

## THE DIATOM SEX CLOCK AND ITS EVOLUTIONARY SIGNIFICANCE

WILLIAM M. LEWIS, JR.

Department of Environmental, Population, and Organismic Biology, University of Colorado,  
Boulder, Colorado 80309*Submitted February 3, 1983; Accepted July 14, 1983*

Some kinds of organisms show continuous sexuality, but others are sexual only intermittently. For organisms with intermittent sexuality, the timing of sexuality presents a significant evolutionary challenge insofar as both the frequency of sex and its coincidence with environmental events must be constrained to prevent sex from becoming disadvantageous. Circumstantial evidence suggests that many species with intermittent sex have evolved timing mechanisms that are based on environmental cues (Williams 1966; Ghiselin 1974). Often the cues are environmental changes that occur once per year as a direct or indirect result of climatic seasonality. Reliability is the great advantage of a seasonal cue.

Environmental cues may be less than ideal for timing sexuality in some organisms. This mechanism will be especially inadequate when the optimal interval for sexuality is not 1 yr. Although there are some reliable environmental periodicities at frequencies shorter than 1 yr (solar, lunar), reliable and biologically useful periodicities with frequencies longer than 1 yr probably do not exist. Among organisms for which sex at intervals longer than one year is optimal, three evolutionary pathways seem possible: (1) evolution of an induction threshold just outside the average annual range of the environmental factor that is serving as a cue (stochastic mechanism); (2) evolution of an endogenous timer based on metabolism (internal clock mechanism); (3) retention of annual sexuality because 1 and 2 are impossible or too costly.

The stochastic mechanism has the disadvantage of irregularity. Short-lived species that rely on this mechanism would be more likely to lose sexual capacity as a result of selection favoring asexual genotypes over the occasionally very long intervals between cues. Since the loss of sex is probably a long-term disadvantage even though it can be a short-term advantage, groups that lose sexual capacity are likely to face a higher probability of extinction (Williams 1975; Maynard Smith 1978). This is a group-selectionist argument against the feasibility of the stochastic induction mechanism in small organisms. An individual-selectionist argument against such a mechanism could also apply: poor control over the timing of sex might nullify the advantages of sexuality in improving the fitness of a particular genotype. Either or both the group or individual disadvantages must be important

under some circumstances, as the stochastic mechanism does not appear to be common in small organisms. In contrast, the stochastic mechanism is found in large organisms whose lifespans are longer than 1 yr. For example, Janzen (1976) cites a number of examples of supra-annual, environmentally cued mast seeding in higher plants. In such organisms the group and individual selective disadvantages are lower because the time scale of irregularities in the induction of sex is shorter in relation to lifespan.

The internal clock mechanism could maintain much greater supra-annual regularity than the stochastic mechanism and probably evolves easily among large organisms in which the clock can be geared to development or growth of a single organism (e.g., biennial plants). The most impressive examples include certain bamboos that have flowering cycles as long as 120 yr (Janzen 1976). These bamboos rely on a physiological clock that continues to function long after development is complete. For very small organisms, especially unicellular ones, the barriers impeding evolution of a comparable supra-annual internal clock are much greater because of the tremendous protoplasmic dilution that must occur over supra-annual intervals. Generalized protoplasmic clocks capable of spanning these extremes of biomass turnover are apparently unknown.

A particular kind of disadvantage is common both to environmental cues and to internal clocks, whether they be annual or not, among unicellular organisms that grow as dispersed clones (e.g., unicellular algae). Since the cells of a clone are, if mutation is ignored, genetically identical, it will be difficult for the clone to evolve a mixed strategy in which some cells commit to sex and others do not. The evolutionary success of iteroparous organisms, which withhold enough reserves from one reproductive event to allow them to remain viable to reproduce again, is good testimony to the evolutionary value of such a mixed strategy, yet it is difficult to see how genetically identical cells separated in an isotropic environment can show controlled differentiation with regard to sex. Again there are three possible evolutionary pathways: (1) development of environmental induction criteria so sensitive that even slight environmental heterogeneity will cause mixed sexual response in a clone (stochastic mechanism); (2) development of an endogenous differentiation mechanism that does not depend on environmental cues (endogenous differentiation mechanism); and (3) abandonment of sex. Abandonment of sex is, for a variety of very compelling reasons, considered to be an evolutionary trap (Maynard Smith 1978). The stochastic mechanism is based on the probability that the phenotype of spatially separated cells, even from the same clone, will show some variety. Similarly, the environmental factor levels to which specific cells of a clone are exposed may vary slightly. If the genotype were appropriately programmed to divide the clone into sexual and asexual parts based on slight random variations in phenotype and environment, then the clone could follow a mixed strategy with regard to sex. This would be a very risky sort of mechanism in most environments, however, because the spectrum of variation is likely to change greatly from one year to the next. An endogenous differentiation mechanism offers greater control of sex but in a unicellular dispersed clone could not occur through genetic differentiation or through communication.

For reasons explained more fully below, it is probably advantageous for some kinds of unicellular organisms to become sexual only at long intervals because the

cost of sex is high and short-term benefits are low. When a clone of such organisms does become sexual, it will also be advantageous in most instances for it to commit only a portion of its cells to sexuality and reserve the rest for vegetative carryover. For dispersed clones, the first of these potential advantages would seem to be difficult to control and the second would seem to be mechanistically intractable. The purpose of this paper is to show how the diatoms, a diverse and abundant group of unicellular algae, have evolved a mechanism that simultaneously solves both evolutionary problems.

#### COSTS OF SEX IN UNICELLULAR EUKARYOTES

Costs of sex fall into two broad categories: those which are related to recombination and those which are not. Genetic recombination accompanying sex can produce a cost if genetic combinations of high fitness are broken up and replaced by combinations whose average fitness is lower. Under such circumstances, sex may still be advantageous if the genetic reshuffling produces a few combinations that are superior to the prevailing combinations before sex. This is the general rationale that is often given for the coincidence of sex with onset of unfavorable conditions, although the reduced cost of sex in small organisms when growth is slow may also be a viable explanation (Lewis 1983). The mean and variance of fitness for sexually produced offspring are critical to the exact balance of costs and benefits (Maynard Smith 1978).

Some costs do not involve recombination. A general cost of this type is the so-called "cost of producing males," which applies to species showing sexual differentiation (Williams 1975; Maynard Smith 1978). This cost is not paid by isogametic species that show no sexual differentiation. Another cost of sex is characteristic of unicellular eucaryotes or very small eucaryotes in which the time required for sexual processes at the cellular level is a substantial fraction of the generation time of the organism. The extra time delay required by sexual reproduction is attributable to mechanical processes inherent in meiosis, the joining of gametes, and the fusion of nuclei, hence it has been called the "cellular-mechanical" cost of sex (Lewis 1983).

There is marked restriction of sexuality in certain groups of unicellular eucaryotes, particularly unicellular algae and especially those which are planktonic. Two division-level taxa, the Euglenophyta and the Cryptophyta, are thought to lack sexuality entirely (Bold and Wynne 1978). Many unicellular chlorophytes show sexuality, but some very prominent and successful genera of this group, particularly the planktonic Chlorococcales, lack sex entirely. An example is the widely cultured genus *Chlorella*. Many placoderm desmids, especially planktonic ones, also lack sex (Brook 1981). Similarly, the Dinophyceae, which include species with sexual phases, also include many freshwater taxa that appear to lack sexual capacity (Fritsch 1935). While some genera of Chrysophyceae that are important in fresh waters show evidence of sexuality, many apparently lack the capacity for sex (Fritsch 1935). Since sex is difficult to document in algae, ignorance may well exaggerate our impression of pervasive asexuality in algae. Nevertheless, there seems little doubt that asexual taxa are much more common than in the Metazoa and Metaphyta.

Even among those unicellular algae which have sexual capabilities, sex is typically infrequent. There is a tendency, which crosses divisional lines, for sex to be induced by nutrient deficiency (Dring 1974), although other cues can cause sex in some instances. Since nutrient deficiencies tend to be seasonal, this mechanism generally causes an annual sexual phase. If a planktonic algal species with annual sex were dividing at the reasonable rate of  $0.3 d^{-1}$ , it would experience sex approximately every 100 generations.

A number of evolutionary puzzles are connected with the low frequency of sex in unicellular algae and with the large number of completely asexual taxa. Without knowing the mechanisms accounting for suppression of sex, it seems reasonable to conclude that the ratio of costs to benefits for sex are especially high in unicellular algae. The genotypes of such organisms may be adjusted to environmental conditions that continually recur with little variation. Recombinational disruption of such a genotype could be disadvantageous, especially if there is no reliable mechanism for insuring that some portion of a clone remains asexual, thus preserving a proven genotype.

The diatoms stand apart from other taxa of unicellular algae in their virtually universal retention of sexual capacity. They resemble or even surpass other taxa, however, in showing sexuality only at long intervals. Their ability to retain sexual capacity while suppressing frequency of sex suggests better control over timing of sex than in most taxa. Incremental, steady reduction in size during vegetative growth is a long-known peculiarity of diatoms. This phenomenon, which has been called the McDonald-Pfitzer Rule (Drebes 1977), is probably a unique evolutionary solution to the problem of clocking sex over many generations extending beyond 1 yr, and simultaneously allows evolutionary control over the degree of investment in sex when sex does occur.

#### THE MACDONALD-PFITZER RULE

Diatoms are both diverse and abundant. They make important contributions to the primary production of fresh waters and oceans, and they are found on substrates and in suspension. The number of species is estimated to be between 10,000 and 20,000 and, principally because of their importance in marine environments, diatoms account for as much as 25% of the total oxygen production of the earth (Werner 1977). Diatoms show some key peculiarities, especially in relation to their use of silicon. These peculiarities must be in part responsible for the success of diatoms, but their underlying adaptive significance, which may be multifaceted, is mostly obscure. Most importantly, diatoms possess a skeleton consisting of opaline silica. This skeleton is typically highly ornamented and consists of two halves, an epitheca and a hypotheca. The two halves (valves) are complementary, as would be the two parts of a pill box: the epitheca fits snugly over the hypotheca. The epitheca and hypotheca are joined by bands of connecting material called the girdle.

When a diatom divides, each of the two valves develops a new valve that fits just inside the old. The old epitheca thus remains an epitheca but develops a new hypotheca. The old hypotheca, however, becomes an epitheca and develops a hypotheca of its own. If none of the valves makes any size compensation, it is

evident that this process of division will have two results, which together are the manifestations of the MacDonald-Pfitzer Rule: (1) there will be a decrease in the average size of cells in a clone; and (2) there will be an increase in the standard deviation of cell size in a clone.

It is always tempting to conclude that a patterned change in morphology such as that described by the MacDonald-Pfitzer Rule is an imperative that arises inexorably from other properties of the organism, in this case the formation of valves. Such an explanation is traditionally implied or stated for the MacDonald-Pfitzer Rule. Conclusions of this sort are often erroneous, however, in that they under-rate the power of natural selection to modify morphology. In the case of MacDonald-Pfitzer Rule, it has been shown that closely related species, and even different clones of the same species, can differ greatly in the valve size decrease that occurs with each division (Geitler 1932). Furthermore, change of dimension across the narrow axis of the diatom is negligible or very small despite very great changes along the long axis (Drebes 1977; Geitler 1932). Finally, and perhaps most importantly, progressive size decrease does not occur in some taxa (Drebes 1977; Geitler 1932). Although some might still argue that decrease in size is a necessary consequence of morphology in diatoms, with some inexplicable exceptions, the more reasonable conclusion would seem to be that the decrease in size is adaptive, and, if it were not, that mechanisms could easily evolve by which it would be circumvented.

#### SEXUALITY IN DIATOMS AND ITS RELATION TO SIZE

Sexuality has been documented in a large number of diatom genera. The older literature is reviewed by Geitler (1957) and Fritsch (1935) and the extensive modern literature is summarized by Geitler (1973) and Drebes (1977). In the centric diatoms (typically cylindrical in cross section, with radial symmetry), oogamy is the rule. Small motile (male) gametes are produced as a result of meiosis in certain vegetative cells, while others produce a single large (female) gamete. In the pennate diatoms (often box or coffin shaped, lacking radial symmetry), which are considered to be of more recent phylogenetic origin (Simonsen 1972), two diploid vegetative cells come together and undergo meiosis. Gametes, which are typically about the same size but may be physiologically anisogamous, migrate between the joined cells to produce zygotes.

The known variation in mechanisms of sexuality both among the centric and the pennate diatoms is very large (Drebes 1977). The number of gametes varies considerably between genera according to the number of mitotic divisions in the mother cell and the number of nuclei that are aborted during meiosis. There is also variation in the relative size of gametes and in the sex determination mechanisms. The literature generally gives the impression that sexuality in diatoms has been extensively diversified by natural selection.

A diatom zygote becomes an auxospore. The zygote escapes the valves of the mother cell and secretes a spherical covering which, although it contains some silicon, is flexible and lacks the detailed ornamentation and shape characteristic of the vegetative cell. The zygote grows, expanding its outer covering as necessary to accommodate its increased bulk. Within the next few divisions, it resumes the

characteristic shape of the vegetative cell. It then divides vegetatively through many generations until the next auxospore formation. It is possible for auxospores to form asexually in some species, but this is the exception rather than the rule (Drebes 1977).

The proximate environmental cue that induces sexuality in diatoms varies greatly among species and even among clones of the same species. In fact, at least in some cases, it appears that a specific genetic stock can be induced to sexuality by any one of a number of environmental cues. The diatoms show some evidence of being less specific in their environmental cues than most other taxa of algae, in that they seem to show sexual induction in response to light and temperature as well as nutrients (Dring 1974).

Diatoms are unlike other taxa of algae in that environmental cues are not sufficient to induce sexuality. As first shown by Geitler (1932), the inducing factors are only effective if a cell has reached, through successive divisions according to the MacDonald-Pfitzer rule, a certain critical minimum size. The critical minimum size is about 30% of the maximum in centric diatoms and in pennate diatoms it is about 40% (Drebes 1977). Although the exact mechanism is unknown, von Stosch (1965) has shown by artificial alteration of size that size rather than age sets the threshold for sexuality.

#### INTERVALS BETWEEN SEXUAL EVENTS

Because sexuality is governed by size reduction, in addition to environmental cues, it follows that division by the MacDonald-Pfitzer rule in effect acts as a chronometer of sexuality. Whether or not this is of major significance depends upon the special advantages that organisms possessing such a chronometer might have. It is convenient to consider first the advantages of a sexual chronometer in species for which annual sexuality is optimum. The chronometer of diatoms provides a mechanism, which is in effect mechanical, by which a clone, composed of cells with identical genotypes and exposed to identical environmental cues, and without any means of communicating with each other, can maintain a mixed strategy with regard to annual sexuality. The MacDonald-Pfitzer rule insures that there will be variance in the size of diatoms after an extended period of vegetative growth. When the appropriate cues occur, a certain proportion of the cells will be within the inducible range and others will not. Obviously the inducible range and the rate of change in size per generation are both subject to selection; the percentage of cells becoming sexual at a given time since the sexual origin of the clone can thus be adjusted by natural selection to a very fine degree of tolerance. So long as sex has even minimal or occasional advantages, the chance that sexuality will be completely eliminated under such a mechanism is very low. This may account for the strong tendency of diatoms to retain sex in all evolutionary lines, in contrast to many other taxa of algae that occupy the same habitats.

Even supra-annual sexuality is within the range of the sexual chronometer of diatoms. It has long been known that populations of planktonic diatoms may pass through several years of vegetative growth before experiencing sexuality and auxospore formation. For example Nipkow (1928) showed by examination of

diatom valves in laminated sediments of Lake Zurich that the average time between auxospore formation in diatom populations in that lake was 4 to 5 yr for three different genera of two different orders. Fritsch (1935) cites other cases from the older literature. Clones may pass through as many as 1,000 generations before a major proportion of cells become sexual, yet sexuality is assured once the time on the chronometer has elapsed. In actuality, some cells are out of phase with the main group, as a result of the variance induced by division, and the really obvious incidence of sexuality occurs when the mean size of an abundant age class reaches the inducible range.

The examples of supra-annual sexuality are especially important in that they provide a means of comparing the sexual chronometer hypothesis with other hypotheses that might rely more on the primary adaptive significance of cell size. For example, Bellinger (1977) has pointed out that decreasing cell size might be of some advantage to a diatom clone as a phenotypic response to changing conditions during the growing season. Advantages that might accrue directly from decrease in cell size would almost certainly induce annual cycles of change in cell size, and not supra-annual cycles. The existence of supra-annual cycles suggests that cell size reduction is principally important as a sexual chronometer rather than as a phenotypic response to environmental conditions. It is quite possible, however, that phenotypic changes are of major supplementary benefit for other reasons among those diatom populations that show annual sexuality. For example, change in cell size and increasing variety in cell size over the progression of a single growing season might help foil size-specific grazers.

#### CONCLUSIONS

The rate of size reduction in diatom appears to be very labile under the influence of natural selection. Retention of size reduction by diatoms therefore must be connected with some evolutionary advantage. It is difficult to imagine what significance this phenomenon could have, other than the timing of sexuality, that could be so pervasive as to cause the retention of size reduction among almost all taxa of diatoms. Insofar as the mechanism provides a unique degree of selective control over sexuality, it reduces the costs of sex and insures that sexuality is less likely to be lost because of its short-term disadvantages. From this viewpoint it does not seem too farfetched to suggest that the sexual chronometer may be the primary explanation for the existence and persistence of the skeletal valves in diatoms, and that the impressive diversity and abundance of the diatom algae is in some significant degree attributable to their refined control over sexuality.

#### SUMMARY

Diatoms, which possess distinctive skeletal valves of opaline silica, show steady incremental reduction of size with successive vegetative divisions. Size reduction is explained by consistent formation of slightly smaller new valves inside the old ones. Diatoms cannot be induced to become sexual until a certain critical minimum size has been achieved through this steady reduction of size. Sex

leads to regeneration of the largest size. There is strong support for the hypothesis that the primary evolutionary purpose of the vegetative size reduction is to serve as a sex clock, thus allowing sex to be spaced optimally even over very long intervals in genetic lines of these unicellular organisms whose lifespans are too short to support the function of long-term physiological clocks. This control over sex, which is unique to diatoms among the unicellular algae, may contribute substantially to the high diversity and worldwide abundance of diatoms.

## ACKNOWLEDGMENTS

This work was supported by a fellowship from the John Simon Guggenheim Memorial Foundation and by NSF Grant 80-03883. I am grateful to J. B. Mitton, Y. B. Linhart, M. C. Grant, and anonymous reviewers for helpful comments.

## LITERATURE CITED

- Bellinger, E. G. 1977. Seasonal size changes in certain diatoms and their possible significance. *Br. Phycol. J.* 12:233-239.
- Bold, H. C., and M. J. Wynne. 1978. Introduction to the algae. Prentice-Hall, Englewood Cliffs, N. J.
- Brook, A. J. 1981. The biology of desmids. University of California Press, Berkeley.
- Drebes, G. 1977. Sexuality. Pages 250-283 in D. Werner, ed. The biology of diatoms. University of California Press, Berkeley.
- Dring, M. J. 1974. Reproduction. Pages 814-837 in W. D. P. Stewart, ed. Algal physiology and biochemistry. University of California Press, Berkeley.
- Fritsch, F. E. 1935. The structure and reproduction of the algae. Vol. 1. Cambridge University Press, Cambridge.
- Geitler, L. 1932. Der Formwechsel der pennaten Diatomeen (Kieselalgen). *Arch. Protistenkd.* 78:1-226.
- . 1957. Die sexuelle Fortpflanzung der Diatomeen. *Biol. Rev.* 32:261-295.
- . 1973. Auxosporenbildung und Systematik bei pennaten Diatomeen und die Cytologie von *Cocconeis*-Sippen. *Osterr. Bot.* 2. 122:299-321.
- Ghiselin, M. T. 1974. The economy of nature and the evolution of sex. University of California Press, Berkeley.
- Janzen, D. H. 1976. Why bamboos wait so long to flower. *Annu. Rev. Ecol. Syst.* 7:347-391.
- Lewis, W. M., Jr. 1983. Interruption of synthesis as a cost of sex in small organisms. *Am. Nat.* 121:825-834.
- Maynard Smith, J. 1978. The evolution of sex. Cambridge University Press, Cambridge.
- Nipkow, H. F. 1928. Ueber das Verhalten der Skelette planktischer Kieselalgen im geschichteten Tiefenschlamm des Zurich- und Baldeggensees, etc. *Z. Hydrologie* 4:71-120.
- Simonsen, R. 1972. Ideas for a more natural system of the centric diatoms. *Beih. Nova Hedwigia* 39:37-54.
- von Stosch, H. A. 1965. Manipulierung der Zellgross von Diatomeen im Experiment. *Phycologia* 5:21-44.
- Werner, D. 1977. Silicate metabolism. Pages 110-149 in D. Werner, ed. The biology of diatoms. University of California Press, Berkeley.
- Williams, G. C. 1966. Adaptation and natural selection. Princeton University Press, Princeton, N.J.
- . 1975. Sex and evolution. Princeton University Press, Princeton, N. J.