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Parasite Diversity and the Evolution of Diploidy, Multicellularity and Anisogamy

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It may be reasonably assumed that a diversity of parasite genotypes in any one cell or organism is more harmful than a population of uniform genotypes. If this is accepted the following consequences follow:

(i) Parasite mixing, due to cytoplasm mixing, at the time of zygote formation is a new and additional cost of sex. The rapid divisions typical of zygotic cleavage may be viewed as an adaptation to minimize the degree of mixing of parasites in each daughter cell. The faster the divisions the less chance parasite populations have to grow and mix. Mitosis is the fastest form of cell division. Prolongation of the diploid phase follows as a consequence of mitosis in a diploid zygote. This view is unusual in that it demands no advantage *per se* to the possession of two chromosome sets.

(ii) The cells of the blastula formed from rapid zygotic divisions are different as regards their symbiotic inclusions. If the right to gametogenesis is restricted, then every replicator symbiont and nuclear genome alike and hence every cell of the developing embryo, will have an incentive to compete. Selection between the clonal blastula cells would result in the cells of low parasite diversity forming the gametes. Thus, germ line restriction is in the interests of the nuclear genome. Controlling the right to gametogenesis is only possible if the blastula remains intact. Hence, multicellularity might have evolved so as to enable the limitation of the right to gametogenesis and hence reduce the parasite diversity of gametes. Inter-cell competition during embryogenesis is central to Buss's seminal notion of the evolution of developmental complexity within the metazoa. The above theory provides the missing motive force behind such competition.

(iii) For a given zygote size, the fittest zygotes are those produced by the gametes most disparate in size because these have a lower diversity of parasites. This may be the advantage of anisogamy. The novelty of this new view of anisogamy is that it puts a premium on sperm being very small, in order to exclude parasites from sperm cytoplasm. The hypothesis is briefly tested by examining if there are alternative means of parasite limitation in organisms with large gametes.

1. Introduction

This paper considers two possibilities. First, that the evolution of multicellularity and of the protracted diploid state (diploidy not just restricted to the zygote) were consequences of a host's attempt to minimize the fitness reduction forced by a diversity of intracellular "unfriendly" symbionts. Second, that the evolution from isogamy to anisogamy was a host's adaptation to minimize costs due to both intracellular and intraorganismic parasite diversity.

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2. Is a Diversity of Parasites a Bad Thing?

Three theoretical reasons for believing that diversity of intracellular and intra-organismic parasitic genotypes is harmful to a host are explored below.

(i) A cell/organism is likely to be fittest if it has as few parasite particles as possible. Competition can limit a population's growth. The degree of competition between two populations of parasites will depend on the closeness of match of their respective niches. Intra-genotype competition tends, as a consequence, to be more intense than inter-genotype competition. If competition between parasite types is weak, the two genotypes deplete the host of different resources and grow independently. Hence, the total parasite population size is best limited by keeping parasite diversity to a minimum. I can find no published empirical data on intracellular competition between symbionts. Information is available on the nature of microbial interactions in free culture, i.e. Gause type mixed culture experiments. These experiments indicate that a mixed culture, even when competing, will produce a population whose total density is at least that of pure cultures (see Fredrickson, 1977).

(ii) In hosts which are not multiply infected the pathogen can be maximally successful by not harming the host unduly. A healthy host is one which can be used as a source of infection for a considerable time. However, in a multiply infected host, even if the strains do not directly compete, they do conflict indirectly in that the growth of one strain enhances the host's mortality, hence reducing the expected success of the other strains. Thus, a conditional strategy of rapid severe exploitation may be followed if a strain detects the presence of other differing strains. This idea, first presented by Axelrod & Hamilton (1981), has been formalized by Sisaki & Iwasa (in press) who conclude that because each strain has no incentive to restrict its own growth, multiple infections are liable to be more damaging than single infections.

(iii) If a host has limited resources with which to defend against invaders, then it makes sense for a potential invader to wait until the host is waylaid by one infection before becoming aggressive. Such behaviour defines the "opportunist pathogens" (see von Graevenitz, 1977 for review).

The last two explanations make similar predictions about parasite behaviour, namely that parasites are to be expected to play conditional strategies which use either the failing health of the host or the presence of other parasites as cues to proliferate. There is a sizeable body of information supporting such a notion. Gledhill *et al.* (1955) showed that a mouse virus can be stimulated out of latency by the introduction of bacteria obtained from a different strain of mouse. Similarly, dual infections of Mollicute-like organisms (MLO) and viruses have been reported (see Banttari & Zeyen, 1973 for a review). For instance, tristeza virus of citrus in combination with a MLO causes a naturally occurring disease in *Citrus reticulata.* Plants infected with only one of the pair reveal no symptoms. Usually the two agents are not observed within the same cell, but occasionally the two have been seen together in one phloem cell (Chen *et al.*, 1972).

Nelson *et al.* (1988) have shown in brain cells, that if HIV-1 and Human Cytomegalovirus (HCMV) coinfect, then HIV-1 proliferates rapidly eventually kill-

ing the host cell. If either virus infects singly then rapid propagation does not occur. The synergism between Potato Virus X (PVX) and Potato Virus Y (PVY) is comparable. PVX and PVY produce in tobacco a severe veinal necrosis instead of the milder mottling or vein-banding seen with either virus separately (Smith, 1931). In potatoes the same combination of viruses often proves lethal. Likewise, dual infection of Cucumber Mosaic Virus (CMV) and blackeye cowpea mosaic virus results in cowpea stunt, whereas infection by either virus singly is less damaging (Pio-Ribeiro *et al.*, 1978).

In the multicellular amoeba *Pelomyxa*, Buchner (1965) reports that if the host becomes diseased, normally mutualistic bacteria can proliferate, kill the host and carry on reproducing after the host is dead.

Klenk & Rott (1987) have established a mechanism for one synergism. They have shown that certain strains of *Staphylococcus aureus* produce serine proteases which cleave surface haemagglutin of influenza virus, thus enabling the virus to enter host cells. Without haemagglutin cleavage the virus is ineffectual. They suggest that *Haemophilus influenzae* may have similar potential.

Not all symbiont-symbiont synergisms are explicable in terms of opportunism or non-co-operation. For instance, the protozoan *Histomonas meleagridis* requires growth factors from intestinal bacteria before it can proliferate and induce disease in turkeys (Lesser, 1961).

Close proximity of differing bacteria permits the potentially damaging exchange of plasmids. The increase in many bacterial species of resistance to penicillin is thought to be a product of such plasmid transfer between compatible bacteria. *Escherichia coli* acts as a reservoir of one such plasmid. From this reservoir the plasmid is transferable to numerous species of several genera including *Shigella*, *Salmonella*, *Klebsiella* and *Enterobacter* (see Watanabe, 1969). Likewise, another penicillinase plasmid, rare in 1940, not only spread through its original host *Staphylococcus* population (Munch-Peterson & Boundy, 1962), but also started newly colonizing both *Neisseria gonorrhoeae* (Phillips, 1976) and *H. influenzae* (Medeiros & O'Brien, 1975). Restricting either intra-cell diversity or intra-organismic diversity could reduce the rate of plasmid spread. Such restriction could result from selection at either individual or group level.

Similarly, the mixing of parasite types allows recombination and the production of novel genotypes. Phages can be inactivated by exposing them to ultraviolet radiation. Such phages can infect cells but cannot multiply. However, Stent (1963) has shown that if two or more U.V.-inactivated phages of closely related strains co-infect a bacterial cell, then recombination of the viral genomes produces phages capable of multiplication. Furthermore, under conditions of mixed viral infection, when the viral particles are formed, DNA of one strain can end up in the protein coat coded for by another. Such "resurfacing" extends viral host range (see Cooper & MacCallum, 1984 for review).

Multicompartment viruses (MCVs) represent a dramatic and clear demonstration of the potential damage intracellular interparasite synergisms can inflict. MCVs are a complex of independent genetically different virions which require each others presence to replicate. Tobacco rattle virus has two such independent units; a long

particle which codes for a replicase and a short particle coding for the coat protein. On their own the individual compartments of MCVs are not pathogenic, but together they are potentially lethal to the host (see Bruening, 1977).

So long as the beneficial effects of a multiplicity of parasites are in the minority, the general statement that parasite diversity is to the detriment of the host, will be valid. The occasions on which a diversity of parasites is beneficial to the host seem to be rare. Kaper *et al.* (1976) report that in tobacco dual infection of CMV and CARNA5, one of CMV's satellite viruses, results in a less severe affliction than expected. However, in tomatoes the same viruses act synergistically (Kaper & Waterworth, 1977). Richie (1988) has reviewed evidence suggesting that closely related *Plasmodium* species can compete so intensely for red blood cells that neither species takes hold immediately. Richie adds however, that a short while after such a bout of competition prolonged malaria is common. Viral infection of parasitic protozoa can inhibit the protozoan's destructive course (see Miles, 1988 for review).

For the rest of the paper I shall explore some of the consequences of the assumption that a high intracellular and/or intraorganismic diversity of parasites is generally costly.

3. The Evolution of Diploidy

Consider a population of unicellular organisms with a life history much the same as *Chlamydomonas snowiae*, i.e. a population of eukaryotic, sexual, haploid, isogamous protists. Such unicells often harbour intra-cellular parasites. For example, numerous cytoplasmic RNA "killer particles" are known in yeast (Bevan & Somers, 1969; Fink & Styles, 1972; Wickner, 1981). At least eight different species of bacterial killer particles are known to infect *Paramecium aurelia* (Beale *et al.*, 1969; Preer *et al.*, 1974). Martin & Benson (1982) review viral, bacterial and fungal infections in algae. Kirby (1941) provides an extensive review of the parasites of protozoa.

Let us focus on the time when the two haploid gametes fuse to produce the diploid zygote. In present day Chlamydomonads the first division after zygote production is meiotic. Meiosis however is a time consuming process (Bennett, 1971). As a consequence, the maternally and paternally derived parasites are afforded the chance to mix. Cytoplasmic mixing is known to occur during cell fusion in a number of protozoans, e.g. *Tetrahymena pyriformis* (McDonald, 1964). Sonneborn (1944, 1945) has shown in *Paramecium* that if separation after conjugation is artificially delayed, then the cytoplasmic bridge between the conjugants widens and cytoplasmic exchange is extensive. French (1978) has argued that the probable cause of abnormal (teratological) embryogenesis in certain mosquito eggs is an adverse interaction between dissimilar paternally and maternally derived symbionts.

If, as is likely in an outcross of the hosts, the genotypes of the maternally and paternally derived parasites are different, then parasite diversity within the four daughter cells is potentially high. As a consequence, the daughter's fitness is at risk. Thus if mixing of parasite types could be avoided, the fitness of the offspring could be enhanced.

One way to limit the degree of mixing of cytoplasmic contents would be to cleave the cytoplasm down the plane of cell fusion soon after the fusion of the nuclei. The simplest and most economical way to do this would be for the zygote to divide producing two nucleate daughter cells. For this to be a viable means of preventing cytoplasmic mixing, the first division must be rapid. Mitosis is relatively rapid compared to meiosis. The degree of parasite mixing can be further reduced by continuing high speed mitotic divisions after the first vital division. The plane of such divisions is less important than that of the first division. Rapid divisions in a constant size blastula producing ever smaller cells, could also have the side effect of leaving some cells parasite free. For some zygotes there is a short delay before the first cytoplasmic cleavage can take place. In many systems (e.g. *Caenorhabditis elegans*) the zygote inhibits the mixing of cytoplasm during this period by "pseudo-cleaving", i.e. by constricting across its centre but not truly dividing (Strome, 1986).

The efficacy of rapid mitotic divisions as a means of eliminating parasites has been empirically shown. Preer (1950) demonstrated in *Paramecium* that duplication of intracellular kappa particles does not always keep step with cell division. As a consequence some cells (and therefore descendant lines) were rendered parasite free. Hammerling (1946) and Schulze (1951) both observed that if a population of the protozoan *Stentor* is provided with abundant resources at an appropriate temperature (30°C) then the division rate of the protozoa accelerates and frequently outpaces that of the symbiotic algae (*Chlorella*) rendering many cells white. Bevan & Somers (1969) provide evidence that cytoplasmic determinants ("killer particles") in yeast can be lost from cell lineages by virtue of asymmetric segregation during mitosis. In Euglenoids division occasionally results in one of the daughters receiving all the chloroplasts or both of the daughter nuclei (Leedale, 1967). French (1970) has demonstrated the segregation of cytoplasmic elements in the germ line of female mosquitoes. The population dynamics of intracellular replicators has been analysed for the condition where the duplication of the particles keeps pace with that of the host cell (Wright, 1969: 351). Whether or not such segregation occurs during cleavage would be straightforward to test.

If the total volume of the zygote remains unaltered during the phase of rapid multiple divisions, then the pool of free nucleotides will necessarily decrease, whereas the nuclear demand for nucleotide bases would exponentially increase. This tendency for the multiplying nuclei to act as metabolic sinks sequestering nucleic acids may be itself inhibit parasite growth.

3.1. OOCYTE COMPETITION

In a rapidly dividing blastula, preventing the elimination of necessary organelles would be of importance, particularly in systems where every cell of the blastula is fated, for example, *Caenorhabditis elegans.* A common and apparently effective tactic to circumvent the problem is for oocytes to be provided with large numbers of randomly distributed organelles (reviewed in Billett, 1979; well documented in *Xenopus*: Dawid, 1966; Callen *et al.*, 1980, 1983; Laskey *et al.*, 1979). Such a large mass of organelles might also crowd out parasites. How it is that oocytes can allow

the expansion of a population of mitochondria but not the proliferation of parasites is unknown. It might be that no such mechanism exists but rather that oocytes are forced to compete for the right to mature, and hence only the parasite free members ever live to reproduce. From an initial 7 000 000 potential egg cells in a human foetus only 2 000 000 primary oocytes will survive till birth. Of these, all but 300 000 will degenerate prior to puberty, and of these only some 400 will mature into secondary oocytes. The notion that oocytes compete for the right to reproduce is not new (see Stearns, 1987). However, the parasite theory makes novel discrete testable predictions as to the source of variation in fitness between oocytes.

3.2. CLEAVAGE POLYEMBRYONY

Dividing a blastula into several subunits (cleavage polyembryony) may reduce within organism parasite diversity. This phenomena is seen as a normal occurrence in various species of parasitic insects (*Hymenoptera* and *Strepsiptera*) (see Imms, 1957 for review), in species of armadillo (Galbreath, 1985) and in species of both gymnosperms (Chamberlain, 1935; Buchholz, 1926) and angiosperms (Lakshmanan & Ambegaskar, 1984). For the parasitic insects, cleavage polyembryony is probably an adaptation to allow the production of a large clutch from few eggs. Why armadillos produce identical offspring is unknown. The plant examples might be explicable in terms of reduction of parasite pressure. Whereas in the animal examples there is little or no competition between the identical sibs, in the plant examples it is observed that where, for instance, five identical embryos are produced they all compete for limited resources and only one ever survives. The survivor then competes with non identical sibs for resources. In this case cleavage polyembryony might act to produce embryos of not only lower than original parasite diversity, but also of different parasite diversity. Competition may be acting to select out the embryo with the least problems with parasites. This hypothesis may be testable by examining the competing embryos and assessing their parasite load. Cooper *et al.* (1984) report that birch seeds formed from virus free gametes have greater success at germination than do infected embryos.

Mitotic cleavage of a diploid zygote would produce a blastula comprised of diploid daughter cells some of which might be parasite free. Diploidy as a long standing condition would have evolved as a side effect of selection to prevent parasites from mixing within any one cell, and to eliminate parasites from some cell lines. Unless infected cell lines commit suicide, rapid divisions do not reduce parasite diversity within the whole organism and any costs associated with this condition still remain.

3.3. THE EVOLUTION OF MULTICELLULARITY AND THE RESTRICTED RIGHT TO GAMETOGENESIS

In a blastula formed from rapid mitotic divisions, the cells would differ in their cytoplasmic constituents. If the cells of the blastula are forced to compete for the right to gametogenesis, then it is probable that the cells with the lowest parasite diversity (often parasite free cells) would win. Thus, it is in the interests of the

nuclear genome to restrict the right to develop into gametes and thus force such inter-cell competition, with the "winning" cell lineage undergoing gametogenesis and "losers" being subjugated. The ability to restrict the right to gametogenesis is likely to be contingent on the blastula remaining as a discrete entity: controlling the reproduction of numerous dispersed free-living unicells is not a viable alternative. As the cells of the blastula have identical nuclear genomes, there is no disadvantage to the nuclear genome in any given cell in allowing a more competitive cell lineage to produce the gametes. This assumes that the number of gametes produced by the multicellular organism is not dramatically lower than the number of gametes produced by the unicells which would otherwise have formed. As the multicellular condition allows the nuclear genes of the subjugated (and therefore heavily parasitized) cells the opportunity of investing in low parasite diversity gametes, such a deficit is unlikely. It follows that the evolution of multicellularity might be a consequence of the advantages of being able to restrict the right to gametogenesis and hence force competition between the cells of a clone, i.e. having gametes of minimal, if not zero, parasite diversity.

Once multicellularity and the restricted right to gametogenesis have evolved, then every cell would have a stake in the competition for gametogenesis. Those cells with parasites would be forced by their parasites to strive to allow the symbionts vertical transfer. Unparasitized cells would be inspired by their nuclei to prevent parasitized cells from gaining such access. It would be in the interests of the nuclei of the subjugated cells (which would be identical to the nuclei of the dominant cells) to serve a somatic function instead. Such differentiation of sterile lineages would increase the fitness of the blastula and promote the long term stability of the multicellular condition. The possible consequences and extensive evidence of cell lineage competition during embryogenesis have been further explored by Buss (1987). In his discussion, Buss assumed that cell lineages derived from the same zygote would compete for the limited right to gametogenesis. The rationale given above suggests a reason for such gametogenic restriction and for the subsequent competition and hence for the evolution of the multicellular condition. Hastings (1989) has conjectured that nuclear genetic asymmetries due to mutation, mitotic cross overs and mitotic gene conversion could also result in cell lineage competition between the sperm of an individual.

The cleavage divisions would not only act to sequester maternal from paternally derived symbionts, but also the maternal and paternal organelles would be isolated. There is a body of evidence supporting the idea that organelles are forcibly isolated from each other (Sager, 1972, 1977; Sager & Ramanis, 1976; Singer *et al.*, 1976; Wilkie, 1973). This may be because when these organelles detect that they are in competition with "foreign" genotypes, they reveal facultative exploitative behaviour, a consequence of which is harm to the host cell.

4. The Evolution of Anisogamy

In this section I argue that anisogamy may have evolved to reduce both intracellular and intraorganismal parasite diversity.

Consider a population of multicellular organisms like the one whose evolution has just been considered, i.e. a diploid, sexually differentiated, dioecious, isogamous simple multicell. Suppose the sizes of the cells involved in gametogenesis are the same for both mating types. The parasite diversity of a gamete is likely to be an increasing but decelerating function of gamete size. An upper limit on parasite diversity in any gamete is imposed by the diversity of the parasites in the cell from which the gamete was produced.

How will selection act on gamete size? Consider a population of + and − type isogametes in which there is heritable variation in gamete size. The mean size of "+ gametes" equals the mean size of "− gametes". If by chance the mean sizes of the two gamete types were to drift apart, will selection restore the population to its isogamous equilibrium or magnify the difference in mean gamete size? Selection pressures will arise both from the size of zygotes and their parasite diversity. For a given zygote size, the fittest zygotes are those with the lowest parasite diversity, i.e. those which are the products of the gametes most different in size. Hence, it is the zygotes produced by the fusion of the smaller of the small type with the larger of the large type that are the fittest. In contrast to the perfectly isogamous condition, this fitness is greater than the fitness of the zygotes which are the products of the fusion of the larger of the small type gametes with the smaller of the large type gametes. If the selection pressure arising from parasite diversity is strong enough then in the next generation of gametes the difference in the mean size of + and − type gametes will be greater than before. Successive bouts of selection, acting to minimize parasite diversity, would increase the difference in mean gamete size. The selection pressure from parasite diversity would be a continuing force for anisogamy. As the zygote size can be maintained through an increase in the size of the large gametes, there is likely to be no strong force initially against divergence of gametic sizes.

The scenario can also be run without mating types. As the pressure forcing bimodality continues there will arise a selective pressure on the gametes to ensure that they mate only with a gamete of the opposite size. Gametic recognition mechanisms might then be expected to evolve. Wastage due to suboptimal zygote formation prior to the formation of gametic recognition mechanisms would inhibit the tendency towards anisogamy.

This theory for the evolution of anisogamy works just as well for Chlamydomonad-like unicell as for multicellular organisms. The advantage a multicellular organism has is that large eggs can more easily be afforded and manufactured. Furthermore, the sperm whose effectiveness as parasite free agents will depend on their small size, can be easily nurtured in a multicell, in a nutrient-rich predator-free environment. On the whole unicells would depend on other cells to provide such protection and nutrition. Such is the tactic adopted by, for example, Eimeriid coccidians when they parasitise host cells (see Noble & Noble, 1972).

5. Are Parasites Vertically Transmitted?

If parasites are not transferred to the zygote from gametic cytoplasm then all of the above theory is irrelevant. Demonstrating that vertical transmission of parasites

is relevant to all sexual organisms is all but impossible. What can be said, is that in the groups most extensively studied vertical transmission is found frequently. There is a consensus of opinion that vertical transmission is an important phenomenon. In seed plants most is known about virus transmission in the agriculturally important species of the Chenopodiaceae, Graminae, Leguminosae, Rosaceae and Solanceae (see Cooper & MacCullum, 1984 for review). Vertical transmission has been shown for many ilarviruses, hordeiviruses, tobamoviruses, nepoviruses, tobraviruses and several other orders of virus. Seed transmission has not been demonstrated in numerous DNA viruses such as the geminiviruses and luteoviruses. In fungi the transmission of viruses through spores has been demonstrated in numerous cases. Lower plants are less well studied than angiosperms and as a consequence fewer examples of vertical transmission have been reported. Matthews (1981) reviews viral infections and transmission in all plant taxa.

There is a sizeable body of information on gametic transfer of symbionts in animals. Grun (1976) and Buchner (1965) between them discuss the evidence regarding vertical transmission from prokaryotes to vertebrates. Viruses, bacteria, mycoplasmas, protozoans, fungi, and spirocheates have all been shown to be capable of vertical inheritance. For the insects, examples of gametic transfer abound (Koch, 1967). In vertebrates fewer examples have been demonstrated but leukaemia, and choriomeningitis in mice, lymphomatosis in chickens, scrapie in sheep, Leber's optic neuritis and Gerstmann-Strussler syndrome (Hsiao *et al.*, 1989) in man are all thought to be vertically heritable. Fenner & White (1970) report the vertical transfer of an arbovirus in snakes. Fine & Sylvester (1978) provide a brief review of vertically transmitted organisms and provide a model for the analysis of transmission rates using data on an aphid borne virus.

6. Are Small Gametes Less Likely to Carry Parasites?

If the propensity of small gametes to transfer parasites is equal to that of large ones, then the theory of anisogamy presented here is invalid. Afzelius *et al.* (1989) sum up the position arguing that "whereas micro-organisms residing within the female gamete are relatively common ... there are few reports on micro-organisms in the male gamete". Afzelius *et al.*'s 1989 review the literature on arthropods and go on to report four new incidences (in a bird spider, a fire bug, a mite and a sandfly). They comment that "these incidences represent only a minute fraction of the total number of sperm cells from insects and arachnids that we studied during several decades of spermatological investigation."

The situation is similar in the plant kingdom. In lettuce, for instance, there is 5% transmission of lettuce mosaic virus through the ovule compared to less than 0·5% through pollen (Ryder, 1964). Hamilton *et al.* (1977) have shown that viruses can infect pollen exine and from there can be transferred to the zygote. Matthews (1981), in reviewing viral transmission in angiosperms, argues that not only is infection by the megagametophytes much more common than infection by pollen, but also that infected pollen competes poorly with normal pollen during fertilization. Cooper *et al.* (1984) make the same observation of birch pollen infected with Cherry Leaf Roll

Virus. Likewise, mosquito and moth spermatozoa infected by rickettsia are incapable of fertilizing eggs (Weiss & Dasch, 1981; Kellen *et al.*, 1981). The poor competitive abilities of infected pollen suggests a possible advantage to long styles. The notion that style length is a means to force pollen competition is amenable to a comparative analysis across the angiosperms, between style length and the mean and variance in viral load in the pollen grain of each species. Interestingly, in the few cases known where transfer through the male gamete is more efficient than that through the female (Frosheiser, 1974; Hemmati & McLean, 1977) the plants have short flowers (e.g. Alfalfa Mosaic Viruses in Alfalfa).

Vertical transmission of parasites within the nucleus of the male gamete (not including the inclusion of DNA into the host genome) is more rare than cytoplasmic transfer. Afzelius *et al.* (1989) report viral particles in the sperm nuclei of a sandfly (*Phlebotomus papatasii*, Diptera). Schrankel & Schwalm (1975) observed virus-like particles in both nucleus and cytoplasm of maturing spermatocytes from kelpfly, *Coelopa frigida*. Carroll (1974) found Barley stripe mosaic virus in cytoplasm and nucleus of barley pollen.

The low efficiency of nuclear transfer is probably due to a combination of factors. The DNA is packaged very tightly allowing the volume of the nucleus to be minimized and free space in sperm nuclei is miniscule hence there is very little room for parasites. Such tight packing of DNA is facilitated by the use of protamines rather than histones (see Baccetti & Afzelius, 1976; Dixon *et al.*, 1985; Mezquita, 1985; Kasinsky *et al.*, 1985). Kirby (1941) points out that for protozoa, whereas cytoplasmic symbionts may be tolerated and not necessarily lethal, nuclear symbionts are almost inevitably lethal. Whether sperm are equally vulnerable to nuclear parasites is uncertain.

The restriction of vertical transmission of symbionts through sperm is a central tenet of the theory of sex ratio manipulation by symbionts. It is argued that as male hosts prevent transfer of symbionts to the next generation, so it is in the interest of symbionts either to force eggs to develop parthenogenetically (Stouthamer *et al.*, in press; Nur, 1972) or to force hosts which would otherwise be male to alter sex or to kill the host if it is male (Werren, 1988; Bull, 1983). In the latter case it is assumed that the symbionts in subsequent offspring are related to those that killed the male eggs.

7. Conclusions and Discussion

I have conjectured that a host cell will suffer if it is exposed to a diversity of parasites. At least in the context of embryogenesis and anisogamy, this emphasis on parasite diversity seems to be new. Unfortunately there is very little empirical data on intracellular interactions.

7.1. DIPLOIDY

Assuming the validity of the conjecture, it was shown that diploidy may be a side product of a method to limit intracellular parasite diversity. Previous theories of

diploidy have concentrated on finding an advantage to possessing two copies of every chromosome. The "hiding" of recessive harmful mutants, the exploitation of loci which are overdominant for fitness, protection against somatic mutants and the ability to correct mistakes which occur on both strands of the same chromosome at the same locus have been suggested by other authors (see Crow, 1988 for a brief review). The theory outlined here is novel in that it demands no advantage *per se* to the possession of two sets of chromosomes.

7.2. MULTICELLULARITY

The cells of the blastula formed by rapid cleavage differ as regards their parasite diversity. Maintaining the blastula as an entity is a necessary condition to allowing the nuclear genome to restrict the right to gametogenesis. This it was argued, is to the advantage of the nuclear genes in that it forces competition for the right to become a germ cell, and hence results in low parasite diversity gametes. This view of cleavage and the evolution of multicellularity explains why it is that cell fate in numerous invertebrates (notably the deuterostomes), is usually not determined until at least several cleavage divisions have taken place. The notion that competition between the cells of a developing blastula acts to allow those cells with low parasite diversity to transform into gametes, can be tested by examining the fates of cells of any organism with indeterminate cleavage.

7.3. ANISOGAMY

With parasite diversity as a hindrance to host cells, it was shown that there is a positive advantage to an egg to fuse with as minute a sperm as possible. I argued that under such a pressure an isogamous population can evolve into an anisogamous one. The hypothesis is unusual in that it proposes that the interests of mother, father and offspring are best served if sperm is very small. Parker *et al.* (1972) view sperm multiplicity as the advantage to small size. Cohen (1973) argues that sperm multiplicity is a consequence of the inaccuracy of crossing over during meiosis. Crossing over, it is argued, results in many "dud" sperm for every "good" one. Hence large number of sperm are required to ensure reproductive success. Several authors have formulated refined versions of Parker *et al.*'s model (Maynard Smith, 1978; Charlesworth, 1978; Schuster & Sigmund, 1982; Hoekstra, 1980; Cox & Sethian, 1985). Under such a model sperm are seen as being parasitic on the resources of the egg (see also Cosmides & Tooby, 1981). Parker (1982) has pointed out that such theories have not been able to explain fully why, when there is no advantage to producing a large number of sperm, for example, in monogamous mammals, the sperm need be extremely small. The above theory does just that.

The final stage in the development of a mammalian free swimming sperm is the detachment of the large cytoplasm rich spermatid from its site of development. Just prior to this detachment a large body of spermatid cytoplasm accumulates near the head of the spermatid. When the slender spermatazoa breaks free, this cytoplasm is left behind. This excess cytoplasm, the residual body, is digested by the sertoli

cells of the testis, presumably at some cost. The existence of residual bodies is not unique to mammalian spermatogenesis. They have been described in spermatogenesis in plants (e.g. ferns, Myles & Hepler, 1977), invertebrates (e.g. nematodes, Ward *et al.*, 1981) and chordates. It appears then, that these organisms prefer to invest energy into ensuring sperm are cytoplasmically sparse, rather than producing more spermatozoa. In cases of meiotic drive in males only one of the two products of the first meiotic division of the pro-sperm go on to form mature sperm, thus the sperm count is half what it might be. The sterile, often larger half (Jazdowska Zagrodinska & Dallai, 1988) functions as a "residual cell" and is degraded. Notably, both Erickson & Acton (1969) and Yanders *et al.* (1968) observed in *Drosophila melanogaster* that symbionts (probably *Rickettsiae*) are forced into the sterile cell. This exclusion of parasites might underlie the persistence of the meiotic drive systems in natural populations. In the few cases where exclusion from the fertile cell was incomplete the granules ended up in the residual body.

Hoekstra (1984) has proposed a motility advantage for small gametes. This hypothesis predicts that sperm should be small. It differs from the hypothesis presented here, in that it predicts neither that sperm volume should be reduced to zero if possible, nor that cytoplasmic mixing during isogamous fusion should be minimized. The hypothesis is countered by observations that sperm motility efficiency sometimes increases when the sperm are paired or in multiples (Sivinski, 1980, 1984). For example, the sperm of the firebrat (*Thermobia domestia*) are immotile while solitary but motile when entwined in pairs (Bawa, 1964).

The parasite diversity hypothesis of anisogamy can be tested by asking if isogamous organisms (and those with larger than predicted sperm) have some alternative means to prevent parasites from mixing. The hypothesis predicts that some means to prevent parasite mixing should be evident. This is examined below.

The Chlamydomonads are remarkable in that their cytoplasm is all but fully occupied by giant organelles. Likewise, Burr & West (1970: pl. 34) have shown in the male gamete of *Bryopsis hypnoides* (Alga: *Siphonales*) that between the nucleus, the single giant chloroplast and the single giant mitochondrion, almost all of the cell volume is occupied. The less densely packed female gamete can be host to bacterial inclusions (Burr & West, 1970: pl. 23). Similarly, mitochondrial crystals compose 90% of the giant sperm of the back-swimmer (*Notonecta glauca*). Could this tight packing of the cytoplasm be an attempt to restrict parasites by simply not giving them any room? *Paramecium aurelia* avoids even modest cytoplasmic mixing during conjugation, but separating shortly (2–3 min) after fusion and by exchanging micronuclei through a cytoplasmic bridge (Preer, 1969). Furthermore, the micronuclei need to constrict to pass through the small gap joining the conjugants (Andre & Vivier, 1962; Vivier, 1965; Inaba *et al.*, 1966). Sonneborn (1944, 1945) has shown that if separation is delayed, then the bridge widens and cytoplasmic exchange is extensive. In *Tetrahymena pyriformis* inclusions the size of mitochondria cannot pass through the small pores in the conjugative plate (Roberts & Orias, 1973; Elliot & Hayes, 1953). The observation that both the macronuclear/micronuclear system and the process of conjugation are exclusively restricted to the members of the Subphylum *Ciliophora* (Kudo, 1977), lends weight to the idea that the micronucleus

evolved as a means to minimize parasite mixing. By minimizing the size of the nucleus which passes between conjugants, so the diameter of the cytoplasmic bridge can be reduced and cytoplasmic spillage all but eliminated.

Numerous species of land plants, particularly lower land plants, have large spermatozoids, however, paternal contribution of plastids (and cytoplasm generally) to the zygote is apparently rare, though the phenomenon has been observed in some gymnosperms. Whatley (1982) has reviewed the methods by which biparental inheritance of plastids is avoided. It is observed across many divisions of plants, that the entrance to the chamber containing the oocyte is narrow and that spermatozoids can only pass through the constriction if they shed their cytoplasm. Such discarding of cytoplasm is understandably common in the light of the theory proposed here.

The conventional explanation for the preservation of isogamy is ecological. Cox & Sethian (1985) have noted that isogamy is commonly restricted to ecological situations in which long lived gametes are at a selective advantage. Masden & Weller (1983: table 1) support this idea by demonstrating a relationship between the degree of gamete dimorphism and the permanence of habit. These two explanations for the preservation of isogamy and the one offered in this paper are not mutually exclusive.

Implicit in the hypotheses presented was the notion that sex involving contact between maternal and paternal cytoplasm is more costly than has been previously assumed. Anisogamy is possibly an attempt to minimize that additional cost.

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