Sexual Diploids of *Aspergillus nidulans* Do Not Form by Random Fusion of Nuclei in the Heterokaryon

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ABSTRACT

The sexual stage of *Aspergillus* (*Emericella*) *nidulans* consists of cleistothecia containing asci, each with eight ascospores. The fungus completes the sexual cycle in a homokaryotic or a heterokaryotic mycelium, respectively. The common assumption for the last 50 years was that different nuclear types are not distinguishable when sexual development is initiated. When cultured on a medium limited for glucose supplemented with 2% sorbitol, sexual development of *A. nidulans* is slowed and intact tetrads can be isolated. Through tetrad analysis we found that unlike haploid nuclei fuse preferentially to the prezygotic diploid nucleus. When heterokaryons are formed between nuclei of different genetic backgrounds, then recombinant asci derived from opposite nuclei are formed exclusively. Strains in the same heterokaryon compatibility group with moderate differences in their genetic backgrounds can discriminate between the nuclei of a heterokaryon and preferentially form a hybrid diploid nucleus, resulting in 85% recombinant tetrads. *A. nidulans* strains that differ at only a single genetic marker fuse the haploid nuclei at random for formation of diploid nuclei during meiosis. These results argue for a genetically determined "relative heterothallism" of nuclear recognition within a heterokaryon and a specific recruitment of different nuclei for karyogamy when available.

THE filamentous fungus *Aspergillus* (*Emericella*) *nidulans* is a model organism for several differentiation processes including asexual and sexual development. The genetic mechanisms controlling the formation of conidiophores, the asexual reproductive organs, have been studied in detail (reviewed in Adams *et al.* 1998). This process terminates in the production of asexual spores, the uninucleate conidia.

The sexual cycle of *A. nidulans* begins with the formation of dikaryotic hyphae by fusion of ascogonia- and antheridia-like structures (Champe *et al.* 1994). This fusion event is strictly regulated by mating types in heterothallic species such as *Neurospora crassa* or *Podospora anserina* (Raju and Perkins 1994; Coppin *et al.* 1997). The mechanism by which formation of dikaryotic hyphae is regulated in *A. nidulans* is unknown. The two nuclei of the dikaryotic cell divide synchronously (Pontecorvo *et al.* 1953). The ascogenous hyphae branch and karyogamy occurs in the penultimate crozier cells of the branches to produce diploid meiocytes, immediately followed by meiosis. After meiosis, the four meiotic products (tetrad) undergo an additional mitotic division to yield eight haploid nuclei, which differentiate into an octad of eight ascospores. All of the asci in a cleistothecium are assumed to descend from the nuclei of a single dikaryotic mothercell (Cove 1977). Therefore, all ascospores from the same cleistothecium are regarded as progeny from one dikaryotic hyphal fusion event. Asci of *A. nidulans* are spherical, and the uncovered tetrads are complete, but unordered. Mature asci are unstable, and rearray of intact asci with fertile ascospores could be done only with difficulty from slightly immature cleistothecia (Strickland 1958).

There are two distinct mechanisms for the recombination of genetic information in *A. nidulans*. One is the parasexual cycle in which haploid nuclei can fuse vegetatively to diploids at a low frequency (Pontecorvo *et al.* 1953) and new genotypes can arise via mitotic recombination. Heterokaryon formation in *A. nidulans* is restricted by a series of at least eight *het* genes (Dales and Croft 1990; Anwar *et al.* 1993). Compatible strains, forming an h-c group, carry the same set of alleles at all of these loci and are usually quite similar, if not identical, in their genetic background (Croft and Jinks 1977; Esser and Blaich 1994). *Aspergillus nidulans* wild isolates of the Birmingham strain collection were classified into 19 h-c groups. The A4 derivatives (Pontecorvo *et al.* 1953) used in most laboratories are known as Glasgow strains and form the 20th group (Anwar *et al.* 1993; Geiser *et al.* 1994). Formation of a viable heterokaryon between members of different h-c groups usually requires strong selection or protoplast fusion (Grindle 1963). Such fused incompatible strains are unaffected in asexual and sexual development, respectively (Butcher 1969).

Meiotic recombination as an alternative mechanism may occur if the heterokaryotic strain differentiates

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TABLE 1

Strains, genotypes, and genetic backgrounds

Strain	Genotype	Genetic background	Reference
A234	*pabaA1 yA2; veA1*	British wild-type strains A4 and M826	Barratt *et al.* (1965); Roberts (1967)
A237	*pabaA1 yA2; trpC801; veA1*	British wild-type strains A4 and M826	Barratt *et al.* (1965); Roberts (1967)
AGB74	*pabaA1 yA2; trpC801; veA1; phleo^R*	British wild-type strains A4 and M826	This work
GR5	*pyrG89; wA3; pyroA4; veA1*	British A4 Glasgow derivative	FGSC
AGB10	*pyrG89; pyroA4*	British A4 Glasgow derivative	This work
R99	Wild type	British Birmingham collection	Anwar *et al.* (1993); Geiser *et al.* (1994)
R99-6	*paba-99.1 y-99.1*	R99 derivative	Anwar *et al.* (1993)
AGB45	*paba-99.1; phleo^R*	R99 derivative	This work
AGB46	*paba-99.1 y-99.1; phleo^R*	R99 derivative	This work

cleistothecia. The dikaryon within the cleistothecium may be composed of the same or different genotypes. Karyogamy between genetically identical nuclei results in selfed diploids (homokaryotic reproduction) while nonidentical nuclei form a heterozygous diploid in which segregation and meiotic recombination can be observed. In a case in which the two nuclear types within the heterokaryon are equal, and if nuclear fusion occurs at random, then 50% of the cleistothecia should contain recombinant tetrads (Pontecorvo *et al.* 1953). For a few heterokaryotic mycelia an increase in the amount of recombinant ascospores was observed. This phenomenon was named "relative heterothallism." A clear genetic determination of this effect seemed to be unlikely for the strains analyzed because of the identification of increased amounts of recombinant ascospores for crossed strains of the same genetic background and a reduced relative heterothallism for crosses using their recombinant progeny (Pontecorvo *et al.* 1953).

We were interested in whether the random use of nuclei for diploid formation can be generalized for crosses of any *A. nidulans* strains and whether the effect of relative heterothallism really is a highly variable process. Therefore, heterokaryotic mycelia were constructed from parental strains of highly divergent genetic backgrounds and strains with stepwise decreased genetic divergences. In each of these strains single meiotic events for each heterokaryon should be analyzed by developing an easy method of tetrad analysis in *A. nidulans.* As a result we found a strong correlation between the increase of divergence and an increase of hybrid fertilization events during sexual development.

MATERIALS AND METHODS

Strains and media: Strains used in this work are listed in Table 1. *A. nidulans* strain GR5 was obtained from G. May (Houston), and strains R99 and R99-6 were obtained from D. M. Geiser (Pennsylvania State University, College Park, PA). Strains A234 and A237 were provided by the Fungal Genetics Stock Center (University of Kansas Medical Center, Kansas City, KS). Cultivation of all *A. nidulans* strains was performed at 30°. Minimal growth medium was used (Bennett and Lasure 1991) except for formation of stable asci.

Strain construction: Strains AGB45 and ABG46 were constructed by crossing R99 and R99-6. Ascospores were selected for a green-spored phenotype auxotroph for *p*-aminobenzoic acid. For additional selection both strains were transformed with plasmid pAN8-1 containing a phleomycin resistance cassette (Punt and van den Hondel 1992). The same plasmid was transformed in strain A237 to construct strain AGB74. Heterokaryon formation was performed as described in Pontecorvo *et al.* (1953) by growing together mycelia from point-inoculum of conidia on nonselective solid media at 30°. Mycelia of the section between both strains were cut out after 3 days and selected for heterokaryon formation on selective media. The phleomycin resistance marker was used for selection for heterokaryotic mycelia with concentrations of 1 μg/ml phleomycin.

Tetrad analysis: *A. nidulans* strains were incubated under suboptimal conditions and stabilization to form stable asci. The glucose concentration of standard minimal medium was reduced from 2 to 0.5% and 2% sorbitol was added. Instead of a reduction of glucose, reductions of supplement concentrations for *A. nidulans* auxotrophic markers were used (in milligrams/liter: pyridoxine, 0.5 instead of 2; p-aminobenzoic acid, 5 instead of 20). These changes lengthened sexual development. In *veA1* strains fruitbody formation was induced by oxygen limitation by taping petri dishes that resulted in stable asci after ~14 days. *veA* wild-type strains formed stable asci in 7–8 days. Cleistothecia were isolated and crushed in 100 μl of sterile water, and the presence of stable asci was confirmed by microscopic examination. Ten microliters of the suspension was spread in a line on supplemented minimal solid medium without sorbitol. After 30 min most of the asci had burst. Opening could be accomplished by knocking with a micromanipulator needle. Five open asci were dissected per plate with a micromanipulator (Labophot-2, J. Nikon). After incubation for 2 days at 30°, colonies were subcultured and tested for genotypes.

RESULTS

***A. nidulans* heterokaryons with divergent nuclei form exclusively hybrid diploid nuclei:** Most *A. nidulans* laboratory strains are derivatives of the wild-type isolate A4. Crosses between these strains are unaffected by the heterokaryotic compatibility system because all strains belong to the same compatibility group 20 (Anwar *et al.* 1993). Progeny of crosses are originated from selfed

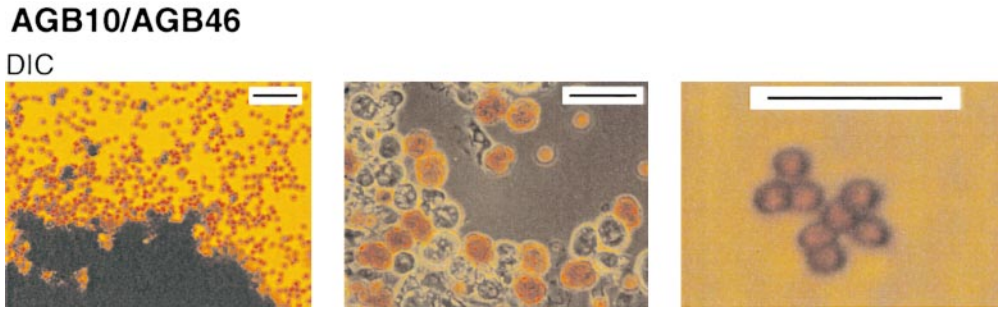

FIGURE 1.—Intact asci of *A. nidulans*. *A. nidulans* AGB10/AGB46 heterokaryon strains were grown with reduced glucose concentrations of 0.5% and halved amounts of supplements for auxotrophic markers on solid, sorbitol-stabilized medium at 30°. Sexual development was induced by oxygen limtation. After 13–15 days cleistothecia of *veA1* strains were isolated and checked for mature asci under a microscope, characterized by their red pigmentation (middle). Mature, intact, and stable asci were diluted in 100 μl H_2O and plated out on an appropriate dry plate without sorbitol. After ~30 min most mature asci walls were burst to make ascospores accessible (right). Mature asci with still intact cell walls could be opened by knocking or sweeping with a micromanipulator needle. Control ascospores of *A. nidulans* grown under minimal conditions are shown (left). Opening of the cleistothecium resulted in the total destruction of ascus walls. Bars, 10 μm. DIC, differential interference contrast.

and hybrid fertilization events in an equal proportion (PONTECORVO *et al.* 1953; CROFT and JINKS 1977). We used British *A. nidulans* strains of different compatibility groups to investigate meiotic events in heterokaryotic mycelia with nuclei of different genetic backgrounds. The strains used are derivatives of the Birmingham wild-type isolate R99 (ANWAR *et al.* 1993) and of the Glasgow isolate A4 (PONTECORVO *et al.* 1953). Strains could form a heterokaryon only when forced with auxotrophic markers *pyroA4* of strain AGB10 and *pabaA1* of strain AGB46. The growth rate of the heterokaryon was reduced to ~30% of the parental strains and formation of conidiophores was 20% of normal. Both haploid components of the heterokaryon could be recovered after removing the selection pressure.

Sexual outcrossing ability of *A. nidulans* strains is independent of the heterokaryon compatibility system (DALES and CROFT 1990). The origin of nuclei involved in meiotic events of crossed heterokaryotic Birmingham and Glasgow strains was analyzed using AGB46 and AGB10 as parents. Strains were grown on specialized medium for tetrad analysis. Under these growth conditions the life span of asci with intact cell walls was markedly prolonged. Stability of intact asci was further increased by addition of 1 to 2% sorbitol to the medium, resulting in up to 80% intact asci within each mature cleistothecium (Figure 1). Fully developed ascospores within intact asci could be identified by their red pigmentation. Intact asci were isolated and opened by a micromanipulator or by incubation for ~30 min on dry agar plates without sorbitol (Figure 1). Ascospores were dissected by micromanipulation. Viability of dissected ascospores was ~94% with respect to ascospores obtained under normal growth conditions and investigated by random ascospore analysis (data not shown). The heterokaryotic mycelium was forced during sexual development by selection for *pabaA1* and *pyroA4*. All cleistothecia were filled with viable ascospores. We selected 10 large and 10 small mature cleistothecia to consider a putative effect of hybrid or selfed dikaryotic mothercells on fruitbody diameter and isolated 10 asci from each cleistothecium. All analyzed tetrads were exclusively derivatives of hybrid diploids (Figure 2A). Similar results were obtained in a cross of AGB10 with the Birmingham strain AGB45 except that 2 of the 20 cleistothecia examined were sterile. Repeated crosses with described strains or their descendants in crosses and backcrosses, respectively, resulted in an identical exclusive formation of hybrid diploids.

Heterokaryons of divergent but compatible *A. nidulans* strains preferentially form hybrid diploids for ascospore formation: Tetrad analyses of crosses with strains of different genetic background show the exclusive use of hybrid diploids for meiosis. We were interested in whether this result is also relevant for heterokaryons of compatible *A. nidulans* strains with moderate genetic differences. We crossed GR5, a derivative of Glasgow wild-type strain A4, and A234, a progeny of a cross of A4 and M826, which differs in its genetic background from A4 (BARRATT *et al.* 1965). Again, we isolated 10 large and 10 small mature cleistothecia of three independent A234/GR5 heterokaryons and analyzed 10 asci from each cleistothecium (Figure 2B). A strong preference for hybrid diploids in formation of ascospores was observed. Approximately 85% of the analyzed asci had ascospores containing genotypes of both parents. This preference to recombine during meiosis was found to be independent of the size of cleistothecia. Selfed diploids were formed with nuclei of both parental strains. Backcrosses of three recombinant ascospores with A234 or GR5, respectively, showed a similar preference for hybrid diploid formation for 5 analyzed cleistothecia each (data not shown).

Compatible *A. nidulans* strains of similar genetic background are unable to distinguish between nuclei and form equally selfed or hybrid diploids during meiosis: The previous results correlate higher genetic divergence of nuclei of a heterokaryon with an increase of hybrid fertilization events during sexual development. Therefore we analyzed the meiotic events of a heterokaryon

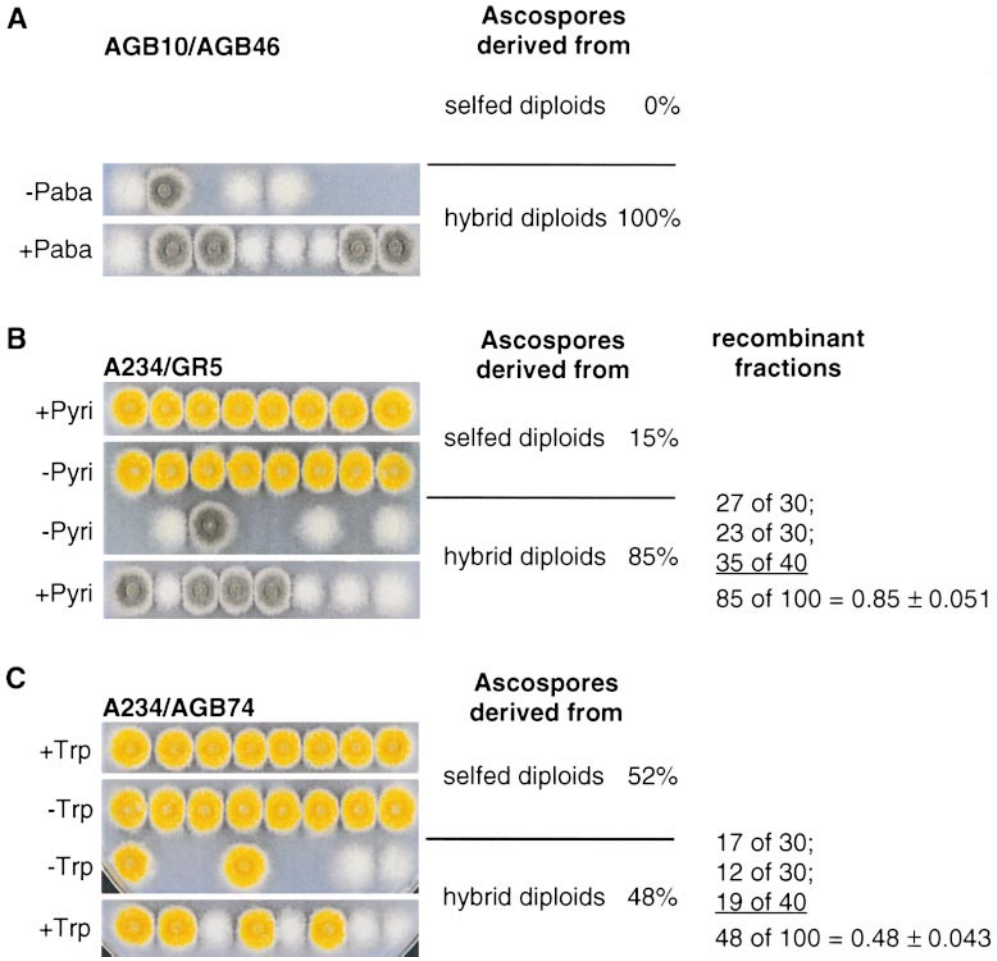

FIGURE 2.—Tetrad analyses reveal nuclear preferences in various heterokaryons of *A. nidulans*. (A) Exclusive formation of recombinant ascospores in fruitbodies of crossed *A. nidulans* strains with strongly differing genetic backgrounds. Heterokaryotic mycelium of a cross between the Glasgow-type strain AGB10 with the Birmingham collection strain AGB46 was incubated on solid minimal medium under oxygen limitation conditions and halved glucose concentrations. Ten stable asci out of 10 large and 10 small cleistothecia each were isolated toward the end of sexual development and ascospores were dissected. Ascospore colonies were tested for recombinant or parental phenotypes as indicators for mating or selfed fertilization events. Growth of ascospores of one ascus is shown on medium without selection (+Paba) and selective for the auxotrophic *pabaA1* marker (−Paba) of the parental strain AGB10. All ascospores tested had a hybrid diploid cell as origin. As a consequence, four spores carried the *pabaA1* auxotrophic marker and four carried the intact gene. Ascospore development derived from a selfing event was not observed. (B) Preference of cross-fertilization events for *A. nidulans* strains with different genetic backgrounds. The A234/GR5 heterokaryon was grown as described in A. Ten large and 10 small cleistothecia were isolated and 10 stable asci from each fruitbody were dissected. Ascospore-specific colonies per tetrad were analyzed for parental or recombinant phenotypes as indicators for selfed or mating fertilization events. One representative ascus derived from a hybrid and one from a selfed diploid cell are shown on medium selecting against the auxotrophic marker *pyroA4* of the parental strain GR5 (−Pyri). As a control, growth of tetrad colonies is shown on medium without selection (+Pyri). Of all asci tested, 85% had a hybrid diploid origin resulting in four ascospores per ascus unable to grow on medium without pyridoxine. No parental genotype was preferred for ascospores from selfed diploid cells. Recombinant fractions in B and C indicate the number of hybrid ascospores for all three independent heterokaryons analyzed for each cross; their percentage and relative standard deviation are according to PONTECORVO *et al.* (1953). (C) Similar amounts of clonal and recombinant ascospores in crosses of *A. nidulans* strains of similar genetic background. The *A. nidulans* strains A234 and AGB74, differing only in one single genetic locus and phleomycin resistance, were crossed and sexual development was induced for the resulting heterokaryon as described in A. Ten large and 10 small cleistothecia were isolated and 10 stable asci of each fruitbody were dissected. Ascospores were analyzed on medium with and without tryptophan for selection against the auxotrophic marker *trpC* of the parental strain AGB74.

of isogenic nuclei derived from strains A234 and AGB74. Both strains differ only by a point mutation in the *trpC* locus of AGB74 and by a phleomycin resistance cassette integrated into the genome of AGB74, which was used for selection. The *trpC* gene product is an enzyme involved in the biosynthesis of the amino acid tryptophan. Altogether, 10 large and 10 small cleistothecia were isolated from three independent heterokaryons after growth under stable tetrad formation conditions. Ten asci of each cleistothecium were dissected and analyzed to determine whether their origins derived from self- or hybrid fertilization events (Figure 2C). The analyzed asci (52%) were formed by selfed diploid cells, while 48% of tetrads had hybrid diploids as their origin, suggesting an equal random distribution. None of the parental strains was preferred for formation of selfed diploids. The same results were observed for crosses of Birmingham R99 derivatives AGB45 and AGB46, respectively, with R99. While these strains formed exclusively recombinant ascospores in crosses with A4 derivatives, no preference in formation of hybrid or selfed premeiotic cells was observed in crosses of unmixed genetic backgrounds (data not shown).

A cleistothecium of *A. nidulans* can be the result of

TABLE 2

Variable amounts of fertilization events per cleistothecium

A234/AGB74[a]						A234/GR5[b]					
Large cleistothecia			Small cleistothecia			Large cleistothecia			Small cleistothecia		
	sd	hd		sd	hd		sd	hd		sd	hd
C1	10^{A234}	—	C1	10^{AGB74}	—	C1	—	10	C1	3^{A234}	7
C2	—	10	C2	—	10	C2	1^{A234}	9	C2	—	10
C3	9^{A234}	1	C3	2^{AGB74}	8	C3	—	10	C3	—	10
C4	—	10	C4	6^{A234}	4	C4	—	10	C4	—	10
C5	7^{AGB74}	3	C5	10^{AGB74}	—	C5	5^{A234}	5	C5	3^{A234}	7
C6	—	10	C6	—	10	C6	—	10	C6	—	10
C7	10^{A234}	—	C7	$4^{3\times A234}_{1\times AGB74}$	6	C7	—	10	C7	—	10
C8	—	10	C8	10^{A237}	—	C8	—	10	C8	—	10
C9	$4^{1\times A234}_{3\times AGB74}$	6	C9	$10^{6\times A234}_{4\times AGB74}$	—	C9	$4^{1\times A234}_{3\times GR5}$	6	C9	—	10
C10	—	10	C10	6^{A234}	4	C10	—	10	C10	4^{GR5}	6

[a] Isogenic strains A234 and AGB74 were crossed and sexual development was induced under suboptimal growth conditions at 30° to induce stable asci formation. Ten large and 10 small cleistothecia (C1 to C10) were isolated and 10 octads per cleistothecium were analyzed. The number of selfed diploid nuclei (sd) or hybrid diploid nuclei (hd) as origin of the 10 octads per cleistothecium is indicated. Additionally, for selfed diploid nuclei the parental genotype is given as superscript.

[b] Analysis as in [a] from 10 large and 10 small cleistothecia of a cross between the genetically different but compatible strains A234 and GR5.

more than one fertilization event: Earlier results based on random ascospore analysis suggest that only one single fertilization event within each protocleistothecium results in a mature cleistothecium. The genotypes of ascospores of all tetrads of each A234/AGB74 cleistothecium were compared (Table 2). Seven cleistothecia contained asci derived only from hybrid diploids. Five cleistothecia contained octads that were genotypically identical to one of the parental strains. The remaining 8 cleistothecia contained asci that originated from selfed or hybrid diploids. Two fruitbodies contained asci corresponding to both parental genotypes as well as asci with recombinant ascospores. The same results were found for cleistothecia from the A234/GR5 heterokaryon. Six of the 20 cleistothecia derived from more than one diploid nucleus. As an example, all dissected asci of the second large cleistothecia analyzed in Table 2 are shown (Figure 3). These data indicate that cleistothecia of *A. nidulans* are not necessarily the result of a single fertilization event but can be the consequence of two or more fertilizations. This seems to be independent of the genetic background of the parent strains and their differences within a heterokaryon.

DISCUSSION

Generally, asci of *A. nidulans* are relatively unstable and only random spore analysis has been described. We developed an easy technique that allows the isolation and analysis of unordered tetrads. Characterization of progenies of single meiotic events allows, *e.g.*, a clear attachment of phenotypes to single gene loci or the identification of essential genes.

Distinction of nuclei in a heterokaryon: For several ascomycetes the first indication of sexual development is the appearance of hyphal ascogonial coils that fuse with other specialized hyphae called antheridia, thus bringing together the nuclei that finally generate the ascospores by meiosis (Coppin *et al.* 1997). In analogy to other Aspergillus species, an *A. nidulans* cell functionally equivalent to an ascogonium presumably fuses to a second cell functionally equivalent to an antheridium (Benjamin 1955; Champe *et al.* 1994). The result is a dikaryotic hypha in which the two composite nuclei reside in close proximity and divide in synchrony (Pontecorvo *et al.* 1953). Our results show that *A. nidulans* can complete the sexual cycle, depending on the nuclei available, in a range between a strict homothallic to partial or even strict heterothallic behavior. Therefore, a mechanism exists that can distinguish between different nuclei at the beginning of sexual development. Pontecorvo *et al.* (1953) described this type of interaction and termed it relative heterothallism. There the phenomenon was found between certain pairs of strains derived from the same original strain. In addition, no differences in relative heterothallism were found when a strain was crossed with isogenic or heterogenic *A. nidulans* strains and frequency of hybrid diploid formation varied strongly in repeated experiments and crosses with descendants. Our results, however, argue for an additional kind of relative heterothallism and suggest that an increase in the differences, rather than similarities, between the nuclei increase the formation of het-

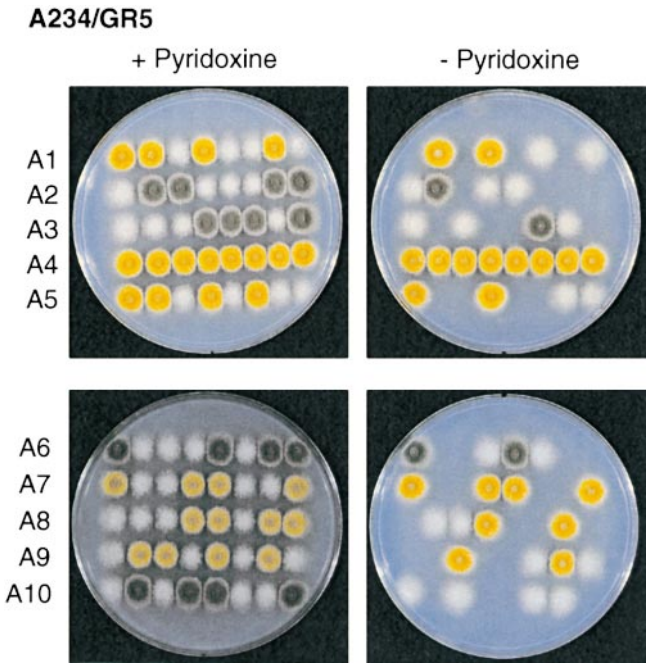

FIGURE 3.—Two fertilization events within the same cleistothecium. Heterokaryotic mycelia of a cross between strains A234 and GR5 were incubated on minimal medium with halved concentration of glucose on solid sorbitol-stabilized medium at 30°. After 15 days stable asci (A1 to A10) were isolated from one single cleistothecium and dissected via micromanipulation on a masterplate containing all supplements of both parental *A. nidulans* strains. After growth, conidia of each ascospore colony were suspended and plated out on medium selective for specific markers. The figure shows the growth after selection for the auxotrophic marker *pyroA1* on minimal medium with (+) and without (−) pyridoxine. Asci A1 to A3 and A5 to A10 contain recombinant ascospores while A4 is a result of selfing of two nuclei from the parental strain A234.

erozygous premeiotic zygotes. Strains forming exclusively hybrid diploids in crosses with genetically different strains fuse nuclei at random when crossed with isogenic strains. Frequency of hybrid diploid preference remained almost constant in repeated experiments and crosses with recombinants of hybrid asci argue for a genetically determined mechanism with putatively more than one locus. Heterokaryon incompatibility and the recognition of different nuclei in relative heterothallism appear to be separate systems because strains in the same h-c group that differ genetically still preferentially form heterozygous zygotes.

Cleistothecia contain asci derived from more than one type of nuclear fusion: At least one dikaryotic cell is a prerequisite for cleistothecia formation in *A. nidulans* (CHAMPE *et al.* 1994). This dikaryon divides to form a population of dikaryotic cells that were assumed to be responsible for all the premeiotic diploids within a cleistothecium. We found that at least 25% of the analyzed fruitbodies contained asci that originated from both selfed and hybrid zygotes. Identical types of fertilization events within the same cleistothecium were indistinguishable from fruitbodies with presumably only one fertilization. Therefore it is very possible than an even higher percentage of cleistothecia are the product of more than one dikaryotic mothercell. The existence of more than one fertilization event within the same cleistothecium does not appear to be determined by a specific genetic background of crossed strains. For crosses of isogenic strains as well as for crosses of strains with considerably different genetic backgrounds an identical amount of cleistothecia with more than one fertilization event were observed. In general, at least two mechanisms could explain several fertilization events as basis for a single cleistothecium. Independent fertilization events in close proximity could induce formation of several adjacent nests of sterile hyphae. Subsequently, these nests could fuse due to their close proximity to a nest of higher order and form a single cleistothecium with mixed dikaryotic cells. A second possibility is that nest formation is not directly induced by any single fertilization event. If nest formation can be induced only during development after the ascogenous hyphae have formed a small mycelium, several independent ascogenous hyphae could be unified within a single cleistothecium and form different asci.

Taken together, the data presented in this work give new insights into the mechanism of relative heterothallism. The data argue for a genetically determined mechanism that favors recombination of different nuclei in a heterokaryon. It is likely that several loci are involved that have no effect on frequency of sexual recombination if homozygous within a heterokaryon. When these loci differ, they seem to influence recombination by preferring hybrid diploids for ascospore formation.

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