

The Place of Morphological* Studies in the Investigation of
Anopheles Species Complexes

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Summary

High standards of comparative morphological work on *Anopheles* were attained early in the 20th Century. The genus now has 401 recognized species and subspecies, about half of which exist as close-knit clusters of similar species. As taxonomic studies progress, many sibling species complexes are discovered in *Anopheles*. In turn, some supposed complexes turn out to be groups of quite well differentiated morphospecies.

Even sibling species of *Anopheles* seldom lack diagnostic morphological characteristics, although their distinctive specific features are often very small, compound or not absolute. Specific differences may also be restricted to only one sex or stage in the life cycle.

The morphological study of *Anopheles* sibling species has not taken enough advantage of technological innovations such as the electronscan microscope - for clarifying minute characters - or electronic computers - for processing morphometric data. The taxonomic future of anopheline complexes should not be monopolized by geneticists and other experimentalists. A thorough morphological knowledge of all *Anopheles* species is essential, if only to corroborate our belief that some sibling species cannot be separated on external characters. When conventional morphological species identification is possible, it is also the cheapest, quickest and usually the simplest method.

*This paper deals only with the morphology of cuticular components of mosquitoes. Discounted are the chromosomes and all other non-chitinized tissues and organs which do not persist in dead specimens without special histological preparation.

Preamble

A species complex consists of sibling species which, by definition, are species with obscure morphological differences. What, then, is the possible role of morphology in understanding species complexes? It is to find out if the seemingly undifferentiated members of a supposed complex are, in reality, a group of species differentiated sufficiently well for some or all of them to be identified morphologically. Until their anatomies have been investigated and their contrasts evaluated to the limits of our abilities, we do not know whether any tell-tale features can be used to separate the species. As our morphological horizon broadens, by the examination of new characters and through technological advances like the scanning electron microscope, our classification becomes a sliding scale. Yesterday's species are today's complexes; today's complexes may be tomorrow's species groups, subgenera or more. But, strictly, the members of true sibling species complexes must always be very hard, if not impossible to identify morphologically.

Background

When Manson (1884) had completed his pioneering work on filariasis transmission, he still knew no conventional mosquito taxonomy, so introduced his own ideas of mosquito morphology by referring to the vector as 'the large brown mosquito'. Ross, too, was quite ignorant of the established mosquito genera *Culex* Linnaeus, 1758, *Aedes* Meigen, 1818, and *Anopheles* Meigen, 1818, in which almost 200 specific names had then been proposed, when he spent the years 1895 and 1896 in India fruitlessly trying to transmit human malaria experimentally with examples of *Culex* and *Aedes* (Ross, 1923; Russell, 1955). Like Manson, he adopted vaguely descriptive names for these culicines, calling them 'grey' or 'plain-winged' mosquitoes. Only when Ross eventually noticed and tested the distinct *Anopheles* (probably *A. stephensi* Liston), which he distinguished as 'dapple-winged' (Ross, 1897), did he begin to make the malariological break-through for which he is medically renowned.

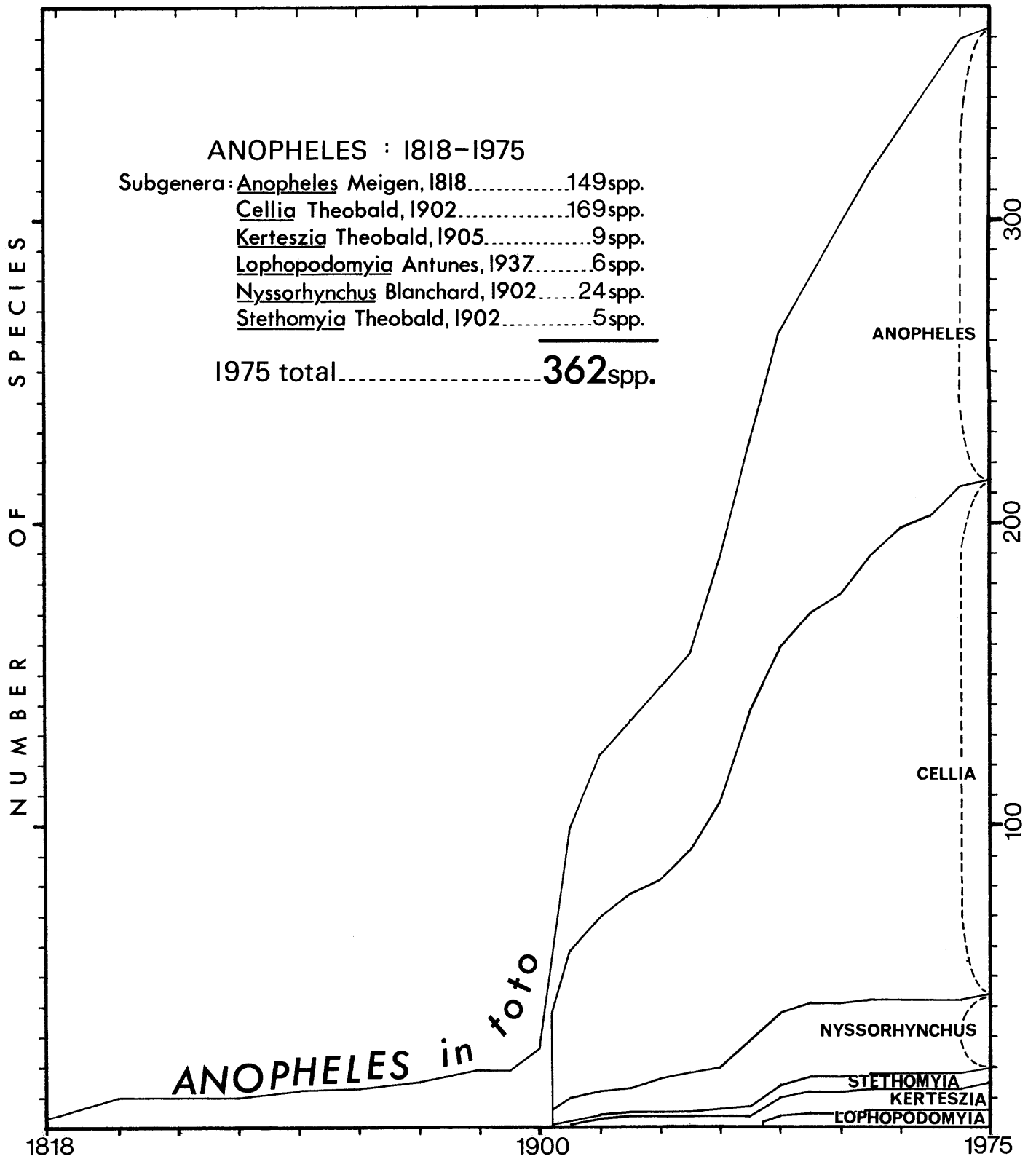
The most celebrated episode linking malariology to anopheline taxonomy concerned the famous and far-flung *maculipennis* group or complex. In the Palaearctic, where 8 or 9 forms are recognized (*atroparvus* Van Thiel, *beklemishevi* Stegnyy & Kabanova, *labranchiae* Falleroni, *lewisi* Ludlow, *maculipennis* Meigen, *melanoon* Hackett, *melanoon subalpinus* Hackett & Lewis, *messeae* Falleroni, *sacharovi* Favr), the species have minimal morphological differences and are widely sympatric. In the Nearctic, on the other hand, at least 4 species (*aztecus* Hoffman, *earlei* Vargas, *freeborni* Aitken, *occidentalis* Dyar & Knab, *quadrifasciatus* Say) are more markedly differentiated and largely allopatric. By the restricted definitions explained below, therefore, we should regard the species cluster based on *maculipennis* as a species group in the New World and as a species complex in the Old World. All this was brought to light because "*maculipennis*" sensu lato, known to be an important malaria vector since the work of Bastianelli, Bignami & Grassi (1898), was also found to be incompetent at malaria transmission in some areas (Hackett, Martini & Missiroli, 1932; Hackett, 1937). Taxonomic relationships and nomenclature of the *maculipennis* complex in Europe were substantially ironed out by Bates (1940), who relied almost completely on morphological and distributional data. His conclusions have been modified, but mainly confirmed, by the advent of hybridization tests and cytogenetical analysis performed on the majority of these cryptic taxa (Kitzmilller, Frizzi & Baker, 1967; Coluzzi, 1970).

Signifying early acceptance of the indispensable place of morphological studies of mosquitoes, so that vectors of malaria and filariasis could be identified and should bear proper Linnaean names, the Secretary of State for the Colonies in 1898 requested every officer of the British Colonial Service to collect Culicidae "in the various tropical Colonies" and to submit them to the British Museum (Natural History) for classification (Chamberlain, 1898). As specimens poured in from all over the world, Fred Theobald purposefully curated and described them with excessive taxonomic thoroughness. He published 65 works on mosquitoes between 1900 and 1914 (Knight & Pugh, 1973); virtually all his descriptions of mosquito species and genera are to be found in a 5 volume monograph having 2536 pages of text and 39 plates (Theobald, 1901-1910). Of the 70 species-group names Theobald proposed for taxa now placed in *Anopheles*, 37 have current usage for accepted species or subspecies. The next most prolific authors in *Anopheles* have been Evans with 26 (22 valid), Edwards with 26 (20 valid), De Meillon with 19 (17 valid) and Reid with 13 (all valid) species-group names.

With 33 (i.e. 47%) of his names not now being accorded priority for their respective species of *Anopheles*, it might be thought that Theobald was a poor morphologist or took insufficient care to check pre-existing descriptions. The latter certainly seems to have been so in 14 cases, 6 of these apparently being redescriptions of species already named by himself! Of the others, 6 were proposed only for varieties, 9 coincided in the press with the priority descriptions, and the remaining 4 cases can be excused because the original descriptions were inadequate and their type-specimens inaccessible. These and other transgressions by Theobald provoked spirited rivalries between him and contemporary Culicid taxonomists, notably Blanchard, Giles and Dönitz in the Old World and Howard, Dyar and Knab in the New. However, for such a fast worker, Theobald's morphological species concept was extremely advanced for mosquitoes in general, being well ahead of his time in its attention to details of the adult morphology. To our lasting advantage he was an unhesitating taxonomic splitter, coining names for new species and varieties based on the minutest of morphological differences. Theobald's productivity cannot be forgotten, since the current upsurge of sibling species detection leads us, as obliged by the Rules of nomenclature, to resurrect appropriate old names from synonymy for application to newly defined biological species. Whenever possible this should be decided on morphological grounds, assuming that long dead type-specimens are useless for genetic or biochemical evaluation. Inferences of identity based on purely distributional evidence may also be feasible, but are not so convincing (c.f. Service, 1976a). Whatever we do with their names, the multiplicity of anopheline species recognized by Theobald has been vindicated to the point where we appreciate that his taxonomic license was properly exercised pretty well to the morphological limit.

As the basis of the world's most comprehensive collection of Culicidae, the type-specimens of Theobald, Evans, Edwards, Reid and many other workers on *Anopheles* are kept in the British Museum (Natural History). The United States National Museum is also rich in *Anopheles* types, while many more of these uniquely important specimens are scattered in depositories elsewhere. Inevitably, the type-material (holotype, lectotype, syntypes, paratype(s), paralectotype(s)) of some *Anopheles* types has been lost or was never designated. To overcome these deficiencies neotypes can be designated when warranted, though this has only been done once for an anopheline*. Types always retain their inviolable taxonomic significance as the

*Reid (1950) designated a neotype for *Anopheles letifer* Sandosham



name-bearing specimens of the taxa they represent. Type-specimens therefore take more than just curatorial pride of place whenever the status or relationships of named taxa are reappraised, or the identity of a taxon disputed.

The number of species included in the genus *Anopheles*, and in its six subgenera, are depicted in Figure 1 for the period since the genus was erected in 1818. Only currently accepted species are counted, being plotted from the date of their original publication. As yet, in terms of the numbers of new species being described, growth of this genus shows little sign of slowing down. The forthcoming edition of "A Synoptic Catalog of the Mosquitoes of the World" (Knight et al., in press) contains 395 valid species-group names applied to 362 species and 33 subspecies of *Anopheles*, together with 293 more available names listed as 30 varieties and 263 junior synonyms. Descriptions of no less than 6 additional *Anopheles* species have been prepared or published since that catalogue closed in 1973. Whether we like it or not, these names exist and, in all but a handful of cases, their ascription to species depends almost entirely on morphological evidence.

Species in *Anopheles*

Whatever their degrees of morphological similarity or dissimilarity, species are incontrovertible biological phenomena for which the most popular definition is "groups of interbreeding natural populations that are reproductively isolated from other such groups" (Mayr, 1969). This 'biological species concept' leaves aside the question of the qualities of species as more or less separable morphological entities. Nevertheless, for the overwhelming majority of species, genotypic isolation can be inferred from their phenotypic discreteness (Simpson, 1961).

As a word, the term 'species' actually has two meanings. Not only are species "an order of classification of living matter" (Dobzhansky, 1962), but also the species is a category in systematics. Parallels between species and speciation phenomena in *Anopheles* mosquitoes and the far better known *Drosophila* fruitflies are so close that reference to the comprehensive writings of Dobzhansky (1951, 1962, 1970) is preferable to covering such ground specifically here.

Embraced by the biological species concept are at least a dozen sorts of species in nature (Sokal, 1973; Scudder, 1974), among which 'morphospecies' can be identified more readily than cryptic 'sibling species'. Steyskal (1972) has suggested the term 'aphanic' to replace the ambiguous word 'sibling' for use in this context. Sibling species have been defined, successively, as "pairs or groups of species that are morphologically indistinguishable, or distinguishable with difficulty" (Mayr, 1942), and "reproductively isolated arrays of populations that show little or no morphological distinctions" (Dobzhansky, 1970). It is a crucial characteristic of sibling species that they tend to be extensively sympatric. For entirely allopatric but extremely similar monophyletic groups of species the term 'superspecies' should be used, but has not been applied to mosquitoes. Quite different mechanisms of speciation are thought to give rise to assemblages of superspecies as opposed to sibling species.

Many of the most infamous *Anopheles* are now seen to be complexes of sibling species. Best known examples, roughly in chronological order of their suspicion

or detection are those based on *maculipennis*, *minus* Theobald, *funestus* Giles, *albimanus* Wiedemann, *hyrcanus* (Pallas), *gambiae* Giles, *punctulatus* Dönitz, *umbrosus* (Theobald), *barbistrostris* Van der Wulp, *leucosphyrus* Dönitz, *aitkenii* James, *subpictus* Grassi, *claviger* (Meigen), *bancroftii* Giles, *annulipes* Walker, *nuneztovari* Gabaldon and so on. For several of these cases, enough morphological work has now been accomplished for us to place the nominate species in a complex of true siblings, while each complex itself stands in a group of rather more strongly differentiated morphospecies and perhaps other complexes. See, for example, how Guy, Salières & Boesiger (1976) extend the *maculipennis* complex from 7 Palaearctic members to embrace 6 Nearctic species, then 4 more neotropical species and finally 3 more Nearctic species, so amalgamating several complexes and more distinctive species in a heterogeneous but monophyletic assemblage. This situation requires a résumé of anopheline systematics.

Systematics of the Anophelinae

Classification of the Culicidae into subfamilies Anophelinae, Culicinae and Toxorhynchitinae, and of the Anophelinae into genera *Anopheles* (368 species), *Bironella* Theobald (7 species) and *Chagasia* Cruz (4 species) has been customary since Stone (1956) elevated the Chaoboridae and Dixidae to the status of separate families. Retention of these two non-biting groups as subfamilies of Culicidae by Belkin (1962) necessitated keeping the Anophelinae down to tribal rank as the Anophelini. In essence, the phylogenetic relationships between these branches of the superfamily Culicoidea are comfortably undisputed. *Anopheles* is subdivided into six stable subgenera: *Anopheles* s.s. (151 species), *Cellia* Theobald (173 species), *Kerteszia* Theobald (9 species), *Lophopodomyia* Autunes (6 species), *Nyssorhynchus* Blanchard (24 species) and *Stethomyia* Theobald (5 species). Respect for this system, which works well and probably reflects the true evolutionary picture, has prevented any attempt to test or tamper with anopheline systematics by modern methods of phylogenetic (Hennig, 1966) or phenetic (Sneath & Sokal, 1973) classification. To undertake such analyses would demand an inordinate amount of the finest morphological observation.

According to the Code of zoological nomenclature (ICZN, 1964), formal categories of classification are restricted to the family, tribe and genus (with optional super- and sub-ranks), species and subspecies. Informal categories employed at the lower levels of anopheline classification are the supraspecific 'Sections' and 'Series' that have become established groupings in the subgenus *Anopheles* (Reid & Knight, 1961) and, regionally, in the subgenus *Cellia* (e.g. Gillies & De Meillon, 1968). For even closer assemblages of *Anopheles* species it is customary to define taxonomic 'groups', 'sub-groups' and 'complexes' of species. The last should be reserved strictly for true sibling species.

Only by exhaustive morphological assessment of apparent sibling species (and superspecies) can the real extent of their physical divergences be evaluated. While our interpretation of the different ranks of species groups and complexes may be subjective (Reid, 1970), the systematic distinction of these two grades depends objectively upon the actual amounts of morphological resemblance among the species concerned. Without denying this qualitative difference between a group and a complex, it is usual to treat any species group as a sibling species complex for as long as the interspecific morphological differences remain undetermined. When all has been done and documented, however, a complex of almost identical sibling species must rank as the lowest conceivable morphologically based category of informal supraspecific classification.

Morphological characters in *Anopheles*

Since Falleroni (1926) hit upon the taxonomic usefulness of egg characters for separating members of the *maculipennis* complex, anopheline taxonomy has become increasingly dependant upon features present on various stages of the life cycle. As a result, the range of morphological characters involved in practical identification of the various *Anopheles* is probably greater than for any other group of insects. Ideally, recognition of a species depends upon a suite of characters that facilitates identification of eggs, larvae, pupae and adults of both sexes. In practise, not all the life stages of closely related or convergent mosquito species differ from each other noticeably. Surprisingly often, specific morphological characteristics are pronounced in only one or two life stages, or one adult sex, of a particular mosquito species. Characters most often used to identify anopheline adults are still many of those adopted by Theobald and even his predecessors (*vide* Ficalbi, 1896). They are confined largely to elements of scale pattern and colour, distribution and structure. Conventional characters for descriptions of immature anophelines lie in the sculpture of eggs, setation and pigmentation of larvae, and the forms of paddles and trumpets as well as chaetotaxy of pupae. Some innovations for characterization of sibling species are detailed below. In general, however, morphological work on *Anopheles* is habituated to a selection of qualitative, quantitative and meristic characters which have been observed and analyzed with progressive refinement. Wastage of outdated information has been minimized by sticking to traditional characters as much as possible.

Primary description of a species may involve much verbiage, but needs only to be a sparing account of salient characteristics by which the new taxon is identified. Experienced taxonomists of particular groups, such as the Anophelinae, tend to anticipate which characters are most likely to repay investigation. This habit undoubtedly inhibits the discovery and utilization of new characters. Gutsevich (1975) has recently shown just how easily *Anopheles* species can be identified with unconventional characters. Unfortunately, original descriptions frequently lack the character details required to separate known species from those discovered later. Most mosquito taxonomists nowadays strive to attain rigorous standards of description and to cover each stage and sex of every species. Where possible, supplementary papers give account of some hitherto unknown egg, larva or pupa, especially in *Anopheles*. Publication of newly described taxa is often kept until a reasonably complete account of the stages and sexes is achieved. To synthesize and balance all this information, successive revisionary taxonomic studies have been made on the Anophelinae of the best known zoogeographic regions. The most outstanding recent monographs on *Anopheles* are those by Grjebine (1966) for Madagascar, by Reid (1968) for Malaya and Borneo, by Gillies & De Meillon (1968) for sub-Saharan Africa and by Harrison & Scanlon (1975) for Thailand.

In all this work, an exceedingly large number of different morphological characters has been invoked for anopheline description and identification. A recent glossary includes the following numbers of definitions of anatomical features on mosquitoes, though some are not present or have not been studied on *Anopheles*: 313 parts of the adult male, 307 parts of the adult female, 93 parts of the pupa and 335 parts of the larva (Knight, 1970; 1971; Knight & Laffoon, 1970a, b, c; 1971a, b; Laffoon & Knight, 1971; 1973). In addition Hinton (1968) cited 52 variations of 6 fundamental egg characters in *Anopheles*. Thus we have well over 1000

named characters per species, all of which can and perhaps should be examined, described and compared for every species. Allowing for the diverse variations of each character, the scope for comparing the morphology of closely related species clearly has an almost inexhaustible resource of conventional characters. If these orthodox features fail to reveal any absolute interspecific contrasts, as between the most similar sibling species, it may still be possible to divine taxonomically reliable differences by means of elaborate, subtle or multivariate statistical comparisons of meristic or morphometric data. In theory sibling species either do or don't have diagnostic morphological characters. In practise, when no differences have been detected, we are still unsure of their absence.

Detection of species complexes

Morphological studies should not come too early in the process of detecting anopheline sibling species, since it might be misleading to attempt to characterize such taxa using specimens not previously identified by cross-mating tests, biochemical or cytogenetical determination, or some similarly trustworthy technique. As with species in general, the members of a sibling species complex are all likely to show clinal variations, seasonal modifications, subspecific differentiation and individual polymorphism resulting in a baffling picture of overlapping phenotypic variation, especially in mixed samples of sympatric populations. Without properly predetermined material, therefore, it is ambitious and risky, but not necessarily impossible, to try to distinguish between intraspecific and interspecific morphological variation.

The usual indicators of a species complex are heterogeneous factors in the biology of a supposed single species. With the sophisticated field technology now at our command for studies of anopheline behaviour, ecology and physiology (WHO, 1975; Service, 1976b), it has become increasingly easy to find out where, when and in what ways each puzzling *Anopheles* varies. Some of the most conspicuous contrasts between sibling species naturally concern their preferences for different hosts and resting places. By checking such medically relevant anomalies in European "*maculipennis*", African "*funestus*" and central American "*albimanus*" the existence of species complexes in the three principal subgenera of *Anopheles* was unmasked and initially elucidated through the ardours and morphological perceptiveness of, respectively, Falleroni and Van Thiel, Evans and Leeson, Rozeboom and Gabaldon, with their many peers and protégés. Once these investigators began using biological clues to guide their choice of material for morphological study, it became possible for them to sort out the real taxonomy of the populations they encountered. Terminologically, members of the first known complexes of *Anopheles* were considered as races or varieties, until their true status as species or subspecies was clarified by the combined progress of their investigation and of evolutionary thinking.

It is already possible to look back on some situations where suspected species complexes have not, in fact, been found. For instance, neither *maculatus* (Reid, Wattal & Peters, 1966; Narang et al., 1973) nor *stephensi* (Rutledge, Ward & Bickley, 1970; Coluzzi, Di Deco & Cancrini, 1973) have rewarded entomologists with the revelation of new sibling species to account for their high incidence of biological and morphological variability. These instances do seem, however, to be exceptions for such notoriously polymorphic and widespread species.

In the majority of cases where sibling anophelines have been detected, their reproductive incompatibility has been demonstrated in the laboratory (Davidson, 1976) and their polytene chromosome rearrangements have become a principal point of attention (White, Coluzzi & Zahar, 1975; Kitzmiller, 1976). Post-copulatory isolating mechanisms, rather than precopulatory factors, are the usual operatives in *Anopheles* speciation, at least in the cases so far explained. Specialized and experimental methods of analyzing sibling species have been so successful with the more amenable *Anopheles* that attention has been increasingly diverted from the proper working out of morphological contrasts, or lack of them, between these species. At the moment it is only with the freshwater-breeding members of the *gambiae* complex (to which the names *gambiae* Giles for species A, *arabiensis* Patton for species B and *quadriannulatus* Theobald for species C have been assigned by White, 1975), that we are approaching the stage of being able to say, from less than a score of studies, that reliable morphological differences cannot be found.

Morphological comparisons of *Anopheles* species: past, present and potential.

a. Adults and general.

The morphological conservatism of anopheline speciation has pushed specific descriptions of *Anopheles* to high standards of precision and perfection ever since the matter became so important to malariologists. While the structures of male terminalia, the female pharynx and some other particulars form the basis of sub-generic classification in *Anopheles*, morphological criteria for species separation depend more on the characteristic scaling of wing spots, leg markings, thoracic and abdominal vestiture and banding of the club or shaft of the sexually dimorphic maxillary palpi. Although differences between adults of the most closely related species are usually restricted to rather small contrasts of scale pattern, shape, colour or density, it is commonplace to describe the adult facies fairly fully - at least to prove that none of the salient characters has been overlooked.

To ascertain specific differences in insect morphology is traditionally a combination of art and science, relying heavily on intuitive interpretation of empirical observations. Taxonomic relationships between sibling species, however, and among any overt infraspecific forms of them, can be properly interpreted only through strictly scientific assessment of all the morphological and ancillary evidence adducible. Anopheline taxonomy suffers inescapably from the limitation that so many species differ in so few, and such small, characteristics of adult or immature morphology. In Malaya, for example, Reid (1970) estimated that about half of the *Anopheles* fauna falls into groups of very similar species; he regarded these still as 'sibling species-groups', despite his success at finding the morphological means of identifying all the species included (Reid, 1968).

An impression of the lengths to which modern mosquito morphology may run can be gained from recent redescriptions of the North American *crucians* complex by Floore, Harrison & Eldridge (1976). This complex contains only 3 species (*crucians* Wiedemann, *bradleyi* King and *georgianus* King), yet this paper on their taxonomy takes 109 pages. Incidentally, its finding call into question the identity of some specimens used for cytogenetic studies. One hastens to emphasize how much taxonomic value lies in most genetical work on *Anopheles* and their chromosomes: genetical determinations have especially facilitated morphological work on the *punctulatus* complex (Bryan, 1974), the *gambiae* complex (Coluzzi, 1964; White, 1973) and a start on the more difficult components of subgenus *Nyssorhynchus*

(Kitzmilller, 1973). In practise, research on somatic karyotypes and the banding patterns of polytene chromosomes is sometimes called micromorphology. Descriptive cytogenetical data have been published for no less than 84 species of *Anopheles*; such information is of the utmost taxonomic importance but really beyond the scope of this paper. No other soft-parts of the internal morphology of mosquitoes have been taxonomically evaluated, or are likely to be.

Some unconventional, external hard-parts of anopheline adults are characters worthy of increased taxonomic attention. For instance, several sorts of chemoreceptors and mechanoreceptors are present on the appendages, but only those on the antennae have been checked for specific differences in *Anopheles*. From one of the very few electronscan studies of any anopheline, Boo & McIver (1976) produced a detailed account of antennal receptors on *stephensi*. Similar sensilla were well described and illustrated for *atroparvus* by Ismail (1962). Variations in the numbers, distribution and forms of such sensilla are much employed for the identification of many Ceratopogonidae and Phlebotominae; their comparative study in Culicidae is required urgently. So far this has been attempted only for the *gambiae* complex, in which the numbers of sensilla basiconica and sensilla coeloconica differ significantly between some samples of certain species (Coluzzi, 1964). This meristic divergence was employed by Ismail & Hammoud (1968) to construct a partial identification key to adults of the *gambiae* complex based on counts of sensilla coeloconica. Prospects for the taxonomic use of qualitative and quantitative contrasts of sensory receptors should not be underrated as a potentially straightforward means of adult anopheline identification. To bring both sexes of a species together for successful mating there must be some specific sensory attribute: this might be manifest in the morphology of sensilla and could be the only phenotypic divergence needed in otherwise morphologically identical sibling species.

Another organ widely employed in entomological taxonomy is the spermatheca. Adult female *Anopheles* have a single, fairly large spermatheca which varies somewhat from species to species (Hara, 1959). Spermathecal diameter differs significantly, on average, between at least four members of the *gambiae* complex (Clarke, 1971; White & Muniss, 1972; Eyraud, Carnevale & Coz, 1973). Overlapping size ranges of the spermatheca in these four species render it unreliable to utilize this measurement for specific identification of individual females, even when compensated for relative size of the whole insect, except in a minority of cases at the extremes of size. Density and distribution of spermathecal gland pores, as well as the basic size and shape of the spermatheca, remain unknown specific parameters for nearly all *Anopheles*.

Spiracular morphology, being functionally adapted to microclimate, offers a further set of features likely to vary in accord with speciation. Vinogradskaya (1941, 1948) investigated thoracic and abdominal spiracles of some Russian *Anopheles* and devised a specifically characteristic prothoracic 'spiracle index' (ratio of thoracic length/spiracle length). Different indices reported for three members of the *maculipennis* complex suggest that adult spiracles may have particular value for sibling species separation.

The maxillary 'palpal index' (length ratio of palp segment 3/segments 4 + 5) is another mathematical expression by which most adults of the *gambiae* complex can be sorted into phenotypic categories of 'freshwater *gambiae*' as opposed to 'saltwater *gambiae*' (Coluzzi, 1964), with an intermediate group of 'mineralwater *gambiae*' represented by species D in Uganda (White, 1973). This novel character has been employed conveniently in the field by Gillies & Wilkes (1969) and their associates in the Gambia for practical identification of *melas* Theobald, the West African saltwater-breeding species, and its separation from either of the local freshwater-breeding members of the *gambiae* complex: *arabiensis* and *gambiae* s.s. A variety of other ratios, sensilla and more abstruse characters (c.f. Menon, 1951; E. T. M. Reid, 1957; Coluzzi, 1964) certainly differ as between species, but have not been adopted as regular taxonomic criteria.

b. Larvae and pupae

Because developmental stages of mosquitoes usually have taxonomic characters rivalling those of the adults, morphological descriptions of larvae and pupae have been extended to cover the complete chaetotaxy (i.e. all setae). Anopheline setal nomenclature has evolved from the first full systems adopted by Senevet (1930) for the pupa and by Puri (1931) for the fourth stage larva, to the present position where the conventions of Belkin (1951, 1962) are generally agreed (e.g. Reid, 1968; Harrison & Scanlon, 1975). Cast larval and pupal pelts, rather than the intact insects, though they require skill to prepare on slides, are the best chaetotaxic material because the entire setation is transparently obvious. Such skins also show taxonomically important aspects of the abdominal tergal plates and distribution of pigment in the head capsule of the larva. Christophers (1906) and Dyar & Knab (1906) independantly launched comprehensive comparative work on mosquito larval morphology. The most important taxonomic advance for *Anopheles* came when Puri (1928) correlated larval thoracic chaetotaxy with the enduring classification by Christophers (1924) of adult Anophelini. By paying attention to fine points of larval setation, pigmentation and chitinization in members of the *funestus-minimus* group of Africa and India, these workers were among the first to appreciate the real value of larval characters for separating otherwise cryptic species (Christophers & Puri, 1931; Leeson, 1937).

For the benefit of control workers over and above taxonomists, comprehensive keys to anopheline larvae have been popular since the beginning of this Century. With the first larval identification key to be published, Dyar & Knab (1906) went so far as to describe and name many sorts of larvae as new species, an undesirable act that still happens occasionally. Not until much later was comparative morphological work on pupae brought to the level of species identification as a general practise, although the earliest pupal identification key was probably that published by Wesché in 1910. Comprehensive keys to pupae, as well as larvae and adults, have been a feature of nearly all the most recent taxonomic monographs and reviews on Culicidae. Marshall (1938) and Dodge (1966) produced the only published key to first stage anopheline larvae, showing that morphological differences between species involve quite another set of characters in the first instar to those upon which identification of species is best achieved in the fourth instar. Unfortunately, the comparative morphology of younger larvae remains completely neglected for about 300 species of *Anopheles*.

By placing increased emphasis these days on the detailed morphology of pupae and fourth instar larvae, virtually all the setae and other characters of these immature stages are examined on each species in due course, so that any distinctive features are bound to become appreciated sooner or later. Failing the discovery of absolute differences between sibling species, it is now the fashion to make statistical comparisons of the amounts of branching of particular larval and pupal setae, either singly or in combination. However, it has to be admitted that, with the exception of Rohlf (1963a, b; 1964), whose work on *Aedes* received immediate rebuttal from Barr & Chapman (1964), and the study of *Psorophora* by Hendrickson & Sokal (1968), mosquito taxonomists have simply not availed themselves of the computer programmes (Cooley & Lohnes, 1962; Sneath & Sokal, 1973; Pankhurst, 1975) and advanced statistical methodology (Fisher, 1950; Simpson, Roe & Lewontin, 1960; Seal, 1964) designed for better storage, retrieval and digestion of morphometric data.

An initial, crude numerical approach to anopheline taxonomy involved summing the numbers of branches on both antepalpal hairs (seta 2) on segments IV + V of the larval abdomen for comparing members of the *maculipennis* complex. This compound character for sibling species diagnosis was first indicated by De Buck, Schoute & Swellengrebel (1930); its laborious graphical analysis by Rioux (1958) serves to demonstrate the unsophisticated statistical procedures then in vogue and still depended on by many a 'progressive' mosquito morphologist. The method of summing the branching values of sequential and homologous setae has rightly gained wide acceptance, because it tends to reduce data overlap for incompletely differentiated species (Reid, 1968). Promising setae on larvae and pupae of various groups and complexes of *Anopheles* have been treated mathematically to some extent, the furthest pursuit of such endeavours being, as one might expect, with the *gambiae* complex (Coluzzi, 1964; Chauvet & Déjardin, 1968; Zahar, Hills and Davidson, 1970; White, 1973; Reid, 1973; 1975a, b). Results are taxonomically disappointing because of much overlap in the ranges of specific values for the various meristic characters evaluated singly and in multiple character combinations. The best setational differences can only facilitate identification of larvae or pupae showing phenotypic extremes of character divergence in a mixed population. To parody this problem, we might imagine being able to distinguish specifically between only the 'most hairy' and 'least hairy' specimens of a pair of 'more hairy' and 'less hairy' species, when the majority of both species are 'moderately hairy' individuals which cannot be morphologically identified. To make matters worse, "the number of branches of nearly all the setae are clearly influenced by ecological factors such as temperature, salinity, and larval density" (Coluzzi, 1964). These effects are more pronounced for some setae than for others, at least in the *gambiae* complex. Branching of setae is also susceptible to clinal modification so that, for instance, prothoracic seta is reliable for separating most larvae of *gambiae* from *arabiensis* (i.e. species A from species B) in West Africa, but less elsewhere. Only Chauvet, Davidson & Déjardin (1969), who relied upon mesothoracic seta 1 of Malagasy *gambiae* and *arabiensis*, have employed this kind of character for practical identification of wild larvae. An inevitable shortcoming of most attempts to evaluate these meristic differences is the use of laboratory-reared samples to assay the sibling species concerned. Discriminatory chaetotactic values determined from broods or strains kept under artificial selection pressures in the laboratory clearly cannot be applied to wild-caught specimens without validation.

c. Eggs

Anopheline eggs have taxonomic importance complementing that of the females which lay them. Fortunately, as first recognized by Stephens & Christophers (1902), "the ovum of each species shows marked specific characters". Classificatory difficulties stem from the many instances of convergent and parallel adaptation in egg shape and design. Egg morphology evidently constitutes a very plastic matrix of highly adaptable characters, so that similar trends of egg modification are associated with speciation in several phylogenetic branches of anopheline evolution. In reviewing over 200 publications on anopheline eggs, Mattingly (1969) superimposed a gradogenetic egg classification onto the accepted taxonomic series in the subgenera *Anopheles* and *Cellia*. This highlights the appeal of egg characters for separation of closely related species in many lines of descent.

The main taxonomic problem with egg morphology arises from the instability of egg characters within species. The form of eggs is sometimes so labile, in response to either environmental (e.g. Deane & Causey, 1943) or genetic (e.g. Coluzzi, Cancrini & Di Deco, 1972) factors, that aberrant eggs may be mixed with normal eggs in a single batch; or in successive batches from one female; or in seasonal broods of one population (e.g. Otsuru and Ohmori, 1960). At least one description of a so-called new species (*hellenicus* Peus, 1954) is based on a few abnormal eggs obtained with a majority of typical eggs laid by a cageful of wild-caught females of a well known variable species (*superpictus* Grassi). Egg polymorphism clearly offers much scope for such taxonomic abuse.

While it is not always easy to obtain, preserve and examine their ova, few *Anopheles* species fail to be well delineated at the level of egg morphology. Since Christophers & Barraud (1931) made special efforts to survey the eggs of all Indian *Anopheles*, the number of specific egg descriptions has increased steadily, but in a sadly disorganized way, to the point where the egg-stage is now known for almost half the Anophelinae. Some faunal treatments, notably those by Lane (1953) for South America, Gillies & De Meillon (1968) for Africa and Reid (1968) for Malaya and Borneo, place great emphasis on eggs; others such as Harrison & Scanlon (1975) for Thailand, Gutsevich, Monchadskiy & Shtakelberg (1970) for the USSR and Carpenter & La Casse (1955) for North America give the eggs little or no attention. Only Hinton (1968) has tried to enlarge the taxonomic utility of anopheline eggs by describing and depicting the excellent array of features revealed by their examination under the scanning electron microscope.

Since the taxonomic mystery of the *maculipennis* complex was solved by Falleroni (1926) and those who followed his lead of employing egg characters for separation of these sibling species (Hackett & Missiroli, 1935; Guy et al., 1976), a succession of similarly elucidated anopheline groups and complexes have repeatedly revealed the early involvement of eggs in the morphological divergence of species. Definite degrees of egg differentiation have been detected between at least some of the sibling species in all of the complexes so far examined. Being visible with direct illumination under the conventional compound microscope, many of these contrasts are gross in relation to the small size of the eggs themselves. Characters usually affected are the overall egg size, deck width, float configuration and elements of the chorionic pattern. Effects of these features on physical properties of anopheline eggs have been nicely discussed by Hinton (1968). In addition to the unsurpassed case of the *maculipennis* complex,

in which all the specific egg differences are effectively absolute, both members of the *claviger* complex have distinct eggs (Coluzzi, 1962). Three kinds of eggs are found among the six members of the *gambiae* complex (Coluzzi, 1964; White, 1973), two kinds of eggs are found among the four members of the *punctulatus* complex (Bryan, 1974) and nearly all the known members of the extensive *hyrcanus* complex have distinctive eggs (*vide* Table 2, p. 39 in Harrison & Scanlon, 1975). The eggs of many other complexes remain imperfectly understood. With the members of more generally differentiated species groups, the eggs are nearly always diagnostic. When they are not, as is undeniably the case with some sibling species, egg similarities may be indicative of coadaptation to mutual oviposition sites. The presence of uniform egg morphology in certain pairs or trios of anopheline sibling species therefore suggests that the species share their breeding sites, with the corollary that speciation factors are most likely to be discerned in the biology and morphology of adults.

Concluding Discussion

Microscopic work on the morphology of small insects demands, above several other things, a great deal of psychological stamina. When such studies are on apparent sibling species, which may well prove impossible to distinguish by any absolutely clear-cut characters, the time and motivation of taxonomists is readily exhausted without reward. Statistical methods of sibling species comparison, such as the computation of discriminant functions based on carefully garnered morphological data, are liable to run up the costs in cash and patience. While it would be unscientific to neglect comparative morphology, for practical purposes it may prove necessary to take a judicious decision eventually to drop the search for diagnostic morphological characters for the identification of anopheline sibling species. This decision will be governed by at least three criteria: the importance of the complex, the facility of non-morphological identification methods (e.g. specific allozymes, chromosomes, crossing characteristics) and, what is often taken for granted, the availability and interest of at least one suitably specialized morphologist.

Regional sampling of sympatric sibling species should raise the prospects of finding morphological contrasts having at least local applicability for practical identification purposes. The possibility of selective character displacement (Brown & Wilson, 1956) between competing sibling species is exemplified by only one known case - that of *campestris* Reid as compared with *barbirostris* (see Reid, 1968, p. 128; Harrison & Scanlon, 1975, pp. 86-91). Such displacement of a particular character on any life stage of any species pair permits sympatric samples (e.g. of *campestris/barbirostris* from India, Malaya or Sri Lanka) to be more readily distinguished than allopatric or scattered samples of the species concerned. By the same token, identification criteria for sibling species may be applicable only within restricted geographical or ecological limits. The size and range of samples for morphological evaluation of sibling species requires much more careful statistical and biogeographical planning than has ever been realized.

Sibling species identification depends on minor morphological characteristics: sometimes quite literally the taxonomic splitting of hairs. To avoid misinterpretation of intraspecific polymorphism, which may be morphologically conspicuous, the formal genetics and morphological mutants of species complexes must be attended to in conjunction with work aimed at discerning differences

which delineate the species. Phenotypically analogous mutants (e.g. melanism) may well be present in several closely related species, tending to obscure their specific distinctions. Since such morphological markers serve to characterize linkage groups and chromosomes, the rising tide of interest in applied mosquito genetics (Wright & Pal, 1967; Pal & Whitten, 1974; Davidson, 1974) will sustain considerable work on detailed mosquito morphology and general phenotypic variation for the foreseeable future. This augurs well for the continuing morphological analysis of *Anopheles* species complexes.

Besides genetics, environmental influences frequently produce taxonomically confusing morphological modifications in anopheline eggs, larvae and adults. Several cases have already been mentioned. The most notorious character of this sort is the 'maxillary index' (Roubaud, 1928). For nearly three decades it was thought that the numbers of serrations on adult female maxillae reflect functional contrasts of host preference: zoophilic mosquitoes having multidentate maxillae, anthropophilic mosquitoes having paucidentate maxillae. The maxillary index was repeatedly employed to characterize anopheline 'varieties', 'races' and species as a whole. Adherence to this doctrine especially obfuscated the taxonomic truth about the *gambiae* complex (Holstein, 1954), until Campbell (1951), Gillies & Shute (1954) and Gillies (1954) debunked the concept, as applied, by demonstrating an overriding influence of the larval environment on the maxillary dentation. Perhaps we have gone too far by abandoning specific morphological studies on the external mouthparts of mosquitoes. For sandflies this approach explains a lot (Lewis, 1975).

There is an entomological obligation not to neglect the morphology of sibling species in any of their life stages, however odious such work may seem in comparison to the more dynamic activities of experimental taxonomy. Whenever they can be accomplished, morphological identifications of mosquito specimens have the supreme advantages of speed and simplicity (Zavortink, 1974). Whatever else is done on the taxonomy of anopheline sibling species, morphologists must always be asked to BOAST: Breed Out And Study Totally.

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