

OPERATIONAL AND SCIENTIFIC NOTES

INDUCED COPULATION OF MOSQUITOES:
EFFECT OF HUMIDITY ON INSEMINATION

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The successful laboratory colonization of most ancline mosquitoes is dependent on a reliable method of manually inducing copulation. The basic technique was developed some years ago at the University of Illinois (McDaniel and Horsfall, 1957). Horsfall and Taylor (1967) modified the technique and used it successfully to mate a number of species of mosquitoes. A variety of anesthetics have been employed from time to time by different investigators. Nitrogen for males and ether for females was used by Spielman (1964). Nitrogen, carbon dioxide, ether, and cyclopropane were successfully used by Jones and Wheeler (1965). Horsfall et al. (1973) reported that high humidity enhanced results.

The inland floodwater mosquito *Aedes vexans* (Meigen) was used in this study. Larval rearing and the technique of induced copulation are described by Horsfall et al. (1973). Additions to the method discussed herein increase the number of females inseminated and the number of spermathecae filled. The changes indicated are (1) management of the humidity in holding cages prior to copulation, and (2) addition of moisture to the stream of carbon dioxide.

Humidity in the holding cages for females gives best results when maintained at saturation. That in the cage holding males should allow variation between saturation and approximately 50% RH. Suitable levels of humidity can be maintained in holding cages made of lucite with vents. Cages for both males and females are provided with a wick of cellulocotton extending upward from a small well of water. Vents in the cages containing males are open to ambient humidity. Vents in the cages containing females are kept closed and additional wicks assure a humidity at or near saturation. Both sexes are provided honey diluted 1:10 with water as a source of carbohydrate. A total of 220 matings and subsequent dissections were made in this study. All matings were 1:1, that is, 1 male to each female. The anesthetic was humidified by passing the CO₂ through water held in a wash bottle fitted with a fritted disc. Two modifications were tested against the standard holding (saturated cage conditions) and anesthetizing technique (dry CO₂) as is indicated in Table 1.

STANDARD HOLDING AND ANESTHETIZING

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METHOD. Results in insemination were erratic when males and females were held in saturated (100% RH) cages and females were anesthetized with dry CO₂ (Table 1). Females joined 2 days after emergence exhibited 60% insemination; of these, 30% had 1 spermatheca filled, and 30% had 2 of them filled. The percentage of females inseminated reached the 90-100% level in the group 3-5 days old; among these, 70-80% had two spermathecae filled. Ninety percent of the females that were joined 6-8 days after emergence were inseminated, and all had two spermathecae filled.

MODIFICATION 1, HUMIDIFIED CO₂. All females were inseminated when the stream of CO₂ was humidified. The percentage of females having two spermathecae filled also increased as compared to those anesthetized with dry CO₂. As the age of the females increased the consistency of filling both spermathecae increased (Table 1). In females 2 days old, 60% had two spermathecae filled. However, 100% of those 6-8 days had two spermathecae filled.

MODIFICATION 2, HUMID CO₂ AND MALES AT 50% RH. The filling of all three spermathecae and 100% insemination occurred when males were kept in cages where humidity ranged from 50 to 100% and females were kept in other cages maintained at 100% RH. Under these conditions all of the 24-hour-old females joined were successfully inseminated. However, not all spermathecae were filled, thus 20% had one filled, 70% had two and 10% had all three (Table 1). Fifty percent of all the females 2 days old and older had all three spermathecae filled. The data indicate that the maximum percentage of insemination where all spermathecae are filled occurred when females were 3-5 days old.

The role of humidity in manually induced copulation significantly affects the degree of insemination. The elimination of erratic insemination and the capability of filling all three spermathecae was established by humidifying the anesthetic and by separating males into cages where optional humidity from ambient to saturation was maintained and females into cages where humidity was held at 100% RH. The adjustment of humidity also produced an increase in response and vigor of both males and females to forced copulation.

Literature Cited

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Table 1. Effect of humidity on the rate of insemination of *Aedes vexans* by manual copulation.

Age of females copulated (days)	Number dissected	Percentage of females inseminated based on number of spermathecae (Sp) filled												
		Humidity of holding cages: males 100%, female 100%					Humidity of holding cages: optional male, 100% female							
		Anesthetic: dry					Anesthetic: humid							
		Sp 1	Sp 2	Sp 3	Total	Total	Sp 1	Sp 2	Sp 3	Total	Total			
1	10	20	70	10	100
2	10	30	0	60	60	0	100	..	10	40	50	100
3-5	30	20	70-80	90-100	..	20	80-100	0	100	10-20	40-70	30-50	100	100
6-8	30	0	90	90	..	0	100	0	100	10-30	20-70	20-50	100	100

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NOTES ON THE HOST RANGES OF SOME PHLEBOTOMINE SAND FLIES IN PANAMA

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Young and Fairchild (1974) have compiled a key to nearly 60 species of New World sand flies (Diptera, Psychodidae) known to bite man. The 7 species *Lutzomyia gomezi* (Nitzulescu), *L. olmeca* (Vargas and Diaz-Najera), *L. panamensis* (Shannon), *L. pessoana* (Barretto), *L. sanguinaria* (Fairchild and Hertig), *L. trapidoi* (Fairchild and Hertig) and *L. ylephiletrix* (Fairchild and Hertig) are major man-biting species in Panama (Chaniotis et al. 1971). The reports of Thatcher and Hertig (1966), Tesh et al. (1971), 1972), Christensen et al. (1972) and Christensen and Herrer (1973) indicate that such species feed on a wide range of vertebrate hosts. The host ranges of many of the more strictly zoophilic species are not well known. Since sand flies transmit American leishmaniasis among animals and from animals to man, the following notes on the host ranges of several species are submitted for the record.

ANTHROPOPHAGY IN *L. camposi* (RODRIGUEZ).
On 22 January 1971 a specimen of *L. camposi* was taken in a biting collection on Farfan Hill at the Pacific Terminus of the Panama Canal. The area is open and grassy with a few scattered trees. The collection was made on a hot, sunny afternoon in the partial shade of a small tree. This species has not been previously reported as biting man. The only other known host is the horse (Christensen and Herrer 1973).

SAND FLIES ATTRACTED TO HORSE-BAITED TRAPS. Christensen and Herrer (1973) listed 12 species of sand flies collected from horses in Panama. The following additional species have been taken from horse-baited mosquito traps operated in the Canal Zone by the Office of the Chief

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