

RECENT ADDITIONS TO OUR KNOWLEDGE OF "ANOPHELES MACULIPENNIS" RACES

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I. INTRODUCTION.

The development of our knowledge of the races or varieties composing the species-complex *Anopheles maculipennis* was carefully summarised with a comprehensive bibliography by HACKETT & MISSIROLI (1935). A brief guide or key to the varieties of *A. maculipennis* was also printed in a *Quarterly Bulletin* of the Health Organisation of the League of Nations, with photographs of the accepted egg-types and maps showing the distribution of the varieties as reported at that time. There is, therefore, no need in this introduction to do more than summarise briefly the status of our knowledge at the end of 1934.

Six different forms were then recognised on the basis of certain characters of the eggs—namely :

(a) The arrangement of the light and dark areas on the upper surface, often called the egg pattern or design, produced by the size and apical development of the papillæ of the exochorion, which in certain areas are reduced in size and height and are rounded on top so that they are transparent to incident light and allow the black chorion to show through, while in

other areas they are tall, with exuberant, spreading, roughened tops irregularly stellate in shape and often coalescing with one another, very refractive to light and giving, in their maximum development, a hoar frost appearance to the lighter spots on the egg.

(b) The size of the float structure as indicated by the number of ribs and rugosity of the intercostal membrane, the floats being superficially described as being large or small, and rough (rugose) or smooth.

Upon carefully comparing the different forms of *A. maculipennis*, secured by breeding imagines under identical conditions from classified eggs with perfectly clear characters, other structural differences of a statistical kind have been found. The races differ in size, in the number of branches and the form of certain larval hairs, and in the form and number of the external spines on the claspette of the male hypopygium. These differences can only be determined by averaging a large number of cases, and do not serve to identify a given specimen.

It was found that the *atroparvus* males would not only mate with females of the same race in small cages, but would also inseminate females of all the other races, with viable results in certain cases. However, these experiments in cross-mating showed that *atroparvus* possesses a different genetic affinity for each of the other races, thus confirming the fact that these races differ genetically among themselves. For example, the product of the cross with *messeæ* is non-viable, whereas the cross with *labranchiæ* produces an F_1 generation with normal females and with males somewhat less than half of which possess well developed organs of reproduction. Crosses with the other races may give a healthy F_1 generation, but one or both sexes are sterile, as in the case of mules. According to this test, *atroparvus* is more remote from the races *messeæ* and *typicus* than it is from *elutus*, which is recognised as a different species. The cross with *elutus* gives non-viable larvæ which succumb, however, at an advanced stage.

The instincts, behaviour and physiology of the races show evident differences of such importance as to make malaria vectors of some and relatively harmless cattle-feeders of others.

II. RECENT OBSERVATIONS ON THE DIFFERENTIAL CHARACTERS.

Biometric Studies.

No character has yet been found which can be used to identify the adults of the various races except in the case of *A. elutus*. SWELLENGREBEL (1936) and his colleagues have reported that, in the absence of *typicus*, the larvæ of *messeæ* can be distinguished from those of *atroparvus* in the Netherlands by the type of palmate hair on the second segment, in conjunction with the number of branches of hair No. 2 on the fourth and fifth segments. The hibernating *messeæ* also differs from the semi-hibernating *atroparvus* by having rod-shaped salivary glands (DE BUCK & SWELLENGREBEL, 1935). SHUTE reports that the external harpaginal spine of the male hypopygium is more constant in shape and length than at first supposed and that it varies most in *messeæ*. He gives a key which differs somewhat from that published by HACKETT & MISSIROLI.

Table 1.

SHAPE OF EXTERNAL HARPAGINAL SPINE, *Maculipennis* RACES.

According to :

	SHUTE (1935)	HACKETT & MISSIROLI (1935)	HACKETT & LEWIS (1935) (Albanian specimens)
Banded eggs :			
<i>Melanoon</i>	—	Variable ¹	—
<i>Messeæ</i>	Variable	Variable	Variable
<i>Subalpinus</i>	—	—	Almost always bluntly pointed or rounded
<i>Typicus</i>	Blunt or round	Blunt or round	Blunt or round
Eggs without bands :			
<i>Atroparvus</i>	Always sharp and tapering	Always pointed	—
<i>Labranchiæ</i>	Always sharp and tapering	Always pointed	—
<i>Elutus</i>	Blunt or rounded	Variable	—

¹ 50% pointed.

It has been the custom, in biometric studies of larval hairs and hypopygial spines, to consider each side of the specimen as a separate case, in view of the lack of symmetry often displayed in these bilateral characters. This leads to the somewhat paradoxical situation that one half of a larva or a hypopygium may lend weight to one classification and the other half to a different one altogether, although the specimen itself may be undoubtedly of pure race and not a hybrid. Furthermore, in the case of the number of branches of hair No. 2 on the fourth and fifth segments, an analysis of a large number of observations shows that the variation between the two sides of the same larval segments is less than that between one segment and the next, or between one individual and another of the same race. Hence, taking the two sides as separate cases does not really double the basis of computation, since there is a high correlation between them. A statistical analysis shows that each larva should be considered a single case, and that with a sufficient number of specimens, the total number of branches of any two hairs may be taken in practice to represent the larva, provided they are located on different segments. It is probable that the same situation of bilateral correlation exists in the male hypopygium as well and that both sides should be taken into account, in tabulating specimens, as regards the bilateral symmetry and form of the external spines.

MARTINI (1933) suggests that the shape of the spine on the dorsal (external) lobe of the claspette may be a function of its position. A twin to the external spine is most like it if the two are side by side springing from the same level. The external spine in *messexæ* is most apt to be blunt at the most dorsal position. The tendency to produce blunt spines decreases from dorsal ventrad.

Egg Characters.

The characters of the egg still constitute the only known method of identifying individuals. Very little confusion has developed in nomenclature. One or two authors continue to employ the original classification of FALLERONI and confuse *atroparvus* with *labranchiæ*. Clear-cut genetic differences have long confirmed the validity of their separation into distinct races, while the eggs themselves have only a superficial resemblance. The brownish *atroparvus* egg is

slender with smooth float membranes, while *labranchiæ* lays a broad egg with very small and very rugose floats. *Atroparvus* is found more and more to be an inland breeder as well as a coastal form, and is now reported as far south as Naples.

...It has been discovered that the egg characters are influenced by temperature, and also show variations between different geographical regions.

(a) *Temperature*.—The early spring eggs, especially those obtained from the generation issuing from hibernation, present the most difficulties in classification. Unpublished observations by D. J. LEWIS in Albania and by M. A. BARBER in Greece point to the influence of low temperatures on the egg or adult female as the main cause of atypical egg batches in spring. BARBER stated in a personal communication to the author in May 1935 :

“ A dark, almost black egg with bands scarcely defined, grown from egg to adult under higher temperatures, will give a mosquito which lays bright *typicus* eggs with very sharply defined bands. ”

R. B. HILL writes from Portugal :

“ From one batch of plain dark grey eggs without a trace of pattern the resulting females laid typical *atroparvus* patterned eggs. ”

RIVERA & HILL (1935) also mention that eggs are lighter in colour and smaller at the height of summer. In Albania in mid-summer, LEWIS obtained darker eggs experimentally in *typicus* and *subalpinus* by subjecting females to temperatures of about 16°C. between ovipositions. The development of the papillæ on the exochorion is apparently retarded at low temperatures, the apical roughening is less exuberant, and the whole appearance of the egg is darker, due to increased translucency of the outer coat.

CORRADETTI (1934) states that the egg-pattern is a Mendelian character, and similar observations have been made before with regard to other insects. For example, JENNINGS (1930) mentions that the race of silkworms with brown eggs differs from the race with slate-coloured eggs in one gene. When the eggs of the brown race are fecundated by slate-coloured males, the resulting eggs are

slate and successive generations (since slate is dominant) follow the simple Mendelian rule. However, *labranchiæ* females inseminated by *atroparvus* males laid *labranchiæ* eggs, since the characters of the anopheline egg are already fixed before its fertilisation from the spermatheca. The eggs laid by the first hybrid generation were mixed, following the Mendelian rule, not only as to pattern, but also in shape, and in the size and rugosity of the floats. All the eggs of a single clutch were alike.

RIVERA & HILL (1935) studied the variations in *atroparvus* through six inbred generations derived from a single female. The variations were not significant in pattern of eggs or rugosity of float membrane. The eggs became somewhat lighter in colour as summer advanced, while the floats were never rough, 4 to 8% showing occasional rugosities. There was no variation in the palmate hair of the second segment, the leaflets being always narrow with little pigment, while the branches of hair No. 2 on the fourth and fifth segments never average more than 4.5. The wing length diminished from 5.2 in the original (spring) female to 4.6 in the sixth (summer) generation, and the maxillary index also decreased with fair regularity from 16.5 in the original female of the spring generation to 14.3 in the last (summer) generation.

(b) *Geographical differences.*—Certain variations are found between one region and another, in eggs presumably of the same race. It is these which tempt observers to make subdivisions of known races, and in certain instances to create new race-names. Several new races have been proposed in addition to the original six:

A. maculipennis var. *fallax* Roubaud, 1934—from Normandy, France. Differs from *atroparvus* in having larger floats and a branched hair instead of an intermediate palmate on the second segment of the larva. Hair No. 2 (fourth and fifth segments) averages more than 4.5 branches. The external harpaginal spine is blunt as in *messeæ* and *typicus*. It is found breeding in fresh water, and has a lower salt tolerance than *atroparvus* (ROUBAUD, COLAS-BELCOUR, TREILLARD, 1935). It differs from *messeæ* in the absence of bands on the egg and rugosities on the floats; the race is stenogamic, and reproduces indefinitely in captivity. It crosses easily with *typicus* from the Netherlands. It is zoophilous and multidentate.

A. maculipennis var. *sicaulti* Roubaud, 1935—from Rabat, Morocco. Allied to *labranchiæ*. Differs in having a narrower white spot on the wing fringe. Branches of larval hair No. 1, second segment, filiform instead of palmate. Eggs more obtuse, darker, but with fewer markings than *labranchiæ*.

A. maculipennis var. *pergusæ* Missiroli, 1935—from Sicily, Italy. Allied to *labranchiæ*. Differs in having broader, shorter eggs almost without markings. Larval and adult characters not described.

[Note.—SERGENT, Et. (1935), has described a form from Algiers intermediate between *labranchiæ* and *sicaulti*. It is evident that *labranchiæ*, like its nearest relative *atroparvus*, shows a number of geographical variations and modifications. Being eurygamic, the genetic affinities between these nearly related forms cannot yet be ascertained, and no attempt has been made to test them in crosses with *atroparvus* males. Their status as new varieties remains to be proved.]

A. maculipennis var. *subalpinus* Hackett & Lewis, 1935—from Albania. Belongs to the barred-egg group. Differs from *messeæ* in having eggs with smooth float membranes and fewer ribs; more branches to larval hair No. 2 on fourth and fifth segments, and invariably a branched hair instead of a palmate on the second segment. The external harpaginal spine is prevailingly blunt and double as in *typicus*. It breeds in fresh-water marshes and is found in the northern Mediterranean basin from Spain to the Balkans, particularly in the uplands.

[Note.—The egg of this variety was first classified as *messeæ* until it was found to have smooth floats, and then as *melanoon*, the description of which was expanded to include both black and banded eggs. It is now proposed to restrict the name *melanoon* to black eggs with smooth floats, and *subalpinus* to banded eggs with smooth floats.]

The American forms of *A. maculipennis* have been described recently by HOFFMANN (1936), DAMPF (1935) and others. The American races are distinguished by the presence or absence of the coppery spot on the wing fringe. According to HOFFMANN, the race with the apical bronze spot should retain the name *occidentalis* (DYAR & KNAB), and its range is given as extending from California to Alaska, across Canada and into Maine and Massachusetts.

The race without the spot is found in high areas in the southern United States and Mexico and has been named by HOFFMANN *aztecus*. This requires further study, for FREEBORN wrote in 1926 :

“ In the valleys and foothills of the Sierra (California) the spot is almost always impossible to distinguish.”

The females which I have seen in California did not have any spot on the wing fringe. No egg, larval or hypopygial characters have been found to distinguish the races. The eggs which I examined were a uniform grey with rather small, smooth fan-shaped floats of about twelve ribs. On the other hand, HOFFMANN (1935) describes the Mexican eggs as having large, rough floats with twenty-five ribs.

It is not unlikely that new local varieties will continue to be found over the vast range of *A. maculipennis*. The reporting, however, of a new race or variety should take into account the special characters which have been found to be useful in separating the races of this species. Hence such reports should pay particular attention to certain points not always included in ordinary descriptions. The following form may be useful as a reminder of these details which unfortunately are lacking in reports of supposedly new races omitted from the above list on account of insufficient data. Our information with regard to the American forms is exceedingly incomplete.

Name : Where and when found ; range.

Characters :

Eggs : Number of batches seen—upper surface (banded or not, dark areas, etc.). Floats : number of eggs examined—date—intercostal membrane smooth or rugose.

Average number of ribs—standard deviation—maximum number, minimum number.

Larvæ : 2nd segment, hair No. 1 : number examined—filiform, intermediate or palmate. 4th and 5th segment, hair No. 2 : number examined—average number of branches—standard deviation—maximum number of branches, minimum number of branches.

External harpaginal spine :

Percentage with spines on both sides rounded, both pointed, and mixed. Percentage single—double—triple.

Bionomics : Larval habitat : water body type :

Depth—permanent or temporary—movement—temperature—salt content—shade—vegetation (submerged, vertical, etc.)—upland or lowland—distance from sea—breeding season.

Habits : Winter behaviour—mating in captivity—cross-breeding.

III. MATING IN CAPTIVITY.

HACKETT & BATES (1936), in Albania, have obtained swarming of males in captive groups of *A. elutus* and of the race *typicus*, first in a very large outdoor cage (10.5 × 5 × 6.2 metres) and later in a smaller laboratory cage (55 × 55 × 110 centimetres). Fertile eggs were obtained, and it is hoped that permanent colonies may be established and kept under observation.

IV. GEOGRAPHICAL DISTRIBUTION OF THE RACES.

As for the geographical distribution of the accepted forms, egg-surveys have been made in regions hitherto unreported :

(1) *Sweden* (EKBLÖM, 1935, with map). *Messeæ* is generally distributed and with it is associated *typicus*, except in the extreme south, where it occurs together with *atroparvus* (which the author continues to call *labranchiæ*, after FALLERONI). The three together are found only on the west coast.

(2) *Yugoslavia* (ZOTTA, 1935). The usual Balkan forms are found—to wit, *messeæ*, *typicus* and, on the coast, *elutus*. In addition, it is stated that *labranchiæ* occurs on the northern Dalmatian coast, and that an atypical egg with bars like *messeæ*, but with a highly contrasted black and white pattern, is found in the uplands. In a private communication, the author agrees that this is probably *subalpinus*.

(3) *North Africa* (Algiers : SERGENT, Et. & TRENSZ, 1935 ; Tunis : GALLIARD, 1935, VILLAIN *et al.* 1935 ; Morocco : ROUBAUD, 1935). The only races are *labranchiæ* and the related form *sicaulli*. GALLIARD says that " it is certain that *elutus* must exist in the coastal region ", but this has never been confirmed.

(4) *Syria* (unpublished report by KLIGLER, 1935). No member of the *maculipennis* group was found except *elutus*, which was breeding in fresh-water streams and marshes. None was found in a coastal marsh near Alexandretta, and none is mentioned as breeding in brackish water.

In the Union of Soviet Socialist Republics, there has been no survey since that of HACKETT & BARBER (1935), but occasional reports from scattered regions indicate that only *messeæ* and *typicus* are found in the interior, while *elutus (sacharovi)* occurs on the Caspian and *atroparvus* on the Black Sea near Odessa.¹

MISSIROLI (1935) suggests that inconstancy of temperature is an important factor in larval ecology and may help to explain the distribution of the races and species. Thus while *plumbeus* is adapted to constant thermic conditions at 15° or 16°C., and so probably is *bifurcatus*, *typicus* prefers a temperature oscillating rather widely between day and night.

V. SYSTEMATIC POSITION.

The word race is still used more frequently than variety, since the genetic and biometric differences between the forms are more striking than the structural. The name *subspecies* has fallen out of use recently, although in their mutual sterility the forms resemble true species. In spite of the difficulties which systematic entomologists find in dealing with the *maculipennis* complex, the distinctions involved have assumed enormous importance in malariology. This is only partly because, in Europe, races like *labranchiæ* and *typicus*, though indistinguishable under the microscope, frequent in many regions a different range of host, making one a dangerous anopheline and the other a non-vector, and, being adapted to different larval habitats, permit the economies of race-control. It is also

¹ See *Medical Parasitology*, 1934, **3**, Nos. 4, 5 and 6.

important because most of the widespread anopheline species of the world have been found on examination to be complexes of this sort. SWELLENGREBEL, in an as yet unpublished report, describes the fresh and salt water forms of *A. ludlowi*, each of which is divided into vectors and non-vectors of malaria. *Hyrceanus*, *barbistrostris* and *junestus* of the Old World and *pseudopunctipennis* and *tarsimaculatus* of the New are examples of species whose biological characters and relationship to malaria differ from place to place. According to DE MEILLON (1935), the *A. junestus* female, which frequents dwellings in South Africa, and that of *A. lesoni*, which does not, can only be told apart by their eggs.

DIEMER and VAN THIEL (1936) suggest that *A. maculipennis* consists of only three fundamental biotypes, each of which has spread over the entire range of the species and has become adapted in different geographical regions to local conditions, forming a group of non-overlapping forms clearly distinguishable from one another, yet closely related and with many common characters. For example, *atroparvus-labbranchiæ-elutus* form such a group or (to use the expression of the authors) *geobiotype*, which divide all of Europe between them, have a similar toleration for salt water, lay non-banded eggs, are more closely akin by genetic tests to each other than to the rest, and possess in common a wide range of hosts on which they feed, making them malaria vectors even in the presence of stabled domestic animals. The authors suggest that *messeæ-melanoon* may form a second geobiotype, for they may not overlap as much as is now believed, while *typicus* stands alone as a third.

This attractive hypothesis gains support from observations of others which the authors have not taken into account. The setting-up of a new race, *subalpinus*, may resolve the difficulty arising from the overlapping between *messeæ* and *melanoon*, with both of which it has been confused and between which it might well serve as a buffer. As for *typicus*, HACKETT & BARBER (1935) found a distinct type in the Caucasus with minute floats, and it is not unlikely that it will eventually be divided up into geographical sub-races. According to this theory of DIEMER & VAN THIEL, one would be apt to find only three *maculipennis* races mingling in any one region, and this, in fact, is fairly in accordance with our present rather deficient knowledge of their distribution. North

of the Alps we find *atroparvus*, *messeæ* and *typicus*, in the Iberian Peninsula *atroparvus*, *subalpinus* and *typicus*, in Southern Italy *labranchiæ*, *melanoon* and *typicus*, and in the Balkans *elutus*, *subalpinus* and *typicus*. The presence of two members of the same group in the same locality has been frequently reported, but either one of the two is relatively uncommon, or there may easily have occurred errors in classification.

VI. RELATION OF THE RACES TO MALARIA.

Virtually all the malaria of Western and Southern Europe due to *A. maculipennis* is transmitted by *atroparvus*, *labranchiæ* and *elutus*. In East Macedonia, where *messeæ*, *typicus* and *elutus* occur together, BARBER & RICE (1935) dissected about 37,000 specimens in 1932, 1933 and 1934. The results were as follows :

	Number dissected	Percentage infected
<i>Elutus</i>	22,200	1.29
<i>Messeæ</i> and <i>typicus</i>	14,713	0.07

Thus, nineteen infected *elutus* were found for every infected *maculipennis*. "*Maculipennis (messeæ, typicus)* in our region", write the authors, "where domestic animals are plentiful, is probably of little or no practical importance in the transmission of malaria." Similarly, SWELLENGREBEL and his colleagues (DE BUCK, SCHOUTE & KRAAN, 1936) dissected *atroparvus* and *messeæ* taken together in the same houses in a malarious village in the Netherlands.

	Number dissected	Percentage infected
<i>Atroparvus</i>	26,521	7.73
<i>Messeæ</i>	1,223	0.0008

Only one infected *messeæ* was discovered in all.

But the early hope that the fresh-water races, *messeæ*, *melanoon*, *subalpinus* and *typicus*, might turn out to be non-vectors of malaria everywhere has not been fulfilled. For example, the malaria of the Volga basin, that of the Danube above the delta, and that of Central Yugoslavia, is undoubtedly due to *messeæ* or *typicus* or both. *Melanoon* and *subalpinus* have not as yet been incriminated. This malaria may be due to the existence of less zoophilic sub-races

of *messeæ* and *typicus*, or, what is more likely, to the fact that social and agricultural conditions do not yet provide in parts of Eastern Europe a sufficient barrier of stabled domestic animals to protect human beings from dangerous contact with zoophilic anophelines. The three factors : number of children, number of anophelines and number of animals, are subject to different values and combinations which seem to determine the level of transmission in the presence of the zoophilic races. The present policy of the Soviet Government to increase as rapidly as possible the number of domestic animals in villages in the Volga basin should permit the local malariologists to evaluate the influence of these factors. On the whole, the moderate intensity and prevailing tertiary type of malaria in these regions must be fairly comparable to that of Western Europe a century ago, when conditions still permitted malaria to maintain itself, with the help of anopheline races now innocuous.

There remains the mystery of *atroparvus*. In Northern Europe, it maintains a mild endemic of *P. vivax* malaria in the Province of North Holland, in the German area around Emden and possibly on the English east coast, but nowhere else. South of the Alps it is harmless on the Mediterranean shores of Italy, France and Spain ; it maintains an intense endemic malaria (of both *vivax* and *falciparum* infections) on the Atlantic coast of Portugal and in Western Spain. In the Netherlands, SWELLENGREBEL and his colleagues believe that *atroparvus* females do not become infected to any extent until after August 15th, when they cease their sexual activities and seek winter quarters for semi-hibernation in dwellings and stables. MARTINI (1934) is not convinced that this is true in general for all the malaria transmitted by *atroparvus* in Northern Europe. His observations indicate that summer infections cannot be excluded in Germany. He also found in records made between 1860 and 1870 that many summer visitors from Leipzig used to be infected with malaria during July and August on the island of Norderney in the North Sea not far from the Dutch border. It is his opinion (personal communication) that the most dangerous period began in mid-July, before anopheline hibernation set in.

This does not explain why *atroparvus* is not a vector in the rest of Northern Europe. It is possible that the reason is to be sought in the quantitative relationships between *atroparvus* densities, methods and extent of animal husbandry and number of children

under 16 in the various regions. In Southern Europe, *atroparvus* inclines to breed in fresh water, and, where it is a vector, it is infected in summer like *labranchiæ* and *elutus*. Here, too, the ratio of anophelines to stabled live-stock may have a critical value above which contact with man is frequent enough to maintain malaria. The situation, however, both in the north and in the south, is far from being well understood.

SUMMARY.

The basis of the division of the *maculipennis* complex into varieties or races still remains the egg, whose pattern and floats permit the setting-up of eight or nine distinct forms of which at least six are well established by biometric, genetic and physiological tests, as well as by field studies on their bionomic characters. A list is suggested of essential points to be observed before reporting new local varieties. The systematic position of the races is still vague, although malariologists have not waited on the entomologists before putting race sanitation into practice. The relationship of the different forms to malaria has been further clarified, especially as regards the distinction to be drawn between the fresh and salt water breeders, although much mystery still surrounds the ecology and epidemiological role of *atroparvus*. In the meantime, many other widespread species of anophelines have been found to be complexes similar to the *maculipennis* group, thus suggesting the solution to a number of baffling epidemiological problems in malaria.

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