assumed. A value of 18 ml/mole was used for the partial molal volume of water and a value of 17.1 ml/mole, for the partial molal volume of sodium chloride (*4*).

For the range of pressures and compositions in Table 1

$$\mu_1 - \mu_2 = RT \ln[(1 - n_1)/(1 - n_2)] + \cdot \bar{v}(p_1 - p_2) \quad (5)$$

and

$$\mu'_1 - \mu'_2 = RT \ln(n_1/n_2) + \bar{v}'(p_1 - p_2) \quad (6)$$

where n_1 and n_2 are the mole fractions of NaCl on each side of the membrane, $\bar{v}$ is the partial molal volume of water, $\bar{v}'$ is the partial molal volume of NaCl, and p_1 and p_2 are the pressures on each side of the membrane.

In Fig. 1, J has been plotted against $\mu_1 - \mu_2$, and J', against $\mu'_1 - \mu'_2$. In each case the data can be represented by a straight line through the origin; this shows that the resistances are constant over the ranges of pressure and concentration given in Table 1. The resistance is the reciprocal of the slope of the line.

The calculations in Table 1 show that

$$RT \ln[(1 - n_1)/(1 - n_2)]$$

is small as compared to $\bar{v}\ (p_1 - p_2)$ for the range of data presented. So the rate of flow of water in effect is proportional to the pressure across the membrane. Conversely, values of $\bar{v}'\ (p_1 - p_2)$ are small as compared to

$$RT \ln(n_1/n_2)$$

so the rate of flow of NaCl depends primarily on the concentration of NaCl on each side of the membrane. At low pressures, water flow is low and salt rejection appears poor. At higher pressures, water flow is higher and salt rejection appears improved. Mahon (*3*) pointed out that water flow was dependent on pressure, and salt flow, on concentration, but he gave no reasons for such dependence.

Data over wider ranges of pressure, membrane thickness, and concentration are necessary to establish the limits of application of the theory introduced here. Within these limits it will be possible to predict a membrane's performance in ultrafiltration, for different thicknesses, pressures, and concentrations, from experiments yielding the values of the various modified diffusion coefficients through the membrane.

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Inter-tissue Complementation: A Simple Technique for Direct Analysis of Gene-Action Sequence

Abstract. Sequence of gene action can be determined from unidirectional complementary interactions between synthetically active but genetically blocked tissues. A linear sequence of gene action has been constructed for anthocyanin synthesis in maize by this approach. This technique probably can be applied to other systems and organisms that have the requisite circumstances.

Application of modern biochemical methods to the many phenotypically established genetic systems in higher organisms can be aided by simple methods for the determination of gene-action sequence. A disarmingly simple prediction can be made that biochemical interactions will occur between excised tissues of complementary genotypes. This prediction is based on the known accumulation of intermediates preceding a genetic block (*1*) and of the utilization of appropriate intermediates by living tissues (*2*). Although these interactions would be analogous to those found in tissue transplants with *Drosophila* and in cross-feeding in microorganisms, they are predictably unidirectional and the results would permit direct determination of gene-action sequence. This report describes such experiments in maize, establishing the sequence of gene action in anthocyanin synthesis.

Anthocyanin synthesis in the aleurone tissue of maize is controlled by several known complementary genes, several modifiers, and a dominant inhibitor, which variously block, reduce, or enhance the formation of pigment. The presence or absence of anthocyanin pigment in this tissue is controlled by the complementary factors A_1, A_2, C_1, C_2, and R, which must be present in dominant condition for the production of anthocyanin. If any one is homozygous recessive or if dominant C^I is present, colorless aleurone results. The factors Bz_1, Bz_2, Pr, and In determine the intensity and the nature of anthocyanin. Previous investigations (*3–6*) have indicated that these factors act in a step-wise manner in the formation of anthocyanin. A gene-action sequence based on indirect reasoning from interactions has been constructed, but it was pointed out that direct studies with active synthetic stages were needed (*7*).

McClintock (*8*) observed that complementary pigment synthesis occurs at the line of contact between genetically colorless (inhibited) C^I Bz cells and nearly colorless (bronze) C bz cells. The relation of this observation to the diffusibility of anthocyanin precursors and to the sequence of gene action has been discussed by Rhoades (*5*). These considerations led to the present repetition of these effects in vitro with excised tissues of complementary genotypes.

The individual testers, a_1, a_2, c_1, c_2, r, bz_1, bz_2, and in, all singly recessive, and homozygous C^I were grown and self-fertilized. In the period 22 to 25 days after pollination, when the tissue would be actively synthesizing pigment if it were genetically competent, aleurone tissue was peeled from fresh kernels. Two distinguishable pieces of different colorless mutants were pressed together and the pair was placed on 0.8 percent agar at 25°C. The results can be illustrated by a specific example: When a_1 and a_2 tissues are pressed together, complementary interaction results in anthocyanin synthesis in 1 to 2 days and is unidirectional—that is, only the a_1 tissue (the "receiver") develops pigment while the a_2 ("donor") tissue consistently remains colorless, as also do control tissues. The pigment was regularly confirmed to be anthocyanin by the standard test with dilute acid. These results clearly suggest that diffused substrate from the donor is used by the receiver to synthesize anthocyanin and that the block in the donor succeeds that in the receiver. The receiver of course would need to carry the dominant factor lacking in the donor as well as the subsequent factors in the sequence.

The "donor-receiver" relation is interchangeable and varies with the combination of mutants. For example, a_1 tissue, receiver in a_1-a_2 combinations, becomes a donor in a_1-r combinations. In any two paired mutant tissues only one develops pigment and the other never does, indicating that little or no diffusion of the pigment occurs from

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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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. 1*b* 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-