DIFFERENTIAL ATTRACTION OF THE YELLOW FEVER MOSQUITO TO VERTEBRATE HOSTS

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INTRODUCTION. It is common experience that in mosquito infested areas some individuals attract more mosquitoes than others. In our screening of humans for degrees of attractiveness to mosquitoes we found gross interhost differences (Khan *et al.*, 1965). Using mosquito probing as the criterion of attractiveness, we recorded the time when 3 to 6 mosquitoes in a small cage probed simultaneously toward the human skin (PT_{50}). Thus, we quantitated the attractiveness of individual humans sequentially. This method, however, did not give the mosquitoes an option to choose between two hosts presented simultaneously.

In this study we compared humans with humans and humans with other vertebrates side by side. We quantitated the mosquito preference when given a choice of hosts. We sought a small mammal or bird of attractiveness to mosquitoes equal to or greater than man. We need such a laboratory animal as a model for pharmacologic studies with oral insect repellents.

METHODS AND MATERIALS. Fifty female *Aedes aegypti* (L.) 6–10 days old, previously fed on sugar water only, were placed in a 30 cm^3 (1 $ft.^3$) cage lined with polyethylene on the sides. The top of the cage was covered with 20 mesh net. There was a small hole in the net for introduction of mosquitoes. The bottom of the cage was fitted with cardboard. Two 9 x 5 cm openings in the bottom, 7 cm apart were used to expose the animals or the forearms of humans to the mosquitoes. The openings were covered with 20 mesh net and a 0.5 cm thick cardboard rim around the openings on the outside prevented the mosquitoes from reaching the skin.

We first made comparisons between the two forearms of the same volunteer. The cage rested on a table touching the forearms and the number of mosquitoes probing on each port over the exposed skin were counted each minute for 10 min. To compensate for any positional bias the subject moved over to the opposite end of the table and again exposed his forearms for 10 min. This constituted one experiment which was replicated on the same subject with different mosquito samples and repeated with three subjects.

To compare two human hosts we first screened 125 individuals for attractiveness by the PT_{50} method. We selected four subjects with PT_{50}'s over 100 sec. and compared them with five others with PT_{50}'s under 25 sec. Most of the subjects screened had their PT_{50}'s under 25 sec. (Fig. 1). One subject with PT_{50}'s over 100 sec. was compared twice to give 5 paired comparisons. The subjects sat at opposite ends of the table facing each other with the left forearm under the cage. Counts of mosquitoes probing were made for 10 min. after which they changed seats. The observations were repeated for another 10 min.

Instead of a random sample we took a discrete sample of human hosts. We did this to ascertain whether we would find the same differences in individual attrac-

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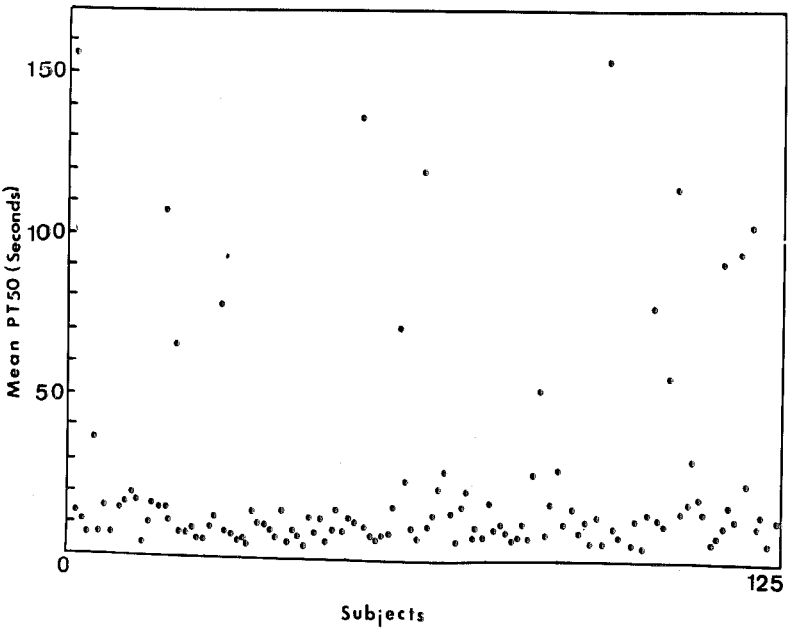

FIG. 1.—Mean PT50 (seconds) of 125 humans screened for attractiveness to *Aedes aegypti* females.

tiveness, using this technique, as we found with the PT50 method. Each human host was tested with the PT50 method at the time of experiment.

Other vertebrate hosts when compared with a human or with each other were strapped with masking tape in the cut-out hollow of a plastic cylinder. Guinea pigs, chickens and pigeons were compared. The guinea pigs were shaved on their backs and the birds on their breasts with electric clippers. They were then exposed under the openings of the cage.

The following comparisons were made:

1. Two forearms of the same human		(3 subjects)
2. Humans	vs. Humans	(5 pairs)
3. Humans	vs. guinea pigs	(10 pairs)
4. Humans	vs. chicken	(10 pairs)
5. Humans	vs. pigeons	(10 pairs)
6. guinea pigs	vs. guinea pigs	(10 pairs)
7. chicken	vs. chicken	(10 pairs)
8. pigeons	vs. pigeons	(10 pairs)
9. chicken	vs. guinea pigs	(10 pairs)

We also quantitated the attractiveness of four rhesus monkeys to mosquitoes tested sequentially with the PT50 method. A human was also tested at the same time. The monkeys were tested on the shaved hind legs and the human on the forearm.

RESULTS. When both forearms of the same subject were exposed to mosquitoes side by side the mosquitoes distributed evenly and no significant differences were observed (Table 1a and b). When two humans of different attractiveness (as measured by the PT50 method) were compared, the mosquitoes sought one subject

TABLE 1a.—Analysis of variance of the *Aedes aegypti* females attracted to the two forearms of each subject tested side by side.

Source of variation	*df*	SS	MS
Total	29	154.2	...
Replicates	5	13.7	2.7
Left vs. right arms	1	0.8	0.8
Error	23	139.7	6.1

Subjects (n=3)

TABLE 1b.—Mean number of mosquitoes attracted to the two forearms of the same subject tested side by side. The figures are means of six replicates.

Subjects	1		2		3	
Forearms	L	R	L	R	L	R
No. mosq. probing	11.2	12.0	12.2	13.0	9.6	9.0

and almost ignored the other. Highly significant differences ($P<.005$) in attractiveness were observed, the mosquitoes preferring subjects with low PT50 and ignoring those with high PT50 (Table 2a and b). During comparisons with humans, guinea pigs attracted significantly more mosquitoes than did the chickens (Table 3).

Among animals, when two of the same kind were tested side by side each attracted equal number of mosquitoes. However, among the various animal hosts tested separately, guinea pigs attracted 3 times as many mosquitoes as did pigeons or the chickens (Table 4a and b). When guinea pigs were compared with chickens, the former attracted roughly twice as many mosquitoes as the chickens (Table 5). No significant difference was found between the mean PT50 obtained on the monkeys (20.8 ± 1.4 sec.) and that on the human (19.8 ± 2.3).

TABLE 2a.—Analysis of variance of the number of *Aedes aegypti* females attracted to the forearms of the attractive and unattractive subjects tested side by side.*

Source of variation	*df*	SS	MS
Total	39	4126.4	.....
Replicates	3	110.6	36.8
Hosts	9	3493.4	388.1**
Error	27	522.4	19.3

* Subjects selected for attractiveness to mosquitoes by the PT50 method (Khan et al., 1965).
** $P<.005$.

TABLE 2b.—Mean number of mosquitoes attracted to the forearms of the attractive and unattractive subjects tested side by side.

Subjects	1	2	3	4	5
Attractive	22.5 (9.5)	27.7 (7.0)	24.0 (5.5)	15.0 (14.5)	22.7 (10.7)
Unattractive	7.0 (174.7)*	8.5 (158.0)	4.7 (98.5)	2.7 (137.2)	2.0 (117.2)

* Fig. in parenthesis are mean PT50 in seconds (4 replicates recorded on the forearms of each subject at the time of comparisons.

DISCUSSION. We did not find significant differences between the two forearms of the same subject. This suggests that there were no qualitative or quantitative differences in the skin emanations from the two forearms.

The overwhelming shift of mosquitoes in favor of an attractive subject and a consequent lack of interest in the unattractive subject must reflect on the nature of the corresponding stimuli emanating from each skin area. This implies both qualitative and quantitative differences. We observed during our experiments that a subject who was unattractive compared to one could become quite attractive when compared with another. The attractiveness of a host can, therefore, be stated only in relative terms, i.e. in relation to another host, and in the present knowledge cannot be estimated in absolute quantities.

Guinea pigs compared to humans were ⅓ as attractive. Separately, they attracted as many mosquitoes as humans did. The differences are therefore not absolute but depend on the availability of an alternate host. Hackett (1937) made similar observations in his studies with *Anopheles maculipennis* complex. This brings into focus the validity of the criticism advanced by Bates and Hackett (1939) of the use of terms "anthropophilous" and "zoophilous"

TABLE 3.—Analysis of variance of the *Aedes aegypti* females attracted to humans compared with animals.

Source of variation	*df*	SS	MS	$\bar{x}$ No. mosq. attracted by animal hosts compared to humans**
Total	49	3177.9	....	guinea pig 6.6[a]
Replicates	9	644.5	71.6	
Hosts	1	2090.9	2090.9*	pigeons 5.8[ab]
(a) Human vs. animals				
(b) Among animals compared to human	2	128.1	64.0*	chickens 4.5[b]
Error	47	314.3	6.7	

* $P<.005$.

** Means followed by the same letter do not differ significantly at 5% level (Duncan's multiple range test).

TABLE 4a.—Analysis of variance of the *Aedes aegypti* females attracted to different species compared with their own kind.

Source of variation	*df*	SS	MS
Total	59	1352.9	....
Replicates	9	164.2	18.2
Hosts*	5	817.5	163.5**
Error	55	371.2	6.7

* (3 different animal species compared with their own kind).

** $P<.005$.

TABLE 4b.—Mean number of mosquitoes attracted to different animal species compared with their own kind.

guinea pig vs. guinea pig		chick vs. chick		pigeon vs. pigeon	
11.5	12.7	4.1	4.7	4.6	4.4

TABLE 5.—Analysis of variance of the *Aedes aegypti* females attracted to guinea pigs compared with chickens.

Source of variation	*df*	SS	MS
Total	19	212.2	...
Replicates	9	65.0	7.2
Hosts	1	72.2	72.2*
Error	9	75.0	8.3

* $P<.025$ (guinea pig $\bar{x}$ attractive 10.2, chicken 6.3).

coined by Roubaud (1920) which they characterize as unfortunate. A curious instance of host preference based on previous blood meals for a few generations from a particular host was reported by Galliard (1936) in his studies on *Culex fatigans* and confirmed by Wanson and Nicolay (1937). In general, attempts to show such a preference have failed and our strain of *A. aegypti* after innumerable generations and years of feeding on guinea pig blood still showed preference for man.

Humans were also found more attractive than pigeons or chickens. The skin temperature of the birds was 37–38° C compared to 33–34° C of humans. If the elevated skin temperature were any one factor responsible for greater attraction, then our results disprove it. While this work was near completion, McIver (1968) published on host preferences and discrimination by the mosquitoes. She compared mice with chicks and found mice more attractive. Her results, therefore, confirm that skin temperature alone does not contribute significantly to mosquito attraction.

The problem of host preference has always been of considerable interest from the point of malaria epidemiology and other mosquito-borne diseases. Investigators have tried different methods. Most studies have been carried out by the precipitin test method of identifying the source of the mosquito blood meal. Hu and Yu (1936) determined host preference by releasing hungry mosquitoes (*Anopheles hyrcanus*

sinensis) in a room containing several possible hosts and subsequently identifying the source of blood meal. Corradetti (1937) exposed a single batch of mosquitoes to a particular host and recorded the number biting. Rice and Barber (1937) interposed a mosquito cage between a man and a cow for ½ to 1 hour and determined the number feeding on each host. More recently McIver (1968) placed the host in sticky traps which revolved on a turntable in a cage full of mosquitoes. Our technique is yet another step in the same direction. We feel it is simple and fast, and can be conveniently used for testing host preferences by mosquitoes. However, as Bates (1949) pointed out, different species of mosquitoes will show different host behavior under given experimental conditions and we would agree with van Thiel (1939) in suggesting that generalizations from laboratory experiments to nature should be avoided.

SUMMARY. Host preferences of *Aedes aegypti* (L.) for several vertebrate hosts were studied using a 2 port cage. Hosts were tested in pairs and the mosquitoes in the cage had the opportunity to choose either host. Mosquito probing was used as the criterion of host preference. Humans, guinea pigs, pigeons and chickens were tested. Each species of hosts was compared with its own kind and with other species. Humans were most attractive compared to other hosts. Among humans highly significant differences in attractiveness were observed but no significant differences were observed when other vertebrate hosts were compared with their own kind. Compared to man, guinea pigs attracted more mosquitoes than did chickens. Among animals themselves guinea pigs were significantly more attractive than chickens or pigeons. It is concluded that attractiveness of a host can be best stated only in relative terms i.e. in relation to other hosts rather than in absolute quantities.

ACKNOWLEDGMENTS. This investigation was supported by the U. S. Army Medical Research and Development Command, Department of the Army, under contract no. DA-49-193-MD-2466.

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