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Stability of the Gene

The concept of the stability of the gene is one of the fundamental principles underlying the theory of the gene. According to Morgan (*1*), "Mendel's theory of heredity postulates that the gene is stable. It assumes that the gene that each parent contributes to the hybrid remains intact in its new environment in the hybrid. . . . If a black guinea pig is bred to a white one, the offspring are black. If these are inbred, the offspring are three blacks to one white. The extracted whites breed as true as the original race of whites. The white gene has not been contaminated by the black gene in their sojourn together in the hybrid."

The conclusion "that the white gene has not been contaminated" is an inference based on assuming a gametic ratio of 2 : 2 at every meiosis. The "area of inference" in current theory is defined in Fig. 1 (*2*). The genotypes of the sperm produced from the spermatocyte of a hybrid male are inferred by analysis of the offspring from a mating with a homozygous recessive female. Approximately one-half of the offspring show the dominant and one-half the recessive phenotype, supporting the view that one-half of the sperm carried the dominant and one-half the recessive gene. The identical result could have been obtained, however, if some spermatocytes were *XXXx*, while a similar number were *xxxX* (*X* and *x* indicating the dominant and the recessive alleles, respectively).

Actually, extensive data seldom show a precise 1 : 1 ratio in the zygotes. Data from an experiment involving 9027 *Drosophila* (*3*) (the female was heterozygous) revealed significant deviation from a 1 : 1 ratio. Small significant deviations from a 1 : 1 ratio are seldom mentioned by geneticists, but when irregular ratios are brought to attention, they are invariably considered to result from differential viability of genetically different classes of individuals (or diminished viability of the sperm or eggs carrying the gene involved). Differences in viability of the different classes of individuals are easily demonstrable and are assumed to be adequate evidence for the view that the stability of the gene has been maintained.

An alternative interpretation is equally probable. The 52 : 48 ratio could have been produced by a few *XXXx* spermatocytes. In Fig. 1, the area of inference is enclosed in a broken line to emphasize that direct information is not available bearing on the genotypes of the sperm produced by individual spermatocytes. The view that the gametic ratio in every heterozygous spermatocyte is 2 : 2 is clearly an inference.

Direct evidence from tetrad analysis of a *Saccharomyces* hybrid heterozygous for the gene controlling maltose fermentation contradicts this inference (*4*). A population of 920 gametes produced 48.5 ± 1.65 percent *X* : 51.5 ± 1.65 percent *x*, which does not differ significantly from a statistical ratio of 50 percent *X* to 50 percent *x*, but direct evidence revealed four unexpected kinds of tetrads: *XXXX*, *XXXx*, *xxxX*, and *xxxx*. The irregular tetrads are of two types—those that contain an excess of dominant genes over the expected 2 : 2 and those that contain an excess of recessive genes over the expected 2 : 2. Each zygote was heterozygous for two pairs of genes, and the tetrads that contained more than two dominant maltose genes also contained more than two dominant α-methyl glucoside genes. This dependence is interpreted to mean that the excess of dominant genes was produced by the segregation of a tetraploid zygote produced by the fusion of cells of like mating type. Specific examples of tetraploid segregations have been described by Lindegren and Lindegren (*5*) and by Roman, Hawthorne, and Douglas (*6*).

Although some tetrads containing an excess of recessive genes are susceptible to this explanation, extensive analysis of a single irregular tetrad (*7*) has shown that polyploidy was not involved, and the irregular ratio has been interpreted to result from gene-conversion. Many ingenious hypotheses have been proposed to explain the unexpected types on the assumption of genic stability. This procedure is based on the assumption that the gametic ratio at every meiosis is 2 : 2. The inadequacy of these hypotheses will be dealt with elsewhere; the purpose of the present paper is to point out that direct evidence takes priority over inference. Genic instability is expressed in the following ways.

1) Some genes are relatively stable and rarely convert. The gene controlling the synthesis of adenine has undergone conversion only rarely (*7*). Others are highly unstable; the *MG mg* alleles undergo conversion frequently.

2) Genes may be markedly stable in one mating and unstable in another. The *MA/ma* and *MG/mg* alleles behaved very differently in different families (*4*).

3) Some genes have several manifestations, some of which are extremely stable, while others convert frequently. The *MZ* allele in *Saccharomyces* (*8*) has at least five manifestations involving ability to adapt to maltose, turanose, sucrose, α-methyl glucoside, and melezitose. The ability to adapt to maltose is lost rarely, while the ability to adapt to melezitose is lost readily. It is possible that most genes have several manifestations that differ in stability and that a gene is usually identified by its most stable manifestation.

4) Some genes are phenomenally stable and have only rarely or never revealed detectable conversion, even after analysis of hundreds of tetrads. (It

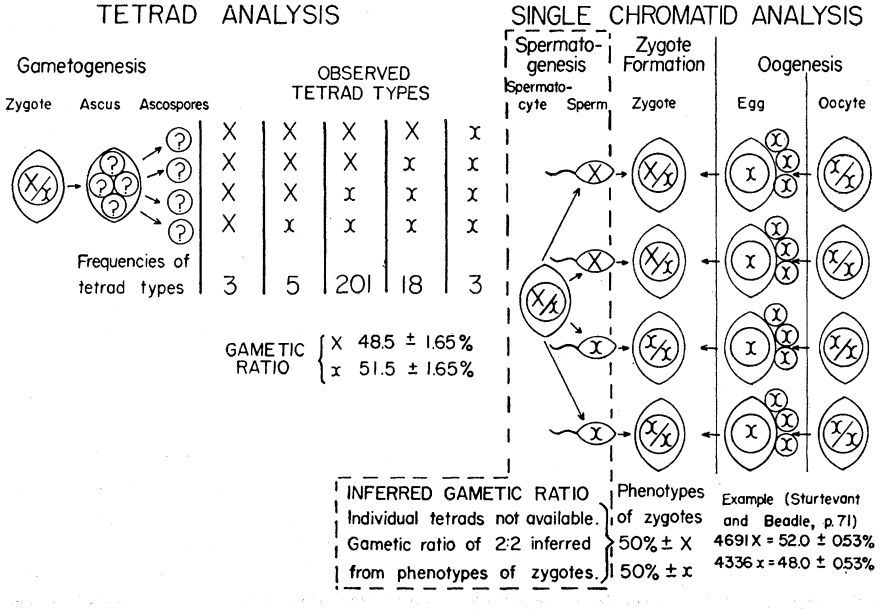

Fig. 1. The differences between tetrad analysis and single chromatid analysis; the "area of inference" in single chromatid analysis is defined by a broken line.

is important to point out again that instability can be detected only by tetrad analysis.) The genes controlling mating type, uracil synthesis, and copper sensitivity are of this type.

Geneticists prefer to work with stable genes, especially in map construction. Genes that occur with higher than the expected frequency in a population are called genes of "high penetrance." It is possible that deviations in "penetrance" may involve gene-conversion. Much of the early work in genetics was devoted to the selection of stocks carrying "good" genes. Although value judgments are rarely expressed in print, it is interesting to note that even the most astute and objective workers (*9*) have made them: ". . . it is a simple matter to determine the relation between different types of double exchanges, though this has not as yet been *satisfactorily* done, because of lack of *good* genetic characters localized within one arm of a single chromosome."

It is difficult to say how much of the attitude toward gene conversion has its origin in the preference of the geneticist for "good" genes that behave "properly." The designation genetical "marker" may provide a key to this psychological problem, since the term implies a relatively solid monument not subject to capricious change.

The theory of gene-conversion proposed by Winkler (*10*) is now known to be invalid in its original form, since it proposed to explain all recombinations by gene-conversion. The experiments presented by Stern (*11*) as refutation of Winkler's theory proved that crossing-over occurred but failed to prove that conversion did not. Stern showed that *all* recombination was not conversion, but his conclusion that this excluded the possibility of gene-conversion was incorrect. The climate of genetical thought in the 1930's was favorable to Stern's argument, and its fundamental fallacy was not discovered. The failure to evaluate it critically resulted in the persistence of the dogma of genic integrity and delayed the demonstration of gene-conversion.

Although geneticists have resisted acceptance of the simpler interpretation of the direct data contradicting the assumed 2 : 2 ratio at each meiosis, they have accepted evidence indicating that genes can be transformed from one type to another (i) by treatment with a nucleic acid extract from cells carrying the complementary allele or (ii) by infection with a virus that previously had infected a cell carrying the complementary allele. These phenomena of transformation (i) and transduction (ii) are gene-conversions in an operational sense (if genes are, in fact, involved) mediated indirectly through chemicals and viruses. The readiness with which these phenomena are accepted as evidence of gene-change and the resistance to the direct evidence that two genes present in the same nucleus can undergo conversion is evidence of the attitude that considers an old accepted concept more reliable than a new one.

The chronological sequence in which scientific discoveries are made has a direct bearing upon the way in which they are interpreted. Because the concept of genic stability preceded the discovery of direct data revealing true gametic ratios, the climate of genetical thought has not been receptive to direct evidence contradicting the concept of genic stability. Data that confirm a well-established theory are generally accepted without critical evaluation. The established concept serves as a fundamental point of reference, and new data are judged by the closeness with which they fit the established point of view. In genetics the stability of the gene is such a point of reference, and other data are evaluated by considering whether or not they support this fundamental concept. This has produced a system of circular reasoning in which experimental data are evaluated by the precision with which they confirm the concept of genic stability and this "confirmation" is taken as evidence for the reality of the concept.

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References and Notes

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Localization and Quantitation of Water in Biological Samples by Historadiography

The development of the interference microscope has made it possible to determine the dry weight and water content of cells and parts of cells (*1*). Although this technique is especially well suited for work on isolated cells, it is perhaps less successful with larger samples, such as microtome sections of tissues fixed by the freeze-drying method. On the other hand, soft x-ray absorption has been applied with particular success to the measurement of dry weight in both tissue sections and cytological preparations (*2, 3*), but heretofore it has not been possible to use this technique for a comparable measurement of water. An instrumental improvement (*4*) by which the sample is permitted to remain at room conditions outside of the x-ray tube has now opened the way for an extension of this method to include the determination of water *in situ*. This report (*5*) is concerned with the theoretical basis for the measurement of the water and the experimental method for each of two procedures that have been devised.

1) If microradiograms are made by the conventional method (*3*), with the newer equipment (*4*), of a fresh-frozen biological sample before and after removal of water by freezing-drying, densitometric measurements of the photographic images will permit calculation of the respective masses and, therefore, of water content by difference. This is based on the following.

$$I_T = I_o e^{-[(\mu/\rho)_s \cdot m_s + (\mu/\rho)_w \cdot m_w]} \quad (1)$$

where the subscripts T, s, and w refer to total sample before dehydration, organic substance in the sample, and water, respectively, so that I_T and I_o represent the x-ray intensities transmitted and incident; and where (μ/ρ) is the mass absorption coefficient (that is, μ is the linear absorption coefficient and ρ the density), and m the mass per unit area. Since the extinction, E_T, equals $\ln(I_o/I_T)$,

$$E_T = (\mu/\rho)_s \cdot m_s + (\mu/\rho)_w \cdot m_w \quad (2)$$

After the removal of the water, the extinction becomes

$$E_s = (\mu/\rho)_s \cdot m_s \quad (3)$$

Likewise we can write

$$E_w = (\mu/\rho)_w \cdot m_w \quad (4)$$

and therefore

$$m_w = \frac{E_T - E_s}{(\mu/\rho)_w}. \quad (5)$$

The values of E_T and E_s obtained by measurement of I_o, I_T, and I_s (the intensity transmitted by the dried sample), permit the calculation of m_w, since $(\mu/\rho)_w$ can be calculated from standard tables.

For the experimental procedure, it would be preferable to freeze the fresh specimen suddenly in isopentane, dichlorodifluoromethane, or any of the other liquids commonly used for this purpose, which has been cooled by liquid nitrogen. The frozen specimen may then be sectioned in a cryostat or cold room and the sections used to obtain a microradiogram with x-ray apparatus inside of the cold chamber. It is essential that the