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INTRASPECIFIC COMPETITION IN *ANOPHELES STEPHENSI* LISTON

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ABSTRACT. When reared in food and space limited environments, the INDIA strain of *Anopheles stephensi* took longer to mature, had increased larval and pupal mortality, produced adults which were smaller in size, had proportionately fewer males in the population, and produced females which imbibed less blood, produced fewer eggs which were shorter in length, and exhibited reduced survival than non-stressed individuals. The percent weight gained from emergence to blood feeding, the proportion of the females taking a blood meal, the percent laying eggs after 48 hours, and the viability of these eggs were independent of larval rearing density. Larvae raised in previously conditioned larval rearing water developed faster than those reared in distilled water, while those reared in water conditioned under uncrowded conditions developed the fastest and had the lowest mortality. All females preferred to oviposit in conditioned water, and those females reared under crowded conditions seemingly preferred to oviposit in uncrowded water. The relationship of these results with population dynamics is discussed.

Recent laboratory studies of intra- and interspecific competition among culicine mosquitoes have suggested that larval crowding elicits a "growth retardant factor" (GRF) which may function naturally to suppress mosquito numbers. GRF was produced only when larvae were crowded and starved (Jones 1960, Moore and Fisher 1969, Moore and Whitacre 1972) and when bacterial flora were present (Jones 1960). Some of the reported effects of larval crowding (presumably due to intraspecific competition for food and space and/or GRF production) included: 1) retarded larval development (Moore and Fisher 1969; many others), 2) increased larval mortality (Ikeshoji and Mulla 1970 a&b), 3) suppressed larval metabolism (Ikeshoji and Mulla 1970b, Barbosa and Peters 1973), 4) decreased pupal and adult sizes (Peters, et al. 1969; Wada 1965), 5) decreased blood feeding and fecundity (Bar-Zeev 1957), and 6) reduced adult survivorship (Ikeshoji and Mulla 1970a).

To date, this mechanism has not been adequately investigated in anopheline mosquitoes, although some negative effects of anopheline larval crowding and diet have been reported by Terzian and Stahler (1949), Galliard and Galvan (1957), Meller (1962), Thompson and Bell (1968), and Nayar and Sauerman (1970). The purpose of this investigation was to evaluate the effects of intraspecific larval competition as a population regulatory mechanism in *Anopheles stephensi* Liston, and to detect possible GRF production.

MATERIALS AND METHODS

STRAIN. The Delhi, India strain of *A. stephensi* was used throughout and was the same strain used in other investigations in this and other laboratories (Gerberg et al., 1968; Thompson and Bell 1968; Nasir 1970; Rutleldge et al., 1970; Clyde et al., 1973; McCarthy and Clyde 1973; etc.). This strain has been maintained at the University of Maryland Medical School for the past 6 years.

LARVAL REARING. First instar larvae within 4 hr of eclosion were pipetted into 16x16 cm plastic boxes (surface area = 256 cm^2) filled with 260 ml distilled water. Numbers per box were 26, 130, 195, 260, 520 and 780 yielding densities of 0.1, 0.5, 0.75, 1.0, 2.0 and 3.0 larvae/cm^2 or

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ml, respectively (referred to hereafter as L/cm^2). Larvae were fed a 1:2 by-weight mixture of edible liver powder (Rich Life Nutritional Laboratory, El Monte, Calif.) and mink chow (Ralston-Purina Co., St. Louis, Mo.) passed through a No. 40 U. S. Standard Sieve (Dual Manufacturing Co., Chicago, Ill.) according to the ration schedule in Table 1. Food rations were calculated by surface area (or volume) as was suggested by Peters, et al. (1969) rather than by larvae. At the uncrowded densities (0.1 and 0.5 L/cm^2), food and space were considered in excess, while at the more crowded densities (1.0, 2.0 and 3.0 L/cm^2) food and space were limited according to the optimum rearing conditions (about 0.7 L/cm^2) suggested by Gerberg, et al. (1968) and Thompson and Bell (1968).

PUPATION AND EMERGENCE. After the first pupae were observed, the rearing containers were inspected at 6-hr intervals at which time the pupae were counted into 1-pint cardboard cages containing distilled water. These cartons were also observed at 6-hr intervals and the numbers of emerging males and females recorded. Adults were transferred to screen cages (30x30x30 cm) where they were offered a 10% sucrose solution on cotton wicks. Mean insectary conditions ($\pm$ standard error of the mean) were: temperature = 26.41 $\pm$ 0.04° C., relative humidity = 74.56 $\pm$ 0.08%, photoperiod = 14:10 (L:D).

ADULT PARAMETERS. After ca. 30% of the adultls emerged, 15 newly emerged males and females were placed in a frost-free freezer (—15° C) overnight in covered petri dishes and weighed. Five days after 90% of the larvae had pupated, between 20 and 40 females per density were offered human blood meals for 30 minutes. Twelve replete females per density were then placed in individual oviposition cages for 5 nights (Nasir 1970). The remaining females were frozen overnight and weighed; those not feeding and those feeding were used to estimate the average pre- and post-blood meal weights, respectively, and the percent feeding. The average weight of the retained blood meal was calculated by subtraction. The total number of eggs laid per female was recorded daily. Approximately 40 eggs from each of 5 randomly chosen females per density were placed on distilled water in individual paper cups and the percent eclosion recorded, while the lengths of 15 fertile eggs from an additional 5 females per density were measured. Adult females were kept in the oviposition cartons for a total of 28 days after blood feeding to estimate survival. In the oviposition cages females were continuously offered 10% sucrose on cotton pads.

GRF ASSAY. Since the delay in larval development could have been caused by either competition for food and space or GRF production, 3 replicates of the water in which 0.1, 2.0 and 3.0 L/cm^2 were reared and distilled water controls were assayed to determine the effect of conditioned water on the rate of larval development. The water was filtered through No. 7760 filter paper (W. H. Curtin Co.) and stored at 5° C until all groups had pupated, therefore, the uncrowded groups were stored the longest. Larvae were assayed in 44 ml of undiluted rearing water in cardboard cartons (surface area = 44 cm^2) at the uncrowded density of 0.5 L/cm^2 surface area, and were fed the non-limiting ration of 0.1, 0.1, 0.2, 0.3, 0.4, and 0.5 mg^2/cm^2 on alternate days.

OVIPOSITION PREFERENCE. Three replicates of 10 blood-fed females reared at 0.5 and 2.0 L/cm^2 were tested for oviposition water preference. Females were placed in plastic containers (16x12x9 cm) and offered 0.1, 2.0 and 3.0 L/cm^2 conditioned rearing water and distilled water in oviposition cups (Nasir 1970). After 96 hours the females were removed and the total number of eggs per cup counted under a stereoscopic microscope.

STATISTICS. The median pupation (P_{50}) and emergence (E_{50}) times, calculated from eclosion, were estimated by probit analysis using the computer techniques described by Dixon (1974). The number of larvae successfully pupating (or pupae

Table 1. Larval ration in mg/larva per 2 days of a 1:2 by weight mixture of mink chow and liver powder; density changes due to larval mortality were not considered.

Day Fed	Ration (mg/cm^2 or ml)	Density (L/cm^2)					
		0.1	0.5	0.75	1.0	2.0	3.0
1	0.1	1.0	0.2	0.13	0.1	0.05	0.033
3	0.2	2.0	0.4	0.26	0.2	0.10	0.066
5	0.4	4.0	0.8	0.52	0.4	0.20	0.132
7	0.6	6.0	1.2	0.78	0.6	0.30	0.198
9 (to end)	0.5	5.0	1.0	0.65	0.5	0.25	0.165

successfully emerging) were used as the total number of individuals upon which the cumulative percent curves were calculated. This reduced mortality-related bias. Comparisons among larval rearing densities were made using the analysis of variance (ANOVA) and regression techniques described in Sokal and Rohlf (1969). Additional rearing containers were used at the original lower densities to provide sufficient numbers of adults, however, these were pooled and considered as a single unit in the analyses. Thus unless specified, the standard error of the means represented within-treatment variability based on randomly chosen subsamples.

RESULTS AND DISCUSSION

LARVAL DEVELOPMENT. The P_{50} values exhibited a significant positive linear regression and the duration of pupation as indexed by the slope of the fitted probit function (β) exhibited a significant negative regression with increasing larval density (Table 2). The relationship of larval mortality to density was not linear (Table 2). Undercrowding and excess food at 0.1 L/cm^2 and competition for food and space at 3.0 L/cm^2 resulted in considerably higher mortality. A surface scum never formed on any of the rearing containers, although considerable yeast and bacterial growth developed. These bacteria were mostly Gram (—) rods of the *Pseudomonas* (Group IV) and *Enterobacter* groups as indicated by their growth patterns on selective media and by biochemical tests. At 3.0 L/cm^2, mortality occurred mostly in the early instars as relatively few dead 3rd or 4th instar larvae were observed. As indexed by the low β value, development was variable and some larvae had developed to 3rd and 4th instar while others were still in 1st or 2nd. Early instar mortality was attributed to cannibalism (Roy 1931a) and/or GRF production (Ikeshoji and Mulla 1970a). As would be expected, crowded larvae required more food (mg/ml) to initiate and complete pupation; however, the efficiency of food utilization was not linear, with fewer pupae produced per mg of food at the most and least crowded densities (Table 2). The concentration of food (mg/cm^2) appeared to be more critical than the amount of food/larvae, agreeing with Peters et al. (1969).

PUPAL DEVELOPMENT AND EMERGENCE. The E_{50} and β values for both males and females exhibited significant positive and negative linear regressions, respectively, with increasing larval rearing density (Table 2). E_{50} and β values could not be calculated for 0.75 and 1.0 L/cm^2 because several pupae were inadvertently mixed. E_{50} and β were consistently less for males than females; therefore, the median development times of male pupae were shorter than those of females, but the duration of pupation was longer. Pupation mortality exhibited a significant positive regression with density (Table 2). Since Thompson and Bell (1965) found that pupal density in emergence containers had no effect on emergence success in *A. stephensi*, mortality was attributed solely to the inability of the pupae to develop and emerge. Many individuals reared at 3.0 L/cm^2 failed to shed their pupal exuviae, and others which completed

Table 2. Some effects of increasing larval rearing density on selected immature and adult atributes.

Attribute	Larval density (L/cm²) 0.1	0.5	0.75	1.0	2.0	3.0	Linear Regression[1]
P_{50} (hours)	184.7	196.0	214.5	213.6	297.5	401.1	+L (0.01)
β (slope of probit line)	31.03	29.52	24.94	17.81	16.99	10.81	−L (0.01)
Larval mortality (%)	27.56	5.38	3.07	4.80	3.08	61.15	NSL (0.10)
Food added until 1st pupation (mg/ml)	0.7	1.3	1.3	1.3	1.8	2.3	+L (0.05)
last pupation (mg/ml)	1.8	2.3	2.3	2.8	4.8	6.3	+L (0.05)
Efficiency (no. pupae/mg food)	0.04	0.21	0.31	0.34	0.40	0.19	NSL (0.10)
E_{50} (hours) males	217.7	227.9	...	...	322.9	409.7	+L (0.01)
females	222.7	235.3	...	...	347.2	448.2	+L (0.01)
β (slope of probit line) males	35.89	38.81	...	...	17.98	8.30	−L (0.01)
females	38.27	38.42	...	...	20.08	13.19	−L (0.01)
Pupal mortality (%)	3.50	6.50	10.68	11.11	10.37	39.93	+L (0.05)
Sex ratio (males/total)	0.514	0.571[2]	0.541	0.534	0.526	0.462	−L (0.10)
Weight at emergence (mg) males	1.095±.027	0.967±.027	0.761±.043	0.589±.035	0.295±.012	0.616±.039	−L (0.01)
females	1.733±.039	1.378±.050	1.051±.031	0.935±.035	0.425±.019	0.864±.023	−L (0.01)
Weight before blood meal (mg)	2.393±.078	1.963±.009	1.803±.189	1.436±.093	0.918±.108	0.894±.054	−L (0.01)
Weight gain (%)	88.44	91.64	75.37	89.94	102.82	77.37	NSL (0.10)
Per cent taking blood meal (%)	59.09	89.28	90.32	75.67	91.83	43.10	NSL (0.10)
Weight of blood meal (mg)	2.117	1.799	1.359	1.209	0.944	0.602	−L (0.01)
Fecundity (# eggs/female)	156.8±6.1	117.0±12.0	98.4±7.9	79.4±5.9	41.8±4.0	45.4±5.1	−L (0.01)
Per cent laying eggs within 48 hrs.	80.0	80.0	80.0	91.0	40.0	100.0	NSL (0.10)
Egg size (microns)	505.20±4.86	517.44±2.05	516.72±1.73	522.00±3.10	520.80±3.13	492.24±3.90	−L (0.05)
Egg hatch (%)	95.55	71.41	90.79	75.98	94.15	71.60	NSL (0.10)
Females surviving to 14 days (%)	83.33	66.67	41.67	75.00	16.67	0.00	−L (0.01)

[1] NSL = no significant linear regression ($P>0.10$), L = significant linear regression ($P<\alpha$), and + or − indicates the slope, the value in parenthesis is the level of signficance (α).

[2] Significantly deviates from expected 1:1 sex ratio ($P<0.01$).

ecdysis seemed unable to fly or climb from the water surface.

Proportionately fewer males were produced at the crowded densities, although only at 0.5 L/cm^2 did the sex ratio significantly vary from the expected 1:1 (Cuéllar 1973) (Table 2). The 19% decrease in the sex ratio from 0.5 to 3.0 L/cm^2 was attributed to greater mortalilty among stressed male larvae. Conversely, in *Aedes aegypti* Weilding (1929) reported a higher proportion of males under crowded conditions, although more recently Hickey (1970) found no density related distortion. In some *Drosophila,* however, the percentage of females has been shown to increase linearly with density (Sokoloff 1955) supporting these findings. The weights of the adults at emergence decreased significantly with increasing density (Table 2). Males were always significantly ($p < 0.01$) lighter than females, and in the ANOVA the main interaction effect of adult weight and density was also significant ($p < .01$), as the difference between male and female weights decreased with increasing rearing density. Adult weights at 2.0 L/cm^2 were less than those at 3.0 L/cm^2 which was attributed to the variability of adult sizes when *Anopheles* are reared under crowded conditions (Terzian and Stahler 1949). Apparently the arbitrary weighing of adults selected at a specified point in emergence (30% in this study) did not eliminate the variability of adult weights. Since food was added continuously according to the schedule in Table 1, the individuals taking longer to develop received more food and produced some adults which were visibly larger. Similarly, Barbosa et al. (1972) found that *A. aegypti* larvae taking longer to develop consistently produced heavier pupae regardless of the degree of experimental crowding.

The weight of the females when they were offered a blood meal also decreased significantly with increasing rearing density Table 2). The percent weight gained since emergence, presumably due to sugar feeding, was independent of density, although the lightest adults (2.0 L/cm^2) exhibited the greatest percent weight gain (Table 2).

BLOOD FEEDING AND FECUNDITY. The percent of the females taking a blood meal was independent of density (Table 2). The lowest percentage feeds were observed for females reared at the least (0.1 L/cm^2) and most (3.0 L/cm^2) crowded conditions (Table 2). As the adults were afforded ample time to mate (Roy 1931b, Nasir 1970), this variability in feeding was difficult to interpret especially since the small, 2.0 L/cm^2 reared females fed avidly. The weight of the blood meal and the number of eggs produced decreased significantly with increasing density (Table 2) and were significantly correlated with each other ($r = 0.974$, $p < 0.01$). The positive relationship between the amount of blood imbibed and the number of eggs produced has been reported for *A. stephensi* by Roy (1931a) and Thompson and Bell (1965), and has been generally accepted as being characteristic for most mosquitoes (Ikesjoji and Mulla 1970a; Colless and Challepah 1960; and others).

The number of eggs produced encompassed the spectrum of values reported by other workers using human blood meals (Nasir 1970) = 88 ± 2.53 eggs/female; Roy (1931b) = range: Dwarf = 31 to Stout = 118). It may be significant that the performance of Roy's dwarf females reared from wild caught larvae approached that of the females reared under crowded conditions in this experiment. Most egg batches were laid on the second night after blood feeding (within 48 hours), although some females required an additional night (Table 2). One female each, reared at 1.0 and 2.0 L/cm^2, retained their eggs for 5 nights before ovipositing. No female took longer than 1 night to lay her entire batch of eggs. These results disagreed with the observations of Nasir (1970), using the same colony, who reported a "72 hour oviposition rhythm" with eggs occasionally laid during several "sittings."

EGG SIZE AND VIABILITY. Egg lengths were essentially the same as those re-

ported for the INDIA strain by Rutledge et al. (1970), although the eggs of females reared under the most (3.0 L/cm^2) and least (0.1 L/cm^2) crowded conditions were significantly smaller (Table 3). All measurements were significantly smaller than those reported for the INDIA strain by Nasir (1970), and were significantly smaller and larger than the lengths reported by Rao et al. (1938) for the TYPE and *mysorensis* varieties (Table 3). In the present experiment, egg size decreased significantly with increasing density (Table 2) and varied significantly among females within treatments ($p < 0.01$). This variability of egg size suggested that the separation of the proposed subspecies *mysorensis* was not feasible using egg length. Even measurements within the same INDIA strain colony were significantly variable (Table 3), as were many of the comparisons with other TYPE strains such as IRAN and IRAQ (Table 3). These results supported Rutledge et al. (1970) who concluded that the "subspecies status for the form and variety *mysorensis* seems inappropriate." Within strain, egg size variability apparently has a simple Mendelian genetic basis (Coluzzi et al. 1972); however, it also appears that crowded larval rearing conditions may exert a significant effect.

The percent egg hatch was independent of density and approached the values reported in the literature (Rutledge et al. (1970) INDIA strain = 79.0 ± 6.8%; Thompson and Bell (1968) = 73.3 ± 3.7%; Meller (1962) = 89.2%; Nasir (1970) = 91.6%) (Table 2).

SURVIVAL. The percentage of the females surviving 14 days after taking a blood meal was significantly higher for those females reared under uncrowded conditions (Table 2). Female survival was also positively correlated with size before the blood meal ($r = 0.838$, $p < 0.05$) suggesting that larger females had a longer life expectancy. All females were dead by 28 days after receiving a blood meal which agreed with the life expectancies reported by Nasir (1970) and Rutledge et al. (1970), but was somewhat less

Table 3. Comparisons of egg lengths with selected estimates reported in the literature using Student's t statistic; .. = significant ($P<0.05$), NS = not significant ($P>0.05$).

Density (L/cm^2)	Egg length in microns (mean ± S.E.)	INDIA 519.±2.7	Rutledge, et al., (1970) IRAN 509.4±3.6	IRAQ 511.6±2.8	Nasir (1970) INDIA 527.5±1.25	Rao, et al (1938) TYPE 548.06±0.26	Rao, et al (1938) *mysorensis* 477.12±0.19
0.1	505.20±4.86	..	NS	NS	..	..	..
0.5	517.44±2.05	NS	NS	NS	..	..	..
0.75	516.72±1.73	NS	NS	NS	..	..	..
1.0	522.00±3.10	NS	..	..	..	..	..
2.0	520.80±3.13	NS	..	..	..	..	..
3.0	492.24±3.90	..	..	..	..	..	..

Table 4. Bioassay of rearing water for possible GRF activity, means of 3 replicates presented.

Parameters	water conditioning larval density (L/cm²)			
	Distilled	0.1	2.0	3.0
P_{50} (hours)	273.7	214.5	211.9	258.6
β (slope of probit line)	13.34	14.23	11.93	10.21
larval mortality (%)	24.1	8.7	50.0	31.6

than that observed by Stahler and Black (1970).

GRF ASSAY. Larvae reared in conditioned water exhibited significantly lower P_{50} values than those reared in the β values ($p > 0.05$) (Table 4). These differences in developmental rates were attributed to the additional food present in the microbiota rich, conditioned water and perhaps the presence of autophagostimulants (Dadd and Kleinjahn 1974). Larvae reared in water conditioned under uncrowded conditions (0.1 L/cm²) had significantly ($p < 0.01$) faster P_{50} times than crowded conditioned water (3.0 L/cm²), although there was little difference between 0.1 and 2.0 L/cm² water. Larval mortality in 0.1 L/cm² conditioned water was significantly less ($p < 0.01$) than either distilled water or 2.0 or 3.0 L/cm² conditioned water. It did appear that GRF was produced by *Anopheles stephensi* under the conditions described in this experiment as indicated by the bioassay of the larval rearing water and the population attributes listed in Table 2. The crowded larval densities used in the present experiment were less than the densities used in other investigations, e.g. 5–7 L/ml by Ikeshoji and Mulla (1970 a&b); 7 L/ml by Moore and Whitacre (1972); and 8 L/ml by Barbosa and Peters (1973), and thus the measurable effects of the larval factors elicited were most likely reduced correspondingly.

OVIPOSITION PREFERENCE. If the females in this experiment laid their eggs randomly, that is, not distinguishing between the different types of water offered in the oviposition cups, 25% of the eggs would be laid in each cup. The percentages of eggs of both 0.5 and 2.0 L/cm² reared females significantly ($p < 0.05$) deviated from the expected proportion as indexed by the G statistic (Sokal and Rohlf 1969) (Table 5). This deviation appeared greatest for the distilled water, or rather, *A. stephensi* females preferred to lay their eggs on the surface of conditioned water. Females reared at 0.5 L/cm² did not seem to distinguish between uncrowded (0.1 L/cm²) and crowded (2.0 and 3.0 L/cm²) conditioned water, while females reared at 2.0 L/cm² seemed to lay more eggs on uncrowded conditioned water. These findings agreed with Ikeshoji (1966) and Soman and Reuben (1970) who found that gravid *Culex pipiens* and *A. aegypti* females also preferred to lay their eggs on, or near, conditioned water. This response was attributed to a series of volatile compounds which were detected by receptors located on the antennae of the female (Ikeshoji 1966, Ikeshoji et al. 1967). Upon locating the oviposition site, the female may then be repelled by the

Table 5. Oviposition preferences of females reared under uncrowded (0.5 L/cm²) and crowded (2.0 L/cm²) conditions. Values presented are mean percentages of 3 replicates of 10 females per container.

Female rearing density (L/cm²)	water conditioning larval density (L/cm²)			
	Distilled	0.1	0.1	3.0
0.5	5.2	30.4	29.5	34.9
2.0	5.2	38.0	26.9	29.7

chemical composition of the water detected by different receptors which are located on the tarsi and/or proboscis (Ikeshoji 1966, Ikeshoji et al. 1967). Perhaps other larval compounds such as GRF may function as a deterrent to oviposition, especially for those individuals reared under crowded conditions.

RELATIONSIP TO BIONOMICS. Since *A. stephensi* breeds in small pools and peridomestic artificial containers subject to desiccation (Christophers 1933, Foote and Cook 1959), intraspecific competition for food and space would seem a natural occurrence in the bionomics of this species. That this phenomenon actually occurs in natural anopheline populations has been suggested by Christie (1950) and is supported by the heterogeneity in size and the reduced fecundity of females reared from wild collected larvae (Roy 1931a). Crowding could have two opposite effects on the population dynamics of this species:

1. Competition could contribute to the collapse of the overall population by facilitating local extinctions in marginal or temporary habitats during periods of unfavourable weather (Andrewartha and Birch 1955). Under competition stress, larval development would be delayed enhancing the chances of the habitat drying out prior to pupation and emergence. Those individuals emerging would be smaller, probably have a reduced flight range (Nayar 1960, Nayar and Sauerman 1970, Schiefer et al. 1973), imbibe less blood, produce fewer eggs, and have reduced survival. These characteristics would tend to reduce dispersal and eventually contribute to localized population extinctions, thus synergistically enhancing the effects of unfavorable weather conditions on the entire population. 2. In permanent breeding sites such as seeps and cisterns, the above-mentioned attributes would help prevent larval densities from exceeding the carrying capacity of the habitat. Crowding could act as an internal regulatory mechanism delaying development, and reducing fecundity, thus helping to reduce the population growth rate when larval density approached critical levels. If females reared under crowded conditions could indeed distinguish between crowded and uncrowded breeding sites, emigration rates could also be increased. During the dry season when alternate breeding sites were scarce, these individuals would most likely perish; however, during the rainy season, these individuals could repopulate the temporary breeding sites and thus eventually cause an overall increase in the population during the favorable weather period.

ACKNOWLEDGMENTS

I would like to thank Mr. J. Klaff, Division of Infectious Diseases, Mr. R. W. Emory, Division of Medical Entomology and Ecology, and Dr. P. L. Canner and Ms. F. Fazio, Division of Clinical Investigation, University of Maryland School of Medicine, for their assistance with the microbiological, entomological and statistical aspects of this investigation, respectively. Dr. V. C. McCarthy, University of Maryland School of Medicine, offered advice and criticism during the preparation of the manuscript. This study was supported by Grant No. AI10049 from the National Institute of Allergy and Infectious Diseases, NIH.

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