Analysis of Frequency Distribution of Adults and Larvae of *Choristoneura fumiferana* (Clem.) and *C. pinus* Free. (Lepidoptera: Tortricidae)[1]

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A rapid means of identifying species of insects on the basis of physical measurements is useful to the field worker. The efficiency of the postclypeal index in separating the larvae of two closely allied species is illustrated herein. Occasionally species fall into overlapping subgroups, making it difficult to determine the characteristics of a given subgroup. A method useful in identifying such subgroups is outlined herein.

Use of Postclypeal Index

The width of the postclypeus and the length of the median dorsal line from the anterior margin of the postclypeus to the termination of the adfrontal sutures (to be called length) were measured for 68 last-instar specimens of *Choristoneura pinus* Free, and 51 last-instar specimens of *Choristoneura fumiferana* (Clem.). These measurements were used in the form of the postclypeal index, L/W, as a rapid means of separating the two species of larvae.

To determine the efficiency of this index the variance and the correlation of the widths and the lengths were determined for each specis. Variance, range, mean, and fiducial limits of the mean were determined for the postclypeal index of each species. The following statistics were obtained:—

	C. pinus	*C. fumiferana*
Variance of width	.1366	.0697
Variance of length	.2963	.1490
Variance of index	.0020	.0016
Correlation coefficient	.9279	.8802
Range of the index	1.49—1.70	1.37—1.52
Mean of the index	1.59	1.47
Fiducial limits of index mean	±.014	±.015

The variance is low for both length and width and of the same order for both species. The correlation coefficient is high in both cases, indicating a close relationship between length and width. The fiducial limits for the index means do not overlap and Student's t test showed the means to be significantly different at the one per cent level.

Six specimens, or 8.8 per cent of the sample of *C. pinus*, and 11 specimens, or 21.5 per cent of the sample of *C. fumiferana*, lay within the range overlap. An overlap as high as 20 per cent indicates that the postclypeal index is not an accurate means of differentiating between the species. However, the index may be sufficiently accurate for general purposes and those larvae occurring within the overlap region may be set aside for more critical examination.

Graphical examination of the data indicated a linear trend. A linear discriminant function (Hoel, 1947, pp. 121-126) of the type $z=aX+bY$ was fitted, where X and Y are the length and width respectively and z is a number characteristic of each larva and thus of the species. The equation so fitted was

$$z=X-1.1782Y.$$

Analysis of z in the same manner as for the postclypeal index gave the following statistics:—

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	C. pinus	*C. fumiferana*
Variance of z	.0469	.0342
Range of z	1.58—2.49	0.99—1.72
Mean of z	2.03	1.34
Fiducial limits of z mean	±.068	±.068

The means of the discriminant function for the two species were significantly different at the one per cent level. Five specimens, or 7.4 per cent of the sample of *C. pinus*, and 5 specimens, or 9.8 per cent of the sample of *C. fumiferana*, lay within the range overlap. The overlap for both species is of the same order and not greater than 10 per cent. Therefore the discriminant function technique of separation of the two species is more accurate than that of the postclypeal index.

For practical purposes the postclypeal index is sufficiently accurate as a means of rapid separation of the two species of larvae. The discriminant function is not so rapid but is more accurate and may be applied when a sharper means of separation is required.

It must be noted that these means and functions were obtained on the basis of last-instar larvae though the latter were a representative sample of the species as a whole. It is possible that other instars of these species have their own characteristic indices and discriminant functions.

Use of Probability Paper

The frequency distribution of the uncus widths of 100 adult males of each species was examined by means of probability paper. The histogram for these had indicated that *C. pinus* was bimodal and *C. fumiferana* unimodal (Freeman, 195 , Fig. 4). The larval measurements were also examined by means of probability paper for the possibility of polymodality.

Harding (1949) dealt with the use of probability paper for separating polymodal frequency distributions in biological problems. Probability paper had been in use for some years but its application to biological problems was new. It may be used to estimate the means and the standard deviations of the groups or populations that are separable and also give an estimate of the fraction of each one present in the sample. A slide rule is sufficiently accurate for the necessary calculations in plotting.

Briefly, the method is one of plotting the cumulative percentage frequency in the sample and drawing a line or lines that will have as a resultant a curve closely approximating the original cumulative percentage frequency curve. If the cumulative percentage curve is a straight line, then the sample is of a single, normally distributed population or group (unimodal). If the cumulative percentage frequency curve is sigmoid (one point of inflexion), then the sample is of two overlapping, normally distributed populations or groups (bimodal). Sigmoidal curves (two or more points of inflexion) are descriptive of three or more overlapping, normally distributed populations or groups.

Inspection of the cumulative percentage frequency curve yields an approximation of the proportionate distributions of the populations or groups present. The point or points of inflexion indicate the approximate line or lines of division. They are not as accurate in the central region because of the nature of probability paper, and the lines describing each population or group must be adjusted to the point of best fit. This is necessary as there are often only two or three points to define one of the lines, and the variation is such that there is a wide choice of slopes. Choose the one that best describes the data.

A certain amount of adjustment must be made when grouping the original frequencies. The size of the groups is fairly important. If the class interval is

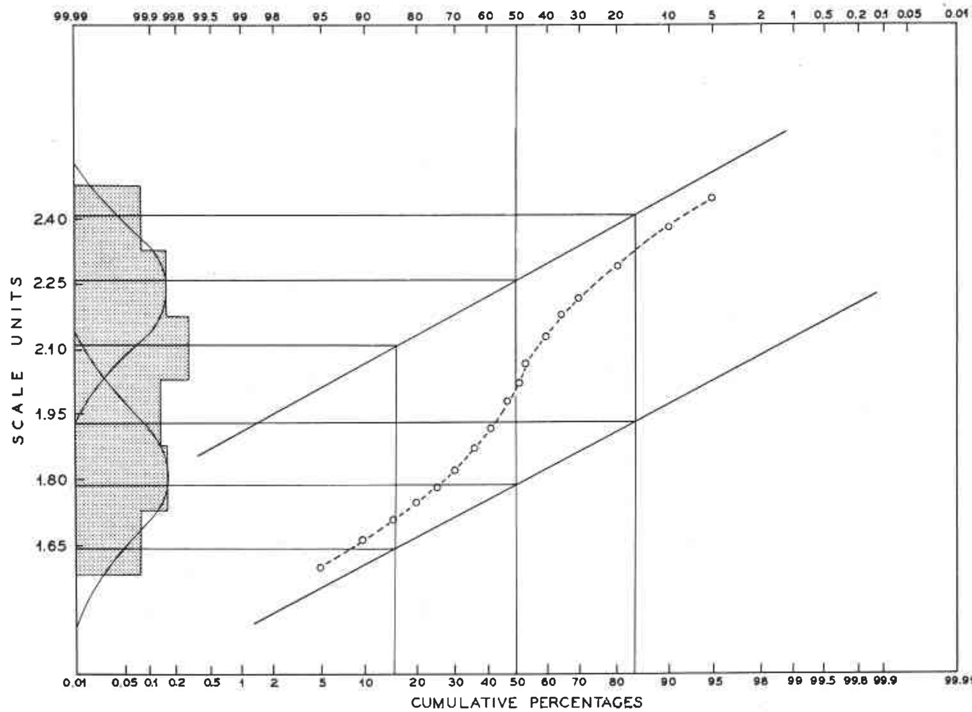

Fig. 1. Cumulative percentage frequency curve for discriminant function of *C. pinus.*

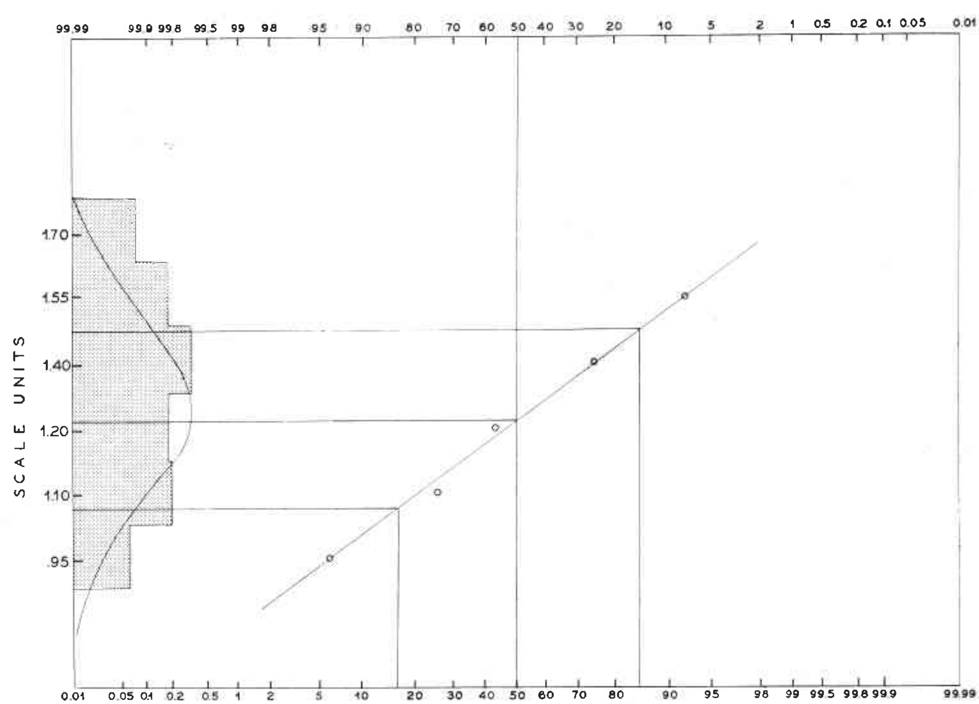

Fig. 2. Cumulative percentage frequency curve for discriminant function of *C. fumiferana.*

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too small, a large number of excessively small values or zeros will apear in the frequency table and interfere with the fitting of the normal curve and also necessitate a large amount of grouping within the frequency table. Grouping is more difficult with a small number of frequencies than with, say, 200 to 500 or more.

Species Subgroups

Larvae.—Initially the lengths and postclypeal widths of the larvae were examined separately for each species. Fitting the cumulative percentage frequency curves showed that *C. pinus* was bimodal and *C. fumiferana* was unimodal. The point of inflexion was somewhere between 40 and 60 per cent for *C. pinus*.

To separate the groups in *C. pinus*, the group of smaller measurement was taken as A and that of larger measurement as B. Resultant cumulative percentage frequency curves were determined where group A was assumed to be 40 per cent, 50 per cent, and 60 per cent of the sample. Means and standard deviations for A and B were determined and normal curves fitted by areas. The χ^2 test of goodness of fit was applied to the theoretical distribution to determine which fraction most closely approximates the observed values. The possibility of *C. pinus* being unimodal was also examined and the fit of the normal distribution tested. Groups A and B were present in equal proportions for the width measurements, whereas group A was present in 60 per cent of the sample for the length measurement. This suggested some discontinuity and some means of using the two measurements together was sought. The linear discriminant function, combining length and width measurements made of the larvae of each species to determine a single representative number, z, seemed appropriate. The values of z were examined (Fig. 1) in the same manner as the original measurements and the χ^2 test made on the fit of the normal distribution obtained from them.

The cumulative percentage frequency curve of z for *C. pinus* showed groups A and B present in equal proportions (Fig. 1). The dotted line between the two solid lines is the resultant. The cumulative percentage frequency curve for *C. fumiferana* showed unimodality for both length and width; similarly, the cumulative percentage frequency curve of z was unimodal (Fig. 2).

Adults.—The uncus widths of 100 adult males of each species were examined for polymodality. In this case the chances of extra variation or hidden points of inflexion were fewer as the sample consisted of males only. The larvae presumably being males and females in equal numbers, sexual differences may have introduced a bimodal effect.

The cumulative percentage frequency curve for uncus width of *C. fumiferana* was linear, indicating a unimodal group (Fig. 3). That for *C. pinus* was sigmoid, and the χ^2 test of goodness of fit showed groups A and B present in equal proportions (Fig. 4).

Figs. 1 to 4 are based on microscope eyepiece scale units. By converting the data to millimetres and microns the following means and standard deviations were determined from the graphs for each species:—

Species	Character	Mean	S.D.
C. fumiferana	postclypeus width	1.16 mm.	.10 mm.
	length	1.68 mm.	.09 mm.
	uncus width	181 μ	27 μ

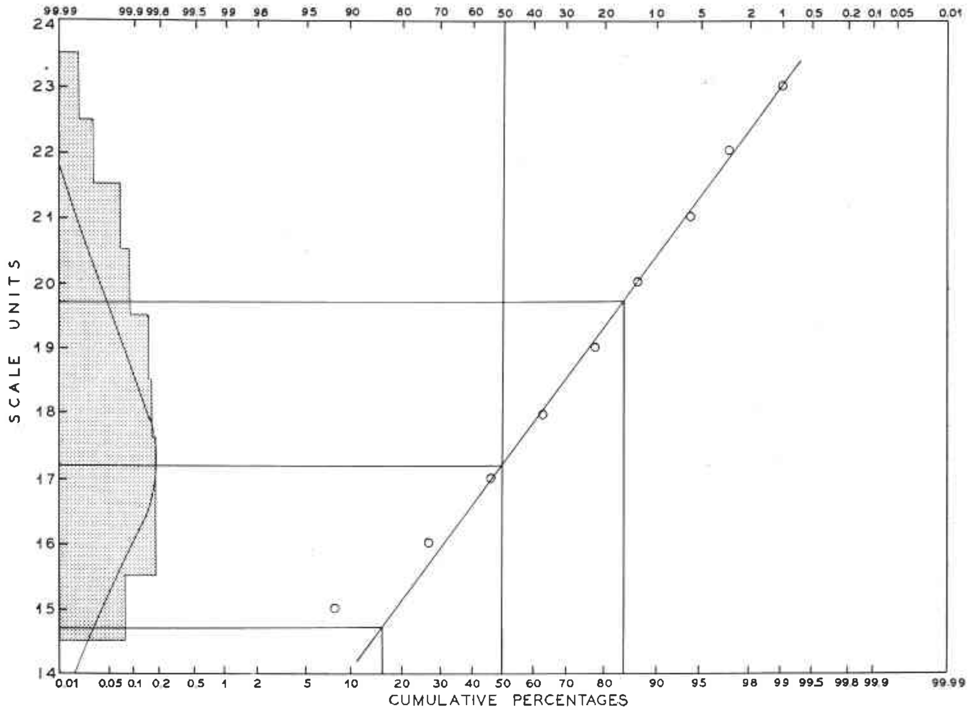

Fig. 3. Cumulative percentage frequency curve for uncus widths of *C. fumiferana.*

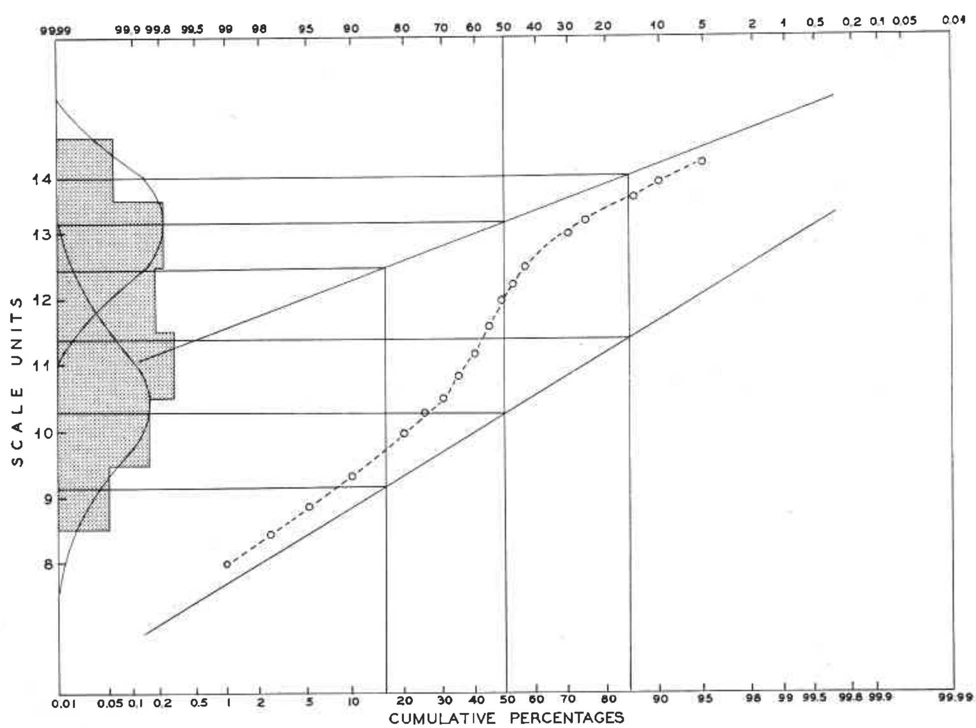

Fig. 4. Cumulative percentage frequency curves for uncus widths of *C. pinus.*

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C. pinus	postclypeus width		
	group A	1.15 mm.	.05 mm.
	group B	1.36 mm.	.06 mm.
	length		
	group A	1.82 mm.	.07 mm.
	group B	2.12 mm.	.07 mm.
	uncus width		
	group A	108 μ	12 μ
	group B	139 μ	7 μ

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Reproductive Isolation and the Integrity of Two Sympatric Species of *Choristoneura* (Lepidoptera: Tortricidae)[1]

By Stanley G. Smith[2]

Introduction

According to Dobzhansky (1951a, p. 262) "Species are . . . groups of populations the gene exchange between which is limited or prevented by one, or by a combination of several, reproductive isolating mechanisms". This definition follows from his concept of a species not as a static unit but as a stage in the process of evolutionary divergence. Limitation or prevention of gene exchange is a property of geographic and reproductive isolation (Mayr, 1942), the various types of which Dobzhansky lists as follows:

I. Geographic or Spatial Isolation
II. Reproductive Isolation
  A. Ecological Isolation
  B. Seasonal or Temporal Isolation
  C. Sexual, Psychological or Ethnological Isolation
  D. Mechanical Isolation
  E. Gametic Isolation
  F. Hybrid Inviability
  G. Hybrid Sterility
  H. Hybrid Breakdown

Several of these have been shown to be effective in various degrees, individually in limiting the interchange of genes between, and collectively in maintaining the integrity of, *Choristoneura fumiferana* (Clem.) and *C. pinus* Free. The factual data that have been accumulated in measuring to what extent the various mechanisms contribute to complete isolation are too numerous to be detailed here: they will therefore only be summarized at present, but will be given at length in a future series of papers.

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