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PROBING AND GORGING RESPONSES OF THREE MOSQUITO SPECIES TO A MEMBRANE FEEDING SYSTEM AT A RANGE OF TEMPERATURES

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ABSTRACT. Membrane feeding of 3 mosquito species, *Aedes aegypti, Anopheles stephensi,* and *Anopheles arabiensis,* with formulated protein meals was carried out at a range of temperatures. The response was evaluated in terms of probing after 5 min, engorgement after 90 min, and 50% feeding time. *Aedes aegypti* showed a satisfactory feeding response across the complete temperature range investigated, 28–40°C, although engorgement was significantly faster between 36 and 40°C. *Anopheles stephensi* fed best between 32 and 40°C. *Anopheles arabiensis* showed a poor probing and feeding response at all temperatures, although the best responses were recorded at 38–40°C.

INTRODUCTION

Investigations of mosquito feeding behavior have identified factors that affect probing, gorging, and egg production. Environmental factors such as CO_2 concentration (Bar-Zeev et al. 1977) and volatile compounds collected from the skin of human subjects (Schreck et al. 1990) have been identified as influencing probing and gorging. Variations in the protein meal taken through a membrane, comparing different bloods or different blood fractions, also affect the fecundity and fertility of mosquitoes (Rutledge et al. 1964, Bunner et al. 1989). The temperature of the protein meal provided in membrane feeders is important to produce the best probing and gorging response within the colony. Membrane feeding systems with the capacity to heat the meal *in situ* have adopted a temperature of about 38°C for feeding *Aedes, Anopheles,* and *Culex* species (Rutledge et al. 1964; Galun et al. 1985, 1988). Whether the authors identified this as the best membrane temperature is not recorded. The present study investigates this question, using 3 species, *Aedes aegypti* (Linn.), *Anopheles stephensi* Liston, and *Anopheles arabiensis* Patton, fed with the membrane system described by Cosgrove et al. (1994), which allows close control of temperature. For this, we have used 2 artificial protein formulations, one for *Aedes aegypti* and another for the 2 species of *Anopheles.*

MATERIALS AND METHODS

All mosquitoes were reared at 28 ± 2°C and 80 ± 5% RH, in a 12-h day/night cycle beginning at 0630 h. *Aedes aegypti* larvae were fed dog biscuit; *An. arabiensis* and *An. stephensi* were fed Tetramin® tropical fish food. The mosquitoes were 7 days old and sugar-starved for 24 h before the start of each experiment. All experiments were carried out between 1000 and 1400 h.

The feeding units are electronically controlled and thermally insulated. They incorporate a detachable 5-ml aluminum reservoir to contain blood or a blood substitute, behind a collagen membrane. Approximately 50 mosquitoes can feed simultaneously on each unit. The temperature of each unit can be maintained within ±0.1°C and varied from 1 to 20°C above ambient room temperature (Cosgrove et al. 1994).

Artificial meals were formulated for each species based on bovine proteins. The type of meal given to the 2 *Anopheles* species was identical. The meal given to *Ae. aegypti* contained less albumin, and ATP was added as a phagostimulant (Galun and Rice 1971). Full details of these and other formulations, and how they were evaluated in terms of fecundity and fertility, are to be published separately. The aim here was simply to investigate the effect of temperature on feeding upon the best artificial media available. Each species was maintained on its appropriately formulated diet for at least 3 generations before the investigation commenced. Experiments were performed in 20-cm³ cages containing 10 mated female mosquitoes. The temperatures of individual feeding units were set at 28, 30, 32, 34, 36, 38, and 40 ± 0.1°C before the experiment with a Fluke 51 electronic thermometer and checked again upon completion. The units were allowed to warm up for 15 min before being placed on the cages. The mosquitoes were then able to probe up through the cage netting and gain access to the protein meal behind the collagen membrane. Observations of the number of mosquitoes probing the membrane, and the number in the process of gorging, were made over a 90-min period. The number of engorged females was recorded at the end of the experiment. Engorgement was defined as being com-

Table 1. Mean number of females probing after 5 min, engorged after 90 min, and 50% feeding time for the 3 species investigated. Each value is based on 7 replicates of 10 females.

Temperature (°C)	Mean no. probing (± SE)	Mean no. engorged (± SE)	50% feeding time (min ± SE)
		Aedes aegypti	
28	6.00 ± 0.53a[1]	9.00 ± 0.43d	29.29 ± 7.27e
30	6.71 ± 1.02ab	8.57 ± 0.68d	22.21 ± 5.13ef
32	8.14 ± 0.51abc	9.42 ± 0.30d	19.00 ± 1.38ef
34	8.29 ± 0.68abc	8.85 ± 0.34d	21.00 ± 2.85ef
36	9.00 ± 0.31bc	9.14 ± 0.40d	13.20 ± 2.15ef
38	8.57 ± 0.57abc	9.28 ± 0.47d	12.21 ± 1.39f
40	9.57 ± 0.20c	9.28 ± 0.26d	12.21 ± 1.62f
		Anopheles stephensi	
28	3.43 ± 0.72g	6.14 ± 0.80hi	92.43 ± 28.3j
30	3.71 ± 0.81g	6.00 ± 1.20h	63.42 ± 14.2jk
32	3.71 ± 0.18g	8.14 ± 0.80hi	37.50 ± 3.99k
34	5.29 ± 0.68g	8.71 ± 0.52hi	41.71 ± 6.16jk
36	5.57 ± 0.48g	7.57 ± 0.57hi	31.64 ± 4.58k
38	5.14 ± 0.91g	8.86 ± 0.26hi	32.86 ± 2.04k
40	5.86 ± 0.63g	9.14 ± 0.34i	27.93 ± 3.20k
		Anopheles arabiensis	
28	2.86 ± 0.91m	4.29 ± 1.34n	65.25 ± 18.40p
30	2.00 ± 0.87m	3.57 ± 1.13n	73.50 ± 7.84p
32	5.00 ± 0.76m	4.43 ± 1.27n	61.20 ± 4.80p
34	5.29 ± 0.57m	5.29 ± 1.06n	76.17 ± 12.30p
36	5.00 ± 1.20m	5.86 ± 0.88n	69.83 ± 9.83p
38	5.00 ± 1.36m	6.29 ± 1.29n	55.17 ± 6.38p
40	5.86 ± 0.74m	6.43 ± 0.68n	55.29 ± 7.73p

[1] Letters in common indicate no significant difference ($P < 0.05$) when tested by one-way ANOVA followed by Tukey's HSD multiple comparison test.

pleted when an undisturbed mosquito, with a clearly expanded scarlet abdomen, withdrew its proboscis and showed no further interest in feeding. The experiment was repeated 7 times at each temperature for each species. Differences in the 5-min probing response, the number of females engorged after 90 min, and the 50% feeding time were tested by one-way ANOVA followed by Tukey's HSD multiple comparison test.

RESULTS

Five-minute probing response: Of the 3 species, *Ae. aegypti* showed the highest probing response after 5 min at all temperatures (Table 1). Each species showed a positive correlation between probing response and temperature (correlation coefficients: 0.94, $P < 0.001$, for *Ae. aegypti*; 0.91, $P < 0.001$, for *An. stephensi*; 0.81, $P < 0.02$, for *An. arabiensis*). In *Ae. aegypti* the extreme temperatures were significantly different, between 28 and 36–40°C.

Mean number of females engorged after 90 min: Engorgement by *Ae. aegypti* on its formulation was always greater than in the 2 *Anopheles* species on their formulation at all temperatures. No significant differences were observed between the mean number of *Ae. aegypti* females engorged at each temperature after 90 min (ANOVA, $P > 0.05$) (Table 1) and there was no correlation between temperature and numbers of females engorged (correlation coefficient: 0.52, $P > 0.05$). Out of 10 females in each replicate, a mean ± SE of 9.07 ± 1.11 females became engorged (all replicates at all temperatures combined). The mean number of *An. stephensi* females engorged at 28 and 30°C was 6.14 and 6.0, respectively, significantly less than the mean number of 9.14 engorged at 40°C (ANOVA, $P < 0.05$) (Table 1). The mean number of females engorged after 90 min was significantly correlated with the temperature of the substitute meal for *An. stephensi* (correlation coefficient: 0.85, $P < 0.01$). Although in *An. arabiensis* there were no significant differences between the mean number of females engorged at the different temperatures after 90 min (ANO-

VA, $P > 0.05$) (Table 1), there was a highly significant correlation with temperature (0.94, $P < 0.001$).

Fifty percent feeding time: Aedes aegypti fed more quickly on its formulation than the 2 *Anopheles* species on theirs, at all temperatures. *Aedes aegypti* and *An. stephensi* showed a significant negative correlation between 50% feeding time and membrane temperature (correlation coefficients: -0.93, $P < 0.01$, for *Ae. aegypti*; -0.85, $P < 0.01$, for *An. stephensi*). The 50% feeding time for *Ae. aegypti* was significantly different between 28 and 38°C (Table 1) and in *An. stephensi* between 28 and 32°C (ANOVA, $P < 0.05$) (Table 1). The shortest 50% feeding time in both species lay within the range 36–40°C. *Anopheles arabiensis* females showed no significant variation within the temperature range investigated (ANOVA, $P > 0.05$) (Table 1) and no significant correlation with temperature. On 5 occasions (out of 49) the 50% feeding time could not be calculated for *An. arabiensis* because no feeding occurred.

DISCUSSION

Prior to this investigation, Khan and Maibach (1966) demonstrated how the probing response of *Ae. aegypti* was increased by the addition of moisture to a heated metal target. Further work (Khan and Maibach 1971) confirmed these results and delineated the range of temperatures conducive to probing within a 3-min exposure period, identifying 34–36°C as the optimum probing temperature for *Ae. aegypti* responding to a moist, heated target within a temperature range of 28–40°C. Gillett and Connor (1976) found no difference in probing response to tubes carrying water warmed to 38 and 41°C. Our results agree in showing a significant increase in the probing of *Ae. aegypti* females between 28 and 36–40°C but no increase between 32 and 40°C. A similar result was reflected in the 50% feeding times, indicating a correlation between increased probing response and faster gorging over the whole range. However, the mean number of females engorged after 90 min showed no correlation with temperature, and there was no significant difference between the numbers of females engorged after 90 min at each temperature. We conclude that with respect to feeding, rather than simply probing, *Ae. aegypti* responds satisfactorily at any temperature within the range 28–40°C, although feeding is faster at 36°C and above.

Our study shows that *An. stephensi* feeds much more slowly than *Ae. aegypti*, although it should be noted that the protein meal presented to *Ae. aegypti* was less viscous and contained ATP. The cheaper *Aedes* meal was unsatisfactory for *Anopheles*, in terms of fecundity and fertility and cannot therefore be recommended. Judged on the index of mean females engorged after 90 min or 50% feeding time, the preferred temperature range for *An. stephensi* is between 32 and 40°C. Temperatures below 37°C have not been previously recommended for feeding *Anopheles* species with membrane systems. This study demonstrates that such a high temperature is not necessarily the most efficient. *Aedes aegypti* and *An. stephensi* can both feed satisfactorily below this temperature. The slower feeding rate is more than compensated by the extra time the protein meal remains in a suitable condition within the feeding units. Feeding at lower temperatures allows a greater number of individuals to be exposed to a potential meal during the period they are most stimulated to feed.

When presented with precisely the same formulation as *An. stephensi, An. arabiensis* showed a poor feeding response at every temperature investigated. However, the significant correlations observed, with respect to the probing response and mean females engorged after 90 min, indicate that the species does respond to increasing temperature. To produce the greatest feeding response from *An. arabiensis*, the temperature of the meal needs to be set at 40°C, a higher temperature than used in the past to feed *Anopheles* species. Membrane feeding at 40°C does, however, require the use of a substitute protein meal. We have shown that defibrinated blood heated to 40°C is no longer in a suitable condition for *An. arabiensis* after 30 min, having formed a viscous layer at the membrane interface that makes it impenetrable to mosquitoes. By contrast, protein meals can be heated to 40°C for 60 min and still remain suitable. The extended period in which individuals can successfully gorge on a formulated protein meal, heated to 40°C, is a significant advantage for feeding the species.

This study was carried out at a consistent laboratory light intensity, at a defined time in the light cycle. The influence of light on feeding behavior was thus controlled to an extent. However, feeding may not have taken place under optimal conditions for each species. The efficiency of the system might be increased if feeding were carried out in different light intensities or at different times in the cycle. We chose these particular conditions because, before the experiments, each of the 3 species had been maintained for several years on animal hosts under the same regime. Improvements in the efficiency of the feeding system are particularly desirable in the case of *An. arabiensis*. Despite the relatively poor response of this species in the pres-

ent experiments, it is routinely and satisfactorily maintained in our laboratory using the formulated protein meal.

A significant increase in 5-min probing response with increasing temperature in all 3 species is consistent with the hypothesis that a temperature differential between the membrane and the environment is influencing attraction, rather than simply the absolute temperature of the membrane. Studies in different ambient temperatures are required to address this point. Price et al. (1979) investigated the attraction of *Anopheles quadrimaculatus* Say to a variety of environmental variables present or absent from an air stream, including temperature, either warmed (32–35°C) or cold (26.4°C). He concluded that factors other than CO_2, water, and heat were involved in the attraction of the mosquitoes to a host. Volatile compounds, such as those Schreck et al. (1990) isolated from human skin, might increase the efficiency of the feeding system.

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