New Concepts of Mosquito Taxonomy

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When we speak of new concepts in mosquito taxonomy we are thinking of the application of new techniques to help us understand the relationships between species. The "new" techniques we have in mind are for the most part not based on morphology. In most cases they are also not new, having been previously used in other groups of organisms. They represent not a substitute for morphological study but in many cases a useful supplement.

There are two purposes for which these techniques are used: firstly to study relationships within a group of closely related forms such as the *Culex pipiens* complex, and secondly, to study more distantly related forms so that evolutionary relationships may be discerned. The two kinds of studies might be called speciational and evolutionary, and different techniques are used for each.

Chromatography has been widely used for both purposes. (Slide 1) This slide from a study by Micks and Gibson (1957) shows an amino acid analysis of representatives of three members of the *Culex pipiens* complex. This technique can be used for analyzing a number of chemical constituents of mosquitoes. The results as shown by the tracings on the right show quantitative rather than qualitative differences in the forms. The technique can be made somewhat more qualitative by using two-dimensional chromatograms as shown in the next slide (slide 2) from another study by Micks (1954). These chromatograms are more qualitative in that amino acids have particular spatial locations and can be judged to be present or absent. The chromatogram on the right for example, European *Culex pipiens,* lacks several spots which are found in other members of the *Culex pipiens* complex.

Spectrophotometry has also been used to analyze mosquitoes. An extract of mosquitoes is used to absorb light of different wavelengths and absorption patterns characteristic of different combinations of chemicals result. The next slide (slide 3) from a study by Micks and Scrollini (1954) shows absorption of infrared light of various wavelengths by four forms in the *Culex pipiens* complex. The differences between the curves are minor and difficult to quantitate.

A third method which has been used is electrophoresis. In this technique a mixture of proteins from a mosquito is separated by differential mobility in a magnetic field. The next slide (slide 4) is from a study by Freyvogel and McClelland and shows a number of bands of alkaline phosphatases in *Aedes vittatus*. This technique can be used for the analysis of many different kinds of proteins and is especially useful for studying enzymes. The results are more or less qualitative since bands are seen to be present or absent.

A somewhat different approach to this problem is the analysis of antigens of mosquitoes by an appropriate antibody producer such as a rabbit. Lawlor studied relationships in the *Culex pipiens* complex by this method some years ago. A variation of this technique is shown in the next slide (slide 5) from a study by Zaman and Chellappah (1964). Antibody to eggs of *Culex pipiens quinquefasciatus* was placed in the center well of the agar plate. Extracts of this *Culex* were placed in three of the outer wells; extracts of *Aedes aegypti, A. albopictus,* and *Armigeres subalbatus* were placed in the other three wells. Antigens diffused through the agar from each of the outer wells. Antibodies diffused through the agar from the center well. Lines of precipitate are seen between the center well and each of the outer wells containing the *Culex* extract. Since the different proteins diffuse at different rates depending on their size, there is a separation of both antigens and antibodies; the several antigen-antibody reactions occur in different places so that there are several lines of precipitate, three in this case. Immunoelectrophoresis is a variation of this technique in which the antibodies are separated by electrophoresis before gel diffusion is begun. The next slide (slide 6) is from a study by Cupp and Ibrahim (1973). The median slot contains antibodies to *C. p. quinquefasciatus* which have been separated by electrophoresis. Extracts of *C. p. quinquefasciatus* and *C. p. pipiens* are added to the upper and lower wells. Lines of precipitate occur where antibodies react with antigens. In the test shown there are six zones of precipitate both to the immunizing and test antigens. The distinct lines in this test give qualitative results although the lines vary in intensity and are quantitatively different.

Hybridization studies have classically been used to resolve doubts about conspecificity in mosquitoes. Members of the *Anopheles maculipennis* group and of the *Anopheles gambiae* group have been shown to be largely intersterile and so are clearly different species even though morphologically similar. Members of the *Culex pipiens* group on the other hand have been shown to be largely interfertile and presumably are conspecific. At this meeting last year, Fukuda and Woodard showed that *Aedes nigromaculis* and *Aedes sollicitans,* two undoubted species, were interfertile. This is one of the few cases in which "good" species of mosquitoes have been shown to have a high degree of interfertility. The hybridization technique is useful for speciation studies but is not very useful for evolutionary studies as earlier defined.

Chromosomal analysis is useful for taxonomic studies. Each of the *Anopheles gambiae* forms, which now number six, has characteristic banding of the giant polytene chromosomes and identification can be made in this way. The chromosomal differences are large enough so that chromosomal pairing is difficult in hybrids. The next slide (slide 7) is from a study by Coluzzi (1966) and shows the chromosome complement of a hybrid between species A and species B of the *Anopheles gambiae* complex. Chromosome 2 shows three inverted regions, chromosome 3 another inverted region, and the sex chromosomes, X, are almost completely asynaptic. Thus the study of cytology of hybrids shows not only whether forms are different but also how different they are.

Finally, we have added a new dimension to mosquito speciation by showing that in *Culex pipiens* crossing barriers which we at first thought were genetic actually were due to intracellular symbionts (slide 8). The "crazy-quilt" pattern of crossing types Laven has described from Europe seems to be due not to racial differentiation of mosquitoes but to racial differentiation of mosquito symbionts. A similar kind of crossing barrier has been described in the *Aedes scutellaris* group so perhaps symbiont analysis will in the future become an essential part of taxonomic studies of mosquitoes.

References

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