isolates on 58-161 and 58-161 F− produced an identical pattern of susceptibility and resistance. Twenty-four plaques were isolated from a plating of sewer water on 58-161 (F+) and 90 plaques from plates seeded with Hfr (Cavalli). Seven of the isolates spotted on 58-161 but not on 58-161 F−. Stocks were prepared from these isolates (labeled f1, f2, and so on), and each was titrated on 58-161 (at no dilution did plaques appear on 58-161 F−).

Although enough experiments have been performed with each of the above stocks to assure that they all distinguish F+ from F− strains, the following tests refer to one stock only, f1.

To determine the sensitivity of various bacteria to f1, f1 was cross-streaked on EMB 0 agar against 21 strains of *E. coli* K-12 of various genotypes and mating types. Each of seven F− strains tested was resistant, whereas nine F+ and four Hfr strains were sensitive. A derivative of Hfr (Cavalli) mating less efficiently than the parental strain was resistant to f1, an exception also demonstrated by cross-streaking against *S. typhimurium*. Cross-streaks against the following strains of *E. coli* were all negative: *E. coli* B, *E. coli* Raper, *E. coli* Wilson, Wg 35 A, W 52 A, and Wg 4A (N8) [the latter three coli strains were isolated by Bernstein (*7*)]. Cross-streaks against the following bacteria were also negative: *S. typhimurium* LT 2, LT 2 F+, *Shigella sonnei*, *Shigella dysenteriae*, and *Aerobacter aerogenes*.

The f1 phage appears to be highly specific for the F+ and Hfr mating types of *E. coli* K-12. This notion is supported by the following experiments.

1) The first experiment was concerned with the transfer of F from *E. coli* to *E. coli*. F− bacteria become F+ when grown in mixed culture with F+ strains. On the other hand, F− strains remain F− when grown with Hfr bacteria. In either case the original F− population can easily be reisolated by using some sugar fermentation as a differential marker. This experiment offers an excellent opportunity to demonstrate that sensitivity to phage f1 is correlated with the F+ mating type.

After growth of Y-10 (F−, lac−) with either 58-161 (F+, lac+) or Hfr (Cavalli) (lac+), the mixtures were diluted and plated on EMB lactose agar. Twenty-one lactose-negative colonies were isolated from the 58-161, Y-10 plates; these were f1 sensitive and F+ (as shown by an ability to mate with the appropriate F− strain). Ten lactose-negative colonies were isolated from the Hfr (Cavalli), Y-10 plates; these were f1 resistant and F− (demonstrated as above).

2) The second experiment was concerned with the transfer of F from *S. typhimurium* to *E. coli*. The design of this experiment was exactly like that of the coli to coli F transfer. LT 2 F+ and Y-10 (F−), two strains resistant to f1, were mated. One hundred clones were isolated and tested for mating type by cross-streaking against *S. typhimurium* (*5*). Of these isolates, 18 selected clones were also tested for susceptibility to f1. Three of these gave positive cross-streaks against f1 and *S. typhimurium*, and 15 gave negative cross-streaks in both tests. Thus, the phage test and salmonella test are perfectly correlated (*8*).

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References and Notes

1. L. L. Cavalli, J. Lederberg, E. M. Lederberg, *J. Gen. Microbiol.* **8**, 89 (1953).
2. W. Hayes, *ibid.* **8**, 72 (1953).
3. Y. Hirota and T. Iijima, *Nature* **180**, 655 (1957).
4. G. A. Maccacaro and R. Comolli, *J. Gen. Microbiol.* **15**, 121 (1956).
5. N. D. Zinder, *Science*, this issue.
6. M. H. Adams, *Methods in Med. Research* **2**, 1 (1950).
7. H. L. Bernstein, *Symposia Soc. Exptl. Biol.* **12**, 93 (1958).
8. I acknowledge my gratitude to Dr. Norton Zinder and Dr. Maurice Fox for their advice.

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Esters Produced by *Chalaropsis thielavioides*

Abstract. *Chalaropsis thielavioides*, a filamentous fungus, produces a characteristic ester-like aroma on potato dextrose broth. By gas-liquid chromatographic techniques, the major aroma-producing substances have been identified as isobutyl and ethyl acetate. Lesser amounts of isoamyl acetate, ethanol, and methanol were also present.

The formation of esters by filamentous fungi has not been extensively investigated. Gordon (*1*) reported the production of ethyl acetate by *Endoconidiophora moniliformis*. *Penicillium digitatum* also synthesizes ethyl acetate (*2*). The formation of isobutyl acetate and methylheptenone by *Endoconidiophora coerulescens* has been reported by Morgan (*3*). Other esters synthesized by filamentous fungi include methyl-*p*-methoxy cinnamate (*4*, *5*), methyl cinnamate (*4*), and methyl anisate (*6*). Longree (*7*) noted an odor resembling that of isobutyl acetate emanating from her cultures of *Chalaropsis thielavioides*. Her observation, however, was not confirmed by analysis of the culture volatiles.

In the spring of 1958 *C. thielavioides* Peyrn. was isolated from dogwood graft failures. During the initial study it was noted that cultures of this organism produced a characteristic ester-like aroma. The purpose of the present study was to identify the volatile constituents responsible for the aroma.

On culturing the organism in various natural media, the production of the characteristic aroma appeared to be best in potato dextrose broth cultures shaken continuously during incubation. Therefore, 25 250-ml erlenmeyer flasks each containing 75 ml of this medium were inoculated and incubated on a shaker at room temperature until a strong aroma could be detected emanating from the flasks. This generally took from 4 to 5 days. The cultures were then pooled aseptically in a 5-lit. round-bottom flask, and this was connected in a gas-train apparatus. The train included flasks for sterilizing and moistening the air before passing it over the cultures and a series of four traps, one cooled with wet ice and alcohol and three with dry ice and alcohol, for condensing the volatiles removed from the cultures.

In different trials air was passed over the cultures for periods varying from 18 to 40 hours. The cultures were agitated during aeration by a magnetic stirring bar. During the longer collecting periods, several milliliters of condensate reached the second dry-ice-alcohol trap and appeared as two layers. In some experiments the condensate was extracted with ether, and the ether was removed by careful distillation; in others, drying the volatiles over anhydrous sodium sulfate provided sufficient material for satisfactory analysis.

Table 1. Gas-liquid chromatographic analysis of the volatiles produced by *Chalaropsis thielavioides* in potato dextrose broth.

Compound	Retention time				Approx. percentage of total
	Carbowax 1500*		Di-*n*-decylphthalate†		
	Known	Volatile	Known	Volatile	
Acetone	1′ 14″	1′ 13″	1′ 10″	1′ 10″	(Trace)
Ethyl acetate	1′ 46″	1′ 46″	2′ 7″	2′ 7″	30
Methanol	2′ 27″	2′ 27″	40″	40″	2
Ethanol	3′ 1″	3′ 2″	1′ 2″	1′ 2″	5
Isobutyl acetate	3′ 56″	3′ 56″	6′ 41″	6′ 43″	58
Isoamyl acetate	7′ 48″	7′ 43″	14′ 36″	14′ 34″	4
Isobutyl alcohol	7′ 55″	‡	3′ 48″	3′ 48″	(Trace)
Isoamyl alcohol	17′ 8″	§	8′ 54″	8′ 58″	(Trace)

* Perkin-Elmer K column; length, 2 m; temperature, 80°C; gas, helium; pressure, 20 lb/sq. in.; flow rate, 46.5 ml/min; sample, 2 μl; sensitivity, 10 mv. † Perkin-Elmer A column; length 2 m; temperature, 100°C; gas, helium; pressure, 20 lb/sq. in.; flow rate, 50.5 ml/min; sample, 5 μl; sensitivity, 10 mv. ‡ Isobutanol not separated from isoamyl acetate. § Trace of isoamyl alcohol not detected.

The volatiles were subjected to gas-liquid chromatographic analysis in a modified Perkin-Elmer vapor fractometer, model A. The retention times of compounds in a typical sample of fungal volatiles as compared to retention times of known compounds, determined on a Carbowax 1500 column and a di-*n*-decylphthalate column, respectively, are reported in Table 1.

The approximate percentages of each of the components of the mixture were determined from estimations of the areas under the peaks recorded during the analysis made on the di-*n*-decylphthalate column. The two major components, with retention times essentially the same as those of authentic isobutyl and ethyl acetate on both columns, accounted for approximately 58 and 30 percent of the mixture, respectively. Components having the same retention times as isoamyl acetate, ethanol, and methanol were present in measurable amounts totaling approximately 11 percent. Traces of components presumed to be isoamyl and isobutyl alcohol were detected.

One milliliter of the volatile mixture was refluxed with 10 ml of 1*N* KOH for 3 hours. The mixture was extracted exhaustively with ether, and most of the ether was removed from the extract by careful distillation. Gas-liquid chromatographic analysis of the extract on the di-*n*-decylphthalate column revealed the absence of the three previously observed ester peaks and revealed marked increases in the isobutyl, isoamyl, and ethyl alcohol peaks.

The alkaline solution used in the saponification procedure was adjusted to *p*H 1.0 with H_2SO_4 and extracted exhaustively with ether. The ether solution was dried over anhydrous Na_2SO_4 and treated with an excess of diazomethane in ether to convert any acids present to their methyl esters. The excess diazomethane was dissipated by the dropwise addition of 2*N* HCl and by vigorous shaking. The ether solution was again dried and then distilled to remove most of the ether. Gas-liquid chromatography of the residue on the Carbowax column revealed a peak at a retention time identical with that of methyl acetate. Since no other methyl esters were detected, and since isobutyl, isoamyl, and ethyl alcohol were liberated during saponification, it must be assumed that the esters in the volatiles collected from cultures of *Chalaropsis thielavioides* are acetates of these alcohols (*8*).

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References and Notes

1. M. A. Gordon, *Mycologia* **42**, 167 (1950).
2. J. H. Birkinshaw, V. Charles, H. Rainstrick, *Phil. Trans. Roy. Soc. London* **B220**, 355 (1931).
3. E. N. Morgan, *Biochem. J.* **43**, No. 3, xxxvii (1948).
4. J. H. Birkinshaw and W. P. K. Findlay, *ibid.* **34**, 82 (1940).
5. F. F. Nord and J. C. Vitucci, *Arch. Biochem.* **14**, 243 (1947); *ibid.* **15**, 465 (1947).
6. J. H. Birkinshaw, A. Bracken, W. P. K. Findlay, *Biochem. J.* **38**, 131 (1944).
7. K. Longree, *Phytopathol.* **30**, 793 (1940).
8. This report is contribution No. 26 from the Institute of Cellular Biology. The work was supported by a grant from the University of Connecticut Research Foundation.

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Statistical Codes for Geometrical Figures

Abstract. A point function may be defined on the "cells" of cellular approximations to plane figures which retains much of the information of the figures and whose distribution function is relatively invariant with respect to cell size; the function is useful in constructing a quantitative field theory in embryology and a nonaddressed system for pattern generalizing in neurophysiology.

It is the purpose of this note to present some of the properties of a coordinate free code for geometrical figures and suggest some applications of such to the theory of embryology and neurophysiological models. The discussion is limited to the two-dimensional case.

Consider the approximation of a plane figure F by a cellular structure of N cells each of area A/N. Call the cells x_i, where $i = 1, \ldots, N$. Let $\mathrm{d}(x_i,x_j)$ be the distance between the midpoints of cells x_i and x_j. A single-valued point function, $\mathrm{r}(x_i)$, is then defined by the set of equations,

$$\mathrm{r}(x_i) = K - \frac{U}{\left[N\left(\frac{A}{N}\right)\right]^{1/2}} \sum_{j \neq i} \frac{\mathrm{r}(x_j)\left(\frac{A}{N}\right)}{\mathrm{d}(x_i,x_j)}, \quad i = 1, \ldots, N, \qquad (1)$$

where K and U are appropriate constants. Before the biological interpretations are given to these functions an important mathematical property will be developed. To do this let $N \to \infty$, in which case the sequence of functions defined over the cellular approximations to the figure F converge to the function $\mathrm{R}(x)$ defined by the integral equation,

$$\mathrm{R}(x) = K - \frac{U}{A^{1/2}} \int_F \frac{\mathrm{R}(y)}{\mathrm{d}(x,y)}\,\mathrm{d}A. \qquad (2)$$

From this it follows that the normalized distribution functions of the $\mathrm{R}(x)$ functions defined over two figures of the same shape but of different areas A and A' will be the same. This follows since if x,y and x',y' are homologous pairs of points of similar figures F and F' of areas A and A' then,

$$\frac{\mathrm{d}(x,y)}{A^{1/2}} = \frac{\mathrm{d}(x',y')}{(A')^{1/2}},$$

and,

$$\frac{\mathrm{d}A}{\mathrm{d}A'} = \frac{A}{A'}.$$

Hence,

$$\mathrm{R}(x') = K - \frac{U}{(A')^{1/2}} \int_{F'} \frac{\mathrm{R}(y')}{\mathrm{d}(x',y')}\,\mathrm{d}A'$$

$$= K - \frac{U}{A^{1/2}} \int_F \frac{\mathrm{R}(y')}{\mathrm{d}(x,y)}\,\mathrm{d}A.$$

Since the point function $\mathrm{r}(x_i)$ defined over a cellular approximation to a figure itself approximates the function $\mathrm{R}(x)$ the above result implies that the distribution functions for $\mathrm{r}(x_i)$ functions defined over a figure are relatively invariant with respect to changes in cell arrangement and size.

It can now be shown how a shaped figure (organ) could develop with the aid of an $\mathrm{r}(x_i)$ code. Let $\mathrm{D}(\mathrm{R}(x))$ be the distribution code defined by the figure, and let us suppose a single original cell contains the information needed to define this function. Suppose the cell divides and segregates in the two daughter cells the information defining the upper and lower halves of the distribution, and that when these cells divide they segregate the information defining the quartile distributions, and so on. Suppose that the rate of division is uniform and that cells grow to a uniform mature size before they divide; then A/N is a constant, and since N depends on the number of cell divisions a simple memory mechanism can provide each cell with the variable A. Suppose also that each cell c_i produces at its center x_i a substance S at a rate $\overline{\mathrm{R}}(c_i)$ equal to the average value of the distribution defined on it. Suppose that S diffuses freely except for a small region Y_i around its point of origin x_i in each cell. Suppose S may both leave and enter Y_i but that a proportion $1\text{-}[U''/(A^{1/2})]$ is destroyed leaving the region. Now, for diffusion in the plane the concentration at any point y is the sum of the components due to each source. The component at y due to $x_i \neq y$ is inversely proportional to the distance between y and x_i and directly proportional to the source strength of x_i. Hence, for a point y_i