A SIMPLE CHROMOSOME SPREAD TECHNIQUE FOR UNARMORED DINOFLAGELLATES AND IMPLICATIONS OF POLYPLOIDY IN ALGAL CULTURES

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LOPER, C. L., STEIDINGER, K. A. & WALKER, L. M. 1980. A simple chromosome spread technique for unarmored dinoflagellates and implications of polyploidy in algal cultures. *Trans. Amer. Micros. Soc.*, 99: 343–346. Chromosome counts can be used to verify stages of dinoflagellate life cycles. However, standard chromosome techniques do not work well with dinoflagellate nuclei. A simple method involving cell lysis, heat, desiccation, and staining has been effective for *Ptychodiscus brevis* (Davis) Steidinger. Two vegetative count ranges were determined with this technique: (1) a haploid number of 121 ± 3 for field and recent culture specimens; and (2) a diploid number of 240 ± 6 for a 25-year old culture. The latter probably is a result of autodiploidy during extended culture conditions. Polyploidy may be occurring in many long-established algal cultures; therefore, the genetic status of present culture collections should be determined and stocks perhaps replaced.

In the study of haplont dinoflagellate life cycles, isomorphic haploid and diploid stages, and other heteroploid conditions, sometimes must be distinguished. Chromosome squashes, or spreads, can be used to detect or verify: (1) planozygotes and hypnozygotes in culture, if the progressive sequence was not observed; and (2) polyploidy, aneuploidy, or other chromosomal irregularities. The sexual cycle, commencing with gamete production, may be difficult to detect because the sequence leading to planozygote formation is short-lived; many species are isogamous, and vegetative and reproductive cells may be indistinguishable. Additionally, although over 70 extant benthic dinocysts have been described as resting stages, their role in dinoflagellate life histories is not clear because few have been verified as hypnozygotes or asexual cysts.

A rapid technique that produces well-spread chromosomes for easy counting would be advantageous in dinoflagellate studies. Standard techniques (Sunderland, 1973; Berlyn & Miksche, 1976), however, do not work well with many dinoflagellate chromosomes, and basic counts in one plane, or from one photomicrograph, are often difficult if not impossible to perform. Chromosome counts or count ranges are available for only about 45 species (Dodge, 1963; Loeblich, 1976), and this low number probably reflects difficulties in technique rather than lack of interest.

A simple technique for chromosome quantification is presented. Using this technique, we observed unexpected diploidy in one of our long-term isolates, which suggests that polyploidy occurred under culture conditions.

MATERIALS AND METHODS

The procedure involves cell lysis to free the nucleus, desiccation under heat to free, spread, and fix the chromosomes, followed by standard staining

of DNA. Neither the initial material nor the final chromosome spread must be chemically prefixed or preserved.

The chromosome spreads are prepared by the following method. Maintain a drying oven at a constant temperature of 49.5°C. Put one drop of concentrated culture material on a saliva-cleaned 76.2 × 25.4 mm (3 × 1″) microscope slide. Add one or two drops of hot distilled water (49.5°C), and spread the drop lengthwise over most of slide. Surface area is critical because it affects the desiccation rate. Place the slide across the rim of a beaker in the oven and let water evaporate until the slide is just dry (usually about 15 min). Add three or four drops of acetocarmine working solution (Humason, 1972), spread the solution over the slide, drain excess, and allow the slide to air dry again. Rinse the slide gently in distilled water and air dry. Observe the material with phase contrast and photograph. Mounting in a medium can increase resolution, but this is not recommended because it can displace chromosomes to lower the recorded count.

Print the photomicrographs on 8 × 10″ paper and gloss. To count the chromosomes, draw a pencil line over each one, and tally on a hand counter. When finished, hold the print horizontal to eye level to determine if any of the chromosomes have been overlooked.

Even with this method, a range of counts will result. Small variations in counts can be attributed to overlapping chromosomes, lost chromosomes, count errors, unequal mitosis (nondisjunction), or fragmentation. However, fragmentation with this technique was low. Comparison between the means of haploid and diploid cells and associated low standard deviations support this. A confidence interval statistic (CI) should be used to express the population mean and standard error (Steel & Torrie, 1960). The standard error is lower with more replicates. Not all nuclei in any one preparation will spread fully. Often within a single spread, outer chromosomes will be distinct and separated, while inner ones may be clumped. Counts should be done only on material where the spread is complete. With this technique, chromosomes may stretch and appear as those illustrated by Haapala & Soyer (1974).

Results and Discussion

The chromosome spread technique described here was designed for *Ptychodiscus brevis* (Davis) Steidinger to aid in the study of its sexual life cycle. Nevertheless, the technique may be applicable to other dinoflagellates, and even to benthic cysts with modifications to the basic procedure; e.g., isolation of spheroplasts (Adamich & Sweeney, 1976), or whole nuclei (Mendolia et al., 1966), and perhaps enzymatic or physical disruption of the nuclear envelope.

A 1953 clonal *Ptychodiscus brevis* isolate (provided by W. B. Wilson, Texas A. & M. University) had a chromosome count of 240 ± 6 at the 95% CI, n = 25, (Fig. 1). A nonclonal 1974 isolate (L. S. Tester, Florida Department of Natural Resources) had a chromosome count of 121 ± 3 at the 95% CI, n = 25, (Fig. 2). Both isolates were from Florida west coast neritic waters during red tides. Two 1976 isolates yielded counts within the range of the 1974 isolates, as did field specimens from the 1976 and 1977 red tide outbreaks.

We interpret the 121 ± 3 count as representative of a haploid cell, and the 240 ± 6 as representative of a diploid cell. However, our research indicates

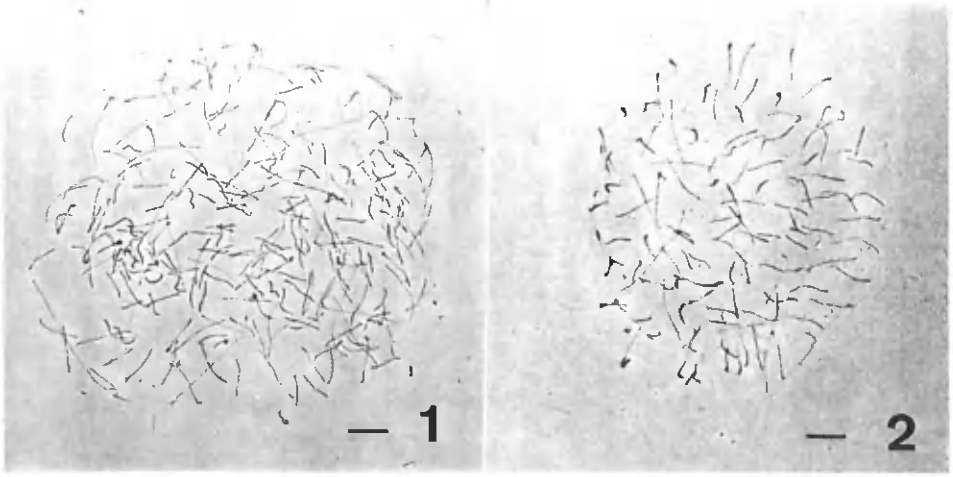

FIG. 1. Chromosome spread of the diploid *Ptychodiscus brevis* 1953 isolate. Scale bar represents 20 μm. FIG. 2. Chromosome spread of the haploid *Ptychodiscus brevis* 1974 isolate. Scale bar represents 20 μm.

that the diploid count represented vegetative cells, not planozygotes or dividing cells. Cells were harvested in the light period (10:14 LD cycle) following mitotic divisions, and were sampled over a period of one year from log and stationary cultures. Planozygotes, as the only cell type, would not be expected for that duration under these conditions. Fusion of gametes was never detected in the 1953 isolate under varied inducement regimes. Contrarily, fusion of isogametes and production of zygotes has been observed in the 1974 isolate.

Based on these results, we believe that the diploid cell type represents a form of autodiploidy, and that the haploid cell from recent culture isolates and field specimens represents normal vegetative material. Dinoflagellates are haplonts (cf. Steidinger, 1975; Loeblich, 1976), there being only one known exception, and therefore meiosis is zygotic.

Chromosomal polyploidy in algae (e.g., Cryptophyceae, Euglenophyceae, Dinophyceae) has long been postulated (Godward, 1966; Leedale, 1966) on the basis of high chromosome numbers, and in one class on viability of daughter cells after amitotic division. Abnormal mitosis or incomplete cytokinesis due to chemical mutagens (e.g., colchicine), viruses, temperature shock, and spontaneous mutation has induced documented polyploidy in plants (Cooper et al., 1964; Kao et al., 1970; Grant, 1971; Sunderland, 1973; Yeoman & Street, 1973; D'Amato, 1975). Polyploidy also may be associated with long-term cultures (Cohn, 1964; Cooper et al., 1964; Godward, 1966; Heinz et al., 1969; Sunderland, 1973). Cohn (1964, p. 285) stated that ". . . Recent findings support the interpretation that polyploidy or aneuploidy in a cell culture is a potential consequence or characteristic of neoplastic growth . . . ." If polyploidy or polyploid aneuploidy is a potential consequence of culturing tissue or single cells, then use of culture isolates over an experimentally determined age should be avoided, and cultures in collections should be replaced with recent isolates. Very few data exist on physiological, ultrastructural, or mor-

phological changes induced by diploidy or polyploidy in microalgae, although more information is available for vascular plants.

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