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Programmed Ascospore Death in the Homothallic Ascomycete *Coniochaeta tetraspora*

Namboori B. Raju and David D. Perkins

Department of Biological Sciences, Stanford University, Stanford, California 94305-5020

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Raju, N. B., and Perkins, D. D. 2000. Programmed ascospore death in the homothallic ascomycete *Coniochaeta tetraspora. Fungal Genetics and Biology* 30, 213–221. Immature asci of *Coniochaeta tetraspora* originally contain eight uninucleate ascospores. Two ascospore pairs in each ascus survive and mature, and two die and degenerate. Arrangement of the two ascospore types in individual linear asci is what would be expected if death is controlled by a chromosomal gene segregating at the second meiotic division in about 50% of asci. Cultures originating from single homokaryotic ascospores or from single uninucleate conidia are self-fertile, again producing eight-spored asci in which four spores disintegrate, generation after generation. These observations indicate that differentiation of two nuclear types occurs *de novo* in each sexual generation, that it involves alteration of a specific chromosome locus, and that the change occurs early in the sexual phase. One, and only one, of the two haploid nuclei entering each functional zygote must carry the altered element, which is segregated into two of the four meiotic products and is eliminated when ascospores that contain it disintegrate. Fusion of nuclei cannot be random—a recognition mechanism must exist. More study will be needed to determine whether the change that is responsible for ascospore death is genetic or epigenetic, whether it occurs just before the formation of each ascus or originates only once in the ascogonium prior to proliferation of ascogenous hyphae, and whether it reflects developmentally triggered alteration at a locus other than mating type or the activation of a silent mating-type gene that has pleiotropic effects. Similar considerations apply to species such as *Sclerotinia trifoliorum* and *Chromocrea spinulosa,* in which all ascospores survive but half the spores in each ascus are small and self-sterile. Unlike *C. tetraspora,* another four-spored species, *Coniochaetidium savoryi,* is pseudohomothallic, with ascus development resembling that of *Podospora anserina.*

Index Descriptors: programmed cell death; ascus development; ascospore differentiation; *Coniochaeta tetraspora; Coniochaetidium savoryi;* homothallism; pseudohomothallism.

Most species of filamentous ascomycetes produce eight-spored asci, following meiosis and a postmeiotic mitosis. However, numerous genera that are predominantly eight-spored include species with four-spored asci. Some species are four-spored because ascospores are cut out immediately following meiosis, prior to a postmeiotic mitosis. Most commonly, species are four-spored because of a genetically programmed alteration of ascus development (Raju and Perkins, 1994). In the four-spored species of *Neurospora*, *Podospora*, and *Gelasinospora*, each ascospore is heterokaryotic, enclosing two nuclei of opposite mating type. Cultures originating from single ascospores are thus self-fertile. This condition was first described in *Neurospora tetrasperma* by Dodge (1927), who coined the term pseudohomothallism (Dodge, 1957), sometimes called secondary homothallism. The developmental and genetic controls that result in pseudohomothallism are different in each of the three genera of Sordariaceae that have been examined (see Raju and Perkins, 1994).

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We have been interested in examining whether ascus development in pseudohomothallic species of other families resembles that of the species already examined or whether it follows other, novel, strategies. For this reason, we examined ascus development in two four-spored species (Coniochaetaceae; Sordariales): *Coniochaeta tetraspora* and *Coniochaetidium savoryi.* We found *C. savoryi* to be pseudohomothallic, resembling *Podospora anserina* rather than *N. tetrasperma* in its ascus programming and spindle behavior. Our expectation that *C. tetraspora* would be pseudohomothallic was incorrect. The species proved to be of interest for a completely different reason, however.

In his original description of *C. tetraspora,* Cain (1961) noted that whereas the mature asci are 4-spored, the immature asci always contain 8 ascospores at the time of spore delimitation. Cain's brief taxonomic description made no mention of the mating system (whether heterothallic, homothallic, or pseudohomothallic), nor did he describe nuclear behavior in the developing asci and ascospores. Other *Coniochaeta* species produce 8, 16, 32, 64, 128, or more mature ascospores per ascus, but very little is known about the genetics or ascus cytology of any of them (Cooke *et al.,* 1969; see Mahoney and LaFavre, 1981 for a synopsis of the genus).

Our present cytogenetic analysis of three *C. tetraspora* strains confirms Cain's (1961) observation that the four-spored condition results from the death of four ascospores in what was originally an eight-spored ascus. Our observations show further that *C. tetraspora* is homothallic and that ascospore death is nonrandom: Two of the four ascospore pairs in every ascus abort and disintegrate, and their positions in the asci are what would be expected if a specific chromosomal locus is responsible. Yet, because the species is homothallic, ascospore death cannot be attributed to an inherited genetic difference that is transmitted from generation to generation. Death of the ascospores is developmentally programmed but the mechanisms underlying it are not clear. Our observations indicate that alteration of a chromosomal locus is responsible and that this occurs before karyogamy, perhaps in the ascogonium. One possible explanation would be that a chromosomal locus might be modified epigenetically in response to developmental cues, and this might be responsible both for nuclear recognition prior to karyogamy and for ascospore death following meiosis. An understanding of the events described here might offer insights into nuclear behavior and ascus development in homothallic fungi that differ from *Coniochaeta* in having all eight ascospores survive.

MATERIALS AND METHODS

Strains and Media

C. tetraspora (Coniochaetaceae, Sordariales) was first isolated by J. H. Warcup from forest soils obtained in Queensland, Australia and was described by R. F. Cain in 1961. The three isolates of *C. tetraspora* used in the present study were kindly provided by D. P. Mahoney. Strain G218 (FGSC No. 8313) was isolated by Mahoney from a soil sample collected in the Galapagos Islands. SA42 (FGSC 8312) and SA451 (FGSC 8311) were isolated by J. LaFavre from postfire chaparral soils in southern California. (FGSC: Fungal Genetics Stock Center, Department of Microbiology, University of Kansas Medical Center, Kansas City, KS.) A cleistothecial Coniochaete, *Coniochaetidium savoryi,* was obtained from John Krug of the University of Toronto. The strains of both species fruit well in petri plate culture on modified Leonian's agar (Malloch, 1981), which we further modified by replacing half the maltose in the original formula by glucose to give the following recipe: maltose 3.12 g, glucose 3.12 g, malt extract 6.25 g, KH_2PO_4 1.25 g, yeast extract 1.0 g, $MgSO_4 \cdot 7H_2O$ 0.625 g, peptone 0.625 g, agar 20 g, distilled water 1000 ml.

The *Coniochaeta* cultures were incubated at 25°C under a 12 h light:12 h dark regimen. Generally, the first perithecia appear at the center of the colony 6 to 8 days after inoculation. Additional perithecia are formed in concentric circles as the colony grows toward the periphery. Mature ascospores were ejected onto the underside of the glass lid from 2 to 3 weeks after inoculation.

Cultures from single conidia and single ascospores were tested to determine the mating strategy. Conidia from a 6-day-old ascospore culture of SA451 were spread on Leonian agar medium. After about 12 h, colonies originating from single conidia were placed onto separate plates containing Leonian agar to induce fruiting. Likewise, 4-week-old ascospores that were ejected from cultures of SA42, SA451, and G218 were spread on 4% water agar and heat-shocked in a 60°C water bath for 30 min to induce germination. After 24 to 36 h, 25 single-ascospore germlings for each strain were placed onto Leonian agar plates and incubated at 25°C for fruiting.

Cytological Methods

For examining ascus development, strips of Leonian agar medium bearing developing perithecia from 10- to 15-day-old cultures were fixed in a freshly prepared solu-

tion containing ethanol, propionic acid, and 10% chromic acid (9:6:2 v/v). Asci were stained using an iron–hematoxylin procedure (Raju and Newmeyer, 1977; Raju, 1978). Because perithecia of *C. tetraspora* are very small (approximately 150 μm diameter compared to 500 μm diameter for *N. crassa*), individual perithecia were not opened under a dissecting scope. Instead, 15 to 20 perithecia were soaked for 2 min in a small drop of ferric acetate mordant on a microscope slide. Excess mordant was blotted out before a drop of hematoxylin solution was added. The perithecia were then gently crushed under a cover glass to extrude asci from perithecia, and the cover glass was sealed with melted dental wax. Mature rosettes of asci (Figs. 1A and 2A) were obtained from 15- to 20-day-old cultures; the asci were lightly stained using ~1/10th dilution of ferric acetate and hematoxylin solutions. Developing asci were also examined using the DNA-specific fluorochrome acriflavin and an Olympus BH2 epifluorescence microscope. For acriflavin staining, unfixed perithecia (on an agar strip) from 10- to 15-day-old cultures were hydrolyzed and stained, as described for *Neurospora* chromosomes (Raju, 1986).

RESULTS

The three strains of *C. tetraspora* (G218, SA42, and SA451) differ from one another in several respects. The perithecia of strain G218 develop faster and ascospores mature several days earlier than those of SA42 and SA451 (12–15 days vs 15–20 days). Perithecia of G218 are thin walled, slightly pigmented, and nearly transparent; mature asci with black ascospores can readily be seen through the perithecial walls. The asci are somewhat broader and shorter than those of SA42 and SA451. Observations were more extensive for SA451, mainly because fertility is consistently good and ascus characteristics are more suitable for cytological observations. Our demonstration of homothallism and ascospore death applies equally well to all three strains, however.

Ascus and Ascospore Development in C. tetraspora

Perithecia from 2- to 3-week-old cultures contained 50 to 100 asci at various stages of development from early prophase through maturing ascospores. The asci at spore delimitation measure 50–60 μm long and 6–7 μm wide (roughly threefold shorter and narrower than those of *N. crassa*). Mature asci are four-spored but the developing immature asci always contained eight spores (Figs. 1A and 1B). Early ascus development, meiosis, and the postmeiotic mitosis appear normal. Nuclear divisions in the developing asci of *C. tetraspora* resemble those of eight-spored *Neurospora* species (Raju, 1978, 1980). Spindles at the second division of meiosis are usually aligned in tandem, one in the proximal half and the other in the distal half of the ascus. At the following postmeiotic mitosis (3rd division), the four spindles are aligned equidistant from one another and oriented across the ascus, as in *N. crassa* (see Raju, 1980, 1992). The eight nuclei are then sequestered into eight ascospores in the narrow ascus. Initially, all eight ascospores and their nuclei appear normal and are indistinguishable from one another. Soon, four of the eight spores (two sister pairs) in every ascus stop developing and degenerate (Figs. 1B–1E). The remaining two ascospore pairs enlarge and mature normally (Figs. 1A and 1E).

Acriflavin, which is DNA specific, also revealed that nuclear morphology and divisions in the developing asci are normal. Then nuclei in the four aborting ascospores become highly condensed. The condensed nuclei in the aborted ascospores fluoresce intensely, indicating that the DNA is not yet completely degraded in the degenerating, shrinking ascospores (not shown).

Evidence That the Factor Responsible for Ascospore Death Is Chromosomal

Whatever is responsible for ascospore death in *C. tetraspora* shows 4:4 Mendelian segregation in individual asci, and the patterns of aborted and viable ascospores in the linear asci indicate that the responsible factor shows about 50% second-division segregation. At a stage when the viable (V) and aborted (A) ascospore pairs are still clearly visible, linear arrangement in the immature asci is diagnostic of first-division segregation (AAAAVVVV), asymmetric second-division segregation (VVAAVVAA), or symmetric second-division segregation (AAVVVVAA) (Figs. 1C–1E). Patterns for strain SA451 were 52 first-division asci, 62 asymmetric second-division asci, and 39 symmetric second-division asci. Patterns for strain SA42 were 23 first-division asci, 47 asymmetric second-division asci, and 24 symmetric second-division asci. In addition, 15 to 20% of asci in both strains had four aborted ascospores that were not arranged in pairs. These asci most likely resulted from nuclear slippage and they will be ignored.

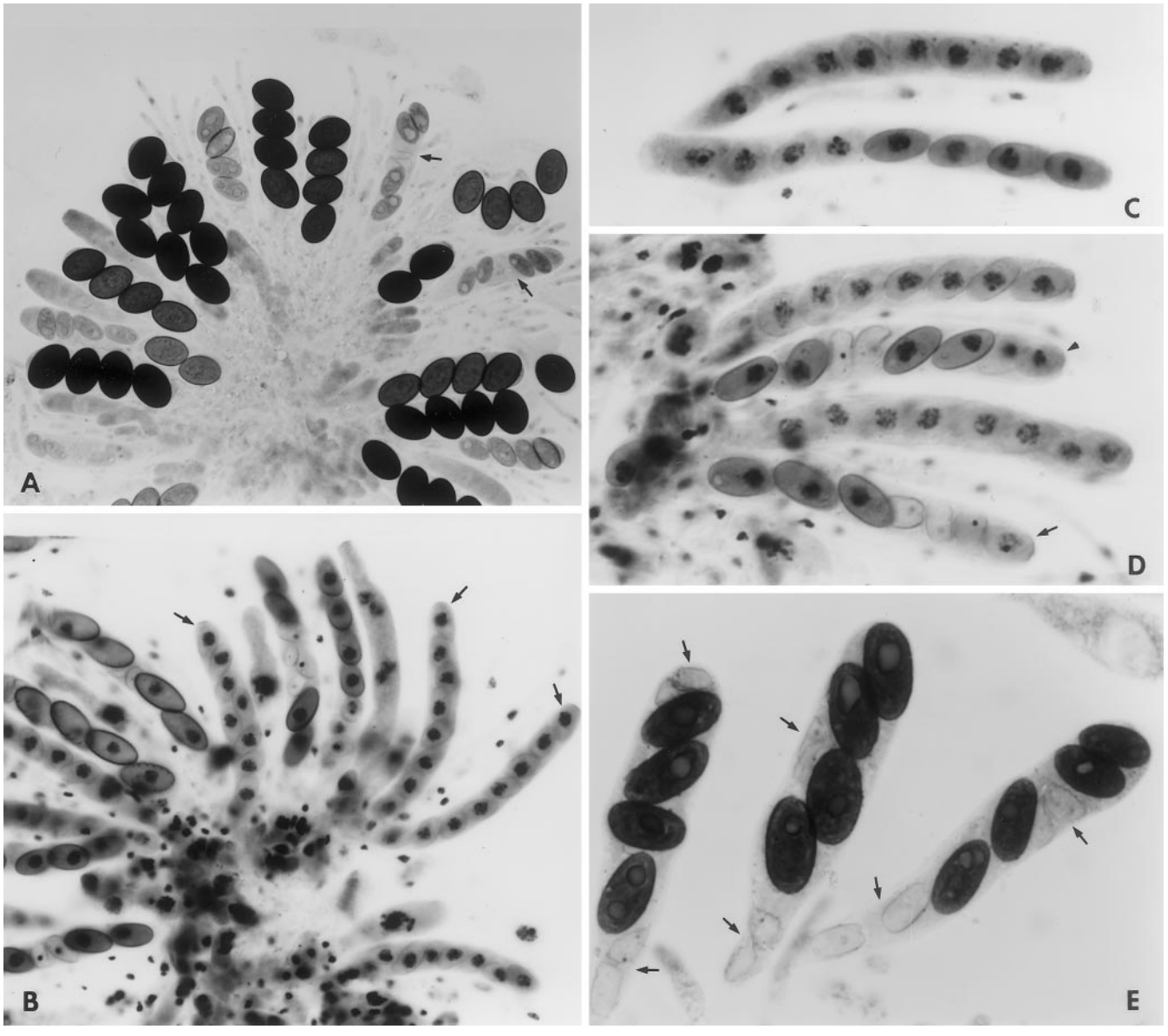

FIG. 1. *Coniochaeta tetraspora.* Hematoxylin staining. (A) A rosette of maturing asci at various stages of development (15-day culture). Mature asci with darker ascospores are clearly four-spored. At least two of the immature asci show four large, developing ascospores and four small, aborted, degenerating ascospores (arrows; ×500). (B) A rosette of developing asci showing nuclear behavior (12 days). Several young asci show eight uninucleate ascospores without any sign of ascospore death (arrows). Some other asci show four large, darker-staining, developing ascospores and four small aborting ascospores (×780). (C) Two eight-spored asci; the top ascus has just cut out eight uninucleate ascospores (12 days). The bottom ascus (more advanced) is beginning to differentiate four aborting ascospores at left and four normally developing ascospores at right (×1180). (D) Two of the four asci show 4V:4A (arrow) and 2V:2A:2V:2A (arrow head) patterns for developing viable (V) and aborted (A) ascospores, reflecting the first- and second-division segregation patterns, respectively (12 days). The remaining two asci are still immature and do not yet show ascospore abortion patterns (×1180). (E) Maturing asci, each with two pairs of large, normally developing, ascospores and two pairs of small, aborted, ascospores (arrows; 15 days). The ascus at left shows a symmetric 2:4:2 arrangement of aborted and viable ascospores. The remaining two asci show an asymmetric 2:2:2:2 ascospore arrangement (×1180).

If ascospore death is due to a centromere-linked chromosomal factor and if there is no slippage of nuclei or ascospores in the developing linear ascus, asci will be of the 4:4 type when the factor segregates at the first meiotic division, whereas asymmetric and symmetric ascus types will result when the factor segregates at the second divi-

sion. The frequency of asci showing second-division segregation provides a measure of genetic map distance between the abortion factor and the centromere.

Asymmetric and symmetric second-division asci are expected to occur with equal probability if linear order is not altered by passage of nuclei or ascospores. The two ascus types are found in equal numbers for fungi such as *Neurospora crassa,* but they differ significantly for *C. tetraspora,* with 109 asymmetric and 63 symmetric second-division asci in a total of 247. The deviation from an expected 86:86 is most readily explained by overlap of second-division spindles, which would convert noncrossover asci from the first-division 4:4 type to the asymmetric AAVVAAVV type. Infrequent asci showing overlapping second-division spindles were indeed observed cytologically. In contrast to the asymmetric type, asci with a symmetric arrangement cannot be produced by a simple spindle overlap, but can result only from crossing over. Therefore, as best shown by Berg (1966) for *Sordaria brevicollis,* a reliable measure of second-division segregation can be obtained by doubling the frequency of asci with symmetric spore arrangements. In the present example, this gives 126/247 = 51%. The factor responsible for ascospore death thus appears to be located in a chromosome arm about 25 map units (centimorgans) from the centromere.

Demonstration That C. tetraspora Is Homothallic

Our cytological observations were initiated with the assumption that *C. tetraspora* is pseudohomothallic, similar to *N. tetrasperma* and *P. anserina.* We were, however, surprised to see that each ascus initially contained eight uninucleate ascospores rather than four binucleate spores. The four-spored condition results only secondarily, by disintegration of two pairs of sister ascospores. *C. tetraspora* is therefore not pseudohomothallic. The species name is accurate in that the mature asci show only four spores and that no sign remains of the missing spores, but it is inaccurate with respect to the initial number of ascospores.

Progeny tests revealed that *C. tetraspora* is not heterothallic, but is truly homothallic. Cultures originating from single uninucleate ascospores are self-fertile. Progeny asci again showed four viable and four inviable ascospores, and the process repeats itself. Self-fertility and ascospore death have been followed through at least five sexual generations using strain SA451.

Timing of Nuclear Modification

All asci in every perithecium show 1:1 segregation of the factor responsible for ascospore death. The event that introduces the factor and differentiates previously identical nuclei into two types must therefore occur prior to meiosis, no later than karyogamy. If alteration occurred before initiation of the sexual phase, two types of nuclei might be demonstrable in vegetative cells—those with the alteration and those without. Altered nuclei might no longer be capable of homothallic behavior. Alternatively, if homothallic capability is not lost by altered nuclei, all eight ascospores might be seen to disintegrate. *C. tetraspora* produces abundant conidia, all of which are uninucleate. Viability was high (over 90%). Vegetative cultures originating from single conidia were examined to determine whether nuclei were stably modified in some and unmodified in others. Fifty single-conidial colonies were isolated to modified Leonian agar medium. Within 2 or 3 weeks, all 50 cultures produced normal numbers of fertile perithecia containing mature asci, all with four ascospores surviving and four degenerated. There is thus no evidence for stable alteration of nuclei prior to perithecial initiation.

Ascus Development in Coniochaetidium savoryi

Unlike *C. tetraspora,* another four-spored member of the Coniochaetaceae family, *C. savoryi,* proved to be pseudohomothallic. Ascocarps of this fungus do not differentiate beaks or ostioles. As with *C. tetraspora,* the perithecia and asci of *C. savoryi* are severalfold smaller than those of *Neurospora* and *Podospora* species. We find that the asci are four-spored, each ascospore encloses two nuclei at inception, and single-ascospore cultures are self-fertile, just as in the better-studied pseudohomothallic species *N. tetrasperma* and *P. anserina.* All four ascospores mature and there is no ascospore death, unlike *C. tetraspora* (Figs. 2A and 2B). Ascus development and spindle behavior in *C. savoryi* resemble those of *P. anserina* rather than those of *N. tetrasperma* (see Raju and Perkins, 1994). As in *P. anserina,* the second-division spindles are aligned in tandem and far apart from one another (Fig. 2C), and the resulting four nuclei are initially in single file in the linear asci (Fig. 2D). The four interphase nuclei subsequently associate themselves in pairs in preparation for the third division (Fig. 2E), where the spindles are aligned pair-wise and oriented across the ascus, as in *P. anserina* and *N. tetrasperma.*

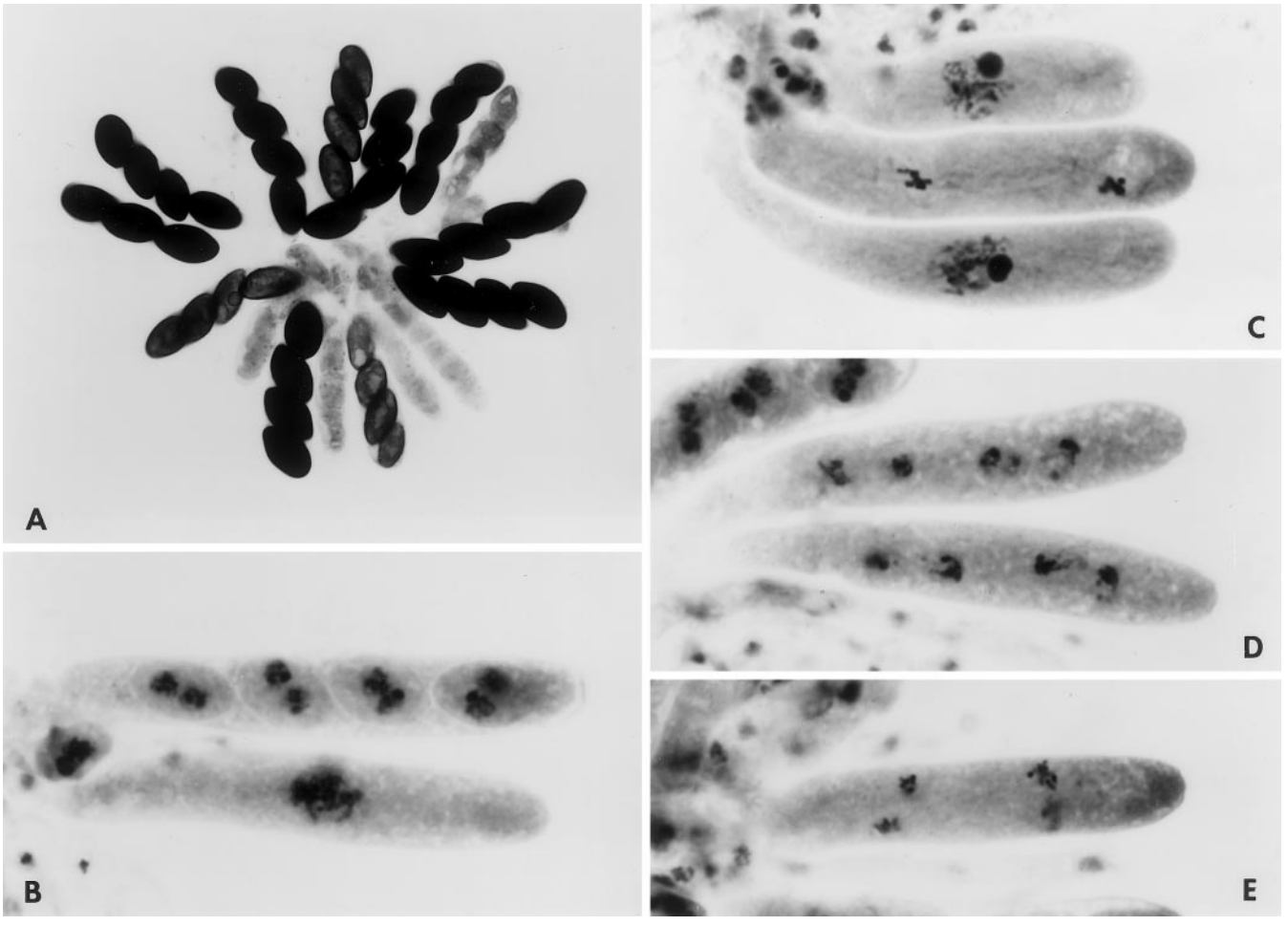

FIG. 2. *Coniochaetidium savoryi*, a four-spored pseudohomothallic species. Hematoxylin staining. (A) A rosette of four-spored asci; only four spores are formed in each ascus and there is no ascospore death such as occurs in *Coniochaeta tetraspora* (16 days; ×590). (B) The upper ascus shows four young ascospores, each of which enclosed two nuclei (9 days). The lower ascus is still in meiotic prophase. The ascus program and spindle behavior during the second and third divisions resemble those of the pseudohomothallic *Podospora anserina* (×1180). (C) Middle ascus is at metaphase II. The two dividing metaphase nuclei are aligned in tandem and are spaced far apart (9 days; ×1180). (D) Early interphase II (9 days). Soon after second division, the four nuclei in each ascus are spaced equidistant and are aligned in single file (×1180). (E) Young ascus at late interphase II, just prior to the postmeiotic mitosis (9 days). The nuclei in each half-ascus associate as a pair and divide together. Subsequently two nonsister nuclei become enclosed in each of the four ascospores (×1180; see B, upper ascus).

DISCUSSION

C. tetraspora, with its novel features, came to our attention during a search for new examples of four-spored pseudohomothallic species in different Pyrenomycete families. It was the first four-spored species to be examined in the family Coniochaetaceae and it proved to be entirely different from what had been expected and of greater interest. Our original objective was realized when another species in the same family, *C. savoryi*, was found to be pseudohomothallic, with asci containing four heterokaryotic, self-fertile ascospores and a program of ascus development similar to that of *P. anserina*, which has second-division spindles in tandem and binucleate ascospores that enclose nonsister products of the second meiotic division. This *Podospora* pattern of ascus development is characteristic of pseudohomothallic species belonging to three of the four genera for which information is available. *N. tetrasperma* differs from all the others in using overlap-

ping second-division spindles to produce heterokaryotic, self-fertile ascospores (see Raju and Perkins, 1994).

C. tetraspora (hereafter called *Coniochaeta*) is homothallic: All cultures originating from single uninucleate conidia or initially uninucleate ascospores are self-fertile. Karyogamy, meiosis, postmeiotic mitosis, and ascospore formation resemble those of other eight-spored Pyrenomycetes, such as *Neurospora crassa, N. africana,* and *Sordaria macrospora* (Raju, 1978, 1980). *Coniochaeta* differs, however, in that four of the eight ascospores invariably die, shrivel, and disappear. This behavior is completely different from that of the autonomous ascospore-color mutants known in many fungi and used most extensively in *Sordaria* and *Ascobolus* (see Raju, 1992). These abnormal ascospores are due to a mutant allele contributed by one of the parents in a biparental cross. These results with *Coniochaeta* cannot be attributed to *Spore killer*-like meiotic-drive elements; four defective spores are produced only if one parent is sensitive to killing and the other parent is a killer (Turner and Perkins, 1979; see Raju, 1994).

The determinant of ascospore death in *Coniochaeta* segregates meiotically as a single Mendelian factor. Second-division segregation frequencies indicate that the factor is located in a chromosome arm at a fixed distance from the centromere. Thus, every ascus must originate from a diploid nucleus that is heterozygous for the factor. Cultures from single haploid, uninucleate conidia or ascospores are invariably capable of producing perithecia within which all asci segregate four viable and four defective ascospores. The two nuclei that fuse in each ascus primordium must differ in the allele that they carry at the critical locus, and they must recognize each other as different, prior to fusion. A unique event must therefore occur in every generation to create a new allele at the critical locus. The new allele could originate early in the sexual cycle, perhaps only once for each perithecium—either prior to the initiation of perithecial development or in the incipient ascogonium. Alternatively, the event creating an allelic difference could occur later, in the ascogenous hyphae or in one haploid nucleus of each crozier, prior to karyogamy. Early occurrence, prior to proliferation of ascogenous hyphae, seems the simplest explanation, requiring only a single change to alter all the asci. The possibility of multiple independent occurrences at a later stage cannot be dismissed out of hand, however. An unstable allele in *Ascobolus immersus,* thought to be due to a mobile element, is programmed to undergo change immediately before meiosis, resulting in asci with four of the eight ascospores altered (Nicolas *et al.,* 1987). Only a small fraction of the asci are affected, however, in contrast to the situation in *Coniochaeta.*

We are largely ignorant as to the nature of the event, which could be epigenetic, involving modification by methylation or acetylation, or mutational, or recombinational through rearrangement, as in mating-type switching of yeast or phase variation by inversion in *Salmonella* (Simon *et al.,* 1980). Whatever the origin, the newly created variant is fated to be eliminated when the ascospores bearing it disintegrate. We do not know whether ascospore death reflects pleiotropic expression of a mating-type gene, activation of which initiates the sexual cycle, or whether the factor is at a locus distinct from mating type, where its developmental activation coincides with activation of a latent mating-type idiomorph.

Other fungi have been described that resemble *Coniochaeta* in having self-fertile haploid ascospores that produce eight-spored asci in which four of the spores are of a morphologically distinct type (see Perkins, 1987; Coppin *et al.,* 1997). *Chromocrea spinulosa* and *Sclerotinia trifoliorum* are examples, with mature asci that contain four large and four small ascospores (Mathieson, 1952; Uhm and Fujii, 1983a). (These will be called *Chromocrea* and *Sclerotinia.*) Cultures from single large spores are self-fertile, and all the asci that they produce are again 4:4. Unlike the abnormal ascospores of *Coniochaeta,* which disintegrate, the small ascospores of these species remain viable. Perhaps they can provide clues regarding the mechanism that underlies ascospore dimorphism in *Coniochaeta.* As with *Coniochaeta,* cultures from the large ascospores show behavior defined as homothallic. In contrast, cultures from the small spores have been interpreted as heterothallic. The small-spore mycelia of *Sclerotinia* are self-sterile but are capable of producing fertile perithecia when fertilized with spermatia from a large-spore culture (Uhm and Fujii, 1983b). Likewise for *Chromocrea,* small-spore cultures are capable of crossing with large-spore cultures.

Molecular analysis of mating type in *Chromocrea* has shown that the self-fertile, large-spore cultures contain both *MAT-1* and *MAT-2* sequences (corresponding to *N. crassa* mating-type idiomorphs *A* and *a,* respectively), whereas the self-sterile, small-spore cultures contain only a single mating-type gene, *MAT-1* (Yun *et al.,* 1999; B. G. Turgeon, personal communication). When mutant markers were used in crosses between large-spore and small-spore cultures of *Chromocrea,* ascospore size and the marker difference both showed 1:1 segregation in biparental perithecia (Mathieson, 1952). The small-spore cul-

tures appeared to be of one mating type and the normally homothallic large-spore cultures behaved as though they were heterothallic and of the opposite mating type. This suggests that the resident *MAT-1* gene of the normally homothallic nucleus is silenced during development of the biparental perithecia.

Unlike the situation in yeast, no evidence has been found for silent, duplicated mating-type sequences in any of the homothallic filamentous euascomycetes that have been examined. It seems unlikely, therefore, that activation of a previously silent gene is responsible for the homothallic–heterothallic switch that appears to take place in the species under consideration here. The molecular evidence from *Chromocrea* suggests instead that a developmentally controlled genetic or epigenetic modification is capable of silencing one or the other of two mating types that are present in large-spore cultures, resulting in a change from homothallic to heterothallic. If altered ascospore size or ascospore viability is a pleiotropic effect of one of the mating-type idiomorphs, and if the effect is expressed only in spores in which the opposite mating-type idiomorph is inactive or absent, this might account both for ascospore dimorphism in *Sclerotinia* and *Chromocrea* and for ascospore death in *Coniochaeta.*

Pleiotropic expression of mating-type genes during the vegetative phase is known to occur for mating-type-associated vegetative incompatibility (see, for example, Saupe *et al.,* 1996). It is reasonable to suggest that the recurring small-ascospore trait in *Coniochaeta, Sclerotinia,* and *Chromocrea* may also be a pleiotropic manifestation of a mating-type gene. Creation of a new allele at the locus responsible for the ascospore trait coincides temporally with a mating-type-mediated function—either initiation of the sexual cycle, formation of perithecia, or association of nuclei in dikaryotic cells. Also, both the mating type and the ascospore trait require precise recognition between differing nuclei prior to karyogamy to ensure Mendelian 4:4 segregation in individual asci.

Hypocrea poronioidea resembles *C. spinulosa* and *S. trifoliorum* in having asci in which 1:1 segregation occurs for self-fertile:self-sterile (Samuels and Lodge, 1996). *H. poronioidea* differs from these species, however, in having both ascospore types equal in size. Spermatia are produced by *H. poronioidea,* but only in cultures that are self-fertile. *C. spinulosa* resembles *Hypocrea* species in most respects and may ultimately be reassigned to that genus (G. J. Samuels, personal communication).

Segregation for self-fertile:self-sterile is also found in various species of the genus *Ceratocystis. Ceratocystis coerulescens,* examined by Harrington and McNew (1997), apparently has no ascospore dimorphism. Ascus analysis is impractical. However, segregation is 1:1 for self-fertile: self-sterile in random-ascospore progeny from a homothallic culture. Mutants have been obtained from the self-fertile type that are self-sterile and that mate as male parent with self-fertile. Although the self-sterile progeny are usually of the same mating type, two opposite, compatible mating types were recovered among the self-sterile progeny of one perithecium in a self-fertile culture. The molecular basis of the transition from homothallic to heterothallic appears similar to that of *Coniochaeta,* with silencing of one idiomorph in the homothallic parent being responsible for production of single-mating-type, self-sterile progeny. Other suggestive examples have been described of unidirectional change from homothallic to heterothallic. (See, for example, Faretra and Pollastro, 1996, using *Botryotinia fuckeliana.*)

If indeed the visible ascospore anomalies in *Coniochaeta, Sclerotinia,* and *Chromocrea* are specified by alterations at the mating-type locus, knowledge gained using the ascospore polymorphisms may prove useful in understanding sexual cycle initiation and selfing in homothallic species. This would be true not only in species such as *Hypocrea poronioidea* and *Cerotocystis coerulescens,* in which there is no ascospore-size dimorphism, but also in typical homothallic species such as *Sordaria macrospora* and *Neurospora africana,* in which there is neither spore-size dimorphism nor loss of self-fertility. Unlike *N. africana,* which contains only the *A* mating-type idiomorph, *S. macrospora* contains both *A* and *a* sequences, and these are contiguous (Pöggeler, 1999; Pöggeler *et al.,* 1997). The fact that germlings from all eight ascospores in these species are uniformly homothallic would seem to favor a model involving epigenetic modification.

Internuclear recognition is essential in heterothallic euascomycetes to ensure that nuclei of biparental origin and opposite mating type coexist in a 1:1 ratio in the ascogenous hyphae and come together at karyogamy, which is essential for the formation of asci and the production of progeny (Zickler *et al.,* 1995; Arnaise *et al.,* 1997; Debuchy, 1999). The observations described for *Chromocrea* and *Ceratocystis* provide a model for *Coniochaeta* and suggest more broadly that karyogamy and ascus development might be preceded by the silencing of one or another mating-type gene in ho-

mothallic fungi that contain copies of both idiomorphs within the same nucleus.

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