AN ANALYSIS OF VARIATION IN A VARIABLE POPULATION OF CLADONIA

EDGAR ANDERSON AND E. D. RUDOLPH [1]

Missouri Botanical Garden, St. Louis, Missouri

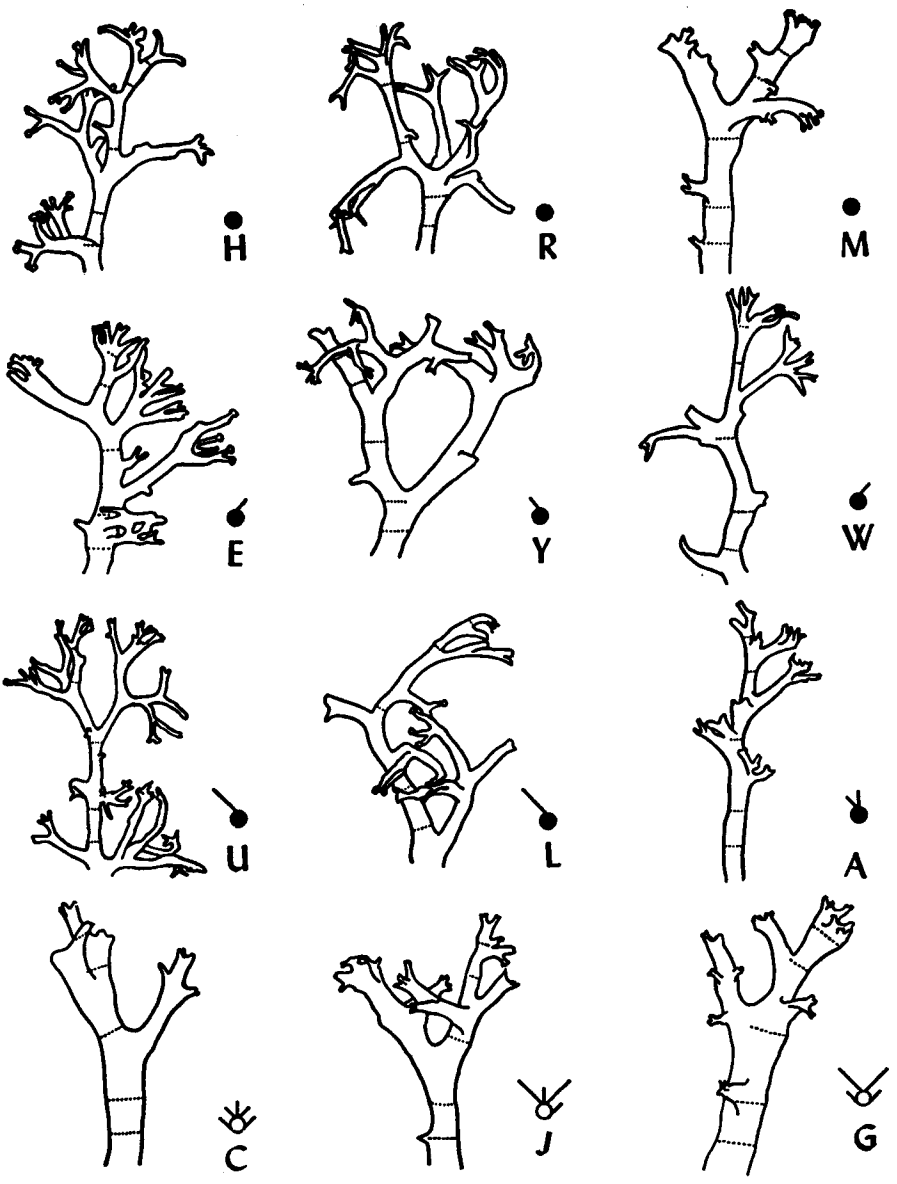
Received July 16, 1955

Fairly early in his studies of variation in natural populations, the senior author realized that he apparently could detect the effects of hybridization in the field even for organisms with which he was unfamiliar. When the suspicion first arose that this might indeed be so, he dismissed it as fantastic. Not until this intuitive judgment had been repeatedly confirmed by the most rigorous experimental tests did he start looking for the objective facts behind the intuition. It was then readily determined that populations which suggested hybridization to the experienced eye had much the same variation pattern as first and second back-cross populations in experimentally produced hybrids. The phenomenon was eventually discussed in print under the title of "Concordant versus discordant variation" (Anderson, 1951) and illustrated by examples which, though hypothetical, were closely modeled upon experimental data (Anderson and Gage, 1952).

The analysis of variation in Cladonia presented below is one of several attempts to test the hypothesis that introgression can be recognized by an experienced observer. The population was discovered by the senior author. Though he knew nothing about the morphology or classification of lichens, the look of entire populations was strikingly "discordant". It presented to the observer that criss-crossing of several variables which is characteristic of introgression whether studied in the field or in the experimental plot. To the senior author it appeared as if two species of lichens had hybridized and the hybrids had back-crossed once or twice to the parental species. The impression was not of a hybrid swarm but of that heightened variability in the participating species which is characteristic of introgression. Accordingly he prevailed upon a trained lichenologist to undertake a joint investigation of this population. It will be seen that the results confirm and extend the working hypothesis. That one was able to make successful predictions of this sort in advance of any scientific literature on the subject, in a group of organisms with which he was wholly unfamiliar suggests that there may be more fundamental similarities in the nature of species in different groups of organisms than we have dared to suspect. If a scientist accustomed to working with hybridizing populations of flowering plants, can recognize a similar situation in lichens, even when he knows nothing about lichens, speciation in lichens and speciation in flowering plants must have certain fundamentals in common.

The markedly "discordant" populations of Cladonia were found a few miles southeast of Pacific, Missouri, in the La Barque Creek area. In ravines at the junction of the St. Peter's sandstone and the Joachim limestone were large Cladonia mats. Within a single mat there usually appeared to be numbers of plants belonging to two distinct species, *C. subtenuis* (des Abbayes) Evans and *C. uncialis* (L.) Web., of different subgenera, with those of each species showing wide plant-to-plant variation. A comparative study of these two species seemed valuable because of what it might tell about the extent of variation not only within each species but also within the genus. The pictorialized scatter diagram (Anderson,

[1] Present address Botany Dept., Wellesley College, Wellesley, Mass.



FIGS. 1 and 2. Camera lucida drawings of the ultimate centimeter of a podetium from each specimen studied. Dotted lines indicate distances ⅛, ¼, ½, ¾ and ⅞ of the distance from the tip. Glyphs indicate scores for characters as given in key of figure 7; additional lower rays to left and right represent abscissa and ordinate characters, respectively (left: no ray, .2–.5 mm.; medium ray, .6–.9 mm.; long ray, 1.0 mm. and up; right: no ray, .2–.5 mm.; medium ray, .6–.7 mm.; long ray, .8 mm. and up). The drawings have been arranged according to the total index value of each specimen as shown in figure 8. As in figures 7 and 8, the color of the center of the glyph indicates the taxonomic determinations made by Dr. Evans. Black represents *C. subtenuis;* white, *C. uncialis;* stippled, *C. uncialis obtusata.*

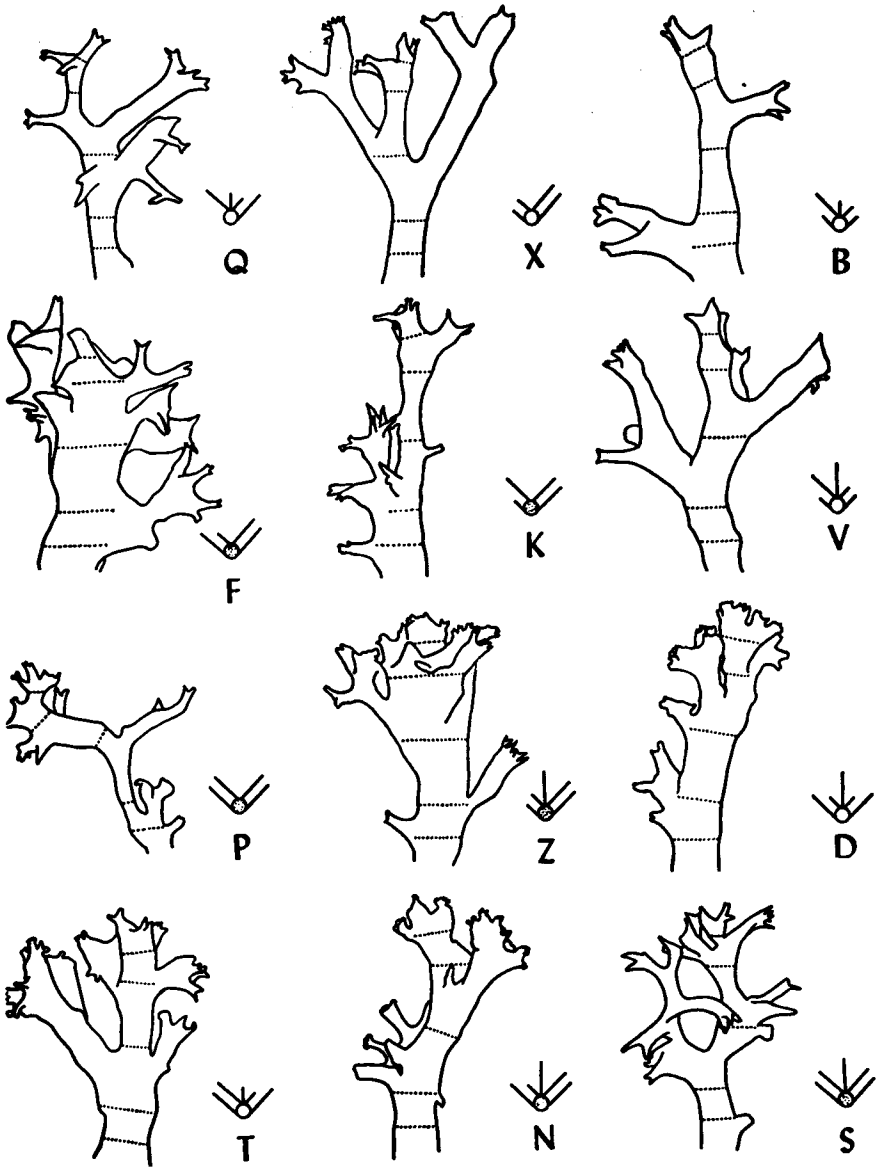


FIG. 2. For caption, see figure 1.

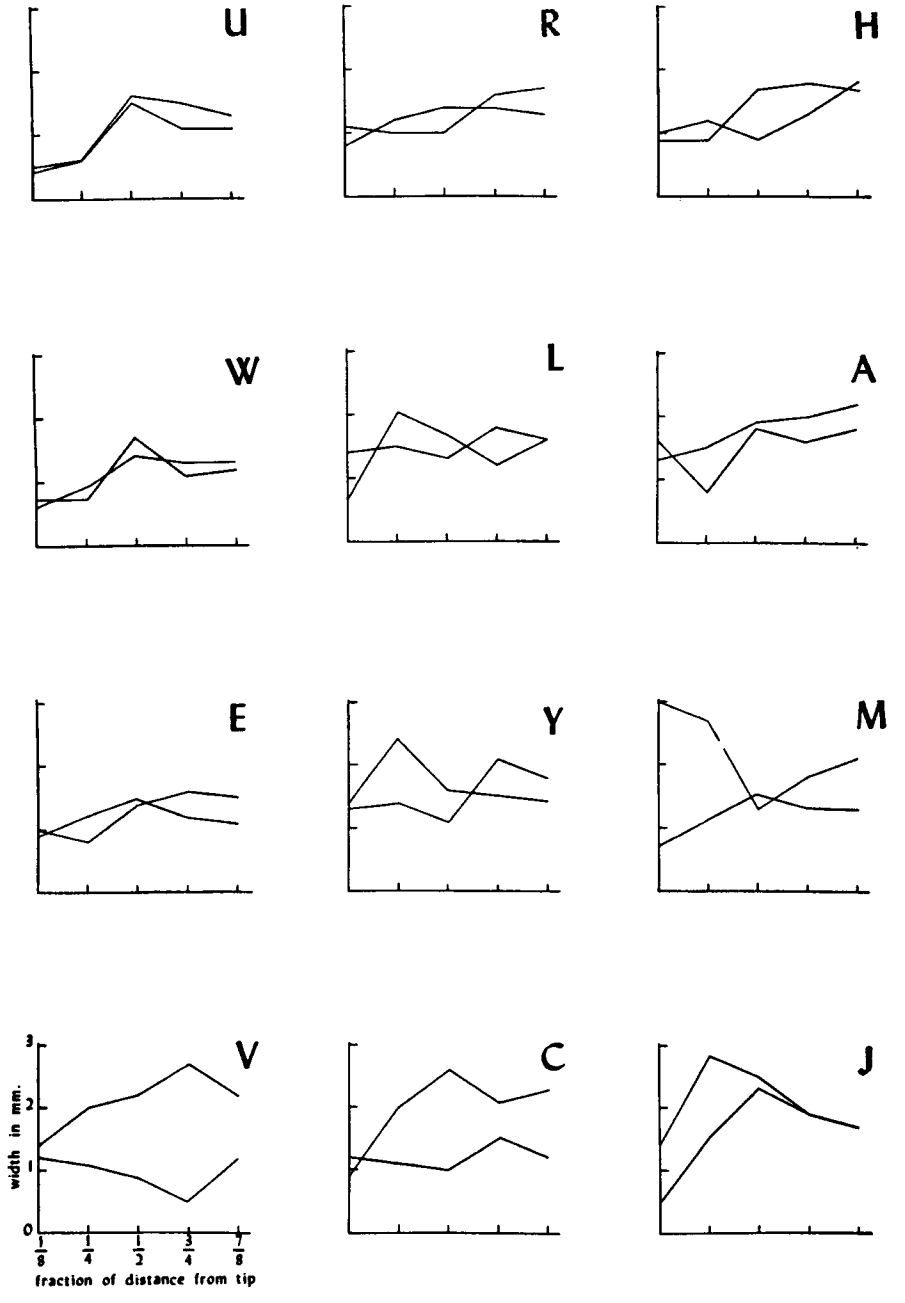
1949) has been used successfully in variation analyses of flowering plants for the comparison of individuals considering more than two characters at one time; it was therefore applied to this problem.

From a single large mat, 24 distinct uniform masses assumed to be individual plants were selected at random. The specimens were all pressed in the same manner. It is of interest to note that since all of the collections were made from a single mat there should not be great habitat differences.

The following characters were compared.

1. *Index of variation in width within the ultimate podetial centimeter.* The best measurable difference between the two species studied was found to be the index of variation in podetial width (the

differences in branching pattern which are characteristic of the species in the subgenus Cladina, des Abbayes 1939, to which *C. subtenuis* belongs could not be used as a comparative character since the branching is of a different type than that found in *C. uncialis*, sub-genus Coenomyce). Such an index was obtained by



FIGS. 3 and 4. Graphs giving relationships of podetial width measurements for the two tips of each specimen. Letters correspond to those of figure 1.

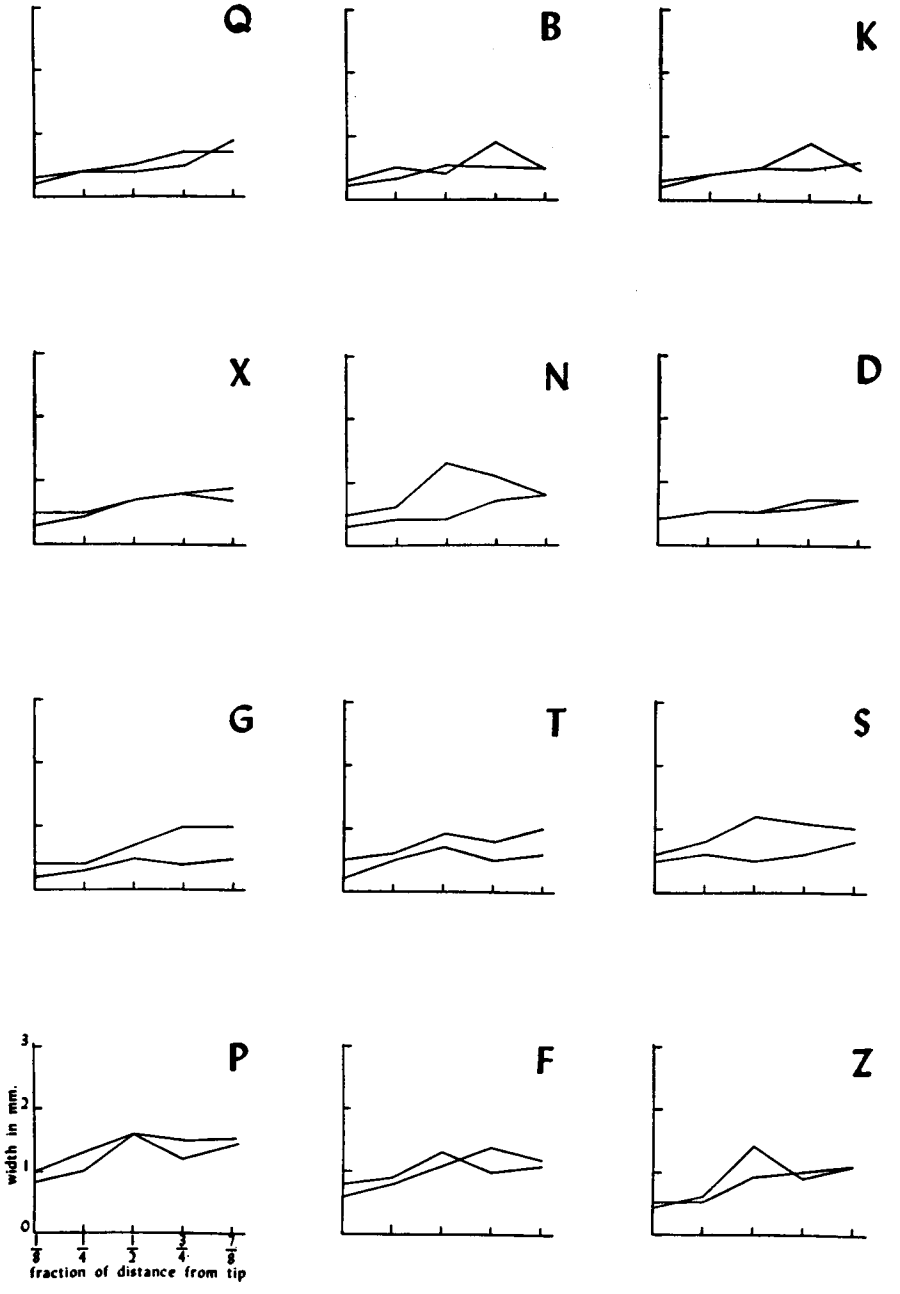


FIG. 4. For caption, see figure 3.

(a) making camera lucida drawings of the ultimate centimeter of two podetia of each specimen, (b) measuring the width on the drawing of each segment at points 1/8, 1/4, 1/2, 3/4, and 7/8 of the distance from the tip and reducing to actual size (to nearest .1 mm), (c) subtracting the smallest measurement from the greatest measurement obtained for each segment to give a number indicating the degree of absolute variation in the width of the individual segments, and (d) calculating

the mean of the two such numbers obtained for each plant. These mean values were used as the ordinant in the scatter diagram. Camera lucida drawings of one podetial tip of each specimen are illustrated in figures 1 and 2. Graphs of the two sets of measurements for each specimen are shown in figures 3 and 4.

2. *Width of podetium ⅛ cm. from tip.* The average of the measurements (determined as in 1 above) made ⅛ cm. from tip in two podetia of each specimen was used for the abscissa.

3. Width of the widest podetium of each specimen, measured to the nearest millimeter.

4. Cortical index. For microscopic study of the cortical region (in *C. uncialis* a true cortex and in *C. subtenuis* the medullary hypae free and external to the algal colonies), free-hand cross-sections taken from the interstitial region of one mature podetial stalk from each specimen were mounted in lacto-phenol containing acid fuchsin. It was apparent from a study of the sections that the cortical region varied in two respects: (a) in the degree of erosion, and (b) in the degree of gelification of the hyphae. In some cases the hyphae were loosely interwoven and the individual hyphae could be determined because the walls were fused showing only occasional cell lumens (the gelified condition). In general the loosely interwoven hyphae were found in those cortical regions which were most eroded. The first of the two differences was measured by determining the difference between the extremes in width in each cross-section (width measured by micrometer eyepiece) giving a value directly related to the degree of erosion. A measure of the second was obtained by giving each specimen a score ranging from 0, loosely interwoven hyphae, to 2, extremely gelified. These two sets of comparisons were then combined in a scatter diagram. The resulting distribution more or less fell into 3 categories, (a) not eroded, heavily gelified; (b) variable; and (c) eroded, not gelified. These were then used as the cortical index in the pictorialized scatter diagram.

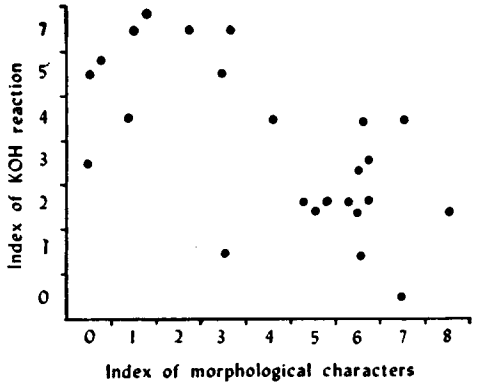


FIG. 5. Scatter diagram showing correlation of KOH reaction with index derived from four morphological characters.

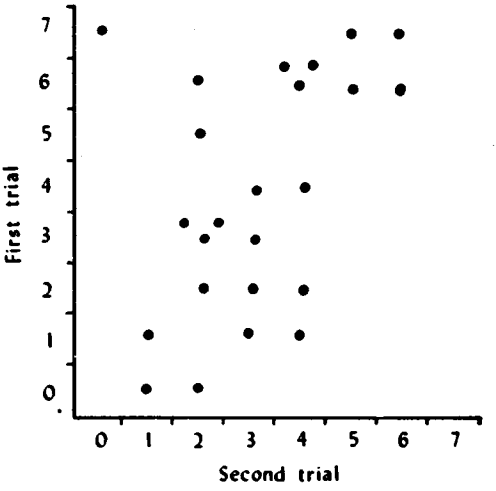


FIG. 6. Scatter diagram showing correlation of first and second KOH reactions.

5. *Reaction to KOH* (figures 5 and 6). A drop of 10 per cent KOH and a drop of tap water were placed in separate spots on the dry podetium. Readings scored from 0 (KOH drop no different from water) to 4 (a very apparent change to yellow in the KOH drop), were made immediately and again after five minutes and the two readings were totaled. A duplicate test was made and the two sets of scores averaged for the final scores. These final scores ranged from 0 to 6.

It should be noted that the strong yellow color reaction common in some Cladonia species was not found in either of the species used here. The measure is of a slighter color change, but one which is apparent when the comparison is made with a spot upon which only tap water was placed.

Of the characters compared the first three are measures of gross morphological variation, the fourth is an anatomical characteristic and the last a physiological reaction.

The twenty-four specimens were scored in the five ways described above and the results combined in the pictorialized scatter diagram (fig. 7). It is apparent that the two species represented, *C. subtenuis* and *C. uncialis,* separate well from each other on the scatter diagram. The same specimens, identified only by number, were sent to Dr. Alexander W. Evans for standard taxonomic determinations independent of those made by the junior author. Taxonomically, *C. uncialis* is subdivided into numerous formae. Ac-

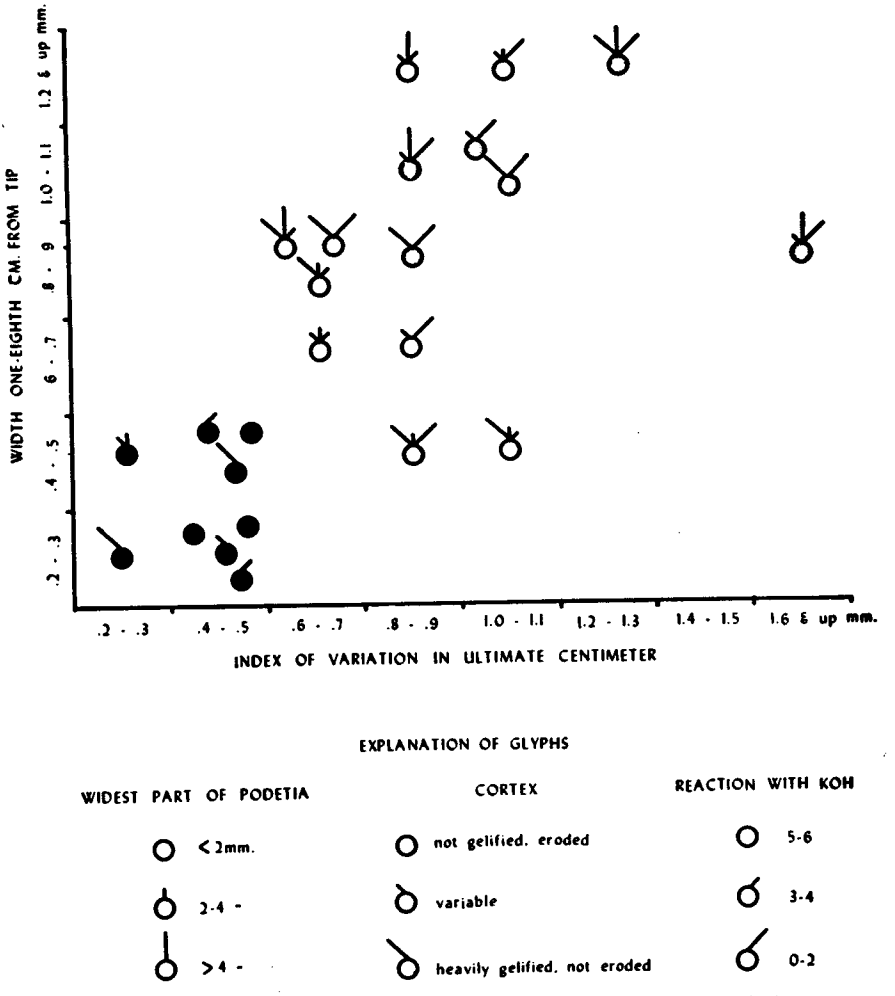


FIG. 7. Pictorialized scatter diagram of Cladonia population (24 samples). Shading of glyphs denotes identification according to Evans. As in figures 1, 2, and 8, black represents *C. subtenuis;* white, *C. uncialis;* stippled, *C. uncialis* f. *obtusata.* See text for explanation of the scoring.

cording to Evans, some of the plants in this study (those appearing in the scatter diagram as the most extreme in both index of variation in podetial width and in width ⅛ cm. from tip) could be referred to forma *obtusata.*

In figure 8 the data of figure 7 have been condensed into a five-fold index. The two characters measured on the horizontal and vertical axes and the three characters diagrammed on the rays have each been scored in three grades, 0, 1, and 2. The three rayless glyphs in the lower left hand corner of figure 7 represent one extreme. The score for each of them is 0 for each of the five characters giving them index values of 0. The other extreme is represented by the specimen in the upper right hand corner of figure 7, the only glyph with three long rays. It scores 2 for each of the five characters giving it a total index of 10.

Figures 7 and 8 (and most particularly the latter) serve as a convenient way to compare our results with Dr. Evans' independent judgments from the same material. It will be seen that there is close agreement. Our scale gives values of from 0 to 3 for all the plants determined as *C. subtenuis* while none of the plants determined as *C. uncialis* scores below 5. Furthermore, *C. uncialis* and its forma *obtusata* have overlapping values, *C. uncialis* from 5 to 8 and forma *obtusata* from 7 to 10. For this population, as seen against the background of various studies of introgression, we would advance as a very likely hypothesis the notion that its ultimate origin was from extreme forms of *C. subtenuis* and *C. uncialis* f. *obtusata.* Hybrids between these two lichens which crossed back to the parental extremes then produced at first or second hand the high-scoring variants of *C. subtenuis* (with index values of 1 to 3) and all the *C. uncialis* which are not in forma *obtusata,* perhaps even the lowest scoring plants of that forma. As in the various other field studies of hybridization which have been undertaken in this laboratory the chief result of hybridization seems to be that greatly enhanced variation in the parental species is presented to natural selection to work upon.

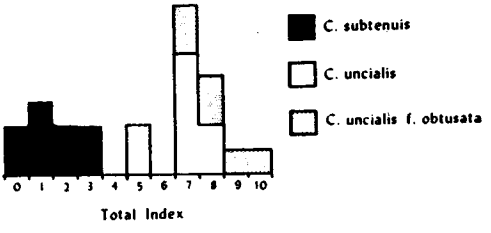


FIG. 8. Frequency distribution of the Cladonia population based on the total index of scores obtained from the pictorialized scatter diagram (fig. 7) by totaling the ray values for each glyph (ordinate and abscissa characters are represented by rays as described in fig. 1 and 2). For each of the five characters, a low value (no ray) is scored 0; a medium value (short ray) is scored 1; and a high value (long ray) is scored 2.

DISCUSSION

The utilization of the pictorialized scatter diagram in studying variation in these two Cladonia species seems significant. With the use of even such a small sample as 24 specimens, by using correlated comparative characteristics, the relative amount of variation can be approximately ascertained. It also permits the comparison of many types of variation, i.e., morphological, anatomical, and physiological at one time. Primarily, it facilitates the graphic measurement of morphological differences which previously have been compared intuitively, e.g., the relative difference in taper of the podetia. The variation found in this study has certain biological implications. Vainie (1897) who has made the most complete study of the genus yet published believes that the species variation found is due mainly to external conditions and not to internal genetic variation. He was of the opinion that sexual reproduction was entirely lacking in this genus. Recent studies establishing sexual reproduction in a related genus (Johnson 1954) indicates that it is also possible in Cladonia. The very common occurrence of spermagonia and

apothecia in Cladonia would of itself indicate sexuality as would also the production of viable ascospores in large numbers (Rudolph, unpublished data). Although it is felt by most lichenologists that various methods of asexual reproduction are much more important in lichen multiplication than is the sexual method, this need not rule out the rare successful sexually reproduced offspring which is all that is necessary for genetic variability. Variation may also be due to heterocaryosis resulting from anastomosis of two mycelial filaments occurring in a manner similar to that found in Neurospora and other Ascomycetes. It is apparent that the large amount of variation in some species could not be produced by habitat variation alone. This study of plants growing in the same mat in an apparently uniform habitat demonstrates considerable variation which could not be due to differences in age, particularly since only mature specimens were studied.

The fact that the variation pattern of this population is so similar to that met with in colonies of the higher plants in which there had been hybridization and consequent back-crossing, suggests not only that there is sexual reproduction in these lichens but that there is introgression. The variation within *C. subtenuis* is all in the direction of *C. uncialis.* The variation in *C. uncialis* is all in the direction of *C. subtenuis.* Introgression is the only known evolutionary force which produces population variation patterns of this kind (Anderson, 1949). There is strong presumptive evidence that the extremely variable population in the La Barque Creek area came originally from hybridization between *C. uncialis* and *C. subtenuis;* that the primary hybrids crossed back to each of the two parental species, thus producing for each species a group of variant forms within which the plant-to-plant variation for one or more characters forms a gradient in the direction of the other species. Cladonia is a notoriously variable and taxonomically difficult genus. The studies here reported suggest that hybridization may well be one of the prime factors responsible for this confusion.

Summary

1. A strongly discordant variation pattern brought large variable populations of Cladonia to our attention.

2. Random collections were made from an essentially uniform habitat and the variation pattern was studied intensively

3. Five characters were eventually chosen for scoring or measurement:

(a) variability in width in the ultimate podetial centimeter.
(b) podetial width ⅛ centimeter from tip.
(c) maximum podetial width.
(d) erosion and gelification of the cortical region.
(e) color reaction to KOH.

4. These five characters were found to be associated in two complexes running from:

little variation in width in podetium tip
distal region of podetium narrow
podetium narrow
cortical region not gelified, eroded
no reaction with KOH

to:

great variation in podetium tip
distal region of podetium wide
podetium wide
cortical region heavily gelified, not eroded
yellow reaction with KOH

5. Analysis by pictorialized scatter diagrams and by the use of the hybrid index method, correlated closely with the judgment of a taxonomic expert, made independently on the same material.

6. The probable sexuality of these species of Cladonia is briefly discussed. It is concluded that they give evidence of sexual as well as asexual reproduction. It furthermore seems most likely that hybridization and subsequent backcrossing between *Cladonia uncialis* and *C. sub-*

tenuis are responsible for the extreme variability of this population.

LITERATURE CITED

ABBAYES, H. DES. 1939. Revision monographique des Cladonia du sous-genre Cladina (Lichens). Bull. Soc. Sci. Bretagne, **16** (2): 1–156.

ANDERSON, EDGAR. 1949. *Introgressive Hybridization,* John Wiley and Sons, Inc., New York.

——. 1951. Concordant versus discordant variation. EVOLUTION, **5**: 133–141.

——, AND AMY GAGE. 1952. Introgressive hybridization in *Phlox Bifida.* Am. J. Bot., **39**: 399–404.

JOHNSON, G. T. 1954. Ascogonia and spermatia of *Stereocaulon.* Mycologia, **46** (3): 336–345.

VAINIO, E. 1887. Monographia Cladoniarum universalis. 3 vols., I: 1–509, Helsinki, 1887; II: 1–498, Helsinki, 1894; III: 1–268, Kuopia, 1897.