Equal-frequency Tolerance Ellipses for Population Studies of *Belonolaimus longicaudatus*

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Abstract: A biometrical method, using x-y plots of measurements of normally-distributed bivariate characters to construct a 95% equal-frequency ellipse representing 95% of the specimens within its boundary, is presented. Comparisons of ellipses of four populations of *Belonolaimus longicaudatus* Rau show mean stylet lengths are relatively stable compared to mean tail lengths and there is greater stylet length variability in short stylet forms. The extent of variability and regression between the populations can be seen by superimposing the bivariate means and orienting the longitudinal axes of the ellipses. To compare ellipses the 95% binomial distribution is used to determine whether a sample population is significantly different from the model. The method is useful for graphic representation of morphological relationships within a nematode population, its relationship to other populations or species and to estimate environmental, ecological and genetic effects upon population morphology. *Key Words:* Sting Nematode, Population Comparisons, Biometrics, Relationships, Equal-frequency tolerance ellipses, *Belonolamius longicaudatus.*

Many ecotypes of the plant-parasitic nematode, *Belonolaimus longicaudatus*, Rau have been collected. A number of morphologically variant populations of *B. longicaudatus* have been described (10). These nematodes have a wide host range. For example, since 1961, we have successfully transferred populations from sweet corn (*Zea mays* L.) to slash pine seedlings (*Pinus elliotii* Engelm.), and from sea oats (*Uniola paniculata* L.) to sweet corn and soybean (*Glycine max* (L.) Merr.).

Bird and Mai (1) reviewed the literature on nematode variability and reported on variations of morphological characters between and within 22 populations of *Trichodorus christiei*. They found host species, host variety, host physiology, and geographic region influenced variability of some characters. Stylet length was least variable but numerical affinities between intraspecific groups and within each population were demonstrated.

Defrise-Gussenhaven (4) used equal-frequency tolerance ellipses to study the morphological relationships of several human and beetle populations. She stated that a population can be characterized by two measurements represented in a scatter diagram. Since the two measurements probably have a normal simultaneous distribution, the population could be represented by an ellipse which graphically outlines the range of variation of the chosen bivariate characters. Ellipses also have been used by Jolicoeur (7) to study morphological variations in the wolf (*Canis lupus* L.). Rau and Fassuliotis reported using 95% equal-frequency tolerance ellipses and regression coefficients to show relationships of *B. longicaudatus* and *B. maritimus*, Rau, in different environments (12).

The purpose of this paper is to demonstrate the method of equal-frequency tolerance ellipses in the morphological characterization of selected populations of *B. longicaudatus*. Known independently normally-distributed pairs of characters (tail length and stylet length) were selected to demonstrate the method.

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MATERIALS AND METHODS

In this paper, we define 'population' as a local population of potentially interbreeding individuals (8), consisting of 50 or more specimens from a specific locality and host plant. Population numbers were assigned as samples were obtained; subsequent samples from the same source were given the same population numbers, followed by a lower case letter, *e.g.,* 1a.

The sample nematode populations were obtained from the following three geographic locations:

Population No.	*Location*	*Host Plant*	*Year Collected*
1a	Sanford, Florida (*B. longicaudatus*, type locality)	corn	1958
12	Tifton, Georgia	corn	1960
12a	Tifton, Georgia	corn	1964
59	Grand Island, Louisiana	corn	1962

Nematodes were extracted with a modified Baermann funnel technique (2), fixed in TAF (3) at least 2 weeks, dehydrated to glycerine and mounted by Baker's rapid method (6). Stylet and tail lengths were measured at 900 × with an ocular micrometer.

CONSTRUCTION OF THE EQUAL-FREQUENCY ELLIPSE: Development and construction of the frequency ellipse was modified from the procedure given in Documenta Giegy (5). Although an ellipse can be constructed for any population percent (except 100%), we have confined our presentation to the construction of 95% ellipses, which by definition is expected to enclose within its boundaries, 95% of the individuals from a sample population. The ellipse representing stylet length (x) and tail length (y) of population 1a is given in Fig. 1.

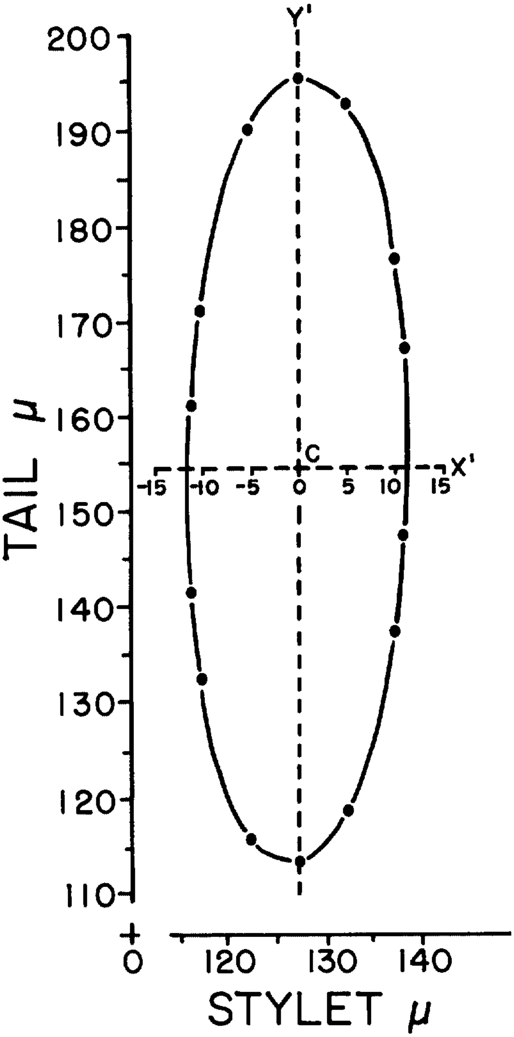


FIG. 1. Location of limiting points for constructing the 95% equal-frequency ellipse for *Belonolaimus longicaudatus* population 1a.

The general mathematical procedure for constructing the 95% tolerance ellipse is as follows: (i) the means of each measured character ($\bar{x} = 127.16$, $\bar{y} = 154.38$); (ii) the corrected sums of the squares of each measured character ($Sx^2 = 2{,}778.4$, $Sy^2 = 37{,}136.5$); (iii) the regression coefficients ($b_{y.x} = 0.26454$, $b_{x.y} = 0.01979$); (iv) the standard error of the regression coefficients

($s_{b_{y.x}} = 0.315$; $s_{b_{x.y}} = 0.02357$); (v) the sample size (n = 136); and (vi) the 5% F value for 2 and (n − 2) degrees of freedom (F = 3.065).

The equations for calculating the frequency ellipse are as follows:

$$\text{(i)}\quad \hat{Y} = \bar{y} \pm b_{y.x}(X - \bar{x}) \pm s_{b_{y.x}} \sqrt{2(n+1)(F)(Sx^2)/n - (n-2)(X-\bar{x})^2}\ (5).$$

Using this equation, it is possible to calculate as many points on the ellipse as necessary from sample estimates of the parameter and various values of X in the equation. For each value of X, two points on the ellipse will be determined. These points become the upper (+) and lower (−) points on the ellipse.

If $X = \bar{x}$ is used in the equation, the term $b_{y.x}(X - \bar{x})$ and the last term under the radical sign both become zero, and the points on the ellipses are equi-distant above and below the mean for Y. However, these points do not necessarily represent minimum and maximum $\hat{Y}$ values on the ellipse.

To determine the minimum and maximum values of X on the ellipse the following formulas were developed. These values occur when the quantity under the radical sign in equation (i) becomes zero. Using the methods of calculus, it may be shown that this quantity becomes zero when the $\hat{X}$ is estimated as follows:

$$\text{(ii)}\quad \hat{X} = \bar{x} \pm \sqrt{2(n+1)(F)(Sx^2)/(n)(n-2)}.$$

Substituting the statistics of population 1a, we calculated the limits to be:

$$\text{(iii)}\quad \hat{X} = 127.2 \pm \sqrt{2(137)(3.065)(2{,}778.4)/(136 \times 134)} = 127.2 \pm 11.3.$$

Although 127.2 ± 11.3 are the minimum and maximum values for X, these do not necessarily occur at the mean value for Y. To obtain the values of X for any value of Y, the following equation must be used:

$$\text{(iv)}\quad \hat{X} = \bar{x} + b_{x.y}(Y - \bar{y} \pm s_{b_{x.y}} \sqrt{2(n+1)(F)(Sy^2)/n - (n-2)(Y-\bar{y})^2}.$$

Inspection of this equation reveals that the $b_{x.y}$ term and the last term under the radical sign becomes zero when $Y = \bar{y}$. We can therefore calculate the point at which the ellipse crosses the line $Y = \bar{y}$ from the following calculation:

$$\text{(v)}\quad \hat{X} = 127.2 \pm .02357 \sqrt{2(137)(3.065)(37{,}136.5)/136} = 127.2 \pm 11.3.$$

Points on the ellipse were calculated for the upper (+) and lower (−) limits by using equation (i). These points were then plotted and connected to form the ellipse shown in Fig. 1 which is expected to contain 95% of the specimens of the sample population.

Binomial Distribution: The 95% binomial distribution tables were used in conjunction with the 95% ellipses to determine the probability that a given population is significantly different from the model population at the 95% confidence level for the two measured characters.

The bivariate distribution of the mean of the given population was derived by plotting the bivariate measurements on the model ellipse, and then calculating the number (%) of individuals falling within the model. Individuals falling outside the 95% ellipse of the model were considered variants. The significance of population variation was then determined from appropriate binomial tables under the 95% column.

RESULTS AND DISCUSSION

Equal-frequency ellipses are effective in outlining the range of variation and degree of correlation of bivariate characters of a given random population. Graphically, the char-

TABLE 1. Required data for construction and comparison of 95% frequency ellipses for stylet and tail length measures of four populations of *Belonolaimus longicaudatus.*

		Stylet (X)		Tail (Y)	
Population number	n	Mean ($\bar{x}$)	Mean square of deviations from the regression $s_{x.y}^2$	Mean ($\bar{y}$)	Mean square of deviations from the regression $s_{y.x}^2$
1a	136	127.16	20.63	154.38	275.69
12	73	127.05	22.59	143.08	161.73
12a	166	126.98	25.94	137.14	155.50
59	135	119.83	34.24	132.36	151.18

Population number	$b_{x.y}$	$s_{b_{x.y}}$	$b_{y.x}$	$s_{b_{y.x}}$
1a	.01979	.02357	.26454	.3150
12	.05934	.04379	.42477	.3135
12a	.14643	.02978	.87767	.1785
59	–.03824	.04113	–.17109	.1816

acters appear as elliptical clusters of points around the bivariate mean. When these points are enclosed within a 95% frequency ellipse, the population is represented in a concise, integrated manner for the two measured characters.

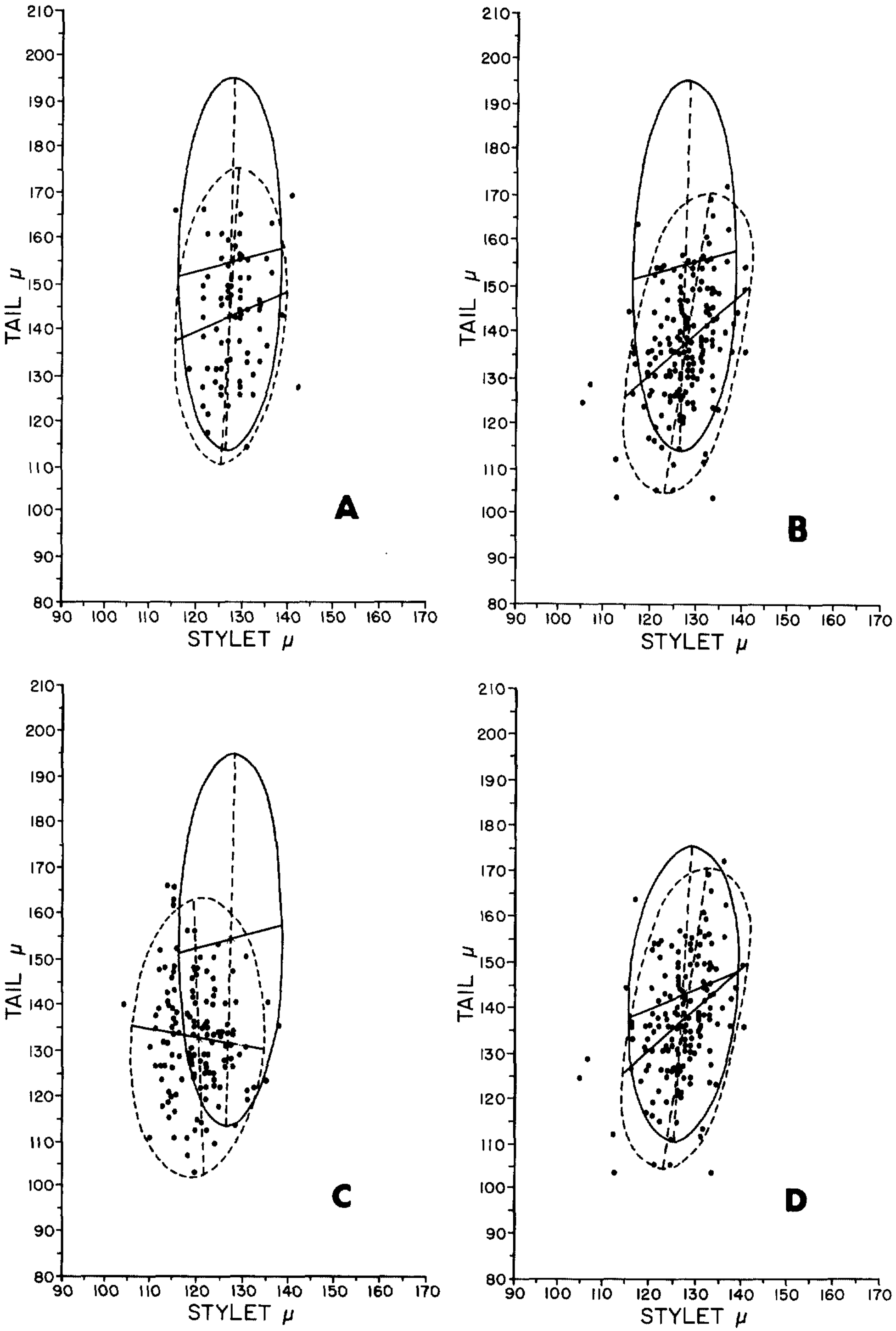
Table 1 presents the sample statistics for the bivariate characters needed to construct 95% frequency ellipses for each of the populations. The larger variability in the tail measurements is indicative of growth. Sclerotized structures, such as the stylet, are formed in the newly moulted adult nematode but do not grow with the rest of the body after the last moult. These differences in variability are reflected in the shape of the ellipses (Figs. 1, 2, and 3). The near vertical orientation of the ellipses indicates small regression and low correlation of these characters. The visual relationships of the sample populations with the model are evident in the overlap and extent of symmetry between them (Figs. 2A–D). Those individuals falling outside of the model ellipse (Population 1a), although identified as *B. longicaudatus,* are considered members of variant or divergent populations.

The relationships of the populations to the models were also determined with the binomial distribution shown in Table 2. Accordingly, Tifton population 12 was judged similar to model 1a, since 68 out of 73 individuals fell within the model with a confidence interval of 97.7 (Table 2 and Fig. 2A). Population 12a, sampled four years later, was found to be variant from 1a for the two characters (Table 2 and Fig. 2B). Grand Island population 59 was also considered a variant population, with only 77 out of 135 specimens falling within model 1a (Table 2 and Fig. 2C).

Tifton populations 12 and 12a were found to be similar (Table 2 and Fig. 2D).

The ellipse also shows the extent of variation from the mean of the bivariate characters both within and between populations. The F value for two and n – 2 degrees of freedom controls the dimensions of the ellipse.

In numerous other studies we have found that bivariate measurements of more than 50 individuals from a population did not alter the limits appreciably. Ellipses constructed from stylet and tail lengths of over 150 pop-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170

TABLE 2. Comparison of *Belonolaimus longicaudatus* populations based upon numbers of bivariate character measurements falling within model equal frequency tolerance ellipses.

Population	No. of specimens in sample	No. of specimens in 95% ellipse	% within ellipse	No. of specimens within model ellipse	% within model limits	Confidence limits of % within model limits
1a (Model)	136	129	94.9	–	–	–
12	73	70	95.9	68	93.2	84.7–97.7
12a	166	157	94.6	139	83.7	77.7–89.3*[a]
59	135	127	94.1	77	57.0	47.3–66.1*
12 (Model)	73	70	95.9	–	–	–
12a	166	157	94.6	152	91.6	86.5–95.4

[a] * *Differs significantly from model populations.*

ulations of *B. longicaudatus* tended to overlap the model ellipse 1a. Based on the amount of overlap two forms, probably falling within the categories of either polytypic or ecotypic origin (8), were readily recognized when populations from the same location were grouped together and ellipses were constructed which represented the bivariate measurements of the specimens and the bivariate means of the populations.

Twenty-three populations of *B. maritimus* Rau, were readily separated from *B. longicaudatus*. The distinctiveness of these species through the use of the ellipses supported the ecological evidence presented in part by Rau (11). Similar ellipses were used to determine whether the variability and mean lengths of stylet and tail of different *B. longicaudatus* populations were affected by conditions of either environmental or genetic origin. The stylet and tail mean lengths remained constant in three variant populations that were raised for three to seven months under both field and greenhouse conditions (13). Figures 2D and 3D show that the bivariate mean values, and the degree of overlap and variability of two Tifton field populations taken four years apart remain constant. These results indicate that the variability and measurements of bivariate characters are controlled by genetic and not environmental factors.

In addition we have been able to separate larvae of *Meloidogyne incognita acrita* Chitwood from *M. javanica* (Treub) Chitwood by ellipses formed from the bivariate measurements of the length of the head to median bulb and the total length. We have found ellipses useful also in showing the extent of variability in measurements of larvae hatched from single egg masses and the effect of resistant and susceptible tomatoes and snapbeans on the variability and measurements of larvae of *M. incognita acrita* over short and long periods of time. In addition, mixed populations of *Pratylenchus zeae* Graham and *P. brachyurus* (Godfrey) Filipjev and Schuur. Stekh. were readily separated using the length of head to vulva or head to anus and total length. The observations cited above will be presented in more detail in future reports.

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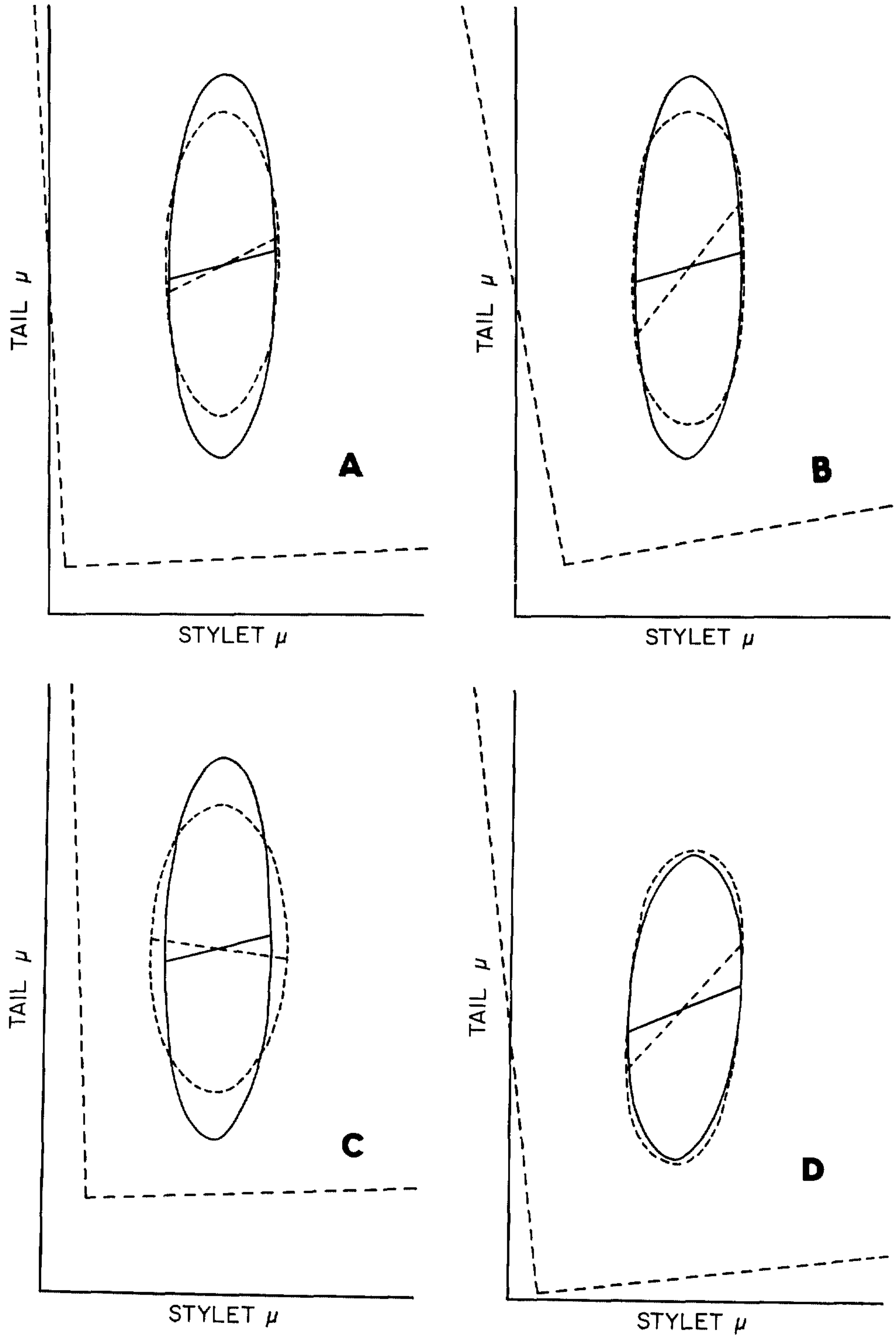
FIG. 2. Scatter diagrams of stylet and tail lengths of sample populations enclosed by a 95% equal-frequency ellipse (broken lines) and compared with the model ellipse of *Belonolaimus longicaudatus* population 1a: **A.** Population 12; **B.** Population 12a; **C.** Population 59. **D.** Comparison of the 95% ellipses of population 12 (model) and population 12a (broken lines) collected at Tifton, Georgia four years apart.


TAIL μ
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Probably 95% frequency ellipses are most valuable because the extent of variability between a model and a sample population is clearly shown. By superimposing the bivariate means and orienting the longitudinal axes of the 95% ellipses, the variability is readily seen between either the bivariate characters or the populations (Fig. 3A–D).

The tail and stylet lengths in populations 12 and 12a (Table 1 and Fig. 3D) are almost similar, indicating these characters had not changed over a period of four years. The stylet lengths are relatively stable, and their variability is approximately the same. Even though the stylet of population 59 is shorter than the model population 1a (Table 1 and Fig. 2C) the variability is greater (Fig. 3C). Increased variability was also found in several short stylet populations of *B. longicaudatus* found on sea oats which grow exclusively on white sand in the Seabrook Beach area of South Carolina. This suggests that the short stylet form might be an incipient species (8).

The 95% frequency ellipse construction is useful in general survey work to demonstrate relationships between bivariate characters of widely-distributed populations. For local populations a model can be constructed for comparison with neighboring populations. It is desirable to have a supply of the model ellipses on hand with coordinates divided into convenient intervals, so that measurements of specimens of other populations of the same or closely related species can be plotted around the outline of the model ellipse.

By using 95% ellipses, 95% distribution tables and the demonstrated method of superimposing ellipses for nematode measurements, it may also be possible to demonstrate open and closed populations (8), polymorphic tendencies, geographic variability, and the morphological shifts produced by ecological factors or chemical treatment of soils.

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FIG. 3. Superimposed bivariate means and orientation of longitudinal axes of the 95% equal-frequency ellipses to compare the variability of the stylet and tail lengths of sample populations (broken lines) with the model ellipse of *Belonolaimus longicaudatus* populations 1a: **A.** Population 12 (Tifton, Georgia); **B.** Population 12a (Tifton, Georgia); **C.** Population 59 (Grand Island, Louisiana). **D.** Superimposed 95% ellipses of populations 12 (model) and 12a (broken lines) collected at Tifton, Georgia four years apart.

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