MAXILLARY INDEX AND BIOLOGICAL RACES IN
ANOPHELES GAMBIAE GILES

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Recently there has been an effort by entomologists in Africa to use maxillary dentition to distinguish races in *Anopheles gambiae*. Holstein's studies (1949, 1954) in the Sehelian and Sudanese savanna zones of French West Africa led him to conclude that *A. gambiae* should be divided into two races: a paucidentate anthropophilic race with a mean maxillary index of 13.5, and a multidentate zoophilic race with a mean maxillary index of 15. Campbell (1951) found that his catches of *A. gambiae* in Gambia represented two races: a paucidentate race with a mean maxillary index of 13.1 breeding in casual water with low chlorophyl content, and a multidentate race with a mean maxillary index of 15.3 breeding in permanent water containing photosynthetic microflora. Maillot (1953) reported on 600 *A. gambiae* collected near Brazzaville, French Equatorial Africa, comparing the maxillary index and the sporozoite rate, concluded that there are two races: a paucidentate anthropophilous race and a multidentate, less anthropophilous, race.

Gillies (1954), working with *A. gambiae* in Tanganyika, takes vigorous exception to Holstein's and Campbell's conclusions on two races and thinks "it would be unfortunate if the impression were to gain credence among malariologists, who have not had first hand experience with *A. gambiae*, that these conclusions are proved and beyond dispute." He found that crowded larval conditions lead to lower maxillary index; he found no difference in the maxillary indices of *gambiae* feeding on cattle and those feeding on man.

We have dissected 621 *A. gambiae* collected in the vicinity of the Liberian Institute in order to study the value of the

maxillary index as a criterion for two races, as well as to ascertain the status in this region of possible infraspecific groupings along the lines advocated by Holstein and by Campbell.

MATERIALS AND METHODS. Of the 621 *A. gambiae* reported here, 162 were from the eighth and ninth generations of the Zopablo strain of our laboratory colony, while 457 were collected in the field by use of man-baited traps, by night collecting and by hut surveys. Thirty-one of the field collected mosquitoes came from labor work camps on the Firestone rubber plantation; 271 came from five villages in Charlesville Township, Marshall Territory, all within a mile of the Institute. Finally, 149 mosquitoes were collected, using the man-baited trap and night collecting, beside a fairly large permanent swamp, with the nearest dwelling some distance away.

The colony mosquitoes dissected had fed on malarious patients during the course of other experiments; previously an unknown but substantial number of these mosquitoes had fed on rabbit blood during their wait for experimental use. As larvae they were reared in distilled water and fed on a mixture of cracker dust and dry yeast.

Ecological conditions in this region are quite different from those pertaining where Holstein and Campbell worked. Briscoe (1952) recorded an average rainfall of 146.28 inches each year for a three-year period. There is no month without some precipitation, even during the dry season. About a mile to the south of the Institute the coastal savanna begins. From here north the country was originally rain forest but now is under sporadic cultivation and is mostly covered with low second growth bush, except the Firestone rubber plantation which is thickly planted with Brazilian *Hevea*. The region

includes several rivers, many creeks and smaller streams and a great deal of swampy land. Casual water is available virtually throughout the year. The nearest brackish and salt water is at least two and a half miles to the south and represents the nearest approach of *A. gambiae melas*, which previous study through egg identification demonstrated was absent from all the collecting sites used. A few goats, dogs and pigs are found in the villages; there is a sparse population of water deer in the bush and some small mammals. There are no cattle or antelope.

To count the maxillary teeth we found a method much simpler than that described by Holstein (1954). The heads were placed in a drop of lactophenol under a binocular dissecting microscope and the mouth parts pressed with the side of a bent needle placed at the proximal end of the proboscis. The pressure causes the elements in the proboscis to separate. The procedure is effective equally with fresh material or with specimens preserved in alcohol, although in the latter case it was found that the mouth parts sometimes disassociated spontaneously while in storage. The mouth parts then were severed from the head, which was discarded, and a cover slip placed over them. The maxillae were located and the teeth counted at about 660 diameters with a compound microscope using 15 x oculars and a 44 x objective; it was found unnecessary to employ immersion objectives.

Our data are grouped into three aggregates: those collected in and around dwellings, both at the Charlesville sites (279 mosquitoes) and on the rubber plantation (31 mosquitoes); those collected over the permanent swamp (149 mosquitoes); those from the laboratory colony (162 mosquitoes).

RESULTS. Table 1. summarizes the data for the collection as a whole and for each of the three aggregates defined above. The mean maxillary index for each aggregate was calculated by the formula $M = \frac{\Sigma (fx)}{N}$. Since the means vary only by 0.2 (Table 1), there is no statistical indi-

TABLE 1.—Frequency distribution of maxillary indices

Maxillary index [1] (A)	Total Collection (B)	Coll. in and near Dwellings (C)	Human bait near swamp (D)	From lab. Colony (E)
11	1	1	0	0
11.5	5	3	2	0
12	6	4	2	0
12.5	19	11	6	2
13	49	25	15	9
13.5	58	27	12	19
14	101	59	18	24
14.5	96	49	25	22
15	101	46	24	31
15.5	72	30	23	19
16	56	25	13	18
16.5	37	19	4	14
17	13	6	3	4
17.5	5	3	2	0
18	1	1	0	0
18.5	0	0	0	0
19	0	0	0	0
19.5	1	1	0	0
Totals	621	310	149	162
Means	14.6	14.6	14.6	14.8

[1] Column A, maxillary index, the sum of the teeth on both maxillae divided by two.

cation that we are dealing with unlike populations.

Histograms, Figures 1 to 4, illustrate the frequency distributions of the respective aggregates as listed in Table 1. From inspection all these except Figure 4, the colony mosquitoes, indicate a distribution fairly close to that of the normal curve and fall into the pattern specified by Quatelet's law. By way of verification, the standard deviation (σ) for each group was computed and the dispersion calculated, with the results shown in Table 2. According to Simpson and Roe (1939), dispersion limits for the normal curve are 68 percent of sample within one σ of the mean, 95.5 percent within 2σ and 99.76 percent within 3σ. The total range should be 6σ, $(M+3\sigma)$ to $(M-3\sigma)$. As will be noted from column B of Table 2, the total collection (Fig. 1) conforms unusually well to the statistical definition for the normal curve. For both the human habitation mosquitoes (Fig. 2) and the swamp mosquitoes (Fig. 3) it will be noted that the 93 percent of specimens

Table 2.—Percentage dispersion of data

	N	σ	$(M\pm\sigma)$	$(M\pm2\sigma)$	$(M\pm3\sigma)$
Total collection	621	1.1	68	94.4	99.8
From habitations	310	1.2	68	93	99
From swamp	149	1.2	68	93	99
From colony	162	1.2	82	100	....

lying within the 2σ range of M is slightly lower than expected for a perfectly normal curve, but this small departure does not seem significant—particularly in view of Simpson and Roe's warning (1939, p. 129) to zoologists that no real distribution ever actually fits exactly any purely mathematical concept.

It is interesting to note that the dispersion in the colony mosquitoes (Fig. 4) shows definite grouping within a range of 4σ. This is to be expected since these specimens were reared from inbred stock under artificial conditions.

All our aggregates are multidentate, with the means of the maxillary indices close to the defined mean of 15 to 15.3 but distant from the paucidentate mean of 13.1 to 13.5.

Discussion. According to the view held by Campbell (1951), these multidentate *gambiae* ought to have developed as larvae in permanent water. One of our aggregates was indeed collected over a permanent swamp and conforms to Campbell's thesis. The breeding water of the field collected mosquitoes is not known, of course, but since both casual and permanent waters are available in the vicinity, this group probably is mixed in that respect, although the histogram of maxillary indices (Fig. 2) does not differ appreciably from the maxillary indices of the swamp group (Fig. 3). The colony mosquitoes developed in distilled water under conditions closer to those of casual water than of permanent water, but identical with neither. Nevertheless the distribution of their maxillary indices (Fig. 4) conforms closely to the other aggregates.

According to Holstein's theory (1949, 1954), our multidentate *gambiae* ought to be zoophilic. But both the field collected aggregates—those from the permanent swamp and those from dwellings—were collected at human bait, hence may be presumed to be anthropophilic, or at least satisfied to feed on human blood. The mosquitoes from the colony all were known to have fed on humans, but many of them previously had taken a meal of rabbit blood. In view of the lack of large herds either of game or of domestic animals in this part of Africa it appears quite unlikely that any race of *gambiae* having a hereditary zoophily could maintain itself. One would expect, then, that the maxillary indices of the local *gambiae* would be paucidentate. Since the opposite is true, it may not be amiss to reexamine the two-race theory.

A pivotal question arises at once: just what did Campbell and Holstein each mean by the word *race*? Was the word used in its specifically correct sense to designate an unnamable infraspecific population of less value than the subspecies, thus more or less synonymously with the word *strain* as frequently used in microbiology and in animal colony work? Or was the word used to designate the principal infraspecific taxon, hence in the sense synonymous with *subspecies*? Although this second usage is no longer considered to be correct, it persists in some quarters and is usual among culicidologists. Campbell (1951) mentions several times his two races as though he thought they were categorically equal to *A. gambiae melas*, a subspecies, although he refers to *melas* as a *variety*. This is another word devoid of systematic precision. In his discussion headed "Systematic position of the populations," Holstein (1954, pp. 142–143) strongly implies, without actually making his position clear, that his races are namable subspecies. Edwards (1954) has called attention to the misuse
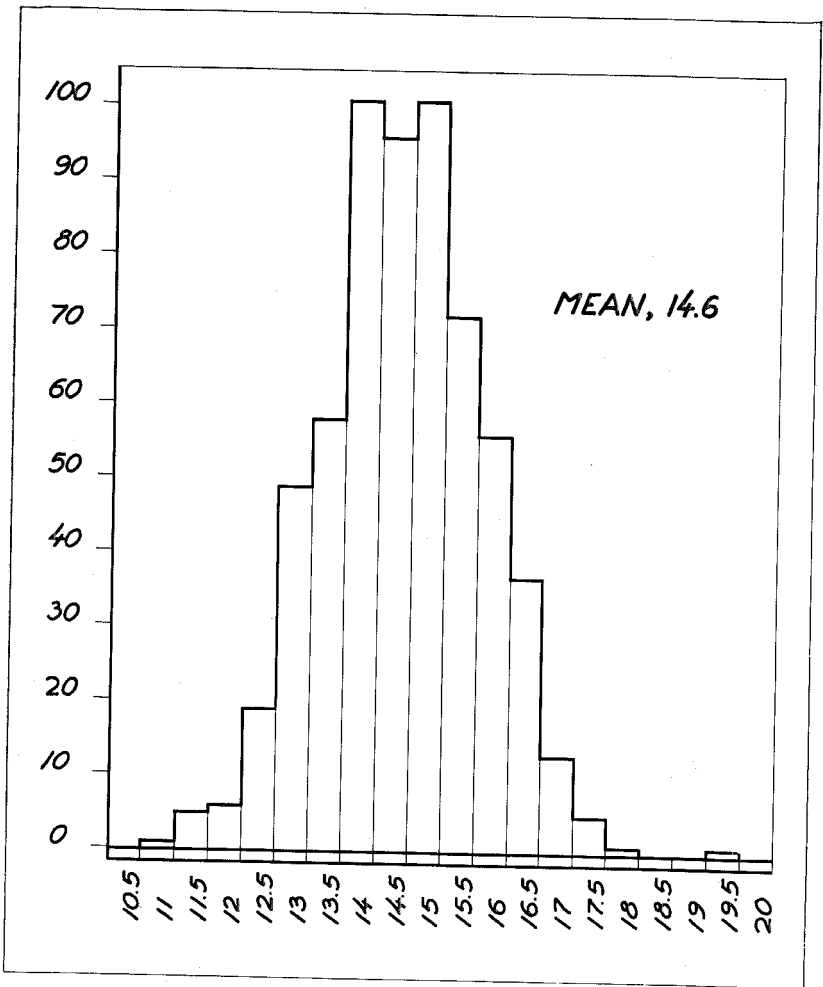
FIG. 1. Frequency distribution of maxillary indices of total collection.

of terms such as *race* and *variety* and to the abuse of statistical methods in defining infraspecific categories. Fox (1955) has emphasized the importance of clarifying the biological concepts underlying infraspecific systematics. There is almost universal agreement that the naming of physiologic populations and of populations recognizable only statistically leads to useless confusion.

That Holstein refrains from naming his races is beside the point; we are interested in his biological thinking. Certainly both Campbell and Holstein felt that they were dealing with two major fractions of *gambiae*, distinguished by feeding tropisms, by preferences in larval water and by maxillary index.

The hitch in each case is that the two races were sympatric. In the light of modern research on speciation and evolution (the literature is enormous, but Dobzhansky (1951), Fisher (1930) and Mayr (1942) are standard references), it is evi-
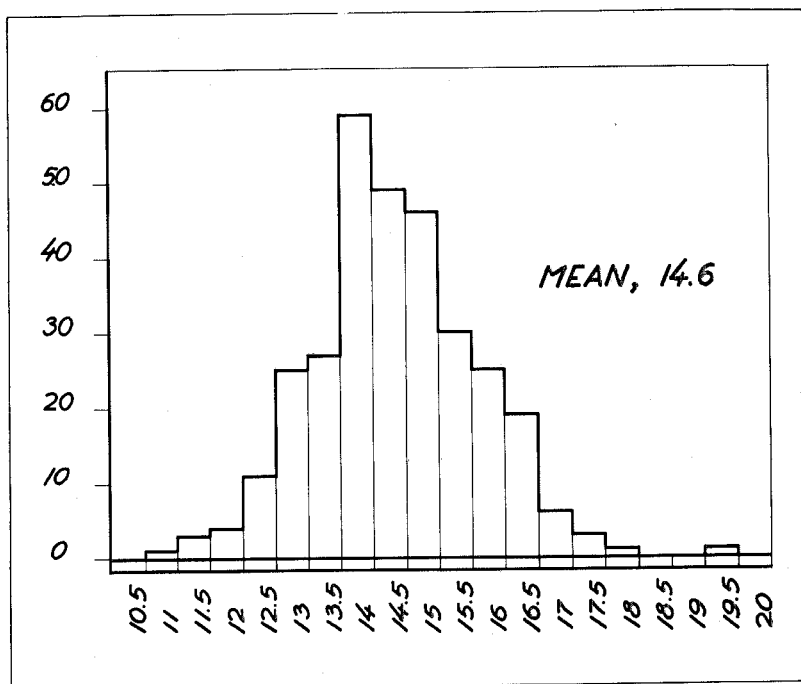
FIG. 2. Frequency distribution of maxillary indices of mosquitoes collected in and near human habitations.
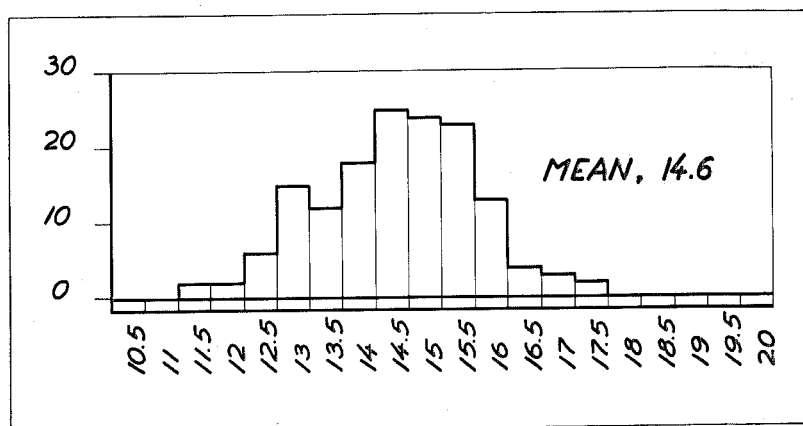

FIG. 3. Frequency distribution of maxillary indices of mosquitoes collected with human bait over a permanent swamp.
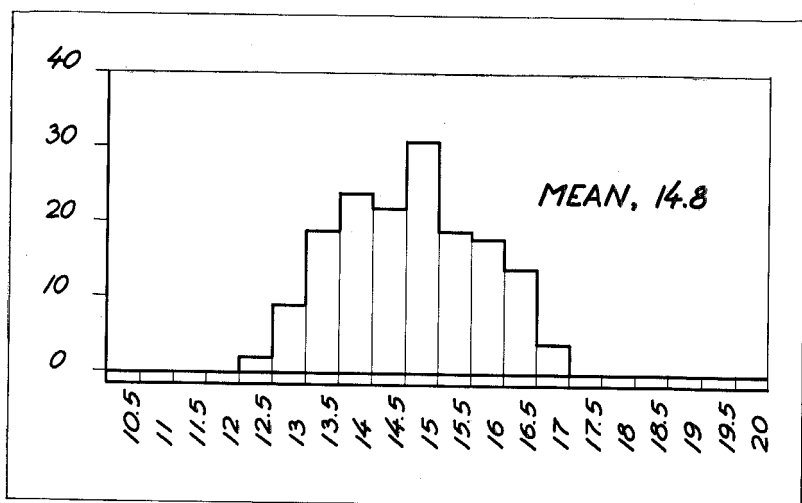
FIG. 4. Frequency distribution of maxillary indices of mosquitoes from laboratory colony.

dent that two genetically distinct populations cannot preserve their separate identities without sexual isolation. Between the different populations of a species, sexual isolation when it exists, is almost invariably the result of geographic isolation and results in the formation of subspecies. But two such populations made sympatric will inevitably undergo gene exchange and their distinctiveness will soon be obliterated. Since neither Campbell nor Holstein demonstrated sexual isolation between their races, it is evident that the explanation for the "significant differences" found in their data must be sought elsewhere than in ascribing them to firm biological populations.

When dealing with living things, statistical results must be interpreted with caution. In analyzing his samples, Campbell calls attention to the fact that his total frequency distribution shows a platykurtic curve, leading him to conclude that two populations were involved. That this conclusion is not necessarily sound is underlined by Simpson and Roe (1939, p. 202) who point out that some homogeneous distributions are platykurtic and merely reflect a high variability within a single natural population. They further call attention to the law of ecological incompatibility—which has nothing to do with mathematics but which is sound zoology: "really separable taxonomic groups of animals such that all their variates have extensive overlapping distributions are seldom found in nature" (1939, p. 200). According to Campbell's data, 75 percent of all his specimens, those with 12.5 to 15.5 teeth, would fall into the netherland of overlapping range. Holstein, too, ignores the law of ecologic incompatibility in establishing two races. The overlap of ranges for his paucidentate and multidentate races is between the maxillary indices of 12 and 15.5. According to his table xxiv (1954, p. 129), 92.1 percent of those mosquitoes listed in the paucidentate column and 70.2 percent of those listed as multidentate fall into this dubious category. Only 7.9 percent of his paucidentates are unquestionably so, and only 29.8 percent of his multidentates cannot be suspected of being incorrectly listed. These very extensive overlaps suggest that the differences between the two populations in each case are both minor and unimportant.

Holstein reports that his populations 1 to 9, chiefly bush captures, have higher maxillary indices, a lower proportion of positive precipitation reactions with human

antiserum and a high proportion of positive reactions with the antisera of other animals; his populations 10 to 19 show the reverse trend and were mainly collected in and around human habitations (1954, pp. 131, 133). Since his populations 1 to 9 have mean maxillary indices ranging from 14.7 to 15.5, whereas his populations 10 to 19 have mean maxillary indices ranging from 12.0 to 13.8, he not unnaturally concludes that there is a correlation—which there is, statistically, perhaps. But the highest paucidentate mean differs from the lowest multidentate mean only by 0.9 teeth, and the futility of defining the vast majority of individuals as either type has already been noted.

Gillies (1954) indicates that crowded conditions during larval life rather than the nature of the breeding water, influences maxillary index. He records lowering the maxillary index of a strain from 15.7 to 13.9 by rearing the larvae under crowded conditions. Further, he found no difference in the maxillary indices of individuals feeding on cattle and those feeding on man.

An alternative explanation for Holstein's 19 populations may be that populations 1 to 9, collected for the most part in sparsely populated areas, passed their larval instars in uncrowded waters, but that populations 10 to 19, collected in and near dwellings, passed their larval instars in waters crowded by the offspring of the better and more regularly fed females frequenting villages and towns.

In *Anopheles gambiae* we have, as might be expected of a common and widely distributed animal, a highly adaptable species with a well developed ability to survive under diverse conditions. The female feeds on mammalian blood; no doubt the choice between animal and man is determined by chance availability. Certainly our own experience with *gambiae* in our laboratory colony, where the insects feed both on human blood and on rabbit blood, whichever is offered, tends to weaken concepts of obligatory feeding tropisms. It is our impression that when *gambiae* females need a blood meal, the first available source—man or animal—will serve.

Nor can we find that a case has been made for an inherited preference for ovipositing in casual versus permanent water in *gambiae,* a concept necessarily embodied in Campbell's theory. When the eggs mature in the female, they must be laid; there is nothing to suggest that the first available water will not be utilized for the purpose. Certainly our colony mosquitoes cooperatively and prolifically deposit their eggs on moist filter paper, suggesting that if there is a tropism in this respect, it is readily overcome by necessity.

To dismiss as abnormal and not significant the behavior of mosquitoes in a colony is a sophistry all too tempting to the field investigator. If a behavior pattern or a tropism is indeed inherent in the animal, it should be manifested in the colony animal as well as under wild conditions.

Campbell defines permanent water as having an established photosynthetic microflora; our swamp collections were made over such a place. These show (Fig. 3) a mean maxillary index of 14.6 with a range of 11.5 to 17.5, not seriously deviating from Campbell's findings. On the other hand, our colony specimens, reared in sterile water, showed (Fig. 4) a slightly higher maxillary index of 14.8 and the narrower range of 12.5 to 17.0. In view of Gillies' findings (1954), this almost certainly indicates that both groups of mosquitoes developed as larvae without being crowded. This is known to be true of the colony, where we are careful not to crowd the larvae and constantly make every effort to afford conditions optimum for development. If indeed there were a true relationship between maxillary index and the photosynthetic microflora as such in the larval environment as Campbell thought, one would expect the colony mosquitoes to exhibit paucidentatism in these eighth and ninth generations developed in water from which microflora has been rigidly excluded. On the contrary, the maxillary index of this group turns out to be, if anything, slightly higher than that of the group collected

from the swamp. Perhaps the presence of the photosynthetic microflora may be taken as an ecological indicator of natural water likely to favor uncrowded conditions for mosquito larvae, rather than as a factor with direct influence on the number of maxillary teeth.

Summary and Conclusions. The maxillary indices of 621 *Anopheles gambiae* Giles collected in Marshall Territory, Liberia, were studied. The collection was divided into three aggregates according to ecological backgrounds: (1) those collected in and near human habitations, (2) those collected over a permanent swamp, (3) those from the laboratory colony.

The mean maxillary index of the total collection was 14.6 with a range from 11 to 19.5. Analysis of the data showed that dispersion of the frequencies lay almost exactly within the limits of a normal curve, hence indicated a pure population. The mean maxillary index for each aggregate was determined. The swamp mosquitoes and those collected at dwellings both showed a mean maxillary index identical with the total collection. The colony aggregate showed a mean maxillary index of 14.8, or a variance of 0.2 teeth. These findings are contrary to expectancy under the two race theories advanced by Campbell and by Holstein, leading us to review the works concerned.

A basic fallacy in these theories lies in the fact that the paucidentate and the multidentate races of Campbell and of Holstein were, in each case, sympatric, precluding the possibility of their maintaining genetic distinctiveness unless sexual isolation exists. Since sexual isolation has not been demonstrated, it is theoretically unlikely that these are races. Both authors appear to have dealt with homogeneous but variable populations. A platykurtic distributional curve does not necessarily indicate the existence of two biologic populations. Use of maxillary index to define the paucidentate and multidentate populations as races (subspecies?) violates the law of ecological incompatibility in that the overlapping range of maxillary indices is much too large. As is to be expected of a widely distributed, adaptable insect, *gambiae* females evidently incline to deposit eggs in the first available water found after the eggs mature and will take blood meals from the most convenient donor—man or animal—with preference being shown for man when a choice is available.

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