INTERRUPTION OF SYNTHESIS AS A COST OF SEX IN SMALL ORGANISMS

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Sex has been the subject of a great deal of evolutionary cost-benefit analysis (Williams 1975; Maynard Smith 1978). The most prominent cost of sex is the "cost of meiosis" (Williams 1975), also called the "cost of producing males" (Maynard Smith 1978). In addition, recombinational load is recognized as a general cost of sex. These two general costs are weighed against the benefits of recombination (genotype diversity in offspring). Various additional but usually minor costs also enter the picture for particular kinds of organisms (Willson 1981). The cost-benefit analysis of sex has centered mainly on multicellular organisms. My purpose here is to describe an apparently unappreciated cost of sex which may exceed in importance the cost of producing males and recombinational load in simple eucaryotes.

Some major groups of organisms, which may be loosely referred to as unicellular eucaryotes, consist of individual cells or small aggregations of undifferentiated cells. The heterotrophic Protozoa, euglenophytes, chlorophytes, diatoms, dinoflagellates, chrysophytes, cryptophytes, and others fit this description. Such organisms are both diverse and abundant. For example, the diatoms account for some 20%–25% of the total photosynthetic oxygen production of the earth and include 10,000–12,000 species (Werner 1977). Unicellular eucaryotes increase numerically almost exclusively by means of asexual reproduction, which often amounts simply to a mitotic cell cycle (variations such as the multiple fission of the Chlorococcales need not be considered here).

Many unicellular eucaryotes show sexuality at times (i.e., they are heterogonic), especially when population growth has just started to decline after sustained growth. Surprisingly large numbers of taxa show no evidence of sex, however, and many of the taxa that show sex do so only infrequently. Sexuality is unknown in the Euglenophyceae and Cryptophyceae (Smith 1950; Fritsch 1965). Even within groups for which heterogonic sex is the rule, certain important genera do not have sexual stages. For example, a number of well-studied genera in the Chlorophyta (e.g., *Chlorella*) and several protozoan lines show no evidence of sex (Fritsch 1965; Hawes 1963). Taxa that do show sex often pass through very large

numbers of cell generations between sexual phases. Planktonic diatoms, for example, do not usually produce gametes every year (Fritsch 1965). The incidence of sex is often further reduced by failure of many cells to be sexually induced even when part of the population is sexual. This is common among phytoplankton species. An explanation for widespread low frequency or absence of sex in unicellular eucaryotes is needed, especially in view of the fact that most are isogametic. For isogametic taxa, the cost of producing males does not apply (Williams 1975; Maynard Smith 1978), so the selective disadvantages to sex would appear to be considerably lower than for many other organisms.

THE MITOTIC CELL CYCLE

The mitotic cycle, whether in the component cells of a multicellular organism or in cells which themselves constitute organisms, consists of a predictable sequence of phases (terminology from Howard and Pelc 1953): G_1, which directly follows the formation of a new cell and during which there is an increase in the mass of cytoplasm; S, during which the amount of DNA doubles; G_2, a typically brief gap between the termination of DNA synthesis and the onset of mitosis; mitosis (M), during which a nucleus with double the G_1 amount of DNA divides to become two nuclei; and cytokinesis, during which the two nuclei are separated into different cells. The minimum mitotic cell cycle time depends on cell size, which is in turn correlated with amount of DNA. In an extensive review of higher plants, Bennett (1972) shows that root tip cells of vascular plants with the smallest amounts of DNA per cell (and thus the smallest cells) complete a cell cycle in just under 9 h. The fastest complete cycle times in cells of multicellular animals are somewhat lower (6.5 h for the fastest, Van't Hoff and Sparrow 1963). Among ciliated protozoans, the shortest cycles are about 3.5 h at 20° C, and possibly less at higher temperatures (as computed from r_m values of Taylor and Shuter [1981] excluding one outlying value of 2.8 h). For eucaryotic algae, the fastest cycles are about 6 h, possibly as short as 4 h (e.g., Eppley 1977). G_1 and S take up the majority of the cell cycle time; mitosis and G_2 usually require much less time. G_1 is highly variable. It ranges from a negligible interval in very rapidly growing cells to many hours in slowly growing cells (Mitchison 1971). S is more rigid, typically requiring a minimum of a few hours, although some cells require less than an hour for S. Mitosis usually does not require more than 40 min in cells with very short cycle times, but can require 1 to 2 h or even more in cells with long cycle times (Prescott 1976; table 1).

The mitotic cell cycle accomplishes four tasks: (1) increase of cellular mass sufficient to provide the cytoplasmic environment for an additional copy of the genome, (2) precise copying of the genome, (3) perfect mechanical separation of the two genome copies, and (4) division of the cytoplasm. Presumably, rapidly growing tissues or individual unicellular organisms represent evolutionarily refined minimum times for each one of these processes. The four processes cannot occur simultaneously. Most importantly for present purposes, mechanical separation of the two genome copies requires condensation of the DNA, which shuts off DNA transcription and requires spatial and biochemical reorganization of the cell.

TABLE 1

DURATION OF MITOSIS AND MEIOSIS IN MULTICELLULAR AND UNICELLULAR TAXA

Mitosis Times (in hours)		Meiosis Times (in hours)	
MULTICELLULAR ORGANISMS			
Mouse fibroblast[a]	.7	Melanoplus differentialis[g]	204
Chinese hamster fibroblast[a]	.4	Mouse[h]	>300
HeLa cells[a]	.5	Man[h]	7,500
Xenopus embryo[e]	.5	Triticum aestivum[e]	24
Chicken erythroblast[c]	.5	Haplopappus gracilis[e]	36
Newt erythroblast[c]	3.5	Vicia faba[e]	72
Sea urchin egg[f]	.7	Allium cepa[e]	96
Zea mays root[f]	2.0	Lilium candidum[e]	168
Mean of 20 higher plants[f]	2.1	Trillium erectum[e]	274
UNICELLULAR ORGANISMS			
Paramecium(micronucleus)[a]	.8	Paramecium bursaria[j]	18
Actinophrys sol(Heliozoa)[k]	.7	Actinophrys sol(Heliozoa)[k]	16
Rhogostoma schussleri(Testacea)[l]	.3	Eimeria maxima(Sporozoa)[m]	9
Eimeria maxima(Sporozoa)[m]	<1.0	Lithodesmium[d]	4.5
Volvocales[b]	.2		
Oedogonium[b]	.5		
Closterium siliqua(desmid)[i]	1.6		

SOURCES.—a, White 1973; b, Pickett-Heaps 1975; c, Prescott 1976; d, Manton et al. 1970; e, Bennett 1972; f, Mitchison 1971; g, Muckenthaler 1964; h, Clermont 1977; i, Brandham and Godward 1965; j, Wichterman 1953; k, Belar 1923; l, Belar 1921; m, Canning and Anwar 1968.

As a consequence of these changes, mRNA synthesis drops to essentially zero during the last half of mitotic prophase (Prescott 1976). This is true not only for component cells of multicellular organisms, but also for unicellular organisms (e.g., *Euglena,* Lovlie and Farfaglio 1965). Strictly from the viewpoint of increase in mass, the synthetic shutdown required for mitosis handicaps the growth potential of the cell.

SEX FROM THE PERSPECTIVE OF THE CELL

Sex requires meiosis, gametic union, and nuclear fusion. Each of these three stages has an important mechanical aspect. "Mechanical" here refers to the movement of discrete bodies or structures too large to be directly influenced by molecular motion. Phenomena with a mechanical component will be subject to time constraints different from those associated with purely chemical phenomena. In meiosis, processes with a mechanical component include synapsis of homologous chromosomes and their subsequent separation. Gametic union requires the directed movement of gametes toward each other, or of one gamete toward another, and sometimes the merger of cytoplasm. Nuclear fusion (karyogamy) requires the closing of distance between the gamete nuclei to form the new diploid nucleus. These three processes can occur sequentially without interruption or can be separated by other events in the life cycle of an organism. In diatoms or ciliates meiosis is immediately followed by formation of a zygote. In chlorophytes or gregarine protozoa, meiosis immediately follows zygote formation and the population growth occurs by mitosis of haploid cells. Formaminifera present an intermediate to these extremes: Meiosis and zygote formation are separated by mitotic population growth, so that haploid and diploid asexual cells alternate (Grell 1973).

The essential point here is that the three processes required by sex are, whatever their arrangement in time, inherently much more time consuming than the corresponding processes of the asexual (mitotic) cell cycle (table 1). Meiosis is probably the most rigid in its minimum time requirements. Meiosis should take longer than mitosis, simply because it includes two divisions instead of one, but the degree of difference in the minimum time for meiotic and mitotic divisions is surprisingly large, and well over twofold. Thus there is a cost of sex associated explicitly with meiosis. Maynard Smith (1978, p. 39) came close to making this point in showing that haploid isogametic species, while escaping the twofold cost of producing males, are still slightly handicapped by the time required for the second meiotic division. Actually meiosis is much more lengthy than two successive mitotic divisions would be, probably because of its additional mechanical components, and this has major implications for the cost of sex.

MEIOTIC VERSUS MITOTIC DURATION FACTORS

The minimum time for meiosis is linearly related to the amount of DNA in the nucleus, which is in turn related to cell size (Bennett 1972). The slope of the linear size-time relationship can differ between evolutionary lines. For example, vertebrates require considerably longer periods to complete meiosis than do higher

plants having similar DNA per cell (Bennett 1971). Despite this variation, meiosis times are universally quite high by comparison with mitosis. Rye requires 51 h and wheat requires 24 h for male meiosis (all of the data used here are for male meiosis), yet these are among the fastest meiosis times in higher plants. Among higher organisms, meiosis times are one to two orders of magnitude higher than the time required for mitosis in the same kinds of organisms (table 1). Comparisons of meiosis and mitosis times would be more straightforward if the two were studied simultaneously in the same species. Unfortunately this is seldom the case, but the consistent differences in meiosis and mitosis times in closely related taxa are so large that the general pattern is clear.

It is obvious at this point that meiosis, as it occurs in higher plants and vertebrates, would be an inefficient mechanism for the perpetuation of unicellular organisms simply because the upper limit set on rate of increase by such a lengthy division process would be far too low. Cells coping individually with the environment would be unable to shut down growth and other aspects of metabolism for the duration required by meiosis without a tremendous selection handicap. In complex organisms, which require a considerable period of time for somatic growth, the germ cells may be allowed the luxury of an extended meiotic period, as they can be maintained and even nourished by somatic cells. Proportionally speaking, the load placed on the entire organism by the inactivation of a relatively small percentage of meiotic germ cells is much less important. Even so, Bennett (1972) has pointed out that the lengths of life cycles of annual and perennial plants are statistically related to their total nuclear DNA and thus to their meiosis time.

Extrapolation of the meiosis times for various cell sizes from higher plants and vertebrates would suggest that meiosis is completely impractical as a continuous or frequent reproductive mechanism in unicellular eucaryotes. This extrapolation may not be well founded, however. Evolutionary pressures may be capable of reducing meiosis time considerably. In other words, the meiosis times observed in higher organisms may not be minimum meiosis times simply because there is a lower premium on quick completion of meiosis in these taxa. Some direct evidence from unicellular organisms is thus required. The existing information of this type is not so rich as one might imagine, but table 1 summarizes measurements for a number of taxa.

The ciliated Protozoa are probably the best single source of information on meiosis and mitosis times, but the presence of a nonmeiotic macronucleus complicates the interpretation for this group: The macronucleus may free the cell of the effects of normal meiotic time constraints, as the macronuclei appear to remain metabolically active during meiosis (Raikov 1969; Nanney 1980). Meiosis times in ciliates may therefore not be minimal in an evolutionary sense. In *Paramecium bursaria* meiosis requires 18 h. This value is similar to meiosis times for protozoan taxa that lack a macronucleus (table 1), suggesting that it is a minimum time. The shortest meiosis time on record for a unicellular eucaryote seems to be 4.5 h for the diatom *Lithodesmium* (Manton et al. 1970). This is short by comparison with higher plant cells and suggests significant reduction of meiotic time under the influence of evolutionary pressures or more efficient mechanics in a simpler genome. *Lithodesmium* may be especially fast, however. The centric diatom

Melosira apparently requires severalfold as much time for meiosis (Erben 1959), although no exact measurements are available; Werner (1971) has found that 36–48 h elapse in *Coscinodiscus* between sexual induction and sperm formation. Even for *Lithodesmium* the time required to produce a specific number of daughter cells by meiosis is considerably longer than for mitosis.

There is great diversity in the form and behavior of nuclei in protists. Some of this diversity may be better understood in view of time costs associated with meiosis. The nuclear dualism of ciliates, for example, has already been mentioned as a possible means of extending synthesis through meiosis. A similar interpretation might apply to nuclear dualism in Foraminifera (Grell 1973). One-step meiosis, a common phenomenon in certain flagellates (Cleveland 1956), is a mechanism by which greater speed might be achieved at the sacrifice of cross-over.

In many unicellular species, including all of the ones listed in table 1, the difference in efficiency between mitosis and meiosis is magnified by loss of one, two, or even three of the four meiosis division products to preserve cell size (e.g., Smith 1950; Fritsch 1965; Brook 1981; Raikov 1972; Grell 1973; Elliott 1973). Thus while mitosis requires a short time to produce two nuclei, each of which will receive cytoplasm during cytokinesis, meiosis requires a much longer time to produce four nuclei, of which one to three are often aborted.

Once the gametes are produced by meiosis, interruption of synthesis imposed by sex is not complete. Each gamete must close the distance between itself and a gamete of the opposite type. The systems that have evolved for this are very efficient but nevertheless require a certain amount of time. In ciliates sexual cells conjugate and nuclei move through a cytoplasmic bridge (Nanney 1980). In the desmids and pennate diatoms, vegetative cells conjugate and the gametes, which lack flagella, come together by ameboid movement to form a simple cytoplasmic mass (Geitler 1932; Brook 1981). In many algae, such as the Volvocales, gametes are motile by flagella and agglutination on contact. Gametic union should be considered to include merger of the two cytoplasmic masses when this occurs. Time required for this aspect of gamete union is highest for isogamous species and lowest for oogamous species; this may have a bearing on the evolutionary trend toward oogamy (i.e., it is an evolutionary force favoring oogamy).

Following the joining of cytoplasm, nuclei must also fuse. Fusion of the nuclei in higher plants can require considerable time (Bennett 1972). Although we might see misleadingly long fusion times in organisms not under evolutionary pressure to complete the process quickly, there must be an irreducible minimum which is possibly related to cell size. The metabolic activity of the separate nuclei prior to fusion is evidently not known, but it could well be negligible. The total time loss in a sexual as opposed to an asexual reproductive mode would obviously be affected by the time required for nuclear fusion.

It is difficult to generalize about time requirements for gametic union and nuclear fusion, but the literature suggests that these cause major delays in resumption of synthesis. In *Paramecium bursaria* total duration of conjugation is 4 days, of which only 18 h is accounted for by meiosis proper. Nanney (1980) has calculated that, for two ciliate species, the ratio of cells produced asexually to

cells produced sexually over the same time interval is 8:1 in one case and 60:1 in the second. The minimum times will vary greatly according to gamete size and reproductive strategy. In the desmid *Cosmarium*, the time between conjugation and fusion of nuclei is about 17 h (Pickett-Heaps 1975); in *Chlamydomonas moewusii* it is 8–10 h (Triemer and Brown 1975). Some taxa may be able to accomplish the tasks more quickly. In the diatom *Gomphonema*, copulation lasts only 20–45 min (Geitler 1932), and in *Haematococcus* (Volvocales) it is 1 h (Schultz 1927), but these figures do not include nuclear fusion.

<div align="center">CONCLUSIONS</div>

Although the data are sketchy, it is clear that the ratio of time required for processes associated at a cellular level with sexual reproduction to the time required for similar processes in an asexual cell cycle easily exceeds two to one and could well exceed ten to one for many organisms. This cost, of which meiosis is perhaps the most inflexible component, is distinct from the cost of producing males and might be called the "cellular-mechanical" cost of sex because the additional mechanical components of the sexual processes appear to be the evolutionary block to greater speed. The cellular-mechanical cost is directly applicable to all unicellular organisms, for which there is no distinction between the vegetative (somatic) and germ lines. The cellular-mechanical cost may also be an important evolutionary force in small multicellular organisms capable of parthenogenesis, if parthenogenesis has been achieved through bypass or simplification of meiosis. For example, rotifers are parthenogenetic via diploid eggs and have a very fast egg-to-egg cycle (Ruttner-Kolisko 1974). Meiosis in such an organism would slow the reproductive rate considerably and this, along with the other more widely recognized costs of sex, will weigh against the feasibility of sex as a continuous or frequent reproductive mode. For organisms whose egg-to-egg time greatly exceeds meiosis time, the cellular-mechanical cost will not influence the frequency of sex.

The cellular-mechanical cost of sex also has an important bearing on the timing of sex. The cellular-mechanical cost is only applicable as long as the organism is growing. If a cell is deprived of a critical resource, causing suspension of growth, then the cellular-mechanical cost becomes zero, as there can be no growth penalty in a growth-arrested organism. The shift from a major cellular-mechanical cost in a growing organism to no cost in a growth-arrested organism will strongly force sexuality to the end of the growing season. Coincidence of sexuality with the end of population growth, as commonly observed in nature, has been attributed typically to temporal optimization of the benefits of genetic variety (however, see Nanney [1980] for a clear statement of the growth-related disadvantages of sex in ciliates at times of good growing conditions, and Willson [1981] for similar reasoning applied to plants). In simple eucaryotes, the cellular-mechanical cost of sex will have a similar effect on the timing of sex and thus may lend unjustified strength to theories that explain the timing of sex solely in relation to recombination. Because sex is dependent on some minimal population density and on organism energy reserves, however, there is a selective pressure that resists delay

of sexuality too much past the growth peak. These selective pressures and the cellular-mechanical cost will thus force sexuality into a narrow time window.

SUMMARY

Three processes associated with sex—meiosis, gametic union, and nuclear fusion—have a mechanical component, i.e., they require movements of supramolecular bodies. Times required for these processes are surprisingly high, probably because of the mechanical component, thus insuring that production of cells by sexual processes will require much longer than production of an equal number of cells by asexual processes. This is especially evident in direct comparisons of meiosis and mitosis. Meiosis requires much longer than two mitotic divisions. For unicellular organisms, and for small multicellular organisms in which the egg-to-egg time is very short, the additional time required by sexual processes at the cellular level lowers the feasibility of sex by slowing the reproduction rate. This is called the "cellular-mechanical" cost of sex. The cellular-mechanical cost of sex is at least twofold and will often exceed 10-fold by comparison with asexual reproduction in unicellular eucaryotes. The cellular mechanical cost of sex thus is a strong selective force acting against sex in unicellular and small organisms and, because it does not apply when the organism stops growing, tends to restrict sexuality to the end of the growth period.

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