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Cytoplasmic fusion and the nature of sexes

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SUMMARY

Binary mating types are proposed to arise in a three-stage process through selection of nuclear genes to minimize cytoplasmic gene conflict at the time of gamete fusion. In support of this view we argue that: (i) in systems with fusion of gametes, the mating type genes are typically binary and regulate cytoplasmic inheritance; (ii) binary sexes have evolved several times independently associated with fusion, although at least twice binary types have been lost, associated with a loss of fusion; further, in accordance with the theory are findings for isogamous species that (iii) close inbreeding may correlate with less than two sexes and biparental inheritance of cytoplasmic genes; and (iv) species with more than two sexes may have uniparental inheritance of cytoplasmic genes, be rare and be afflicted by deleterious cytoplasmic genes which attempt to pervert normal cytoplasmic genetics. Such facts and their rationale support a new and unified definition of sexes based on the control of the inheritance of cytoplasmic genes. For the common cases, the male sex is that which resigns attempts to contribute cytoplasmic genes to the next generation. We differentiate between sexes and the incompatibility types of ciliates, basidiomycetes, some angiosperms and a few other organisms which are independent of organelle contribution.

1. INTRODUCTION

Why in a sexual population are there different sexes? In the most rudimentary condition the gametes are isogamous but are still differentiated into mating types. Following Hoekstra (1987), we extend the view that sperm evolved to prevent the mixing of cytoplasmic genes from different parents (Cosmides & Tooby 1981; Hurst 1990; see also Grun 1976; Hastings 1992; Law & Hutson 1992), and further propose that the fundamental asymmetry of the sexes evolved as a means to minimize the damage caused by conflict between cytoplasmic genes in the zygote. A new definition of sexes based on the control of the inheritance of cytoplasmic genes clearly excludes the incompatibility types of ciliates, basidiomycetes, some angiosperms and a number of other species. With very few exceptions, sexes as newly defined are not multiple. They are either non-existent or binary, and these classes correlate well with the presence or absence of cytoplasmic fusion of gametes. The rare outliers to these generalities illustrate unusual conditions that render them also compatible with the theory.

2. THE MODEL

Consider first a population of haploid sexual chlamydomonad-like algae. The gametes of the algae are isogamous and in their primitive condition are not differentiated into mating types. Any gamete can mate with any other. Let us assume that each gamete carries one chloroplast (any similar cytoplasmic organelle would be equivalent for the following argument), and that at first these chloroplasts are all passive, by which is meant they do not react to the presence of other chloroplasts introduced from the partner's cytoplasm during gametic fusion. Now consider the fate of a mutant chloroplast gene which destroys the chloroplast of the partner. Such a destroyer type will go to fixation so long as any costs incurred by its actions do not outweigh the twofold transmission advantage obtained by eliminating the competitor chloroplast. 'Homozygous' destroyer zygotes will be especially prone to costs due to balanced but still damaging activities. At worst, mutual destruction and death of the new zygote might occur.

Imagine next a nuclear Suppressor gene which leaves its organelle both unable to destroy its opponent and vulnerable to annihilation by that opponent. Its opposite allele in the genome is the wild-type Non-suppressor. Let the relative fitness of cells produced from a zygote formed by fusion of two unsuppressed cells be β, where $0 < \beta < 1$. Let there also be a cost to suppression such that the relative fitness of cells produced by the fusion of two suppressor cells is α, where $0 < \alpha < 1$. Cells which are the product of fusions between Suppressors and Non-suppressors suffer only half the cost of suppression and avoid the potential damage inflicted by two Non-suppressors. They have relative fitness unity.

The two costs specify a situation of heterozygote advantage which has a well-known equilibrium when, if p is the gene frequency of Suppressor and q that of Non-suppressor (with $p+q = 1$),

$$p(1-\alpha)-q(1-\beta) = 0. \qquad (1)$$

Consider now an unlinked choosy gene which prefers its haploid cell to fuse only with one of the opposite suppressor type and thus tends to avoid non-optimal matings. We assume that Suppressor and Non-suppressor cells are somehow recognizable before irreversible fusion. Suppression is likely to be mediated

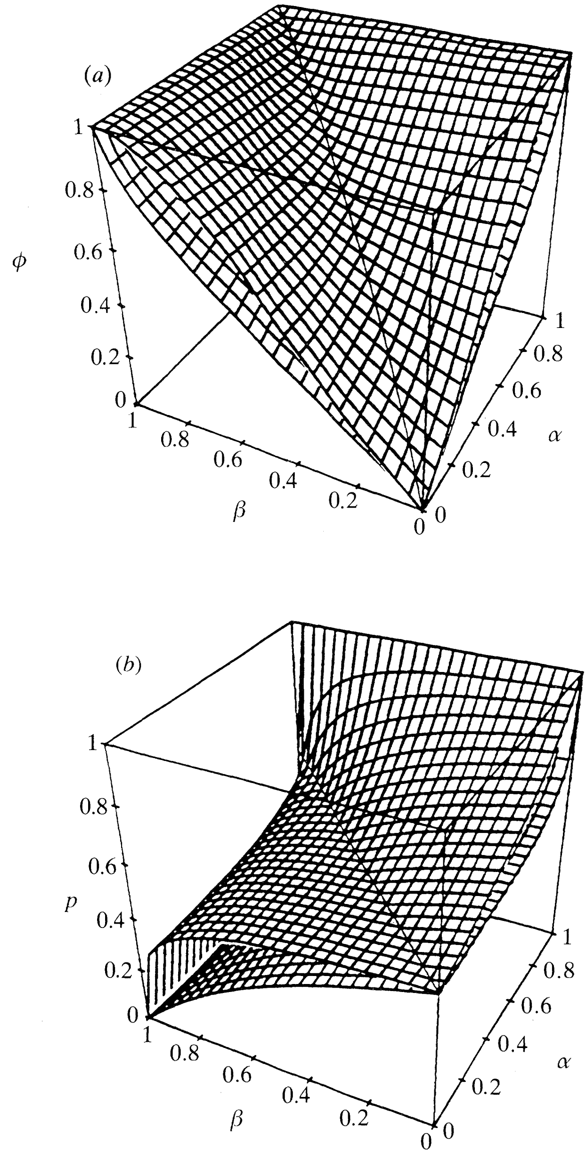

Figure 1. (*a*) Invasion and counter-invasion conditions for a gene causing choice of mating type, and (*b*) stable frequencies of a gene causing suppression of cytoplasmic conflict under choice and non-choice. (*a*) Critical ϕ^* for entry of a nuclear Chooser gene into a population of a random mating gene is given by the solution for ϕ of $V = W$ (equations (1) and (3), see text). This is

$$\phi^* = (p^2\alpha + q^2\beta)/[p^2(p\alpha + q) + q^2(p + q\beta)], \tag{4}$$

and is shown as the upper sheet. When Choosers are universal, (2) and (3) still give the mean fitness of Non-Choosers and Choosers but equilibrium is no longer as in (1); instead, frequency-dependent selection as in (3) holds, giving equilibrium condition

$$p(1 - p\alpha\phi) - q(1 - q\beta\phi) = 0. \tag{5}$$

Thus for a critical ϕ' for re-entry of Non-chooser, (5) instead of (1) must be solved with $V = W$ for ϕ. The resulting quartic solution, obtained iteratively, is shown in the lower sheet of (*a*). Sheets coincide along $\{\alpha = 1, \phi = 1\}$, $\{\beta = 1, \phi = 1\}$ and $\{\alpha = \beta, \phi = 2\alpha/(\alpha + 1)\}$. (*b*) shows equilibrium frequencies p of a nuclear gene suppressing cytoplasmic conflict under two conditions: the more uniformly slanted sheet applies to random mating (absence of Chooser) and displays the classical solution of (1): $p = (1 - \beta)/(2 - \alpha - \beta)$. The more terrace-like sheet emerging into full view to the left applies to universal presence of choice for mating-type choice and displays $p = (A - \sqrt{AB})/(A - B)$, where $A = 1 - \beta\phi$ and $B = 1 - \alpha\phi'$ (by quadratic solution of (5)). Equilibrium frequencies under the two conditions have identical fixations along $\alpha = 1$, and $\beta = 1$, and are also identical along $\{\alpha = \beta, p = q = 0.5\}$.

by the production of a protein which might be directly assessed or which might have side consequences which reveal its presence. Alternatively, substances related to the preparedness of the chloroplast for aggressive fusion might be detected. Let the haploid cells or gametes mix and meet at random. For convenience we will consider a situation in which all cells have only two opportunities for mating. After the first encounter, if both of the cells accept the mating then it proceeds. If one or both of the cells reject union then the pair breaks apart and all those cells which did not mate in the first round make another attempt to find a mate. In this second phase, no cells demonstrate choosiness, hence mating is random. Entering this second round of mating has a cost that applies a fitness factor, ϕ.

The choosy gene starts to spread if its fitness in the average zygote that it forms when rare is greater than fitness in the non-choosing population at large. As in the standard theory above, this is

$$W = p^2\alpha + 2pq + q^2\beta. \tag{2}$$

Assuming the Choosers are so uncommon that their meetings with each other are negligible, and that they occur by mutation in random association with Suppressors and Non-suppressors, their mean fitness is

$$V = p^2(p\alpha + q)\,\phi + 2pq + q^2(p + q\beta)\,\phi. \tag{3}$$

It is easily seen that for any internal equilibrium of the suppressor type by heterozygote advantage, if $\phi = 1$ then $V > W$ and the Chooser gene can enter. Lowering ϕ, however, eventually brings a condition where invasion is barred. The critical ϕ^* at which entry is just prevented, obtained by solving $V = W$, is shown as a function of α and β in figure 1*a*.

Assuming that ϕ is above the critical value and Chooser enters, does it go to fixation? As Chooser invades it always moves q towards 0.5, hence reducing the frequency of necessity for repairing, although never to below 0.5. Thus if invasion is possible it proceeds to fixation. This point is illustrated by the lower sheet in figure 1*a* which shows the critical ϕ' for invasion by Non-chooser if Chooser is universal: clearly also a threshold is implied such that if $\phi' < \phi < \phi^*$, Chooser can still enter and be established if sufficiently assisted initially by genetic drift or hitch-hiking.

If Chooser occurs inseparably linked to Suppressor or to Non-suppressor we have, replacing (3),

$$V = p^2(p\alpha + q)\phi + 2pq + q^2\beta$$

or

$$V = p^2\alpha + 2pq + q^2(p + q\beta)\,\phi.$$

These give $V > W$ under similar conditions. Chooser invades if ϕ is large enough but now does not go to fixation even if $\phi = 1$, for clearly Chooser cannot carry the frequency of either linked suppression type to above 0.5. However, it is noteworthy that Chooser mutations of all three linkage conditions – unlinked,

linked to Suppressor, linked to Non-suppressor – converge in building a bisexual system in which Fisherian selection of sex ratio holds. For the case of non-linkage, the onset of a stable sex ratio situation is shown in figure 1 *b* in the wide 'terrace' around $q = 0.5$ for the states of completed invasion. Still wider, flatter terraces are observed if more than one round of choice is allowed. Other simpler but related choice systems that begin the same tendency to a typical sex ratio situation are mentioned below.

Realistic values of the three parameters are unknown. It is, however, reasonable to presume that α and β will be less than unity. In a parallel indication of cost, the gene determining insensitivity to the meiotic driver Segregation Distorter in *Drosophila melanogaster* is known to inflict a cost relative to the wild-type Non-suppressor (Wu *et al.* 1989). Similar costs have been conjectured to be involved in the suppression of various sex ratio distorters (Gouyon *et al.* 1991 references therein). The cost of choice would be dependent on the ecology, and could possibly be anywhere in the range zero to one, but under numerous circumstances is likely to be closer to zero.

The model can be elaborated for three or more rounds of mating for more complex choosy genes which have a strength of preference which declines with time. By extension of the above result, there is a trade-off such that the decline over time of the strength of preference balances the cost of choice. Unless there is no cost to choice a number of residual cells which have failed to find an opposite type mate will pair without the exercise of preference.

As detection that the host cell and another are not alike is difficult to imagine occurring by a single mutation, it is interesting to note that approaches to similar states to the one described can be attained with simpler choices. A gene could simply determine a preference for a Suppressor cell, or another mutant for Non-suppressor. Such preference genes can be selected because they will tend to establish themselves in linkage disequilibrium with the Suppressor genes such that Non-suppressor preference genes have linkage to cytoplasm Suppressor genes and vice versa. This is because when they are in such association they get an advantage. Subsequent selection favours increasingly tighter linkage.

The above model was originally outlined by Hoekstra (1987), who then commented that 'this scenario appears to work only if the selective differences between the various genotypes are very great, which makes the hypothesis less plausible.' We believe the model to be both plausible and consistent with a sizeable body of data.

3. THE FUNDAMENTAL ASYMMETRY OF THE SEXES: DISCUSSION

The above model shows that if sex involves gametic fusion, a species should evolve nuclear genes to prevent conflict between cytoplasmic genes. In any given pairwise gametic fusion there should be an asymmetry between the cells to mediate the prevention of conflict. We propose that this asymmetry is the fundamental asymmetry of the sexes and is quite different from the system of incompatibility types exhibited by ciliates (see Grell 1973) and basidomycetes (see Day 1978) in which nuclei are exchanged but cytoplasm is not (in basidiomycetes: see Casselton & Economou 1985; May & Taylor 1988; Hintz *et al.* 1988; Baptista-Ferreira 1983; Smith *et al.* 1990; in ciliates: see Preer 1969). These incompatibility systems are thought to have evolved to prevent inbreeding and maximize the number of potential reproductive mates, and thus are comparable to the incompatibility alleles of angiosperms (see de Nettancourt 1977). Significantly, the angiosperms have sexual differentiation as well as incompatibility alleles, supporting the view that sexes and incompatibility types are two separate phenomena. Similarly, in discussing the Foraminiferan *Metarotaliella parva*, Grell argues that the organisms have a system of multiple incompatibility alleles governing choice of mate, but when fusion is initiated the interaction is mediated by a binary mating system. A similar system governs mating in tunicates (see Grosberg 1988).

Table 1 presents the incidences of various numbers of sexes and incompatibility types. In the table, having zero sexes is interpreted as not fusing in any way that allows genetically different cytoplasmic genes to become mixed, whereas having '< 1' incompatibility type means being compatible for nuclear DNA exchange or donation with every individual. In the majority where fusion takes place there are just two sexes. When fusion does not occur our argument suggests that sexual compatibility is best thought of as controlled not by 'sexes'.

Analysis of species with gametic fusion suggests that there exist two means for the prevention of cytoplasmic gene conflict. First, and most commonly, there is the uniparental inheritance of cytoplasmic genes, and second, there is the rapid segregation of cytoplasmic genes from the two parents after gametic fusion. Uniparental inheritance of organelles can be mediated either by making sperm very small or by the unisexual destruction of cytoplasmic genes just after or before entry into the zygote. The nuclear mating type genes of Chlamydomonads determine the inheritance of organelles such that mitochondria are inherited from the minus type parent but chloroplasts are inherited from the plus type, despite the fact that the zygote inherits both types (Bennoun *et al.* 1991). Supporting the view that cytoplasmic gene conflict is central to the process, Chiang (1976) has shown that the chloroplasts from + and − type parents fuse, and chl DNA from both types attempt to destroy each other by means of restriction endonuclease digestion. Typically, however, the + type DNA is destroyed more slowly than the − type DNA. Other reports claim a unilateral disarmament of the chloroplast genes by nuclear genes in the − type gamete (Sager 1977). The same association of isogamy and binary mating types with uniparental inheritance of cytoplasmic genes has been described in a large number of other protists (Whatley 1982; Eberhard 1980; Mirfakhrai 1990; Sears 1980).

In yeast, the zygote which is heteroplasmic for mitochondria produces buds which are homoplasmic.

Table 1. *The incidences of sexes and incompatibility types*

(Common, convergently attained systems are boxed. Systems without gametic fusion are boxed in thin lines. The table is not exhaustive but conveys the general patterns. Notes on the incidences: (*a*) It is uncertain whether any organism has only one sex but this might be an intermediary stage between the possession of two sexes and their absence. (*b*) Wild-caught samples of *Alexandrium excavatum* have been shown to have more than two sexes. However, it has not been shown that within one population there are more than two sexes, and hence the position of this species in the table is uncertain.)

		number of incompatibility types	
		< 1	> 1
number of sexes	0	acellular slime moulds during plasmodial fusion	(*a*) most ciliates (Grell 1973) (*b*) hypotrich ciliates if conjugating (Takahashi 1977; Yano 1985) (*c*) basidiomycetes (Day 1978)
	1	possibly a few homothallic fungi	
	2	(*a*) most organisms (Whatley 1982; Eberhard 1980; Grell 1973) (*b*) peritrich ciliates (Raikov 1972) (*c*) possibly hypotrich ciliates if fusing (Takahashi 1977; Yano 1985)	(*a*) *Metarotaliella parva* (Foraminifera) (Grell 1973) (*b*) tunicates (Grosberg 1988) (*c*) angiosperms (de Nettancourt 1977)
	> 2	*Alexandrium excavatum* (Dinophyta) (Destombe and Cembella 1990)	acellular slime moulds during gamete fusing, e.g. *Physarum polycephalum* (Kawano *et al.* 1987; Meland *et al.* 1991)

Random segregation would be too weak a process to allow this (Dujon 1981). The above model predicts that a nuclear gene with mating type-specific behaviour controls this process. Diploid a/α cells produce buds in different positions from both haploid cells and α/α and a/a diploid cells (Drubin 1991). The bipolar positioning of buds in the a/α cells is probably contributary to the non-random return to homoplasmy.

Examination of the mating patterns of ciliates reveals more than one phylogentically independent evolution of binary sexes associated with fusion. Peritrich ciliates have lost conjugatory nuclear exchange and have evolved sex by fusion of gametes (Raikov 1972). Significantly, sexes are binary and fusion is anisogamontic between a macro- and microconjugant (Raikov 1972). Isogamontic gametic fusion has been described in at least five genera of hypotrich ciliates (Takahashi 1977; Yano 1985). Normal ciliate conjugation and gametic fusion occur in the same population (Takahashi 1977; Yano 1985). Significantly, in every locality there can exist multiple mating types with respect to conjugatory mating, but exclusively binary types with respect to gametic fusion. In one instance (Takahashi 1977) within a syngen there exist five 'sexes', four inducers of fusion and one superclass of non-inducers. The inducers are mutually compatible, but are not found in the same locality. The viability of between-locality hybrids is typically significantly lower than within-population progeny (Takahashi 1983), suggesting genetic divergence. Alternatively, these hypotrich ciliates might represent a further example of a specialized class of binary neo-mating types which are associated with an asymmetry in the possession and donation of genetic material (Hoekstra 1990). The best-known example of this is the bacterial F plasmid which initiates genetic transfer and only those without the plasmid act as recipients.

The outliers in table 1 are interpretable such that they are consistent with the theory presented above. Acellular slime moulds such as *Physarum polycephalum* have more than two sexes. Laboratory studies show that any *Physarum* gamete can mate with any other so long as they are genetically non-identical at all of three different polymorphic loci (Kawano *et al.* 1987). The inheritance of mitochondria is determined by what appears to be a linear hierarchy of at least thirteen alleles at one of these three loci. The fact that cytoplasmic genes are uniparentally inherited is support for our theory. Meland *et al.* (1991) provide further support for our view in arguing that uniparental inheritance involves unilateral digestion of cytoplasmic genes. The problem posed by the *Physarum* case history is why, if multiple sexes can be competent at preventing cytoplasmic gene conflict, do most species have only two sexes? The most obvious explanation is that a system in which the asymmetry of sex is established only after zygote formation is inherently more vulnerable to selfish, zygote-injurious cytoplasmic genes than one in which the asymmetry is established before fusion (as is the case in all other instances). One prediction of this view is that *Physarum* will be vulnerable to selfish cytoplasmic genes which attempt to prevent uniparental inheritance of cytoplasmic genes. It is thus significant that one such unusual gene has already been identified in *Physarum* (Kawano *et al.* 1991). A plasmid in the mitochondria of one of the gametes forces fusion of mitochondria at zygote formation. After splitting apart, all of the mitochondria have this plasmid. If this sort of effect is costly for the nuclear genes, then a gamete which preferred to avoid this problem by destroying its own cytoplasmic genes and mating with those gametes which are prepared to let their cytoplasmic genes be transmitted (proto-females) can spread if the advantages outweigh the cost. Hence multiple sexes systems might be expected to collapse to binary types. An alternative view of the collapse of multiple sexes has been proposed by Iwasa & Sasaki (1987) who argue that, depending on the mating kinetics, either two or an infinite number of sexes are to be expected. In contrast to the model presented here, these authors do not allow the possibility that gametes might not have any sexual differentiation, and hence they do not explain the origin of binary mating types. However, Iwasa & Sasaki's models are not incompatible with the one presented here and their explanation for the preservation of binary mating types might go some way to explaining why *Physarum*-like 'relative sexuality' is so rare (see also Bull & Pease 1989; Power 1976).

In consistently inbred isogamous organisms a conflict-reducing mutant can spread, regardless of whether the mutant is a nuclear or cytoplasmic gene. This is because in inbred organisms all such genes behave as if they are linked. As a consequence, there is no need for nuclear genes to be asymmetric with respect to the control of the inheritance of cytoplasmic genes. Fusion of plasmodia of the slime mould *Physarum polycephalum* is restricted to individuals which are genetically identical at an array of loci (except when one plasmodia can parasitize the other, as reported by Lane & Carlile (1979)). It is thus significant that, as we predict, there is no evidence of anything other than biparental inheritance of cytoplasmic genes (M. Carlile, personal communication) and hence no indication of any asymmetry between the partners. Inbreeding homothallic fungi (e.g. *Neurospora africana*: Metzenberg & Glass 1990) and algae would be good candidates for further study. We predict that, if highly inbred, these organisms could have biparental inheritance of cytoplasmic genes. We also predict that microsporidians which lack both mitochondria and chloroplasts have less likelihood of having binary mating types.

Hoekstra (1982, 1984, 1987) has considered the possibility that the evolution of mating types was forced by problems in finding mates. Hoekstra argues that some cells could evolve to specialize as attractor gametes whereas others specialize to become attracted gametes. This view is not incompatible with the one presented here. Indeed, at the point of invasion it is in the interests of every cell to honestly signal its suppressor type. This could be achieved by amplifying the signal which was used at the outset to discriminate between Suppressor and Non-suppressors. Such a system does not require, as Hoekstra's model does, that some cells specialize as pheromone producers whereas others specialize to become receivers. Yeast's system in which both cell types produce and respond to mating type-specific pheromones (Jackson & Hartwell 1990) is equally understandable.

An alternative view of the relationship between mating types and uniparental inheritance has been proposed by Charlesworth (1983). Charlesworth argues that if an isogamous species already has sexes, then a selfish cytoplasmic gene which acts to guarantee its transmission by interacting with the + mating type locus to exclude the − type cytoplasmic DNA will spread to fixation. The model does not address the issue of the evolution of sexes.

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