

Experimental Hybridization of Geographic Strains of *Anopheles stephensi* (Diptera: Culicidae)^{1,2}

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ABSTRACT

Differences in egg structure, fecundity, feeding behavior, susceptibility to *Plasmodium cynomolgi* malaria, and longevity were demonstrated in 3 laboratory strains of *Anopheles stephensi* Liston originating from India, Iran, and Iraq. The 6 reciprocal genetic crosses of the 3 strains were made, and the fecundities of the 6 parental crosses and of the 6 types of F₁ hybrids were determined and compared with those of the 3 parent strains. An intricate pattern of interstrain fertility was observed. Although increased fecundity was observed in certain paren-

tal crosses, reduced fecundity was observed in others. Similar effects were observed in the F₁ hybrids. These effects were found to be due mainly to decreased or increased egg development or hatch rate, or both. Oviposition rates did not vary greatly, and no abnormality in gonadal development was found. The findings are discussed with respect to taxonomy, population genetics, evolution, and possibilities of genetic control of the species.

The genetics of mosquitoes has been reviewed by Kitzmiller (1953), Rozeboom and Kitzmiller (1958), Davidson and Mason (1963) and Wright and Pal (1967). Although it is a standard laboratory species and a major vector of malaria in southern Asia, *Anopheles stephensi* Liston remains genetically obscure. Mating behavior in this species has been studied by Russell and Mohan (1939a), Quraishi and Arthur (1963) and Quraishi (1965). Spermatogenesis, karyotype, and larval salivary gland morphology have been described by Rishikesh (1955, 1959a, b) and Sharma et al. (1969). Genes for dark larva, DDT resistance, and dieldrin resistance have been characterized by Davidson and Jackson (1961a, b), Singh and Mohan (1965) and Mason and Davidson (1966).

Sweet and Rao (1937) and Rao et al. (1938) distinguished sympatric "races" (designated "*A. stephensi*, type form" and "*A. stephensi* var. *mysorensis*") on the basis of differences in egg length, egg width, length of the egg float, and number of ridges on the egg float. These traits appeared to breed true within the parental lines and to exhibit dominance/recessiveness in the hybrids and recombination in succeeding inbred generations (Sweet et al. 1938). Additional measurements of the egg of *A. stephensi* have been recorded by Afridi et al. (1958) and Davidson and Jackson (1961b), and details of the structure of the egg have been described by Christophers and Barraud (1931) and Hinton (1968). The 2 races have been further compared with respect to wing length and breadth (Subba Rao et al. 1938); maxillary indices (Senior-White 1940); amenity to colonization, i.e. hardiness, longevity, feeding behavior, oviposition behavior, and fertility (Sweet and Rao 1937, Senior-White 1940, Russell and Mohan 1941); and malarial vector capacity (Sweet and Rao 1937, Roy et al. 1938, Russell and Mohan 1939b, Senior-White 1940,

Covell 1944). Sweet and Rao (1937) and Sweet et al. (1938) demonstrated a degree of sterility in the reciprocal parental crosses and in the hybrids, which they inbred through the F₄ generation. Instances of ovarian (but not of testicular) atrophy were reported in the hybrid generations. The 2 forms were given subspecific status by Puri (1949), and this status was accepted by Stone et al. (1959).

Several authors have suggested that the taxon *Anopheles stephensi* comprehends a species complex analogous to the *Anopheles maculipennis* Meigen, *Anopheles gambiae* Giles, and *Culex pipiens* L. complexes. The purpose of the present study was to assess the degree of cross and hybrid fertility of 3 laboratory strains of *A. stephensi* originating in India, Iran, and Iraq. Davidson and Jackson (1961b) and de Zulueta et al. (1968) have shown that such crosses yield fertile offspring.

MATERIALS AND METHODS

General.—The study was conducted from March 1968 to October 1969 at the Walter Reed Army Institute of Research, Washington, D.C. In conducting the research described in this report, the investigator adhered to the "Guide for Laboratory Animal Facilities and Care" as promulgated by the Committee on the Guide for Laboratory Animal Facilities and Care of the Institute of Laboratory Animal Resources, National Academy of Sciences-National Research Council.

Three geographic strains of *A. stephensi* were utilized: (1) INDIA STRAIN from the colony established in 1947 by Shute and Maryon (1966) with material collected near Delhi. This strain was received in 1962 from Mr. P. G. Shute, Malaria Reference Laboratory, Horton Hospital, London (Thompson and Bell 1968). During the course of the present study, it was periodically replaced or supplemented with material from a daughter colony established at Insect Control and Research, Inc., Baltimore, Md., in 1967 (Gerberg et al. 1968). Strain replacement reinforces standard insectary procedures for the prevention of cross-contamination of strains and mitigates the effects of insidious selection and genetic drift. (2) IRAN STRAIN from a colony established ca. 1957 with material collected near Kazerun. This

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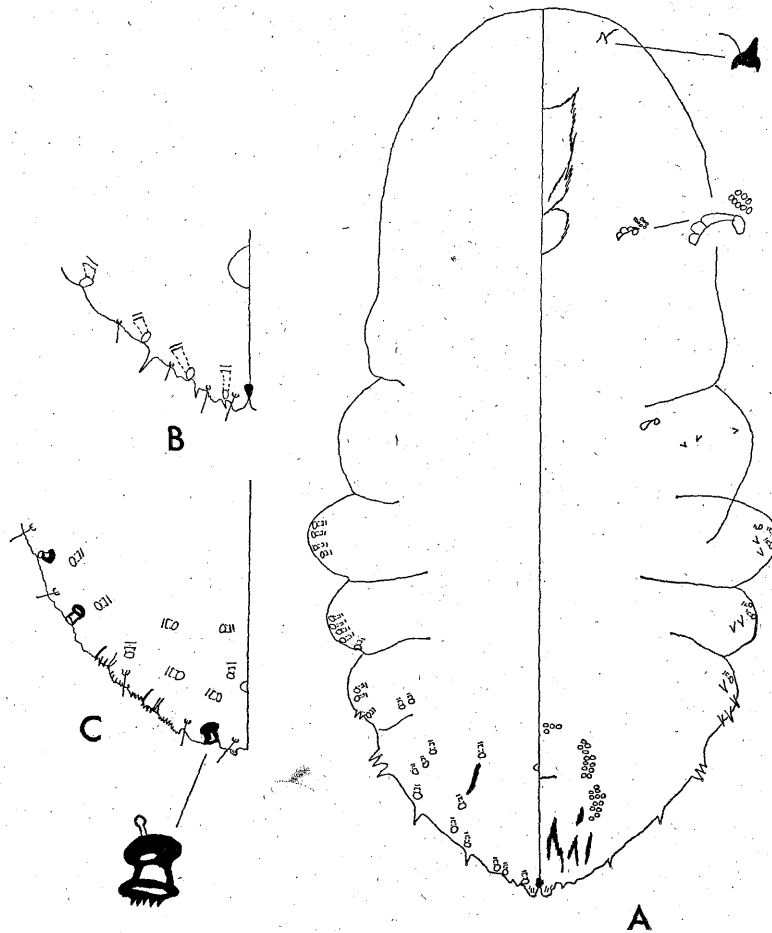


FIG. 2.—*Chionaspis betulae*, n. sp.: A, general features of adult female, bark form; B, pygidium of 2nd instar female; C, pygidium of 2nd instar male.

a well developed glandular system, reduced processes of pygidial margin, and cuplike ducts on what appear to be segments 4, 5, and 7 (Fig. 2,C). Female with a reduced glandular system but otherwise similar to adult female.

Remarks.—Combining the leaf and bark forms under 1 name and rejecting *Phenacaspis* for the leaf form seem justified not only by the work of Takagi and Kawai (1967), but also by the discovery of a specimen which is intermediate between the 2 forms. This specimen (collected on a leaf) has 1 of the median lobes identical to the bark form while the other median lobe is similar to the leaf form (Fig. 1,C).

The bark form of this new species resembles *C. acericola* Hollinger but differs as follows: (1) median lobes of *C. acericola* symmetrical and laterally notched, in *C. betulae* asymmetrical, not notched; (2) gland spines absent on the 1st abdominal segment in *C. acericola*, always present in *C. betulae*; (3) several small submedian macroducts on abdominal segments 3 and 4, these lacking in *C. betulae*.

The leaf form of *C. betulae* resembles the leaf form

of *C. gleditsiae* Sanders (= *Phenacaspis spinicola* Deitz & Morrison) more than any other North American species. It differs in having fewer (less than 10) dorsal macroducts, while *C. gleditsiae* reportedly has more than 20.

Described from 8 adult females of the bark form, 7 adult females of the leaf form, 1 adult female of intermediate form, and immature material. Type-locality, east bank of Ocmulgee River near State Route 16, Jasper Co., Ga. Additional material on the same host from Spalding Co., Ga., is at hand. The holotype, an adult female of the bark form, will be deposited in the University of Georgia collection at Athens. Paratypes will be deposited in the U.S. National Museum of Natural History collection and the authors' collections.

We are grateful to Dr. Michael Kosztarab for the loan of specimens of *C. acericola*.

REFERENCE CITED

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strain was received in March 1968 from Dr. E. R. Shagudian, Teheran University. It was replaced in January 1969 with material from the parent colony. (3) IRAQ STRAIN from a colony established in 1963 with material collected near Basrah. This strain was received in August 1968 from Dr. E. J. Gerberg, Insect Control and Research, Inc., Baltimore. It was replaced in January 1969 with material from the Baltimore colony.

Three insectary rooms were utilized for maintenance of the strains and conduct of the experiments. The colonies and the experimental activities were intermittently shifted among the rooms. This procedure moderates environmental and selection effects in data intended for genetic analysis. Sample means and standard deviations for temperature and humidity within the rooms were $26.8 \pm 1.2^\circ\text{C}$ and $73.2 \pm 11.6\%$ RH, respectively. Natural sunlight was excluded; 12 to 16 hr of fluorescent lighting were intergraded with darkness by simulated twilight using the controller developed by Levin et al. (1958).

Larvae were reared on ground "D. & G." brand dog biscuits⁵ supplemented with locally produced wheat straw. The adults were maintained on sucrose solution approximating 10% in pads of cotton wool. White laboratory rabbits were used as the blood meal source for maintenance of the colonies. New generations of each strain were hatched weekly.

Biological Comparisons of the Strains.—To demonstrate genetic disparity among the strains, to facilitate comparison of the strains with those of previous authors, and to monitor the purity of the strains, the egg structure, fertility, feeding behavior, malarial susceptibility, and longevity of the 3 strains were compared.

The length and width of 60 eggs of each strain (on wet filter paper) were measured with an ocular micrometer in a stereoscopic microscope at 15 \times power, and the number of ridges along 1 side of the egg float was counted at the same time. This was done in 2 replicates of 30 eggs each, representing separate generations of the strains. The means and standard errors for length, width, and number of float ridges were computed for the 60 eggs of each strain. Data on the number of eggs developed in the ovaries following a single blood meal, the proportion of developed eggs deposited (percent oviposition) and the proportion of deposited eggs hatching (percent hatch) were derived from the hybridization experiments described below.

Rhesus monkeys infected with the malaria parasite *Plasmodium cynomolgi* Mayer were utilized for comparisons of the laboratory feeding responses and malarial susceptibilities of the adult females of the 3 strains. Only nulliparous females approximately 1 week old were used in the feeding trials. For a single trial, mosquitoes of the India strain and either the Iran or the Iraq strain were placed in screen-topped, half-liter ice cream cartons, which were taped to the shaved abdomen of a restrained monkey. The

mosquitoes were allowed to feed for approximately 30 min. After feeding, the mosquitoes were anesthetized with diethyl ether vapor, the numbers of fed and unfed mosquitoes were counted (by naked eye), and the proportion of mosquitoes feeding was calculated for each strain. There were 22 such comparisons of the India and Iran strains and 17 of the India and Iraq strains. The numbers of mosquitoes used in a trial averaged 171.4 for the India strain, 108.7 for the Iran strain, and 126.5 for the Iraq strain. The mean and standard error for proportion feeding (over all trials) were calculated for each strain in each of the 2 comparisons.

The fed mosquitoes from the feeding trials were held for 7–9 days in the insectary (ordinarily for 8 days) to allow development and growth of the malaria parasite. At this time a sample (avg 17.4) of the mosquitoes in each lot was dissected in methyl blue-tinted 0.85% saline solution. The stomachs were examined with a bright-field microscope at 100 \times in cover-slipped mounts, the oöcysts on each were counted, and the average per mosquito was obtained for each lot. The linear regressions of mean oöcyst count in the Iran and Iraq strains on the corresponding mean oöcyst counts in the India strain were computed by the least squares method. The linear regression coefficients were taken as the measure of relative susceptibility to *P. cynomolgi* malaria.

For comparisons of adult longevity, pupae of the 3 strains were placed in 15-liter cylindrical cages of plastic and wood with wire-screen tops. Non-emerging pupae were removed the following day. On each succeeding day the dead adult mosquitoes were removed and their numbers and sex were recorded. Sucrose solution was provided during the course of the test; blood meals were not provided. When all mosquitoes had died, the cumulative percent mortality by day of life and sex were calculated for each strain. From these data the median longevity and its standard error was computed for each sex of each strain by the methods of probit analysis.

Fertility of the Parental Crosses.—Each of the 6 reciprocal crosses of the 3 strains and each of the 3 within-strain matings were conducted in the same manner. For a given mating experiment, pupae of the same day from 2 strains were allowed to emerge from wet filter paper on wet cotton wool in screen-topped half-liter ice cream cartons. On the 1st and 2nd days after pupation, the emerged adults were anesthetized with diethyl ether vapor and sorted into screen-topped 1-liter ice cream cartons for the 4 possible matings of males and females of 2 strains. Repeated dissections of spermathecae made at this time during the study demonstrated that mating did not occur within the first 24 hr of adult life. The mosquitoes were held in the 1-liter containers for 2–9 days to allow time for the desired matings to occur.

On the last day or last 2 days of the mating period the mosquitoes were offered a blood meal. Because the number of eggs developed by *A. stephensi* depends on the host species (Roy 1931), white laboratory mice were used as the blood meal source for all mat-

⁵ Price-Wilhoite Co., Frederick, Md. 21701.

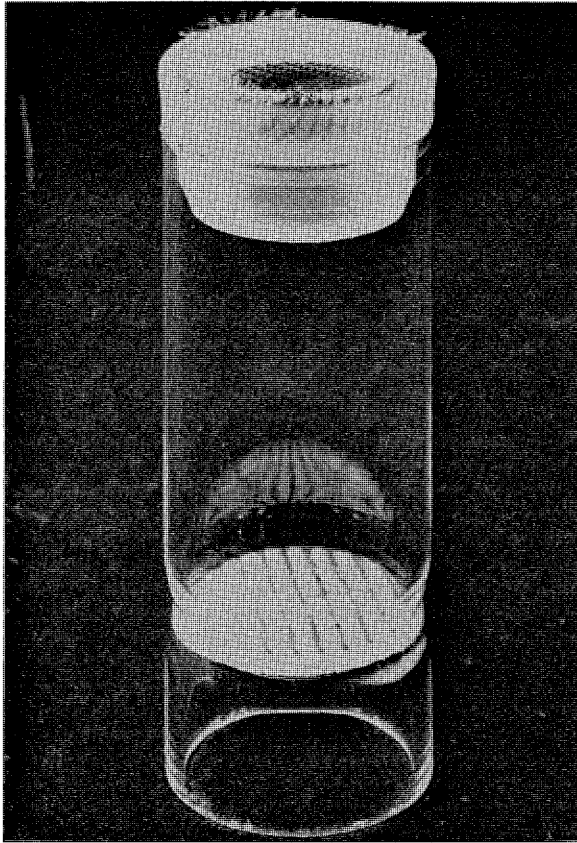


FIG. 1.—Oviposition vial used in mating experiments.

ing experiments. The donor mice were restrained in the supine position, shaved over the abdomen, and placed on the screened top of the cage to allow the mosquitoes to feed. After completion of feeding, the fed females were segregated with an aspirator, or with forceps after anesthetization with diethyl ether vapor, and isolated in screen-topped 7.5×3.0-cm-diam glass vials having fused sintered-glass floors (Fig. 1). These vials were placed in plastic racks to which water could be added to rise through the sintered glass (Fig. 2). Water for oviposition was added 1–4 days after feeding.

After 4–8 days, mosquitoes that had oviposited were dissected in 0.85% saline solution to determine the number of eggs retained in the ovaries, if any. A moderate suction was then applied to the oviposition vials to withdraw the water and immobilize the larvae, and the number of eggs deposited and the number of larvae hatched were counted with a hand-held reciprocating counter using a stereoscopic microscope. Egg development (the number of eggs retained plus the number deposited), percent oviposition (percent of developed eggs deposited) and percent hatch (percent of deposited eggs hatched) were calculated for each mosquito.

Mating experiments of this type were repeated 2–4 times, until data on 20 or more mosquitoes in each of the 9 possible matings had been accumulated. An oc-

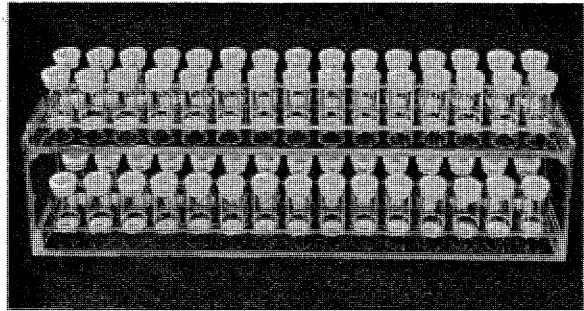


FIG. 2.—Rack of oviposition vials.

casional replicate gave disparate results. When this occurred, the mating experiment was given further replication, but the disparate data were not discarded. Means and standard errors for egg development, percent oviposition, percent hatch, and numbers of hatched larvae produced were computed for each of the 9 matings.

In the case of *A. stephensi*, unmated females develop eggs if given a blood meal (Roy 1940). As an aid in the interpretation of the results, egg development and percent oviposition in unmated females of the 3 strains were determined as described above. In addition, the insemination rate among ovipositing females of the 3 strains was determined by dissection.

Fertility of the F₁ Hybrids.—Larvae from the eggs hatched in the 6 reciprocal parental crosses were reared, and the pupae were placed in cylindrical or cubic cages (15–28 liters) for emergence. Periods of 9–14 days were required for complete pupation, emergence, and mating to occur. On the last day or two of this period the mosquitoes were allowed to feed on white laboratory mice, and the procedures for determining egg development, percent oviposition, and percent hatch were then carried through as in the parental crosses. In addition, 15 of the hybrid males and 15–17 non-ovipositing hybrid females of each cross were dissected to verify that gonadal development was normal.

RESULTS

Biological Comparisons of the Strains.—As shown in Table 1, while the 3 strains are intermediate between *A. stephensi* type form and *A. s. mysorensis* with respect to egg length, they more nearly resemble *mysorensis* with respect to egg width and number of ridges on the egg float and the type form with respect to oviposition. The Iran strain resembles *mysorensis* with respect to laboratory feeding response and adult longevity, but the India and Iraq strains more nearly resemble the type form in these respects.

The India and Iraq strains are generally quite similar, differing mainly in egg width, percent hatch, and adult male longevity. The Iran strain is more exceptional, differing from both the India and the Iraq strains in the number of ridges on the egg float, egg development, laboratory feeding response, susceptibility to *P. cynomolgi*, and adult female longevity.

Fertility of the Parental Crosses.—Data on egg

Table 1.—Biological comparisons of 3 geographic strains of *A. stephensi*. See Materials and Methods section for detailed explanation of entries.

Item	Mosquito strain					
	India		Iran		Iraq	
	No.	Mean ± SE	No.	Mean ± SE	No.	Mean ± SE
1. Egg length	60	519.6 ± 2.7 μ	60	509.4 ± 3.6 μ	60	511.6 ± 2.8 μ
2. Egg width	60	161.8 ± 3.1 μ	60	145.3 ± 2.3 μ	60	137.9 ± 0.5 μ
3. No. ridges on egg float	60	15.0 ± 0.2	60	12.4 ± 0.2	60	14.1 ± 0.2
4. Egg development (virgin ♀)	42	95.0 ± 7.0 eggs	20	46.8 ± 8.2 eggs	20	80.2 ± 6.7 eggs
5. Egg development (mated ♀)	21	118.5 ± 9.7 eggs	20	70.6 ± 9.6 eggs	22	118.0 ± 10.7 eggs
6. % oviposition	21	99.9 ± 0.1%	20	97.8 ± 1.3%	22	93.9 ± 4.0%
7. % hatch	21	79.0 ± 6.8%	20	53.6 ± 8.4%	22	47.4 ± 8.2%
8. Feeding response						
a. India vs. Iran	22	92.6 ± 1.0%	22	69.0 ± 4.4%		—
b. India vs. Iraq	17	88.4 ± 2.4%		—	17	85.1 ± 3.2%
9. Susceptibility to <i>P. cynomolgi</i>						
a. India vs. Iran	17	1.00 ± 0.00	17	0.34 ± 0.02		—
b. India vs. Iraq	16	1.00 ± 0.00		—	16	0.68 ± 0.03
10. Longevity (♀)	39	16.2 ± 0.8 days	36	9.6 ± 1.1 days	88	14.6 ± 1.7 days
11. Longevity (♂)	85	24.4 ± 1.4 days	44	11.5 ± 1.5 days	89	13.8 ± 2.6 days

development, percent oviposition, and percent hatch in the 6 parental crosses and the 3 within-strain matings are given in Table 2. Egg development in females of the India strain was reduced by cross-mating with males of either the Iran or Iraq strains but egg development in females of the Iran strain was increased by cross-mating with males of either the India or Iraq strains. Egg development in females of the Iraq strain was not significantly affected by cross-mating with males of either the India or Iran strains.

Although percent oviposition did not vary greatly among the 9 matings, females of the India strain tend to retain more eggs when cross-mated with males of either the Iran strain or the Iraq strain. Percent hatch of eggs from females of the India strain was

reduced by cross-mating with males of either the Iran or Iraq strains. Percent hatch of eggs from females of the Iraq strain was also reduced by cross-mating with males of the Iran strain.

The overall fecundity of a given mating, or the average number of hatched larvae produced, is determined by 3 factors: egg development, oviposition, and eclosion. Data on progeny are given in Table 3 for each of the 3 within-strain matings and each of the 6 parental crosses. Although fecundity was reduced in females of the India strain cross-mated with males of either the Iran or Iraq strains and in females of the Iraq strain cross-mated with males of the Iran strain, fecundity was increased in females of the Iran strain cross-mated with males of the India strain.

The insemination rate among females ovipositing in the glass oviposition vials was 89.9%, based on dissections of 20–26 ♀ of each strain. This proportion did not vary significantly from strain to strain. Unmated females develop 20–34% fewer eggs than mated females (Table 1). It was observed that ovipositing virgins were nearly always mosquitoes that were moribund and that they deposited an average of only 59.4% of the eggs developed. Such eggs ordinarily do not hatch. However, a record was obtained of an unmated female (an F₁ hybrid of the India ♀ × Iran ♂ cross) which developed 150 eggs and deposited 76, 1 of which hatched. The larva died in the 1st stadium.

Two possible mutations were observed in the course of the study. In the 1st instance, a female of the Iraq strain mated with a male of the Iraq strain deposited 171 eggs, all of which were floatless. The hatch rate was 95% and the larvae were viable. In the 2nd instance, a female of the India strain mated with a male of the Iraq strain deposited 121 eggs, of which 112 or 93% hatched. The larvae were albinistic (a pale reddish yellow with wine-red eyes); all perished in the 1st stadium. Both these supposed mutations would seem to be selectively negative.

Table 2.—Egg development, percent oviposition, and percent hatch in reciprocal crosses and within-strain matings of 3 geographic strains of *A. stephensi*. Numbers of mosquitoes used are given in parentheses under egg development.

Origin of female	Origin of male		
	India	Iran	Iraq
<i>Egg development (mean ± SE)</i>			
India	118.5 ± 9.7 eggs (21)	82.0 ± 8.4 eggs (27)	84.1 ± 5.9 eggs (24)
Iran	97.4 ± 7.3 (21)	70.6 ± 9.6 (20)	101.1 ± 9.3 (20)
Iraq	109.9 ± 11.2 (23)	106.0 ± 9.3 (24)	118.0 ± 10.7 (22)
<i>% oviposition (mean ± SE)</i>			
India	99.9 ± 0.1%	88.5 ± 5.4%	88.4 ± 5.7%
Iran	97.7 ± 1.5	97.8 ± 1.3	96.4 ± 2.8
Iraq	90.2 ± 4.7	88.8 ± 6.1	93.9 ± 4.0
<i>% hatch (mean ± SE)</i>			
India	79.0 ± 6.8%	52.6 ± 7.5%	52.5 ± 9.0%
Iran	63.3 ± 7.4	53.6 ± 8.4	46.4 ± 9.0
Iraq	51.1 ± 7.6	28.3 ± 7.2	47.4 ± 8.2

Table 3.—Fecundities (no. of progeny) of parental crosses, within-strain matings, and F_1 hybrids of 3 geographic strains of *A. stephensi*. Figures following each mean are SE of the mean.

Origin of female	Origin of male		
	India	Iran	Iraq
<i>Parental crosses</i>			
India	101.7±12.4	46.7± 9.6	50.5±10.0
Iran	64.4± 8.8	46.4±10.3	41.4± 9.9
Iraq	56.0±11.8	31.5± 9.5	51.1±11.1
<i>F₁ hybrids</i>			
India	101.7±12.4	64.9± 9.5	73.0±11.3
Iran	80.2±12.3	46.4±10.3	42.4± 7.6
Iraq	59.0±11.4	36.3± 8.2	51.1±11.1

Fertility of the F₁ Hybrids.—Egg development, percent oviposition and percent hatch in the 6 F_1 hybrid types and the 3 within-strain matings are summarized in Table 4. Egg development equal to or exceeding that of both parent strains was observed in the F_1 hybrids from the Iran ♀ × India ♂ cross and in the F_1 hybrids from the India ♀ × Iraq ♂ cross. Egg development in F_1 hybrids from the reciprocal crosses of the Iran and Iraq strains and in F_1 hybrids from the India ♀ × Iran ♂ cross was intermediate between that of the 2 parent strains. Egg development was less than that of either parent strain in F_1 hybrids from the Iraq ♀ × India ♂ cross.

Percent oviposition did not vary greatly among 6 F_1 hybrid types and 3 parental strains. Values intermediate between those of the 2 parent strains or less than that of either parent strain were observed for the 6 F_1 hybrid types. In each instance, percent hatch in eggs of the 6 F_1 hybrid types either approximated that of 1 parent strain or was intermediate between that of the 2 parent strains.

Overall fecundity, or number of progeny (hatched larvae) produced, was intermediate between that of the 2 parent strains in F_1 hybrids from reciprocal crosses of the India and Iran strains and also in F_1 hybrids from reciprocal crosses of the India and Iraq strains (Table 3). It was less than that of either parent in F_1 hybrids from reciprocal crosses of the Iran and Iraq strains.

No obvious abnormality in gonadal development was observed in dissections of 15 ♂ of each hybrid type or in dissections of 15–17 non-ovipositing females of each hybrid type. Motile sperm were observed in the testes of all males dissected. In a few females, the ovaries seemed smaller than the usual for *A. stephensi*, but follicular development in these appeared entirely normal. In some, follicular development did not proceed beyond Stage II; however, this condition also occurs in some blood-fed individuals of the parent strains. One F_1 hybrid of the India ♀ × Iran ♂ cross was found to have developed 1 egg in the left ovary and 45 in the right; the undeveloped follicles were in Stage I.

DISCUSSION

Data on egg length and width, number of ridges on the egg float, laboratory feeding and oviposition behavior, fecundity, longevity, and susceptibility to malaria are presently available for 5 populations of *A. stephensi*: the type form and var. *mysorensis* of Sweet and Rao (1937) and the laboratory strains from India, Iran, and Iraq used in the present study (Table 1). It appears that no two of these forms are precisely equivalent. Until now laboratory crossing experiments have invariably produced viable, fertile offspring without recourse to the forced-mating technique, although a degree of sterility occurs in certain crosses (Sweet and Rao 1937, Sweet et al. 1938, Davidson and Jackson 1961b, de Zulueta et al. 1968, Table 3 of present study). In this connection, Russell and Mohan (1939a) reported that males of the type form inseminate females of *A. annularis* van der Wulp in cages, but it is not known whether this cross is fertile. Both the type form and var. *mysorensis* are widely distributed on the Indian subcontinent, but the population of a given locality is usually either one or the other, the former being chiefly urban and the latter chiefly rural (Sweet and Rao 1937, Rao et al. 1938, Senior White 1940, Covell 1944, Afridi et al. 1958).

It seems unlikely that cryptic species exist within the taxon *Anopheles stephensi*, since interbreeding is possible among all forms tested thus far. In addition, subspecies status for the type form and var. *mysorensis* seems inappropriate since these 2 forms are sympatric. On the basis of present information it seems best to regard these and other forms as local population variants. The adoption of Linnaean race, form, or variety names to specify the numerous localized populations of a species is not warranted. Such populations can be adequately specified by a statement

Table 4.—Egg development, percent oviposition, and percent hatch in F_1 hybrids and within-strain matings of 3 geographic strains of *A. stephensi*. Numbers of mosquitoes used are given in parentheses under egg development.

Origin of female	Origin of male		
	India	Iran	Iraq
<i>Egg development (mean ± SE)</i>			
India	118.5± 9.7 eggs (21)	94.7±7.6 eggs (25)	133.1± 6.3 eggs (22)
Iran	124.5±12.7 (22)	70.6±9.6 (20)	70.1± 7.5 (26)
Iraq	91.5±11.4 (23)	81.8±6.9 (22)	118.0±10.7 (22)
<i>% oviposition (mean ± SE)</i>			
India	99.9± 0.1%	98.0±1.4%	92.8± 4.0%
Iran	84.9± 7.0	97.8±1.3	97.7± 1.5
Iraq	97.3± 1.9	93.5±4.6	93.9± 4.0
<i>% hatch (mean ± SE)</i>			
India	79.0± 6.8%	65.8±6.2%	54.3± 7.9%
Iran	61.0± 8.0	53.6±8.4	54.6± 7.1
Iraq	59.2± 7.1	40.8±8.2	47.4± 8.2

of time and place of occurrence, history (if in culture), and known characters (egg dimensions, breeding sites, insecticide susceptibility, host preference, etc.). A method for quantitative nomenclature is also available (duPraw 1965).

The evolutionary significance of genetic variation and partial intersterility at the population level in *A. stephensi* is difficult to assess. Certain alleles, such as those expressed in variant feeding and oviposition behavior, fecundity, and longevity, would seem to be adaptive in nature. The adaptive value of others, such as those expressed in egg dimensions and susceptibility to malaria, is not apparent. These latter may represent selectively neutral mutations that have become fixed in the population by genetic drift (King and Jukes 1969). The existence of partial sterility between certain populations is suggestive of incipient speciation, since a cordon sanitaire at interpopulation boundaries would promote reproductive isolation. Speciation in localized populations in the absence of barriers to gene flow is discussed by Ehrlich and Raven (1969).

In *A. stephensi*, crossmating effects on fecundity are due primarily to altered egg development and hatch rate (Table 2). It is known that the semen of *Aedes aegypti* (L.) contains a substance ("matrone") which inhibits repeated mating of the female (Craig 1967; Fuchs et al. 1968, 1969). The presence of semen in the spermatheca of the female ("spermathecal stimulation") is a prerequisite to egg development in several anophelines (Roy 1940; Muirhead-Thomson 1941, 1948; Bates 1949). Unmated females of *A. stephensi* develop fewer eggs than do mated females, and seldom deposit those that are developed (Table 1). It is now known also that the semen of a foreign strain may be either more or less effective than the native semen in promoting egg development and oviposition (Table 2).

Hatch rates were reduced by 20–25% in 3 of the 6 parental crosses (Table 2). Cross sterility can be due to genic, chromosomal, or cytoplasmic mechanisms. Chromosomal sterility is known in interspecies crosses of members of the *Anopheles maculipennis* complex (Kitzinger et al. 1967), and cytoplasmic sterility is known in intra-species crosses of populations of the *Culex pipiens* complex (Laven 1967).

A similar range of variation in egg development, oviposition, and hatch was observed in the F_1 hybrids. Moreover, the effects observed in a given hybrid type (Table 4) did not always agree with those observed in the corresponding parental cross (Table 2). In the case where the hybrid is more fecund than either parent, the operation of heterotic factors is evident. The case where the hybrid is less fecund than either parent can be interpreted in terms of sterility factors analogous to those operating in the parent crosses. Intermediate fecundity can be interpreted in terms of incomplete dominance, multiple factors, and the interaction of heterotic and sterility factors.

From the forgoing discussion, there appear to be 2 approaches to the genetic control of *A. stephensi*. In the 1st approach, the seminal factor controlling egg

development and oviposition would be identified. If this factor proved to be chemical (as in the case of "matrone"), it would be evaluated as a potential chemical control agent. In the 2nd approach, advantage might be taken of partial interstrain sterility to breed strains that are completely sterile when crossed. In theory, appropriately staged mass-release of reproductively incompatible strains could successively replace and eliminate a natural population. Alternatively, a strain which breeds true for nonsusceptibility to infection with human malaria might be produced for use in replacement of natural populations (see Table 1). The feasibility of population replacement has been evaluated by The World Health Organization (Wright and Pal 1967, App. A). Procedures for mass-rearing *A. stephensi* are available (Gerberg et al. 1968).

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Lutrochus arizonicus New Species, with Notes on Ecology and Behavior (Coleoptera, Dryopoidea, Limnichidae)¹

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ABSTRACT

Lutrochus arizonicus, described from Yavapai County, Arizona, is morphologically and ecologically similar to *L. luteus* LeConte of Texas. Only adults have been taken. The microhabitat and some aspects of behavior are strikingly reminiscent of *Psephenus*, the beetles being found in fast streams at or near the water line on the moist downstream sides of stones or other objects protruding above the water surface. They are less agile than

Psephenus both afoot and in flight, and creep beneath the water more readily than males of *Psephenus*. Little courtship, if any, appears to precede copulation. Details of ecology and behavior are essentially the same as in other species of *Lutrochus* ranging from Oklahoma to northern South America, but these details have not hitherto been published for any species of limnichid.

To date, only 2 species of *Lutrochus* Erichson (1847) have been described from the United States:

L. luteus LeConte (1852) from central Texas, and *L. laticeps* Casey (1893) from Michigan. The range of the latter extends south and east, at least to eastern Oklahoma and Tennessee. Other species have been described from Cuba, Guatemala, and several South American countries (Blackwelder 1944). One of us (H. P. B.) has collected and observed *Lutrochus* from Missouri and Oklahoma to Brazil, and is

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