochoerus hydrochaeris*), human (*Homo sapiens sapiens*), pig (*Sus domesticus*), raccoon (*Nasua nasua*), rodent (*Rattus rattus*), and frog (*Bufo bufo*).

Analysis. Based on definitions by Chaves et al. (2010), we analyzed presence–absence patterns using null-model analyses to test whether mosquito foraging occurred randomly or whether mosquitoes obtained bloodmeals only from certain host species. Each hypothesis can respectively be supported either by random, segregated patterns in which some host species were fed upon only by some mosquito species, or by aggregated patterns in which some host species were fed upon by all mosquito species. A fixed equal probability algorithm with 5,000 randomizations was used, and simulations were carried out using the Ecosim 7.72 software (Gotelli and Entsminger 2004).

Results

Of the 918 moquitoes collected, 325 were bloodfed adult females that were analyzed (Table 1). Of these, 273 (84.0%) reacted to the antisera, whereas 52 (16.0%) did not react. The specimens with a positive reaction to a single feeding source were distributed in the following proportions: bird (39.2%), rodent (22.5%), opossum (13.2%), capybara (6.6%), horse (5.7%), frog (6.2%), human (4.0%), and lizard (2.6%). The specimens that were positive for more than one blood source ($n = 46$) most frequently presented the following combinations: bird + rodent and bird + frog (17.4%), followed by bird + human (13.0%) (Fig. 1).

Regarding the feeding patterns of each mosquito species, the predominance of positive results for birds suggested that host prevalence influenced the feeding patterns of the culicids. The greater abundance of hosts (birds) may contribute to the feeding patterns observed for the following species: *Aedes scapularis* (Rondani),

Table 1. Total number of species of mosquitoes collected in REGUA and total number of mosquitoes that reacted to the precipitin and their respective food sources: Bird (B), Horse (Ho), Lizard (L), Opossum (O), Capybara (C), Human (Hu), Rodent (R), Frog (F)

Species	B	Ho	L	O	C	Hu	R	F	B+C	B+Ho	B+Ho+Hu+L	B+O	B+Hu	B+L	B+R	B+F	C+R	C+F	Ho+O	Ho+Hu	Ho+L	Ho+F	O+Hu	O+L	O+R	Hu+R	L+R	L+F	Sum of reactive specimens	Total of collected mosquitoes
Ae. scapularis	8	0	0	2	2	0	4	1	0	0	0	0	0	0	2	1	1	0	0	1	1	0	1	1	0	0	1	1	27	45
Ae. serratus	5	0	0	1	0	0	1	1	0	1	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	11	24
An. albitarsis	13	1	0	2	2	1	5	3	0	0	0	0	1	0	1	1	0	0	0	0	0	0	0	0	1	1	0	0	32	72
Cq. venezuelensis	7	3	0	4	2	1	4	0	0	0	0	0	1	1	0	0	0	0	1	0	0	0	0	0	0	0	0	0	24	39
Cx. pleuristriatus	9	1	0	2	1	2	6	1	0	0	0	0	1	2	0	1	0	0	0	0	0	0	0	0	0	0	0	0	26	32
Cx. bastagarius	4	2	3	2	3	1	12	1	1	0	0	1	2	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	34	90
Cx. declarator	9	5	1	2	3	2	3	0	0	0	0	0	0	1	0	1	0	0	0	0	0	1	0	0	0	0	0	0	28	202
Cx. usquatus	3	1	1	1	1	0	3	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	12	203
An. aquasalis	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	1	1
An. argyritarsis	1	0	0	1	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	3	4
An. benarrochi	2	0	0	1	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4	6
An. cruzii	3	0	0	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	6	7
An. eiseni	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	3
An. evansae	3	0	0	1	0	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	8	38
An. strodei	3	0	0	2	0	0	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	8	9
An. triannulatus	4	0	0	2	0	1	4	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	13	23
An. intermedius	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	3	4
An. minor	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	2
An. rangeli	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	4
Cq. juxtamansonia	5	0	0	3	0	0	1	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	11	88
Ma. titillans	6	0	0	2	0	0	3	2	0	0	0	1	0	0	1	1	0	0	0	0	0	0	0	0	1	0	0	0	17	22
Total	89	13	6	30	15	9	51	14	1	1	1	2	6	4	8	8	1	1	1	1	1	1	1	1	4	1	1	1	273	918

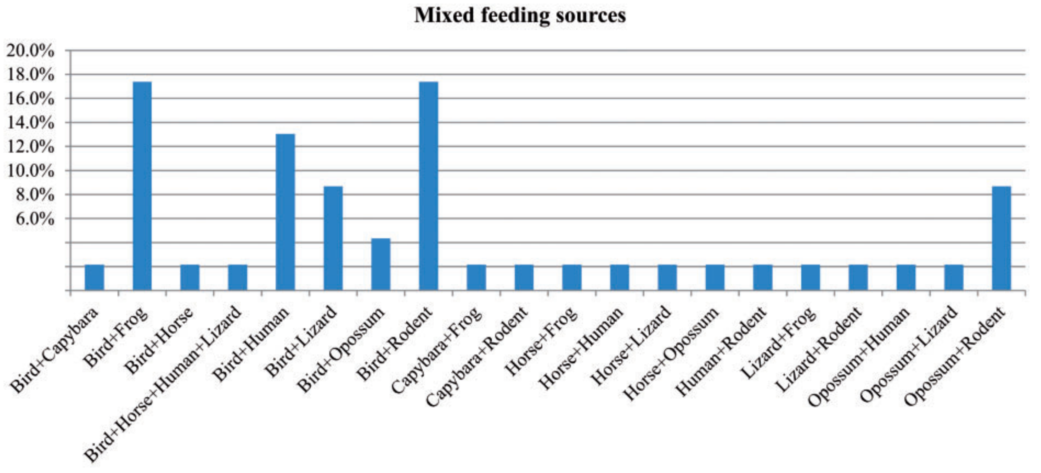

Fig. 1. Identification of blood ingested by female mosquitoes for two blood sources in the Ecological Reserve of Guapiaçu, municipality of Cachoeiras de Macacu, state of Rio de Janeiro, Brazil.

Aedes serratus (Theobald), *Anopheles albitarsis* Lynch-(Arribálzaga), *Coquillettidia venezuelensis* (Theobald), *Culex pleuristriatus* Theobald, *Culex declarator* Dyar & Knab, *Anopheles benarrochi* Cova Garcia & Lopez, and *Anopheles cruzii* Dyar & Knab (Table 1).

The mosquito species were analyzed according to their hosts in REGUA, to assess whether the feeding patterns of different species in the same area were random or structured. According to this analysis, the species from REGUA presented an aggregate pattern (Table 2).

Table 2. Feeding patterns of different species of mosquitoes in the Ecological Reserve of Guapiaçu, municipality of Cachoeiras de Macacu, state of Rio de Janeiro, Brazil

Area	REGUA
C-score	0.319
Mean ± variance	1.505 ± 0.015
$P <$ exp	0.000
$P >$ exp	1.000
Pattern	Aggregate

Discussion

In the present study, the bloodmeal profiles of the species caught in an area of the Atlantic forest, located in the Ecological Reserve of Guapiaçu, municipality of Cachoeiras de Macacu, state of Rio de Janeiro, were determined.

Deane et al. (1949) and Lourenço-de-Oliveira and Heyden (1986) found a high degree of zoophilia (vs. anthropophily) with *An. albitarsis*, showing a preference for attacking animals such as horses. In the present study, *An. albitarsis* fed predominantly on birds; however, it also fed on all other hosts tested except the lizard. These observations are consistent with those by Silva et al. (2012), who reported that both in the Iguaçu National Park and on the Trans-Pantanal and Pantanal Park highways (Mato Grosso and Mato Grosso do Sul), there was predominance of feeding on birds. Just as in REGUA, *Anopheles evansae* (Brethes) was also found to feed predominantly on birds in areas of the Atlantic forest, Cerrado (savanna) and Pantanal, associated with host availability (Silva et al. 2012). Although *An. cruzii* also presented greater reaction to bird antiserum, this could be related to the small size of the sample analyzed. Silva et al. (2012) reported that this species fed mostly on birds in two conservation areas in the states of São Paulo and Rio de Janeiro.

Species in the tribe Mansoniini have shown broad feeding patterns (Forattini 2002). In Florida, Edman (1971) found that *Mansonia titillans* (Walker) fed predominantly on birds and mammals. The predominance of positive results from *Ma. titillans* for birds was similar to that reported by Alencar et al. (2005) and Silva et al. (2012), thus corroborating information in the literature that considers *Mansonia* spp. to be ornithophilic (Aitken 1968). *Coquillettidia venezuelensis* and *Cq. juxtamansonia* (Chagas) presented behavior similar to *Mansonia*.

As noted by Forattini et al. (1990), Consoli and Lourenço-de-Oliveira (1994), Teodoro et al. (1994), Forattini (2002), and Silva et al. (2012), *Ae. scapularis* exhibits an eclectic host feeding pattern. This information concurs with the present analysis demonstrating a tendency to feed on bird blood, i.e., from the host that was most abundant in the area. According to Forattini (2002), *Ae. serratus* also presents eclectic behavior regarding the host, and just as in REGUA, in the Serra do Mar State Park and the Iguaçu National Park, this species presented an "aggregate pattern" (Silva et al. 2012), feeding mostly on birds.

In Brazil, most species of the subgenus *Culex* seem to be ornithophagic, but also feed on mammals (Consoli and Lourenço-de-Oliveira 1994). This behavior was observed in REGUA, where *Cx. declarator* and *Culex usquatus* Dyar fed mostly on birds. The species

Culex bastagarius Dyar & Knab can be considered zoophilic: in REGUA, it fed mostly on rodents. According to Lourenço-de-Oliveira and Heyden (1986), *Microculex* tends to bite amphibians, but in REGUA, *Cx. pleuristriatus* fed mostly on birds and rodents.

The null-model test for community feeding patterns indicated that the species in REGUA shared at least one host group (aggregate pattern). The aggregate pattern indicated that the community host-feeding patterns was closely related to host availability (Edman and Spielman 1988). Other authors have also reported that in each habitat, the feeding pattern of most species corresponded to the abundance of primary hosts (Tempelis and Washino 1967, Edman 1971, Mitchell et al. 1987, Forattini et al. 1989, Silva et al. 2012). These results support that the patterns may be associated with the host availability, but with no preference for a particular host.

This behavior shows that there is no greater preference for birds, but this behavior is directly influenced by higher availability, species richness, and avifauna diversity whereby birds become a primary feeding source for these mosquitoes (REGUA 2013).

According to Dégallier et al. (1993), birds play an important role in the intercontinental spread of several arboviruses, and population density and seasonal variations are important in understanding cycles and dynamics of arbovirus transmission in the Amazon. Although no migratory bird species collected in the Amazon has been positive for arbovirus, the role of these intercontinental hosts has been suspected for some viruses.

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