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Mitochondrial inheritance in *Aspergillus nidulans*

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Summary

Mitochondrial chloramphenicol and oligomycin resistance mutations were used to investigate mitochondrial inheritance in *A. nidulans*. Mitochondrial RFLPs could not be used to distinguish between paternal and maternal mitochondria because none were detected in the 54 isolates investigated. Several thousand ascospores from each of 111 hybrid cleistothecia from 21 different crosses between 7 heterokaryon incompatible isolates were tested for biparental inheritance. All mitochondrial inheritance was strictly uniparental. Not one instance of paternal inheritance of mitochondria was observed. The implications of our results for the theory that uniparental inheritance evolved to avoid cytoplasmic conflict are discussed. Possible explanations for the maintenance of strict uniparental inheritance of mitochondria in an inbreeding homothallic organism are suggested. The chloramphenicol resistance marker was inherited preferentially to the oligomycin resistance marker probably due to the inhibited energy production of mitochondria with the oligomycin resistance mutation. The maternal parent was determined for 93 hybrid cleistothecia from 17 crosses between 7 different strains. Contrary to previous reports *A. nidulans* strains functioned as both maternal and paternal parent in most crosses.

1. Introduction

An explanation for the evolution of uniparental inheritance of mitochondria is that uniparental inheritance prevents conflict between mitochondrial and nuclear genes (Cosmides & Tooby, 1981; Hoekstra, 1990). Biparental inheritance of mitochondria would cause competition between maternal and paternal mitochondria in progeny. The competition will be won by the mitochondrial genome that optimizes its own reproduction even if this genome carries mutations that are disadvantageous for the reproduction of nuclear genes. Uniparental inheritance of mitochondria limits the spread of such selfish mitochondria to the maternal line (Grun, 1976). Hurst & Hamilton (1992) argued that biparental inheritance may occur in inbreeding homothallic organisms. In such organisms, paternal and maternal mitochondria will usually be identical eliminating the danger of cytoplasmic conflict and allowing relaxation of the costly maintenance of uniparental inheritance. *A. nidulans* is a homothallic organism with no preference for outcrossing. For this reason we expect selfing to occur much more frequently than outcrossing in natural *A. nidulans* populations. Consequently *A. nidulans* is an ideal candidate for an organism with biparental inheritance. However Reboud & Zeyl (1994) pointed out that the above argument is only correct if the genes that regulate cytoplasmic inheritance are nuclear and not cytoplasmic. If the regulatory genes are cytoplasmic then there will be selection for biparental inheritance in outcrossing species because this will enhance the spread of cytoplasmic genes. Selection for biparental inheritance could be relaxed in selfing species resulting in uniparental inheritance in inbreeding homothallic organisms. This was made clear by Hurst (1994) who demonstrates that both nuclear maintenance of uniparental inheritance as well as cytoplasmic resistance against uniparental inheritance might decay under inbreeding conditions. Consequently we would

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only expect to find biparental inheritance in *A. nidulans* if mitochondrial inheritance is under nuclear and not cytoplasmic control.

A. nidulans is a filamentous ascomycete that can reproduce both asexually (conidiospores) and sexually (ascospores). The initial step (fertilization) in fruitbody (cleistothecium) formation in ascomycetes is the formation of a dikaryon (Alexopoulos & Mims, 1979; Burnett, 1976). All the ascospores in a cleistothecium are formed by repeated mitosis, fusion and meiosis of the two nuclei in the dikaryon. Dikaryon formation by fusion of differentiated male and female reproductive structures has never been observed in *A. nidulans* (Benjamin, 1955). Zonneveld (1988) observed hyphae differing from vegetative hyphae and suggested that these were dikaryotic. Rowlands & Turner (1976) used mutations of the mitochondrial genome to determine from which parent the ascospores in a cleistothecium inherited their mitochondria. This parent they called the maternal parent. They demonstrated that the maternal parent determines the colour of the ascospores and the cleistothecial wall. These observations suggest that although they have never been observed *A. nidulans* has differentiated maternal and paternal structures (anisogamy). The maternal parent contributes the cytoplasm and mitochondria to the dikaryon and forms the ascospore and cleistothecial wall, the paternal parent donates only a haploid nucleus to the dikaryon. Because *A. nidulans* is a homothallic organism all colonies must be capable of producing both maternal and paternal structures (hermaphroditism).

While demonstrating the extra-nuclear character of an oligomycin resistance mutation Rowlands & Turner (1973) observed the uniparental inheritance of this mitochondrial marker. Their crosses were all performed with two different mutants of the same (Glasgow) wild-type. An extensive search for biparental inheritance in crosses of different (heterokaryon incompatible) wild-types has never been performed. Rowlands & Turner (1976) performed crosses between two heterokaryon incompatible strains, hc-Glasgow and hc-B (Croft, 1985). In four separate crosses between these strains they examined a total of 79 hybrid cleistothecia. They found that, despite being homothallic, in sexual crosses of *A. nidulans* one strain would consistently act as only maternal or only paternal parent. One possible explanation of this result is some form of mating type differentiation in *A. nidulans*. However, these results have never been confirmed nor has a possible explanation been suggested.

The present research was undertaken to answer the following two questions:

(1) Is there biparental inheritance of mitochondria in *A. nidulans* as has been suggested to be possible in inbreeding homothallic organisms when mitochondrial inheritance is under nuclear control (Reboud Zeyl, 1994; Hurst, 1994)?

(2) Can *A. nidulans* strains, despite being homothallic, only function as maternal or paternal parent in outcrosses as was observed by Rowlands & Turner (1976)?

2. Materials and methods

(i) *Strains*

All *A. nidulans* isolates used in this study are listed in Table 1. The isolates are numbered according to the Birmingham collection of Dr J. H. Croft. the 54 isolates belong to at least 40 different heterokaryon compatibility groups. Isolate 7 is the original Glasgow isolate described by Pontecorvo in 1953. In our strain notation the numbers before the slash refer to a particular isolate and the numbers after the slash refer to a genotype. So strain 7/2 is the *cnx* mutant of isolate 7. Isolation of /2 (*cnx*) and /3 (*y*; *nia*,) mutants has been described previously (Coenen *et al.* 1994). /4 (*cnx*; mt*Oli*^{r}) and /5 (*y*; *nia*; mt*Cam*^{r}) mutants were constructed by protoplast fusion. All isolates used in crosses were heterokaryon incompatible.

(ii) *Media*

Minimal medium (MM) was prepared as described previously (Coenen *et al.* 1994), urea was used as a nitrogen source in all plates. Oligomycin medium (MMU-Oli) contained 1 mg/l oligomycin, chloramphenicol medium (MMU-Cam) 4 g/l chloramphenicol.

(iii) *Transfer of mitochondrial markers by protoplast fusion*

/4 (*cnx*; mt*Oli*^{r}) mutants were constructed by protoplast fusion of /2 mutants and strain WG274 (*y*; *paba*; *mtoli*^{r}). /5 (*y*; *nia*; mt*Cam*^{r}) mutants were constructed by protoplast fusion of /3 mutants and strain AN1007 (*w*; *pyro*; *mtcam*^{r}). The mitochondrial oligomycin resistance was originally isolated by Rowlands & Turner (1973), the mitochondrial chloramphenicol resistance by Gunatilleke (Gunatilleke *et al.* 1975), strains WG274 and AN1007 are both mutants of the Glasgow isolate.

250 ml flasks containing 100 ml liquid supplemented MM were inoculated with 10^6 conidiospores per ml and incubated in a shaker at 30 °C, after 24 h mycelium was harvested by suction filtration. 1 gram of mycelium was suspended in 20 ml novozym solution (10 mg/ml Novozym in 0·7 M-NaCl + 0·2 M-$CaCl_2$, sterilized over a 0·2 μm bacteria filter) and gently shaken at 30 °C for 2–4 h. Protoplasts were loosened from the mycelium with a sterile 10 ml pipette. When microscopic observations showed the presence of enough protoplasts the mycelial suspension was filtered over sterile glass wool. Protoplasts were centrifuged at 3500 rev/min for 10 min, the pellet

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resuspended in 4 ml STC (1·4 M sorbitol, 10 mM-Tris ph 7·5, 50 mM $CaCl_2$) and the protoplasts counted in a haemocytometer. Equal amount of protoplasts from the donor and the acceptor strain (10^6–10^7) were mixed and centrifuged for 10 min at 3500 rev/min. For fusion the pellet was resuspended in 1 ml PEG (30% w/v polyethylene glycerol in 50 mM-$CaCl_2$), incubated for 20 min at 30 °C and then diluted twice with 0·5 ml STC at a 5 min interval. Protoplasts were diluted in 1·4 M sorbitol and plate in 5 ml topagar (7·5 g agar/l, 1 M sucrose). A 10^{-4} dilution was plated on supplemented MM as a control that protoplasts from both strains were in the fusion mixture. Dilutions of 10^0–10^{-4} were plated on MMU containing either chloramphenicol or oligomycin. This medium selects for the nuclei from the acceptor strain and the mitochondrial marker from the donor strain. Colonies growing on the selection plates were transferred to new selection plates, purified by isolating a colony grown from a single conidiospore and the nuclear and mitochondrial mutations and heterokaryon compatibility group verified (Coenen *et al.* 1994).

(iv) *Mitochondrial restriction patterns*

Mitochondrial restriction patterns were obtained by restricting total DNA with *Hae* III and *Hae* III + *EcoR* I as described by Varga *et al.* (1993).

(v) *Crosses*

Crosses were performed between /4 (*cnx*; *mtOli*^{r}) and /5 (*y*; *nia*; *mtCam*^{r}) mutants and between wild type and /5 mutants. Except when mutants of the same isolate were crossed, all crosses were between heterokaryon incompatible strains to prevent the vegetative transfer of mitochondrial markers. Crossed cleistothecia were selected as described previously (Coenen *et al.* 1994).

To determine which parent was the maternal parent ascospores from /4 × /5 crosses were plated on MMU-Oli and MMU-Cam and ascospores from wild-type × /5 crosses were plated on MMU and MMU-Cam. To test for biparental inheritance the entire contents of hybrid cleistothecia from /4 × /5 crosses were plated half on MMU-Oli and half on MMU-Cam. Uniparental inheritance would result in wild-type and yellow colonies on the medium selecting for maternal mitochondria and no colonies on the medium selecting for paternal mitochondria. Colonies with the maternal colour on the plate selecting for the paternal mitochondria could only be caused by paternal inheritance of the mitochondrial marker and would be accepted as evidence of paternal inheritance. In crosses between wild-type and /5 mutants progeny could only be tested for presence of the paternal mitochondria if the wild-type strain was the maternal parent. The reason for this is that there is no selective medium for wild-type mitochondria.

(vi) *Radial growth measurements*

Strains were inoculated on MMU at the centre of a petri dish with a 9 cm diameter. Growth in four directions was marked at 48 and 120 h after inoculation. The growth between two marks (72 h) was measured in millimetres. Each growth experiment was performed in triplicate giving a total of twelve measurements per strain. However, in some cases growth was obstructed by new colonies growing from conidiospores. These measurements were discarded.

3. Results

(i) *Mitochondrial restriction patterns*

The *Hae* III restriction pattern of all 54 *A. nidulans* isolates in Table 1 was determined. All 54 isolates had the same seven bands of approximately 7·7, 7·1, 4·9, 3·7, 2·6, 2·0 and 1·6 kb, no restriction fragment length polymorphisms for *Hae* III were observed. For isolates 5, 6, 7, 85, 109, 257, 261, 284, 291, 706, 722 and 727 the *Hae* III + *EcoR* I restriction pattern was determined. All 12 isolates had the same 8 bands of approximately 7·5, 6·4, 3·7, 2·6, 2·0, 1·6, 1·5 and 1·2 kb, no restriction fragment length polymorphism for *Hae* III + *EcoR* I were observed.

(ii) *Transfer of mitochondrial markers by protoplast fusion*

The mitochondrial oligomycin resistance mutation was transferred by protoplast fusion from strain WG274 to the /2 (*cnx*) mutants of isolates 701, 704, 705, 711, 712, 713, 715 and 723. The mitochondrial chloramphenicol resistance mutation was transferred by protoplast fusion from strain AN1007 to the /3 (*y*; *nia*;) mutants of isolates 700, 701, 704, 705, 711, 713, 715, 716, 723 and 726. All attempted transfers of the mitochondrial markers were successful. Because of the lack of mitochondrial RLFP's between the isolates fused it could not be determined whether entire mitochondrial genomes were transferred or whether the mitochondrial genomes recombined as is the case in inter-species transfer of mitochondrial markers in *Aspergillus* (Croft *et al.* 1979).

(iii) */4* (cnx; mtOli^r) × */5* (y; nia; mtCam^r) *crosses*

In cleistothecia from /4 × /5 crosses there were in theory three possibilities for the inheritance of mitochondria. (1) Uniparental inheritance in which case one cleistothecium contains only chloramphenicol resistant ascospores or only oligomycin resistant ascospores. (2) Biparental inheritance in which case one cleistothecium contains chloramphenicol resistant ascospores as well as oligomycin resistant ascospores. (3) Mitochondrial recombination in which case a cleistothecium may contain ascospores with both mutations and/or ascospores with neither mutation.

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Table 1. *Isolates used*

Isolate number	Place of Isolation	Year
1	Birmingham, England	1954
5	Bombay, India	1960
6	Rhodesia	1960
7		±1939
28	Birmingham, England	1962
33	Birmingham, England	1962
34	Birmingham, England	1962
43	Durham, England	1962
49	Durham, England	1962
51	Durham, England	1962
66	Birmingham, England	1962
67	Birmingham, England	1962
68	Birmingham, England	1962
80	Pembrokeshire, Wales	1962
85	Pembrokeshire, Wales	1962
89	Cardiganshire, Wales	1962
94	Hampshire, England	1962
99	Hampshire, England	1962
106	Warwickshire, England	1962
109	Kent, England	1962
114	Pembrokeshire, Wales	1962
161	Cardiganshire, Wales	1962
257	Hungary	1981
261	California, USA	1984
277	India	1986
284	India	1986
290	Barbados	1987
291	Barbados	1987
292	Barbados	1987
293	Barbados	1987
700	Birmingham, England	1992
701	Birmingham, England	1992
702	Birmingham, England	1992
703	Birmingham, England	1992
704	Birmingham, England	1992
705	Pembrokeshire, Wales	1992
706	Pembrokeshire, Wales	1992
707	Pembrokeshire, Wales	1992
708	Pembrokeshire, Wales	1992
709	Pembrokeshire, Wales	1992
710	Cardiganshire, Wales	1992
711	Cardiganshire, Wales	1992
712	Cardiganshire, Wales	1992
713	Cardiganshire, Wales	1992
714	Cardiganshire, Wales	1992
715	Cardiganshire, Wales	1992
716	Cardiganshire, Wales	1992
717	Cardiganshire, Wales	1992
718	Cardiganshire, Wales	1992
722	Pembrokeshire, Wales	1992
723	Cardiganshire, Wales	1992
724	Cardiganshire, Wales	1992
726	Birmingham, England	1992
727	Birmingham, England	1992

A total of 82 hybrid cleistothecia from 22 different crosses were examined. For five isolates (701, 704, 705, 713 and 723) /4 and /5 mutants of the same isolate were crossed. In these crosses asexual transfer of mitochondrial markers could occur. This phenomenon was observed by Rowlands and Turner (1976). In one heterokaryon compatible cross (705/4 × 705/5) two cleistothecia of which none of the ascospores would grow on either MMU-Oli or MMU-Cam were found. This was most probably caused by recombination of the mitochondrial genomes resulting in loss of both resistance markers. Apparently this recombination did not occur in the sexual cycle.

Table 2. *Maternal parent in /4 × /5 crosses*

Strains crossed	/4[a]	/5[b]
701/4 × 701/5	—	2
701/4 × 704/5	1	2
701/4 × 705/5	1	—
701/4 × 713/5	1	—
704/4 × 701/5	—	3
704/4 × 704/5	—	8
704/4 × 713/5	4	8
704/4 × 715/5	—	1
705/4 × 701/5	—	1
705/4 × 704/5	—	12
705/4 × 713/5	—	6
705/4 × 715/5	1	3
711/4 × 701/5	—	2
711/4 × 704/5	1	—
711/4 × 705/5	—	3
711/4 × 713/5	1	—
713/4 × 701/5	—	1
713/4 × 704/5	—	3
713/4 × 713/5	—	1
715/4 × 701/5	—	1
715/4 × 704/5	—	3
715/4 × 705/5	—	1
715/4 × 713/5	—	1
723/4 × 701/5	—	1
723/4 × 704/5	—	1
723/4 × 705/5	—	3
723/4 × 713/5	—	2
723/4 × 723/5	—	1

[a] Number of cleistothecia with /4 strain as maternal parent.
[b] Number of cleistothecia with /5 strain as maternal parent.

Pontecorvo (1953) estimated that a cleistothecium contains between 10^2 and 10^6 ascospores. We measured an average of 10^4 ascospores per cleistothecium. This means that several thousand ascospores from each hybrid cleistothecium were tested for presence of one of the mitochondrial markers. In the 80 hybrid cleistothecia examined mitochondrial inheritance was strictly uniparental. There was no biparental inheritance in any of the crosses.

Table 2 shows which parent was the maternal parent of 80 cleistothecia from 28 crosses of /4 × /5 strains. The /5 mutant was the maternal parent significantly more often (70 cleistothecia) than the /4 mutant (10 cleistothecia). Using a χ^2 test we can reject the hypothesis that there is no preference for inheritance of the chloramphenicol resistance mutation above inheritance of the oligomycin resistance mutation ($\chi^2 = 45$, D.F. = 1, $P < 0{\cdot}001$).

From these results we conclude that mitochondrial inheritance in *A. nidulans* is strictly uniparental. These results also established that when a strain with

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Table 3. *Radial growth in mm in 4 days*

Strain	Mean	*n*
701/2	24·1 (1·04)[a]	11
701/3	24·3 (1·77)	12
701/4	20·5 (0·85)	10
701/5	24·4 (0·67)	12
704/2	24·1 (1·24)	12
704/3	24·0 (1·41)	12
704/4	18·4 (1·44)	12
704/5	23·9 (1·44)	12
705/2	26·1 (0·52)	12
705/3	25·5 (0·80)	12
705/4	19·1 (0·90)	12
705/5	26·7 (0·89)	12
713/2	24·4 (0·97)	10
713/3	22·2 (1·17)	11
713/4	16·4 (0·53)	9
713/5	22·6 (0·53)	9

[a] Standard deviation with *n* measurements is given between brackets.

chloramphenicol resistant mitochondria is crossed with a strain with oligomycin resistant mitochondria the strain with chloramphenicol resistant mitochondria is preferred as maternal parent.

(iv) *Radial growth measurements*

To determine the effect of the mitochondrial markers on the growth of the strains the radial growth rates of the /2 (*cnx*), /3 (*y*; *nia*;), /4 (*cnx*; *mtOli*^{r}) and /5 (*y*; *nia*; *mtCam*^{r}) mutants of isolates 701, 704, 705 and 713 were measured (Table 3). A two tailed T-test with unequal variances was used to test the hypothesis that the growth rate of the /4 mutant did not vary significantly from that of the /2, /3 and /5 mutants. The results were: isolate 701 $P = 6{\cdot}9 \times 10^{-8}$, isolate 704 $P = 1{\cdot}1 \times 10^{-8}$, isolate 705 $P = 8{\cdot}4 \times 10^{-12}$, isolate 713 $P = 6{\cdot}6 \times 10^{-20}$. The results show that the /4 mutants grows significantly slower than the /2, /3 and /5 mutants. Because the only difference between the /2 and the /4 strain is the mitochondrial oligomycin resistance this mutation must be the cause of the reduced growth rate. This is in accordance with Rowlands and Turner (1973) observation that the mitochondrial oligomycin resistance impaired growth.

(v) *Wild-type* × */5* (y; nia; mtCam^r) *crosses*

These crosses were performed to eliminate the influence of the mitochondrial oligomycin resistance marker on the outcome of the experiments. Because only one of the parental strains had a mitochondrial marker mitochondrial recombination could not be observed in these crosses. Because there is no selective media for wild-type mitochondria biparental inheritance could only be observed when the wild-type strain was the maternal parent. Table 4 shows which parent

Table 4. *Maternal parent in wild type* × */5 crosses*

Strains crossed	wt[a]	/5[b]
701 × 701/5	2	—
701 × 704/5	—	11
701 × 705/5	—	2
701 × 713/5	—	2
701 × 715/5	—	1
701 × 723/5	1	—
704 × 704/5	1	2
704 × 705/5	1	1
704 × 713/5	1	1
704 × 723/5	—	5
705 × 701/5	1	2
705 × 713/5	1	3
711 × 715/5	2	—
711 × 723/5	5	—
713 × 701/5	—	2
713 × 704/5	1	1
713 × 705/5	1	3
713 × 713/5	2	3
715 × 711/5	4	4
715 × 715/5	3	1
715 × 723/5	—	4
723 × 701/5	—	7
723 × 704/5	1	—
723 × 711/5	2	3
723 × 715/5	2	1
723 × 723/5	—	3

[a] Number of cleistothecia with wild-type strain as maternal parent.
[b] Number of cleistothecia with /5 strain as maternal parent.

Table 5. *Maternal parent in reciprocal wild-type* × */5 crosses*

Strains crossed	1st[a]	2nd[b]
701 × 704	0	11
701 × 705	2	3
701 × 713	2	2
701 × 715	0	1
701 × 723	8	0
704 × 705	1	1
704 × 713	2	2
704 × 723	0	6
705 × 713	4	4
711 × 715	6	4
711 × 723	8	2
715 × 723	1	6

[a] Number of cleistothecia with first strain as maternal parent.
[b] Number of cleistothecia with second strain as maternal parent.

was the maternal parent of 93 cleistothecia from 17 crosses of wild type × /5 strains. The 31 cleistothecia with the wild type strain as maternal parent did not contain any ascospores with the paternal chloramphenicol resistant mitochondria. In all 31 cleistothecia the mitochondrial inheritance was strictly uniparental.

Table 5 shows which parent was the maternal parent in the total of the reciprocal crosses (for

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example $701 \times 705/5 + 701/5 \times 705$). All seven isolates tested (701, 704, 705, 711, 713, 715 and 723) could function as both maternal and paternal parent in outcrosses. The three crosses between isolates 701, 704 and 723 had only one of the parental strains as maternal parent. In the other 9 crosses all strains functioned as both maternal and paternal parent.

Of the 25 crosses between isolates 701, 704 and 723, 23 had the /5 strain as maternal parent and only two the wild-type strain. This gives the impression that there is preference for the inheritance of the /5 mitochondria when in fact there is a preference for maternal parent. If we neglect the results from crosses between isolates 701, 704 and 723 from the results in Table 5, because in crosses between these isolates there appears to be a preference for maternal parent, then there are 29 cleistothecia with the wild type strain as maternal parent and 39 cleistothecia with the /5 strain as maternal parent. A χ^2 test establishes that in these nine crosses we do not have to reject the hypothesis that there is no preference for inheritance of the chloramphenicol resistance mutation above inheritance of the wild-type mitochondria $\chi^2 = 1{\cdot}5$, D.F. $= 1$, $0{\cdot}2 < P < 0{\cdot}3$).

From these results we conclude that in crosses of *A. nidulans* all isolates can function as both maternal and paternal parent. However, in some crosses, notably between isolates 701, 704 and 723, there is a preference for which isolate functions as maternal parent. Also these results supply additional evidence to the results from the $/4 \times /5$ crosses that mitochondrial inheritance in *A. nidulans* is always strictly uniparental.

4. Discussion

To our knowledge the only previous large scale investigation into mitochondrial restriction fragment length polymorphism (RFLP's) within a fungal species was carried out with *N. crassa* (Taylor *et al.* 1986). Twenty *N. crassa* isolates could be divided into nine groups by comparing their *EcoR* I mitochondrial restriction pattern. This is in marked contrast with our own investigation of the mitochondrial restriction pattern of 54 isolates of *A. nidulans* which revealed no RFLP's. The lack of mitochondrial RFLP's and nuclear polymorphism (Geiser *et al.* 1994) within the *A. nidulans* population indicates that all *A. nidulans* isolates share a recent ancestor.

To investigate mitochondrial inheritance we have to be able to differentiate between maternal and paternal mitochondria. Because no natural mitochondrial genetic markers (RFLP's) could be found mitochondrial antibiotic resistance mutations were introduced into *A. nidulans* by protoplast fusion. The advantage of using resistance markers above RFLP's to follow mitochondrial inheritance is that very large numbers of progeny can be screened for presence or absence of the marker. If one ascospore in a cleistothecium has a paternal resistance marker then this will be observed on the selection plates. Therefore, we can be absolutely certain that no paternal inheritance of mitochondria took place in our experiments. Consequently mitochondrial antibiotic resistance mutations are a much more powerful tool than molecular markers for investigating mitochondrial inheritance.

In fungi the only examples of paternal inheritance of mitochondria have been described in *Neurospora crassa* and *N. intermedia* (May & Taylor, 1989; Yang & Griffiths, 1993). In two out of four crosses of *N. intermedia* and three out of six crosses of *N. crassa* paternal transmission of mitochondrial plasmids was observed (Yang & Griffiths, 1993). Eighty out of 4303 ascospores were found to contain paternal mitochondria. Analysis of mitochondrial restriction patterns showed that the mitochondrial genomes were recombined. In *A. nidulans* uniparental inheritance was first observed by Rowlands and Taylor (1973) while performing crosses between two mutants of the Glasgow isolate to determine whether a mutation was nuclear or cytoplasmic. In our research several thousand ascospores from each of 111 hybrid cleistothecia from 26 different crosses involving seven different heterokaryon incompatible isolates were tested for presence of the paternal mitochondrial marker. An investigation of this size into the paternal inheritance of mitochondria in fungi has never been reported before. Not one incident of paternal inheritance was observed.

Uniparental inheritance can be achieved by two mechanisms. If the dikaryon from which the ascospores are formed contains both maternal and paternal mitochondria uniparental inheritance can be achieved by active degradation of one mitochondrial type. Active degradation of plastids has been observed for mitochondria in the myxomycete *Physarum polycephalum* (Meland *et al.* 1991) and for chloroplasts in *Chlamydomonas* (Kuroiwa, 1985). Alternatively uniparental inheritance can be accomplished by a fertilization mechanism whereby paternal mitochondria are excluded from the dikaryon. Complete absence of the paternal mitochondrial marker from all the ascospores in a cleistothecium demonstrates that the dikaryon from which the ascospores are formed does not contain both maternal and paternal mitochondria for any length of time. This means that the regulation of uniparental inheritance in *A. nidulans* by active degradation is extremely unlikely. The complete absence of the paternal mitochondrial marker in ascospores strongly supports the mechanism of exclusion of paternal mitochondria. Consequently this means that sexual reproduction in *A. nidulans* is initiated by introduction of a paternal nucleus into a maternal structure.

There is a potential conflict between nuclear and mitochondrial genes in the question of mitochondrial inheritance. Nuclear genes wish to prevent the spread

of selfish mitochondria by enforcing uniparental inheritance and mitochondrial genes wish to stimulate their own spread by inheriting biparentaly (Grun, 1976; Cosmides & Tooby, 1981; Hoekstra, 1990). In a predominantly inbreeding organism the spread of mitochondria is not deterred by uniparental inheritance or enhanced by biparental inheritance as most progeny effectively only have one parent. The observation of inactive mating types genes in homothallic *Neurospora* species (Beatty *et al.* 1994; Glass & Smith, 1994) indicates that homothallic *Neurospora* evolved from heterothallic *Neurospora* by inactivation of the mating type genes. The possibility of evolution from heterothallism to homothallism has been demonstrated in a model by Nauta & Hoekstra (1992). If the nuclear/mitochondrial conflict was won by the nucleus in the heterothallic fungus then in the homothallic fungus enforcement of uniparental inheritance could be relaxed resulting in biparental inheritance (Hurst & Hamilton, 1992). If the nuclear/mitochondrial conflict was won by the mitochondria in the heterothallic fungus then in the homothallic fungus mitochondrial resistance to uniparental inheritance could be relaxed resulting in uniparental inheritance (Reboud & Zeyl, 1994; Hurst, 1994). Although paternal inheritance of mitochondria has been observed in heterothallic *Neurospora* (May & Taylor, 1989; Yang & Griffiths, 1993) mitochondrial inheritance in *Neurospora* was found to be predominantly uniparental. This is evidence for the nuclear regulation of uniparental mitochondrial inheritance in heterothallic fungi. Our results show that *A. nidulans* has not adapted to homothallism by relaxing uniparental inheritance of mitochondria. On the contrary, we have demonstrated that mitochondrial inheritance in *A. nidulans* is always absolutely uniparental.

There are several possible explanations for the maintenance of uniparental inheritance in a homothallic fungus. Perhaps the assumption that maintenance is costly is incorrect. However, it would seem reasonable to assume that such strict maintenance of any mechanism must involve some form of energetic input. Perhaps *A. nidulans* has not had enough time to adapt to homothallism by relaxing uniparental inheritance. The lack of variation in the nuclear and mitochondrial genomes of *A. nidulans* (Geiser *et al.* 1994, this paper) is evidence that all *A. nidulans* isolates share a recent ancestor. Alternatively it may be that despite being a homothallic primarily selfing organism enforcement of uniparental inheritance is the best strategy should an individual happen to have a rare outbred mating.

Strains containing mitochondria with the oligomycin resistance mutation were maternal parent significantly more often than strains containing mitochondria with the chloramphenicol resistance mutation (Table 2). Strains containing mitochondria with the oligomycin resistance mutation also grew significantly slower than strains containing the wild type mitochondria or mitochondria with the chloramphenicol resistance mutation (Table 3). Impaired growth as a result of the mitochondrial oligomycin resistance was first observed by Rowlands & Turner (1973). Apparently mitochondria with the oligomycin resistance mutation are inhibited in their energy production. This is consistent with the fact that the oligomycin resistance is a mutation of an ATPase subunit (MacLannan & Tzagoloff, 1968). From these results we can conclude that in *A. nidulans* the energetic investment of the maternal parent in sexual reproduction is significantly larger then that of the paternal parent. A strain with limited energy production will primarily invest in asexual conidiospores rather than sexual ascospores.

To eliminate the influence of the mitochondrial oligomycin resistance mutation on determining which parent is maternal parent crosses between wild-type and /5 strains were performed. The object of these crosses was to establish whether in crosses only one strain could function as maternal parent as reported by Rowlands and Turner (1976). This is interesting because preference for maternal parent will influence the spread of cytoplasmic genetic material in a population. All seven isolates used in our experiments could function as both maternal and paternal parent in outcrosses. In nine of the 12 crosses examined both parents did function as both maternal and paternal parent, contrary to the findings of Rowlands & Turner (1976). In crosses between isolates 701, 704 and 723 only one isolate functioned as maternal parent. The relationship between these three isolates is unclear. Isolate 704 was maternal parent to isolate 701, isolate 701 was maternal parent to isolate 723 and isolate 723 was maternal parent to isolate 704. The preference for maternal parent in crosses of isolates 701, 704 and 723 is the subject of further research.

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