

systematics, it should be feasible to develop a composite of composites for the evaluation of uniformity or variation in the total biota. Differentiations in purely physical factors (e.g., temperature, elevation, rainfall, etc.) may also be evaluated by this methodology, and employed either in comparison with the biologic function or integrated with it to approximate the total environmental differentiation. However, in the development of such supercomposites, subjective evaluations play an increasingly important role in choosing from among the multitude of variables the relatively few it is practical to include in an analysis.

The subtleties of human culture and the intricate relationships of its elements pose problems for which, as yet, there appears to be no adequate mathematics. However, the quantification of even the grosser variables would permit the development of a systemic function which should better define the spatial and temporal structuring of culture fields and their patterns of interaction than do any of the current culture area and culture sequence classifications.

Dialects show analogies to subspecific phenomena. Like biologic varieties, they are involved in the uneasy dynamic equilibrium of their universes of interaction. In isolation they may "speciate" as distinct languages. This methodology would seem well suited to the ordering of the many variants of dialect geography.

It is not feasible at this time to foresee all possible applications of differential systematics. Essentially, it is a development in logic rather than a specialized technique. Consequently, it may be employed in the ordering of numerous realities exhibiting multiple variations through time and space. However, significant interpretation of differing systemic functions requires that individual attention be given to the processes involved in maintaining similarities and developing differences.

In addition to whatever value this formulation may

have within various fields of study, it should open new avenues for the analysis of covariation and probable interaction between phenomena now departmentalized among separate, and often mutually incomprehensible, disciplines.

Differential systematics is a methodology for synthesizing multiple measurements, indices, and frequencies into a composite variable, the *systemic function*, which, for all loci, evaluates the average change with distance of a total reality. It is applicable to the analysis of any type, number, and combination of quantifiable traits, provided only they are related as to means of variation, diffusion, and interaction.

This technique does not completely exclude arbitrary elements from the ordering of complex phenomena, but it reduces them to a minimum and makes explicit much that is often implicit in the "feeling" of an authority. The necessity for assaying numerous continuous variables, which previously has proved a liability for the development of a clear systematic, is transformed into an asset in broadening the statistical base and minimizing effects of errors in data and in judgment.

Systemic space and systemic time are introduced as conceptual tools. Also, they serve to define an analytic manifold for future developments in space-time genetic calculus.

References

1. MAYR, E. *Systematics and the Origin of Species*. New York: Columbia Univ. Press (1942).
2. ALPATOV, W. W. *Quart. Rev. Biol.*, **4**, 1 (1929).
3. CALHOUN, J. B. *Am. Naturalist*, **81**, 203 (1947).
4. SUMNER, F. B. *Biologiographia Genetica*, **9**, 1 (1932).
5. DICE, L. R. *Contribs. Lab. Vert. Genetics Univ. Mich.*, **15**, 1 (1941).
6. HUXLEY, J. S. *Bijdr. Dierk.*, **27**, 491 (1930).
7. BIRDSSELL, J. B. *Cold Spring Harbor Symposia Quant. Biol.*, **15** (1951).
8. HALDANE, J. B. S. *Evolution*, **3**, 51 (1949).

Technical Papers

Contaminating Bacteria in Plant Tumor Tissues¹

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In the course of investigations on plant tumors bacterial growth was noted when a tumor (P II) believed to be bacteria-free was grown on a peptone agar. None was observed when the tumor tissue was grown on a basal medium of mineral salts, cane sugar,

and three vitamins (thiamine, pyridoxine, and nicotinic acid) used for the cultivation of the tumor tissues. In fact, this tumor tissue had been cultivated in this laboratory for several years with no evidence of bacterial contamination.

As a consequence of our observation, all the tumor tissues in our collection have been examined for sterility. None had shown bacterial growth on the medium used for growing the plant tumor tissue, although under cultivation for considerable periods of time. The plant tumors examined are shown in Table 1.

A portion of each of these tissues was mashed in a small amount of sterile distilled water, streaked on slopes of AC agar, and incubated at 36° C. The AC

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TABLE 1

H 13	<i>Helianthus</i> tumor	Isolated from secondary sunflower tumor. July 1941. P. R. White
K I	" "	From a secondary tumor on an <i>in vitro</i> graft. Subcultured June 28, 1946. R. S. de Ropp.
K II	" "	Isolated from induced tumor. R. S. de Ropp.
K III	" "	Isolated from induced tumor. R. S. de Ropp.
P I	" "	Isolated from primary tumor 14/12/45. R. S. de Ropp.
P II	" "	Isolated from a different segment of same primary tumor as P I (14/12/45). R. S. de Ropp.
P III	" "	Isolated from primary tumor Feb. 1946. R. S. de Ropp.
V 60	<i>Vinca rosea</i> tumor	Isolated from heat-treated plants of <i>Vinca rosea</i> . Apr. 1943. P. R. White.
PN	<i>Parthenocissus</i> normal	G. Morel
PT	" tumor	" "
PA	" accustomed	" "
NT	<i>Nicotiana</i> tumor	" "

agar contained, per liter, 20 g proteose-peptone #3, 3 g yeast extract, 3 g malt extract, 5 g dextrose, 3 g beef extract, and 15 g Difco agar. All the tumor tissues showed the presence of bacteria except PN and PA.

The bacteria isolated from our cultures of plant tumor tissues are more fastidious in their nutritional requirements than *Agrobacterium tumefaciens*, which grows readily on the tissue culture medium at room temperature. The contaminating bacteria are gram-negative rods. They grow on AC agar but not on the tissue culture medium, and they grow more rapidly at 36° C than at room temperature. The bacteria isolated from each tumor were placed on disks from carrots that had been tested for ability to form tumors when inoculated with virulent *A. tumefaciens*. No tumors were produced.

The plant tumors were retested for bacteria by fragmenting bits on slopes of AC agar or on the medium supplemented with yeast extract described by Theis, Riker, and Allen (1). Again bacteria grew from all but PN and PA.

Thirteen different plant tumor tissues were obtained from another laboratory. Bacteria were demonstrated in four of these. This group of tumor tissues included duplicates of five of the strains of tumor tissues in our collection—namely, K I, NT, P I, P II and H 13. The P I, P II, and H 13 were sterile, the K I and NT were contaminated.

The contaminating bacteria would not seem to be causally associated with the tumorous condition of the tissue, since our P I, P II, and H 13 contained bacteria, whereas the same strains of tumors from another laboratory did not. However, plant tumor tissues that appear to be bacteria-free when grown on a medium suitable for the cultivation of tumor tissues

may contain bacteria. This possibility must be considered and eliminated by the use of suitable test media and incubation temperatures when sterile plant tumor tissues are essential for the purposes of a particular investigation.

Reference

1. THEIS, T. N., RIKER, A. J., and ALLEN, O. N. *Am. J. Botany*, 37, 792 (1950).

The Isolation of *Histoplasma capsulatum* from Soil in an Unused Silo¹

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The epidemiology of histoplasmosis in man is largely unknown. The fungus *Histoplasma capsulatum* has been isolated by Emmons from a variety of wild and domestic animals and from 6 of several hundred samples of soil collected from farms on which animals harboring the fungus had been trapped (1, 3). There is no direct evidence, however, that infected animals serve as vectors in the case of histoplasmosis in man or that contaminated soil is important in the epidemiology of the disease.

This report concerns the study of the possible source of the fungus (*H. capsulatum*) responsible for extensive pulmonary infections in 3 male members of a farm family living near Lowell, Ind. The father (A. E., age 53) and his two sons (E. E., age 20, and A. E., Jr., age 5), had been well until about Sept. 20, 1950, when they became ill at the same time with fever, chills, non-productive cough, and weakness. X-rays of the chest revealed widespread noncalcified pulmonary infiltrates in all three. E. E. and A. E., Jr., were clinically ill for only 2 weeks, but the father failed to recover immediately and was admitted to Billings Hospital on Oct. 28, 1950. He had a stormy course but gradually improved and was discharged after 2 months' hospitalization. Sputum specimens were examined for the presence of fungi and acid-fast bacilli by culture and animal inoculation. Specimens collected on the fifth and twelfth hospital day yielded *H. capsulatum*, as did a bone marrow culture taken on the tenth hospital day. In all three, skin tests were positive to histoplasmin 1:100 dilution but negative to tuberculin (PPD 0.0001 mg) and coccidioidin 1:100. Sera from all three yielded highly positive complement fixation titers (A. E., 1:160; E. E., 1:320; and A. E., Jr., 1:160) in a test employing yeast phase histoplasma as antigen (4).

During the 2 months preceding their illness the two men had undertaken in addition to the usual farm chores (a) removal of "blow sand"—a fine shifting silt from driveways; (b) grinding feed composed of

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