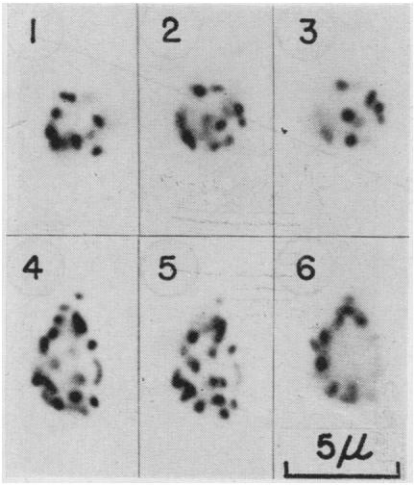
Reports

Colchicine-Induced Polyploidy in Chlamydomonas

Although the alkaloid colchicine has proved to be an effective agent for the induction of polyploidy in higher plants, comparable success has not been achieved with microorganisms. In a recent review of the literature, Eigsti (*1*) concluded that no substantial evidence existed for the induction of polyploidy in microorganisms with colchicine. However, a paper by Tschermak (*2*), not listed in Eigsti's review, reported mitotic metaphases with the doubled chromosome number in cultures of *Oedogonium* after treatment with colchicine.

The establishment of polyploid strains of microorganisms suitable for genetic analysis would be of considerable significance for studies of the physiology of polyploidy. In spite of previous unsuccessful attempts reported in the literature (*3*), a series of colchicine treatments was made (*4*) as part of a larger study of inheritance in *Chlamydomonas*.

Haploid vegetative cells of *C. eugametos* Moewus were exposed to concentrations of 0.01, 0.1, and 1.0-percent colchicine using standard microbiological spot-plate technique. Essentially no inhibition of growth or cell division was noted at 0.01 or 0.1 percent, but, in a narrow zone around the 1.0-percent solution, cell division was completely inhibited, yet cell enlargement proceeded, producing cells up to 10 times normal cell volume.



Figs. 1–6. Photomicrographs of two mitotic metaphase nuclei of *C. Reinhardi*: (1–3) three focal levels of a normal haploid nucleus; (4–6) three focal levels of a nucleus from a colchicine-induced diploid strain.

A study of this effect was made by fixing cells, in a solution of 3 parts ethanol to 1 part acetic acid, at intervals during a 55-hour continuous exposure to 1.0-percent colchicine. Using the selective staining method of De Lamater (*5*) it was possible to observe clearly, C-mitoses, polyploid nuclei, and multinucleate cells. A high degree of polyploidy was evident by 24 hours. The majority of treated cells had not undergone cytokinesis by the end of 55 hours and were many times the size of the untreated cells, which had essentially completed a series of five divisions by the end of 18 hours. These observations confirmed the efficacy of colchicine and suggested the possibility of obtaining polyploid strains in culture.

Haploid vegetative cells of *C. Reinhardi* Dang. were cultured under optimum growing conditions, in a nutrient solution (*6*) containing 1.0-percent colchicine. Cells were removed and plated in colchicine-free media hourly. Cells arising from single, treated cells were subcultured for further study. Optimum exposure time was not established, but 8 hours proved sufficient to induce a doubling of the chromosome number. On the basis of the results of prolonged exposure of *C. eugametos* to colchicine, it is felt that viable strains possessing higher degrees of polyploidy can be obtained.

Exact determinations of chromosome number were difficult, owing to the small size of the chromosomes and the lack of flatness of the metaphase plates. Schaechter and De Lamater (*5*) reported a chromosome number of 18 ± 2 for *C. Reinhardi* Dang. In the present study, approximately 16 chromosomes were observed in the normal, haploid, vegetative cells, while the number in the colchicine-induced diploid was in excess of 28 and not more than 34. Figures 1–3 show a typical metaphase plate of the control photographed at three focal levels. A metaphase plate of the colchicine-induced diploid, photographed at three focal levels is shown in Figs. 4–6.

Growth rates were determined for these two strains, both in light on an inorganic medium (*7*) and in the absence of light in the same medium plus 0.2-percent sodium acetate. Rapidly growing cultures were adjusted to a standard optical density and were used to inoculate test-tube cultures that were grown at a light intensity of 700 foot-candles and with continuous agitation. Growth was measured as optical density at 5600 A. Maximum growth rate was essentially equal for all cultures; however, growth of both strains, in the absence of light, was preceded by a 48-hour lag phase. Dry weight of cells centrifuged from illuminated, rapidly growing cultures was 2.8 mg/10 ml of culture for both strains. Calculations of dry weight per cell and volume per cell were made from cultures of the two strains which were in the logarithmic phase of development. The ratio, induced diploid to haploid, for these values was determined as 1.68 for dry weight and 1.65 for volume.

Preliminary studies have shown that the rate of synthesis of cell material per unit of dry weight of inoculum was essentially the same in both strains. However, when calculated on the basis of rate of synthesis per genome, the diploid strain is only 70 percent as efficient as the haploid strain. This was demonstrated by starting with inocula containing equal numbers of cells for both strains and with inocula of equal dry weights. In both cases, analysis of parallel cultures, interrupted in the logarithmic phase of growth, showed that synthesis in the diploid strain attained only 65 to 70 percent of that possible if the rate of synthesis per genome had been equal. This loss of efficiency may be a consequence of the lower surface to volume ratio of the diploid cells or may simply be the result of the alteration of the nucleus to cytoplasm ratio (*8*). In a recent comparison of autotetraploid and diploid yeasts (*9*), it is stated that 4N cultures produced about 1.76 times the dry weight of 2N cultures under equal circumstances. However, standardization of inoculum seemed poor in this test.

These preliminary studies are being expanded, and determinations of x-ray sensitivity, pigment production, and biochemical fractions of the haploid and colchicine-induced diploid strains are in progress.

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References and Notes

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5. M. Schaechter and E. D. De Lamater, *Am. J. Botany* 42, 417 (1955).
6. D. F. Wetherell and R. W. Krauss, *Carnegie Inst. Wash. Yearbook* No. 53, 180 (1954).
7. R. Sager and S. Granick, *J. Gen. Physiol.* 37, 729 (1954).
8. Although the number of genomes per cell is doubled, the dry weight per cell increases only about 1.68 times.
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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

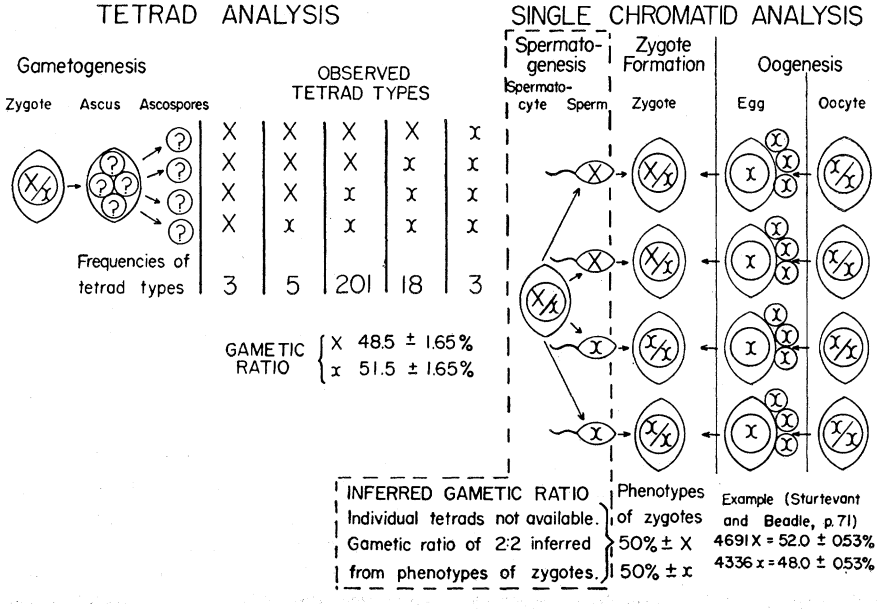

Fig. 1. The differences between tetrad analysis and single chromatid analysis; the "area of inference" in single chromatid analysis is defined by a broken line.