THE UNIVERSITY OF OKLAHOMA

GRADUATE COLLEGE

STUDIES ON THE FECUNDITY OF A LABORATORY STRAIN OF *ANOPHELES STEPHENSI* LISTON

A DISSERTATION

SUBMITTED TO THE GRADUATE FACULTY

in partial fulfillment of the requirements for the

degree of

DOCTOR OF PUBLIC HEALTH

BY

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Oklahoma City, Oklahoma

1970

STUDIES ON THE FECUNDITY OF A LABORATORY STRAIN OF

ANOPHELES STEPHENSI LISTON

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ACKNOWLEDGMENTS

This study was made possible through the cooperative arrangement between the Department of Environmental Health, University of Oklahoma Medical Center and Dr. H. C. Barnett, Professor of Medical Entomology and Ecology, the University of Maryland, Institute of International Medicine, Baltimore, Md.

I would like to express my sincere gratitude and deep appreciation to Dr. H. C. Barnett, who suggested the problem and directed the work and for his invaluable assistance and advice throughout the research.

With reverence and gratitude, I express my sincere indebtedness to these people who gave me immense insight and knowledge: Professors Cluff Hopla, Michael H. Ivey, Charles H. Lawrence, Raymond A. Mill, and my Major Professor, Dr. Carl A. Nau.

For the assistance given by Mrs. Pamela Lankford, I express my deep appreciation and sincere thanks.

Recognition and appreciation is extended to the World Health Organization during whose fellowship this work was done.

I also thank my wife for her patience and endurance of the lonely confinement throughout my busy schedule.

With appreciation and gratitude I also acknowledge the assistance given to me by all the people with whom I have been associated during the course of my stay in this country.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

STUDIES ON THE FECUNDITY OF A LABORATORY STRAIN OF *ANOPHELES STEPHENSI* LISTON

CHAPTER I

INTRODUCTION AND LITERATURE REVIEW

Anopheles stephensi Liston, 1901, was first described from Ellichpur in India (1). Since that time it has been recorded from several other countries in the Middle East and the Far East. Sweet and Rao (2) distinguished two types of *A. stephensi* on the basis of the measurement of the ova, *A. stephensi* type form and *A. stephensi* variety *mysorensis*. They reported that the type form was readily colonized in small cages in the laboratory but variety *mysorensis* was extremely difficult to maintain in captivity. Sweet, Rao and Subba Rao (3) reported existence of natural barriers to successful crossbreeding between the two forms, but de Zulueta, Chang, Cullen and Davidson (4) found no evidence of hybrid sterility between five populations of *A. stephensi* from India, Iraq and Iran.

A. stephensi is widely found in the plains of the Indo-Pakistan sub-continent. From India its distribution extends eastward into Burma, Thailand and China and westward through the Bahrein Islands in the Persian Gulf to Iran, Iraq and Eastern Arabia (5). *A. stephensi* has also been reported from Afghanistan (6). It has not been recorded from either Ceylon or the Andaman Islands (5).

The notoriety of A. stephensi as a malaria vector in the Middle East, the Persian Gulf and in the Indo-Pakistan sub-continent is well known. Daggy (7) reported that A. stephensi was the only malaria vector in the oases of Eastern Saudi Arabia. Pringle (8) considered A. stephensi as an important malaria vector in certain parts of Iraq. Dow (9) and Qureshi et al. (10) stressed the importance of this mosquito as a malaria vector in Iran. Dow (9) considered A. stephensi to be a vector of great importance in Abadan in southwestern Iran. Afridi and Majid (11) found A. stephensi prevalent in Bahrein Islands in the Persian Gulf, where it was the only naturally infected mosquito in the area. In recent years, A. stephensi has been responsible for several malaria epidemics in Iran and Iraq (4).

Covell (12) reported that A. stephensi was an important malaria vector found throughout India. He referred to this mosquito as the notorious carrier of malaria in the city of Bombay, in Delhi and in other Indian cities. Afridi, Majid and Singh (13) reported A. stephensi to be the only infected mosquito in the state of Kutch on the southwestern shoreline of India. According to Horsfall (14), Roy et al., reported an infection rate of 5.4 per cent in A. stephensi in Madras Province in South India. Bentley (15), and Christopheres (16), reporting on the famous malaria epidemic of Bombay in 1908, incriminated A. stephensi as solely responsible for malaria epidemics in that city. They remarked on its unusual adaption to breed in cisterns and wells throughout the city of Bombay.

Although A. stephensi is equally abundant and perhaps important in the rural areas of India and Pakistan, this mosquito has been labelled

mainly as an urban vector of malaria in the Indo-Pakistan sub-continent. According to Afridi (17), ". . . it is a potent vector in the coastal tracts of India from Karachi through Kathiawar to Bombay and Banglore and around Vizagapatam." Subba Rao and Apa Rao (18), however, reported A. stephensi to be the chief rural malaria carrier in Vizagapatam in Madras Province, and Neogy and Sen (19) incriminated A. stephensi as a vector of malaria in rural Bengal. Rafi (20) referred to A. culicifacies and A. stephensi as the well established malaria vectors of the Punjab province of both India and Pakistan. Ansari and Nasir (21) reported that A. stephensi constituted about 30 per cent of all anopheline mosquitoes collected in and around the city of Lahore, West Pakistan during spring and summer. The peak population of this mosquito is observed from March to May. This writer has observed A. stephensi as the most abundant mosquito in some rural areas of the Punjab during the spring and early summer of most years.

In recent years, the city of Karachi in West Pakistan, has been in the grip of a serious malaria epidemic. A survey of 62 localities in the city revealed that 9.5 per cent of 5,000 blood slides were malaria positive (22). It has further been reported that A. culicifacies, which has been considered to be the chief malaria vector throughout West Pakistan including Karachi (23), was totally absent, and A. stephensi showed a gland infection rate of 1.2 per cent (22).

Most workers have failed to designate the form of A. stephensi in their investigations. Although the vectorial role of variety mysorensis has been controversial, Russel and Mohan (24, 25) failed to detect any significant difference in susceptibility of the two forms to

P. falciparum.

Chlorinated hydrocarbon insecticides account for the largest volume among all other methods of chemical control of insect vectors of human diseases. Consequently, in some vectors, resistance against these insecticides appeared earlier. DDT resistance in A. stephensi was noticed as early as 1953, in Saudi Arabia, where this species continues to be an important malaria vector (26). Subsequent reports from other countries where A. stephensi is a problem mosquito confirmed high vigour, tolerance and physiological resistance to DDT (27). BHC and Dieldrin resistance in A. stephensi was confirmed in 1959 (28).

In West Pakistan A. stephensi from a number of places in the country including the city of Karachi was subjected to WHO standard tests for resistance to DDT and Dieldrin. These tests showed that high tolerance to resistance existed both to DDT and Dieldrin in different areas (29). It has also been reported that A. stephensi from West Pakistan colonized at the Ross Institute, London, was found resistant to both DDT and Dieldrin (22).

As stated by de Zulueta, Chang, Cullen and Davidson (4), physiological resistance to DDT and Dieldrin in A. stephensi is the most serious technical problem encountered in the malaria eradication campaign in the Middle East. In India a similar situation has arisen in areas where A. stephensi is a malaria vector (30).

A nation-wide malaria eradication program, considered to be the second largest program in the world, was launched in Pakistan in 1961 (31). This program has been seriously threatened by the malaria situation in Karachi where the disease has established itself in an endemic

form with recurrent epidemics exclusively due to DDT and BHC resistant A. stephensi. In other areas of West Pakistan where the eradication of malaria had already been achieved, several localized malaria epidemics have since appeared. The origin of infection in all such cases was traced back as having been imported from Karachi, local transmission having been propagated by the local vectors which, at one time, had been free of infective agents.

The World Health Organization (WHO) co-ordination group on Genetic Control of Insects of Public Health Importance (32) noted with deep concern that malaria eradication programs are seriously hampered by inability to control by conventional methods, such species as A. stephensi, A. albimanus and A. balabacensis. This group therefore recommended that extensive genetic and ecological studies on these species be undertaken. Their report also stated that "interest in the biological control of insects has been heightened by the development of insecticide resistance and the consequent partial failure of the chemical approach to control as well as by problems of environmental contamination and associated health hazards."

The hazards accompanying the use of insecticides against vectors of diseases has been a subject of great controversy in recent years. Although no direct evidence is available to prove the ill effects of DDT and related insecticides on human health when applied in permissable dosage, the circumstantial evidence and public opinion is sufficiently strong to warrant only the judicious use of these insecticides, observing strict safety measures. In a malaria eradication program this seems rather difficult to maintain, especially when the application of the in-

secticide is invariably in the household. Widespread use of these insecticides has already created problems of possible contamination of the environment and its potential hazard to fish and wild-life has amply been witnessed (33). A ban on the use of DDT has already been imposed in Sweden and other Scandinavian countries following reports of harm to people (34). This has further strengthened the public opinion in the United States and other free countries for a restricted use of the synthetic insecticides. In view of these drawbacks and pressing demands, the era of the chemical control of pests seems closing. Thus, there is an immediate need to make available methods of insect control other than those based on insecticidal strategy.

CHAPTER II

PURPOSE AND SCOPE

The WHO Scientific Group on Genetics of Vectors (35) upon reviewing the problem of development of resistance to insecticides by certain species, as well as on consideration of environmental contamination as a result of insecticidal strategy, expressed the opinion that the most promising answer to the problem lies in the development of genetic control methods. This group defined genetic control as "any condition or treatment that can reduce the reproductive potential of noxious forms by altering or replacing the hereditary material."

"A number of features in the insect biology suggest that they should be amenable to control by the application of genetic principles. Their high biotic potential and short life cycle makes selection possible from large numbers in short time" (36).

During the past few years many advances have been made indicating that genetic manipulation could be successfully utilized toward insect control. The eradication of the screw-worm fly from Curacao and from southeastern and southwestern parts of the United States by utilizing the sterile male technique (autocidal control) has already been accomplished. Cytoplasmic incompatibility has been employed with success in the control of Culex pipiens fatigans in a small village in Burma (37).

Craig (38) and the WHO Expert Committee (37) has discussed in detail the feasability of genetic control, which includes, among others, methods such as hybrid sterility, meiotic drive, lethal genes, chromosomal mutations and population replacement. The basic principle in genetic control of insects is to utilize factors which will lead to reproductive failure. For such a purpose, an intimate knowledge of the reproduction biology of the species concerned and also of the strain to be used as the tool of control is, therefore, an essential pre-requisite to planning genetic control. Explorations of techniques for mass production under laboratory conditions is another avenue of no less importance, since in genetic control methods an overflooding of the field population with defective gametes may be necessary (32).

The presently available information on the reproductive biology of mosquitoes is relatively small. Moreover, the sexual behavior of mosquitoes varies remarkably from species to species and generalizations cannot always be made. Reproduction in a species of mosquito has an important bearing on the control measures of any kind. Such studies may furnish details for the selection of appropriate genetic strategy (39).

It is now well established that in mosquitoes, susceptibility to Plasmodium infections is a genetically controlled phenomenon (40, 41). Jones (42) has suggested isolation of strains refractory to malaria from among the vector species, which may be used to replace vector strains, thus rendering disease vectors harmless even though the population size is not reduced. Such a strain would establish itself only when it is highly competetive, productive and adaptive. It is important therefore, that knowledge and techniques for inducing the desired qualities in a

species are available. The study of fecundity in a species may lead to resolving some of the basic questions arising in planning a genetic control program. A highly productive laboratory strain can be an invaluable asset in operating mosquitoe factories for the mass production necessary for control through the sterile male technique, exemplified by the control of the screw-worm fly. Additionally an acute need exists for the development of innocuous forms with higher fecundity which may be used to replace vector species and to occupy the vacated ecological niche. Detection of sex distorters and deleterious genes causing sterility, for use in genetic control program, is only possible by studying reproduction in a species.

The objective of this study was a comprehensive investigation of the reproduction potential of A. stephensi under laboratory conditions. In addition, attempts were made to select a strain for greater egg production.

Further, it is generally thought that mosquitoes will develop more eggs after feeding on certain hosts than others. It was desired to compare the egg production capacity of A. stephensi on the bloods of rabbits, mice, man or chicken, in order that a suitable laboratory host for mass production of this species be determined.

Specifically, this study was designed to investigate the following areas of the reproductive biology of A. stephensi:

1. The reproduction potential under standard laboratory conditions.
2. Selection of a high fecundity and fertility strain from the laboratory strain.

3. The influence of blood meals from different species of animals on egg production.
4. The variation in fecundity and fertility in consecutive egg batches.

CHAPTER III

EXPERIMENTAL MATERIALS AND METHODS

The Insectary

The experiments were carried out in the insectary of the University of Maryland, Institute of International Medicine, Division of Medical Entomology and Ecology, in the city of Baltimore, Maryland. In the insectary a temperature of 27°C and a relative humidity of 70 to 80 per cent was constantly maintained. In addition there was overhead artificial lighting, which gave 14 hours of light followed by 10 hours of darkness. Dusk and dawn simulations were provided.

Origin of the Strain

The mosquito used in this study was Anopheles stephensi Liston, which came originally from India and was maintained at the London School of Tropical Medicine and Hygiene, under reference No: 1-STSSDP I, New Delhi, 1950. The culture of this colony was received in Baltimore in January, 1969, through the courtesy of Dr. G. Davidson, of Ross Institute of Tropical Hygiene, London, U.K., and the colony has since flourished. This strain has been under colonization for almost 20 years.

General Techniques

All the experiments were initiated from the stock colony by ran-

domly picking pupae from the larval rearing trays. Paper cartons of 1-gallon capacity, screened on the top and sleeved on the side, served both for adult emergence and further maintenance for 6 to 9 days for mating and initial collective feeding of the female mosquitoes on the vertebrate host. Only the first blood-meal was offered twice at 24 hour intervals. On feeding, the fully engorged females were individually isolated in specially designed holding cages of the following description:

A plastic coated Nestyle paper cup of 1 pint capacity was fitted by a chimney made from a pint-size round paper carton. The base of the paper carton was removed and the top was replaced by nylon netting, held under the ring of the top cover. The female mosquito was introduced in the holding cage by means of a hole punched in the side of the Nestyle cup.

Blood Feeding

The age of the mosquito on first blood feeding ranged between 6 and 9 days. A rabbit was chosen as host in all experiments except in the study relating to comparative egg production on blood-meals from different species of animals. Unlike initial blood feeding, repeat feeding was done only once, starting on the day following each oviposition. Blood was offered each day until the mosquito fed. Some females did not feed for days and died, others fed with a gap of 1 to 10 days after oviposition, although blood was offered on each day following egg laying. Most females, however, fed on blood on the day following oviposition.

Blood feeding of the mosquitoes was accomplished by inverting the gallon cage or the holding cage on the shaved abdomen of a rabbit. Each cage was held in this position for at least 30 minutes. Feeding on

a chick was done by placing a chick tied to a wooden block with elevated wings in the gallon cage. For individual feeding, mosquitoes were released one by one in the gallon cage, allowing 30 minutes feeding time to each mosquito. Later, each mosquito was returned to their respective individual holding cages. Feeding on mice was done in similar manner except that the mouse was enveloped in a soft wire screen to restrict mobility.

Maintenance Sugar Feeding

In the initial stages of the study, maintenance feeding of the adult mosquitoes was done on sugar cubes. It was soon discovered that feeding 100 per cent sugar interfered with oviposition rhythm and it prolonged the interval between blood meals and oviposition. Five per cent sugar solution was therefore substituted for the maintenance diet. Cotton pads dipped in sugar solution were continuously provided before offering the first blood meals, but in the case of individual holding cages, the sugar pads were wet daily with tap water, and replacement was performed every third or fourth day after each blood meal following oviposition.

The Oviposition Receptacle

The standard technique of obtaining eggs on moist filter paper placed over water soaked cotton pads was unsuccessful. It was observed that mosquitoes preferred to oviposit in the bottom of the holding cage, whenever a drop of water was available on the non-absorbant bottom of the holding cage. This created problems, since eggs were destroyed on evaporation of the water, rendering counting and hatching of eggs impos-

sible. An efficient method was discovered which served well for oviposition as well as for obtaining a full larval hatch.

The bottom of the holding cage was lined with filter paper, which was replaced after each oviposition. This prohibited fungal growth, parasitic mites, and it also absorbed any water droplets which condensed in the bottom. The oviposition receptacle was comprised of a 3-ounce paper cup, a 1-ounce plastic cup and a 0.75-ounce wax coated jelly cup. The 3-ounce paper cup was placed in the bottom of the holding cage in an inverted position with the base removed. Thus, it served as a pedestal for the receptacle. The base of the paper cup was fitted with the plastic cup (glass) half filled with tap water. The water in the plastic cup served as a water jacket and it curtailed evaporation of water from the jelly cup. Over the plastic cup was fitted the small jelly cup with its bottom touching the water in the plastic cup. The jelly cup was filled with 3 to 5 milliliters of tap water. Oviposition occurred on the water contained in the jelly cup.

Egg Count

Egg counting was facilitated by a thin scum which formed on the surface of the water in the jelly cup, provided the water did not exceed 5 milliliters in quantity. All the eggs were arranged in rows by manipulation with a needle under a stereoscope and an accurate counting was accomplished.

Larval Hatch

After oviposition, the entire receptacle was removed after giving it an identification number on the pedestal. Counting of the larvae

was done 72 to 96 hours after egg laying. The scum did not interfere with the hatching of the eggs. Accurate larval count could be performed by examining under the stereoscope the eggs still arranged in rows and the larval escape slit could be observed on the shells of all viable eggs. In this study, however, larval hatch was determined by emptying the contents of the jelly cup over a funnel lined with a gridded filter paper. Accurate counting of the larvae was accomplished by examining the filter paper under the stereoscope.

Larval Rearing and the Larval Food

Larvae desired to be reared were transferred to enamel photo trays (8 inches by 12 inches) allowing about 2 milliliters of tap water per larva. The larval food consisted of mink chow and powdered pork liver mixed in equal proportions.

Proof of Insemination

Results have been recorded only from those females which proved to be inseminated. This was confirmed either by the viability of the eggs or by observing, at the time of death, sperm in the spermathecae of the females which laid non-viable eggs. To observe sperm reserve depletion, the spermathecae of the majority of the females under study were examined following their natural death.

For the maintenance of mosquitoes in general, the above plans were followed in all the experiments. For the sake of relevancy, any special techniques employed in individual experiments are mentioned under the results of those studies.

Definition of the Terms Used

Fecundity

This term has generally been used to denote the average number of eggs produced by the females under study in a single oviposition, instead of the average number of eggs per oviposition, calculated on the basis of the total number of eggs laid by the females over the entire life time.

La Chance, North and Klassen (43) have given the following definition for fecundity and related terms:

> Fecundity is the number of eggs produced regardless of their fertility, whereas fertility is the percentage of eggs deposited that develop into viable progeny. The biotic potential (reproductive capacity) is the product of fecundity and fertility.

Mayr (44) gave the following definition to the terms fecundity and fertility:

> Fecundity. Reproductive potential as measured by the quantity of gametes, particularly eggs produced.
> Fertility. Reproductive potential as measured by the quantity or percentage of developing eggs or of fertile matings.

In consideration of the aforesaid definitions, various terms used in this study are defined as follows:

Oviposition and Egg Batch

All egg layings are designated as ovipositions. The first oviposition of one or several mosquitoes is Batch I, subsequent ovipositions in series are Batch II, III, IV and so on.

Batch Fecundity

It is the average of eggs per oviposition, derived after taking into account all eggs deposited by all the females under study in the

corresponding egg batch.

Overall Fecundity

It denotes the number of eggs deposited per oviposition, taking into account all the eggs deposited by all the females under study over their entire life times.

Hatch Rate

Hatch rate denotes the percentage of larvae that hatch out of all eggs observed for hatching in a batch.

Overall Hatch Rate

This term denotes the percentage of larvae that hatched from all egg depositions of all females under study over their entire life times.

Life Time

Life time in this context means the length of productive life in terms of egg batches, based upon a single blood-meal per oviposition.

Gonotrophic Cycle

Broadly this term denotes the period between blood-meal and subsequent oviposition, without another blood-meal in the intervening period. The dissociation of ovarian development and feeding, but digestion of blood is termed gonotrophic dissociation.

Reproduction Potential or Reproductive Capacity

This is defined as the overall fecundity multiplied by the overall hatch rate of a population of females. The reproduction potential of the stock colony based on the study of 42 females, with an average of

103 eggs per oviposition and a hatch rate of 84.6 per cent, is 87.14 viable eggs per oviposition. This definition of reproduction potential reflects on the capacity of the species to reproduce (per oviposition) given optimum environmental conditions.

CHAPTER IV

OBSERVATIONS AND DISCUSSION

Reproduction Potential

Observations

This phase of the investigation involved the study of total egg production in the life time of 42 randomly selected females from the stock colony. On emergence from pupae, these females were given 6 to 7 days for maturation and mating before the first blood-meal was offered. Each female was isolated after the first blood-meal and no further opportunity for mating was given. A repeat blood-meal was offered beginning the day following each oviposition. Eggs were obtained over the total life time of each female. Other techniques employed in this experiment were detailed under material and methods.

The 42 females under study oviposited a total of 185 times, and the eggs laid totaled 19,120. The number of eggs in a single oviposition ranged from two to 195. The mean number of eggs per oviposition (overall fecundity), was 103. The mean number of eggs per female in a life time was 455. Number of ovipositions averaged 4.4 per female, with a range of 1 to 11.

The total number of eggs observed for hatching was 18,978. Of these, 16,057 larvae hatched. This gave an overall hatch rate of 84.6

per cent.

The highest number of eggs laid by a single female was 1,523 in nine ovipositions, with an overall hatch rate of 97.3 per cent. The longevity under the conditions of this experiment averaged 31.6 days, with a range of 10 to 65 days. Females dying without egglaying (first batch) were not included in this calculation. Fifty per cent of the ovipositing females died by the 30th day from the day of emergence from pupae.

The reproduction potential of this laboratory strain of A. stephensi based on the study of 42 females under the conditions of this experiment was 103 x 84.6 = 87 viable eggs per oviposition. Details of these observations are shown in Table 1.

Discussion

The survival of mosquitoes under laboratory conditions is no measure to their longevity in nature. But since the discovery of the techniques for determining the physiological age of female mosquitoes from the follicular relics (45), it has been shown on many occasions that wild populations of Anopheles comprise a high proportion of females which have passed through two or more gonotrophic cycles (46, 47). Detinova (48) reported having observed females of A. maculipennis messeae caught in nature which had passed through ten to 13 gonotrophic cycles.

In the present study, the egg production capacity of the laboratory adapted strain of A. stephensi was based on the average egg production per oviposition determined by the total egg production of these females in the life time. Such a definition seems appropriate for the following reasons: (a) Fecundity is not limited to the first batch of eggs alone. (b) Each natural population comprises different age groups. (c)

TABLE 1

OBSERVATIONS ON FECUNDITY AND FERTILITY (HATCH RATE) IN FEMALES OF A LABORATORY STRAIN OF A. STEPHENSI

Total Females 42	Batch I	Batch II	Batch III	Batch IV	Batch V & onward	Total all Batches
No. of Ovipositions	42	32	27	25	59	185
Eggs laid (total)	4252	3671	2984[a]	2590	5623	19120
Larvae Hatched (Fecundity)	3898	3461	2515	2213	3970	16057
Avg. eggs per Oviposition (Fertility)	101.2	114.7	110.5	103.6	95.3	103.3
Hatch rate Per cent	91.6	94.5	88.4	85.4	70.5	84.6

Mean eggs per oviposition: 103
Median: 108
Range: 2-195
S.D.: 33.13
S.E.: 2.43
Reproduction Potential: 87 viable eggs per oviposition

[a]Only 2842 eggs examined for larval hatch in this batch.

In mosquitoes, a progressive decline in egg production with each gonotrophic cycle has been demonstrated (49, 50, 51).

The number of eggs laid by a female during one gonotrophic cycle varies greatly between species and even strains. In the first gonotrophic cycle, A. maculipennis melanoon lays up to 500 eggs (52), whereas A. maculipennis messeae lays about 280 eggs (45). In the case of A. stephensi, Roy (53) reported a maximum of 100 eggs with an average of 80 eggs per batch from females fed on the rabbit. Meller (54) reported a range of 100 to 150 eggs per batch and Thompson (55) observed a mean of 76 eggs per batch in the same strain of A. stephensi used in the present study. Gerberg et al., (56) found a range of 90 to 158 eggs and a mean of 117 eggs per oviposition. In the present study the 42 females which oviposited 185 times in their life times exhibited a mean egg production of 103 (see Table 1).

Hatching of eggs was complete in 72 to 96 hours after oviposition and the overall hatch rate from 19,120 eggs was 84.6 per cent. It may be pointed out that the hatch rate related to the eggs from those females alone in which fertilization was confirmed. The hatch rate in A. stephensi as reported by Meller (54) was 89.2 per cent based on the observation of 12,000 eggs. Thompson (55) obtained a hatch rate of 71.25 per cent from 1800 eggs of A. stephensi.

The overall hatch rate of 84.6 per cent was relatively less than the hatch rate shown against egg batches I, II, III and IV (Table 1). This was due to some females having laid partially fertile or totally infertile batches in later ovipositions. Examination of all such females showed that their spermethecae had either been completely emptied of

sperm or the amount of sperm remaining was extremely small.

Usually oviposition followed a blood-meal with a time lag of 72 hours. In a few cases however, further delay of an additional 24 to 48 hours was noticed. In some instances the blood-meal was digested and oviposition did not take place despite the fact that the female was fully engorged. Under the normal laboratory conditions, no explanation for this gonotrophic dissociation was discovered. Some females did not feed on blood for 3 to 10 days after oviposition, although blood was offered every 24 hours. But upon feeding, these females maintained the 72 hour oviposition rhythm following blood-meal. Occasionally, oviposition was not complete in one sitting and additional eggs were laid a second time, 24 to 48 hours later. Such females did not feed on blood during the intervening period. A similar experience during studies on reproduction in A. stephensi was reported by Roy (53).

Earlier it was noticed that the effect of carbohydrate diet on oviposition was pronounced. Females maintained on sugar cubes showed a delay of as much as 20 to 30 days between blood-meal and oviposition. Eggs laid by such females were generally discolored and deformed and fertility in these cases decreased proportionately with dealy. Kuhlow and Garms (57) have shown that in Culex pipiens fatigans, constant excess to sugar delayed oviposition and made it erratic and that such females continued to lay for some 2 months after a blood-meal. But in Genus Aedes total absence of supplementry carbohydrate feeding resulted in a decline in fecundity and an increase in the number of degenerating follicles (58).

In a single oviposition, the maximum number of eggs laid by a female was 195, and the highest number of eggs laid by a female was 1,523,

deposited in nine ovipositions, with a hatch rate of 97.3 per cent. The highest number of ovipositions by a female was 11. The maximum number of ovipositions in A. stephensi, was eight, reported by Roy (53).

Though longevity in mosquitoes is unpredictable even under conditions of extreme care, in the present study, the average longevity of the female was 31.6 days, ranging from 10 to 65 days. According to Detinova (48) females which are allowed to reproduce have shorter life spans than females fed on sugar solution. Gerberg et al., (56) reported that when adult A. stephensi females were maintained on sucrose alone, 30 per cent mortality occurred in 2 weeks. In the present study, highest mortality among the ovipositing females was observed between the first and second oviposition (24 per cent). Fifty per cent of the females died by the 30th day from the time of emergence and between the 3rd and 4th ovipositions.

This laboratory strain of A. stephensi, under the conditions of this experiment, demonstrated a reproduction potential of 87 viable eggs per oviposition.

Selection for High Fecundity Trait

Observations

This phase of the study was designed on the presumption that high fecundity is a genetically controlled trait. All other environmental factors being equal, selection of a line with high egg production would be possible on the basis that extreme phenotypes on selection would result in selection of extreme genotypes. In this context phenotype means females producing highest number of eggs in a batch.

The parent generation was obtained from the stock colony. Newly emerged females were isolated individually, following the first blood-meal after mating. From each female, egg batches were obtained over the total life time. The single egg batch showing highest number of eggs from any female was selected to parent the next generation. If sufficient number of females from any egg batch failed to oviposit, the next batch from the same female (mother) was utilized to form a total of 15 to 25 ovipositing females in the next generation. Failing this, an egg batch from another female in the generation of the mother and showing equally high egg production was used. Females that failed to lay eggs were usually unfertilized, nevertheless, a few unfertilized females laid non-viable eggs. These were eliminated from the records if the examination of the spermetheca on the death of the female showed an absence of sperm. In each generation, mating was strictly restricted between sisters and brothers. All matings took place before the first blood-meal and no mating opportunity was provided subsequently.

Lines A and B both emanated from the parent generation. Selection in line A was carried out for seven generations and in line B for five generations. The criterion for high fecundity was a statistically significant increase at the 0.05 level in the overall egg production, compared to the preceding generations. In each generation, the mean egg production per oviposition through the entire life of all females was used to denote the overall fecundity of the respective generation. Since high fecundity with low fertility, resulting in low reproduction potential, was not desirable, the overall hatch rate in each generation was also studied. The results of this study are presented in Table 2.

TABLE 2

OVERALL FECUNDITY AND HATCH RATE IN THE PARENT GENERATION AND IN SUCCESSIVE GENERATIONS OF SELECTION LINES A AND B

Generation	P	F1		F2		F3		F4		F5		F6		F7	
Line		A	B	A	B	A	B	A	B	A	B	A	B	A	B
No. of females	20	20	16	17	10	21	25	40	19	24	25	30	-	20	-
No. of oviposi-tions	42	57	47	69	55	74	79	126	79	66	107	85	-	57	-
No. of eggs	3743	5290	4359	7425	6476	9469	10777	15137	10585	9433	10820	8734	-	5726	-
Avg. per oviposi-tion	89	93	93	108	118	128	136	120	134	143	101	103	-	100	-
No. of eggs obs. for hatching	-	-	-	3592	6476	9098	10733	14829	10585	9433	10820	8734	-	5726	-
Hatch Rate Percentage	-	-	-	85.2	89.0	74.3	84.5	79.1	93.4	91.8	82.2	86.8	-	87.5	-

P = Parent generation

The effect of selection on mean egg production in various generations, and comparison of the egg production of the final generation with the mean egg production of the stock colony (Experiment 1), was assessed by t tests. Results and P values are shown in Table 3.

During the course of these experiments, it was discovered that the number of eggs in the first egg deposition of an A. stephensi female could be correlated to the number of eggs in the consecutive egg depositions. The stability of high fecundity trait in a selected line was judged on this character in the following study.

In line B, generation F3, eight females raised from a single egg batch showed an exceedingly high egg production, averaging 176 eggs (first batch fecundity). The progeny from the eight females were allowed to breed in a population cage. After a lapse of 90 days (three generations), samples of pupae from the breeding trays were drawn at 14 day intervals. Each time, on the emergence of the adults, 25 females were individually isolated in the usual manner and egg batches were obtained. Counts for egg production and hatch rate were made on the first egg depositions (Table 4).

Discussion

The mean egg production in the parent generation was 89 eggs per oviposition. Selection, therefore was started from a line low in fecundity, compared to the mean of 103 eggs of the stock colony as shown in Experiment 1.

Results arrived at in both the selection lines A and B, showed little consistency in mean egg production in parallel generations after F3 (Table 2). Selection, however, seemed to operate up to the 5th gen-

TABLE 3

COMPARISON OF MEAN EGG PRODUCTION (OVERALL FECUNDITY) BETWEEN VARIOUS GENERATIONS IN SELECTION LINES AND WITH THE MEAN OF STOCK COLONY

Line	Test Between Generations	P=	Trend in Selection Line
A	Parent & F3	0.001[b]	Increase
A	F3 & F5	0.01[a]	Increase
A	F7 & SC	0.90[c]	Decrease
B	Parent & F3	0.001[b]	Increase
B	F3 & F4	0.75[c]	Stable
B	F4 & F5	0.001[b]	Decrease
B	F5 & SC	0.60[c]	Decrease

[a]Significant

[b]Highly significant

[c]Not significant

SC = Stock colony

TABLE 4

AVERAGE EGG PRODUCTION AND HATCH RATE IN THREE SAMPLES DRAWN FROM THE POPULATION CAGE OF GENERATION F3 OF LINE B

Progeny	Females Isolated No.	Females Oviposited No.	Eggs Produced			Hatch Rate Percentage
			No.	Mean±SE	Range	
B, F3 (Parent)	12	8	1404	176± 5.0	150-196	96.7
Sample I	25	16	2629	164± 5.2	138-215	91.2
Sample II	25	16	2478	172±10.0	83-238	92.0
Sample III	25	20	2247	112± 4.4	81-132	86.8

eration in line A and 4th generation in line B. The average egg production in F5 line A, was 143 eggs, an increase of 60 per cent and 39 per cent compared to the parent generation and the stock colony, respectively. The overall fecundity dropped to an average of 103 eggs in F6 and further to 100 eggs in F7, which is approximately equal to the average of the stock colony. Similarly, in line B, generation F3, the average egg production showed an increase of 53 per cent (32 per cent compared to stock colony), but in F5, the drop brought the mean egg production in line with the stock colony.

Craig and Hickey (59) stated that reduced vigour, fecundity and longevity may appear in inbred lines. They also observed that inbreeding was most difficult in generations F4 to F7 of Aedes aegypti because of sterility and reduced viability, which were overcome in the lines surviving to F8. Interestingly, reduced fertility and viability was not exhibited, especially in the later generations (Table 2).

A similar situation of sudden drop in fecundity was observed in the study on the stability of fecundity in a highly fecund selected line (Table 4). From the three samples drawn at fortnightly intervals after a lapse of 90 days, the mean egg production in the first and the second samples was 164 and 172 eggs, respectively, based on counts of the first egg batch. In sample I, one female laid 215 eggs in the first egg batch and, in sample II, four females exhibited more than 200 eggs in one batch. One of these females laid 238 eggs, out of which 217 larvae hatched. In sample III, however, the mean egg production dropped to 112 eggs and the maximum number of eggs laid by a female was only 132. The size of the females in sample III, also appeared smaller compared to females in samples I and II.

Roy (53) observed that in A. stephensi, the size of the female exhibited a positive correlation to the number of eggs. Such observations have also been made by some workers in other species of mosquitoes (48, 52). Similar correlation of size to number of eggs was reported in Aedes hexodontus (60). Colless and Chellaph (61) observed that the number of ovarioles in the two ovaries of Aedes aegypti show a positive correlation with the size of the female. They also observed that only small and medium sized blood meals influenced fecundity and in fully engorged females; the amount of blood had no effect on the number of eggs she lays. From this evidence Clements (62) concluded that "within any species, the size of the individual female, which varies with the larval breeding conditions, will have a pronounced effect on the number of eggs she lays, acting partly through the number of ovarioles available for development".

In the experiment, the breeding conditions were uniformly maintained, throughout. Nevertheless, there was noticeable difference in the female sizes in some generations compared to others. No visible reason could be assigned to such an occurrence.

In this experiment a directional selection for high fecundity was not accomplished. But the significant increase in fecundity in the first few generations of lines A and B, and the presistence of high fecundity for at least 120 days after releasing the selection pressure in the experiment on the stability of this trait, both gave a positive indication toward the heritability of the high fecundity trait.

Effect of Different Blood-Meals

Observations

This experiment was designed to determine, if the quality of blood influenced fecundity in A. stephensi. In each of the four replicas, progeny from a single egg batch of a female from the stock colony was raised. When the females were 7 days old, they were divided into equal lots and each of the four lots was fed on a different species of animal- rabbit, mouse, man or chicken. Only the fully engorged females were isolated individually for oviposition. Successive blood-meals were offered beginning the day following each oviposition. Egg production was followed throughout the life time of each female and averages were determined per oviposition based on the total egg production in the life of all females. The experiment was repeated four times to arrive at the desired number of 25 females fed on each kind of blood. Standard procedures mentioned under materials and methods were followed for rearing,

feeding, mating and oviposition.

The detailed results are shown in Table 5. Mean egg production on different species of hosts and their 95 per cent confidence limits are depicted for comparison in Figure 1.

TABLE 5

MEAN EGG PRODUCTION IN A. STEPHENSI FEMALES FED ON DIFFERENT SPECIES OF ANIMALS

Host	No. of Females	No. of Ovipositions	No. of Eggs	Avg. No. of Eggs Per Oviposition ± SE
Mice	25	73	10,661	146±4.71
Rabbit	25	118	15,438	131±3.16
Chicken	25	67	6,955	104±4.25
Man	25	109	9,556	88±2.53

Discussion

Woke (63) made a detailed study of the effect of different sources of blood on egg production in Aedes aegypti. Mosquitoes that fed on man and monkeys laid significantly fewer eggs than mosquitoes that had fed on rabbit, guinea pig or canary. Roubaud and Mezger (64) observed similar results in the case of Culex pipiens and Roy (53) reported that A. stephensi laid only about half as many eggs after feeding on man as after feeding on rabbit, guinea pig or rat.

From the results obtained under this experiment, the mean egg production appears to vary significantly from one kind of blood-meal to another. The highest averages for egg production were obtained with the blood of mice. Rabbit blood ranked next in order of merit, while chicken

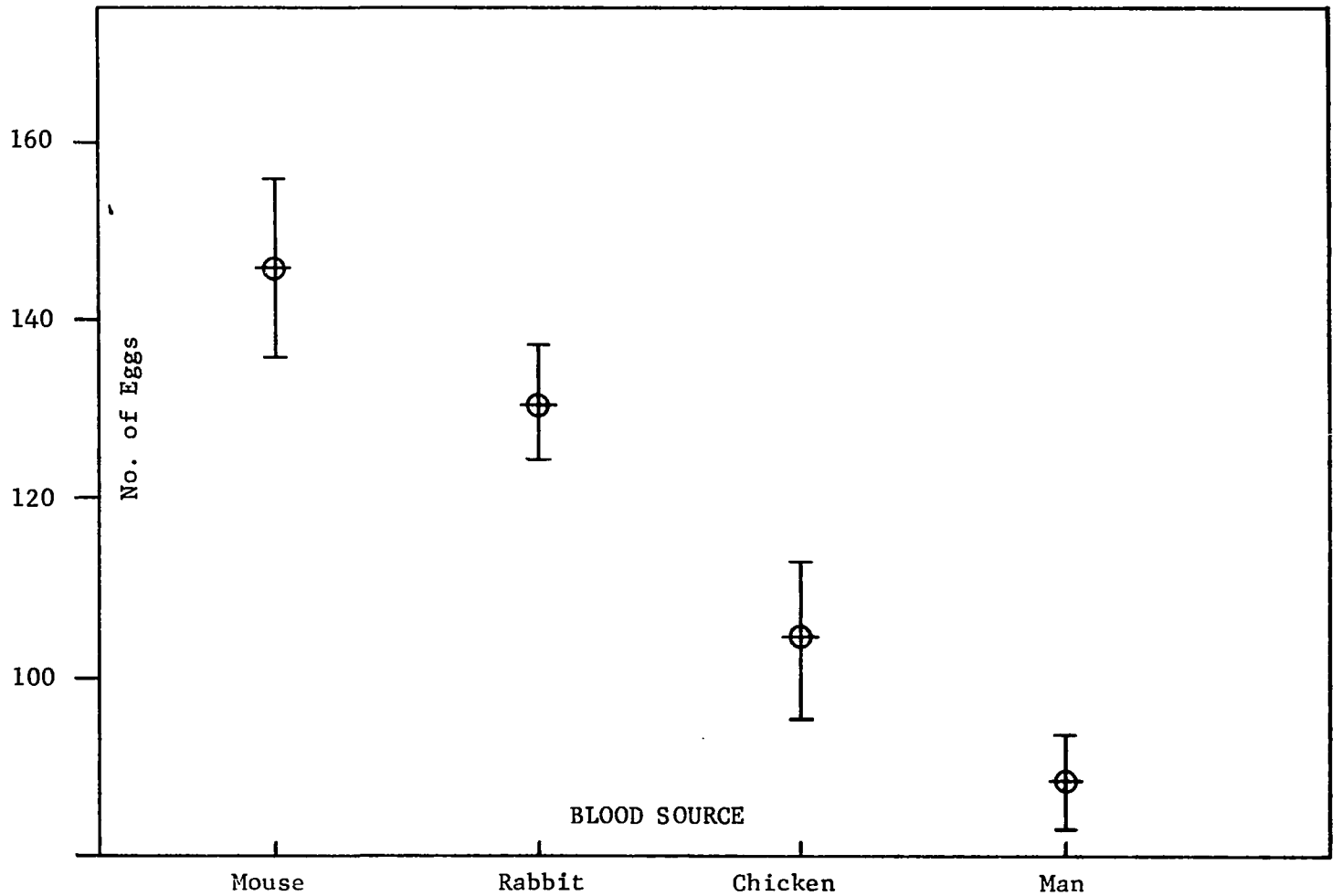

Figure 1. Mean egg production and their confidence limits (95 per cent) in A. stephensi fed on different species of animals.

and human blood stand lowest for egg production in descending order. The mean egg production on different bloods and their 95 per cent confidence limits (Figure 1) depict these differences clearly.

The mean egg production per oviposition in mosquitoes fed on human blood was 40 per cent and 33 per cent less than the egg production with the mice and rabbit bloods, respectively. These findings are in agreement with the observations reported by Roy (53).

Although a little higher egg production was indicated in feeding A. stephensi on mice, compared to the rabbit, the convenience and ease in handling this animal and feeding on large numbers, makes the rabbit a suitable laboratory host for obtaining a reasonably high egg production in this mosquito. It is pointed out that the observed difference in the mean egg production from the mice and the rabbit blood was only 2.6 times the standard error of their combined means. It is possible that due to the small size of the test, the increased level of fecundity in feeding A. stephensi on rabbit blood was not fully manifest. It may also be noted that for the same number of mosquitoes, the number of total life time ovipositions in rabbit-fed females was 118 as against 73 ovipositions in females fed on mice. This statement, however, does not imply that longevity was also influenced by different blood sources.

Variation in Fecundity and Fertility in Egg Batches

Observations

Some studies on reproduction in various species of mosquitoes have indicated that with each successive oviposition, there was a decrease in the number of eggs laid. This has been attributed to an in-

crease in the number of degenerating follicles, along with increasing physiological age of the female mosquito.

How far this applies in the case of A. stephensi and whether the quantitive decrease in egg production also effects fertility (hatch rate) in successive egg batches, has been studied. In addition, data have been analysed to demonstrate the oviposition frequency, together with the percentage of A. stephensi females surviving after each oviposition. All the data in this study related to A. stephensi fed on rabbit only.

The results from successive egg batches of 354 A. stephensi females are shown (Table 6). Egg production in batches I through VIII, has been treated individually, but on account of fewer observations in batch IX and onward, the data were combined. The variation in the mean number of eggs in each egg batch has been compared by graphically illustrating 95 per cent confidence limits of each batch (Figure 2).

Similar to the presentation of data on fecundity, Table 7 shows hatch rates observed in successive egg batches of 294 A. stephensi. The oviposition frequency, the death rate and the percentage surviving after each egg deposition, among 343 A. stephensi females is illustrated in Table 8.

Discussion

It has been estimated that in Aedes aegypti, each successive egg batch contained 15 per cent fewer eggs than the batch immediately preceding (51). Volozina (58) reported that when old and young females in Genus Aedes ingested the same amount of blood, fewer eggs matured and more follicles degenerated in the older females. In A. maculipennis

TABLE 6

BATCH FECUNDITY AND OVERALL FECUNDITY OF *A. STEPHENSI*

Total Females 354	Batch I	Batch II	Batch III	Batch IV	Batch V	Batch VI	Batch VII	Batch VIII	Batches IX-XVI	Total
No. of Ovipositions	354	276	204	134	86	65	46	28	56	1,249
No. of Eggs	41,421	33,422	23,430	15,379	9,372	7,058	5,096	2,783	4,525	142,486
Avg. per Oviposition	117.01 ± 1.98	121.09 ± 2.26	114.85 ± 2.66	114.76 ± 3.17	108.97 ± 3.99	108.58 ± 4.85	110.78 ± 5.15	99.39 ±4.09	80.80 ±4.10	114.09

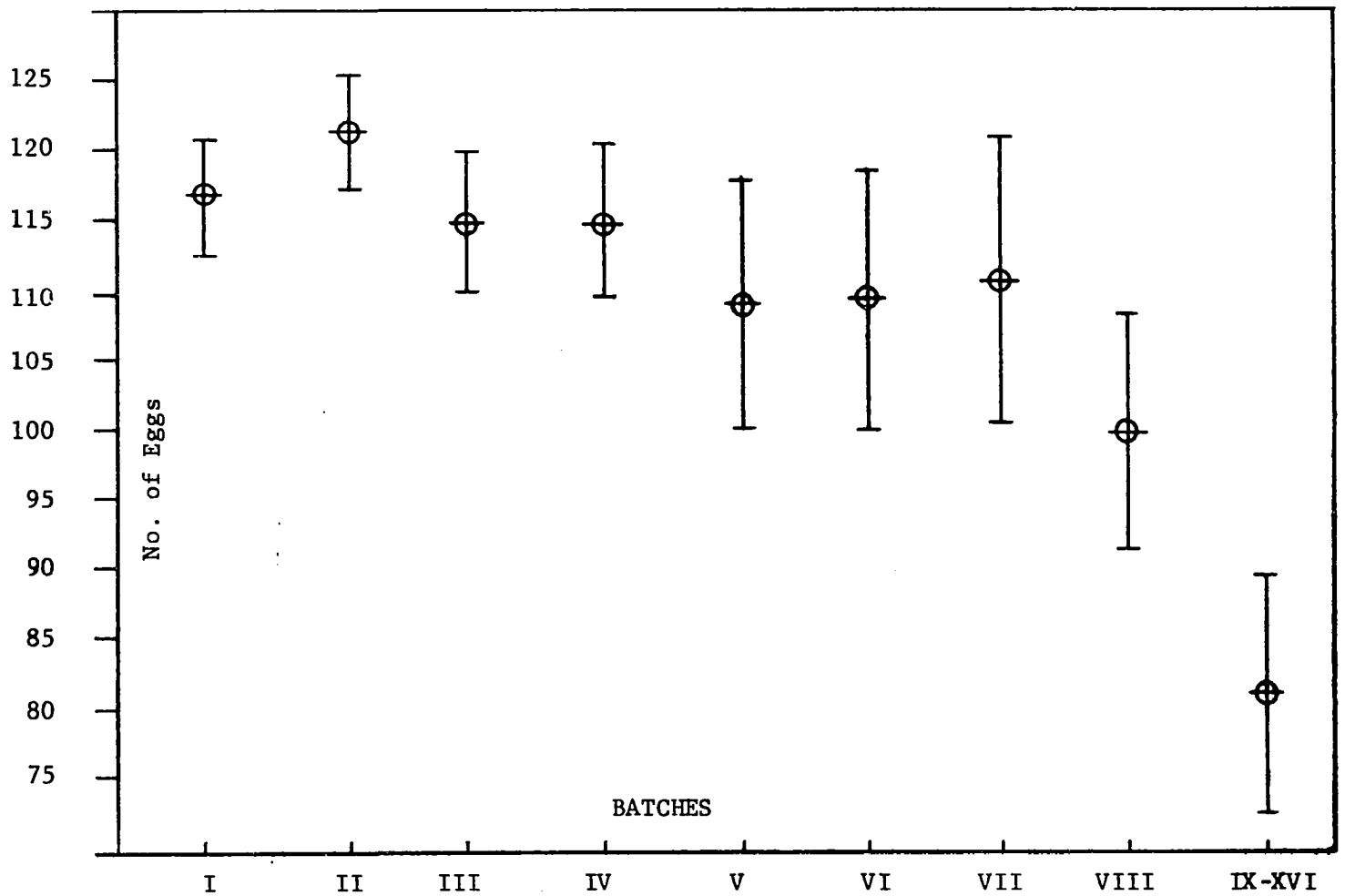

Figure 2. Mean number of eggs and their confidence limits (95 per cent) in consecutive egg batches of A. stephensi.

TABLE 7

HATCH RATE IN SUCCESSIVE EGG BATCHES AND OVERALL HATCH RATE OF *A. STEPHENSI*

Total Females 294	Batch I	Batch II	Batch III	Batch IV	Batch V	Batch VI	Batch VII	Batch VIII	Batches IX-XVI	Total
No. of Eggs Examined	33856	28323	20881	13921	8841	6723	4913	2678	4301	124,437
No. of Larvae Hatched	29010	23868	17995	11978	7250	5625	3964	2058	2859	104,607
Hatch Rate Percentage	85.68	84.27	86.13	86.0	82.0	83.66	80.68	76.84	66.47	84.06

TABLE 8

OVIPOSITION FREQUENCY AND DEATH RATE IN A. STEPHENSI FEMALES FED ON RABBIT BLOOD

No. of Ovipositions During Life-time	No. of Females	Per Cent of Total Died	Cumulative Per Cent Dead	Per Cent Females Survived
1	76	22.7	22.7	77.3
2	67	19.5	42.2	57.8
3	67	19.5	61.7	38.3
4	48	14.0	75.7	24.3
5	19	5.5	81.2	18.8
6	21	6.1	87.3	12.7
7	17	4.9	92.2	7.8
8	9	2.6	94.8	5.2
9	8	2.3	97.1	2.9
10	1	0.3	97.4	2.6
11	3	0.8	98.2	1.8
12	2	0.5	98.7	1.3
13	4	1.0	99.7	0.3
14	0	0.0	99.7	0.3
15	0	0.0	99.7	0.3
16	1	0.3	100.0	0.0
TOTAL:	343	100.0	100.0	0.0

messeae the increase in the number of degenerating follicles eliminated the relationship between body weight and the size of the egg batch which was found during the first two cycles (48).

Results given in Table 6 show that the egg production in batches I and II was highest compared to all other egg batches. Between batches I and II the mean egg production was higher in batch II. A slight downward trend in mean egg production from batches III to VII, is seen. This decline, however, does not appear to have any statistical significance (Figure 2). From batch VIII onward, the decrease in the mean number of eggs was sharp. In this context, it may be stated that, by the time the seventh egg batch was laid, almost 92 per cent of all the ovipositing females had died (Table 8). This left only 8 per cent females which survived to oviposit batch VIII and beyond. Table 8, therefore, not only depicts how far, but also how few A. stephensi females survived to demonstrate the maximum egg laying capacity.

Despite the sharp decline in fecundity in batch VIII and beyond, it may be observed that the overall fecundity was not distinctly influenced. This is due to fewer females surviving batch VII onward. The overall fecundity stayed at 114 eggs per oviposition, whereas fecundity in batches I and II was only 117 and 121 eggs, respectively. It may be observed, therefore, from this study, that no visible decrease in fecundity occurred up to batch VII, in this strain of A. stephensi.

From the results on hatch rate in successive egg batches of 294 A. stephensi (Table 7), it is evident that the hatch rate did not vary markedly from batch I to batch VII. Beyond batch VII, however, there is a noticeable decline in the hatchability of the eggs. Due to fewer eggs

after batch VII, the overall hatch rate was not visibly affected. Meller (54) reported a hatch rate of 89.2 per cent in A. stephensi based on the observation of 12,000 eggs. In the present study, the overall hatch rate, determined by the counting of the larvae from 124,437 eggs, was 84.06 per cent which is about 6 per cent less than the finding of Meller.

The decline in hatch rate after batch VII can be attributed to the insufficiency of sperm. It was observed that some females which initially laid highly fertile egg batches laid subsequent egg batches which were partially fertile and later wholly sterile. The examination of the spermethecae in these females showed severe depletion to complete exhaustion of the sperm reservoir. Conversely, it was also observed that, even after laying 1,000 or more eggs, in some females, the depletion in the amount of sperm in the spermethecae was only slight. One female oviposited 16 times in 71 days and laid a total of 1612 eggs. Of these eggs, 1518 larvae hatched. On the death of the female the spermetheca was found 33 per cent full with motile sperm. Kuhlow and Garms (57), reported a progressive decrease in percentage hatch from successive egg rafts laid by isolated females of Culex pipiens fatigans. From this evidence, they postulated that the amount of sperm transmitted to the females in a number of matings was not sufficient to ensure production of viable eggs throughout life. In this connection it may be recalled that the A. stephensi females under study were provided mating opportunity only, before the first blood meal on the assumption that on insemination the female would acquire sufficient sperm for its life time requirements.

In recent years the concept of monogamy in mosquitoes has become popular among some mosquito biologists. Contrary to his earlier ob-

servations that in laboratory populations of Aedes aegypti multiple insemination was quite common (65), Craig (66) has shown that in this species copulation may take place many times, but insemination occurs only once in life.

Among the anophelines, on the other hand, French and Kitzmiller (67), indicated occurrence of multiple fertilization in A. quadrimaculatus. Qureshi and Arthur (68) reported that virgin females of A. stephensi and those having oviposited once, both showed evidence of radioactive material in their spermethecae when these females were caged together with males labelled with radioactive phosphorus P^{32}. Qureshi (69) also reported that more A. stephensi females, caught from male swarms, were blood engorged and their dissection showed that some females were nulliparous while others had completed one or two gonotrophic cycles. From this study, he concluded that A. stephensi females mate more than once and that they mate after completion of each gonotrophic cycle. In the opinion of the writer, the latter part of the statement does not seem to hold true, in view of the fact that the female spermetheca has the capacity to hold enough sperm for laying fertile eggs throughout life, received during one or several initial matings.

When males were introduced in the holding cages containing females which had initially deposited fertile eggs but which subsequently laid only nonviable eggs, mating did not take place. In these females neither was the viability in subsequent egg batches regained, nor did the spermethecae show evidence of additional sperm.

From the study on hatch rate in consecutive egg batches, several observations may be summarized:

a) That the hatch rate in all successive egg batches remains approximately uniform, subject to the availability of sufficient amount of sperm in the spermetheca of the female.

b) That the spermetheca once filled to capacity, holds sufficient sperms enabling the female to lay fertile eggs throughout its productive life.

c) That one mating or insemination does not necessarily fill the spermetheca of the female to its capacity.

Additional studies were conducted in order to resolve some basic questions which arose during the course of major experiments.

Oviposition in Virgin Females

Observations

Some A. stephensi females were observed to lay only non-viable eggs. The number of eggs and the oviposition rhythm in these females was identical with those laying viable eggs. Examination of the spermethecae of these females revealed absence of sperms indicating that these females had not mated. This study was undertaken in order to determine what percentage of virgin A. stephensi females would lay eggs. Female pupae from the stock colony were picked on the criterion of size and were transferred to a gallon cage. On emergence of the adults, the absence of males was ensured. When 7 days old, these females were offered blood-meal using the rabbit. A second blood-meal was offered 1 week after. Eggs obtained after the first and second blood-meals were kept under observation for 1 week to ensure that the eggs were not viable and that the females had no contact with any stray male. A week after the

second blood-meal the cage was discarded. Results of the experiment are shown in Table 9.

TABLE 9

THE NUMBER AND PERCENTAGE OF A. STEPHENSI VIRGIN FEMALES LAYING EGGS

Replica	No. of Females	1st Blood-Meal No. of Eggs	2nd Blood-Meal No. of Eggs	Total
1	40	-	112	112
2	30	-	-	-
3	30	95	320	415
Presumptive No. of Females Oviposited		1	4[a]	5
Per Cent Females Oviposited		1	4	4[b]

[a]Presuming an average of 108 eggs per female.

[b]Presuming that one female laid twice both after the first and second blood-meal.

Discussion

The fact that ovulation follows ingestion of blood and is independent of insemination in A. stephensi was first reported by Chalam (70). Roy (71) observed that females of A. stephensi will lay eggs irrespective of mating, one blood-meal leading to egg formation even in virgin females. On the contrary, virgin females of A. minimus and A. gambiae (72), and A. subpictus (71), digest blood without parallel development of

the ovaries.

Results shown in Table 9 indicate that only 1 per cent of the 100 virgin females laid eggs after the first blood-meal, but when a second blood-meal was offered, the presumptive number of such females rose to five. Assuming that one female laid twice (once with each blood-meal), at least 4 per cent of the virgin females oviposited.

Gillies (73, 74) reported that A. gambiae and A. funestus emerging from undernourished larvae, need at least two blood-meals to develop their first batch of eggs. This possibility may not be ruled out for more females oviposited on second blood-meal than on the first in this study.

Single Pair Mating

Observations

When wild populations of mosquitoes are kept under captivity, single pair mating rarely occurs, but among the laboratory adapted strains of certain mosquitoes such matings are common. Among Anopheles mosquitoes, this phenomenon is rare compared to others, hence, certain traits demonstrated by individuals in a species cannot be isolated.

During the course of the study on selection for high fecundity, single pair mating, attempted for gaining greater genetic uniformity in the progeny, was not successful. This study was undertaken to determine whether A. stephensi single females isolated with a male in the holding cage, would mate and produce viable progeny. The consideration of large or small mating space (eurygamy and stenogamy) was ignored on the evidence that matings invariably took place when several newly emerged males

and females were placed together in a single holding cage.

One hundred holding cages were stocked with two pupae each, a male and a female differentiated on size. On emergence of adults, 80 holding cages had one pair each. Seven days were allowed for maturation and mating. In the event of death of any male, another male from the stock colony was introduced into the holding cage to ensure that each female had a contact of at least 6 days with one male. On the seventh day all males were discarded and females were pooled and fed on a rabbit. After feeding, each female was singly isolated and an oviposition receptacle was placed in each cage. A week after feeding, all surviving females which failed to oviposit and those which laid infertile eggs were dissected and their spermethecae were examined for the presence of sperm. Results of the study are shown in Table 10.

TABLE 10

RESULTS OF SINGLE PAIR MATING IN A. STEPHENSI

No. of Pairs under obs.	No. of Females Oviposited	No. of Females with Viable Eggs	No. of Females with Non-viable Eggs
(1)	(2)	(3)	(4)
80	13	8	5

No. of Females Dissected for obs. Sperm	No. of Females $^{+}$ve for Sperm	Total $^{+}$ve for Sperm	Total $^{-}$ve for Sperm
(5)	(6)	(3 & 6)	(5 - 6)
47[a]	3	11	44

[a]Includes 5 females which laid non-viable eggs.

Discussion

Out of the 80 females under observation, only 13 females oviposited after the first blood-meal. Eggs from eight of these females hatched. The remaining five females which laid non-viable eggs, were dissected and found negative for sperm in their spermethecae. The total number of females dissected for detection of sperms was 47. Among these, three females showed sperm and 44 females were negative for sperm. Therefore, when isolated in single pair, 11 females were inseminated and 44 females were not inseminated (Table 10). From a close examination of the results, it is estimated that 15 per cent of the females were inseminated and the remaining 85 per cent were not inseminated. Out of the unfertilized females, only 8 per cent laid(non-viable)eggs.

Space requirements for mating in mosquito species is of prime importance (75). Roy (53) in stating the requirement of mating space in A. stephensi, demonstrated that with greater height and larger space, more A. stephensi females are inseminated than in smaller space. Kitzmiller (76) reported that in Culex pipiens, it has been possible to select both stenogamous and eurygamous strains from a single population.

In view of these observations, it may be argued that A. stephensi females would have mated in single pairs, if the space provided under this experiment were enlarged. Further study is therefore necessary to resolve the amount of optimum space required for this purpose.

Varietal Status

Observations

Two forms of A. stephensi have been recognized on the basis of

egg measurements, namely, A. stephensi stephensi or type form, and A. stephensi variety mysorensis. To resolve the varietal status of the laboratory strain, length and breadth measurements of 500 ova from 20 females (25 ova from each female isolated individually) were made. The mean length of 500 eggs was 527.50 microns and the mean breadth was 172.50 microns. These measurements together with the measurements obtained by Rao, Sweet and Subba Rao (77), while designating the two forms, are shown in Table 11:

TABLE 11

AVERAGE MEASUREMENTS (LENGTH AND BREADTH) OF THE OVA OF LABORATORY STRAIN AND TYPE FORM AND VARIETY MYSORENSIS OF A. STEPHENSI

A. stephensi	No. of Females	No. of Ova	Mean length (microns) ± SE	Mean breadth (microns) ± SE
Lab strain	20	500	527.50±1.25	172.50±0.50
Type form[a]	105	4707	548.06±0.26	200.26±0.12
Var mysorensis[a]	147	6916	477.12±0.19	160.65±0.12

[a]Measurements reported by Rao, Sweet and Subba Rao (77).

To study the statistical significance of variation in the size of the ova within females and variation between females, analysis of variance of both length and breadth measurements was done (Tables 12 and 13).

Discussion

The measurements made on 500 ova of the laboratory strain of A. stephensi indicated the average length to be 527.50 microns, with a

TABLE 12

ANALYSIS OF VARIANCE OF EGG-LENGTH AMONG AND WITHIN
A. STEPHENSI FEMALES

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Square	F=
Among females	19	2097	110.368	71.80[a]
Within a female	24	36	1.500	0.97[b]
Error (Residual	456	701	1.537	
Total	499	2834		

[a]Highly significant

[b]Not significant

TABLE 13

ANALYSIS OF VARIANCE OF EGG-BREADTH AMONG AND WITHIN
A. STEPHENSI FEMALES

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Square	F=
Among females	19	110	5.80	9.70[a]
Within a female	24	13	0.54	0.90[b]
Error (Residual)	456	277	0.60	
Total	499	400		

[a]Highly significant

[b]Not significant

standard deviation of 30 microns, and the average breadth (including floats) to be 172.50 microns with a standard deviation of 11.1 microns. Since these measurements are not in conformity with length and breadth measurements of either forms, it has not been possible to assign a definite category to the laboratory strain on this basis alone.

The analysis of variance on the measurements has, however, shown that both the length and breadth measurements are highly consistent within a female, but among females the difference is wide and highly significant (Tables 12 and 13).

CHAPTER V

SUMMARY AND CONCLUSIONS

There is little doubt now that Anopheles stephensi, a notorious malaria vector of the Middle East and the Indo-Pakistan sub-continent, is no longer amenable to conventional methods of insect control. The genetic control is perhaps the safest and the most promising method of controlling disease vectors posing the resistance problem. The research presented was conducted in order to add necessary information on the reproduction biology of A. stephensi, which is fundamental to the application of genetic control.

In order to facilitate these experiments, a new and rapid procedure for egg recovery was developed and applied to more than 180,000 eggs laid by approximately 500 females over their total life span. In addition, by the application of this technique, hatching and individual counting of nearly 120,000 larvae from 142,000 eggs were facilitated.

The study on the reproduction potential of the laboratory strain was based on the total life time egg production and hatch rate in 42 A. stephensi females. Following the modified definition given to the term, these females demonstrated a reproduction potential of 87 viable eggs per oviposition, under the existing laboratory condition.

The experiment on the selective breeding for high fecundity, oc-

cupied 9 months of breeding and counting life-time egg productions (totaling 107,974 eggs) for seven and five generations in the two lines. Although high fecundity was developed in the laboratory strain, stability of this characteristic could not be maintained and fecundity returned to the normal levels. However, the experiment did furnish evidence that high fecundity is an inheritable trait and that maintenance of this characteristic could be improved by the selection of optimum environmental factors in the laboratory.

The experiment on the effect of blood meal from different host species, on life time egg production in 100 females, demonstrated the inherent disadvantage of a highly reduced fecundity in A. stephensi when fed on human blood. In this connection it may be pertinent to point out that in Karachi, West Pakistan, A. stephensi assumed the vectorial role only, after the enforcement of a prohibition against keeping domestic cattle in the city limits. Additionally, this study indicated that the rabbit was a suitable host for the mass production of this species.

The study of fecundity in successive egg batches was based on the analysis of data from 354 A. stephensi females which laid 1249 egg batches, comprising 142,486 eggs. Highest egg production was observed in batches I and II, with batch II being the larger. Observations indicated no significant decline in the number of eggs up to batch VII. Fertility in successive egg batches of 294 females was studied by observing hatch rate in 124,437 eggs. The results relating to fertility demonstrated a pattern similar to fecundity. In this case, however, the decline in hatch rate after batch VII has been attributed to the failure of some females to receive their full share of sperm from the limited

number of caged males as against an overwhelming number of vigorous males found in the natural swarms. For this reason, monogamy in A. stephensi, implying complete filling of the spermetheca once only, seemed conceivable. Because of the few surviving females after batch VII, the decline in fecundity and fertility in the later egg batches showed little effect on the overall fecundity and fertility. The maximum reproduction observed in A. stephensi was 16 egg depositions, comprising 1,612 eggs with a hatch rate of 94 per cent.

The rate of oviposition following blood meal in 100 virgin females was found to be 4 per cent. On the other hand, in another experiment where males were kept with females but failed to fertilize them, 8 per cent of the unfertilized females oviposited. Single pair mating in the amount of space provided, occurred only in approximately 15 per cent of the females.

The length and breadth measurements performed on 500 ova from 20 females of the laboratory strain did not conform with the measurements of either A. stephensi stephensi or A. stephensi variety mysorensis. Hence, the laboratory strain could not be assigned a definite category. Measurements observed were highly consistent within females, but were highly variable among females.

Further research on the reproductive biology of A. stephensi is needed in order to bring to light many more possibilities leading to the application of other approaches of genetic control. The following areas of study are therefore suggested.

1. Attempts should be made for improving breeding methods for studying, raising and colonizing highly competetive, fecundative and

adaptive strains from field populations.

2. Studies are needed to resolve whether A. stephensi is a monogamous species. This will determine the feasibility of the application of sterile male technique against this mosquito.

3. Tests should be carried out to set crosses between different populations to discover mating types and hybrid sterility.

4. Studies on host preference and egg production should be conducted to demonstrate why A. stephensi is not primarily a rural vector.

5. In many genetic control programs, success is assessed on the extent of infertility induced in the field populations. It is necessary therefore, to find if virgin A. stephensi females also oviposit under natural conditions. Studies are required to enable discrimination of virgin's eggs from fertile eggs to disregard eggs laid by virgin females.

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