

in natural infections but are recorded because they illustrate that under given conditions bacteriophage is of protective value and further that bacteriophagy can occur *in vivo*.

A strain of freshly-isolated *Bact. typhosum* ('Silloo' strain) isolated from the blood of a patient on the seventh day of the disease was used for the study of the different typhoidphage types. The M. L. D. of this strain for white mice was found to be 0.2 c.cm. of an 18-hour-old broth culture when injected intraperitoneally. Series of five animals were given 0.5 c.cm. of mixed typhoidphage one hour before and at different periods after the injection of typhoid bacilli (1½ M. L. D.). All the injections were given intraperitoneally. The results are given in tabular form.

There is complete protection when the bacteriophage is injected one hour before or one hour after the injection of 1½ M. L. D. of *Bact. typhosum*. When the bacteriophage is injected later the protection is poor. These experiments were repeated with similar results. When separate types of typhoidphage were used individually no protective value was demonstrated for the different types. Two preparations of commercial typhoid bacteriophage were used in similar experiments but possessed no protective value.

Summary

The characters of four types of typhoidphage are described. Type 1 appears to be similar to Craigie and Brandon's V bacteriophage.

Experiments demonstrating the protective value of typhoidphage when injected intraperitoneally into mice are reported.

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RACES OF *A. STEPHENSI* LISTON, 1901\*

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SINCE the finding in Europe of varieties of *A. maculipennis* (Hackett and Missiroli, 1935, and Hackett, 1937a), interest in the possibilities for similar findings in other parts of the world has been widespread, and increasing numbers of articles carry references to such possibilities. During a study of village malaria in Mysore State (Sweet, 1937), several anophelines were studied, including *A. stephensi*, from various parts of the State, but no evidence of races of Indian anophelines based on egg patterns could be found and it was concluded that other methods to distinguish possible differences would have to be used. Such a new approach to the subject was suggested by Walch and Walch-Sorgdrager (1935 and 1936), who found considerable differences in the measurements of ova of the Indian and Malayan forms of *A. subpictus*. [We are indebted to Strickland and Roy (1936) for the reference.]

Several references have been made at various times to the possibility of the existence of two races of *A. stephensi*, amongst which may be mentioned Knowles and Basu (1934), Ramsay and Macdonald (1936), and Mulligan and Baily (1936). Our own experience in Mysore seemed to indicate two races and for over a year we have been making measurements of ova of this species obtained from various parts of India. Since the results of these measurements seem to point to the existence of two races of *A. stephensi*, this preliminary report seems justified, although much more work remains to be done before definite conclusions can be drawn.

Measurements of *A. stephensi* ova

Stephens and Christophers (1902), who gave the measurements of ova of *A. metaboles* Theobald as 0.51 mm. in length and 0.19 mm. in greatest breadth including floats, stated that the float ridges numbered 15, and that the floats occupied the middle half of the ova or slightly less. They

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Controls 1 M.L.D. of <i>Bact. typhosum</i>		TIME OF ADMINISTERING TYPHOIDPHAGE IN RELATION WITH THE INJECTION OF 1½ M.L.D. OF <i>Bact. typhosum</i>							
		1 hour before		1 hour after		2 hours after		3 hours after	
Alive	Dead	Alive	Dead	Alive	Dead	Alive	Dead	Alive	Dead
0	5	5	0	5	0	4	1	2	3

apparently got their specimens from either Lahore or Nagpur, or both, since they mention that larvæ were found in pots and tins in Lahore and 'in cattle hoof-marks and other similar small pools by the side of the nullah' in Nagpur. These measurements are repeated by Christophers and Barraud (1931) for *A. stephensi* ova, as the species is now named.

The ova which we measured were laid by gravid wild females confined in lamp chimneys over wet filter paper, the measurements being made by direct light under the low power of the microscope while the eggs were on the thoroughly wet paper. Tables I and II give the average measurements of two types of ova of *A. stephensi* obtained from various places. The measurements recorded were the length, the breadth at greatest point including the floats, the length of the floats, the number of ridges on one side of the float, and the proportion of the total length covered by the floats.

The *A. stephensi* of 'B' type from Mysore State came from Bangalore and Mysore cities

small numbers of eggs, a fact which was not true of any other of the 'B' type *A. stephensi* whose ova were measured. The Calcutta *stephensi* will be mentioned again when cross-breeding is discussed.

Using the 1,828 ova from 40 Mysore females as a standard, it was found that the measured ova from 'B' type *A. stephensi* caught in other places did not vary from the Mysore ova in their means by more than the limits set by twice the standard deviations of the Mysore means. All the 'B' type of ova averaged 555 microns in length, 204 in breadth, 294 in length of float, averaged 18 ridges on one side of the float, and the floats covered over half of the total length. It should be noted that the number of ridges may be different on the two floats of one ovum but that this does not occur often nor is the difference in number ever large.

The *A. stephensi* of 'M' type from Mysore were caught in the Chitaldrug and Mysore districts but were not found in either Bangalore or Mysore city. The other specimens were

TABLE I  
Measurements of *A. stephensi* ova  
'B' type

	LENGTH (MICRONS)		BREADTH (MICRONS)		LENGTH OF FLOAT (MICRONS)		NUMBER OF RIDGES ON FLOAT		PROPORTION: LENGTH OF FLOAT OVER LENGTH	
	Mean	Standard deviation	Mean	Standard deviation	Mean	Standard deviation	Mean	Standard deviation	Mean	Standard deviation
Mysore State— 40 females 1,828 ova	553.62 ± 0.40	25.15 ± 0.28	200.09 ± 0.19	11.89 ± 0.13	286.73 ± 0.36	22.58 ± 0.25	17.92 ± 0.03	1.74 ± 0.02	0.516 ± 0.0004	0.028 ± 0.0003
Delhi— 21 females 895 ova	558.09 ± 0.45	20.14 ± 0.32	212.92 ± 0.19	8.35 ± 0.13	309.17 ± 0.42	18.47 ± 0.29	18.43 ± 0.03	1.27 ± 0.02	0.553 ± 0.0005	0.021 ± 0.0003
Poona— 1 female 45 ova	585.33 ± 1.12	11.10 ± 0.79	205.33 ± 0.66	6.57 ± 0.47	293.56 ± 1.07	10.60 ± 0.75	17.24 ± 0.10	0.97 ± 0.07	0.503 ± 0.001	0.014 ± 0.001
Calcutta— 6 females 220 ova	544.59 ± 0.99	21.78 ± 0.70	205.95 ± 0.32	6.96 ± 0.22	291.27 ± 0.80	17.58 ± 0.57	17.90 ± 0.05	1.08 ± 0.03	0.536 ± 0.001	0.028 ± 0.0009
All 'B' type— 68 females 2,988 ova	554.92 ± 0.30	24.27 ± 0.30	204.44 ± 0.15	12.04 ± 0.11	293.89 ± 0.29	23.24 ± 0.20	18.06 ± 0.02	1.58 ± 0.01	0.528 ± 0.0004	0.031 ± 0.0003

only. Those from Delhi were sent by Major Afridi and Doctor Puri, to whom we are greatly indebted, as we also are to Doctors Barber and Rice for assistance in the catches at Poona. From Calcutta we received specimens reared in the School of Tropical Medicine and very kindly sent to us by Doctor B. C. Basu to whom we are grateful. These Calcutta specimens were the only ones recorded in tables I and II which were not caught wild and it should be noted that it was only with great difficulty and after repeated blood meals that a very few of the Calcutta specimens could be induced to lay

caught by Doctor C. Cheluvareyan of the Mysore State Department of Health during a trip north and we are indebted to him as well as to all the local health officers and sanitary inspectors who were of great assistance to him. Again using the Mysore average as a standard (112 females and 5,258 ova), there was, with one exception, no variation in the means greater than twice the standard deviations of the respective Mysore means. The exception was in the case of the ova laid by one female from Poona which averaged in length 49.21 microns less than the Mysore ova, 2.51 times the

TABLE II  
Measurements of *A. stephensi* ova  
'M' type

	LENGTH (MICRONS)		BREADTH (MICRONS)		LENGTH OF FLOAT (MICRONS)		NUMBER OF RIDGES ON FLOAT		PROPORTION: LENGTH OF FLOAT OVER LENGTH	
	Mean	Standard deviation	Mean	Standard deviation	Mean	Standard deviation	Mean	Standard deviation	Mean	Standard deviation
Mysore State— 112 females 5,258 ova	469.96 ± 0.18	19.58 ± 0.13	157.17 ± 0.09	10.15 ± 0.07	213.80 ± 0.17	18.34 ± 0.12	13.40 ± 0.01	1.28 ± 0.008	0.454 ± 0.0003	0.033 ± 0.0002
Sukkur, Sind— 11 females 535 ova	504.92 ± 0.57	19.39 ± 0.40	177.36 ± 0.29	10.00 ± 0.21	242.79 ± 0.41	14.19 ± 0.29	13.81 ± 0.03	0.99 ± 0.02	0.479 ± 0.0006	0.020 ± 0.0004
Hyderabad, Sind— 7 females 303 ova	489.44 ± 0.67	17.26 ± 0.47	159.04 ± 0.42	10.76 ± 0.29	229.27 ± 0.51	13.19 ± 0.36	13.95 ± 0.04	0.90 ± 0.02	0.467 ± 0.0007	0.017 ± 0.0005
Poona— 11 females 530 ova	505.72 ± 0.50	16.95 ± 0.35	170.74 ± 0.28	9.46 ± 0.20	232.62 ± 0.48	16.51 ± 0.34	13.52 ± 0.04	1.21 ± 0.03	0.458 ± 0.0008	0.028 ± 0.0006
Poona— 1 female 40 ova	420.75 ± 1.68	15.77 ± 1.19	139.25 ± 1.14	10.66 ± 0.80	194.00 ± 1.20	11.12 ± 0.84	14.03 ± 0.08	0.77 ± 0.06	0.458 ± 0.002	0.019 ± 0.001
All 'M' type— 142 females 6,666 ova	476.20 ± 0.20	23.71 ± 0.14	159.86 ± 0.10	12.06 ± 0.07	218.20 ± 0.17	20.02 ± 0.12	13.47 ± 0.01	1.24 ± 0.007	0.457 ± 0.0003	0.032 ± 0.0002

standard deviation of the Mysore mean length. These ova, however, were included in the general means to make the measurements for the 'M' type of *A. stephensi* ova average 476 microns in length, 160 in greatest breadth, and 218 in length of float, with 13 ridges on one side of the float, and the floats covering less than half of the total length of the ova. After considering the standard deviations of the various means, there seems no question of the significant differences in measurements between 'B' and 'M' ova.

It is of interest to note that from Poona we received eleven females which laid ova definitely of the 'M' type, one female with ova considerably shorter in length than the 'M' average but otherwise not significantly different, and one female which laid ova characteristically 'B' in type. From the data we have, we cannot say that this would not have been true in the other places from which we received specimens, had longer searches been possible. In Calcutta also it seems probable that both types exist, as will be mentioned later.

The measurements given by Stephens and Christophers (1902) and repeated by Christophers and Barraud (1931) do not fit in well with the measurements of the two types of ova here described. The length they report of 0.51 mm. might be in either 'M' or 'B' type, but the breadth of 0.19 mm. is quite definitely of the 'B' type; the number of 15 for ridges on the float might be in either 'M' or 'B', but the

statement that the floats cover half or less of the ova sounds more like the 'M' type. It should be noted that they mention two distinct types of breeding places and it seems quite possible that their measurements were of ova from different females, some 'M' and some 'B', and that their averages present a composite picture of the two types of ova.

#### *Influence of climate and breeding conditions*

It is not possible as yet to be very definite as to the effect of climate on *A. stephensi* ova but what evidence there is would seem to be against its having any effect on their measurements. From as widely different climatic conditions as obtain in Bangalore, Delhi, Poona and Calcutta we have received specimens of 'B' type, with the same being true of 'M' type specimens and, furthermore, there has been no evidence in the Mysore State specimens of any change in measurements of either 'B' or 'M' in different seasons of the year. Only 'B' type specimens are found in Bangalore and Mysore cities, 87 miles apart, but only 'M' type in an area between them.

Variations in breeding places of *A. stephensi* were noted as long ago as 1902 when Stephens and Christophers published their original ova measurements and have been mentioned many times since, most references being to a well and artificial container, city breeder as opposed to a more general breeder in rural areas. From our experience we can say that the city breeder is

as a rule of the 'B' type while the rural specimens are usually 'M'. However, this is not always true as in one small town in Mysore where *A. stephensi* breed in wells we have been able to find 'M' type specimens only. In another area, 'M' type *A. stephensi* breed in wells while the river is high but go back to the river and nullahs during drier seasons. As far as we can tell at present, breeding places and conditions are not a determining factor in egg size.

That such a variation in ova is not a phenomenon common to other anophelines is shown by our measurements of 1,994 ova from 56 female *A. culicifacies* caught in Mysore State and several places in North and Central India. A report on these measurements will be published later but it may be said here that we could find no significant differences in measurements of *A. culicifacies* ova from any part of India from which we got specimens.

#### *Differences in larvæ and adults*

We have not been clever enough as entomologists to detect any constant differences between the larvæ and adults of types 'M' and 'B' *A. stephensi*. It seems possible that if such differences are found they will be in two or more characteristics and of average value only, as is the case in the varieties of *A. maculipennis* of Holland, as described by Swellengrebel *et al.* (1936). No measurements were made of wing lengths, nor were male genitalia studied.

There was apparently some difference in size of adults, type 'B' usually being the larger of the two, but this was by no means absolute and size had no determinable effect on the measurements of the ova. When the Sukkur specimens were first received they were judged to be of 'B' type from their size but, nevertheless, they laid 'M' type ova. Further, when 'B' type larvæ receive poor food they hatch into small adults but still lay eggs well within the average measurements of the ova of large 'B' specimens, hatched from well-fed larvæ.

*A. stephensi* of 'B' type seem to be the hardier specimens and are longer-lived than those of 'M' type. The former take human blood meals with avidity and are easily fed in captivity by putting the hand or arm in a suitable position, while it is always difficult to get 'M' females to take blood, either human or other, although there is an impression that they will feed on a rabbit more readily.

Unfortunately, it has not as yet been possible to test the source of blood meals in stomachs of wild *A. stephensi* of either type. It may be difficult to get sufficient specimens of 'B' type for such tests, since control work is being done in so many of the places in which this type has so far been found.

#### *A. stephensi as a carrier of malaria*

Covell (1927 and 1931) in two reviews of anopheline carriers of malaria gives information

as to infections reported in *A. stephensi*. Experimental infections have been reported from Nagpur, Ennur, Bombay and the Punjab and natural infections from Delhi, Lucknow, Bombay, Mopad in Madras, and Kohat. Laboratory reared *A. stephensi* have been used in Calcutta and these specimens are found readily infectible with malaria, as Knowles and Basu (1934) report infections with *P. vivax* in colony *A. stephensi* varying in different seasons of the year from 22.0 to 72.0 per cent. According to Covell's lists, negative results were reported by Ross in dissecting 70 *A. stephensi* in Cuddapah, by Mayne in dissections of 248 of this species in Saharanpur, and by Sur and Sur in 21 specimens dissected in Krishnagar, Bengal. In Mysore, Sweet and Rao (1931) and Nursing, Rao, and Sweet (1934) reported examinations of 3,198 *A. stephensi* stomachs with five (0.2 per cent) having oöcysts and of 3,162 glands of which none showed sporozoites. All of these Mysore *A. stephensi* were from areas since found to have only type 'M' *stephensi*, as no dissections were done in Mysore and Bangalore cities where type 'B' is found.

Specimens of *A. stephensi* received from Delli and from the Calcutta colony were of type 'B' but unfortunately it was not found possible to get any specimens from Bombay and the other places from which natural infections have been reported. It is suggested, however, that these places have type 'B', and that Saharanpur, Cuddapah, and Krishnagar will be found to have type 'M' *stephensi* as is the case in those parts of Mysore where largely negative dissections of this species have been made.

Mulligan and Baily (1936) report the dissection of 719 *A. stephensi* in Quetta with a finding of six gut and two gland infections. The infection rate of 609 specimens caught in villages and rural areas was 0.5 per cent as against a rate of 3.7 per cent in 109 captured in the railway and police lines, the military area, and the civil hospital, presumably all more or less in the city areas. Mulligan and Baily say, 'It is well known that, in certain parts of India, *A. stephensi* is a highly efficient malaria carrier, while in other parts it appears to be less important. So far as Quetta is concerned, the relative inefficiency of *A. stephensi* as a malaria carrier may be attributable to the presence of a "race" of *stephensi* which is less androphilic than those which occur in certain other places'. A member of our staff who visited Quetta was unable to find *A. stephensi* at that time of year, but it seems possible that Quetta may have the two types of *A. stephensi*, 'B' in the city and 'M' in the villages. We could not determine from the tables in the Quetta report whether any gland infections were found in the village dissections.

No records can be found as to the relation to malaria of the *A. stephensi* of Poona and Hyderabad Sind but none of the surveys and studies made in the Sukkur Barrage area has

considered that this species was concerned in transmission there. From the specimens received from these three places, the *A. stephensi* are mainly of type 'M' and quite possibly do not carry malaria. The whole position is still vague and much more work must be done, but there seems a possibility that the two types of *A. stephensi* differ, from one cause or another, in their malaria-carrying potentialities.

#### *A. stephensi* in captivity

Efforts to establish laboratory colonies of the two types of *A. stephensi* began early in this study but no success has been achieved with the 'M' type. Early in the work 'M' males and females were put in a large cage and the males were observed swarming in the top of the cage. However, most of the females died early, in spite of taking blood meals after considerable urging, and only three laid eggs. The ova of one were sterile and did not hatch out and of the other two all the larvæ died. After that six more attempts were made with this type, once in the large cage and later in a small cage. None of

these females could be induced to lay eggs, most of them dying early. Finally on 12th July, 1937, four females, fertilized in the small cage, laid ova which mostly hatched out and the larvæ are developing slowly. Up to date it has not been possible to get beyond this first generation with the 'M' type. The measurements of the ova of these seven females of the first generation are given in table III and their averages are not significantly different from those of the original Mysore 'M' type as given in table II. This type breeds true at least for one generation.

That there is a fundamental difference in the two types in their reaction to captivity is shown by the history of the 'B' type colony. A first attempt to start a colony in the large cage failed, largely because of lack of proper attention, but a later start in the small cage was at once successful. Average measurements of ova from the fifth to the ninth generation are given in table III.

The means of the measurements of 70 ova from two females of the fifth generation were

TABLE III

Measurements of ova of first and succeeding generations of laboratory-bred *A. stephensi* 'M' and 'B' types

	LENGTH (MICRONS)		BREADTH (MICRONS)		LENGTH OF FLOAT (MICRONS)		NUMBER OF RIDGES ON FLOAT		PROPORTION: LENGTH OF FLOAT OVER LENGTH	
	Mean	Standard deviation	Mean	Standard deviation	Mean	Standard deviation	Mean	Standard deviation	Mean	Standard deviation
'M' type, 1st generation— 7 females 297 ova	472.76 ± 0.63	16.17 ± 0.45	157.04 ± 0.29	7.37 ± 0.20	201.82 ± 0.42	10.78 ± 0.30	12.00 ± 0.04	0.97 ± 0.03	0.424 ± 0.0008	0.020 ± 0.0006
'B' type colony, 5th generation— 2 females 70 ova	590.00 ± 1.35	16.75 ± 0.95	219.71 ± 0.71	8.79 ± 0.50	306.71 ± 1.22	15.19 ± 0.87	18.61 ± 0.07	0.85 ± 0.05	0.513 ± 0.001	0.018 ± 0.001
'B' type colony, 6th generation— 8 females 369 ova	612.28 ± 0.54	15.48 ± 0.38	232.07 ± 0.31	8.79 ± 0.22	326.02 ± 0.53	15.14 ± 0.38	19.24 ± 0.04	1.25 ± 0.03	0.534 ± 0.0007	0.019 ± 0.0005
'B' type colony, 7th generation— 16 females 729 ova	609.97 ± 0.77	30.65 ± 0.54	233.83 ± 0.27	10.88 ± 0.27	316.54 ± 0.57	22.84 ± 0.40	18.90 ± 0.03	1.19 ± 0.02	0.519 ± 0.0005	0.021 ± 0.0004
'B' type colony, 8th generation— 21 females 1,024 ova	616.49 ± 0.52	24.68 ± 0.37	231.41 ± 0.21	10.06 ± 0.15	321.66 ± 0.37	17.56 ± 0.26	19.53 ± 0.03	1.31 ± 0.02	0.521 ± 0.0004	0.017 ± 0.0003
'B' type colony, 9th generation— 8 females 385 ova	622.60 ± 0.67	19.57 ± 0.48	227.90 ± 0.40	11.58 ± 0.28	314.83 ± 0.57	16.69 ± 0.41	19.52 ± 0.05	1.59 ± 0.04	0.510 ± 0.0006	0.018 ± 0.0004

not significantly different from those of the Mysore 'B' type but in later generations the lengths varied from 2.2 to 2.7 times greater than the standard deviation of the mean length of Mysore 'B', and breadths varied from 2.3 to 3.0 times greater; the means of the other measurements were not significantly different from those of Mysore 'M'. The comparisons are made with Mysore 'B' since it was from one female (St. 4 My.) of this series that all the original ova were obtained to start the colony. There seemed to be some tendency for reduction in breadth to the type mean of this measurement in the successive generations, but the lengths tended to increase. Since the colony was entirely an in-bred one from one female any dominant tendency to increased length would naturally show up but it should be noted that there was no tendency to decrease in averages towards the 'M' means and that in the main the colony bred true in the measurements of its ova.

#### Cross-breeding

Hackett (1937b) gives a table of the results of crossing of the varieties of *A. maculipennis* in Europe and summarizes it by saying, 'The results were thus of two different sorts: either no viable  $F_1$  generation could be obtained, as is the case, for instance, in attempted crosses between the water buffalo and the cow; or healthy hybrid offspring could be bred from the eggs but they were sexually defective and hence unable to continue the race, as in the case of mules, which are usually, but not in every instance, sterile'. We have attempted cross-breeding with both 'B' females and 'M' males (BM) and *vice versa* (MB).

Two original attempts for a BM cross with very small numbers of insects were unsuccessful, the females refusing to lay eggs in spite of repeated blood meals. Later, however, when 40 'B' virgin females were placed in a cage with 52 'M' males we got ova from nine BM females. Of these the ova from only two specimens appeared entirely normal, hatched out, and produced a  $BMF_1$  generation, the average ova measurements of these two females being entirely within the limits set by the standard deviations of the means of the measurements of the eighth generation of the 'B' colony (table III) from which the 'B' larvæ came. The 'M' males had apparently had no effect on the measurements of these ova in spite of the successful cross. Another BM female laid eggs with type measurements but none of them hatched at all, while from two other females only a small minority of the ova hatched out. The remaining females laid two kinds of ova, a few coming within 'M' type average measurements and the majority being of 'B' type, with only a small minority of either type hatching. With the exception of the first two BM females mentioned, the others laid only a small number of ova, a point which is not true of 'B' females in general.

Dissections were made of five BM females just before they died and active sperms were found in the spermatheca but the remaining 26 BM females refused to lay eggs in spite of repeated blood meals. This again is not characteristic of 'B' females which, as a rule, lay eggs in captivity without difficulty.

Up to the time of writing, four  $BMF_1$  females have laid eggs, the average number per female being much below the usual 'B' average and in one most of the ova being sterile. The average measurements of these  $BMF_1$  ova showed that they were of 'B' type in length and length of float, and in either 'B' or 'M' type in breadth, number of ridges on the float, and proportion of length covered by the floats. Of four  $BMF_1$  females dissected, one had completely undeveloped ovaries and would have been sterile; the other three, of the same age, had normal ovarian development.

The first attempt at a MB cross was made with 10 females of 'M' type and 20 males of 'B' type but none of the females laid eggs in spite of repeated blood meals; eight of these females were dissected just before death and no sperms were found in the spermatheca. A second attempt was also unsuccessful in that all 30 females died without laying eggs although in 20 of them active sperms were found. At a third attempt eighteen died before ova were obtained, two laid ova and one was still alive at the time of writing. The average measurements of the ova of these two MB females were entirely within the limits of the 'M' type and the ova hatched out into larvæ, still undergoing development. No  $MBF_1$  generation has yet been raised.

These few preliminary cross-breeding experiments gave comparable results to those reported for the crosses of varieties of *A. maculipennis* in Europe. Some of the first crosses were sterile, some laid sterile eggs, and the minority laid fertile eggs which developed into an  $F_1$  generation in which some specimens were fertile, some had totally undeveloped ovaries, and others laid sterile ova. Such a state of affairs in nature would mean that crosses of 'B' and 'M' would probably not survive and would seldom or never be found; of the 204 wild females whose ova we have measured there has not been one which had average measurements which made its typing difficult. All this would not seem to indicate a true breeding between members of the same race or variety but much more work remains to be done along this line.

#### The Calcutta *A. stephensi*

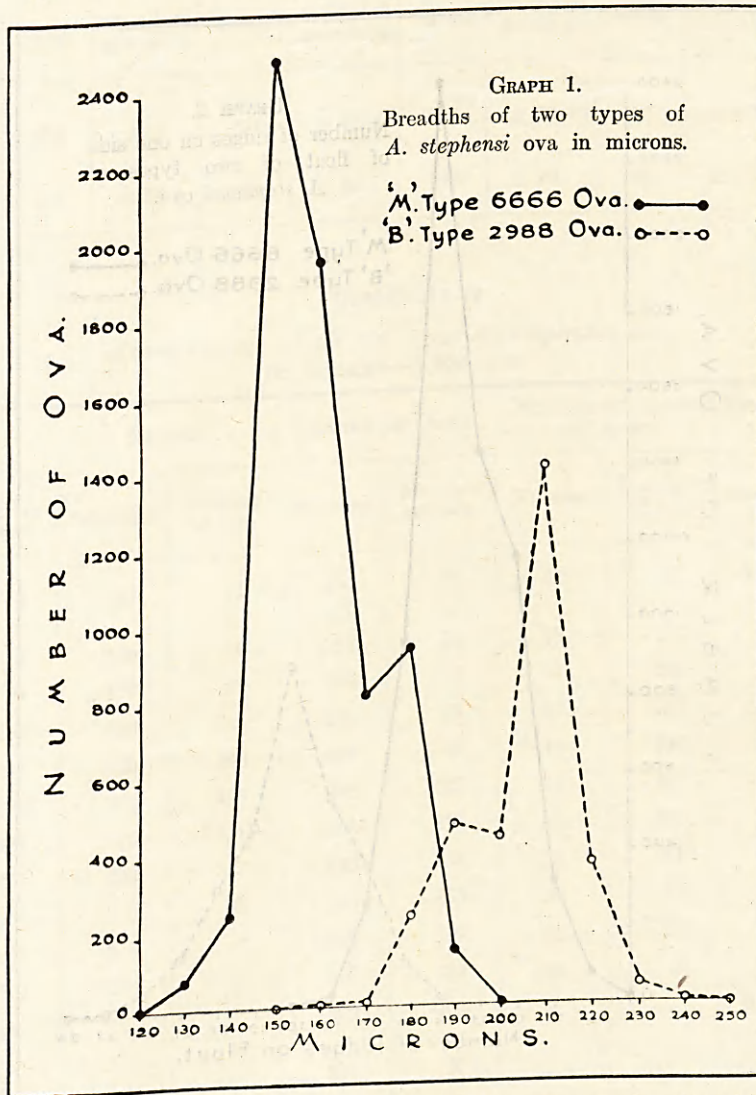
As can be seen from table I, six female *A. stephensi* from Calcutta were of type 'B' but it was also mentioned that only a very few of these Calcutta females laid eggs and the number of ova was small. One other Calcutta female (not included in table I) laid eggs which were of 'M' type in length, breadth, and length of float, were of 'B' type in number of ridges on

the float and might have been of either type in the proportion of length covered by the floats. An eighth female laid very few ova, most of them not fertile, and of two quite distinct types, about half being 'M' and the other half 'B', except for number of ridges on the float which was of 'B' type throughout. Knowles and Basu (1934) report on longevity that some of their *A. stephensi* lived 14 to 20 days and some only 7 to 9 days, a difference we have noted in our 'B' and 'M' type specimens. This whole

be due to unconscious use of 'M' type one time and 'B' type another, or to the occasional use of laboratory-bred crosses about whose reaction to malaria infection we know nothing?

Conclusion

On the whole, the evidence available seems to point to two distinct types of *A. stephensi*. So far, the only demonstrable anatomical difference between these types is in the average measurements of length of ova, their greatest breadth

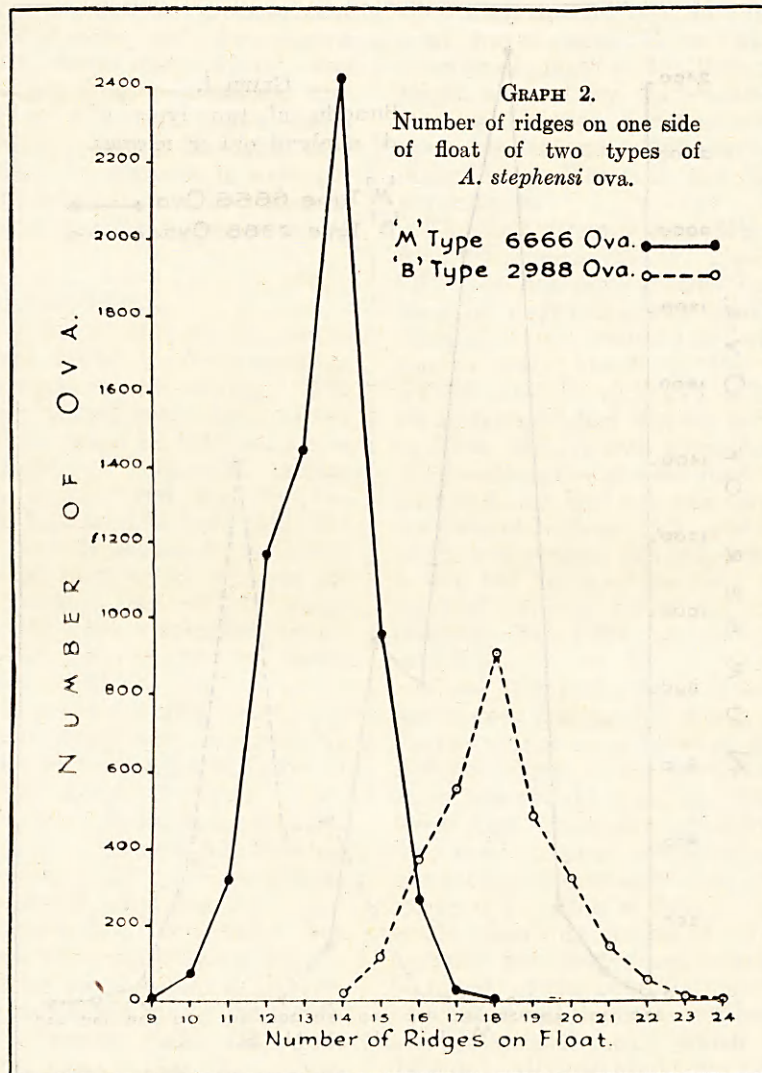


picture is much like that we have found in our BM crosses and BMF<sub>1</sub> generation, and it seems a possibility that both 'B' and 'M' types exist in Calcutta so that laboratory rearing of larvæ obtained from various sources has resulted in some cross-breeding. It is also of interest to note that Knowles and Basu (1934) reported varying rates of infection in *A. stephensi*, a fact they ascribed to temperature and humidity changes. Since it seems possible that there are the two types of *A. stephensi* obtainable in Calcutta, might this difference in infection rates

including floats, the length of the floats, the number of ridges on the float, and the proportion of the total length which is covered by the floats, the 'B' type having the larger measurements in each instance. There seems to be no evidence that climate, breeding place, food of the larvæ, or size of female have any effect on the measurements of the ova, and measurements of *A. culicifacies* ova do not show that in this species there are any significant differences in averages. 'B' type *A. stephensi* seem to be hardier than 'M' type, to live longer in captivity, and to feed

avidly on human blood, while 'M' type females are induced with difficulty to feed on any blood and leave the impression that they prefer animal blood. There is a difference between the two types in willingness to lay eggs in captivity, the 'B' type being easy layers. In captivity, a 'B' colony is easily established, carries on readily, and breeds true at least through the ninth generation, while we have not succeeded in establishing an 'M' colony and only seven

A word of caution is necessary. The differences in ova measurements are average differences and a glance at graphs 1 and 2, which give the numbers of ova in each class of breadths, and numbers of ridges on the float, will show that individual ova may be misrepresentative of the type to which the female belongs. However, we have never found a *wild* female *A. stephensi* which could not be immediately placed in one type or the other on the basis of two or more of



females of the F<sub>1</sub> generation have laid ova; these seven bred true in egg measurements. In cross-breeding of 'B' females with 'M' males, the results are quite comparable to those reported for the crosses of the varieties of *A. maculipennis* in Europe. Evidence as to the malaria-carrying potentialities of the two types is still vague but seems to point to 'B' being a good carrier and to 'M' not being so. Work is continuing along all these lines and in other directions.

the average measurements of from 20 to 50 ova, and usually on the averages of even less than 20. There is, moreover, a difference in the physical appearance of the ova, under the microscope, which enables one with experience immediately to classify a group of ova as either 'B' or 'M'; the measurements then become confirmatory.

These two types of *A. stephensi* ova have been referred to as 'B' and 'M' (Bangalore and



Marikanave, from which places the first females were obtained), but considering all the evidence and to avoid confusion, it seems best to suggest that the 'B' type retains the name of

*A. stephensi*, type form, and that the 'M' type be called *A. stephensi* var. *mysorensis*. The average ova measurements of these two types are as given in table IV.

TABLE IV

Mean ova measurements and their standard deviations of two types of *A. stephensi*

	LENGTH (MICRONS)		BREADTH (MICRONS)		LENGTH OF FLOAT (MICRONS)		NUMBER OF RIDGES ON ONE SIDE OF FLOAT		PROPORTION OF LENGTH COVERED BY FLOATS	
	Mean	Standard deviation	Mean	Standard deviation	Mean	Standard deviation	Mean	Standard deviation	Mean	Standard deviation
<i>A. stephensi</i> (type form)	555	± 24	204	± 12	294	± 23	18	± 1.6	0.53	± 0.03
<i>A. stephensi</i> (var. <i>mysorensis</i> )	476	± 24	160	± 12	218	± 20	13	± 1.2	0.46	± 0.03

## APPENDIX A

Measurements of all 'B' type *A. stephensi* ova  
68 females—2,988 ova

LENGTH		BREADTH		LENGTH OF FLOAT		NUMBER OF RIDGES ON FLOAT		PROPORTION: LENGTH OF FLOAT OVER LENGTH	
Microns	Number of ova	Microns	Number of ova	Microns	Number of ova	Number	Number of ova	Proportion	Number of ova
480	4	150	1	210	7	14	17	0.40 and 0.41	4
490	4	160	6	220	15	15	116	0.42	13
500	24	170	14	230	43	16	366	0.44	42
510	136	180	235	240	11	17	553	0.46	105
520	117	190	466	250	49	18	904	0.48	252
530	199	200	435	260	105	19	481	0.50	541
540	687	210	1,401	270	318	20	341	0.52	750
550	337	220	367	280	354	21	141	0.54	713
560	498	230	53	290	855	22	55	0.56	406
570	502	240	9	300	288	23	11	0.58	128
580	127	250	1	310	353	24	3	0.60	33
590	182	..	..	320	406	..	..	0.62 and 0.63	1
600	105	..	..	330	97	..	..	..	..
610	20	..	..	340	50	..	..	..	..
620	26	..	..	350	12	..	..	..	..
630	8	..	..	360	2	..	..	..	..
640	3	..	..	370	13	..	..	..	..
650	7	..	..	380	4	..	..	..	..
660	0	..	..	390	4	..	..	..	..
670	2	..	..	400	2	..	..	..	..
TOTAL	2,988	..	2,988	..	2,988	..	2,988	..	2,988

## APPENDIX B

Measurements of all 'M' type *A. stephensi* ova  
142 females—6,666 ova

LENGTH		BREADTH		LENGTH OF FLOAT		NUMBER OF RIDGES ON FLOAT		PROPORTION: LENGTH OF FLOAT OVER LENGTH	
Microns	Number of ova	Microns	Number of ova	Microns	Number of ova	Number	Number of ova	Proportion	Number of ova
400	7	120	6	150	15	9	10	0.32 and 0.33	3
410	20	130	77	160	42	10	74	0.34	22
420	26	140	244	170	54	11	318	0.36	105
430	233	150	2,483	180	341	12	1,168	0.38	118
440	374	160	1,959	190	421	13	1,440	0.40	373
450	316	170	816	200	485	14	2,415	0.42	879
460	1,256	180	934	210	1,535	15	947	0.44	1,614
470	1,087	190	140	220	1,372	16	261	0.46	1,528
480	1,257	200	7	230	1,116	17	29	0.48	1,377
490	651	..	..	240	675	18	4	0.50	490
500	559	..	..	250	356	..	..	0.52	139
510	549	..	..	260	243	..	..	0.54	14
520	136	..	..	270	10	..	..	0.56 and 0.57	4
530	111	..	..	280	1	..	..	..	..
540	77	..	..	..	..	..	..	..	..
550	6	..	..	..	..	..	..	..	..
560	1	..	..	..	..	..	..	..	..
TOTAL	6,666	..	6,666	..	6,666	..	6,666	..	6,666

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## A MODIFIED VILLAGE MOSQUITO TRAP

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ACTING on a valuable suggestion made by the Editor of the *Indian Medical Gazette*, to use a hoop, I have attempted to simplify the working of the village mosquito trap described by Gore (1936) with regard to the arrangement of the pieces of woollen cloth each night, and catching of the mosquitoes every morning, and I have found it much easier to operate than it formerly was.

A bamboo hoop is made to rest just on the outer edge of the mouth of an earthen pot. A piece of woollen blanket such as is used by villagers, the sides of which are hemmed, and two inches shorter than the circumference is sewn on to the hoop (figure 1). The dimensions of the cloth are such that, when introduced into the pot, the lower edge nearly touches the bottom, and a two-inch vertical gap is left open (figure 2), which serves as a passage for mosquitoes to the darker spaces in the pot. Three 12-inch pieces of string are tied to the hoop, the free ends being tied in a knot. This serves to lift the cloth.

The trap is kept at night in a corner which has been previously ascertained to be a resting place of mosquitoes. This is determined by disturbing them from different places and observing where they alight, for a day or two. One trap is kept in each room. The following morning situations other than those where the trap is