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Selfish Genetic Elements and their Role in Evolution: The Evolution of Sex and Some of What that Entails

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Selfish genetic elements and their role in evolution: the evolution of sex and some of what that entails

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SUMMARY

An individual is often considered (sometimes implicitly) to be the product of a well functioning mutualism between its constituent genes. This however need not be so. One consequence of sexual reproduction is that costly competition within an individual between genes that are effectively allelic can provide the conditions for the spread of suppressors of such competition. The spread of both these ultracompetitive alleles (alias selfish genetic elements) and their suppressors is evidence of a 'conflict of interests' within the genome. That this conflict is a potentially important force in the evolution of genetic systems is illustrated by consideration of the problem of the evolution of sexes (alias mating types). One hypothesis holds that sexes are the result of selection on nuclear genes to coordinate the inheritance of cytoplasmic genomes (usually this means the enforcement of uniparental inheritance) so as to prevent competition between unrelated cytoplasmic genomes. This hypothesis is tested against five comparative predictions and shown to receive considerable empirical support.

1. INTRODUCTION

The production of a diploid zygote through the amalgamation of two haploid gametes (i.e. sex) is not only a marvel of communication and coordination, it is also one of the most risky endeavours performed by eukaryotic organisms. The risks include the possibility that the two genomes fail to adequately coordinate the subsequent development of the zygote, thus resulting in inviability or sterility, of contracting a disease from the partner or even of not finding a mate. A more subtle risk perhaps is that your partner's genome might try to parasitize yours, so tending to prevent your genetic contribution to the progeny from being transmitted to further generations.

Like the Montagues and Capulets whose coexistence in Verona could only end in disaster, the bringing together into one territory (the zygote) of unrelated genomes through sexual reproduction should result in tragedy. Seen from this perspective, it is perhaps one of the abiding mysteries of genetics that two genomes can ever come together into one cell, reside harmoniously together and then peaceably disperse (i.e. go through Mendelian segregation). Here I consider what might happen if coexistence was not peaceful. In particular I discuss the possibility that, following the fusion of two unrelated gametes, competition between cytoplasmic factors might break out and how the evolution of sexual differentiation may be the consequence of attempts to restrict this competition.

Genes that out-compete their allelic counterparts in the struggle for transmission from an individual are usually referred to as selfish genetic elements (alias selfish genes, ultraselfish genes etc.). Well described examples include meiotic drive genes such as segregation distorter of *Drosophila* and the *t-complex* of mice (reviewed in Lyttle 1991). Males that are heterozygous at the drive locus transmit almost exclusively the drive allele and hence the wild type allele is recovered at frequencies well below the expected Mendelian proportion. This is achieved by the drive allele specifically interfering with the functioning of sperm containing the wild type allele. Even if deleterious the drive allele spreads through a population because of its transmission at greater than Mendelian rates (assuming that the fitness of heterozygotes is not too low).

Selfish elements need not only be nuclear alleles. Biparental inheritance of cytoplasmic heritable factors (organelles, symbionts etc.) after the fusion of two cells provides the conditions for competition between these factors (Eberhard 1980; Cosmides & Tooby 1981). Fast replicating mitochondrial genomes are one form of selfish cytoplasmic factor that has been reported in fungi (e.g. petite mutants of yeast) and is suspected in animals (see: Rand & Harrison 1989; Wallace 1992). Alternatively, we may envisage 'killer' organelles that specifically eliminate their competitors (much as SD bearing sperm inhibit wild type sister sperm). Only circumstantial evidence can be provided for the existence of such organelles (Anderson 1968; Chiang 1976; Eberhard 1980; Koslowsky & Waaland 1987). There exist however numerous precedents for such directed 'spite' by cytoplasmic factors, e.g. cytoplasmic incompatibility factors in arthropods (Rousset & Raymond 1991), ciliate cytoplasmic mate killers (Beale & Jurand 1966), and cytoplasmic killer factors in yeast (Somers & Bevan 1969) and in *Ustilago* (Puhalla 1968). Plasmid-encoded colicin factors (Eberhard 1990) may be considered a prokaryote analogue of the above.

Here I consider the hypothesis that sexes/mating types are the result of selection on nuclear genes to coordinate the inheritance of cytoplasmic genomes so as to limit competition between unrelated cytoplasmic genomes.

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2. SELFISH CYTOPLASMIC FACTORS AND THE EVOLUTION OF MATING TYPES

(*a*) *The models*

Imagine a population of unicellular organisms that have sex involving the fusion of two gametes. The gametes in the primitive condition are assumed to be the same size as each other (i.e. isogamous) and are not differentiated into mating types: any gamete can mate with any other. Let us assume that each gamete carries one mitochondrion (any similar cytoplasmic organelle would be equivalent for the following argument). At first these mitochondria are all *passive*, by which is meant they do not react to the presence of other mitochondria introduced from the partner's cytoplasm during gametic fusion nor does one replicate faster than the other. Now consider the fate of an ultra-competitive mutant mitochondrial gene (i.e. one that either destroys the mitochondrion of the partner or replicates faster than the others, as discussed above). Either way we may envisage that there is some cost to the individual to the possession of the selfish element. Let then the fitness of cells with the ultracompetitive mitochondrial genome be $1-U$ (i.e. they suffer some cost, U). A zygote that is the product of the fusion of a cell with the new mutant and the wild type transmits the mutant to a proportion k of its progeny ($k > 0.5$ would mean that the mutant is ultracompetitive). In an outbred population it trivially follows that invasion of the mutant is possible if $k(1-U) > 0.5$. These provide very broad conditions for invasion.

The spread of the deleterious selfish element reduces the average fitness of the population and provides the context for the invasion of a nuclear gene that enforces uniparental inheritance. The precise dynamics of the spread of the mutant nuclear allele depend on just what one envisages as the costs associated with the selfish cytoplasmic factor (Hoekstra 1987; Hastings 1992; Hurst & Hamilton 1992; Law & Hutson 1992; Hutson & Law 1993). Two costs on individual fitness have been considered to accompany over-representation. First, there is the cost to the act of attempted over-representation (e.g. if two mitochondria attempt to annihilate each other the cell may end with a depleted mitochondrial population). Second, there is the cost to the possession of the selfish factor. For fast replicating cytoplasmic factors it is this latter cost that is possibly the more significant, whereas for 'killer' factors both may be realistic.

If the only cost is to possession of the selfish cytoplasmic factor then the nuclear allele that enforces uniparental inheritance (the suppressor) must (1) arrive before the selfish mitochondrial genome goes to fixation, (2) act to destroy the mitochondria from the partner, not its own, and (3) be introduced in the population in a cell with the relatively unselfish mitochondrial genomes (but see Hutson & Law 1993). Under these circumstances, the nuclear suppressor allele maintains itself in linkage disequilibrium with (and hence hitchhikes with) the 'fit' mitochondrion, whereas alternative alleles will have their fitness reduced as they tend to associate with the selfish mitochondrion (Hastings 1992).

Alternatively, we can consider a cost to the act of gaining over-representation that can be reduced if the process of over-representation is not enacted (e.g. with

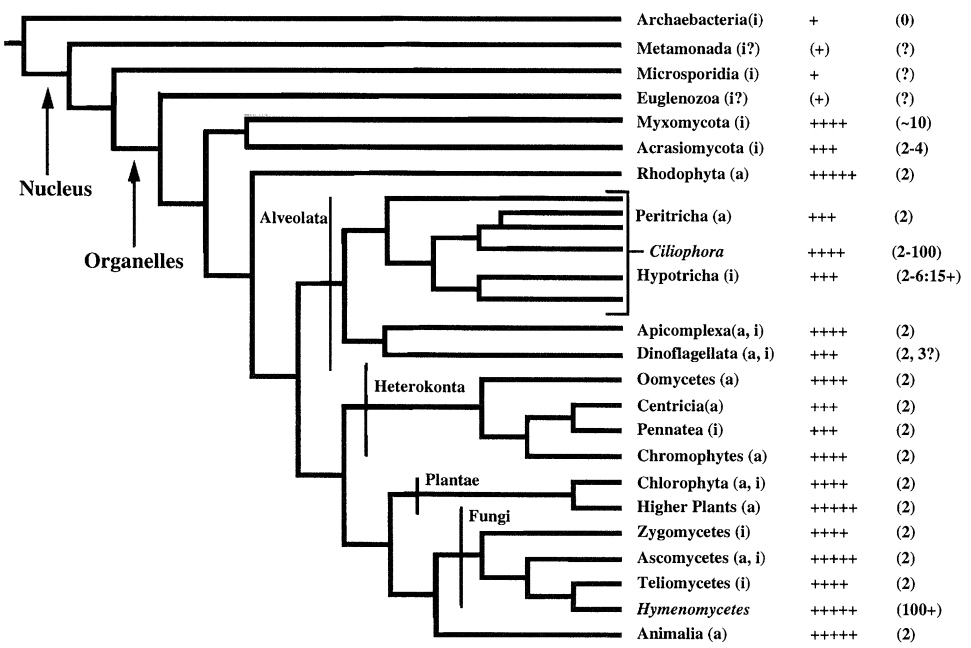

Figure 1. The phylogeny of eukaryotes, the incidences of sex and of sexes (mating types). Number of +s indicates the amount of sex that has been reported and with what confidence (a bracket indicates a questionable report). Those taxa in italics have sex but no cytoplasmic fusion. Letters in parentheses after the taxa indicate whether gametes are generally isogamous (i) or anisogamous (a). Numbers in parentheses indicate number of mating types/sexes. Two sets of figures are given for hypotrich ciliates as both fusion and conjugation occur within the same population. The first figure is the number of mating types that induce fusion within a syngen (i.e. species), while the second is the number that coordinate conjugation. Data on phylogeny from Cavalier-Smith (1983); data on sex and sexes from Grell (1967, 1973), Margulis (1990) and other papers detailed in the text.

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uniparental inheritance two mitochondria will not eliminate each other). Under this circumstance, even when the selfish factor is at fixation the nuclear modifier that forces uniparental inheritance can still invade (Hurst & Hamilton 1992).

What will be the fate of a nuclear allele that forces uniparental inheritance? It is reasonable to suppose that it will not go to fixation. This will be because it too is associated with certain costs. When two gametes with this allele meet, the zygote may end up with no (or depleted) mitochondria. There may also be a metabolic cost to the enforcement of uniparental inheritance. Either way, a population will go to a stable polymorphic equilibrium maintaining both suppressor and non-suppressor alleles.

At this equilibrium, non-suppressors can still mate with non-suppressors (so suffering the costs associated with the selfish mitochondria) while at the same time two cells each of which annihilate mitochondria can mate. This position is one in which relatively strong selection for the evolution of assortative mating can occur (Hurst & Hamilton 1992; Hutson & Law 1993). We could hence consider a nuclear allele that acts to prevent its haploid cell from fusing with one of the same suppressor type and thus tends to avoid non-optimal matings.

Several different forms of preference allele can be envisaged. There may be a general purpose recognition allele that 'knows' both the suppressor type of its host cell and that of the potential mates and permits fusions between unlike cells only. Alternatively, and more realistically, we could consider an allele that prevented the cell containing it from mating with cells with the same allele. The invasion conditions for the latter are more stringent than those for the former and require close linkage (which can be maintained by a block to recombination) of the choosy allele with the allele controlling mitochondrial inheritance.

By either model we end up with a population in which two types of cells coexist. One half of them prefer to mate with the other half and vice versa. That is, two mating types have evolved in a population in which there were originally none.

Binary mating types (i.e. +/− mating types) are commonplace among isogamous protists (see figure 1) but the alternative sexual differentiation (male/female) can be interpreted in the same light. No doubt the production of small gametes (sperm) has the advantage that many of them can be produced (Parker *et al.* 1972), but the small dimensions of sperm can be easily considered an adaptation to prevent the transmission of organelles and cytoplasmic parasites (Grun 1976; Cosmides & Tooby 1981; Hurst 1990; Hastings 1992; Law & Hutson 1992). Although sperm competition could vie as an alternative explanation for the same fact (Parker 1982) it is not consistent with the frequently reported elimination of cytoplasm just before gametic fusion (Sears 1980; Hurst 1994*a*).

(i) *Why only two sexes?*

The above is a possible explanation of the evolution of sexuality (see also Hoekstra 1987). As noted, one of the risks of sexual reproduction is the possibility that an individual cannot find a partner. This must be especially true for single celled protists that may be relatively immobile. That sexual species typically have two gametic types (+/− types in numerous single celled protists, sperm and eggs in others) makes the possibility of not finding a mate all the more problematic. Were there no sexes (as envisaged as the starting population) then any gamete could mate with any other; were there 100 sexes then any gamete could mate with 99/100 of the population; were there an infinite number, then, as with no sexes, any gamete could mate with any other. But with only two types, any gamete can only mate with half of the population. In fact, with two sexes the probability that a randomly encountered gamete is a viable mate is at a minimum and hence the state is that which we might least expect to regularly encounter. It is then remarkable that so many organisms have two sexes. So why did mating types evolve in the first place (the transition from zero to two) and why do so many organisms have only two sexes? The above model provides an answer to the first quandary. The same logic can be extended to considering the second question.

Imagine a population with three sexes (numbered 1, 2, 3). An individual that is of type 3 can mate with one of types 1 or 2. Similarly one that is type 2 can mate with types 1 or 3 etc. In any pairwise mating the inheritance of cytoplasmic factors needs to be coordinated. Let us suppose a hierarchy exists for the control of cytoplasmic gene inheritance: mitochondria of type 3 are always inherited; those of type 1 are never inherited. In a mating between type 1 and type 2, then, mitochondria of type 2 are inherited and in a mating between type 2 and type 3 those of type 3 are inherited. A precedent for such a hierarchy has been described (Kawano & Kuroiwa 1989; Meland *et al.* 1991). The mating between type 2 and type 3 is the problematic one. Unlike the mitochondria of type 1, in any given process of zygote formation mitochondria of type 2 cannot be 'primed' to be destroyed (were the mating between type 2 and type 1, it is the organelles of type 2 that would be inherited). Differential methylation of the chloroplast genomes of + and − type gametes of *Chlamydomonas* (for references see Matagne 1987) can be interpreted as evidence of such priming. One consequence of the absence of priming in a mating between type 2 and type 3 is that two sets of potentially heritable mitochondria will be present in the cytoplasm. This position is vulnerable to the spread of a selfish mitochondrial gene that somehow prevents itself being destroyed.

The spread of such a 'resistant' genome has a dynamic very similar to that described above and can in turn create the conditions for the spread of a novel type 3 cell that prefers to mate exclusively with type 1 (so permitting consistent uniparental inheritance). A model of this process will be presented at a future date. The spread of such a preference gene can, it is suggested, send the population back to having two sexes, or possibly, when the pressure to have more than two sexes is particularly intense, maintain a stable low number of (but more than two) sexes.

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Above the assumption is made that (1) sexual encounters are pairwise and (2) mating types function in such a manner that an individual of a given mating type can mate with all others that are not of the same mating type. This need not be so. A system of three mating types could be one such that type 1 can mate with type 2 but not type 3, while type 3 could mate only with type 2 (Bull & Pease 1989). This would make mate finding especially difficult, as would the necessity to find more than one partner (Power 1976). That such systems are not usually found suggests that the pressure to ease the finding of a mate is a realistic selective pressure (Bull & Pease 1989).

(*b*) *Testing the hypothesis*

The above models provide parsimonious explanation for the evolution of sexes (i.e. mating types) that is open to testing by comparative analysis. Below I test five predictions of the hypothesis.

Prediction 1. *The mating type of organisms with fusing gametes should define both who can and who cannot be a potential mate, but should also define which partner should and which should not transmit a particular class of cytoplasmic genes.*

In the anisogamous condition sexuality is typically associated with a bias in the transmission of a particular class of cytoplasmic factors. Typically it is the male that is not a transmitter, although in outbred gymnosperms and angiosperms paternal inheritance is described (Reboud & Zeyl 1994). In isogamous organisms uniparental (or heavily biased) transmission of organelles is typically coordinated by alleles that cosegregate with the alleles controlling mating preferences. This is true not only of the best described case, the mating type locus of *Chlamydomonas* (Armbrust *et al.* 1993), but also of acellular slime moulds (Kawano & Kuroiwa 1989), cellular slime moulds (Mirfakhrai *et al.* 1990), teliomycete fungi (Wilch *et al.* 1992) and numerous others (Sears 1980; Whatley 1982). One possible exception to this rule has however been described (Silliker & Collins 1988) and deserves further study.

The theory above can be employed to explain features of the organization of the mating type locus in *Chlamydomonas* (reviewed in Goodenough *et al.* 1995). The mating type locus contains, as expected, two types of genes: those coordinating who mates with whom and those coordinating organelle inheritance (Armbrust *et al.* 1993). The theory also makes sense of three other features of the mating type alleles. First, it is to be expected that selection should act to minimize the recombination rate between the numerous genes in the + and − cluster so keeping the two groups as intact units. Significantly then, the mating type loci have several features associated with the minimization of the recombination rate. These include a small absolute size and numerous rearrangements and inversions (Ferris & Goodenough 1994). Second, it is expected that the mating type allele responsible for enforcing uniparental inheritance of organelles should cause the destruction of the organelles of the opposite cell. The best available model of mating type function is consistent with this expectation as it proposes that the + type locus acts to destroy the chloroplast genome of the − type (U. Goodenough, personal communication). Finally, the two sets of self-avoidance alleles, while being allelic in segregational terms, need not code for the same product. This is indeed what is found at the mating type allele of *Chlamydomonas* (i.e. they are idiomorphic (Ferris & Goodenough 1994)) (for alternative interpretation see Bell 1993).

Prediction 2. *Fusion between cells that are closely related need not be associated with incompatibility types (mating types) and need not have controls over cytoplasmic gene inheritance.*

If the enforcement of uniparental inheritance is costly then it is not expected where, in a population with biparental inheritance, either selfish cytoplasmic genes cannot spread or the cost of those that do spread is always below the cost of nuclear suppression. These conditions are met when the rate of inbreeding is adequately high (Hurst 1994*a*). Biparental inheritance may thus be expected in fusions between closely related cells. Somatic cell fusion, as described in myxomycetes, ascomycetes and possibly rhodophytes, and also between muscle cells within developing animals, is usually restricted to cells that are very closely related (Hurst 1994*a*). This may be either because the nearest neighbour is always a relative (as with muscle cells and probably also rhodophytes (see below)) or because fusion is only permitted if cells are identical at highly polymorphic compatibility loci, as shown for ascomycetes and myxomycetes. These compatibility alleles can be contrasted to what are variously referred to as mating types, sexes and incompatibility alleles that permit fusion only if the two parties are *different* at the relevant locus. The high polymorphism and requirement for similarity is adequate to guarantee that fusions usually only occur between extremely closely related individuals and the system may have evolved so as to prevent parasitism by, and the transfer of parasites from, less well related cells (Caten 1972).

In these systems then, when there is fusion between compatible cells (rather than aggressive invasion (see, for example, Lane & Carlile 1979)), nuclear controls on cytoplasmic inheritance should be absent and biparental inheritance could be stably maintained. In the above instances there is no evidence of nuclear controls of organelle inheritance.

Genetic evidence demonstrates biparental transmission after somatic cell fusion in ascomycete fungi (see, for example: Jinks 1964; Gunatilleke *et al.* 1975). Cytological evidence suggests biparental transmission of organelles in somatic cell fusion in myxomycetes (M. Carlile, personal communication reported in Hurst & Hamilton 1992). Cytological evidence also demonstrates an absence of plastid degradation in naturally occurring intrastrain fusion in *Griffithsia* (red algae) (Goff & Coleman 1990). Perhaps significantly, artificial fusions between somatic cells of *Griffithsia* result in unilateral chloroplast destruction in between-strain fusions but not in within-strain artificial fusions (see: Koslowsky & Waaland 1984, 1987; Goff & Coleman 1990). Polymorphic compatibility loci have

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yet to be demonstrated in this species. These need not be expected. The fusion response in these organisms is a natural wound response stimulated by the breakage of a stem. The repair is coordinated by the two closest cells attracting each other and fusing. If the closest cell is always the broken cell then a close kinship between fusing partners is guaranteed and extra genetic means to ensure this are unnecessary (cf. the fusion of muscle cells in embryogenesis).

Note also that numerous species employ polymorphic compatibility loci to coordinate fusion between organisms (but not cells) to constitute a colony (e.g. sponges, bryozoans, colonial tunicates (Crampton & Hurst 1994)). That such fusions only go on between close relatives can again be understood through consideration of the possibility of competition between cells of unrelated individuals.

A variety of inbred species seem to have relaxed controls on organelle inheritance. Yeast for instance are almost certainly inbred (extrapolated from the fact of mating type switching) and have biparental inheritance (Dujon 1981). This biparental inheritance is however associated with relatively rapid sequestration of mitochondria into homoplasmic lineages. Some of this homoplasmization may be random, but some may also be enforced (e.g. those of the first buds from the mother cell) (for discussion see Hurst & Hamilton 1992; see also Sears & VanWinkle-Swift 1994).

It should be noted that it is not an absolute prediction that inbred organisms will have biparental inheritance, just that it can be stable if they do (Hurst 1994*a*). Under other conditions the prediction will indeed be that uniparental inheritance could be very strictly adhered to (Hurst 1994*a*).

Prediction 3. *Organisms in which sex involves the full fusion of cells but that lack cytoplasmic organelles need not have mating types.*

As cells with full fusion but no organellar DNA would not have a problem with over-replicating cytoplasmic DNA it has been predicted that such organisms need not be differentiated into sexes. It has hence been suggested that a variety of early protists, such as microsporidians, might not have sexes (Hurst & Hamiton 1992). Nothing is yet known of the presence or absence of mating types in microsporidians. However, one sexual species is known with no mating types, this being *Halobacterium volcanii* (Archaebacterium) (R. Ortenberg *et al.*, in preparation). Originally it was considered that the cells of this species do not fuse but just mutually exchange DNA to end up in the diploidic condition (Mevarech & Werczberger 1985; Rosenshine *et al.* 1989; Rosenshine & Mevarech 1991). However, more recent analysis shows that fusion does occur and that the resident plasmids cannot over-replicate (Tchelet & Mevarech 1994; R. Ortenberg *et al.*, in preparation). This species is not only the one described prokaryote with full sexual fusion between cells, but also the only species with full fusion and no organelles (or mobile plasmids) about which mating type information is known.

This data point is all the more significant in that the archaebacteria are considered to be the ancestors of the eukaryote nucleus (reviewed in Dyar & Obar 1994). The finding of sex by fusion suggests that sex by fusion may be an ancestral character within the eukaryotes. Further it supports the assumption that an absence of sexes is the primitive condition (for the alternative possibility see Hoekstra 1990).

Prediction 4. *Organisms in which sex does not involve the full fusion of cells, just the exchange of nuclear DNA, need not be restricted to two mating types.*

Sex by exchange of nuclear material but not of cytoplasmic material has evolved independently at least twice, in the ciliates and in the hymenomycetes (e.g. mushrooms). In both instances sex is coordinated by multiple ($\geqslant 2$) incompatibility types.

In the ciliates such as *Paramecium aurelia* two conjugants will pair up, open a small bridge between them and reciprocally pass a small haploid nucleus (a micronucleus) through the gap. Cytoplasmic mixing during conjugation is usually avoided by the two conjugants separating shortly (2–3 min) after first joining. Furthermore, the gap between the two is often very restrictive and the micronuclei need to construct to pass through (Andre & Vivier 1962; Inaba *et al.* 1966). Sonneborn (1944) has shown that if separation is delayed, then the bridge widens and cytoplasmic exchange is extensive. Similarly, in *Tetrahymena pyriformis* inclusions the size of mitochondria cannot pass through the small pores in the conjugative plate (Elliot & Hayes 1953; Robert & Orias 1973). It is parsimonious to suppose that the micronuclear is so small as to ensure a minimization of cytoplasmic mixing (Hurst 1990). Ciliates have anything up to about 100 mating types and often have between ten and 30 (Grell 1973; Afon'kin 1990).

The hymenomycetes have sex mediated by the anastomosis of two hyphae. After this anastomosis, the nuclei are typically passed up to the end of the hyphae where recombination and meiosis can occur. In contrast, the cytoplasmic genes are held in the zone of anastomosis and hence, as required by the models, are typically not transmitted to the progeny (Baptista-Ferreira *et al.* 1983; Casselton & Economou 1985; Hintz *et al.* 1988; May & Taylor 1988; Smith *et al.* 1990). Hymenomycetes typically have a very high number of mating types, often in the tens of thousands (Webster 1980).

Prediction 5. *Organisms with full fusion of cytoplasms should tend to have two or just a few sexes, and those with more than two may have problems with 'aggressive' cytoplasmic factors.*

Even among isogamous organisms the most common condition is that of two sexes (see figure 1). Indeed, Grell (1973) comments that as regards isogamous species 'no case of more than two sexes has ever been found'. While a few exceptions now exist to this rule (discussed below) the vast majority of isogamous species do indeed have two sexes. The number of independent evolutions of this condition is however unknown and phylogenetic analysis (see figure 1) suggests that it might actually be rather limited. If however we ask about the association between having a low number of sexes (two to ten; as opposed to the

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typical number found in ciliates and hymenomycetes) then there seems to be a good correlation between two or a few sexes and cellular fusion.

Suggestive evidence that this might be a real convergent state comes from analysis of ciliates that have re-evolved sex by fusion (see figure 1). This has occurred at least twice, once in peritrichs and once in hypotrichs. In petritrichs sexes are binary and fusion is anisogamontic between a macro- and a micro-conjugant (Raikov 1972). Within the hypotrich ciliates the system is somewhat confused. Isogamontic fusion has been described in at least five genera of hypotrich ciliates (Takahashi 1977; Yano 1985). Normal ciliate conjugation and gametic fusion may occur in the same population (Takahashi 1977; Yano 1985). Within every population there exist multiple mating types with respect to conjugatory mating (as is typical of conjugatory mating types). In many populations there are binary types with respect to gametic fusion. In one instance (Takahashi 1977) within a syngen (i.e. species) there exist five 'sexes', four inducers of fusion and one superclass of non-inducers. The inducers are mutually compatible but not found in the same locality (Yano 1985) and whether they would ever come to mate is unclear. The viability of between-locality hybrids is typically significantly lower than within-population progeny (Takahashi 1983), indicating genetic divergence.

In at least one species of hypotrich sex by fusion is the exclusive means of sexual propagation (conjugation is not found) and in laboratory cultures six mating types can be identified (Jerka-Dziadosz & Dubielecka 1985). I am unaware of any studies of the cytoplasmic genetics of this or other hypotrichs.

Although within the hymenomycetes there are no well described instances of reversion to full gametic fusion, their sister group, the teliomycetes, do have sex by anastomosis of two cells and full confluence of cytoplasm. However, unlike gametic sex in the 'standard' isogamous organism, that within teliomycetes (e.g. *Ustilago*) is unusual in that the two cells do not fully fuse but are instead joined by a conjugation bridge that is anatomically equivalent to the conjugation bridge in hymenomycetes. This is consistent with the notion that the teliomycetes are derived from an ancestral group that had standard hymenomycete sex. If so, then the evolution of two sexes and their involvement in the regulation of cytoplasmic gene inheritance (Wilch *et al*. 1992; Hurst 1994*a*) within the teliomycetes would be a further independent evolution of two sexes associated with zygote formation in which competitor cytoplasmic genomes could obtain biparental transmission. However, the hymenomycete conjugatory tube could have been derived from that of teliomycetes and hence, without sound knowledge of the ancestral condition, these data are ambiguous.

Apart from the above, only a few exceptions to the rule of two sexes are now known. Other than one possible report of three mating types in a dinoflagellate (Destombe & Cembella 1990), multiple mating types are only regularly reported in slime moulds. In both the acellular (myxomycetes) and cellular slime moulds (acrasiomycetes) sex involves the fusion of isogametes. A number of cellular slime moulds have two mating types, a few have three or four (Chang & Raper 1981; Urushihara 1992). In the acellular slime moulds the number of alleles coordinating the inheritance of cytoplasmic genes can be up to about 13 (Kawano & Kuroiwa 1989; Meland *et al*. 1991). It is unclear whether the multiple mating types of the two classes of slime moulds represents one or two independent evolutions of multiple sexes. Phylogenetic analyses sometimes place these two as sister groups (Cavalier-Smith 1993) but at other times do not (see, for example, Knoll 1992).

It was predicted that having a large number of mating types leaves a system vulnerable to usurption by selfish cytoplasmic factors (Hurst & Hamilton 1992). One unambiguous incidence of such usurption has been found and it may hence be significant that it is in the acellular slime mould *Physarum* (Kawano *et al*. 1991). A plasmid in the mitochondria of one of the gametes forces fusion of mitochondria at zygote formation. After the mitochondria has split apart all of the resultant organelles have this plasmid. Note also that this intermitochondrial fusion forced by a selfish gene is an example of the evolution of sex (not sexes) and lends considerable credence to the view that sex initially evolved because of takeover by selfish elements (Hickey & Rose 1988; Hurst 1991). Paternal transmission of plasmids has also been reported in *Neurospora* (Yang & Griffiths 1993) but it is unclear whether this is due to the action of the plasmids.

3. ALTERNATIVE HYPOTHESES

The match of the above predictions to the data seems adequate for us to suppose that mating types and cytoplasmic genetics are probably not independent. However, at least one other force must also be of great importance in explaining the above data, this being what has been termed the mating kinetic (Iwasa & Sasaki 1987). Put simply, one assumption of models that suppose that individuals would be better off having many different partners to choose between (and hence multiple mating types) is that there exists a cost to not being able to find a mate or in finding one but discovering that it is the same mating type as you and hence not a viable partner. This will almost certainly be true for relatively immobile organisms (Power 1976). Hymenomycetes depend on growing into another set of hyphae to engage in sexual propagation. If the number of mating types were low then the probability of finding a mate would also be lower than it might be. It is probably for this reason that mushrooms have so very many incompatibility types. It might hence be a necessary adaptation of these organisms that they have a high number of mating types, and the controls on cytoplasmic genes follow from this.

It is probably for this sort of reason that the slime moulds too have multiple sexes. In these species the isogamous gametes must swim or crawl over a terrestrial habitat. With movement so difficult, choice of mate is possibly quite limited. It is unclear whether this sort of force can explain why ciliates have multiple

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mating types as they are extremely mobile. They are however often found in fragmented habitats (small ponds) and may suffer from the consequences of having only a few initiators of a population. By the same measure many other organisms should then have more than two sexes, which seems not to be so.

If however there is no cost to failed meetings between gametes, then neutral drift should remove the excess mating types and the population should return to two (Iwasa & Sasaki 1987). This theory for the dynamic of mating types has a number of weaknesses. It cannot explain the transition from zero to two sexes (Hurst & Hamilton 1992). Further, even if we assume two sexes to be a primitive condition, considerations of the mating kinetic cannot explain all the data. First, the analysis predicts either two or an infinite number of sexes (Iwasa & Sasaki 1987). However the slime moulds have few but more than two mating types. Second, as the presence of more than one locus controlling mating preferences can only make the problem of finding a mate more difficult, the model predicts that organisms should never have more than one locus controlling compatibility. Many organisms however have both two sexes and multiple incompatibility alleles at a separate locus (e.g. angiosperms (de Nettancourt 1977), tunicates (Grosberg 1988), ascomycetes and teliomycetes (Fincham *et al.* 1979)).

That multiple loci exist but only one set coordinates cytoplasmic gene inheritance is also consistent with the notion that some incompatibility loci evolve for reasons quite independent of the coordination of cytoplasmic genetics. The second locus is often assumed to prevent inbreeding, although control of pathogenicity and possible pathogen resistance might also be of importance. In for example the genus *Ustilago* many species have two sets of incompatibility loci. One set has two alleles and coordinates uniparental transmission of mitochondria (Wilch *et al.* 1992). The second set is typically multiallelic and heterozygosity at this locus is necessary for the initiation of pathogenic development (Banuett 1992). Whether the forces responsible for the evolution of cytoplasm-independent incompatibility types are relevant to the initial evolution of two mating types is unresolved, but is not supported by theoretical consideration (Hoekstra 1987; Hoekstra *et al.* 1991).

Third, and related to the above, close examination of the action of incompatibility alleles in *Physarum* (myxomycete) reveals that one set of these alleles acts after gametic union (Bailey *et al.* 1990). Laboratory studies indicate that any *Physarum* gamete can successfully mate with any other so long as they are genetically non-identical at all of three different polymorphic loci (Kawano *et al.* 1987). For at least one of these, fusion of gametes will not occur unless the two gametes are different at the locus. This is not the locus that coordinates the inheritance of cytoplasmic genes. These cytoplasmic control alleles instead act as postfusion incompatibility alleles. Two cells may fuse if they are the same at the relevant locus, but if they are the same then the cells subsequently split apart again and the two will go off to find other mates (Bailey *et al.* 1990). If multiple mating types exist solely to allow an increased probability of finding a mate then this fusion and rejection should never occur. That these alleles coordinate cytoplasmic gene inheritance instead suggests that the coordination of cytoplasmic genetics is itself a motive force in the evolution of mating types and not superimposed on pre-existing mating types as has been argued (Charlesworth 1983).

A third and possibly important consideration in the evolution of mating types and mating systems is the issue of net zygotic size. If the above theory is correct we might ask why slime moulds have not invented what the ciliates invented. One possible explanation is that zygotic size matters for slime moults but not for ciliates and hence nuclear exchange is not a viable option for slime moulds. Although possible this hypothesis seems hard to test, although we could examine the fitness of zygotes of various sizes and see whether small ones are particularly unfit.

Even if full fusion is required for size purposes, one quandary remains. It was assumed above that the coordination of cytoplasmic genes is problematic in species like *Physarum* as in certain matings the cytoplasmic genomes could not be primed for destruction. But why could not two individuals/gametes communicate before fusion to determine who is to be the donor of cytoplasm and who not? In the oomycete *Achyla* there is good evidence for just such a phenomenon (sometimes known as relative sexuality) (see discussion in Webster 1980). Two individuals will communicate via phenomones and one will be induced to become a female and the other a male. The sex of most individuals is not fixed and an individual can be male for one interaction and female for another. There exist claims of a similar phenomenon in other oomycetes (see, for example: Galindo & Gallegly 1960; Sherwood 1971), in *Ectocarpus* (brown algae) (see discussion of Hartmann's work in Waddington 1939), in *Spirogyra* (Conjugaphyta = Zygnematales) (Kniep 1928) and in *Dasycladus* (Chlorophyta: Ulvophyceae) (Jolos 1926). Claims that such a phenomenon is found in a few protists are contentious (for critical and historical analysis of this literature see Sapp 1990). Sexual development in bonnelid worms (see discussion of Blaltzer's work in Bull 1983) is comparable (though not identical) in that male development is conditional upon exposure to a female. If there is no such exposure the individual becomes male. Relative sexuality is surely an ideal solution: sexual differentiation and hence control of cytoplasmic genetics is possible, while at the same time just about any individual is a potential mate. It is unclear why this is not a more common strategy.

In conclusion, the evidence suggests that the evolution of mating types and the restriction of the number of mating types to two (or a few) may well be intimately connected to problems that cells have in preventing potentially antagonistic cytoplasmic genomes from coming into costly competition. The costs of not finding a mate must almost certainly force a number of species into having more than two sexes.

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4. UNIPARENTAL INHERITANCE AS THE GENERATOR OF NEW CONFLICTS

Given that cytoplasmic genes are uniparentally inherited can we conclude that the competition between cytoplasmic factors must be under control and hence over? The answer must be a resounding no. First, cytoplasmic mutants, such as that in *Physarum*, that can force transmission when they are due for destruction/non-transmisson can spread in a population. This sort of resistance to destruction is consistent with some patterns of cytoplasmic genetics in angiosperms and gymnosperms (Hurst 1994*a*; Reboud & Zeyl 1994).

Second, if cytoplasmic factors are forced to receive uniparental transmission then there exist other means by which any given cytoplasmic mutant can spread in a population and out-compete competitor alleles while still being deleterious. Typically cytoplasmic factors in anisogamous species are preferentially transmitted through eggs. Consider then a cytoplasmic factor that converts males into females. The mutant will be in a transmitting host every generation, whereas the competing cytotype is transmitted only half the time. The conditions for the spread of such a factor are hence very broad (Bull 1983; Taylor 1990).

Just such a pattern of events has been observed. Isopod crustaceans, such as *Armadillidium vulgare*, bear maternally transmissible bacterialparasites that convert their male host into a female host (see, for example: Juchault *et al.* 1992; Rigaud & Juchault 1993). The inverse has also been described, i.e. an agent with paternal transmission that masculinizes females (Werren *et al.* 1987). The spread of such factors biases the sex ratio and hence creates the conditions for the spread of autosomal suppressors. This too has been reported in the isopods and is closely linked with the evolution of sex determination in this species.

A. vulgare has as its native sex determining system ZW females and ZZ males. Some populations are infected with the cytoplasmic feminizing bacteria (F) which renders would-be males female. The feminizing factor may spread in the population and standard ZW females can then be entirely replaced by feminized ZZ males. In such populations the sex determining system is no longer female heterogamety, but one in which nuclear genes support male development while specific cytoplasmic genes force the host to become female (Juchault & Legrand 1989; Juchault *et al.* 1992; Juchault & Mocquard 1993). The sex ratio is then controlled, not by the segregation of Z and W in females, but by those nuclear genes that regulate the transmission of the cytoplasmic factor from mother to progeny (Rigaud & Juchault 1993).

The process does not, however, stop there. This F factor can spontaneously change into a different feminizing factor, f, which acts as a mobile (possibly nuclear) genetic element. The f factor has at least two modes of inheritance. First it can be transmitted as a cytoplasmic gene, though possibly with some paternal leakage. Second it has been known to integrate into a Z chromosome, thereby converting ZZ+f individuals into ZZ_f females and in the process rebuilding the recipient Z chromosome into what functionally is a new W chromosome (Juchault & Mocquard 1993). In this later mode the f factor undergoes Mendelian transmission and the carrier females can be considered as normal 'genetic females' (Juchault & Mocquard 1993). In such populations where all females end up having f stably incorporated into the Z chromosome,

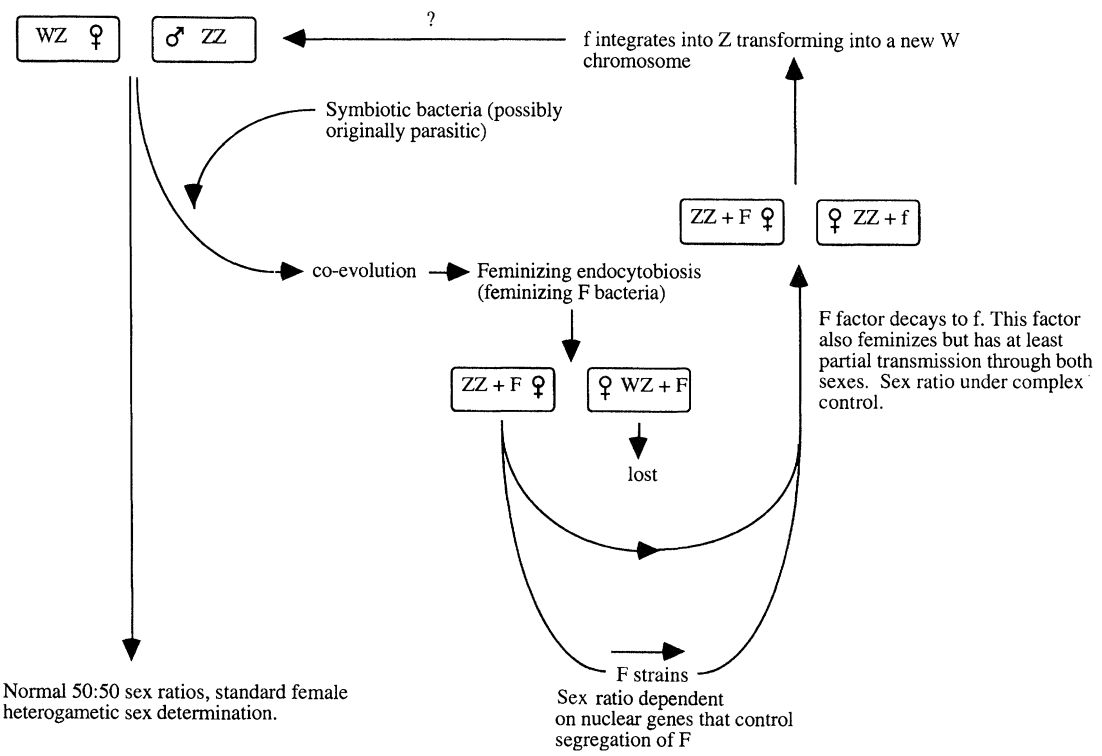

Figure 2. A scheme for the evolution of sex determination in *Armadillidium vulgare* (Isopoda). Based on Juchault & Legrand (1989), Juchault *et al.* (1992) and Juchault & Mocquard (1993).

the sex ratio returns to 50:50 and all females are genetic females. This is probably where the population started before the cycle of conflict; the system has hence gone full circle (see figure 2). (Note however that it is unclear whether the newly formed W chromosome could or does spread in the population.)

The above isopod case history has drawn considerable attention because, not only is it one of the best understood systems involving cytoplasmic sex ratio distorters, but it is an instance in which the sex determining system of a population can be seen to change over time. In general as so many conflicts are over the sex ratio and hence sex determination, a general paradigm for the turnover of sex determining systems is one in which a selfish element causes a bias to the sex ratio and thus creates the contexts for restorer genes to invade. Either distorter or restorer could interfere with the sex determining pathway to affect the sex ratio.

Many similar examples of cytoplasmic sex ratio distorters could be given. Trichogammid wasps for instance have cytoplasmic bacteria that convert normal sexual females (producing both males and females) into ones producing exclusively parthenogenetically derived female progeny (Stouthamer *et al.* 1990). These progeny in turn are capable of producing parthenogenetically derived female progeny, and so on. Other wasps have cytoplasmic elements that increase the proportion of eggs that are fertilized (being haplo-diploid, fertilized eggs are females) (Skinner 1982).

Perhaps the least subtle strategy (and possibly also the most common) is that of the so-called cytoplasmic male-killers (reviewed in: Hurst & Majerus 1993; Hurst 1993). These simply kill the host when they find themselves in a male. This appears not to result in the horizontal transmission of the causative agent. Instead, as shown in for example ladybirds, the death of males results in enhanced fitness of the sisters (Hurst *et al.* 1995). The frequency of infected females increases as the sisters contain the clonal relatives of the bacteria that killed the males. These male-killers can be contrasted with maternally transmissible microsporidians of mosquitoes which kill males, but not females, but having done so escape from the dead host to an alternative host (reviewed in Hurst 1993).

A very closely related phenomenon to male-killing with the absence of horizontal transmission is so-called cytoplasmic male sterility (CMS) in hermaphroditic plants. Here male tissue is sterilized by cytoplasmic elements. The plants may then redistribute resources to the ovules (reduced inbreeding levels may also enhance fitness of ovules) (Lewis 1941; Frank 1989). The ovules however contain clonal relatives of the genome that sterilized the male tissue and hence the trait spreads. Unlike the animal examples which are typically caused by maternally transmissible microorganisms, CMS is more often than not caused by a mitochondrial mutation (Gouyon & Couvet 1987).

The above illustrations (which are by no means exhaustive; see also Hurst 1993) show that to a large degree intragenomic conflicts never really go away. One conflict can be converted to another but the problem in general remains. This conclusion can be further strengthened through consideration of ciliate cytoplasmic genetics. We might imagine that the route taken by ciliates to restrict cytoplasmic genome competition (i.e. not to let two unrelated genomes into the same cell and hence not to have a transmitting and non-transmitting sex) should be the best alternative, as such a system would not be vulnerable to parasitization by cytoplasmic sex ratio distorters. While this is true it conceals the fact that ciliates still have potential competition between cytoplasmic genomes. This is expressed in the form of cytoplasmic mate-killers. In *Paramecium* for example, when two conjugants join together although their cytoplasms do not mix, the cytoplasmic genomes are every bit as unrelated as they would be were fusion to occur. A cytoplasmic mutant that could kill the conjugant partner and by so doing gain some advantage (e.g. reduced resource competition or reduced probability of inbreeding) could easily spread in the population. Such ciliate cytoplasmic mate-killers have been described (Beale & Jurand 1966), although the presumed advantages have yet to be demonstrated. The ciliate resolution to competition between cytoplasmic genomes, like the enforcement of uniparental inheritance, is not a complete solution.

5. CONCLUSION

It has been argued that the evolution of sex creates the conditions for the spread of selfish genetic elements. In turn has been proposed that what this entails may be the evolution of novel features of the organization of eukaryote genetic systems that may be considered as responses to limit the spread and deleterious effects of selfish elements. In particular the possibility that the control of ultracompetitive cytoplasmic factors is of central concern in the evolution of mating types has been discussed. It has been further noted that in an unconstrained sexual system the spread of selfish elements is always a potential and hence there exists no state in which outbreeding and selfish elements could not coexist. Selfish elements are thus good candidates to explain the turnover of components of genetic systems when, from a purely adaptive point of view, such turnover would seem unlikely. An involvement of selfish elements can hence be considered as a strong candidate explanation in instances of the evolution of biased sex ratios (Hamilton 1967), of sex determination systems (Hamilton 1967; Bull 1979; Haig 1993*a*, *b*; Hurst 1994*b*), of speciation (Powell 1982; Kidwell 1983; Rose & Doolittle 1983; Thompson 1987; Breeuwer & Werren 1990; Frank 1991; Hurst & Pomiankowski 1991; Levy 1991; Beeman *et al.* 1992; Pomiankowski & Hurst 1993; Taylor 1994) etc. The problem of the maintenance of Mendelian segregation of autosomes is explicitly a question of the evolution of a genetic system that is not parasitized by selfish elements. Whether this evolution is a consequence of the spread of selfish elements (Haig & Grafen 1991) or a fortunate side effect of the evolution of various mechanisms, such as recombination, has yet to be decided.

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The conflict based perspective can be contrasted with the more classical analysis of adaptive evolution in general, and of eukaryotic cell processes in particular. When considering the evolution of many eukaryotic cellular process, for example components of intermediate metabolism, it is reasonable to suppose that the phenomena under consideration are the consequence of selection favouring those organisms with the more efficient operation and hence higher absolute fitness. The reverse side of this coin is the assumption that genes that are deleterious cannot deterministically spread in a population. Selfish genetic elements are however of this nature. The above analysis suggests that some of the characteristics of eukaryotic cells may be better viewed, not as exquisite adaptations, comparable to those of the beaks of Darwin's finches, but rather as a form of internally driven evolution in which the cell evolves to be able to control its own constituents and to prevent one constituent from parasitizing the rest. The general applicability of such notions has yet to be resolved.

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