Use of the Siphonal Index to Separate *Culex pipiens* Subspecies and Hybrids

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ABSTRACT. Siphonal indices of *Culex pipiens* complex field strains were compared. Collection locales included those where only *Culex pipiens pipiens* or *Cx. p. quinquefasciatus* occur and a study area (Memphis, Tennessee) within the zone where intermediates occur between these subspecies. It proved possible to identify single egg rafts as *Cx. p. pipiens*, *Cx. p. quinquefasciatus* or one of two types of intermediates. The method of precisely measuring siphonal index is described and uses of the method are discussed along with questions that need to be answered about the *Culex pipiens* complex.

It is generally recognized that *Culex pipiens pipiens* and *Cx. p. quinquefasciatus* occur in the United States as subspecies with a zone of intermediate forms observed between approximately 36° N and 39° N latitudes (Barr 1957; Jakob et al. 1980a, b). Studies of the *Culex pipiens* complex elsewhere in the world (Sirivasakarn in Southeast Asia 1976; Miles in Australia 1976; Jupp in South Africa 1976) have led Knight (1978) to list *Cx. pipiens* L. and *Cx. quinquefasciatus* Say as separate species. The importance of the *Cx. pipiens* species complex in arbovirus diseases makes clarification of taxonomic relationships essential.

As part of a larger study of St. Louis encephalitis (SLE) vectors in Memphis, Tennessee, extensive egg raft collections were made during the 1979 and 1980 breeding seasons. One goal of the study was the biochemical and morphological discrimination of *Cx. pipiens* subspecies and intermediates, with an ultimate goal being the development of simple field methods for analysis of populations.

Morphological characters have long been used to attack the problem of *Cx. pipiens* systematics, with the DV/D ratio (male genitalia) showing some usefulness (Barr 1957; McMillan 1958; Jakob et al. 1980a, b). However, the process of preparing and reading DV/D ratios is time intensive and considerable experience is required to overcome subjectivity in measurement. Therefore, other morphological characters were evaluated.

Although McMillan (1958) discounted the usefulness of the siphonal index in determining *Cx. pipiens* subspecies, this technique has been reevaluated with certain modifications. Reported herein are the results of the comparison of pure *Cx. pipiens pipiens* and *Cx. p. quinquefasciatus* field strains and intermediates from Memphis, Tennessee, using siphonal index measurements from late fourth instar larvae.

MATERIALS AND METHODS

Field strains of *Culex pipiens quinquefasciatus* were obtained from Houston, Texas; New Orleans, Louisiana and Port-au-Prince, Haiti. Field strains of *Cx. p. pipiens* were obtained from Ft. Collins, Colorado; Dayton, Ohio and Chicago, Illinois. Intermediates were collected throughout the 1979 Memphis, Tennessee breeding season from individually reared egg rafts. Late fourth instar larvae were collected in 25-30 larvae/egg raft lots, dehydrated in methyl cellusolve and mounted in balsam.

Precise measurement of siphonal indices requires consistent orientation of siphons with the micrometer. A net micrometer is essential for siphonal index measurements. The most useful net micrometer (100 10 mm squares within the field) has at least the median rows running in either direction further divided into 2 mm fifths. Use of a net micrometer allows one to orient the base and the dorso-anterior surface of the siphon along perpendicular lines of the micrometer. Length is measured from the siphon base to the midpoint of the siphon tip, since the entire surface of the tip end is seldom parallel to the siphon baseline. Siphonal width is measured at the widest point, which is usually in the area of greatest curvature about midway along the siphon in *Cx. p. quinquefasciatus* and nearer the base in *Cx. p. pipiens*. Since the dorso-anterior surface may be strongly recurved from the base of the siphon in some specimens (particularly *Cx. p. quinquefasciatus*), it is essential to align the dorso-anterior limit of the siphon base and the widest point of the siphon along the same micrometer line to obtain consistent measurements. Division of the length in units by the units of width yields the siphonal index. For statistical purposes, a single egg raft is characterized either by the mean and standard deviation of siphonal indices from 25-30 larvae from that egg raft or by a 95% probability ellipse representing the lengths and widths of the same individual siphons. The probability ellipses are derived from programs written for a Hewlett Packard 9845B desk top computer.

RESULTS

In Figure 1 are represented the means and standard deviations for siphonal indices of the mosquito strains studied. The data for pure *Cx. p. pipiens* and *Cx. p. quinquefasciatus* are derived from single egg rafts of three widely separated strains for each subspecies. The data from Memphis, Tennessee, is derived from multiple egg rafts and represents many more individuals. These egg rafts were collected at approximately two week intervals throughout the 1979 breeding season.

It is clear from these data that *Cx. p. pipiens* and *Cx. p. quinquefasciatus* egg rafts may be easily distinguished from each other. Overlap of individual siphonal index values may occur, but even small field larval samples would suffice for subspecies determinations where only the pure subspecies occur.

Intermediates pose a more difficult problem. Individuals within the same intermediate egg raft may be found with indices which fall well within the range

of either subspecies. However, the data from Memphis shows that the mean and standard deviation of intermediate egg raft indices falls neatly between the means and standard deviations of the subspecies indices. Moreover, the early and late season (breakpoint is August 6) intermediate material was of two discrete types in 1979. The early season material more closely resembles *Cx. p. pipiens* and that from late season collections resembles *Cx. p. quinquefasciatus*.

These data may also be expressed in the form of probability ellipses representing length and width measurements of individual siphons. In Figure 2 is shown the 95% probability ellipse (including 95% probability, ellipse midpoint) for all individuals of the purely *Cx. p. pipiens* and *Cx. p. quinquefasciatus* strains. Ellipses in Figure 3 represent the two subspecies plotted separately on the same graph. Note the orientation of the long axes of these ellipses along the index ratio 4.0 line drawn at a 45° angle to the ordinate and abscissa. The contrast of these ellipses with that (in Figure 1) representing the total *Culex pipiens* populations is further evidence that the siphonal index reflects a true distinctiveness of the two subspecies populations. Finally, in Figure 4 are shown ellipses for typical early and late season *Culex pipiens* intermediate populations from Memphis. Comparison of these with the subspecies ellipses shows that two types of intermediates may be distinguished: early season ones more similar to *Cx. p. pipiens* and late season ones more similar to *Cx. p. quinquefasciatus*.

DISCUSSION

The roles of intermediates and subspecies of the *Culex pipiens* in transmission of St. Louis encephalitis (SLE) are imperfectly understood. Since virus transmission ability, man-biting preference, vertical stratification, response to temperature and light, and seasonal occurrence of the *Culex pipiens* subspecies may be quite different, determination of these characteristics in intermediates becomes essential. This is especially important in view of the shift in character of intermediate populations during a single breeding season. The siphonal index shows promise as the needed method for field differentiation of species components. Moreover, siphonal indices may be read rapidly and consistently by unskilled workers with minimal training.

The findings of this study in Memphis, Tennessee, a zone of intermediate *Culex pipiens* populations, are consistent with those of other workers in similar areas. McMillan (1958) in Kansas, Rosay and Nielsen (1973) in Utah and Jakob et al (1980a, b) in Memphis have observed the late season replacement of *Cx. p. pipiens*-like by *Cx. p. quinquefasciatus*-like populations. Whether this change in population characteristics represents the oft postulated northward incursion on *quinquefasciatus* or is due to more subtle factors is addressed in a paper in preparation by this worker.

Given that certain segments of the *Culex* complex (due to man-biting preference, stratification or whatever other reason) might be more important in SLE transmission to humans, use of the siphonal index might provide further information. For example, might periods of increased public health risk from

SLE be recognized by seasonal shifts in the characteristics of vector populations? The dynamics of population shifts might be followed using the siphonal index, especially where an adequate egg raft surveillance scheme already exists. Indeed, an important component of the SLE vector study in Memphis, Tennessee, is thorough egg raft surveillance throughout the season. A major question to be answered is whether seasonal shifts in population (*Cx. p. pipiens*-like to *Cx. p. quinquefasciatus*-like) differ in time and extent between breeding seasons and, more importantly, between epidemic and non-epidemic years.

ACKNOWLEDGMENTS

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REFERENCES 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.

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

Jakob, W. L., D. B. Francy and S. A. Taylor. 1980b. Studies of male offspring from overwintering *Culex pipiens* complex mosquitoes. Mosquito 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. Mosquito Systematics 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 *C. 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 australicus, C. p. quinquefasciatus* and *C. globocoxitus*. Syst. Ent. 1:263-270.

Rosay, B. and L. T. Nielsen. 1973. The *Culex pipiens* complex in Utah. Proc. 26th Ann. Mtg. Utah Mosq. Abatement Assoc., pp. 28-31.

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

Figure 1. Means and standard deviations for 25 individual siphons from single egg rafts from three geographic locations for *Cx. p. pipiens* (bracket P, n = 75) and *Cx. p. quinquefasciatus* (bracket Q, n = 75); intermediate individuals from the 1979 Memphis breeding season (bracket MI, n = 2025 from 81 egg rafts); intermediate individuals from June 6-August 6, 1979 Memphis collections (bracket E, n = 1075 from 43 egg rafts); intermediate individuals from August 30-October 30, 1979 Memphis collections (bracket L, n = 950 from 38 egg rafts).

Figure 2. Probability ellipse (95%) for lengths and widths of *Culex* complex individual siphons (n = 2175) used in this study. The smaller ellipse is the 95% probability ellipse for the midpoint of the larger ellipse.

Figure 3. Probability ellipses (95%) for lengths and widths of *Cx. p. pipiens* (ellipse P, n = 75) and *Cx. p. quinquefasciatus* ellipse Q, n = 75) siphons.

Figure 4. Probability ellipses (95%) for lengths and widths of Memphis intermediate populations of *Culex pipiens* early (lower ellipse, n = 1075) and late (upper ellipse, n = 950) in the 1979 breeding season.

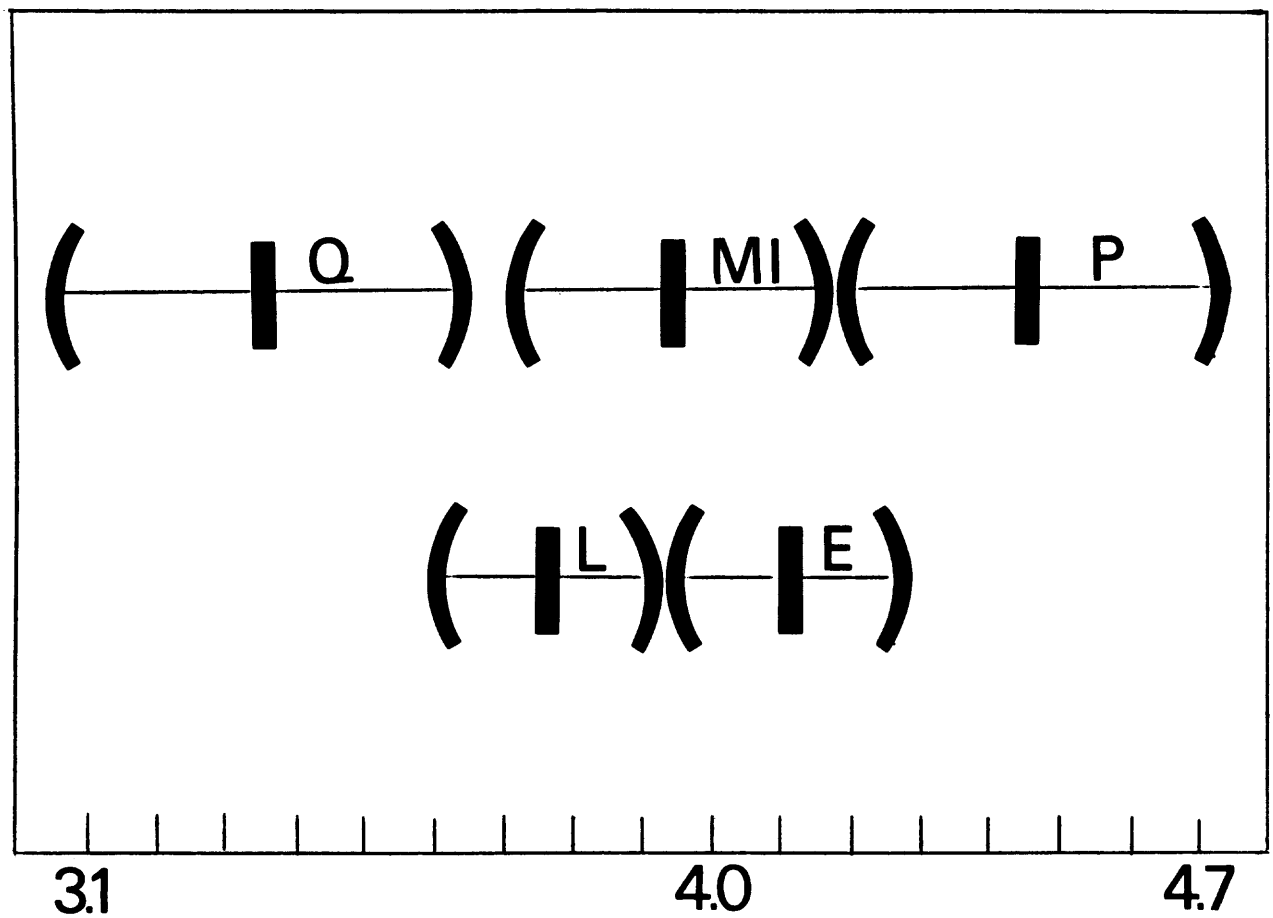
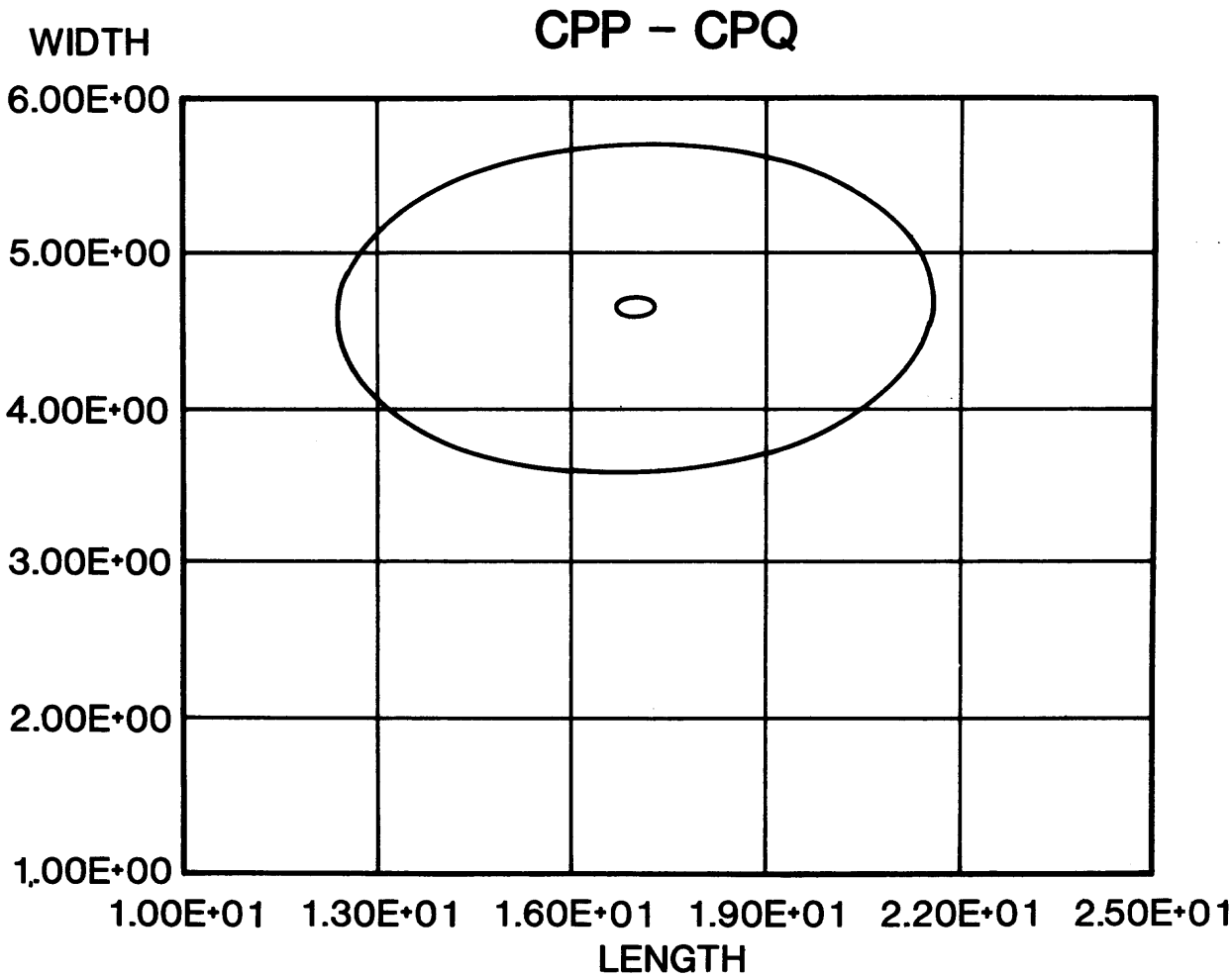


Figure 1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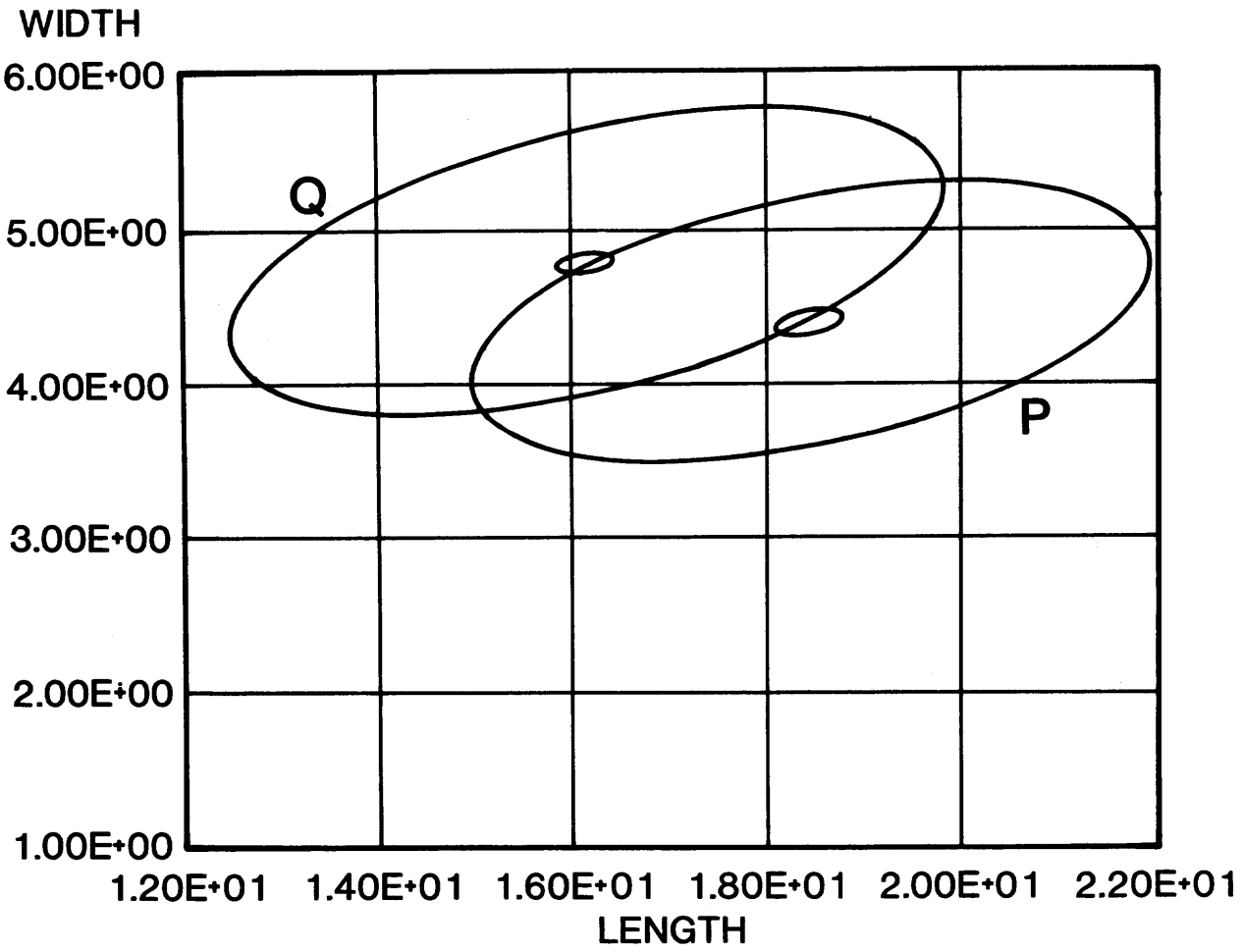
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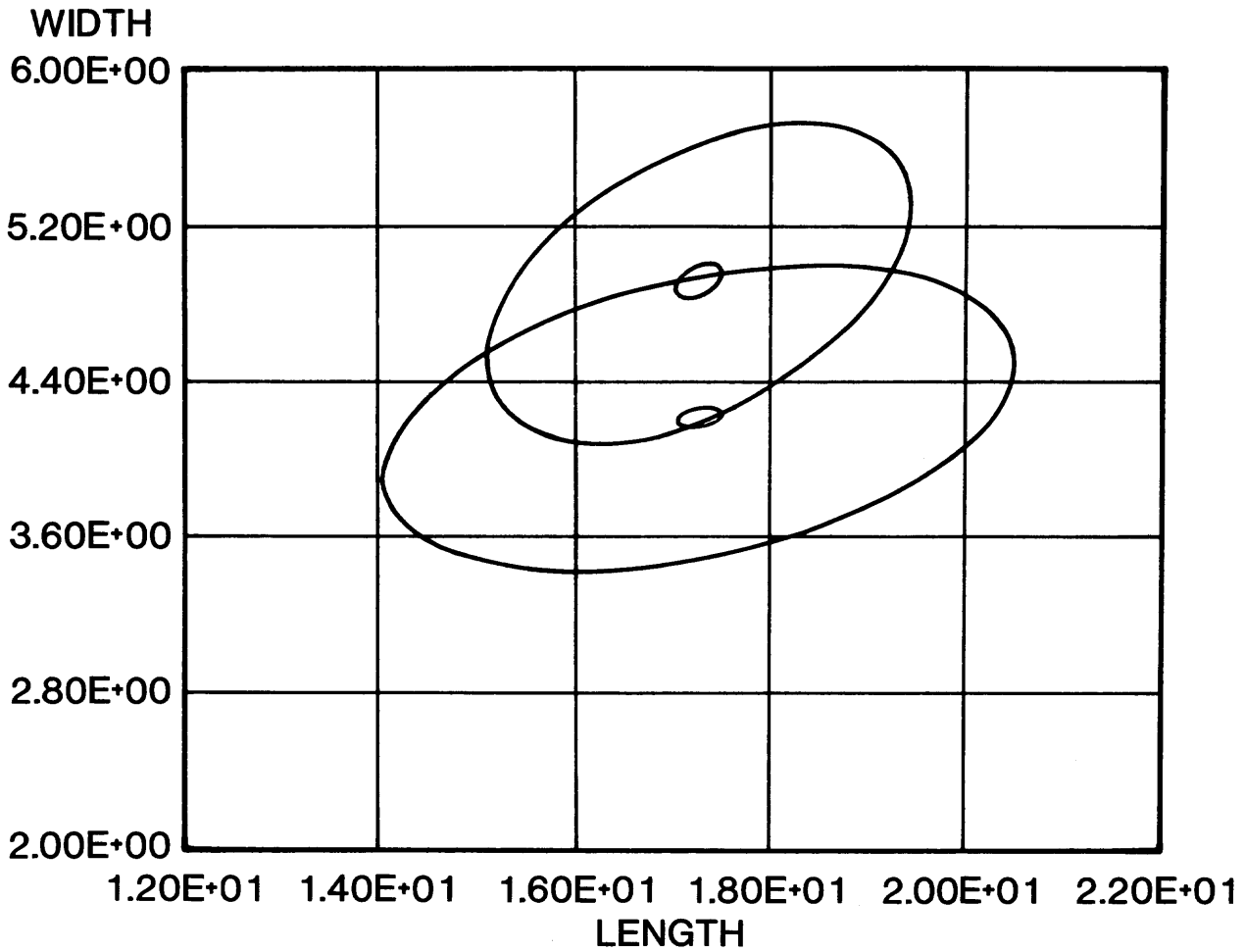
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