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The Secretary of the Malaria Commission has the honour to communicate herewith to the members of the Commission the following document:

The geographical distribution of the varieties of anopheles maculipennis in Spain, with short remarks on their biology,

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On the appearance of the first work of Professors Martini, Missiroli, and Hackett on the varieties of A. maculipennis, based on the characteristics of the egg, and on the relation between these forms and malaria, which gave fresh topical importance to the breeds discovered by Van Thiel, de Buck, and Swellengrebel, in Holland, I was commissioned by the Central Anti-Malaria Commission to study their distribution in Spain and their biology.

During the years 1932 and 1933, the different Sections of the above-mentioned Commission sent me specimens of pregnant females so that I might study their eggs in the laboratory. Notwithstanding, during this early period, I used, in order to obtain them, lamp-chimneys into which I introduced the mosquitoes on a Petri dish with water.

With this process I could not go beyond the 200 annual batches of eggs, seeing that the major part of the females died without laying, until, through Dr. Hackett's visit to Spain and a journey to Italy subsidised by the League of Nations Malaria Commission to study this problem, I found myself in a position to complete my preparations and acquire the technical data indispensable to the carrying-out of this work.

I should like to take this opportunity of acknowledging my gratitude to Professors Missiroli and Hackett, and to Dr. Pampana, of the League of Nations, and, in particular, to the doctors of the Anti-Malaria Commission, provincial dispensaries and Provincial Anti-malaria organisations as well as of the "Confederacion del Guadalquivir", who, by supplying me with material from their respective Sections, made it possible for me to collect about 3,500 batches of eggs from a wide variety of regions during the past year. In short, it may be said that only the northern part of Spain remains to be completely studied.

I. THE DISTRIBUTION AND THE NUMBER OF VARIETIES IN SPAIN.

Four varieties of A. maculipennis have been met with in Spain so far: basiliei Fall., atroparvus Van Thiel, labranchiae Falleroni, and melanoon Hackett.

These forms show notable differences in their distribution. One of them, A. m. atroparvus, is spread over all the great central plateau and the south of Spain, and has also been found in the north-west; which seems to indicate that it must likewise be distributed through the northern region, in spite of our present lack of data on this point.

A. maculipennis basiliei has been found up to the present in only one place in the province of Orense, near the Atlantic coast; A. m. melanoon is distributed through the eastern region, giving way to A. m. labranchiae in the south of Alicante and in Murcia, the only provinces in which the last variety has yet been found.

The data referring to the detailed distribution are given later on in the text, the number of batches of eggs studied of each variety in the following table:

Distribution of A. maculipennis varieties in Spain.

Provinces	Localities	Ovipositions obtained atr.	mel.	labr.	bas.	Total
Barcelona	Prat del Llobregat	90				90
Huesca	Alrededores	95				95
Burgos	Miranda de Ebro	22				22
Orense	Castrelo de Mino	77			13	90
Tarragona	La Cava	33	40			73
Valladolid	Medina del Campo	11				11
Salamanca	Campocerrado	160				160
"	Ciudad Rodrigo	41				41
"	Retortillo	215				215
Castellon	Alrededores		63			63
Madrid	Capital	51				51
Valencia	Saler		162			162
Toledo	Talavera de la Reina	25				25
Caceres	Caminomorisco	128				128
"	Torrequemada	93				93
"	Coria	18				18
Ciudad Real	Daimiel	152				152
"	Puertollano	201				201
Badajoz	Pantano de Cijara	627				627
Alicante	San Fulgencio			172		172
"	Oliva		12			12
"	Orihuela			11		11
"	Pego		96			96
"	Orba		7			7
Murcia	Zona de huerta			42		42
Jaen	Capital	15				15
"	Camporredondo	49				49
Cordoba	Zona de riego	136				136
"	Cabra	5				5
"	Hornachuelos	119				119
"	Fuenteovejuna	17				17
Sevilla	Utrera	57				57
"	Lebrija	75				75
Huelva	Gibraleon	150				150
Granada	Genes de la Vega	10				10
Cadiz	Arcos de la Frontera	519				519
"	Ubrique	1				1
"	Sanlucar	4				4
Total ovipositions		3,546	370	225	13	3,824

II. THE MORPHOLOGICAL CHARACTERISTICS OF THE SPANISH VARIETIES.

The forms found in our country are similar to those of Italy, so I will not repeat the characteristics set out by the authors mentioned above, who give excellent descriptions of them, which may be consulted for their distinguishing marks; I have, however, to make some remarks about certain morphological details which have not been mentioned, or have been referred to only cursorily, but which help to distinguish them.

A. maculipennis basiliei corresponds exactly to the descriptions given. I have never met in this variety the hexagonal network found in some others, and the protuberances of the exochorion seem very irregular, with a tendency to be triangular, as in labranchiae, and, like those of labranchiae, they are completely devoid of a dark spot in the centre. These protuberances are big, so that the egg seems pure white, contrasting with the black bands, on which they are much smaller but equally irregular.

The larva agrees likewise with the description given by Dr. La Face, and in the adults the external spine of the harpago is blunt.

The A. maculipennis labranchiae of our country varies in colour, specimens existing with well-defined black spots, although this is unusual; the floats are characteristically grooved, and very narrow, bearing out more in narrowness than in length the distinction from the atroparvus floats. The protuberances of the exochorion are coarse, and of the irregular shape already described. It may be affirmed with Hackett that the Spanish variety is generally darker than the Italian form. In the larvae the hair No.1 of the second abdominal segment is always palmate. In the adults the harpago is invariably sharp-pointed.

A. maculipennis atroparvus, perhaps because it is the most widespread and commonest sort, is the one that shows the greatest variations among its specimens. The floats, apart from the very few grooves which occur in the zone of their insertion, are smooth; but in some eggs I have seen them heavily grooved. This always coincided with an abnormally reduced size of float and with considerable differences of dimension among different specimens; that is to say that I was always dealing with non-typical eggs. As to the floats, I would point out that amongst our atroparvus there are some in which the ribs are quite clear and the rest of the egg perfectly smooth, whilst in others there are very obvious grooves in addition to and parallel with the ribs, which give them a corrugated appearance, so that it is sometimes difficult to count them.

The differences in the protuberances of the exochorion are considerable. In the normal type where the colour contrast is strong, the pale protuberances are fairly large and rounded, with festooned edges in the light areas, while those on the black parts are very small and irregular in outline. The colour of the egg depends on the size of the protuberances; the larger these are, the whiter the eggs appear; in the palest ones they look as if they were caked on them.

In a second type, the protuberances on the dark parts are slightly larger, while those in the pale area are of normal size, giving the egg a uniform greyish tinge; in a third type, the protuberances on the pale areas have a ravelled appearance; they are small, and have lost their usual shape, giving the egg a uniformly dark appearance. Nevertheless, in a single batch in which nearly all the eggs are completely uniform and dark, a few may have normal protuberances with a greater colour contrast.

The black spot in the centre, mentioned by Hackett, is found only in the first two types, and not in the third, probably owing to the stellate shape of the former.

The greyish tint of the chorion, described by the above-mentioned authors in their first reports, is hardly ever found in our specimens, although they are not blue-black like labranchiae.

The hexagonal network described by Hackett in messeae is found in a fairly large proportion of our atroparvus, being more or less clearly marked at both extremities of the egg; in some specimens it is large enough to cover almost the whole surface, Owing to the absence of protuberances, this network has a dark appearance.

A special all-black type with the net work covering the whole surface was found in 0.5 per cent of the specimens of eggs examined. E. de Buen told me that he had obtained from these eggs, which appear mainly in the winter, ♀ ♀, whose eggs were entirely normal in appearance.

The larvae are indistinguishable from those of labranchiae, as in many cases Hair No.1 of the second abdominal segment is of the typical palmate form, which at first led me to think that this variety must exist in the central plain, until I obtained larvae with the same palmate hair from typical eggs of atroparvus which convinced me that this feature is by no means exclusive; as the hairs of the fourth and fifth segments are practically the same in both varieties, there is, in my opinion, no real distinguishing mark.

The adult males, of which I observed several hundred, always have the external spine of the harpago pointed, although in some specimens it is sharper than in others. As regards the maxillary index of the females, there is a higher percentage of mosquitoes whose index varies between 14.5 and 17 teeth. These data, like the previous ones, are based on specimens caught in nature at the same season as the other varieties, i.e., in June and July. Very complete statistics of their variations in the province of Cáceres are given in the recently-published work of E. de Buen.

A. maculipennis melanoon. In its general shape the egg closely resembles that of atroparvus. The floats are approximately the same as in that species, but in some batches a considerable percentage of eggs possess corrugated floats, not so marked as in labranchiae, but similar to those of the messeae which we observed in Rome. In other batches all the eggs are more or less corrugated, contrary to Hackett's finding. The hexagonal network is always clearly marked, and, as that author observed, the spots follow its outline fairly closely. The protuberances on the exochorion are much smaller than in atroparvus, and are always round, with a darker spot in the centre. As a rule, the light-grey colour of certain specimens is due not to the size, but to the number, of the protuberances, so that a slight increase in the number of the latter always gives them a smooth appearance. The shade varies considerably, appreciable differences being found among eggs in the same batch; while some are almost completely black, others have pale-grey areas standing out in marked contrast.

Hair No.1 of the second segment of the larva is nearly always branched, as in messeae. The adults, as I had occasion

to demonstrate to Dr. La Face in the course of our tour, sometimes have the apex of the external spine of the harpago rounded, or rather, blunt; in other specimens it is definitely pointed, and in some the apex comes to a sharp point, i.e., midway between the two previous shapes.

The maxillary index of the ♀ is midway between atroparvus and labranchiae, usually varying between 14.5 and 16.5. The majority of specimens have an index of 15 to 15.5. As a rule the length of the wing varies between 4 and 4.3; the most usual wing length is 4.2 (24 per cent).

A comparison of these data with those relating to messeae in other European areas shows that the characteristics of the specimens closely coincide, and that the only difference is in the $\frac{\text{length of float}}{\text{length of egg}}$ index, which is definitely lower in the Spanish specimens, and in the number of ribs, which is smaller in our variety.

It is interesting to compare the maxillary indices obtained in the three varieties; this is lowest in the case of labranchiae, in which the highest percentage varies between 13.5 and 14.5, 14 predominating; on the other hand, atroparvus shows an index of 15.5 to 16.5, while our melanoon is midway between the two, 15 to 15.5 predominating. The dental index shows that, generally speaking, the figures vary between 28 and 29 in labranchiae, between 34 and 37 in atroparvus, and between 36 and 39 in melanoon, i.e., in the first case the figures are quite different, whereas in the second and third they overlap slightly. I was unable to ascertain these indices in the case of basiliei, as up to the present the pure variety has nowhere been found, and the data obtained from laboratory cultures may be influenced by artificial breeding conditions. The number of batches of basiliei eggs was so small that I was unable to obtain it from various ♀ ♀.

III. The Distribution of Varieties in Relation to Environment.

The atroparvus variety is obviously the most robust, and hence the one best adapted to any environment. It is found both in mountainous districts and at sea level, in dry as well as in damp areas, and is the only one able to stand the very barren conditions prevailing in our great central plateau, where the water almost disappears during the summer season and the relative humidity is very low. Its breeding-places vary widely, and the biological observations made by nearly all Spanish authors relate to this breed; as it has been studied in a paper by Dr. S. de Buen, I need not deal with it any further here.

The labranchiae variety is confined to the irrigated zone, where the humidity is relatively high owing to the evaporation of the water used for cultivation purposes, and where the temperature is mild in winter and fairly hot in summer. In one part (Murcia), the breeding-places of larvae are extremely difficult to find, as they are confined to small stagnant patches of water intended for irrigation purposes and to a few pools. In the houses and stables of this zone, adult specimens are very rare. San Fulgencio affordsanother example of an irrigated area where S. de Buen

found in the drainage channels, and in certain receptacles used for boiling hemp, larvae which are doubtless of this variety, as the pure strain exists there. The rice-fields also occasionally serve as breeding-places, without, however, modifying the general conditions; nevertheless, they afford an excellent breeding-place for larvae.

I was unable to study the breeding-places and habitat of the basiliei variety; Castrelo de Mino is situated in a cold region, with an Atlantic climate, an abundant rainfall, and considerable humidity, so that conditions are more like those prevailing in Northern Europe.

The melanoon variety is generally found in the rice-fields in the eastern part of the country, where the temperature is mild in winter and comparatively hot in summer, with a relatively high humidity, which is checked during the day by the rise in temperature, but almost saturates the atmosphere at sunset. The breeding-places are in the rice-fields, their drainage channels, and the so-called "préstamos", which are channels dug out for irrigation purposes. The water in these rice-fields is always pure; in many cases there is plentiful horizontal vegetation, the temperature is comparatively warm, and there is a very abundant fauna. In some parts of the Ebro delta, the water in the drainage channels is slightly brackish, but it cannot be definitely asserted that this species develops there; it is probably atroparvus, also found in this area, which has a preference for brackish water.

It is of interest to note that only melanoon is found in the rice-fields running all along the coast from the northern part of Alicante as far as Castellón; in the Ebro delta it is mingled with atroparvus, which replaces melanoon at Prat de Llobregat, near Barcelona.

It might be assumed that melanoon is replaced by atroparvus in the north owing to the more severe climatic conditions, but in my opinion that assumption is incorrect - it is the outcome of a biological struggle between the two for predominance in the rice-fields. In Valencia, and also in Castellón and Alicante, where - thanks to the irrigation system, in some cases dating back to the Arabs - rice has been grown for a long time past, the melanoon variety has had time, with the help of particularly favourable environmental conditions, to oust atroparvus. This is probably what is happening in the Ebro delta, on one side of which rice has been grown for about eighty years, while on the left bank it has been cultivated for some twenty years. On the other hand, in the Llobregat delta, rice cultivation is of more recent date, and consequently melanoon does not exist, or at any rate is sufficiently rare for us not to have come across it among the several hundred batches of eggs collected. This view is, in my opinion, much more likely to be correct than the assumption that the disappearance of melanoon and its replacement by atroparvus is due to the difference in temperature, which is really very slight, between Castellón and Barcelona.

IV. The different varieties and their relations with man.

Owing to its epidemiological importance, the more or less close relations of the varieties of mosquito with man constitutes one of their most interesting biological peculiarities; unfortunately, though anxious to do so, I have been unable to study this point on the spot in various regions for the purpose not only of collecting blood from stable and house mosquitoes for subsequent study by means of precipitins, but also of making practical observations as to the food they prefer in each of these zones. I have no data whatsoever regarding maculipennis basiliei and labranchiae, and although those relating to melanoon are not very plentiful, there are certain detailw which I should like to record.

Hackett states that melanoon shows a deviation from man, so that this variety does not transmit malaria. Nevertheless, in Valencia, Dr. Hill and I made a point of staying out of doors to see whether the local anopheles bit in the open air, and in less than half an hour five arrived, of which we were able to capture three. Luengo, in 1924, stated that in this region these culicidae frequently bit man. This statement appears to be borne out by the comparatively large number of these insects in the houses and stables, as I observed in a short note published in the Boletín del Instituto de Higiene of that province. Whereas, in the districts where atroparvus predominates, the number of mosquitoes in dwellings is always much smaller than the number in the stables, in this region the two are almost equal.

In the whole district around Albufera, cattle are very rare, as I had occasion to observe, and at Saler - a place I have already mentioned - during my first visit with Dr. Hill I found one shed containing cows and another goats, whereas on my second visit the latter had disappeared. It is hardly likely, therefore, that the thousands of anopheles in the houses there could keep themselves alive on the four cows in the shed. In Castellón, according to Dr. Cámara's observations, there were nearly as many mosquitoes in the houses as in the stables.

V. The different varieties and their relation to malaria in Spain.

A comparison of the malaria map of our country with the map showing the distribution of varieties will lead us, I think, to interesting conclusions. We will leave out of account the Cantabrian zone, for which we have no data - except for Castrelo de Miño, where malaria exists, the fauna consisting chiefly of atroparvus with a small number of basiliei - and will confine ourselves to the three following very dissimilar zones.

(a) Rice-field zone, inhabited by melanoon.

In this zone, malaria is rare, and is decreasing almost everywhere while districts of intense anophelism without malaria are being formed. Castellón is an example of the former, and the places near Albufera de Valencia of the latter.

In the Ebro delta, where this variety, although it predominates slightly according to my data, is mingled with atroparvus, malaria, which, thirty years ago, was very prevalent and severe (on which account the district is feared by the Valencian rice-growers who go there to plant and harvest the rice), has gradually declined, until it is now much less frequent. In my opinion, this decline is closely associated with the biological struggle, as the outcome of which atroparvus is being replaced by this variety, the latter being more vigorous in this environment.

It would appear, therefore, that melanoon is not an active vector of malaria, and that districts where it is found alone are often free from the disease.

(b) Labranchiae zone.

As S. de Buen observes, the region inhabited by this variety is marked by the existence of what might be called epidemic malaria.

This is clearly proved by the fact that in the district of San Fulgencio and Rojales, in the province of Alicantes, its endemicity is checked if a vigorous campaign is conducted, but as soon as this is slightly relaxed - as occured in 1934-35 - veritable epidemics break out.

In the irrigated part of Murcia, which is situated within this zone, malaria persists, although the number of mosquitoes is incredibly small; however, the anti-malarial measures employed for the treatment of patients get the better of the disease fairly easily. Hence the Spanish observations coincide with those of the Italian authors, who state that this variety is the most active vector of the human plasmodium.

(c) Atroparvus zone

If we regard atroparvus as a single variety, and attach no importance to the differences in the colouring of the egg, taking them to be simply individual variations, we may say that it covers almost the whole of our country, and is found in pure strain in the central plateau and Andalusia.

The differences in the percentage of infection are considerable; it is always less intense north of the Carpeto-Vetónica range separating the two Castiles, where it is rare or non-existent except at Salamanca and in the Aragonese irrigated zone, probably owing to the bleaker climate; south of that range there are certain districts where malaria is very troublesome, as in the Extremena region and Western Andalusia, others where its endemicity is lower, and also, here and there, places in which anophelism exists without malaria, as at Aranjuez, Padul, etc., although the number of mosquitoes is considerable.

We see, therefore, that the labranchiae zone is marked by intense malaria, the melanoon zone by its rarity or absence, and the atroparvus zone by the considerable differences in the prevalence of the disease.

In the case of separate varieties, their greater or lesser epidemiological importance can easily be explained by the biological differences between them; nevertheless, as we have already observed, the reason why melanoon is not an active vector is to be found not in its deviation by animals, as Hackett asserts, but in physiological peculiarities. For instance, whereas in the laboratory atroparvus may produce three or four batches of eggs, melanoon can hardly be made to produce a second batch, although the number of eggs in its first batch is usually considerably larger than in the case of atroparvus; if this also happens in nature, it would mean that its adult stage is shorter than that of atroparvus, and that the risk of infection is therefore less.

On the other hand, the different behavious of atroparvus according to the locality is due to factors extraneous to the mosquito and capable of modifying its biology. What are these factors?

Some years ago, Missiroli and Hackett discussed the various theories which had been put forward up to that time to explain anophelism without malaria, and I agree with them that none is altogether satisfactory. Moreover, this phenomenon must have a definitely local character, certain factors which are decisive in some places being absent in others. In the case of Spain, climatic conditions might explain the absence of malaria on the Cantabrian coast and north of the plateau, but cannot account for the position at Algodor and Aranjuez, which are very near and possess the same climate - the former being malarious and the latter not.

As regards deviation by animals, the interesting experiments of Roubaud have shown that there is a clearly-marked distinction in the zoophilism of the anopheles of Camporredondo (Jaen); nevertheless, although cattle are found in that zone which might attract them, endemic malaria is widespread.

I should now like to record some personal observations I have made in the course of my work, which may possibly throw some light on the matter. My object is not to put forward a new theory, but to draw attention to certain somewhat obscure points in the biology of mosquitoes, from the detailed study of which it may be possible to draw valuable conclusions.

It is a noteworthy fact that in our country malaria does not appear to have any direct connection with the large number of ponds to be found in so many places, which are capable of feeding a hugh and vigorous swarm of anopheles; on the contrary, the prevalence of the disease is in inverse ratio to the number of these ponds. For instance, in Cáceres, where there is possibly more malaria then anywhere else in Spain, there are many places where, owing to the excessive drought, there is hardly enough water during the summer months to maintain small larval breeding-places in what remains of the streams and wells; nevertheless, malaria is widely prevalent.

These larval breeding-places produce mosquitoes which are small in size and usually have a rather low maxillary index. This denotes a morphological change due to the unfavourable

conditions of the aquatic environment, of which the following may be mentioned: (a) the excessively high temperature of the water, which, although it accelerates the cycle of evolution, is prejudicial to the anopheles; (b) the scarcity of aquatic vegetation and plankton; (c)over-population by anopheles; (d) prolonged stagnation with too rich a bacterial flora; (e) salinity.

It is not going too far to assume that the considerable morphological changes caused by development under these conditions are accompanied by physiological changes in these mosquitoes, regarding which little or nothing is known.

Of what nature are those changes likely to be? It is highly probable that in the case of a badly-nourished mosquito, or one which has passed through precarious conditions during its aquatic life, the development of the ovaries will be retarded, and it will also have a greater need of food to make good to some extent the deficiency during its larval stage.

Both these factors would be highly conducive to the propagation of the parasite, both because it would have a longer time in which to complete its evolution, and because of the greater risk of infection to which the mosquito is liable if it feeds more frequently.

This is not the first time that attention has been drawn to the small ponds where conditions are unfavourable, in their relation to malaria. The brackish water areas have been regarded as more malarious for some time past. Moreover, Alessandrini pointed out that mosquitoes which develop in a favourable aquatic environment are less likely to transmit malaria than those bred under adverse conditions during their larval stage. Nevertheless, the explanation given by this author, that it is owing to their vigour that they are immune to the parasite, is untenable, because it has been proved that they are just as likely to become infected as weakly specimens.

The question is deserving of study, not only on account of its theoretical interest, but in view of the practical consequences which might ensue if my observations proved to be correct, as this would confine the campaign against anopheles to ponds where conditions are definitely unfavourable, and where greater precautions would be taken, if it were not possible to drain them.

My investigations into this question were cut short suddenly before I was able to draw any conclusions, but I trust that it will soon be possible for me to resume my work, once the causes which prevented me from continuing it have been removed.