The Siphonal Index

I. A Method for Evaluating *Culex pipiens* Subspecies and Intermediates

William G. Brogdon
Division of Parasitic Diseases, Center for Infectious Diseases,
Centers for Disease Control, Public Health Services,
U. S. Department of Health and Human Services, Atlanta, Georgia 30333

ABSTRACT. The siphonal index and the parameters used in its determination were compared for late fourth instar larvae of *Culex pipiens* subspecies reference strains, field-collected intermediates, and laboratory hybrids. Groups of larvae from egg rafts of *Culex pipiens pipiens* (CPP) and *Culex pipiens quinquefasciatus* (CPQ) could be distinguished from each other by measurement of length or width of their siphons as well as by the ratio of length to width (the siphonal index). Groups of larvae from egg rafts of intermediates similar to *Cx. p. pipiens* (CPP*) differed primarily in siphon width from those similar to *Cx. p. quinquefasciatus* (CPQ*); intermediates differed primarily in siphon length from the subspecies each resembled. Laboratory hybrids (F_1) obtained from crossing the two subspecies were indistinguishable from the CPQ* intermediate under the rearing conditions used. Siphon width was positively correlated with siphon length, and the slopes of the regressions for these correlations were similar for all subsets of the *Culex pipiens* complex.

INTRODUCTION

This work does not address the taxonomic status of the *Culex pipiens* complex, an issue which provokes disagreement among specialists. The work of Sirivanakara in Southeast Asia (1976), Miles in Australia (1976), and Jupp in South Africa (1978) led Knight (1978) to list *Culex pipiens* and *Culex quinquefasciatus* as separate species. Barr (1957), McMillen (1958), Jakob et al. (1979), and Brogdon (1981) provided evidence that the *Culex pipiens* complex is represented throughout North America by the subspecies *Culex pipiens pipiens* (in the North) and *Culex pipiens quinquefasciatus* (in the South), which occur as intermediates between 36° N and 39° N latitudes.

Within the complex of *Culex* species are important vectors of St. Louis encephalitis (SLE) in the Mississippi and Ohio River valleys. The dynamics of the vector population must be studied to allow fuller understanding of SLE transmission for disease control purposes. Unfortunately, certain *Culex* species (such as *Culex pipiens* and *Culex restuans*) are difficult to distinguish from each other as field-collected adults, and *Culex pipiens* subspecies and intermediates cannot be determined in the field in the adult stage.

The author (Brogdon 1981) previously described a method for distinguishing egg rafts of the *Culex pipiens* complex as to subspecies by measuring the siphonal index on groups of sibling late fourth instar larvae reared from these

rafts. In addition, intermediates collected in a Memphis, Tennessee, study area seemed to be of two types resembling, respectively, *Cx. p. pipiens* and *Cx. p. quinquefasciatus*. As an extension of that study, the present paper reports a comparative analysis of siphonal lengths, widths and indices determined on sibling larvae for the following: *Cx. p. pipiens* (CPP), *Cx. p. quinquefasciatus* (CPQ), field collected intermediates resembling *pipiens* (CPP*), field-collected intermediates resembling *quinquefasciatus* (CPQ*) and hybrids from laboratory crosses of subspecies reference strains (CPH).

MATERIALS AND METHODS

Field strains of CPQ were obtained from Houston, Texas, New Orleans, Louisiana, and Port-au-Prince, Haiti. Field strains of CPP were obtained from Fort Collins, Colorado, Dayton, Ohio, and Chicago, Illinois. CPQ* and CPP* were from individually reared egg rafts collected in Memphis, Tennessee, throughout the 1979 and 1980 breeding season. CPH were obtained from reciprocal crosses of CPP (Chicago strain) and CPQ (New Orleans strain). Pupae were isolated in individual emergence chambers, and egg rafts from matings were individually reared. Late fourth instar larvae were collected in 25-30 larvae/egg raft lots, dehydrated in methyl cellusolve and mounted in balsam.

Siphonal length, width, and index were determined as previously described (Brogdon 1981). Briefly, a binocular microscope equipped with a net micrometer allowed measurement of the length and width of siphons from late fourth instar larvae. Length was measured from the siphon base to the midpoint of the siphon tip, since tips were seldom parallel to the baseline. Siphonal width was measured at the widest point, which was usually in the area of greatest curvature about midway along the siphon in CPQ and nearer the base in CPP. The index equals siphon length in units divided by siphon width in units. The net micrometer used here measured units of 0.07 mm, so that lengths and widths in units should be multiplied by 0.07 for measurements in millimeters. Data for egg rafts from CPP (Colorado, Ohio, Illinois) or CPQ (Texas, Louisiana, Haiti) were pooled for purposes of comparison.

RESULTS

The CPP and CPQ subspecies were significantly different[1] in siphon length, while the intermediates CPP* and CPQ* were virtually identical (Fig. 1). The laboratory hybrid CPH showed a length distribution similar to that of the Memphis intermediates.

---

[1]Significant difference is defined for the purposes of this work as P±95% in a two-tailed t test.

The analysis of siphon widths showed that subspecies CPP and the intermediate CPP* are similar and are significantly different from CPQ and CPQ*, which likewise resemble each other (Fig. 2). The hybrid CPH is similar to CPQ and CPQ*. Siphonal length and width were positively correlated (Table 1), and the slopes of the regression lines for each subset of the *Cx. pipiens* complex were similar.

The ratios (siphonal indices) of each type of intermediate were significantly different from each other and from both subspecies (Fig. 3). The hybrid was not significantly different from the CPQ* intermediate. Particularly interesting is the symmetry of the frequency distributions for the intermediate strains. Each is skewed to the same degree away from the subspecies it resembles and toward the other intermediate.

DISCUSSION

The data show that CPP and CPQ egg rafts can be distinguished from each other retrospectively through measurements of larval siphonal length or width, but that more complete characterization of the subspecies may be accomplished using the siphonal index. The two types of intermediate strain egg rafts may be discriminated through analysis of siphon width of their larvae. Either intermediate may be distinguished from CPP or CPQ using siphon length or the index, but not siphon width.

It is interesting that the hybrids from reciprocal crosses of the two subspecies more closely resembled the CPQ* intermediate. The fact that hybrids were reared under conditions more favorable to CPQ (32°C,†90%RH) would be consistent with the hypothesis that climatic factors produce the distribution of intermediate types observed in Memphis. Hayes and co-workers (Hayes 1975; Hayes and Hsi 1975; Hayes and Downs 1980) have shown the fundamental importance of temperature in all stages of CPQ life history.

Jakob et al. (1979; 1980a and b), interpreted their DV/D ratio data to mean that a large self-sustaining, intermediate population with considerable gene flow occurs in Memphis. The work described herein is consistent with this hypothesis with the further observations that two intermediate types may be distinguished and that CPP and CPQ probably do not occur in Memphis (or did not in 1979 and 1980).

The unanswered questions about these intermediates involve the vector ability of CPP* as contrasted to CPQ*. Does the CPQ* intermediate show the human feeding preference of CPQ? Do the intermediates serve as a superior bridge for virus circulating in the bird population to spill over into the human one? Certainly, further research on these issues is needed.

The aim of this work is to facilitate the identification of field samples of members of the *Cx. pipiens* complex so that potential disease vector populations can be recognized more readily.

ACKNOWLEDGMENTS

The efforts of Jake Mullenix of the Memphis/Shelby County Health Department in collecting and mailing egg rafts were essential to this work. Special thanks go to Jake and to Bill Kelly who headed vector control and surveillance activities. Thanks also go to Lauren Sheeler who measured many siphons in the course of this study.

LITERATURE CITED

Barr, A. R. 1957. The distribution of *Culex p. pipiens* and *Culex p. quinquefasciatus* in North America. Am. J. Trop. Med. Hyg. 6:153-165.

Brogdon, W. G. 1981. Use of the siphonal index to separate *Culex pipiens* subspecies and hybrids. Mosq. Syst. 13:129-137.

Hayes, J. 1975. Seasonal changes in population structure of *Culex pipiens quinquefasciatus* Say (Diptera: Culicidae): study of an isolated population. J. Med. Entomol. 12:167-178.

Hayes, J. and B. P. Hsi. 1975. Interrelationships between selected meteorologic phenomena and immature stages of *Culex pipiens quinquefasciatus* Say: study of an isolated population. J. Med. Entomol. 12:299-308.

Hayes, J. and T. D. Downs. 1980. Seasonal changes in an isolated population of *Culex pipiens quinquefasciatus* (Diptera: Culicidae): a time series analysis. J. Med. Entomol. 17:63-69.

Jakob, W. L. S. A. Daggers, D. B. Francy, J. Mullenix, and K. Moseley. 1979. The *Culex pipiens* complex in Memphis, Tennessee. Mosq. Syst. 11:179-185.

Jakob, W. L., D. B. Francy, J. Mullenix, and S. A. Taylor. 1980a. Further studies on the *Culex pipiens* complex in Memphis, Tennessee. Mosq. Syst. 12:371-376.

Jakob, W. L., D. B. Francy, and S. A. Taylor. 1980b. Studies of male offspring from overwintering *Culex pipiens* complex mosquitoes. Mosq. News 40:524-526.

Jupp, P. G. 1978. *Culex (Culex) pipiens pipiens* L. and *Culex (Culex) pipiens quinquefasciatus* Say in South Africa: morphological and reproductive evidence in favour of their status as two species. Mosq. Syst. 10:461-473.

Knight, K. L. 1978. Supplement to a catalog of the mosquitoes of the world. Thomas Say Foundation Suppl. to Vol. VI.

McMillan, H. L. 1958. Study of a naturally occurring population intermediate between *Culex p. pipiens* and *Cx. p. quinquefasciatus*. Am. J. Trop. Med. Hyg. 7:505-571.

Miles, S. J. 1976. Taxonomic significance of assortive mating in a mixed field population of *Culex pipiens asutralicus*, *Cx. p. quinquefasciatus*, and *Cx. globocoxitus*. Syst. Ent. 1:263-270.

Sirivanakarn, S. 1976. Medical entomology studies. III. A revision of the subgenus *Culex* in the Oriental Region (Diptera: Culicidae). Cont. Am. Entomol. Inst. (Ann Arbor) 12(2).

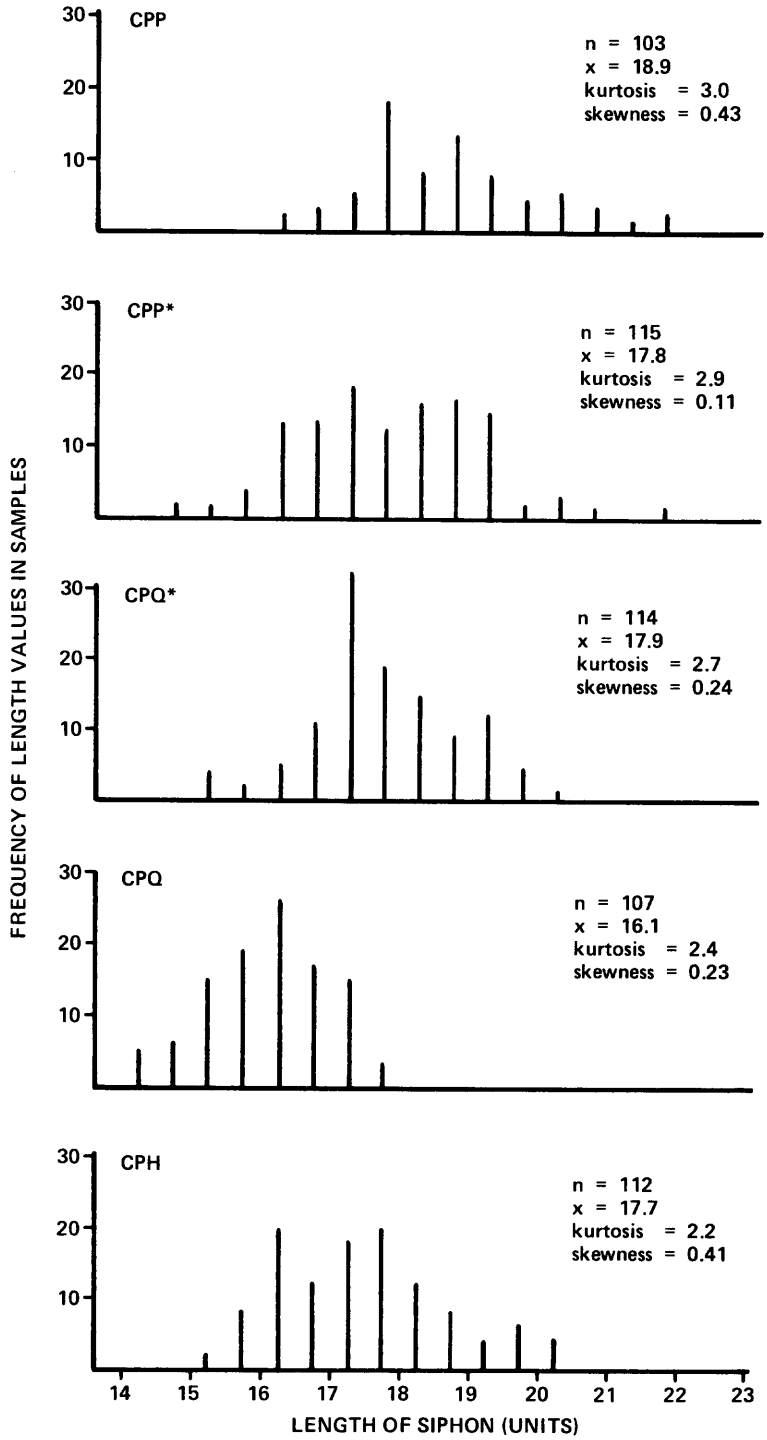

Fig. 1. Frequency distributions with means and moments for siphon lengths of *Cx. p. pipiens* (CPP), *Cx. p. quinquefasciatus* (CPQ, intermediates resembling *Cx. p. pipiens* (CPP*) and *Cx. p. quinquefasciatus* (CPQ*), and hybrids (CPH) of subspecies CPP and CPQ.

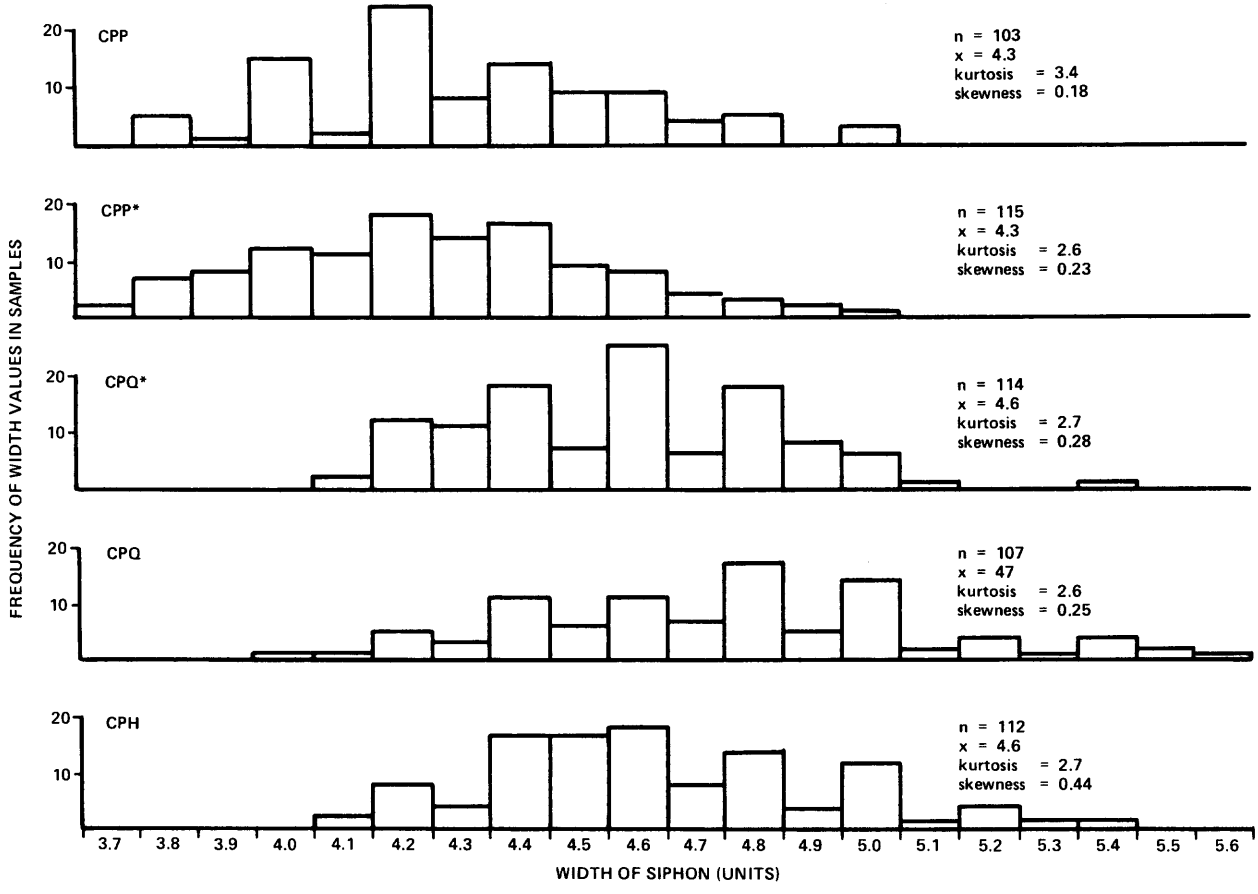

Fig. 2. Frequency distributions with means and moments for siphon widths of *Cx. p. pipiens* (CPP), *Cx. p. quinquefasciatus* (CPQ), intermediates resembling *Cx. p. pipiens* (CPP*) and *Cx. p. quinquefasciatus* (CPQ*), and hybrids (CPH) of subspecies CPP and CPQ.

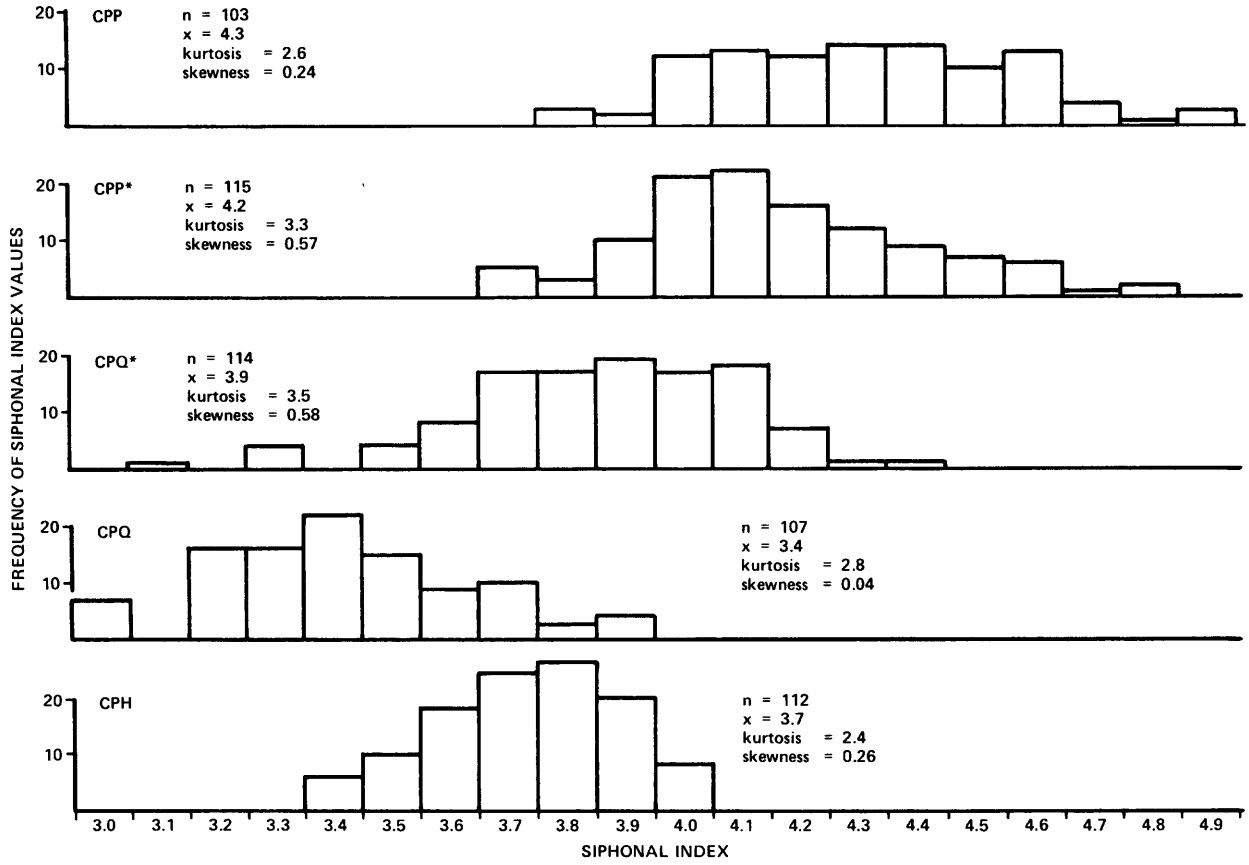

Fig. 3. Frequency distributions with means and moments for siphonal indices (index = length in units divided by width in units) of *Cx. p. pipiens* (CPP), *Cx. p. quinquefasciatus* (CPQ), and intermediates resembling *Cx. p. pipiens* (CPP*) and *Cx. p. quinquefasciatus* (CPQ*), and hybrids (CPH) of subspecies CPP and CPQ 10.

SUBSET OF COMPLEX	n	LENGTH WIDTH	y INTERCEPT	SLOPE	CORRELATION
CPP	103	19.0 ∓ 1.7 4.3 ∓ 0.3	1.6	0.14	0.73
CPP*	115	17.7 ∓ 1.6 4.2 ∓ 0.3	2.0	0.13	0.74
CPQ*	114	18.0 ∓ 1.3 4.6 ∓ 0.2	2.2	0.13	0.82
CPQ	107	16.0 ∓ 1.1 4.7 ∓ 0.2	2.1	0.16	0.77
CPH	112	17.7 ∓ 1.4 4.7 ∓ 0.3	1.3	0.19	0.87

Table 1. Regression data for siphon lengths and widths of *Cx. p. pipiens* (CPP), *Cx. p. quinquefasciatus* (CPQ), intermediates resembling *Cx. p. pipiens* (CPP*) and *Cx. p. quinquefasciatus* (CPQ*), and hybrids (CPH) of subspecies CPP and CPQ.