INBREEDING OF *AEDES AEGYPTI* AND *ANOPHELES STEPHENSI*[1]

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ABSTRACT. Twenty-five lines of the IRAN strain of *Anopheles stephensi*, 32 of the MOYO INDOOR strain of *Aedes aegypti* and 37 of the MASAKA strain of *Ae. aegypti* were inbred by the single-pair, brother-sister mating technique. Two lines of the IRAN strain of *An. stephensi* were successfully carried through six generations of inbreeding, and five lines of the MOYO INDOOR strain and two lines of the MASAKA strain of *Ae. aegypti* were successfully carried through 10 generations of inbreeding. Four inbred strains derived from the MOYO INDOOR strain and two derived from the MASAKA strain were compared with respect to size, color pattern and life table characteristics. Each strain exhibited a distinctive and unique combination of deleterious and adaptive traits. Comparison of the inbred strains with the respective parent strains demonstrated reduced fitness in all of the inbred strains.

INTRODUCTION

The form of inbreeding most often employed in experimental biology has been single-pair brother-sister mating. Various aspects of mosquito biology, including morphology, development, variation and laboratory bionomics, have been studied by this technique. Although a number of inbred lines and strains of mosquitoes have been established, few descriptions of the biological characteristics of the lines and strains produced have been published.

Boesiger (1978) inbred 100 lines of *Culex pipiens* Linn. by single-pair brother-sister mating to the 4th to 11th generations, and reported on the rates of extinction of the lines under three different inbreeding schemes and on the life table characteristics of each line in each generation of inbreeding. In a similar study (Boesiger 1982), she subsequently reported on the effects of inbreeding on several morphological characters of *Cx. pipiens*. Fergusson-Laguna and Machado-Allison (1979) inbred a strain of *Aedes aegypti* (Linn.) by single-pair brother-sister mating to the 5th generation. They presented life table data for each generation and demonstrated that the intrinsic rate of increase of the strain was reduced by inbreeding effects.

From September 1975 to June 1976, we established 69 lines of *Ae. aegypti* from eggs laid by individual colony females, and successfully carried seven of the 69 through the 10th generation of single-pair brother-sister mating. In this paper, we describe the techniques employed, report the pattern of extinction of lines observed in the inbreeding process, and present data obtained on morphology and life table characteristics of the surviving strains. Limited information from inbreeding 25 lines of *Anopheles stephensi* Liston for one to six generations at the Walter Reed Army Institute of Research, Washington, D.C., from November 1969 to June 1970 is also presented for comparison. Inbreeding of *Ae. aegypti* was performed in connection with studies on the heritability of levels of sensitivity to mosquito repellents. Inbreeding of *An. stephensi* was performed in connection with studies on the heritability of susceptibility of mosquitoes to infection by *Plasmodium cynomologi* Mayer (Haemosporida, Plasmodiidae).

MATERIALS AND METHODS

PARENT STRAINS. Two strains of *Ae. aegypti* and one strain of *An. stephensi* were used. The MOYO INDOOR and MASAKA strains of *Ae. aegypti* were obtained from Dr. G. B. Craig, University of Notre Dame, in July and September 1974. The MOYO INDOOR strain is a laboratory strain originating in Kenya; the MASAKA strain is a laboratory strain originating in Uganda. Standard conditions and procedures for rearing mosquitoes at this Institute were described by Rutledge et al. (1978).

The IRAN strain of *An. stephensi* was obtained from Dr. E. R. Shagudian, University of Teheran, in March 1968. Selected biological characteristics of the strain and the standard conditions and procedures for rearing mosquitoes at the Walter Reed Army Institute of Research were reported by Rutledge et al. (1970).

INBREEDING SCHEME. Inbreeding of *Ae. aegypti* was initiated with 32 clutches[2] of eggs from individual colony females of the MOYO INDOOR strain and 37 clutches of eggs from individual colony females of the MASAKA strain. The eggs deposited by each female were reared separately to the adult stage, and the resulting

[1] Opinions or assertions contained herein are the private views of the authors and should not be construed as official or as reflecting the views of the Department of the Army or the Department of Defense. Use of a trade name does not imply official approval or indorsement of the product mentioned.

[2] In this paper, the term "brood" is used to mean the offspring produced by a single female in a single gonotrophic cycle. A brood in the egg stage is referred to as a "clutch" of eggs.

males and females, representing the first (F_1) generation of inbreeding, were allowed to mate at liberty within their respective sibships. This procedure was considered to be equivalent to single-pair mating, since females of *Ae. aegypti* are ordinarily monogamous (Craig 1967; see also Jones 1973). The mated females were then blood-fed, and, where possible, two replete females were chosen from each sibship for oviposition. Subsequently, one of each of the resulting duplicate broods was chosen on the basis of apparent vigor to represent the F_2 generation of each line.

The foregoing scheme was modified in subsequent generations to minimize losses of the lines as inbreeding progressed. Beginning with the 5th generation, we retained three replete females of each line of the MASAKA strain for oviposition where possible instead of only two. In the 6th generation we retained five females of each line of both the MASAKA and the MOYO INDOOR strains, and beginning in the 7th generation, we retained ten females of each line. Beginning in the 7th generation, also, male and female pairs were segregated for egg production instead of females only.

Inbreeding of *An. stephensi* was initiated with 25 clutches of eggs from individual colony females of the IRAN strain. The inbreeding scheme employed was similar to that employed for *Ae. aegypti* except that only one replete female of each line was retained for oviposition in each generation of inbreeding. Although available evidence indicates that monogamy is the rule in female mosquitoes (Barr 1974), this has not yet been demonstrated for *An. stephensi.*

INBREEDING TECHNIQUES. Craig and Vandehey (1962) have discussed the special equipment and methods needed for rearing mosquitoes in genetic investigations. Our study required equipment and procedures for rearing, holding and blood-feeding single broods of mosquitoes and for obtaining the eggs of single female mosquitoes separately.

Larvae were reared in white, enameled pans ($5 \times 18 \times 29$ cm for *Ae. aegypti* and $6 \times 26 \times 41$ cm for *An. stephensi*). Adults of *Ae. aegypti* were maintained in aluminum-frame, wire-screen, insect cages measuring $15 \times 15 \times 15$ cm (Insect Control and Research, Inc., Baltimore, MD). *Anopheles stephensi* adults were maintained in pint (0.5 liter) cardboard ice cream carton cages similar to those described and illustrated by Craig and Vandehey (1962).

Craig and Vandehey (1962) recommended chicks for blood-feeding large numbers of single broods of *Ae. aegypti.* We used white laboratory mice for blood-feeding both *Ae. aegypti* and *An. stephensi* because they can be kept indefinitely without growing to an inconveniently large size. Mice used in blood-feeding *An. stephensi* were bound to small restraining boards with adhesive tape, shaved over the belly with an electric razor, and placed on the cages to be fed (Rutledge et al. 1970). Mice used in blood-feeding *Ae. aegypti* were anesthetized by intraperitoneal injection, shaved as before, and placed on the mosquito cages without mechanical restraint. Anesthetics used in the study were Nembutal™ Sodium Solution (sodium pentobarbitol; Abbott Laboratories, North Chicago, IL), Innovar-Vet™ (fentanyl and droperidol: Pitman-Moore, Inc., Washington Crossing, NJ) and CI 744 (tiletamine hydrochloride and zolasepam: Ward et al. 1974). Although anesthetization was more convenient and efficient than physical restraint, none of the anesthetics used were entirely satisfactory. The mice often developed tremors that disturbed the mosquitoes, or they revived prematurely (in 15 or 20 minutes) and had to be reanesthetized. In conducting this study, the authors adhered to the "Guide for the Care and Use of Laboratory Animals" of the Institute of Laboratory Animal Resources, National Research Council.

Blood-fed female *Ae. aegypti* chosen for oviposition were placed in individual amber snap-cap plastic vials 6 cm high and 3 cm diam. To provide ventilation, 13 mm diam holes were punched in the caps, and the caps were snapped on over small squares of organdy. A. rectangular section of paper toweling was placed in each vial as an oviposition site. Two days after the mosquitoes were blood-fed and placed in the vials, sufficient water was injected into each vial with a hypodermic needle and syringe to moisten the paper toweling and induce oviposition. Two days after oviposition, the parous mosquito was discarded and the eggs were hatched or dried for storage. All eggs of a given generation (F_1 to F_{10}) were hatched and reared concurrently.

Blood-fed females of *An. stephensi* chosen for oviposition were isolated in screen-capped, 7.5 cm high $\times$ 3.0 cm diam glass vials with fused sintered-glass floors. These vials were designed for use in specially constructed plastic racks to which water for oviposition could be added to rise through the sintered glass. The oviposition vial and a rack of vials were illustrated by Rutledge et al. (1970). Water for oviposition was added to the rack one to four days after blood-feeding. Hatching and rearing of the different generations (F_1 to F_6) of the 25 lines were not concurrent.

MORPHOLOGICAL COMPARISONS. After ten generations of inbreeding, the surviving inbred lines of *Ae. aegypti* were brought to colony strength using the same rearing conditions and

procedures as those employed for the parent strains (Rutledge et al. 1978). Approximately seven generations were needed to bring the lines to colony strength. Samples of the eggs, larvae and adult males and females of the parent and inbred strains were subsequently examined microscopically, and the following comparisons were made: egg length, 4th larval instar head capsule width, and adult male and female wing length and color patterns. The results obtained were interpreted in terms of data from a standard reference (Christophers 1960). Morphological comparisons of the parent and inbred strains of *An. stephensi* were not performed.

LABORATORY BIONOMICS. The following biological data were obtained on the parent and surviving inbred strains of *Ae. aegypti:* clutch size, hatching time, duration of the larval and pupal periods, percent mortality in the egg, larval and pupal stages, sex ratio and longevity of the adult males and females. The specific experiments conducted and the respective sample sizes will be given in connection with the results obtained. Except as noted, the materials and methods used were similar to those used in the inbreeding program. The data obtained were utilized in strain comparisons, comparison with data from a standard reference (Christophers 1960) and construction of abbreviated life tables (Harcourt 1969, Southwood 1978). Bionomic comparisons of the parent and inbred strains of *An. stephensi* were not performed.

RESULTS AND DISCUSSION

SURVIVAL OF LINES AND BROODS. Five lines of the MOYO INDOOR strain and two lines of the MASAKA strain of *Ae. aegypti* were successfully carried through 10 generations of inbreeding; two lines of the IRAN strain of *An. stephensi* were successfully carried through six generations of inbreeding (Fig. 1). Percent survival of lines derived from the IRAN strain of *An. stephensi* was linear with respect to number of generations of inbreeding ($r^2 = 0.94$). Percent survival of lines derived from the MOYO INDOOR and MASAKA strains of *Ae. aegypti* increased in the later generations, as shown by the leveling off of the respective curves. This increase presumably reflects the greater number of replete females retained and the longer mating periods provided in the later generations of inbreeding of the MOYO INDOOR and MASAKA strains.

Support for this interpretation of Fig. 1 is provided by the brood survival rates for the F_1 to F_5 (80%, N = 153) and F_6 to F_{10} (59%, N = 133) generations of the MOYO INDOOR strain. Although brood survival declined in the F_6 to F_{10} generations of inbreeding (chi-square = 13.14, $P < .001$), the decrease was apparently offset sufficiently by the greater number of replete females retained and the longer mating periods provided to produce the observed increase in survival of the inbred lines. The observed decline in brood survival in the F_6 to F_{10} generations can be attributed to the progressive expression of deleterious recessive genes as the degree of homozygosity of each line was increased. Brood survival rates for the F_1 to F_5 (73%, N = 145) and F_6 to F_{10} (64%, N = 106) generations of the MASAKA strain did not differ significantly at the 5% level.

Most brood losses in the 10 generations of inbreeding occurred in the immature stages of development (Table 1). However, 12 broods of the MOYO INDOOR and MASAKA strains of *Ae. aegypti* produced adult males or adult females only. In small broods unisexuality can be explained in terms of random variation of the normal sex ratio. However, two broods of 30 and 41 males, respectively, were observed in the 3rd generation of inbreeding of the MASAKA strain, and it seems more likely that the sex ratio

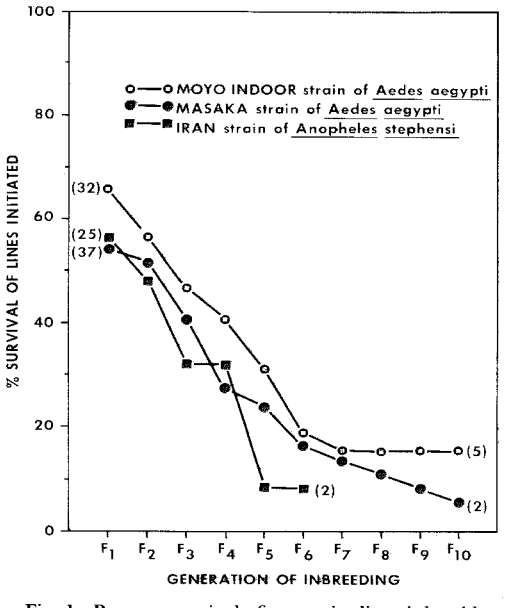

Fig. 1. Percent survival of mosquito lines inbred by sib-mating for six (*Anopheles stephensi*) or 10 (*Aedes aegypti*) generations. Figures in parentheses indicate the number lines initiated and successfully carried to the 6th or 10th generation.

Table 1. Survival data for broods reared in 10 generations of inbreeding of *Ae. aegypti* and six generations of inbreeding of *An. stephensi*.

Item	MOYO INDOOR strain (*Ae. aegypti*)	MASAKA strain (*Ae. aegypti*)	IRAN strain (*An. stephensi*)
No. broods reared	286	251	69
No. broods surviving to adult stage[a]	233(81%)	194(77%)	64(93%)
No. broods bisexual	228(80%)	187(75%)	64(93%)
No. bisexual broods successfully blood-fed	201(70%)	174(69%)	63(91%)
No. replete females retained for oviposition	319[b]	303[b]	63
No. clutches of eggs obtained from replete females	254(80%)[b]	214(71%)[b]	46(73%)

[a] For *An. stephensi*, number surviving to the pupal stage.
[b] Data obtained for F_1 to F_9 generations only.

distorter factor (Hickey and Craig 1966) was present in that strain.

A substantial number of broods were lost through failure of the adult females to take blood, but our data do not establish that this was due to anything other than technique. Similarly, a number of the blood-fed females that were retained for oviposition either died or failed to deposit eggs. An extreme example of failure to oviposit occurred in the 8th generation of inbreeding of Line S-20, derived from the MASAKA strain of *Ae. aegypti*. Ten replete females retained from three different broods of this line failed to deposit any eggs. Subsequently, the four remaining females were fed and set up to oviposit, but only one of these deposited eggs.

SIZE COMPARISONS. One of the five inbred strains derived from the MOYO INDOOR strain of *Ae. aegypti* was lost subsequent to the inbreeding phase of the study. The four surviving inbred strains derived from the MOYO INDOOR strain were designated Strains R-3, R-13, R-21 and R-44; the two surviving inbred strains derived from the MASAKA strain were designated Strains S-15 and S-22. Comparative data on the sizes of the eggs, larvae and adult males and females of the parent and inbred strains are presented in Table 2.

There were no statistically significant differences in overall size among the eight strains (Friedman's test, Steel and Torrie 1980), although Strain R-3 was the smallest or next smallest in all stages of development. Although there were numerous statistically significant differences in the sizes of the egg and adult stages, there was no evidence that cognate strains were more similar than unrelated strains. These observations can be interpreted in terms of the segregation and fixing of one or more genetic factors for size that were originally present in the two parent strains. The postulated factors for size would be incompletely penetrant, since size is known to be affected by nutrition and crowding (Christophers 1960).

ADULT COLOR PATTERNS. The MOYO INDOOR strain of *Ae. aegypti* and its cognate inbred strains resembled the "type form" of the species, as defined by Mattingly (1957) and McClelland (1974). Most specimens examined were brown with pale scales on the tergite of abdominal segment I. The upright forked scales of the occiput were characteristically light orange-brown. In addition, all specimens of strain R-3 were marked with pale scales on the submedian areas of the occiput, although this marking was not seen in the parent strain.

The MASAKA strain and its cognate inbred strains were distinctly darker than the MOYO INDOOR strain, but differed from ssp. *formosus* (Walker) (Mattingly 1957, McClelland 1974) in that most individuals had pale scales on the tergite of abdominal segment I. The upright forked scales of the occiput were characteristically dusky. None of the strains were marked with pale scales on the submedian area of the occiput.

LABORATORY BIONOMICS. Six replete females of each parent and inbred strain of *Ae. aegypti* (4 of strain R-21) were observed individually to determine the clutch sizes of the eight strains. All individuals of the two parent strains deposited eggs, but the numbers of individuals of the six inbred strains ovipositing ranged from 2 (strain R-13) to 6 (strains R-44 and S-15). The mean clutch sizes for parent and inbred strains other than strain S-22 ranged from 65.2 to 90.0 eggs/female, whereas that for strain S-22 was only 7.3 eggs/female. Although clutch sizes in *Ae. aegypti* are highly variable (Christophers 1960), that observed for strain S-22 was clearly exceptional.

The hatching times of the two parent and six inbred strains were determined by flooding the eggs obtained in the clutch size experiment and

Table 2. Size data for parent and inbred strains of *Aedes aegypti*.

	Strain							
	MOYO INDOOR	R-3	R-13	R-21	R-44	MASAKA	S-15	S-22
	Egg Length (mm)							
Number	10	10	10	10	10	10	10	10
Range	0.54–0.62	0.46–0.60	0.60–0.66	0.56–0.64	0.48–0.60	0.44–0.64	0.56–0.58	0.58–0.64
Mean[a]	0.59bc	0.54a	0.62c	0.59bc	0.54a	0.58b	0.61bc	0.62c
	Head Width, Larval Instar IV (mm)							
Number	10	10	10	10	10	10	10	10
Range	0.86–0.98	0.82–0.98	0.78–0.96	0.74–0.98	0.76–1.00	0.82–0.96	0.86–1.00	0.82–0.96
Mean[a]	0.91a	0.87a	0.87a	0.89a	0.91a	0.90a	0.92a	0.92a
	Wing Length, Adult ♂ (mm)							
Number	15	10	13	9	8	15	11	8
Range	2.3–2.8	2.0–2.4	2.1–2.6	2.5–2.6	2.5–2.7	2.4–2.7	2.2–2.7	2.1–2.7
Mean[a]	2.5b	2.2a	2.3a	2.6bc	2.6bc	2.6c	2.5bc	2.5bc
	Wing Length, Adult ♀ (mm)							
Number	15	11	14	6	10	15	8	9
Range	2.5–3.2	2.7–3.1	2.8–3.4	3.3–3.5	2.8–3.3	2.7–3.3	2.8–3.6	2.8–3.6
Mean[a]	2.8a	2.9ab	3.1cd	3.4e	3.1cd	3.0bc	3.3e	3.2de

[a] Means not followed by the same letter differ at the 5% level of significance by Fisher's (protected) lsd test. (Steel and Torrie 1980).

counting the resulting larvae after 1, 2, 3, 4 and 7 days of immersion. Larval periods and pupal periods were determined by rearing samples of 64 newly hatched larvae of each strain in 250-ml glass beakers containing 100 ml of demineralized water. The larvae were fed 0.6 mg of a prepared ration (4 parts dried yeast: 4 parts guinea pig feed: 1 part dried liver) each work day until pupation was complete. The pupae and adults obtained were counted each work day until both pupation and emergence were complete. Median hatching times, larval periods and pupal periods were calculated by the method of Arkin and Colton (1970) for grouped data.

The median hatching times and larval and pupal periods of the parent and inbred strains are given in Table 3. Although the hatching times of the two parent strains were within normal limits for the species (Christophers 1960), those observed for inbred strains R-13, S-15 and, especially, S-22 were much longer than normal. According to Christophers (1960), eggs that are slow to hatch (residual eggs) are subvital. The studies of Gillett (1955) indicated that the condition is heritable, and this finding was supported by the present study. In contrast to hatching times, all larval and pupal periods observed in the study were normal for the species (Table 3).

The mortality and survival rates of the eight strains in the egg, larval and pupal stages, were determined in conjunction with the hatching and rearing studies described. The MASAKA strain exhibited greater mortality than the MOYO INDOOR strain in all stages of development (Table 4). (The relative weakness of the MASAKA strain was also evident in the inbreeding phase of the study; see Table 1.) More importantly, mortality rates in the six inbred strains were invariably greater than in the respective parent strains. This is a clear demonstration of inbreeding depression in the six inbred strains. The most extreme mortality rates observed in the study were 77% (strain S-22) in the egg stage, 82% (strain R-21) in the larval stage and 50% (strain R-44) in the pupal stage.

The sex ratios of the eight parent and inbred

Table 3. Median development times of parent and inbred strains of *Aedes aegypti*.

	Strain							
Item	MOYO INDOOR	R-3	R-13	R-21	R-44	MASAKA	S-15	S-22
Median hatching time (days)	0.9	0.5	2.2	0.6	0.9	0.9	2.0	5.5
Median larval period (days)	6.9	7.1	7.2	6.5	6.6	6.4	6.8	6.6
Median pupal period (days)	2.2	2.4	2.3	2.2	2.2	2.1	2.0	2.4
Total development time (days)	10.0	10.0	11.7	9.3	9.7	9.4	10.8	14.5

strains of *Ae. aegypti* were determined by counting the adult males and females emerging in the respective colonies over a period of several generations. The number of mosquitoes counted ranged from 304 for strain R-44 to 1,730 for strain R-13 (mean = 1,096.5). The sex ratios of both the MOYO INDOOR strain (42% female) and the MASAKA strain (44% female) differed significantly from nominal (i.e., 50% female) (Table 4). This type of sex ratio is normal in *Ae. aegypti* (Christophers 1960). However, the sex ratios of inbred strains R-3 (56% female), R-13 (50% female) and R-21 (49% female) differed significantly from that of the parent (MOYO INDOOR) strain. Since the departures from the parental norm were in the direction of a greater proportion of females, the sex ratio distorter factor (Hickey and Craig 1966) was evidently not involved in producing the three aberrant ratios.

Table 4. Stage-structured life tables for parent and inbred strains of *Aedes aegypti*[a]. See text for actual sizes of the egg, larval/pupal and adult cohorts.

x	lx	dx	qx	Sx
MOYO INDOOR Strain				
Eggs	1000	20	0.020	0.980
Larvae	980	153	0.156	0.844
Pupae	827	30	0.037	0.963
Adults	797	465	0.583[b]	0.417[b]
Adult females	332	—	—	—
Strain R-3				
Eggs	1000	479	0.479	0.521
Larvae	521	350	0.672	0.328
Pupae	171	33	0.190	0.810
Adults	138	61	0.443[b]	0.557[b]
Adult females	77	—	—	—
Strain R-13				
Eggs	1000	47	0.047	0.953
Larvae	953	640	0.672	0.328
Pupae	313	45	0.143	0.857
Adults	268	133	0.495[b]	0.505[b]
Adult females	135	—	—	—
Strain R-21				
Eggs	1000	446	0.446	0.554
Larvae	554	459	0.828	0.172
Pupae	95	26	0.273	0.727
Adults	69	35	0.506[b]	0.494[b]
Adult females	34	—	—	—
Strain R-44				
Eggs	1000	440	0.440	0.560
Larvae	560	350	0.625	0.375
Pupae	210	105	0.500	0.500
Adults	105	62	0.589[b]	0.411[b]
Adult females	43	—	—	—
MASAKA Strain				
Eggs	1000	101	0.101	0.899
Larvae	899	253	0.281	0.719
Pupae	646	98	0.152	0.848
Adults	548	305	0.556[b]	0.444[b]
Adult females	243	—	—	—
Strain S-15				
Eggs	1000	281	0.281	0.719
Larvae	719	517	0.719	0.281
Pupae	202	67	0.333	0.667
Adults	135	75	0.558[b]	0.442[b]
Adult females	60	—	—	—
Strain S-22				
Eggs	1000	773	0.773	0.227
Larvae	227	146	0.641	0.359
Pupae	81	17	0.217	0.783
Adults	64	33	0.519[b]	0.481[b]
Adult females	31	—	—	—

[a] x = age class; lx = number living at beginning of age interval x; dx = number dying within age interval x; qx = mortality rate within age interval x; Sx = survival rate within age interval x.

[b] Sex ratio applied as a mortality/survival factor (Harcourt 1969).

The median longevity of the adults was determined from samples of 25 newly emerged adults of each sex and strain. The mosquitoes were maintained on 10% sucrose solution in 15 × 15 × 15 cm wire-screen cages at 27°C and 80% RH. Blood was withheld from the females to permit comparison of the longevity of the males and females on an equal basis. Mortality was determined at 15-day intervals for 90 days; medians were estimated by the method of Arkin and Colton (1970) for grouped data.

The longevity of the adult males and females of the parent and inbred strains of *Ae. aegypti* is shown in Table 5. The longevity of the two parent (MOYO INDOOR and MASAKA) strains was considerably greater than that reported for other laboratory strains by other workers (Putnam and Shannon 1934, Kershaw et al. 1954, Liles and DeLong 1960, Crovello and Hacker 1972, Briegel and Kaiser 1973).

Contrary to the observations of other workers (Kershaw et al. 1954, Liles and DeLong 1960, Crovello and Hacker 1972, Briegel and Kaiser 1973), males of *Ae. aegypti* were not consistently shorter-lived than the females in our study (Table 5). Males of strains R-13 and S-15 lived more than two weeks longer than their respective females. The specific genetic and/or environmental factors favoring male survival in the study are unknown.

Adult longevity was reduced in the inbred strains except for the males of strain R-13 and the females of strain S-22 (Table 5). In most instances, this reduction was more than 50% compared to the same sex of the cognate parent strain. The most extreme reduction observed (81%) occurred in the males of strain R-21, for which median longevity was only 13.4 days. The observed reductions in adult longevity in the inbred strains are in keeping with the increased mortality observed in the egg, larval and pupal stages (see above) and the expectation of inbreeding depression in sib-mating programs.

Table 5. Median longevity of adult females and males of parent and inbred strains of *Aedes aegypti*.

Item	Strain							
	MOYO INDOOR	R-3	R-13	R-21	R-44	MASAKA	S-15	S-22
Median male longevity (days)	71.2	28.0	68.2	13.4	25.6	68.2	38.7	53.9
Median female longevity (days)	89.1	26.2	50.4	21.7	22.5	64.5	24.5	85.5

The several strains of *An. stephensi* and *Ae. aegypti* established in the study were produced under conditions of intense inbreeding (sibmating) and intense selection pressure (single-pair selection). [For a discussion of selection processes in inbreeding programs see Fisher (1965).] Thus, although the overriding result of the inbreeding process was the expression of deleterious recessive traits, the surviving strains exhibited both deleterious and adaptive traits. Deleterious traits observed in the study included reduced fertility, increased egg, larval and pupal mortality, and reduced adult longevity. The adaptive effects of selection included not only preservation of more or less normal values for those characteristics but also, in three strains, an actual shift of the sex ratio in favor of the egg-producing sex.

Since segregation and recombination are predominantly random processes, each of the inbred strains produced in the study was distinctive and unique. Strain R-3, for example, was distinctive in having pale scales on the submedian area of the occiput, a female-dominated sex ratio, small eggs, small males and a short life span. An additional effect of inbreeding is to greatly increase the homozygosity of the inbred strains (Fisher 1965). Homozygous strains are useful in the analysis of quantitative behavioral characters because the among-strain variance is expressed by genetically uniform genotypes (Fuller and Thompson 1960, Ehrman and Parsons 1976). In a subsequent paper we will describe experiments with the inbred strains produced in this study on the inheritance of the responses of *Ae. aegypti* to repellents.

References Cited

Arkin, H. and R. R. Colton. 1970. Statistical methods, 5th ed. Barnes and Noble Books, New York.

Barr, A. R. 1974. Symposium on reproduction of arthropods of medical and veterinary importance. V. Reproduction in Diptera of medical importance with special reference to mosquitoes. J. Med. Entomol. 11:35–40.

Boesiger, B. 1978. Comparison de plusieurs caracteres en fonction du degre de consanguinite chez *Culex pipiens*. Arch. Genet. 51:193–216.

Boesiger, B. 1982. Effets de la consanguinite sur des caracteres morphologiques quantitatifs chez *Culex pipiens autogenicus* Roub. Genetica 59:99–111.

Briegel, H. and C. Kaiser. 1973. Life-span of mosquitoes (Culicidae, Diptera) under laboratory conditions. Gerontologia 19:240–49.

Christophers, S. R. 1960. *Aedes aegypti* (L.), the yellow fever mosquito: Its life history, bionomics and structure. Cambridge Univ. Press, Cambridge.

Craig, G. B. 1967. Mosquitoes: Female monogamy induced by male accessory gland substance. Science 156:1499–501.

Craig, G. B. and R. C. Vandehey. 1962. Genetic variability in *Aedes aegypti* (Diptera: Culicidae). I. Mutations affecting color pattern. Ann. Entomol. Soc. Am. 55:47–58.

Crovello, T. J. and C. S. Hacker. 1972. Evolutionary strategies in life table characteristics among feral and urban strains of *Aedes aegypti* (L.). Evolution 26:185–96.

Ehrman, L. and P. A. Parsons. 1976. The genetics of behavior. Sinauer Associates, Sunderland, MA.

Fergusson-Laguna, A. and C. E. Machado-Allison. 1979. Efecto de la endogamia en sucesivas generaciones de cruces hermana-hermano en *Aedes aegypti* (L.). Acta Cient. Venez. 30:507–10.

Fisher, R. A. 1965. The theory of inbreeding, 2nd ed. Oliver and Boyd, Edinburgh.

Fuller, J. L. and W. R. Thompson. 1960. Behavior genetics. John Wiley and Sons, New York.

Gillett, J. D. 1955. The inherited basis of variation in the hatching-response of *Aedes* eggs (Diptera: Culicidae). Bull. Entomol. Res. 46:255–65.

Harcourt, D. G. 1969. The development and use of life tables in the study of natural insect populations. Annu. Rev. Entomol. 14:175–96.

Hickey, W. A. and G. B. Craig. 1966. Distortion of sex ratio in populations of *Aedes aegypti*. Can. J. Genet. Cytol. 8:260–78.

Jones, J. C. 1973. Are mosquitoes monogamous? Nature 242:343–44.

Kershaw, W. E., T. A. Chalmers and M. M. J. Lavoipierre. 1954. Studies on arthropod survival. I. The pattern of mosquito survival in laboratory conditions. Ann. Trop. Med. Parasitol. 48:442–50.

Liles, N. N. and D. M. DeLong. 1960. The longevity and productivity of adult male and female *Aedes aegypti* when reared separately and together on three different diets. Ann. Entomol. Soc. Am. 53:277–80.

Mattingly, P. F. 1957. Genetical aspects of the *Aedes aegypti* problem: I. Taxonomy and bionomics. Ann. Trop. Med. Parasitol. 51:392–408.

McClelland, G. A. H. 1974. A worldwide survey of variation in scale pattern of the abdominal tergum of *Aedes aegypti* (L.) (Diptera: Culicidae). Trans. R. Entomol. Soc. Lond. 126:239–59.

Putnam, P. and R. A. Shannon. 1934. The biology of

Stegomyia under laboratory conditions. Proc. Entomol. Soc. Wash. 36:217–42.

Rutledge, L. C., M. A. Moussa, C. A. Lowe and R. K. Sofield. 1978. Comparative sensitivity of mosquito species and strains to the repellent diethyl toluamide. J. Med. Entomol. 14:536–41.

Rutledge, L. C., R. A. Ward and W. E. Bickley. 1970. Experimental hybridization of geographic strains of *Anopheles stephensi* (Diptera: Culicidae). Ann. Entomol. Soc. Am. 63:1024–30.

Southwood, T.R.E. 1978. Ecological methods, with particular reference to the study of insect populations, 2nd ed. John Wiley and Sons, New York.

Steel, R. G. D. and H. H. Torrie. 1980. Principles and procedures of statistics, 2nd ed. McGraw-Hill Book Co., New York.

Ward, G. S., D. O. Johnsen and C. R. Roberts. 1974. The use of CI 744 as an anesthetic for laboratory animals. Lab. Anim. Sci. 24:737–42.