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The evolution of non-reciprocal nuclear exchange in mushrooms as a consequence of genomic conflict

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Heterothallic mushrooms accomplish sex by exchanging nuclei without cytoplasm. Hyphal fusions occur between haploid mycelia resulting from germinated spores and subsequent reciprocal nuclear exchange without cytoplasmic mixing. The resulting dikaryon is therefore a cytoplasmic mosaic with uniformly distributed nuclei (two in each cell). Cytoplasmic inheritance is doubly uniparental: both mated monokaryons can potentially transmit their cytoplasm to the sexual spores, but normally only a single type per spore is found.

Intracellular competition between mitochondria is thus limited, but at the dikaryon level, the two types of mitochondria compete over transmission. This creates the conditions for genomic conflict: within the dikaryon, a selfish mitochondrial mutant with increased relative transmission can be favoured, but selection between dikaryons will act against such a mitochondrial mutant. Moreover, because nuclear fitness is directly dependent on dikaryon fitness, a reduction in dikaryon fitness directly conflicts with nuclear interests.

We propose that genomic conflict explains the frequent occurrence of non-reciprocal nuclear exchange in mushrooms. With non-reciprocal exchange, one monokaryon donates a nucleus and the other accepts it, but not vice versa as in the typical life cycle. We propose a model where non-reciprocal nuclear exchange is primarily driven by mitochondria inducing male sterility and the evolution of nuclear suppressors.

Keywords: nucleo-mitochondrial conflict; mitochondrial inheritance; doubly uniparental inheritance; basidiomycetes; cytoplasmic male sterility

1. INTRODUCTION

(a) *Uniparental transmission of cytoplasmic genomes and genomic conflict*

In most sexual life cycles, transmission of cytoplasmic genomes, such as plastids and mitochondria, is uniparental (for an overview see Birky 1995, 2001). The evolution of uniparental transmission may have been mediated by genomic conflict (Grun 1976; Cosmides & Tooby 1981; Hoekstra 1990). Genomic conflict occurs when a trait is favoured at one level but selected against at another, or when different genes affecting the same trait experience contradictory selection pressures because they follow different transmission rules (Hurst *et al.* 1996). With cytoplasmic mixing, the fitness of a cytoplasmic element not only depends upon the effect on its bearer's fitness, but also on its success *relative* to unrelated elements within the same host. In the absence of partitioning mechanisms that ensure a fair cytoplasmic segregation over the daughter cells of a heteroplasmic cell, selfish cytoplasmic variants may evolve that give an intra-individual advantage, but lower the fitness of the host individual. Cytoplasmic mixing is therefore expected to lead to genomic conflict: natural selection favours a gene at the level of the cytoplasmic genome but disfavours it at the level of the organism. By contrast, with uniparental transmission the fate of a cytoplasmic mutation depends only upon its effect on the individual fitness of its carriers. Therefore, uniparental transmission prevents competition between cytoplasmic elements over transmission, and thereby genomic conflict.

(b) *Mushrooms: doubly uniparental transmission of mitochondria*

Heterothallic mushrooms (Homobasidiomycetes, Basidiomycotina) accomplish sex by exchanging nuclei without cytoplasm (Buller 1931; Casselton & Condit 1972; Hurst 1995). In a typical life cycle (figure 1), hyphal fusions occur between two monokaryons (haploid mycelia arising after spore germination) and subsequent reciprocal exchange of the haploid nuclei without cytoplasmic mixing (Casselton & Economou 1985; Elliott 1994). The resulting dikaryon is therefore a cytoplasmic mosaic with uniformly distributed nuclei (two in each cell). Filamentous fungi are modular organisms, and potentially every cell of the dikaryon can reproduce sexually through the production of fruiting bodies (basidiomata or mushrooms) bearing basidia. In these basidia nuclear fusion occurs, followed by meiosis and the formation of basidiospores. Because the cytoplasms of the two parents are not mixed, cytoplasmic inheritance is doubly uniparental: both monokaryons involved in a mating can potentially transmit their cytoplasm to the sexual spores, but normally only a single type per spore. In such a life cycle, within-cell competition

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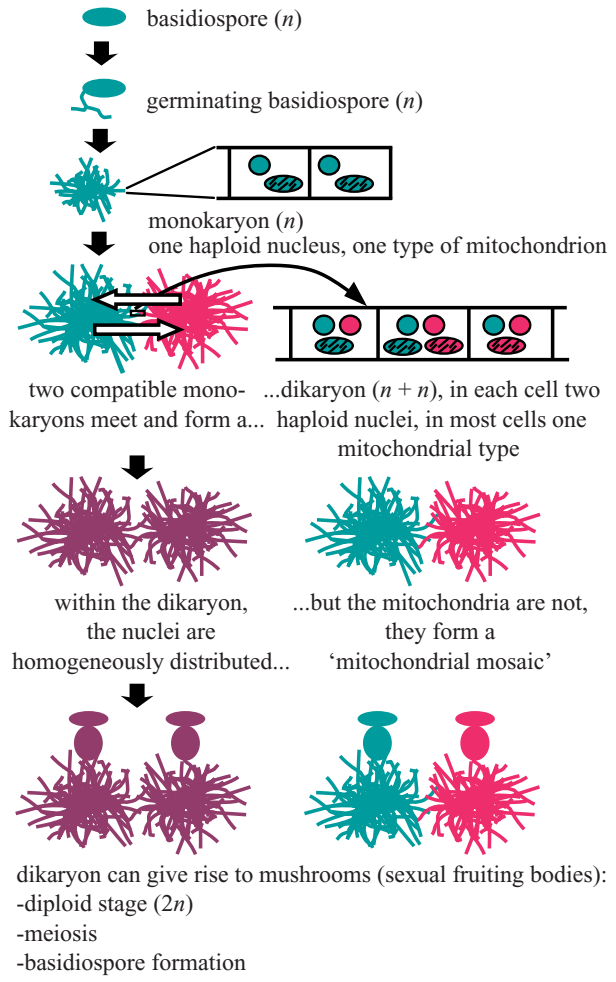

Figure 1. General life cycle of a heterothallic hymenomycete.

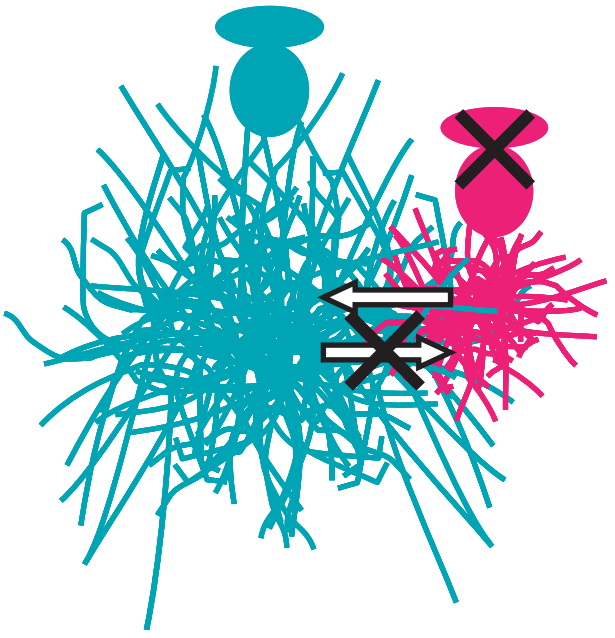

Figure 2. CMS and its consequences: monopolization of spores by blue mitochondria and higher growth rate.

between mitochondria is limited (heteroplasmy occurs only in the fused cells). Mushrooms typically have multiple mating types, often in the thousands (for example,

	9516	9522	9523	9524	9526	9529	9532	9534	9535	9540	9556	9623	9625	9639	9643	9647	9653
9504	B	L	B	L	B	B	L	B	B	B	B	B	B	B	B	B	B
9516		B	B	B	B	B	L	O	B	B	B	–	B	B	B	B	B
9522			U	L	U	U	O	O	O	B	B	U	U	B	O	U	B
9523				L	B	B	L	L	O	B	U	–	B	B	–	B	B
9524					B	U	–	O	B	B	B	B	B	U	B	B	U
9526						B	L	L	–	B	B	B	–	–	B	B	B
9529							L	L	B	B	B	B	–	B	L	B	B
9532								O	B	–	B	U	U	U	O	U	B
9534									B	B	U	U	B	U	U	B	U
9535										B	B	B	B	–	L	B	B
9540											B	B	B	B	B	B	B
9556												B	B	B	B	B	B
9623													B	B	L	B	B
9625														B	L	B	B
9639															L	B	–
9643																B	–
9647																	B

Figure 3. Results of pairing tests in biological species 17 of the *Hebeloma crustuliniforme* complex (Aanen & Kuyper 1999). Key: B: both monokaryons become dikaryotized; L, left monokaryon becomes dikaryotized; U, upper monokaryon becomes dikaryotized; O, dikaryotic outgrowth (no nuclear migration observed); –, no dikaryon formation. The pairings involving monokaryons 9522, 9524, 9532, 9534 and 9643 are shaded (see text).

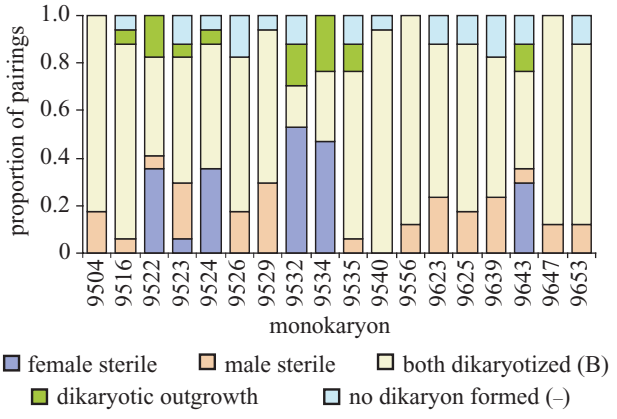

Figure 4. Summary of the nuclear behaviour in pairings for individual monokaryons.

an estimated 23 328 in *Schizophyllum commune*; Kothe *et al.* 2003), which makes outbreeding highly efficient (Burnett 2003). This is in strong contrast to most other sexual eukaryotes, which are differentiated into two sexes/mating types, where typically one of the two transmits the cytoplasmic genes. It has been proposed that mushrooms need not be restricted to two sexes or mating types because they do not need a bisexual system to regulate (uniparental) cytoplasmic inheritance (Hurst & Hamilton 1992; Hurst 1995).

(c) *Genomic conflict in mushrooms?*

Intracellular competition between mitochondria is absent, because normally only a single type is found within a dikaryotic cell. However, the dikaryon as a whole is a mosaic for its mitochondrial composition, consisting of two pieces with genetically different mitochondria. This is a peculiar situation: although there is restricted cytoplasmic exchange, there is nevertheless enduring physical contact. Therefore, at the dikaryon level, the two types of mitochondria compete over transmission. If individual mitochondria can increase their relative chance to be included in the spores *and* if this occurs at a cost of dikaryon fitness, this leads to genomic conflict for two reasons.

(i) A mitochondrial gene can be selected at the level of the cytoplasmic genome but selected against at the level of the dikaryon.
(ii) Because nuclei are homogeneously distributed in the dikaryon, nuclear fitness is directly dependent on dikaryon fitness. A reduction in dikaryon fitness because of intra-dikaryon mitochondrial competition is therefore directly in conflict with nuclear interests.

Several studies have shown that non-random segregation of mitochondria occurs (e.g. Hintz *et al.* 1988; Smith *et al.* 1990; Jin *et al.* 1992; Fischer & Seefelder 1995; De la Bastide *et al.* 2003). The question is whether these mitochondria, which apparently bias their transmission, do so at a cost to the dikaryon fitness. It is these types of mitochondria that will cause genomic conflict. Several types of biasing could be associated with costs for the dikaryon. In this paper, we focus on one possible mode of selfish action, namely the induction of male sterility. First, we will derive some general predictions for the evolution of selfish mitochondria in the absence of nuclear suppressors. We then look in detail at one possible example, cytoplasmic male sterility (CMS) and propose a genetic model.

2. THE EVOLUTION OF SELFISH MITOCHONDRIA IN THE ABSENCE OF NUCLEAR SUPPRESSION

Assuming mitochondria have the possibility to increase their relative transmission, we derive some general predictions for the evolution of such mitochondria in the absence of nuclear repression. We use a population genetic approach to derive conditions for invasion of selfish mitochondria. Consider two types of mitochondria: F (fair) and S (selfish). We assume that monokaryons are either F (contain exclusively F mitochondria) or S (contain only S mitochondria) and that all monokaryons in a population are involved in pairwise encounters. In FS dikaryons, most of the spores produced are of the S-type, owing to some suppressive effect from the S mitochondria. We suppose that this suppression entails a cost, reflected in a reduced total spore output of the dikaryon. In addition, SS dikaryons will suffer a reduced spore output due to the action of the S mitochondria. We specify the model as follows:

dikaryon type	FF	FS	SS
relative frequency	p^2	$2pq$	q^2
relative spore production	1	$1-a$	$1-s$

A fraction $k(k > 1/2)$ of the spores produced by an FS dikaryon is of type S.

The recurrence equation connecting the relative frequency of S monokaryons in two successive generations is

$$q' = q\,\frac{(1-s)q + 2k(1-a)\,p}{p^2 + 2(1-a)\,pq + (1-s)q^2}.$$

Standard stability analysis of the trivial equilibria $\hat{q} = 0$ and $\hat{q} = 1$ yields the following conditions:

S monokaryons increase in frequency when rare if

$$k > \frac{1}{2(1-a)};$$

F mitochondria increase in frequency when rare if

$$k < \frac{1-2a+s}{2(1-a)}.$$

Therefore, if both these inequalities are satisfied, a stable (F,S) polymorphism will exist. This requires in any case $s > 2a$, implying that a selfish monokaryon reduces fitness more in interaction with another selfish monokaryon than with a fair monokaryon.

We next consider what possibilities mitochondria have to increase their relative transmission rate, and whether this occurs at a cost to the dikaryon fitness. One such possibility is CMS.

3. CYTOPLASMIC MALE STERILITY

A monokaryon normally both accepts its partner's nucleus and donates its own, and these two actions can be considered as female and male roles, respectively. Theoretically, a mitochondrion that can prevent the male role of the monokaryon it resides in while maintaining its female role (CMS) will have a selective advantage over a partner mitochondrion that does not do so. This relative advantage has two causes (which are not mutually exclusive; figure 2).

(i) Such a mitochondrion will monopolize the spores, because fruiting in the other section of the mycelium will be prevented.
(ii) The relative growth rate of a dikaryon is higher than that of a monokaryon (e.g. Swietzynski & Day 1960; Kües 2000). Therefore, even postponing male function relative to female function can be advantageous for an individual mitochondrion.

Male sterility of a monokaryon is a relative phenomenon: male sterility for one monokaryon in a pairing is female sterility for the other monokaryon in that pairing. Few studies have systematically investigated the direction of nuclear migration. However, from these few studies it appears that non-reciprocal nuclear exchange frequently occurs (e.g. Kemp 1976: several species of *Coprinus*; Hintz *et al.* 1988: *Agaricus bitorquis*; May & Taylor 1988: *Coprinus cinereus*; Petersen & Ridley 1996: *Pleurotus*; Aanen & Kuyper 1999: *Hebeloma crustuliniforme* complex). Within a species, the occurrence of non-reciprocal nuclear exchange can be very common. May & Taylor (1988), for example, found such occurrence in a third of the pairings in *Coprinus cinereus*.

Male sterility is likely to be associated with costs for the dikaryon. First, a part of the dikaryon will not be used optimally. More importantly, as the frequency of male sterility increases, more and more pairings will be between male sterile monokaryons. If male sterility were absolute, such pairings would have zero fitness: no dikaryon would arise, and hence no sexual spores. However, dikaryotic outgrowth from the contact zone might still be possible in such combinations. Dikaryotic outgrowth without any accompanying nuclear migration is common in pairing tests between monokaryons (e.g. in *Coprinus*, Swietzynski & Day 1960; *Pleurotus*, Petersen & Ridley 1996; *Hebeloma*, Aanen & Kuyper 1999).

No genetic models have been formulated to explain patterns of non-reciprocal nuclear exchange in mushrooms. For Ascomycetes, Esser (1956) and Kemp (1976) have proposed genetic models based on two nuclear loci. However, no selective explanations have been proposed. The questions that need to be addressed are as follows.

(i) Is non-reciprocal nuclear exchange primarily male or female sterility?
(ii) Is non-reciprocal nuclear exchange induced by nuclei or by mitochondria?
(iii) Is there a selective explanation for non-reciprocal nuclear exchange or is it a deleterious phenotype?

(a) *Non-reciprocal nuclear exchange in* Hebeloma crustuliniforme

Aanen & Kuyper (1999) studied sexual compatibility in the *Hebeloma crustuliniforme* complex. They found 21 intercompatibility groups or biological species. Most of these had multiple mating types, so that pairings between non-sib monokaryons were generally compatible. In 12 out of the 21 species, pairings were observed that resulted in non-reciprocal nuclear exchange. The authors did not propose any explanation for the observed phenomenon. We propose a hypothesis to explain the results of the pairing tests in biological species 17 (*Hebeloma velutipes*), to which most strains belonged. In figure 3, the results are shown (from Aanen & Kuyper 1999). Three types of result can be seen:

(i) bidirectional nuclear migration (B(oth) monokaryons in figure 3 became dikaryotized);
(ii) unidirectional nuclear migration (to the L(eft) or U(pper) monokaryon in figure 3);
(iii) no nuclear migration resulting in:
  (1) dikaryotic outgrowth (O(utgrowth)),
  (2) no dikaryon at all (−).

There are no absolute differences between monokaryons: most monokaryons show some of the above behaviours in different pairings. Because male sterility is relative, the total number of pairings where a monokaryon behaved male sterile equals the number of pairings where a monokaryon behaved female sterile (35). However, the distribution of male sterility over monokaryons is very different from the distribution of female sterility (figure 4): with one exception (9523 × 9556), female sterility is restricted to five monokaryons (pairings involving those five are shaded in figure 4). Male sterility, by contrast, is more evenly distributed over the monokaryons and (with one exception) restricted to pairings with the five, relatively female sterile, monokaryons. Finally, pairings between members of the five relatively female sterile monokaryons generally result in an absence of nuclear migration (mostly dikaryotic outgrowth from the contact zone between the two monokaryons, but sometimes no dikaryotic growth at all).

(b) *A genetic model of non-reciprocal nuclear exchange*

To explain the *Hebeloma* observations, we propose a genetic model where non-reciprocal nuclear exchange is primarily mitochondrion-driven male sterility. In the model a mitochondrion can induce male sterility (S, selfish) or not (F, fair). Under many circumstances, S will reach a high frequency (see above). This high frequency of S creates the conditions for the invasion of nuclear mutants that are able to break CMS (resistance). In our model, resistance is coded by a nuclear gene R (resistant), present in the partner monokaryon, whereas N (non-resistant) is the wild-type that is not resistant. We thus have four possible genotypes: *SR*, *SN*, *FR* and *FN*, and 10 possible different combinations between these.

	SR (9529)	*SN* (9522)	*SN* (9532)	*FR* (9540)	*FR* (9556)	*FN* (9524)
SR (9526)	B	L	L	B	B	B* (L)
SR (9529)		L	L	B	B	L
SN (9522)			O	B	B	L
SN (9532)				—*(B)	B	—*(L)
FR (9540)					B	B
FR (9556)						B

Figure 5. Genetic model with crosses between seven monokaryons that are deduced to have the four different genotypes. Broken diagonal lines imply nuclear migration, whereas unbroken diagonal lines symbolize the dikaryotic outgrowth from the meeting zone between two monokaryons. — Implies a completely negative pairing without any dikaryotic outgrowth. The three pairings indicated by an asterisk are not in accordance with the model and the expected behaviours are indicated between brackets.

The model is illustrated with crosses between seven monokaryons that are deduced to have the four different genotypes (figure 5). Most combinations result in reciprocal nuclear exchange (*SR–SR*, *SR–FR*, *SN–FR*, *FR–FR*, *FR–FN*). Pairings where one monokaryon has an *S* genotype and the other an *N* genotype result in unidirectional nuclear migration, into the monokaryon with the *S* genotype (*SR–FN*, *SN–FN*, *SR–SN*). The combination between two *SN* monokaryons will not result in any nuclear migration. This will either result in a dikaryotic outgrowth (O) or no dikaryon formation at all (—).

The model explains, to a large degree, the *Hebeloma* observations. Most monokaryons are of genotype *SR*, and pairings between those generally result in reciprocal nuclear exchange. Moreover, as predicted by the model with a high frequency of *S* and *R*, non-reciprocal nuclear exchange has a skewed distribution over monokaryons in the population, with female sterility restricted to the monokaryons of genotype *SN* and *FN*, and male sterility spread more evenly over the monokaryons (the monokaryons of genotype *SR* in combinations with those monokaryons of genotype *SN* or *FN*). With the exception of one pairing, male sterility is indeed restricted to pairings with the supposed *SN* and *FN* genotypes. Finally, as predicted by the model, the combinations between *SN* genotypes generally do not result in any nuclear migration. Although the model predicts the overall patterns well, it does not explain every individual mating. For example, in a fraction

of the pairings between supposed *SN* and *SR* genotypes, bidirectional migration occurs, instead of the predicted unidirectional migration. Furthermore, some of the negative pairings are not explained by the model. This suggests one of two possibilities.

(i) The proposed mitochondrion-induced male sterility and nuclear resistance are not absolute qualitative properties of a monokaryon, but statistical properties instead.
(ii) There are additional modifier genes or pairs of genes.

4. DISCUSSION

In this paper, for the first time to the authors' knowledge, the possibility that intra-mycelial competition between mitochondria leads to genomic conflict in mushrooms was explicitly considered. We paid special attention to one particular possibility of a conflict between nuclei and mitochondria, namely the induction of male sterility by mitochondria. We argued that the induction of male sterility by mitochondria might be selected at the mitochondrial level as a consequence of between-mitochondria competition over transmission within the dikaryon. However, CMS is disadvantageous for nuclei and hence nuclei are selected to suppress the selfish behaviour of mitochondria. The predicted result is an arms race between mitochondrial mutants inducing male sterility and nuclei suppressing it. These theoretical considerations provide a selective explanation for the frequent observation of non-reciprocal nuclear exchange instead of the 'standard' reciprocal exchange in homobasidiomycete life cycles. Previous models to explain unidirectional nuclear migration (for Ascomycetes; Esser 1956; Kemp 1976) have considered only nuclear genes. However, unlike our model, these models did not provide any selective explanation for the occurrence of unidirectional migration.

The coevolution between mitochondria and nuclei at the local scale that our model predicts can be a strong divergent force between populations. We suggest that partial sterility in the form of non-reciprocal nuclear exchange could be a first step in speciation, i.e. the evolution of complete reproductive isolation. Therefore, the evolution of non-reciprocal nuclear exchange in mushrooms might be an explanation for the high numbers of closely related biological species that have been found in many mushrooms (for reviews, see Petersen & Hughes 1999; Burnett 2003).

(a) *Alternative models*

The genetic model presented here provides a testable hypothesis for the evolution of non-reciprocal nuclear exchange in mushrooms. In our model, resistance to a CMS-inducing mitochondrion is coded by the nucleus in the partner monokaryon. Because both nuclei in a pairing with a CMS-inducing mitochondrion suffer a cost (and especially so in a pairing where both monokaryons have such a mitochondrion), resistance could also be coded by the nucleus of the same monokaryon. However, this would not explain the *Hebeloma* data. This is because with such a model, and high frequencies of both CMS and resistance, male sterility, and not female sterility, would be concentrated in a few monokaryons, whereas female sterility would be spread over more monokaryons.

It is difficult to envisage any selective explanations for the evolution of non-reciprocal nuclear exchange, other than mitochondrion-induced male sterility. Advantages of female sterility cannot be expected for the nucleus or for the mitochondrion. Non-reciprocal nuclear exchange could also be a deleterious phenotype that is being selected against. However, the high frequency with which non-reciprocal nuclear exchange occurs suggests that there is a selective explanation. Alternatively, non-reciprocal nuclear exchange is due to genetic variation in species-recognition mechanisms, or in inbreeding avoidance mechanisms.

(b) *Proximate explanations for CMS*

How could a mitochondrion induce male sterility? We see two possible mechanisms. One is by cell death in the region of contact that is slow enough to allow the import of a nucleus, but fast enough to prevent the emigration of a nucleus. Usually 'barrages' (or 'aversion zones' (Burnett 2003) where there is reduced growth and increased cell death) do not occur in pairings between compatible monokaryons (Burnett 2003). However, in some species they occur at a low frequency, for example in pairings between monokaryons of *Polyporus* spp. Interestingly, for these species, in all instances of unidirectional nuclear migration, barrages were found (Hoffmann & Esser 1978).

The other possible route would be the interaction of a mitochondrion or of a mitochondrial gene product with one of the mating factors. Most mushrooms are tetrapolar, which means that mating is regulated by two factors, *A* and *B*, which together determine the mating type. For a pairing to be fully compatible, both factors need to be different. The *B* factor is involved in nuclear migration and clamp cell fusion (Raper 1966). It has recently been shown that the *B* locus of *Schizophyllum commune* and *Coprinus cinereus* (the species that have been studied most extensively) codes for both a receptor and several pheromones (Wendland *et al.* 1995; O'Shea *et al.* 1998). The interaction of the pheromone produced by monokaryon I with the receptor of monokaryon II results in nuclear migration into II, whereas the interaction of the pheromone produced by II with the receptor of I results in migration into I (Vaillaincourt & Raper 1996; Olesnicky *et al.* 2000; Kothe 2002). Experiments have shown that if a monokaryon fails to induce the receptor of a partner monokaryon, it will not be able to donate its nucleus, but it will still accept a nucleus, when its own B receptor is induced (Kothe *et al.* 2003). Therefore, if a mitochondrial gene product (or a gene product of a mitochondrial plasmid) can inactivate the pheromone (or inhibit its production), while maintaining the receptor function (or receptor synthesis), this will result in male sterility. Alternatively, a mitochondrion or mitochondrial plasmid could produce a product that blocks the receptor of its partner monokaryon, but not that of its own.

(c) *Other ways of biased transmission rates and the consequences for the dikaryon*

In this paper, we have assumed that mitochondria can behave selfishly, and studied one possibility, the induction

of CMS in more detail. However, there are other possibilities that we did not consider.

An obvious way for a mitochondrion to increase its relative transmission rate is by increased growth rate and/or early induction of fructification. It is difficult to predict what consequences such behaviour has for dikaryon fitness. It is likely that under many circumstances, selection on the mitochondrion and the dikaryon will work in the same direction and there will be no conflict: increased growth will be to the benefit of the mitochondrion and the dikaryon. However, under some circumstances the optimal life-history characters of an individual mitochondrion in competition with another mitochondrion may be different from those for a dikaryon. However, this hypothesis is difficult to test and no data exist in the literature.

A second way in which mitochondria could increase their relative transmission rate would be to invade into a partner monokaryon's domain and replace the mitochondria there. For *Pleurotus ostreatus* and *P. pulmonarius* evidence has been found that such 'hostile take-overs' take place, because within a few weeks after establishment dikaryons comprised only a single mitochondrial type (Fischer & Seefelder 1995; Fischer & Wolfrath 1997). However, these experiments were performed in liquid culture with an artificially high level of cell–cell contacts. Also, in Ascomycetes evidence has been found for invasive behaviour of mitochondria (Lee & Taylor 1988). As invasive mitochondria kill the resident mitochondria, it is probable that the dikaryon suffers a cost associated with such mitochondria.

(d) *A comparison with CMS in plants*

CMS has been shown to occur in many plant species. With CMS, a fraction of the population of an otherwise hermaphroditic plant species is unable to produce functional pollen (for reviews see Frank 1989 and Budar *et al.* 2003). In most cases CMS is caused by mitochondrial genes, and in all documented examples, the male-sterility-inducing cytoplasm effect can be counteracted by nuclear genes. To explain the occurrence of CMS, increased female fertility in CMS plants has been suggested. The mitochondria in a plant are in conflict over resource allocation with nuclei: nuclei have an equal interest in a plant's male and female function, whereas mitochondria have an interest in only the female function. If the unused male resources in CMS plants transfer to female function, CMS plants would have a higher female fertility, and CMS-inducing mitochondria would therefore be selected. In many cases, increased female fertility has indeed been found in CMS plants.

In fact, this closely parallels the situation in the mushroom life cycle: the mitochondria have an interest in only the female function of their monokaryon (nuclear immigration) and not in its male function (nuclear emigration). If, as we showed is probable, a decrease in male function is accompanied by an increased female function, such a decrease can be selected at the level of the mitochondrion. In contrast to the plant life cycle, however, in the mushroom life cycle two mitochondrial types are in direct competition over a resource.

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REFERENCES

Aanen, D. K. & Kuyper, T. W. 1999 Intercompatibility tests in the *Hebeloma crustuliniforme* complex in northwestern Europe. *Mycologia* **91**, 783–795.

Birky Jr, C. W. 1995 Uniparental inheritance of mitochondrial and chloroplast genes: mechanisms and evolution. *Proc. Natl Acad. Sci. USA* **92**, 11 331–11 338.

Birky Jr, C. W. 2001 The inheritance of genes in mitochondria and chloroplasts: laws, mechanisms and models. *A. Rev. Genet.* **35**, 125–148.

Budar, F., Touzet, P. & De Paepe, R. 2003 The nucleo-mitochondrial conflict in cytoplasmic male sterilities revisited. *Genetica* **117**, 3–16.

Buller, A. H. R. 1931 *Researches on fungi*, vol. 4. London: Longmans Green.

Burnett, J. 2003 *Fungal populations and species*. New York: Oxford University Press.

Casselton, L. A. & Condit, A. 1972 A mitochondrial mutant of *Coprinus lagopus*. *J. Gen. Microbiol.* **72**, 521–527.

Casselton, L. A. & Economou, A. 1985 Dikaryon formation. In *Developmental biology of higher fungi* (ed. D. Moore, L. A. Casselton, D. A. Wood & J. C. Frankland), pp. 213–229. Cambridge University Press.

Cosmides, L. & Tooby, J. 1981 Cytoplasmic inheritance and intragenomic conflict. *J. Theor. Biol.* **89**, 83–129.

De la Bastide, P. Y. & Horgan, P. A. 2003 Mitochondrial inheritance and the detection of non-parental mitochondrial DNA haplotypes in crosses of *Agaricus bisporus* homokaryons. *Fung. Genet. Biol.* **38**, 333–342.

Elliott, C. G. 1994 *Reproduction in fungi: genetical and physiological aspects*. London: Chapman & Hall.

Esser, K. 1956 Die Inkompatibilitätsbeziehungen zwischen geographischen Rassen von *Podospora anserina* (Ces.) Rehm. I. Genetische Analyse der Semi-inkompatibilität. *Z. Indukt. Abstamm. Vererbungs* **87**, 595–624.

Fischer, M. & Seefelder, S. 1995 Mitochondrial DNA and its inheritance in *Pleurotus ostreatus* and *P. pulmonarius*. *Bot. Acta* **108**, 334–343.

Fischer, M. & Wolfrath, H. 1997 Mitochondrial DNA in mon–mon and di–mon pairings of *Pleurotus ostreatus*. *Bot. Acta* **110**, 172–176.

Frank, S. 1989 The evolutionary dynamics of cytoplasmic male sterility. *Am. Nat.* **133**, 345–376.

Grun, P. 1976 *Cytoplasmic genetics and evolution*. New York: Columbia University Press.

Hintz, W. E. A., Anderson, J. B. & Horgan, P. A. 1988 Nuclear migration and mitochondrial inheritance in the mushroom *Agaricus bitorquis*. *Genetics* **119**, 35–41.

Hoekstra, R. F. 1990 Evolution of uniparental inheritance of cytoplasmic DNA. In *Organizational constraints on the dynamics of evolution* (ed. J. Maynard & G. Vida), pp. 269–278. Manchester University Press.

Hoffmann, O. & Esser, K. 1978 Genetics of speciation in the basidiomycete genus *Polyporus*. *Theor. Appl. Genet.* **53**, 273–282.

Hurst, L. D. 1995 Selfish genetic elements and their role in evolution: the evolution of sex and some of what it entails. *Phil. Trans. R. Soc. Lond.* B **349**, 321–332.

Hurst, L. D. & Hamilton, W. D. 1992 Cytoplasmic fusion and the nature of sexes. *Proc. R. Soc. Lond.* B **247**, 189–194.

Hurst, L. D., Atlan, A. & Bengtsson, B. O. 1996 Genetic conflicts. *Q. Rev. Biol.* **71**, 317–364.

Jin, T., Sonnenberg, A. S. M., Van Griensven, L. J. L. D. & Horgan, P. A. 1992 Investigation of mitochondrial transmission in selected matings between homokaryons from commercial and wild-collected isolates of *Agaricus bisporus* (= *Agaricus brunescens*). *Appl. Environ. Microbiol.* **58**, 3553–3560.

Kemp, R. F. O. 1976 A new interpretation of unilateral nuclear migration in fungi with special reference to *Podospora anserina*. *Trans. Br. Mycol. Soc.* **66**, 1–5.

Kothe, E. 2002 Sexual development in basidiomycetes. In *Molecular biology of fungal development* (ed. H. Osiewacz), pp. 245–273. New York: Dekker.

Kothe, E., Gola, S. & Wendland, J. 2003 Evolution of multispecific mating-type alleles for pheromone perception in the homobasidiomycete fungi. *Curr. Genet.* **42**, 268–275.

Kües, U. 2000 Life history and developmental processes in the basidiomycete *Coprinus cinereus*. *Microbiol. Mol. Biol. Rev.* **64**, 316–353.

Lee, S. B. & Taylor, J. W. 1988 Uniparental inheritance and replacement of mitochondrial DNA in *Neurospora tetrasperma*. *Genetics* **134**, 1063–1075.

May, G. & Taylor, J. W. 1988 Patterns of mating and mitochondrial DNA inheritance in the agaric basidiomycete *Coprinus cinereus*. *Genetics* **118**, 213–220.

Olesnicky, N. S., Brown, A. J., Honda, Y., Dyos, S. L., Dowell, S. J. & Casselton, L. A. 2000 Self-compatible *B* mutants in *Coprinus* with altered pheromone-receptor specificities. *Genetics* **156**, 1025–1033.

O'Shea, S. F., Chaure, P. T., Halsall, J. R., Olesnicky, N. S., Leibbrandt, A., Connerton, I. F. & Casselton, L. A. 1998 A large pheromone and receptor gene complex determines multiple *B* mating type specificities in *Coprinus cinereus*. *Genetics* **148**, 1081–1090.

Petersen, R. H. & Hughes, K. W. 1999 Species and speciation in mushrooms. *Bioscience* **49**, 440–452.

Petersen, R. H. & Ridley, G. S. 1996 A New Zealand *Pleurotus* with multiple-species sexual compatibility. *Mycologia* **88**, 198–207.

Raper, J. R. 1966 *Genetics of sexuality in higher fungi*. New York: Ronald Press.

Smith, M. L., Duchesne, L. C., Bruhn, J. N. & Anderson, J. B. 1990 Mitochondrial genetics in a natural population of the plant pathogen *Armillaria*. *Genetics* **126**, 575–582.

Swietzynski, K. M. & Day, P. R. 1960 Heterokaryon formation in *Coprinus lagopus*. *Genet. Res. Camb.* **1**, 114–128.

Vaillaincourt, L. J. & Raper, C. A. 1996 Pheromones and pheromone receptors as mating-type determinants in basidiomycetes. In *Genetic engineering*, vol. 18 (ed. J. K. Setlow), pp. 219–247. New York: Plenum Press.

Wendland, J., Vaillancourt, L. J., Hegner, J., Lengeler, K. B., Laddison, K. J., Specht, C. A., Raper, C. A. & Kothe, E. 1995 The mating type locus *B*α1 of *Schizophyllum commune* contains a pheromone receptor and putative pheromone genes. *EMBO J.* **14**, 5171–5178.

As this paper exceeds the maximum length normally permitted, the authors have agreed to contribute to production costs.