Further Studies on Protoplast Fusion and Interspecific Hybridization within the *Aspergillus nidulans* Group

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Hybridization of eight species of the *Aspergillus nidulans* group was attempted using auxotrophic mutants and protoplast fusion methods. Viable fusion products were obtained from eight crosses. Allodiploid hybrids were recovered from crosses involving *A. nidulans* with *A. rugulosus*, *A. quadrilineatus*, *A. nidulans* var. *echinulatus* and *A. violaceus*, although some mutants only gave heterokaryons. Crosses involving these latter species also gave heterokaryons. Crosses between *A. nidulans* and *A. unguis*, *A. stellatus* and *A. heterothallicus* were unsuccessful. Fusions involving three parents gave heterokaryons made up of only two of them.

INTRODUCTION

Protoplast fusion techniques, first described by Anné & Peberdy (1975, 1976) and Ferenczy *et al.* (1975*b*, 1976) in fungi, have proved to be a valuable method for interspecific hybridization. So far only two genera of filamentous fungi have been studied in detail using these techniques. In the penicillia both closely related species (Anné & Peberdy, 1976; Peberdy *et al.*, 1977; Anné & Eyssen, 1978) and taxonomically diverse species (Anné *et al.*, 1976) could be hybridized. The patterns of hybridization and subsequent segregation resemble a normal parasexual cycle (Pontecorvo *et al.*, 1953). In the aspergilli, when taxonomically distantly related species were hybridized (Ferenczy, 1976; Ferenczy *et al.*, 1977), unstable complementation products were recovered at very low frequencies. There was no evidence of a regular parasexual cycle. Fusion of protoplasts of the closely related *A. nidulans* and *A. rugulosus* (Raper & Fennell, 1965) resulted in interspecific heterokaryons as primary fusion products. These heterokaryons may lead to hybrid formation following nuclear fusion, as we reported earlier (Kevei & Peberdy, 1977, 1979). Protoplast fusion was successfully applied in producing heterokaryons between vegetatively incompatible strains of *A. nidulans* in which the incompatibility factors prevent hybridization via anastomosis (Dales & Croft, 1977; Croft & Jinks, 1977). This finding has allowed the investigation of the genetic basis of the incompatibility system (Dales, 1978; Dales & Croft, 1980). Similarly, successful protoplast fusion experiments have been carried out between *A. nidulans* and *A. quadrilineatus* (Dales & Croft, 1980; Croft *et al.*, 1980) and between *A. nidulans* and *A. nidulans* var. *echinulatus* (Croft, 1985). In the latter cross, mitochondrial transfer and the recombination of mitochondrial DNA have also been studied. Interspecific hybrids exhibited the same phenotype as diploids of incompatible *A. nidulans* strains. In all these experiments, auxotrophs were used leading to the recovery of balanced heterokaryons under selection pressure. Recently a new selection method has been suggested to select for products of interspecific hybridization, using various antifungal agents as post-fusion selective agents (R. E. Bradshaw & J. F. Peberdy, unpublished data).

Here we report the results of attempted protoplast fusions in all possible crosses amongst eight species from *A. nidulans* group, which is comprised of 18 species and several varieties (Raper & Fennell, 1965). The species studied were as follows: *A. nidulans* (N), *A. rugulosus* (R), *A.*

quadrilineatus (Q), *A. nidulans* var. *echinulatus* (E), *A. violaceus* (V), *A. unguis* (U), *A. stellatus* (S), and *A. heterothallicus* (H). In our previous experiments (Kevei & Peberdy, 1977), hybrid formation was not observed in every cross between N + R, therefore our aim was to utilize various auxotrophs to carry out more crosses in certain species-pairing combinations to eliminate the possible unfavourable consequence of mutagenic treatment. The fact that mutation can modify the *het* gene expression was clearly demonstrated by Croft (1985) in the intraspecific incompatibility system.

METHODS

Organisms and growth conditions. The origin and the genotypes of the *Aspergillus* species used in these experiments are listed in Table 1. Cultures were grown and maintained on complete medium (CM) containing (g l⁻¹): yeast extract (Oxoid), 5.0; malt extract, 5.0; glucose, 5.0; agar 15.0. Minimal medium (MM) according to Pontecorvo *et al.* (1953) was used for selection of fusion products. In protoplast regeneration and fusion experiments both media were supplemented with osmotic stabilizer, 0.7 M-KCl, and designated CMS and MMS respectively.

Trichoderma harzianum CBS-345-33 obtained from Professor J. G. H. Wessels (University of Groningen, The Netherlands) and SZMC 0592 (Szeged Microbial Collection, Dept of Microbiology, University of Szeged) were used for lytic-enzyme production. All incubations were at 30 °C.

Isolation of auxotrophic mutants. Conidia were harvested from 5–7 d cultures and irradiated by UV light (Philips TUV 15 W; intensity 45 µJ mm⁻² from 50 cm) or treated by ethyl methanesulphonate (EMS) at 30 g l⁻¹, or *N*-methyl-*N*'-nitro-*N*-nitrosoguanidine (NTG) 250 µl ml⁻¹, respectively, to achieve 2–3% viability. The filtration enrichment technique (Catcheside, 1954) was used to increase the frequency of mutant isolation.

Protoplast isolation, fusion and regeneration. The protoplast methodology applied in these experiments has been described previously (Ferenczy *et al.*, 1975a; Kevei & Peberdy, 1977). For protoplast isolation, cultures were grown on cellophane sheets for 14–22 h. Cellophane sheets were inoculated with a multipoint inoculator (developed for this purpose) resulting in 50 colonies per 9 cm diameter Petri dish. KCl, 0.7 M, was used as osmotic stabilizer without buffer, except with *A. stellatus*, where 0.8–0.9 M KCl was used.

Helix gastric juice (Dept of Microbiology, University of Szeged) at 15 mg ml⁻¹ was used regularly as lytic enzyme. In several cases it was combined with *Trichoderma* culture filtrate produced by a slight modification of the method of Peberdy & Isaac (1976). Fresh *Aspergillus* mycelium from a 48 h culture was homogenized by a Braun homogenizer (Cell Homogenizer MSK, B. Braun, Melsungen, FRG) and then washed several times. The residue of the homogenate (20 g l⁻¹), supplemented with chitin (Sigma; 2.5 g l⁻¹), served as carbon source for enzyme induction. After 4 d cultivation at 30 °C, the *Trichoderma* culture filtrate was harvested by centrifugation at 220 r.p.m., evaporated to a fivefold volume reduction and stored at –30 °C. Protoplast fusion was performed using polyethylene glycol 4000 (PEG) at 300 g l⁻¹, in the presence of 0.1 M-CaCl₂. Fusion frequency was calculated as the ratio of colonies growing on MMS and CMS.

Nuclear staining. Protoplasts were fixed in glutaraldehyde solution (40 g l⁻¹) in 0.8 M-mannitol for 1 h, and stained by Chromomycin A3 (Sigma), as described by Slater (1976).

Induced segregation of hybrids and characterization of segregants. The haploidization and analysis of segregants was performed as described earlier (Kevei & Peberdy, 1979). Small discs of mycelium, 2 mm diameter, were placed onto CM containing 1.0–1.05 µg benomyl ml⁻¹ (Chinoin, Budapest, Hungary). After 7–10 d incubation, one sector from each colony was randomly isolated. After subsequent purification and ploidy determination (Upshall *et al.*, 1977), segregants were assembled onto master plates and replicated onto MM supplemented appropriately for phenotypic characterization.

RESULTS

Two-parent crosses

Crosses were set up so that each species was fused with all the other members of the group. Altogether 28 main pairing combinations were involved. At least two auxotrophic mutants from each species were utilized in the fusion experiments, except for *A. nidulans* var. *echinulatus*, and each species-pairing combination represents the results of at least four independent crosses. Where complementation occurred, heterokaryons and 'hybrids', in some instances, were obtained from the crosses N + R, N + Q, N + E and N + V. From the crosses R + Q, R + E, R + V and V + E, only heterokaryons were recovered. The 'hybrids' arose as a consequence of nuclear fusion and the combination of the two genomes (Kevei & Peberdy, 1977).

Fusion colonies were not obtained from crosses involving *A. unguis*, *A. stellatus* and *A.*

Table 1. *Strains used in hybridization experiments*

Species	Symbol	Strain no.*	Genotype†	Origin‡
<i>A. nidulans</i>	N1	FGSC 407	<i>adE20 biA1; AcrA1; phenA2; pyroA4; lysB5; lacA1; choA1; riboB2 chaA1</i>	Dr A. Upshall
	N2	MSE	<i>suA1adE20 yA2 adE20; wA3; galA1; pyroA4; facA303; sB3; nicB8; riboB2</i>	Prof. J. A. Roper
	N3	1101	<i>paba-1; y; pro-1; ts6</i>	Dr R. F. Rosenberger [induced (UV) in <i>A. nidulans</i> R-21]
	N4	0442	<i>paba-1; y; met-1; ts6</i>	
<i>A. rugulosus</i>	R1	1093	<i>met-1</i>	Induced (UV or EMS*) in <i>A. rugulosus</i> wild-type CMI 91020a
	R2	1248*	<i>met-2</i>	
	R3	1249	<i>met-3</i>	
	R4	1250	<i>ribo-1</i>	
	R5	1095	<i>nic-1</i>	
	R6	1283*	<i>nic-2</i>	
	R7	1094	<i>pro-1</i>	
	R8	1253	<i>pyro-1</i>	
	R9	1247*	<i>ad-1</i>	
<i>A. quadrilineatus</i>	Q1	1312	<i>ad-1</i>	Dr J. H. Croft [induced (UV) in <i>A. quadrilineatus</i> wild-type no. 12]
	Q2	1313	<i>ad-2</i>	
	Q3	1301	<i>met-1; com</i>	
	Q4	1314	<i>met-2</i>	
	Q5	1302	<i>ribo-1</i>	
	Q6	1311	<i>ribo-1</i>	
	Q7	1315	<i>ribo-3</i>	
<i>A. nidulans</i> var. <i>echinulatus</i>	E	25-30	<i>y-25.2; pyro-25.1</i>	Dr J. H. Croft
<i>A. violaceus</i>	V1	1304	<i>met-1</i>	Induced (UV or NTG*) in <i>A. violaceus</i> wild-type CMI 61449
	V2	1305	<i>ino-1</i>	
	V3	1337*	<i>ino-2</i>	
	V4	1316	<i>ade-1</i>	
	V5	1338*	<i>ade-2</i>	
	V6	1339*	<i>ade-3</i>	
	V7	1340*	<i>lys-1</i>	
<i>A. unguis</i>	U1	1297	<i>arg-1</i>	Induced (UV) in <i>A. unguis</i> wild-type 136767
	U2	1298	<i>ino-1</i>	
	U3	1296	<i>met-1</i>	
<i>A. stellatus</i>	S1	1242*	<i>ad-6; whi</i>	Induced (UV or EMS*) in <i>A. stellatus</i> wild-type CMI 60316
	S2	1228	<i>ad-1</i>	
	S3	1229	<i>met-1</i>	
	S4	1232	<i>met-3</i>	
<i>A. heterothallicus</i>	H1	1306	<i>met-1</i>	Induced (UV) in <i>A. heterothallicus</i> wild- type CMI 136767
	H2	1307	<i>ribo-1</i>	

* FGSC, Fungal Genetic Stock Center, Humboldt State University Foundation, Arcata, California, USA; MSE, Master strain E. (McCulley & Forbes, 1965). Other numbers derived from the Collection of the Department of Microbiology, University of Szeged (SZMC).

† Gene symbols used according to the suggestion of Clutterbuck (1973).

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heterothallicus, either in crosses with each other or in pairings with other members of the species group. No fusion products were recovered from the crosses of Q + E and Q + V, despite the fact that these strains could be successfully hybridized in other species combinations.

Detailed results of crosses between *A. nidulans* and *A. rugulosus* strains are shown in Table 2. More crosses were carried out than previously reported (Kevei & Peberdy, 1977). In a few cases, pairings failed to give fusion products, but this was probably due to allelism in the strains used,

Table 2. *Consequences of protoplast fusion crosses between A. nidulans and related species*

	N1			N2			N3			N4		
	F%	Het	Hyb	F%	Het	Hyb	F%	Het	Hyb	F%	Het	Hyb
R1	2.75	+	M	1.51	(+)	L	2.05	+	M	2.28	+	L
R2	2.07	+	L	0.89	X	M	2.14	+	—	*	—	—
R3	2.23	X	H	1.68	non	—	2.20	non	—	4.33	+	L
R4	2.55	+	L	1.53	+	L	2.71	X	H	1.32	+	H
R5	2.05	+	N	1.15	(+)	—	3.28	+	L	1.36	+	L
R6	2.05	+	L	2.64	+	L	1.21	+	L	0.97	+	L
R7	3.33	+	M	1.03	(+)	L	1.18	X	L	1.04	(+)	—
R8	*	—	—	•	—	—	2.76	+	M	1.23	+	L
R9	*	—	—	0.08	(+)	—	1.83	+	L	1.89	+	—
Q1	0.009	B	—				*	—	—			
Q2	0.69	+	M				0.83	+	—			
Q3	1.17	(+)	—				1.21	non	—			
Q4	0.87	+	L				1.02	+	M			
Q5	0.75	+	M				1.34	+	L			
Q6	1.09	B	—				1.24	B	—			
Q7	*	—	—				0.96	non	—			
E				0.12	X	L	0.28	(+)	L	0.17	(+)	L
V1	8.1×10^{-3}	(+)	L				*	—	—			
V2	NT						12.5×10^{-4}	(+)	L			
V3	5.5×10^{-4}	non	—				11.2×10^{-4}	(+)	L			
V4	2.5×10^{-4}	non	—				4.8×10^{-4}	non	—			
V5	*	—	—				*	—	—			
V6	*	—	—				*	—	—			
V7	*	—	—				*	—	—			

Key:

NT, Not tested.

F%, fusion frequency; *, no complementation.

Het, heterokaryons recovered from fusion products: +, can be maintained on MM with varying ability for hybrid formation; (+), very weak growing; X, all isolated fusion colonies gave rise to hybrids directly; non, fusion product cannot be cultured.

Hyb, hybrid formation: —, no formation; L, low frequency; H, high frequency; M, moderate frequency; B, isolated heterokaryons developed into brown hybrid-like colonies but more slowly growing and remained unstable on CM.

e.g. crosses N4 + R2, N1 + R8 and N1 + R9, where the markers *met*; *pyro*; *ad*, common to both parental strains, did not complement each other. Fusion frequency was relatively high, mostly around 1–2%, and only one cross showed a lower value of one order of magnitude. In this particular cross the N2 strain possessed a suppressor mutation *suA1 ade20* on *ade20* which may interact with *ad-1* marker on the R9 strain.

In crosses where complementation occurred, the first colonies isolated from the fusion plates were heterokaryons. They exhibited a great variation in growth on MM and in hybrid development. The heterokaryons could be cultured, in most cases, by frequent subculture of young mycelium. Exceptions to this situation were crosses such as N1 + R3, N2 + R3, N2 + R2, N3 + R4 and N3 + R7, where practically all colonies isolated from fusion plates developed immediately into hybrids.

Other pairing combinations, e.g. N2 + R3 and N3 + R3, showed a different character. The fusion colonies could not be maintained on MM even though a reasonably high complementation frequency was observed in repeated experiments. Mycelia from young fusion plates transferred to CM gave rise to parental segregants. These heterokaryons may have been unbalanced and failed to achieve the required nuclear ratio for stabilization. An interesting comparison can be made between the three crosses N3 + R1, N3 + R2 and N3 + R3, where the same *A. nidulans* mutant was crossed with *A. rugulosus* strains bearing *ad* mutations from different complementation groups. Each of these crosses represents a different level of compatibility and a different ability for hybrid formation. In a few crosses, e.g. N3 + R2 and

N4 + R7, the heterokaryons obtained could be maintained indefinitely, by selection, but nuclear fusion leading to hybrid formation was never observed. Of all the strains of *A. nidulans* and *A. rugulosus* used in crosses, N2 and R3 proved to be the least effective in hybrid formation.

Hybridization between *A. nidulans* and *A. quadrilineatus* (Table 2) showed similar phenomena to the N + R crosses. The fusion frequency was slightly lower; the average value was about 1%. This fact does not reflect a lower hybridization ability than with N + R, it is rather a consequence of technical problems, e.g. protoplast isolation, the nature of the protoplast population and how the fusion was performed. When fusion frequency is consistently lower by at least one order of magnitude, this may reflect real compatibility differences. There was one instance where complementation occurred with very low frequency in a N1 + Q1 cross. The *ad-1* mutation of Q1 was complemented only weakly by *adE20* in the N1 parent. There is no explanation why N2 + Q1 did not give fusion products. In comparison with the N + R crosses, the limited number of pairings that resulted in stable hybrid colonies might be due to certain characteristics of the particular *A. quadrilineatus* mutations. Thus Q3 possesses restricted colony morphology with very slow growth; Q5, Q6 and Q7 are *ribo* dependent, the latter is possibly allelic with a *ribo* gene of N1. Each riboflavin-requiring *A. quadrilineatus* strain showed a different ability for hybrid formation.

In three crosses, N1 + Q1 and particularly N1 + Q6 and N3 + Q6, heterokaryons subcultured on MM produced a brown pigment, but hyphal branching did not become so regular as in hybrids, and their growth rates were lower. These colonies exhibited a heterokaryotic nature at the early period of their existence. However, a transient diploid probably developed because during subsequent cultivation they became aneuploid, losing the majority of one of the parental genomes. Mostly *A. quadrilineatus* parents were recovered from these colonies, labelled 'B' in Table 2.

Hybridization between *A. nidulans* and *A. nidulans* var. *echinulatus* was studied in less detail (Table 2) and only one mutant of the latter was used. Complementation frequency showed markedly lower values than observed in previous pairings. Each cross gave fusion products which ensured hybrid formation at a low rate in all cases. The general observation was that the interspecific heterokaryons could be cultured for a short period only. In the N2 + E cross all the fusion colonies transferred to MM gave hybrids very readily, but in the other two cases heterokaryons grew poorly and those which did not form hybrids after 10–14 d had not survived.

Hybridization between *A. nidulans* and *A. violaceus* gave fusion colonies in less than half of the pairings attempted, and at the lowest frequency of the crosses studied (Table 2). Only three combinations resulted in the formation of hybrid type colonies. This may have been due to possible allelism: V4, V5 and V6 carry *ad* mutations belonging to various complementation groups, N1 has an *ad* requirement also. N1 + V4 complemented one another, but N1 + V5, and N1 + V6 did not. This observation does not explain the absence of complementation between these particular V mutants and N3, nor why N1 + V7 and N3 + V7 crosses were not successful. Heterokaryons from the crosses N1 + V1, N3 + V2, N3 + V3 always gave hybrids. Those cultures which did not develop into hybrids failed to survive similarly to N + E. In the N1 + V3, N1 + V4, and N3 + V4 combinations the primary fusion products could not be maintained even though these colonies showed a heterokaryotic nature, an identical situation to that found in certain N + R combinations. Hybrids derived from N + V crosses exhibited a basically similar phenotype to N + R hybrids, but their stability, growth rate and viability was noticeably reduced.

Table 3 shows the results of crosses amongst those species which gave hybrids when paired with *A. nidulans*. Fusions of all possible combinations involving *A. rugulosus*, with *A. quadrilineatus*, *A. nidulans* var. *echinulatus* and *A. violaceus* were successful except for Q + E, Q + V (Table 2). In a few crosses, e.g. R1 + Q3, R4 + Q5 and R1 + V1, the absence of complementation might be due to allelism where common *met* and *ribo* mutations were involved. Heterokaryons were recovered from the successful crosses and in most cases could be maintained in culture. Heterokaryons recovered from R + V and E + V were the most vigorous, R + Q heterokaryons were less so, and those from R + E usually failed to grow after subculture. There was no correlation of fusion frequency with the vigour of the fusion products.

Table 3. *Consequences of protoplast fusion crosses involving A. rugulosus and between A. nidulans var. echinulatus and A. violaceus*

	R1			R4			R6			R7		
	F%	Het	Hyb	F%	Het	Hyb	F%	Het	Hyb	F%	Het	Hyb
Q2	3.0×10^{-2}	(+)	—	1.0×10^{-2}	(+)	—						
Q5	1.0×10^{-2}	(+)	—	*	—	—						
Q3	*	—	—		NT							
V1	*	—	—	8.0×10^{-4}	(+)	—						
V2	12.0×10^{-4}	(+)	—	9.0×10^{-4}	(+)	—						
E	19.0×10^{-4}	non	—	28.0×10^{-4}	non	—	36.0×10^{-4}	non	—	16.0×10^{-4}	non	—
	V1			V2			V5					
	F%	Het	Hyb	F%	Het	Hyb	F%	Het	Hyb			
E	15.0×10^{-4}	(+)	—	12.0×10^{-4}	(+)	—	7.0×10^{-4}	(+)	—			

For key to symbols, see Table 2.

Three-parent crosses

Fusion crosses between three species of the *A. nidulans* group were carried out (i) to investigate the possibility of tripartite hybridization and the subsequent segregation that might ensue, and (ii) to assess the possibility of obtaining hybridization of an 'incompatible' species in combination with a compatible pair.

In the first instance, crosses involving totally compatible species, N + R + Q and N + R + V, were investigated. Heterokaryons were obtained and proved to be biparental at the first subculture. The N + R + Q fusion gave predominantly N + Q heterokaryons, N + R types being found only rarely; both heterokaryons gave hybrids. The fusion of N + R + V produced heterokaryons with N and R genomes which also gave hybrids, and the N + V combination was detected extremely rarely. R + Q or R + V combinations were never observed.

In the second experiment, *A. stellatus* was used as the 'incompatible' species in N + Q + S and N + R + S fusion mixtures. Heterokaryons were isolated and proved to be N + Q and N + R, respectively, and gave hybrids after prolonged culture. Clearly the genome of *A. stellatus* cannot be introduced in this manner.

Influence of the nuclear ratio of the heterokaryon on hybridization

The effect of the heterokaryon nuclear ratio on the frequency of hybridization or the success of hybridization was investigated. The nuclear content of protoplasts was determined following staining with chromomycin. The average nuclear content of different strains studied ranged ($\pm$ SE) from 1.50 ± 0.18 to 4.5 ± 0.62 (100 protoplasts were counted in each case). The nuclear content of protoplasts appeared to depend on the nature of the particular mutant strains, the age of mycelia or the methodology of protoplast isolation, rather than on the original characteristics of wild-type parental species. Differences in average numbers of nuclei of protoplasts might result in an alteration in nuclear ratio from the presumed ideal 1:1 after mixing equal volumes of protoplast populations with similar density. In certain cases, when fused protoplasts failed to give viable fusion products in the usual 1:1 ratio, e.g. in N + H, N + S and N + U combinations, further experiments were carried out where the ratios were changed to 1:2, 1:5 and 1:10. Hybridization was not obtained in these cases. Where compatible strains were used there were no significant changes in the fusion frequency when the ratio of protoplasts was altered from 1:1.

Segregation in the hybrids

To confirm the allodiploid character of interspecific hybrids, induced-haploidization experiments were carried out. Disregarding the detailed haploidization patterns, the results can be summarized as follows. All hybrids derived from various species pairings behaved similarly. Besides the parental types, a series of non-parental segregants were isolated. Non-parental can

be classified as stable haploid or unstable aneuploid isolates. Haploid non-parental types showed various combinations of parental genetical markers and parental morphology. When one of the partners was a multimarked strain, the hybrids generated extensive variations of the parental markers. Back-crossing the non-parentals parasexually with the parent strains showed differing abilities for hybrid formation. All types of hybrids gave segregants in ageing colonies without induction. Small clusters of segregant-type conidia formed mainly in the central part of the colony and showed haploid parental or non-parental characters.

DISCUSSION

A total of 28 possible variations of protoplast fusion crosses were carried out amongst eight closely related *Aspergillus* species; eight gave fusion products (Fig. 1). The primary fusion colonies proved to be interspecific heterokaryons. Prolonged cultivation of these heterokaryons under selective conditions, in some cases, resulted in interspecific hybrid formation as a consequence of somatic nuclear fusion. The haploidization experiments confirmed that the hybrids were the phenotypic expression of a nucleus with both parental nuclear genomes and so corresponding to an allodiploid (Kevei & Peberdy, 1985; Croft, 1985).

N + R, N + Q, N + E and N + V combinations resulted in such allodiploids following heterokaryosis. From the analysis of complementation patterns it would appear that *A. nidulans*, *A. rugulosus*, *A. quadrilineatus* and *A. nidulans* var. *echinulatus* form a close association; *A. violaceus* also belongs to this sub-group but the boundaries of this species appear to be more pronounced. *A. heterothallicus*, *A. stellatus* and *A. unguis* are incompatible with each other and other species; possibly their genomes are greatly different from other members of the species group, especially those that can be hybridized, which may have extensive homology. N + R, N + Q and N + E showed similar complementation ability, fusion frequencies were around 1% or slightly lower in N + E. There is no explanation at this stage why the other crosses amongst these species did not form allodiploids following heterokaryosis, e.g. R + Q and R + E, or why Q + E did not produce any fusion product.

The N + V pairing gave heterokaryons with the lowest complementation frequency, and hybrid formation was rare. In contrast, R + V and E + V heterokaryons were formed at a low frequency but showed more pronounced viability than R + Q or R + E heterokaryons. Some fusion combinations failed to give fusion products, possibly due to the allelism between parental mutants (Tables 2 and 3). Alterations of complementation ability from the average value within a species pairing combination are probably due to the modification of *het* gene expression in consequence of mutagenic treatment.

In conclusion, where hybrids can be formed between species of the *A. nidulans* group, they share the same characteristics as those described previously (Kevei & Peberdy, 1977, 1979;

Fig. 1. Synopsis of hybridization crosses. Heavy lines indicate the formation of allodiploid hybrids, double lines indicate heterokaryon formation, and single lines indicate unsuccessful crosses.

Dales & Croft, 1980; Bradshaw *et al.*, 1983). Only N + V hybrids appear different, exhibiting a noticeably reduced viability.

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