MOSQUITO ATTRACTION TO SUBSTANCES FROM THE SKIN OF DIFFERENT HUMANS[1]

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ABSTRACT. Mosquito attraction responses to substances collected from human skin and placed on glass petri dishes were studied. Mosquito response varied according to the source of the substance. Substances removed from the head and hands elicited the greatest attraction response in laboratory-reared mosquitoes. Mosquito response lasted up to 6 h when the substance was aged and was increased by warming the samples from ca. 25°C to 37°C. Of the 12 mosquito species studied, attraction response was greatest in *Aedes aegypti*. It is cautioned that residues deposited by handling traps or other apparatus used in mosquito studies may influence test results.

INTRODUCTION

Many efforts to determine how and why mosquitoes are attracted to a host are documented. Now and again the human skin has been studied as a source of mosquito attractants. Brown et al. (1951), Rahm (1958) and others found that axillary and palm sweat were attractive. Maibach et al. (1969) reported airborne odors, solvent washes and sweat originating from dry or moist skin were attractive. Our experiments with sweat from hands in polyethylene gloves led to studies of mosquito response to substances collected from glass beads previously rubbed between the hands (Schreck et al. 1981). We showed that the attractant was transferable from skin to a glass surface, which then became attractive to mosquitoes. We also showed that the attractant could be transferred from the glass into a solvent. Bioassays were performed in a dual-port olfactometer (Schreck et al. 1967) where mosquitoes flew upwind to the attractive source. The attractive source was air passed over the glass beads after the beads had been handled, or air passed over glass beads treated with the attractive substances in a solvent.

We believe that these attractive substances will require extensive analyses to define both their chemical identity and their biological activity. The purpose of our current study was to identify some of the factors that will affect experimental error in the bioassay segment of these analyses.

Specifically, we sought to determine: 1) whether substances collected from the skin of different human beings varied in attractiveness to the same species of mosquito; 2) whether the aging or heating of these substances on a glass surface altered mosquito response; 3) the relative attractiveness to mosquitoes of these substances when they are collected from different body areas of the same human; and 4) the responses of different species of mosquitoes, and of mosquitoes of different ages and varied rearing conditions, to these substances.

MATERIALS AND METHODS

We tested a new bioassay procedure to study mosquito response to attractive vapors in still air. An adult mosquito holding cage (37 × 38 × 46 cm) was used as a test chamber. The cage was made of aluminum (bottom), clear acrylic plastic (2 sides) and fiberglass screen (back and top). A stockinette sleeve was attached to the front of the cage to provide access. Contact of the test cage, or of any of the experimental apparatus, with human skin during the bioassay was averted by using disposable polyethylene gloves. Additional apparatus used included a battery powered aspirator, 100 × 15-mm glass petri dish bottoms (dish), 11 × 14-cm squares of white bond paper, liquid dish washing soap (unscented), Cole-Parmer digital thermister thermometer and a vacuum oven maintained at 80°C for holding clean, washed dishes.

Bioassay procedure: The bioassay comprised 6 steps: 1) Fifty 6- to 7-day-old laboratory-reared female *Aedes aegypti* (Linn.) or *Anopheles quadrimaculatus* Say mosquitoes fed 10% sucrose *ad libitum*, but not blood, and which responded to attractive vapors from the hand (Posey and Schreck 1981) were collected from a holding cage. Mosquitoes were reared under standardized conditions after the method of Dame et al. (1978) and held in cages at 27°C and 70% RH until testing. It was assumed that each test population would respond in a similar manner. 2) Mosquitoes were placed in the test cage without food or water for 30 min at ca. 25°C and 44% RH. 3) A clean dish was removed from the vacuum oven and inverted on a paper square in the room for ca. 10 min (the time required for a heated dish to cool to room temperature or 24–25°C). 4) The untreated dish was then inverted

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on a paper square in the test cage for 3 min and then removed. During this time, the number of mosquitoes which landed on or attempted to probe the dish were counted. 5) A second clean dish from the vacuum oven was prepared as in (3), then was rubbed in the bare hands of a volunteer for 3 min, allowed to cool to room temperature for 3 min, then placed in the same test cage for 3 min, and the number of mosquitoes landing/attempting to probe the dish were counted. 6) All mosquitoes were aspirated from the test cage and counted. Mosquito response to each dish was calculated as the proportion:

$$\% \text{ responding} = \frac{\text{No. responding to treatment} - \text{No. responding to control}}{\text{No. in cage}}$$

In the first experiment, we determined whether *Ae. aegypti* and *An. quadrimaculatus* mosquitoes responded differently to substances from the skin of different humans and whether aging and temperature of these substances on glass affected mosquito responses. Two male volunteers each handled a dish. In separate cages, each dish was tested after it had cooled for 3 min to room temperature (24–25°C). Each handled dish was then rewarmed and retested in its respective cage after 3, 30 and 60 min, and at 1-h increments thereafter up to 6 h. Untreated control dishes were also tested at the corresponding time intervals. Between tests the dishes were placed in the room on a clean paper square. Dishes were rewarmed using a 7.7 × 1.2-cm aluminum disc heated to 80°C and covered by (but not touching) the inverted dish at the start of the 3-min test. A thermal surface probe from the thermister thermometer was placed on the dish to measure the radiant heat from the aluminum disc. The surface temperature of the dish rose from 24°C to 33°C in the 1st min, 33°C to 35°C in the 2nd min, and 35°C to 37°C in the 3rd minute. Using the same time regimen each day, tests were replicated 6 times by making new collections from the hands of each volunteer and bioassaying them as described above each day for 6 days.

In the 2nd experiment, we attempted to characterize mosquito responses to substances from the skin of different body areas on the same volunteer. The experiment employed a split plot design to characterize mosquito responses to 5 body parts of 8 volunteers (4 white and 4 black). Substances were removed from the hands, forearm, face, lower leg and abdomen by rubbing each body area with a separate clean dish for 3 min and tested at room temperature. Eight male volunteers aged 38 to 60 years were used in the experiment, and each volunteer rested for ≥ 20 min before the collections were made. Except for the collections from the hands, each volunteer wore gloves when transferring the material from the different body areas to a dish. Logistics required that collections from the various volunteers and their body areas be made in a nonexplicit randomized sequence; thus, some people did not always participate at the same time or on the same day. Collections were bioassayed in a cage of mosquitoes assigned to each volunteer for that day, and a series of bioassays was performed each day for 6 days for each volunteer. Diet of the test volunteers was not taken into account in this study.

In the 3rd experiment, we tested the responses of 12 species of laboratory-reared or wild mosquitoes (collected with sweep nets or with CO_2-baited traps) to substances from the hands of the volunteer that, in previous tests, showed the highest attractiveness to mosquitoes. Tests were made at room temperature (24–25°C) and when dishes were warmed to 36–37°C.

The percent response data from the first 2 experiments was transformed using the inverse sine and analyzed using the analysis of variance procedure (PROC ANOVA, SAS Institute 1985). For experiment 2, differences in race, subject, and body part were determined using Duncan's multiple range test. Because of the nonstandard error structure produced by the split plot design used in experiment 2, SAS test statements were used in order to specify the use of nonresidual error terms in the F value and means discriminatory procedure calculations for the main plot effects of race (TEST H = RACE E = SUBJECT (RACE)), subject (TEST H = SUBJECT E = REP (SUBJECT)) and subplot effects of body part (TEST H = PART E = SUBJECT* PART (RACE)). Sample size in the 3rd experiment was too small to permit statistical analysis.

RESULTS

Experiment 1: There was a significant difference ($P = 0.0001$) in the attraction responses of *Ae. aegypti* to substances from the hands of different volunteers. At room temperature, these differences remained significant throughout the 6-h test period (Fig. 1). When dishes were warmed, mosquito attraction responses among substances from each volunteer were significantly different (Fig. 1). Mean attraction response to the control dishes ($n = 96$) was 0.34 ± 0.88% at room temperature, and 3.3 ± 3.8% when dishes were warmed.

Attraction responses of *An. quadrimaculatus* varied significantly among volunteers, regardless of the temperature of the dish, up to 4 h. The data indicate *An. quadrimaculatus* was less sensitive overall to the attractant than was *Ae. aegypti.* In these tests, mosquito response to the control dish at room temperature was 0.0; when

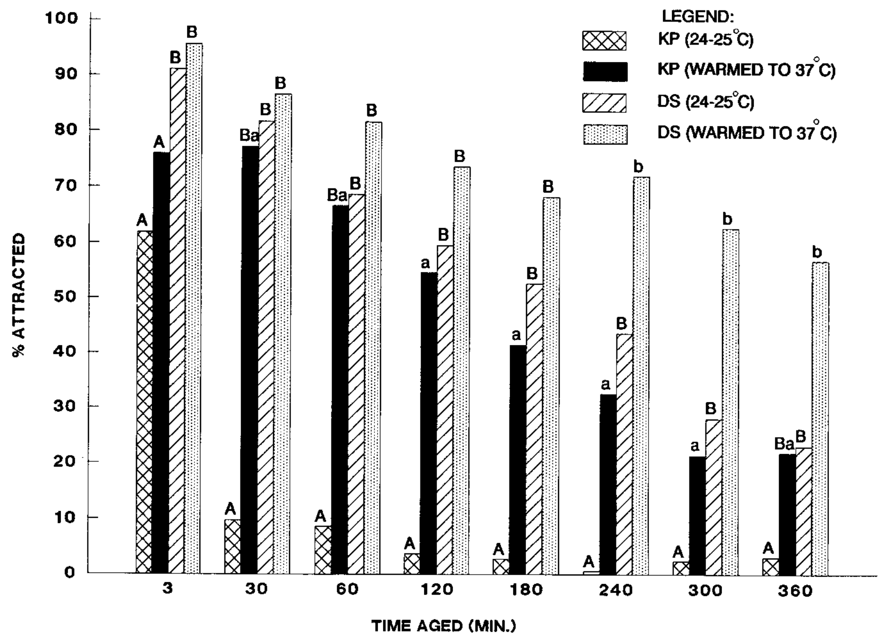

Fig. 1. Attraction responses of *Aedes aegypti* mosquitoes to warmed or unwarmed substances (aged 3–360 min) from human skin of 2 different subjects. Bars within each time interval with the same upper case letter are not significantly different ($P = 0.05$).

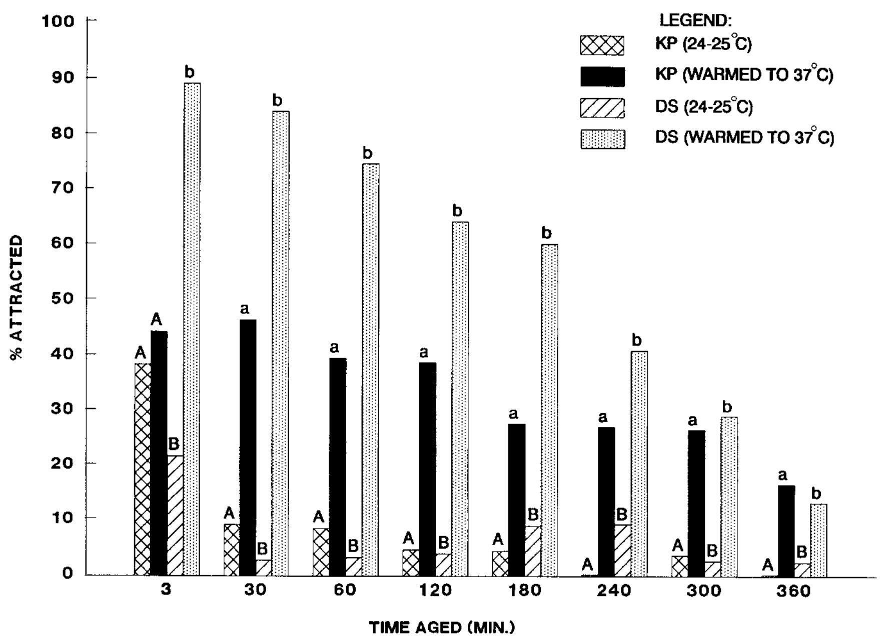

Fig. 2. Attraction responses of *Anopheles quadrimaculatus* mosquitoes to warmed or unwarmed substances (aged 3–360 min) from human skin of 2 different subjects. Bars within each time interval with the same upper case letter are not significantly different ($P = 0.05$).

warmed, the mean response was 1.6 ± 2.8% (Fig. 2).

Experiment 2: Mosquito attraction responses to substances collected from different body areas on human subjects were significantly different ($P = 0.001$) (Table 1). Responses to substances from subject DS were consistently higher than for other participants. Responses to substances

Table 1. Mean attraction responses (± SE)* of *Aedes aegypti* to substances from the skin on 8 body areas deposited on glass petri dishes.

	Mean % attraction to (substances from) the indicated body region					
Subject	Arm	Face	Hand	Leg	Abdomen	x̄
ds**	77.5 ± 6.1 a	89.7 ± 2.4 a	72.3 ± 15.1 a	63.8 ± 14.4 a	46.7 ± 5.0 a	70.0 ± 5.9 A
fw**	51.5 ± 12.5 ab	69.5 ± 6.5 ab	68.7 ± 13.9 a	40.0 ± 10.0 ab	20.2 ± 5.3 a	49.9 ± 5.5 B
mb**	38.8 ± 9.3 b	50.7 ± 6.9 bcd	33.8 ± 15.5 b	14.3 ± 3.7 cd	45.2 ± 10.5 a	36.6 ± 4.7 BC
jm**	32.7 ± 12.0 bc	56.0 ± 16.3 bc	34.3 ± 11.2 ab	43.0 ± 9.5 ab	43.0 ± 12.9 a	41.8 ± 5.5 BC
hm***	28.2 ± 7.0 bc	38.0 ± 7.7 cde	47.8 ± 9.3 ab	27.7 ± 5.4 bc	44.7 ± 4.0 a	37.3 ± 3.3 BC
jw***	23.7 ± 12.1 bc	20.3 ± 4.2 e	39.5 ± 8.4 ab	22.3 ± 10.1 bcd	42.5 ± 10.0 a	29.7 ± 4.3 CD
kp***	23.5 ± 3.5 bc	24.3 ± 7.0 e	32.7 ± 4.4 ab	22.0 ± 5.6 bcd	35.3 ± 4.5 a	27.6 ± 2.4 CD
cs***	9.5 ± 4.8 c	26.0 ± 13.2 de	36.5 ± 4.2 ab	7.7 ± 3.1 d	33.7 ± 5.5 a	22.7 ± 3.0 D
x̄	35.7 ± 4.1 BC	46.8 ± 4.2 A	45.7 ± 4.2 AB	30.1 ± 3.4 C	38.9 ± 3.2 ABC	

* Data transformed by arc sine. Means among subjects (columns) followed by the same letter are not significantly different. Means among body areas within subjects (rows) followed by the same letter are not significantly different ($P = 0.05$).

** Black (Negro) volunteer subjects.

*** White (Caucasian) volunteer subjects.

Table 2. Attraction responses of 12 species of mosquitoes to substances from the skin of humans.

	Mean attractive response in 3 min	
Species	25°C	36–37°C
*Aedes albopictus**	+++	+++++
Ae. aegypti	+++++	+++++
*Ae. aegypti***	+++	+++++
Ae. taeniorhynchus	+	++
Anopheles quadrimaculatus	++	+++++
An. freeborni	+	++++
An. albimanus	+++	++++
*An. crucians**	N	N
*Coquillettidia perturbans**	N	N
*Culex quinquefasciatus**	+	+
*Cx. salinarius**	+	+
*Cx. nigripalpus**	N	N
Mansonia spp.*	N	N

* Field collected.

** Field collected but data represent only 1 test. + = <10%; ++ = >10%; +++ = >30%; ++++ = >50%; +++++ = >80%. N = No response.

from CS were significantly lower than for all other participants. There was significant variation in mosquito response to substances from different body areas on the same subject except for the abdomen. Responses were generally highest for substances removed from the face (46.8%) and hands (45.7%) and lowest for substances removed from the leg (30.1%). The statistical analysis did not provide sufficient evidence to suggest racial differences in attractiveness of the volunteers. Mosquitoes responded to control dishes in 0.33 ± 0.99% of the tests (n = 240).

Experiment 3: The attraction responses of 12 mosquito species to substances removed from human skin are shown in Table 2. Except for *Ae. albopictus* (Skuse), which was field-collected then reared in the laboratory for several generations, all field-collected mosquitoes were adults of unknown age. Only *Ae. albopictus* and *Ae. aegypti*, of the species we collected in the field, responded to the warmed dish. This may in part be due to host preference characteristics of some species. Among laboratory-reared species, responses ranged from < 10 to > 80% at room temperature and > 10% to > 80% when dishes were warmed. *Aedes aegypti* responded (> 80%) to test samples at both room temperature and when warmed.

DISCUSSION

Differences among humans in attractiveness to host-seeking mosquitoes is documented. Collectively, these differences are attributed to variations in skin temperature, skin color, sex, age, body odor and other factors (Khan 1977). However, the unique role, if any, of each of these factors in the mosquito attraction response is not understood. The results of our study suggest that differences in the mosquito attraction response to substances from the skin of different human beings is evident in the substance isolate alone. Therefore, it should be possible to characterize, chemically and biologically, some or all of these substances.

Wright (1975) described mosquito attraction as a response to warmth and humidity but doubted the need to search for a particular skin odor as a mosquito attractant. Furthermore, Wright (1975) suggested that "... an optimum combination of warmth and humidity is more attractive than the most attractive arm or hand." Price et al. (1979) concluded that female *An. quadrimaculatus* were attracted to human hosts primarily by chemicals emanating from the skin. Our tests demonstrate that heat is not necessary to elicit an attraction response in mosquitoes. Over time, in some cases, heat does increase the mosquito attraction response to dishes (*Ae. aegypti*) or to recently handled dishes (*An. quadrimaculatus*). However, warmed control dishes do not evoke this response.

Anthropophilic feeding preferences of one species over another may explain the difference in magnitude of response between *Ae. aegypti* and *An. quadrimaculatus.*

Our data suggest that substances from human hands may contaminate test equipment, mosquito cages and other apparatus used to study mosquito behavior. Attractive residues deposited by handling may affect mosquito responses to traps. Thus, traps handled frequently or recently may catch a disproportionate share of mosquitoes. This possibility should be considered by those involved in studies of the host-seeking behavior of mosquitoes.

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