



Computer as Aid in Describing Form in Gastropod Shells

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receiver to donor after synthesis is completed. This also indicates the expected localization of the enzymes or catalysts within the cell; only the substrates are diffusible. If either the catalyst or the final product is diffusible, both of the combined tissues should develop pigment. This was observed rarely but only in older, fungus-infected pairs. A preliminary gene action sequence has been constructed by using this technique and simple reasoning (9).

The inhibitor C^I develops pigment in all pairs; c_1 tester develops pigment in all pairs except with C^I ; c_2 tester develops pigment in all pairs except with c_1 and C^I (however, the behavior of c_2 is erratic); r with all except C^I , c_1 , and c_2 ; a_1 with all except C^I , c_1 , c_2 , and r (weak response to in); a_2 develops pigment only with bz_1 and bz_2 (weak response to in).

The mutants bz_1 , bz_2 , and in have anthocyanin pigment, and when they are combined with colorless mutants, pigment appears in the colorless tissue. Simple diffusion of the pigment already synthesized possibly could simulate interaction in pairs involving these factors. Some further experiments were performed to circumvent this possibility. When tissues of the colorless double recessives $r\ bz_1$ and $r\ bz_2$ are combined in the four possible combinations with the single recessives bz_1 and bz_2 and subjected to the standard conditions, only $r\ bz_1$ develops pigment in $r\ bz_1$: bz_2 pair, while all others remain colorless (or the original bronze color), despite even the greater intensity of anthocyanin in bz_1 mutant as compared with bz_2 . These observations clearly suggest that simple diffusion of anthocyanin from bronze tissues does not occur and that R and Bz_1 precede Bz_2 in their action. A similar test was carried out with the double recessive $bz_1\ bz_2$, which has practically colorless aleurone tissue and could not transfer significant quantities of anthocyanin to a pair mate. In pairs of this type with a_1 and a_2 testers, only the tester develops pigment; this can be interpreted, as before, to mean that the action of A_1 and A_2 precedes that of both Bz_1 and Bz_2 . In the case of intensifier (in), which causes enhancement of the pigment over the normal purple (probably not changing the nature of anthocyanin), it is not clear whether the diffusion of anthocyanin is involved in combinations.

All these observations can be combined consistently into one linear sequence of action: $(C^I)-C_1-(C_2)-R-(In)-$

$A_1-A_2-Bz_1-Bz_2$ -anthocyanin. This sequence confirms unequivocally and considerably extends previously postulated sequences (4, 7). The parentheses indicate doubt based on the possible lack of straightforward relation of C^I and In to the sequence and on contradictory results with c_2 tester.

These studies point out clearly that a definitive gene-action sequence can be established directly even without extracting or isolating intermediates from the active tissues. The characterization of these diffusible substrates can be expected to reveal the intermediates and reaction steps in the biosynthesis of anthocyanin and to lead to further analysis of the mechanism of gene action and interaction in this system. Finally, this method can be extended to other organisms and systems having gene-controlled, step-wise reactions involving complementary factors in the biosynthesis of a final product if the intermediates are diffusible and are not cell-limited, if the substrates are readily utilized and a unidirectional effect obtains, and if the product-identification technique is simple. The ease of allelism tests in diploid organisms has resulted in the accumulation of many series of complementary factors to which this technique may apply, for example, eye and body colors, carotenoids and chlorophyll, leaf wax, and stature (10).

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10. This report is a contribution from the Crops Research Division, U.S. Agricultural Research Service, and the Missouri Agricultural Experiment Station (Journal series, No. 2415). It is also part of a doctoral thesis submitted to the University of Missouri by one of us (G.M.R.). Our work was aided by National Science Foundation research grants G5535 and G12965.

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Computer as Aid in Describing Form in Gastropod Shells

Abstract. The basic form of many coiled gastropod shells can be defined by four parameters. A digital computer with automatic plotting equipment can be used to make graphical reconstructions of a shell from any given values of the four parameters.

The study of external morphology of coiled snails requires a scheme for describing the basic form of the shell. To be effective, this scheme must not only describe the shell but also place it in some conceptual framework which implies comparison with other known forms—just as description of a color in terms of wavelength of light fixes that color in relation to other colors. In snail description, form categories based on overall aspect are commonly used (*turbiniform*, *naticiform*, *biconical*, and *obconical*, for example) but this system is far from ideal because it tends to split continuously varying spectra into rather arbitrary types that are difficult to compare rigorously.

In 1961 I proposed an alternative method based on a mathematical model defined by four measurable parameters of shell growth (1). The four parameters are reviewed below. Each of them represents some generalization, and thus the model should not be expected to be reproduced exactly in nature.

The first parameter is the shape of the generating curve. It is illustrated in Fig. 1b and is defined (2) as the cross-sectional outline of the hollow tube (helicocone) which coils about a fixed axis as the shell grows. The generating curve becomes progressively larger with each revolution about the axis but retains an essentially constant shape. In a plane of cross section that contains the axis of a snail, we see replicas of the generating curve at intervals of 180° in its path about the axis.

The second parameter is the position of the generating curve relative to the axis of coiling; the third, the rate of increase of this curve in size (which is exponential); and the fourth, the curve's rate of translation along the axis (also exponential). The first and second parameters can be defined by a sketch showing the generating curve and the axis; the third, by a constant, w , the factor by which any linear dimension of the generating curve is enlarged during one full revolution; and the fourth (translation), by a constant, t , which is the proportion of the height of a generating curve (measured parallel to the axis) which is covered by the succeed-

ing generating curve on the same side of the axis. The definition of the four parameters is illustrated in Fig. 1c. (In this figure translation is shown graphically also, by the position of the horizontal dashed line.)

It has been shown that the information in Fig. 1c is sufficient to reconstruct views of the snail comparable to Figs. 1a and 1b (1, pp. 606, 607). The reconstruction can be done most quickly and accurately, however, with a digital computer. By the computer method, the shape of the generating curve is expressed in terms of the coordinates of a convenient number of points on the generating curve (determined graphically). The position of the generating curve is fixed by using an x - y coordinate system such that the y -axis coincides with the axis of coiling. A simplified example is shown in Fig. 1d; here, each of 20 points is defined by its x and y values taken from the figure. The 20 pairs of coordinates combine with the constants w and t to form the input data for the computer. With this information, the computer can be programmed to produce x and y values for points determining the shape and position of any other generating curve on the cross section. For example, if we consider the x , y input data to represent the smallest gen-

erating curve on the right side of the cross section, the x -coordinates of points for the next larger replica on the same side of the axis are obtained by multiplying each of the original x values by w . The y -coordinates are similarly obtained, except that a value for translation must be added to each result. Machine time (with an IBM-7090) and therefore cost are negligible. In this study, the output data were plotted automatically on a Calcomp x - y plotter with the result shown in Fig. 1e. A perspective drawing can be constructed readily from this cross section (3). Figure 2 shows five hypothetical snails drawn from computer-produced cross sections. Only the rate of enlargement of the generating curve (w) and the rate of translation (t) are varied along the series. Thus, the morphological extremes in Fig. 2 can be described and related to each other by a few definable changes in basic parameters.

The method can also be applied to many species which do not adhere strictly to the "rules" of coiling. For example, the rounded spire of the pupiform snail can be produced by replacing the constant w by a variable w which decreases regularly with each revolution of the generating curve about the axis. This assumes, of course, that the depart-

ure from the "rules" is systematic. In cases where such departures cannot readily be expressed mathematically, the model loses its effectiveness.

It must be emphasized that the mathematical model applies only to the basic form of the shell (as does the system based on form categories). Equally important to the morphologist are the many features of shell ornamentation, and so forth, and soft part anatomy, but these are effectively treated by other means.

If the method described here is to be used successfully, problems must be chosen with an eye toward the limitations as well as the strengths of the basic model. For example, precise description of a single shell is still accomplished most effectively by a photograph because of the inevitable departures from geometric perfection. On the other hand, it would be appropriate to use this model to define limits for turbini-form and comparable categories or even to replace them by a flexible system based on differences in the four parameters.

At present, a project is under way to "map" the total spectrum of possible variation in snail form. The computer method is being used to produce a series of ideal snails which vary in w

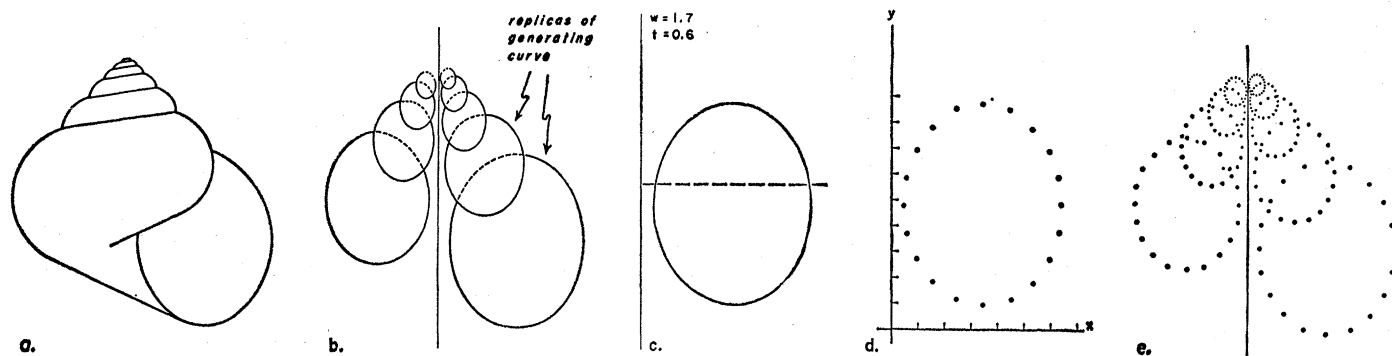


Fig. 1. Stages in the reconstruction of snail form from the four basic parameters.

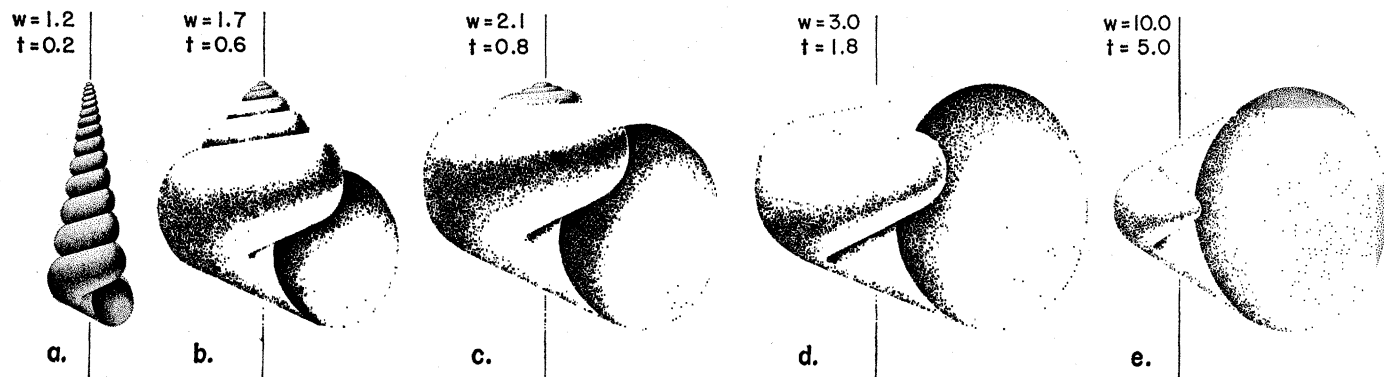


Fig. 2. Hypothetical snail forms drawn from cross sections made by the computer method.

from 1.0 to 20.0, in t from 0.0 to 20.0, and which include several generating curve shapes, at varying distances from the coiling axis. The resulting array will be an extension of Fig. 2 and may find application to a variety of problems of gastropod evolution and ecology. For example, the problem of the efficiency with which a given snail uses skeletal material may be studied by this means, because shell geometry has a pronounced effect on the amount of skeletal material necessary to produce a given volume of living space (4).

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3. The same approach can be applied to coiled cephalopods and to the individual valves of pelecypods and brachiopods. In general form, these differ from snails only in values of the four parameters.
4. I am indebted to all those who gave helpful advice and criticism at various stages of this study. In particular, I wish to thank Erle G. Kauffman, Lynton S. Land, Harald A. Rehder, and Aaron C. Waters for reading early drafts of the manuscript, M. Patricia Power for aid in computer methods, John S. Spurbeck for preparing the illustrations, and Mary Gill for typing the manuscript.

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Two-Dimensional Gel Electrophoresis

Abstract. Improved resolution of serum protein mixtures is effected by electrophoresis, first in a 5 percent acrylamide gel following which a strip of the resolved pattern is embedded in 8 percent gel and subjected to a second electrophoresis separation at right angles to the first. Lactic dehydrogenase enzymes appear as small rectangular spots lying on an oblique straight line passing through the point of initial application.

Two-dimensional electrophoresis, employing paper medium in the first direction and starch gel in the second direction, has been effectively applied by Poulik and Smithies (1) to the resolution of serum proteins. Hermans, McGuckin, McKenzie, and Baird (2) described a similar technique employing paper in the first direction and cyanogum gel in the second direction. Ashton (3) also used agar in the first direction combined with starch gel in the second direction with good resolution.

Experimental studies of the factors affecting electrophoretic migration and resolution in acrylamide gels, now in progress in this laboratory, have demonstrated significant nonlinear effects of gel concentration on the electrophoretic mobility of various proteins. These studies suggest that two-dimensional gel electrophoresis employing a low-concentration gel in the first direction followed by a high-concentration gel in the second direction, will produce separations analogous to those obtained in two-dimensional paper chromatography. The resolution should be obtained without change in pH, composition, ionic strength, or electrophoretic conditions, inasmuch as they depend solely on the concentration of the gel itself.

The procedure employed in the experiments reported here was as follows. A 5 percent concentration of acrylamide gel was prepared in pH 9.0 tris buffer by the method of Raymond and Nakamichi (4) and then poured into the vertical gel electrophoresis cell described by Raymond (5). A sample of fresh human serum 0.04 to 0.06 ml in volume was applied to a 2-cm wide slot in the top of the gel. Electrophoresis was effected at 300 v, 50 to 150 ma for 1½ hours at which time the albumin (rendered visible by prestaining with a small crystal of bromphenol blue) had migrated approximately 10 cm. The gel slab was removed from the apparatus and a 1-cm wide strip was excised longitudinally through the pattern. This strip was replaced transversely in the gel compartment of the cell. The compartment was then filled with an 8 percent acrylamide gel solution in the same buffer. The solution gelled within 30 min, with the 5 percent slab embedded across the top of the gel. Electrophoresis was again effected at 300 v and 50 to 150 ma, increasing the time to 3 hr to compensate for the reduction in average migration velocity of the migrating components.

Following electrophoresis the entire gel slab can be stained for protein and other components in the usual way. It is particularly effective to use multiple stains in sequence. Figure 1 shows lactic dehydrogenase isozymes stained by the procedure of Latner (6), which form a linear series of spots that do not coincide with any of the protein components demonstrated by amidoblack stain. The straight line through the lactic dehydrogenase spots passes

through the point of initial application, suggesting that these isozymes all have the same molecular size but differ by equal increments of charge. The components of the haptoglobin complex, on the other hand, fall on a smooth curved line which is asymptotic to the 5 percent direction, suggesting a constant ratio of charge to molecular size with increasing size of the molecule.

The initially thin layer of sample applied in the first direction diffuses as the components migrate through the gel so that the resolved components form spots 1 to 3 mm long (in the direction of travel) at completion of the first electrophoresis. (Albumin, because of its greater concentration, spreads 10 to 15 mm). At the start of

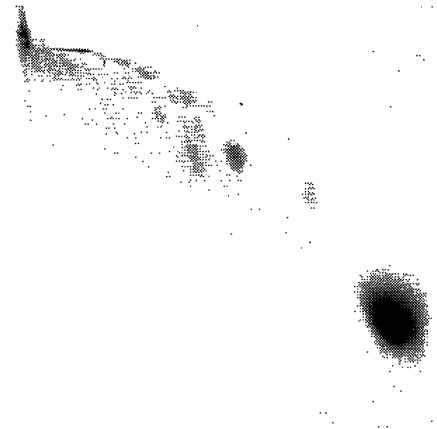


Fig. 1. Two-dimensional electrophoresis in acrylamide gel, 5 percent and 8 percent, tris buffer pH 9.0. Three rectangular spots on an oblique line near the center are lactic dehydrogenase isozymes, others are proteins stained with Light Green SF. Origin at upper left, 5 percent direction across, 8 percent direction down.

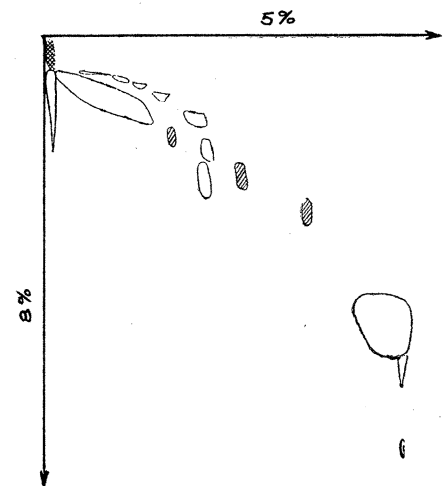


Fig. 2. Tracing of the gel shown in Fig. 1. Cross-hatched spot is origin, diagonal-hatched spots are lactic dehydrogenase isozymes.