Journal of Vector Ecology 21(1): 1-5

The Blood-Feeding Habits of *Aedes sollicitans* (Walker) in Relation to Eastern Equine Encephalitis Virus in Coastal Areas of New Jersey[1]

II. Results of Experiments with Caged Mosquitoes and the Effects of Temperature and Physiological Age on Host Selection.

W. J. Crans[2], D. A. Sprenger[3], and F. Mahmood[2]

[1]New Jersey Agricultural Experiment Station Publication No. D-40101-02-95 supported by U.S. Hatch Act funds with partial support from the New Jersey State Mosquito Control Commission.
[2]Department of Entomology, Cook College, Rutgers University, P.O. Box 231, New Brunswick, NJ 08903.
[3]Corpus Christi Health Department, Vector Control Division, 3041 Morgan Avenue, Corpus Christi, TX 78404.

Received 27 December 1990; Accepted 24 October 1995

ABSTRACT: Because of the extremely low incidence of avian feedings reported from field collected *Aedes sollicitans* (Walker), host-seeking mosquitoes were collected in the field, placed into outdoor cages and offered a restrained bird (Japanese quail) and restrained mammal (guinea pig) to determine if the species would accept or reject an avian host. No significant difference in feeding response was observed between groups of mosquitoes offered only the bird or the mammal. A majority of the mosquitoes accepted the host that was offered suggesting that *Ae. sollicitans* is an opportunistic blood-feeder rather than a fixed mammalophilic species. When mosquitoes were offered a choice of a mammal or bird, one-third of the mosquitoes accepted the bird as the blood-meal host when the mammal was present as an alternative. Physiological age dissections showed that both nulliparous and parous mosquitoes fed preferentially on the mammalian host but significantly more parous mosquitoes accepted the guinea pig when a choice was available. Reduced temperatures decreased the number of mosquitoes that attempted to blood-feed under laboratory conditions but did not alter host selection in these tests. Results are discussed in relation to acquisition of eastern equine encephalitis virus and the role of *Ae. sollicitans* as an epidemic vector of the disease.

Keyword Index: Aedes sollicitans, host preference, bird, eastern equine encephalitis.

INTRODUCTION

Considerable evidence has been accumulated to incriminate *Aedes sollicitans* (Walker) as the epidemic vector of eastern equine encephalitis (EEE) virus in New Jersey (Kandle 1960, Hayes et al. 1962, Goldfield et al. 1966, Crans 1977, Crans et al. 1986). Birds, however, are considered to be the primary amplification hosts for the virus (Stamm et al. 1962, Stamm 1963) and blood-meal identification studies with *Ae. sollicitans* have shown that this species only rarely feeds on avian hosts in nature (Thompson et al. 1963, Crans 1964, Edman and Downe 1964, Schaefer and Steelman 1969, Edman 1971). Crans et al. (1990) tested numerous wild-caught specimens collected from study sites in New Jersey where EEE virus was known to be enzootic. The results indicated that 98.2% of the specimens tested had fed on a mammalian host and only 1.4% fed on birds.

Blood-feeding patterns determined solely by precipitin tests on wild-caught specimens may not be indicative of the true host preference for a given species. Host availability within collection sites strongly influences the range of potential hosts that are ultimately exhibited in the results. The methods used to collect the engorged specimens and the habitat that is sampled could further bias the sample. Nayer and Sauerman (1977) successfully fed *Ae. sollicitans* on a chicken, dove, and owl during experiments designed to test the effects of blood source on oocyte development. Their studies suggest that *Ae. sollicitans* may be more of a catholic feeder than the literature indicates.

In an effort to explore avian feeding by *Ae. sollicitans*, a series of experiments were conducted to determine 1) if the mosquito would accept a bird as a blood-meal host when no other host was present, 2) if the mosquito would accept a bird when a mammal was present as an alternative, and 3) if the ratio of mammalian to avian feedings approached the results obtained by precipitin testing. The animals were restrained to eliminate any variability in defensive behavior by the hosts which may have interfered with the success of engorgement once the mosquitoes initiated blood feeding. In addition, the effects of temperature and physiological age were measured to determine if these factors influenced host selection during the experiments. Since the experiments were designed to investigate avian acceptance by *Ae. sollicitans*, unavoidable biases in methodology were purposely shifted in favor of the mammalian host (host initially used to attract specimens for the tests, comparative size of the caged animals used in the experiments, and source of the blood meal that produced F_1 progeny).

MATERIALS AND METHODS

Host Selection at Ambient Temperatures

Once each week from July to October 1982 and again in July and August 1983, 400 *Ae. sollicitans* of unknown physiological age were collected by aspiration as they attempted to bite the authors at a coastal site in New Jersey. Collections were made during the late afternoon in an old field in Belleplain State Forest, Cape May County, New Jersey, where EEE virus had been isolated from field-collected specimens in 1982 (Crans et al. 1986). Care was taken to assure that none of the specimens had successfully penetrated skin prior to capture. The host-seeking mosquitoes were placed, 100 each, in paper pint cartons and sealed in plastic bags with moist paper toweling to maintain humidity until the tests were run. In no cases were the mosquitoes held longer than two hours from collection to exposure to the test animals.

Mosquitoes were exposed to potential blood-meal hosts in 1 ft^3 cages that were set on a portable table in the old field where the mosquitoes had been collected. Four cages were used in each feeding trial. The experimental design included a guinea pig (representing a mammalian host) in one cage, a Japanese quail (representing an avian host) in another, and a combination of both animals in the remaining two cages. A purposeful effort was made to eliminate defensive behavior of the host as a variable during the trials. As a result, the animals were restrained separately in nylon stockings and were firmly taped to the floor of the cage to minimize movement. This design was repeated on 11 separate dates.

Two hours before sunset, 100 mosquitoes were released into each cage and allowed to engorge on the restrained animals throughout the twilight period. After four hours of exposure, the animals were removed and the mosquitoes were anesthetized, placed into vials, and frozen on dry ice. Individual mosquitoes were examined for physiological age and host selection at a later date. Blood meals were identified using the agar gel diffusion technique described by Crans (1969). Parity was determined by examining tracheolation of the ovaries as described by Detinova (1962).

Host Selection at Controlled Temperatures

The F_1 progeny of wild-caught *Ae. sollicitans* were used to determine host selection at controlled temperatures. The mosquitoes were collected by the methods described earlier, given a blood meal on a guinea pig in the laboratory, and allowed to lay eggs on moist cotton. Adult mosquitoes for the experiments were then reared from the eggs by the methods of Khoo and Sutherland (1983). A 10% sucrose solution was made available as a source of nutrition while the specimens were being held for blood-feeding tests.

Host selection experiments were conducted in an incubator at three controlled temperatures (15°, 20°, and 27°C). Each temperature regime was replicated five times. On the day of each test, a guinea pig and a Japanese quail were restrained separately in a nylon stocking and taped, side by side, to the floor of a 1 ft^3 cage, duplicating the methodology used in the field trials. Fifty 4-6 day old *Ae. sollicitans* were placed in a paper pint carton just prior to each test. The restrained animals and the mosquitoes were then allowed to acclimate to the test temperature for two hours. Pans of water were placed in the incubator where the tests were conducted and the cages were draped with cheese cloth to maximize humidity in the artificial environment.

After acclimation, 50 mosquitoes were introduced into each cage with the restrained animals and allowed to engorge over a four-hour period. When the test was completed, the animals were released, the entire cage was placed in a freezer, and the mosquitoes were separated into vials. Instead of using antisera to determine the host, each blood meal was smeared on a microscope slide, stained with giemsa, and identified to host by the presence or absence of nuclei in the red blood cells.

RESULTS

Host Selection at Ambient Temperatures

TABLE 1 lists the results of 11 paired exposures when caged *Ae. sollicitans* of unknown physiological age were presented with a single animal (either a guinea pig or quail) at ambient temperatures out-of-doors. No significant difference was found between the feeding responses to the mammal or bird when the mosquitoes had the choice of accepting or rejecting the host. A total of 54.9% of the mosquitoes fed upon the quail (n = 395) and 52.9% upon the guinea pig (n = 372). In 7 of the 11 tests, the cage containing the bird produced the greatest number of blooded mosquitoes.

TABLE 2 lists the results of 22 tests where mosquitoes were exposed simultaneously to both animals and then dissected to correlate physiological age with host selection. Of those mosquitoes that accepted a host, 66.4% fed on the guinea pig and 33.6% fed on the quail. Ovarian dissections showed that out of the total 981 mosquitoes that accepted a blood meal from one of the hosts, 343 (35%) were nulliparous and 638 (65%) were parous. To determine if physiological age affected host selection, nulliparous and parous mosquitoes were tested by a 2x2 Yates χ^2 contingency test. The results indicated that significantly more mosquitoes in both groups fed on the mammal when the choice of hosts was available.

Host Selection at Controlled Temperatures in the Laboratory

At controlled temperatures, a total of 254 nulliparous mosquitoes that were reared from eggs accepted a blood meal from one of the two animals in the cage under the conditions of the experiment (TABLE 3). The mammal was fed upon by 59.5% of the mosquitoes in these tests

TABLE 1. Contingency table of feeding responses of *Aedes sollicitans* when offered a single bird or mammal as a blood meal host ($\chi^2 = .5844$, $\alpha = .05$, df = 1, not significant).

	Host Selected			
	Bird		Mammal	
Status	Number	Percent	Number	Percent
Fed	395	54.9	372	52.9
Unfed	324	45.1	331	47.1
TOTAL	719	100.0	703	100.0

TABLE 2. A comparison of host acceptance by parous and nulliparous *Aedes sollicitans* when offered the choice between a bird or a mammal ($\chi^2 = 6.545$, $\alpha = .05$, df = 1, significant).

	Bird		Mammal	
Status	(n*)	%Fed	(n*)	%Fed
Nulliparous	134	39.1	209	60.9
Parous	196	30.7	442	69.3
TOTAL	330	33.6	651	66.4

* Total number of mosquitoes fed in 22 tests

TABLE 3. Feeding responses of F_1 nulliparous *Aedes sollicitans* when offered the choice between a bird or a mammal at three controlled temperatures in the laboratory.

Host Selected	Average No. Feeding/ 50 Exposed Temperature Regime (°C) 15°	(n)	20°	(n)	27°	(n)
Bird	4.4	22	6.4	32	9.8	49
Mammal	7.0	35	8.6	43	14.6	73
TOTAL	11.4	57	15.0	75	24.4	122

(n = 151) and the bird was the host of choice by 40.5% (n = 103). A two-way analysis of variance showed that significant differences existed in blood-feeding response due to temperature ($P < 0.05$) and host ($P < 0.05$). The interaction of temperature and host was not significant. Analysis of the data by temperature regime indicated that lowered temperatures decreased the number of mosquitoes that attempted to blood feed but did not change the pattern of host selection in these tests.

DISCUSSION

Edman et al. (1972) categorized blood-feeding patterns of mosquitoes as fixed or opportunistic. The results obtained in these experiments suggest that *Ae. sollicitans* is not a fixed mammalophilic species but an opportunistic feeding species that will readily accept a bird as a blood-meal source. When specimens were captured from the wild and offered a restrained host, the bird was not rejected. Data indicated that, under the conditions of the experimental design, birds and mammals were fed upon with nearly equal avidity. The experiments were conducted under caged conditions, but the mosquitoes were not starved before the hosts were offered, and the trials were held out-of-doors during the crepuscular period when the mosquitoes would normally be seeking a blood-meal host. The results indicate that *Ae. sollicitans* has the potential of accepting birds at a rate well above the 1-2% indicated by precipitin testing.

When *Ae. sollicitans* was offered a choice between a quail and a guinea pig, one-third of the mosquitoes fed on the bird even though a mammal was present as an alternative. Temperature did not appear to affect the overall pattern or cause a shift in feeding behavior from bird to mammal as has been described for *Culex tarsalis* (Tempelis et al. 1965) and *Culex nigripalpus* (Edman and Taylor 1968). Low temperatures merely depressed the feeding response in the specimens that were included in the test series.

Precipitin tests conducted on wild *Ae. sollicitans* have consistently indicated a strong preference for large mammals and minimal acceptance of birds as a blood-meal host . Our results with caged mosquitoes suggest that blood-feeding patterns that are based solely on precipitin tests may not reveal the potential host range of this species. Collecting blooded mosquitoes from open field habitats may give more information on the animals that passed through that field than on the host preferences of the mosquitoes that were resting in that field. Alternatively, a lack of avian blood in wild-caught specimens may indicate that the mosquitoes are either not host-seeking in areas where birds are abundant or that the available birds are exhibiting behavior that rejects mosquito attack.

Edman et al. (1972) stated that caged mosquitoes frequently accept a wider range of hosts than they would normally feed upon in nature. Although we support that observation, it would be reasonable to assume that *Ae. sollicitans* would probably not reject an opportunistic encounter with a bird under natural conditions. The design of our experiment eliminated host defensiveness as a variable. It is doubtful, however, that host-defensive behavior alone is responsible for the paucity of bird blood found in field-collected specimens. Isolation of EEE virus from wild-caught *Ae. sollicitans* (Crans et al. 1986, see also Crans 1977) supports the concept that this species acquires EEE virus as a result of avian-feeding behavior. Our results suggest that avian host acceptance by *Ae. sollicitans* may be more common than the 1-2% avian feedings that have been suggested by precipitin testing of wild-caught specimens.

Acknowledgments

Sincere appreciation is extended to the Cape May County Mosquito Control Commission and their staff for providing storage space for field equipment and care for the animals used in these experiments. Without their help and interest, the field aspects of this study would have been difficult to conduct on a weekly basis.

REFERENCES CITED

Crans, W. J. 1964. Continued host preference studies with New Jersey mosquitoes. Proc. N. J. Mosq. Exterm. Assoc. 51: 50-58.

Crans, W. J. 1969. An agar diffusion method for the identification of mosquito blood meals. Mosq. News 29: 563-566.

Crans, W. J. 1977. The status of *Aedes sollicitans* as an epidemic vector of eastern equine encephalitis in New Jersey. Mosq. News 37: 85-89.

Crans, W. J., J. McNelly, T. L. Schulze, and A. J. Main. 1986. Isolation of eastern equine encephalitis virus from *Aedes sollicitans* during an epizootic in southern New Jersey. J. Amer. Mosq. Control Assoc. 2: 68-72.

Crans, W. J., L. J. McCuiston, and D. A Sprenger. 1990. The blood-feeding habits of *Aedes sollicitans* (Walker) in relation to eastern equine encephalitis in New Jersey. I. Host selection in nature determined by precipitin tests on wild-caught specimens. Bull. Soc. Vector Ecol. 15(2): 144-148.

Detinova, T. S. 1962. Age-grouping methods in Diptera of medical importance with special reference to some vectors of malaria. WHO Monograph no. 47, Geneva, 216 pp.

Edman, J. D. 1971. Host-feeding patterns of Florida mosquitoes. I. *Aedes, Anopheles, Coquillettidia, Mansonia,* and *Psorophora.* J. Med. Entomol. 18: 687-695.

Edman, J. D. and A. R. Downe. 1964. Host-blood sources and multiple feeding habits of mosquitoes in Kansas. Mosq. News 24: 154-160.

Edman, J. D. and D. J. Taylor. 1968. *Culex nigripalpus:* Seasonal shift in the bird-mammal feeding ratio in a mosquito vector of human encephalitis. Science 161: 67-68.

Edman, J. D., L. A. Webber, and H. W. Kale. 1972. Host-feeding patterns of Florida mosquitoes. II. *Culiseta.* J. Med. Entomol. 9: 429-434.

Goldfield, M., O. Sussman, and R. P. Kandle. 1966. A progress report on arbovirus studies in New Jersey. Proc. N. J. Mosq. Exterm. Assoc. 53: 47-51.

Hayes, R. O., L. D. Beadle, A. D. Hess, O. Sussman, and M. J. Bonese. 1962. Entomological aspects of the 1959 outbreak of eastern encephalitis in New Jersey. Amer. J. Trop. Med. and Hyg. 11: 115-121.

Kandle, R. P. 1960. Eastern encephalitis in New Jersey. Proc. N.J. Mosq. Exterm. Assoc. 47: 11-15.

Khoo, B. K. and D. J. Sutherland. 1983. The susceptibility status of *Aedes sollicitans* adults to topically applied malathion. Mosq. News 43: 441-444.

Nayar, J. K. and D. M. Sauerman. 1977. The effects of nutrition on survival and fecundity in Florida mosquitoes. Part 4. Effects of blood source on oocyte development. J. Med. Entomol. 14: 167-174.

Schaefer, R. E. and C. D. Steelman. 1969. Determination of mosquito hosts in salt marsh areas of Louisiana. J. Med. Entomol. 6: 131-134.

Stamm, D. D. 1963. Susceptibility of bird populations to eastern, western and St. Louis encephalitis viruses. Proc. XIII Intern. Ornithol. Congr. 1: 591-603.

Stamm, D. D., R. W. Chamberlain, and W. D. Sudia. 1962. Arbovirus studies in south Alabama, 1957-1958. Am. J. Hyg. 76: 61-81.

Tempelis, C. H., W. C. Reeves, R. E. Bellamy, and M. F. Lofy. 1965. A three-year study of the feeding habits of *Culex tarsalis* in Kern County, California. Amer. J. Trop. Med Hyg. 21: 238-245.

Thompson, E. G., D. E. Hayes, and K. W. Ludham. 1963. Notes on the feeding habits of *Aedes sollicitans* in the Chincoteaque-Assateaque Island area of Virginia. Mosq. News 23: 297-298.