Loss of Meiosis in Aspergillus

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If strictly mitotic asexual fungi lack recombination, the conventional view predicts that they are recent derivatives from older meiotic lineages. We tested this by inferring phylogenetic relationships among closely related meiotic and strictly mitotic taxa with Aspergillus conidial (mitotic) states. Phylogenies were constructed by using DNA sequences from the mitochondrial small ribosomal subunit, the nuclear ribosomal internal transcribed spacers, and the nuclear 5.8s ribosomal gene. Over 920 bp of sequence was analyzed for each taxon. Phylogenetic analysis of both the mitochondrial and nuclear data sets showed at least four clades that possess both meiotic and strictly mitotic taxa. These results support the hypothesis that strictly mitotic lineages arise frequently from more ancient meiotic lineages with Aspergillus conidial states. Many of the strictly mitotic species examined retained characters that may be vestiges of a meiotic state, including the production of sclerotia, sclerotium-like structures, and hülle cells.

Introduction

Asexual lineages are believed to be susceptible to the progressive accumulation of deleterious mutations in the absence of recombination (Muller 1964; Lynch et al. 1993). This view leads to the prediction that asexual species are recently derived from sexual lineages, because asexual species should be more susceptible to extinction (Maynard Smith 1978). However, possibly ancient meiotic lineages have been identified on inferences from mitochondrial DNA sequences in unisexual vertebrates (Hedges, Bogart, and Maxson 1992; Quattro, Avise, and Vrijenhoek 1992; Spolsky, Phillips, and Uzzell 1992). Other organisms not known to undergo sex, such as Archaeabacteria and Euglenas, may represent very ancient asexual lineages as well. Fungi are uniquely suited for the study of the evolution of sexual and asexual modes of propagation within a lineage, because many fungal species reproduce strictly mitotically (asexually), whereas their close relatives are capable of meiotic (sexual) reproduction. Establishing evolutionary patterns between meiotic and strictly mitotic species should provide evidence as to the evolutionary fate of strictly mitotic lineages.

The genus Aspergillus is a diverse and familiar group of ascomycetes which is mostly saprobic, but includes human pathogens, plant pathogens, and species useful in industrial processes and genetic research. Of the 186 species with Aspergillus asexual states listed by Samson (1994), 114 are not known to produce a sexual meiotic spore (ascospore). These include some of the species most frequently encountered by humans: A. flavius, A. niger, A. terreus, and A. fumigatus. These species propagate solely via asexual mitotic spores (conidia). The remaining 72 species do produce meiotic spores, and are placed in nine sexual genera, the most common being Emericella, Eurotium, and Neosartorya. All but two meiotic species are known to produce conidia as well as ascospores (fig. 1).

Key words: Aspergillus, meiosis, fungi, asexuality.

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The roles of clonal and recombinant modes of propagation in nature are unknown. Evidence for sexual recombination has been identified in the meiotic species Emericella nidulans (Geiser, Arnold, and Timberlake 1994), while strictly mitotic species such as A. flavius are believed to propagate in mostly or completely clonal fashion (Cotty et al. 1994). The production of recombinant mitotic spores is possible in the laboratory (the parasexual cycle; Pontecorvo et al. 1953), but it is not known to occur in natural populations. No studies have been attempted to quantify the level of recombination that occurs in meiotic or strictly mitotic species.

If strictly mitotic lineages are clonal, the conventional view (Maynard Smith 1992) predicts that they should be short-lived and recently derived from older meiotic lineages. Asexuality may provide a short-term benefit to fungi, with a long-term cost evident in such a phylogenetic pattern (Berbee and Taylor 1993a). Phylogenetic analysis of Penicillium, a genus closely related to Aspergillus, showed close relationships between sexual and asexual taxa, suggesting recent derivation of asexual lineages (LoBuglio, Pitt, and Taylor 1993). We used mitochondrial and nuclear ribosomal DNA sequences to infer phylogenetic relationships among meiotic and strictly mitotic taxa to determine whether strictly mitotic Aspergilli represent multiple independent derivatives of meiotic lineages.

Materials and Methods

We examined seven strictly mitotic and seven meiotic Aspergillus species (table 1). Talaromyces flavus was chosen as an outgroup species based on the evidence that Aspergillus is part of a monophyletic group derived from it (Berbee et al. 1995). Talaromyces is sexual, and it was previously shown that many Penicillium asexual states are independently derived from Talaromyces ancestors (LoBuglio, Pitt, and Taylor 1993). The 14 species were chosen to represent a diverse array of species with Aspergillus asexual states, representing 5 subgenera (Samson 1994) and 13 sections or species groups (Samson 1994; Raper and Fennell 1965). The internal transcribed spacers (ITS1 and ITS2) + the 5.8s ribosomal DNA (nuclear rDNA), and part of the mitochondrial ribosomal small subunit (mtSSU), were se-
quenced in each species. In keeping with the current taxonomic schemes (Klich and Pitt 1988; Samson 1994), meiotic species with *Aspergillus* asexual states will be referred to by their sexual names in this paper (Emeriella, etc.), while strictly mitotic species will be referred to by their *Aspergillus* names (table 1). Both meiotic and strictly mitotic species as a whole will be referred to as “Aspergillus” or “Aspergilli.”

DNA Extraction and Polymerase Chain Reaction

Mycelium was grown by inoculating dry spores and mycelium into 25 ml of liquid minimal medium (MM; Barratt, Johnson, and Okata 1965) in petri plates. The cultures were incubated at either 30°C or 37°C for 1 day to 1 week, until a floating mass of mycelium was present. Mycelium was harvested, followed by a DNA extraction protocol adapted from Yelton, Hamer, and Timberlake (1984) and suspension in 100 μl of water. Although 10× or 100× dilutions were used in some cases, 1 μl of genomic template was used as template. Reactions were performed in 50-μl volumes, using 5 units of *Taq* DNA polymerase, 15–30 pmol of each primer, 0.2 mM dATP, dCTP, dGTP, and TTP, and 2 mM MgCl₂, according to the enzyme manufacturer’s instructions (Promega). For the nuclear rDNA, primers designed for use in higher plants were used (Hodges and Arnold 1994). These primers differ slightly from those designed specifically for fungi (White et al. 1990). We designed new primers that amplify an approximately 715-bp region of the *Aspergillus* mtrssu: AspMS5 (5'-AAGAAAGGAAAAAGAAGGTC-3') or AspMS3 (5'-CGGTGAGTAGTAGTAAAGGT-3') and AspMS4 (5'-AAATAACATACTTCACT-3') were used as external primers, and AspMS6 (5'-TTCTTCTTCTTTACATAAA-3') and AspMS7 (5'-TTATGTGAGAAGAAAGAA-3') were used as internal primers. The primers were designed from regions of similarity identified between the aligned mtrssu sequences from *E. nidulans* and *Saccharomyces cerevisiae*, with standardized conditions for automated sequencing taken into consideration.

DNA Sequencing and Analysis

PCR products were purified on Wizard PCR Prep columns (Promega) and both strands were sequenced directly using an Applied Biosystems automated sequence (Model 373A) at the University of Georgia Molecular Genetics Instrumentation Facility. Sequences were edited using SeqEd (v. 1.0.3, Applied Biosystems), and initially aligned using the PILEUP option in GCG (Wisconsin Package, version 8). Only sequences that could be read from two strands were kept for analysis. Alignments were then optimized manually. Phylogenetic analysis was performed using PAUP (Phylogenetic Analysis Using Parsimony) version 3.1.1 (Swofford 1993), with general heuristic searches and bootstrap analyses using 500 replicates. Random addition and tree-bisection-reconnection (TBR) branch-swapping were used. Indels of two or more nucleotides were encoded according to their presence or absence (0/1) if the inserted sequence was conserved. Gaps were treated as missing data. Additional branch support analysis was performed by using the Bremer support index, saving all trees of increasing length in a step-wise manner, increasing one step at a time (Bremer 1994). For example, for the nuclear
Table 1
Isolates Used in This Study

<table>
<thead>
<tr>
<th>Asexual Statea</th>
<th>Sexual Statea</th>
<th>Strain Number</th>
<th>Source</th>
<th>Classification (Raper and Fennell 1965)</th>
<th>Classification (Samson 1994)</th>
<th>Nuclear rDNA GenBank Accession No.</th>
<th>mt rssu GenBank Accession No.</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>A. compatibilis</em> Samson and Gams</td>
<td><em>Emericella heterothallica</em> (Kwon et al.) Raper and Fennell</td>
<td>222</td>
<td>R. B. G. Dales</td>
<td><em>Aspergillus nidulans</em> group</td>
<td>Subgenus <em>Nidulantes</em>, section <em>Nidulantes</em></td>
<td>L76743</td>
<td>L76688</td>
</tr>
<tr>
<td><em>A. ustus</em> (Bain.) Thom &amp; Church</td>
<td>None</td>
<td>H611</td>
<td>R. T. Hanlin</td>
<td><em>Aspergillus ustus</em> group</td>
<td>Subgenus <em>Nidulantes</em>, section <em>Usti</em></td>
<td>L76744</td>
<td>L76689</td>
</tr>
<tr>
<td><em>A. nidulans</em> Samson and Gams</td>
<td><em>Emericella nidulans</em> (Eidam) Vuill.</td>
<td>FGSC4</td>
<td>Fungal Genetics Stock Center</td>
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<td>Subgenus <em>Nidulantes</em>, section <em>Nidulantes</em></td>
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<td><em>A. versicolor</em> (Vuill.) Traboschi</td>
<td>None</td>
<td>226</td>
<td>R. B. G. Dales</td>
<td><em>Aspergillus versicolor</em> group</td>
<td>Subgenus <em>Nidulantes</em>, section <em>Versicolores</em></td>
<td>L76745</td>
<td>J09914</td>
</tr>
<tr>
<td><em>A. flavipes</em> (Bain and Sart.) Thom and Church</td>
<td><em>Fennellia flavipes</em> Wiley and Simmons</td>
<td>NRRL5202</td>
<td>S. Peterson</td>
<td><em>Aspergillus flavipes</em> group</td>
<td>Subgenus <em>Nidulantes</em>, section <em>Flavipes</em></td>
<td>L76775</td>
<td>L76918</td>
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<td><em>A. terreus</em> Thom</td>
<td>None</td>
<td>GA02</td>
<td>D. Geiser</td>
<td><em>Aspergillus terreus</em> group</td>
<td>Subgenus <em>Nidulantes</em>, section <em>Terrei</em></td>
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<td>L76917</td>
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<td><em>A. niger</em> van Tieghem</td>
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<td>H125</td>
<td>R. T. Hanlin</td>
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<td>Subgenus <em>Circumdati</em>, section <em>Nigri</em></td>
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<td>L76916</td>
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<td><em>A. flavus</em> Link</td>
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<td>H142</td>
<td>R. T. Hanlin</td>
<td><em>Aspergillus flavus</em> group</td>
<td>Subgenus <em>Circumdati</em>, section <em>Flavi</em></td>
<td>L76747</td>
<td>L76915</td>
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<tr>
<td><em>A. vitis</em> Novobranova</td>
<td><em>Euroti um amstelodami</em> Mangel</td>
<td>H38</td>
<td>R. T. Hanlin</td>
<td><em>Aspergillus glaucus</em> group</td>
<td>Subgenus <em>Aspergillus</em>, section <em>Aspergillus</em></td>
<td>L76777</td>
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<tr>
<td><em>A. restrictus</em> Smith</td>
<td>None</td>
<td>H701</td>
<td>R. T. Hanlin</td>
<td><em>Aspergillus restrictus</em> group</td>
<td>Subgenus <em>Aspergillus</em>, section <em>Restricti</em></td>
<td>L76776</td>
<td>L76919</td>
</tr>
<tr>
<td><em>A. coryzeides</em> Samson and Gams</td>
<td><em>Chaetosartorya chrysella</em> (Kwon and Fennell) Subramanian</td>
<td>H267</td>
<td>R. T. Hanlin</td>
<td><em>Aspergillus cremeus</em> group</td>
<td>Subgenus <em>Circumdati</em>, section <em>Cremei</em></td>
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<td>L76922</td>
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<tr>
<td><em>A. wentii</em> Wehmer</td>
<td>None</td>
<td>H631</td>
<td>R. T. Hanlin</td>
<td><em>Aspergillus wentii</em> group</td>
<td>Subgenus <em>Circumdati</em>, section <em>Wentii</em></td>
<td>L76806</td>
<td>L76921</td>
</tr>
<tr>
<td><em>A. warcupii</em> Samson and Gams</td>
<td><em>Warcupiella spinulosa</em> (Warcup) Subramanian</td>
<td>NRRL4376</td>
<td>S. Peterson</td>
<td><em>Aspergillus ornatus</em> group</td>
<td>Subgenus <em>Ornati</em>, section <em>Ornati</em></td>
<td>L76807</td>
<td>L76923</td>
</tr>
<tr>
<td><em>Aspergillus fischerianus</em> Samson and Gams</td>
<td><em>Neoasartorya fischeri</em> (Wehmer) Malloch and Cain</td>
<td>NRRL4161 (mtrssu), FRR 1833 (mtDNA)</td>
<td>R. T. Hanlin</td>
<td><em>Aspergillus fumigatus</em> group</td>
<td>Subgenus <em>Fumigati</em>, section <em>Fumigati</em></td>
<td>U18355</td>
<td>L76924</td>
</tr>
<tr>
<td><em>Penicillium dangeardi</em> Pitt</td>
<td><em>Talaromyces flavus</em> (var. macrosporus) Stolk and Samson</td>
<td>FRR 2386</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>U18354, L14508, L76925</td>
</tr>
</tbody>
</table>

* The names used in this paper are those in boldface; "sexual states" (those referring to the meiotic state) were used in meiotic species, and "asexual" *Aspergillus* states were used as names for strictly mitotic species.
* The nuclear ITS + 5.8S rDNA sequence from *N. fischeri* isolate FRR 1833 (CSIRO culture collection, North Ryde, NSW, Australia) was provided by Mary Berbee and John Taylor.
* The nuclear ITS + 5.8S rDNA and part of the mitochondrial sequence from the outgroup species *Talaromyces flavus* isolate FRR 2386 were kindly provided by Katherine LeBuglio, Mary Berbee, and John Taylor.
data, the most parsimonious tree had 139 steps. General heuristic searches were performed saving all trees of length ≤140 steps, then all trees of ≤141 steps, up to six steps longer than the most parsimonious tree. A strict consensus tree was identified with each increment, and the number of increased steps necessary to collapse each branch on the most parsimonious tree was determined. When the number of trees saved at each increment exceeded computer memory limits, the consensus of three sets of 8,000 trees generated by random sequence addition was determined. The "Trace Character" option in MacClade (v. 3.0.1; Maddison and Maddison 1992) was used to superimpose the evolution of sexual and asexual taxa on the parsimony trees. The tracings were performed using both the ACCTRAN and DELTRAN methods, which maximizes early gains and forces subsequent reversals, and the DELTRAN method, which delays changes away from the ancestors. The "constraints" option in PAUP was used to constrain tree topologies, forcing asexual taxa to be monophyletic, and heuristic searches were performed as described above to find the most parsimonious constrained trees. Maximum-likelihood estimates for the most-parsimonious and constrained most-parsimonious trees were calculated using the DNAML program in the PHYLIP package (v. 3.57; Felsenstein 1993). To test whether constrained trees were significantly less likely than unconstrained trees, the Kishino-Hasegawa test was performed using DNAML (Kishino and Hasegawa 1989). Neighbor-joining analyses were performed by using the DNADIST and NEIGHBOR programs, and the SEQBOOT program for bootstrapping (Felsenstein 1993). The Jukes-Cantor (Jukes and Cantor 1969) and Kimura two-parameter distances were employed, both of which gave nearly identical branching order. A transition:transversion ratio of 2.0 was used in estimating the Kimura two-parameter distance for the entire group.

Results

In each species, a minimum of 460 bp of double-stranded region was derived from both strands of the nuclear rDNA region, and a minimum of 565 bp of sequence was derived from the mtrssu gene. The ITS regions of the nuclear rDNA were highly variable among Aspergillus species, so we removed four sections totalling ~90 bp of 11S1 and ~12 bp of 11S2 because alignment was problematic due to insertions and deletions. This left a total of 354 nucleotide positions for analysis. Two small indels were included as data, encoded as single characters. Forty informative sites were found in the 354-bp region. The mtrssu provided another 50 phylogenetically informative characters. A 12-bp indel was found in the mtrssu of A. wentii and C. chrysella, the presence of which was coded as a single character. Heuristic searches yielded single most-parsimonious trees for the mitochondrial (140 steps) and nuclear data sets (139 steps) (fig. 2). The topologies of the most parsimonious trees from the two data sets differed slightly, but mostly in branches that had poor (<50%) bootstrap support and Bremer support indices of 1 or 2. If branches with less than 70% bootstrap support are collapsed into polytomies, trees for the two data sets are identical with one exception. The nuclear data place A. versicolor and Emericella nidulans together as a strongly supported clade, while the mitochondrial data place Emericella nidulans basal to A. versicolor. This inconsistency may reflect a real difference in the genealogies of these two genes, as might be expected in closely related taxa (Avise and Ball 1990). Except for this inconsistency, all of the inferred trees showed three strongly supported groupings of strictly mitotic and meiotic taxa: A. ustus and Emericella heterothallica, A. restrictus and Eurotium amstelodami, and A. wentii and Chaetosartorya chrysella. Since two independent losses (or gains) of meiosis can be inferred in the A. ustus/E. heterothallica/A. versicolor/E. nidulans clade, at least four independent losses (or gains) of meiosis can be inferred for the entire group.

In contrast to the parsimony analysis, neighbor-joining of both the mitochondrial and nuclear data produced two clades grouping Emericella heterothallica/A. ustus and Emericella nidulans/A. versicolor, although the latter clade was not strongly supported by bootstrap analysis of the mitochondrial data (65%). With this exception, differences between the topologies of the parsimony and neighbor-joining trees were on branches with poor (<50%) bootstrap support on the parsimony trees. The Jukes-Cantor and Kimura two-parameter distance estimates both gave trees with identical branching order for both data sets, except for the branching order on the A. flavus/A. terreus/A. niger/Fennellia flavipes clade using the nuclear data. All of the differing branches had very weak (~32%) bootstrap support. Both data sets show multiple independent losses or gains of meiosis.

When the seven asexual taxa were constrained to form a monophyletic ingroup, heuristic searches using the mitochondrial data set produced nine most-parsimonious trees of 191 steps, 51 steps longer than the unconstrained most-parsimonious tree. Heuristic searches using the nuclear data set produced two trees of 191 steps, 52 steps longer than the unconstrained most-parsimonious tree. The log likelihood scores of the unconstrained most-parsimonious trees and the constrained most-parsimonious trees with the highest log likelihood are given in table 2. Both constrained most-parsimonious trees are significantly less likely than the unconstrained trees: the nuclear constrained tree is more than seven standard deviations less likely, and the mitochondrial constrained tree is more than five standard deviations less likely. Differences of more than 1.96 standard deviations are considered significant (Kishino and Hasegawa 1989).

Discussion

The inferred phylogenies confirm conclusions based on morphology (Raper and Fennell 1965; Samson
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The data show that meiosis has been independently lost and/or gained at least four times in *Aspergillus* among the sampled taxa. The nuclear and mitochondrial data sets both strongly group two meiotic/strictly mitotic species pairs, *A. restrictus/Eurotium amstelodami* and *A. wentii/Chaetosartorya chrysella*, indicating that these asexual *Aspergillus* species are derived from meiotic lineages (fig. 2). Parsimony analyses of the mitochondrial and nuclear data sets do not give a consensus as to the origins of the asexual species *A. versicolor* and *A. ustus*. The DELTRAN method indicated two independent losses of meiosis leading to these taxa, while the ACCTRAN method indicated one loss and one gain from the mitochondrial data, and two gains of meiosis from the nuclear data. Neighbor-joining analyses of both data sets lead to an unambiguous inference of two independent losses.

Fig. 2.—Phylogenetic analysis of mitochondrial and nuclear data sets. Dark lines represent inferred asexual lineages, light lines represent sexual lineages, and striped lines represent lineages for which ACCTRAN and DELTRAN methods produced different inferences. Numbers below branches represent bootstrap values (500 replicates), and numbers above branches are Bremer support indices. Tree branches are drawn proportional to inferred branch lengths. Consistency indices (CI), retention indices (RI), and rescaled consistency indices (RC) are given for the parsimony trees (Farris 1989). Neighbor-joining trees shown are based on the Jukes-Cantor measure of genetic distance (Jukes and Cantor 1969). (A) Parsimony analysis of the mitochondrial ribosomal small subunit (mtrssu). (B) Parsimony analysis of the nuclear ITS + 5.8S ribosomal region. (C) Neighbor-joining analysis of the mitochondrial data. (D) Neighbor-joining analysis of the nuclear data.
Model for the loss of meiosis in Aspergillus

Meiotic (sexual) modes

<table>
<thead>
<tr>
<th>Homothallism</th>
</tr>
</thead>
<tbody>
<tr>
<td>Emericella, Chaetosartoya, Eurotium, etc.</td>
</tr>
</tbody>
</table>

Strictly mitotic (asexual) modes

- irregularly shaped hüle cell masses
  - Aspergillus ustus
- scattered hüle cells
  - Aspergillus versicolor
- no apparent vestiges
  - Aspergillus restrictus
- hyphal masses resembling immature cleistothecia
  - Aspergillus wentii

![Diagram of meiotic sexual modes and strictly mitotic asexual modes]

Our sample of taxa was wide, but it was not a complete analysis of the possible 186 species with Aspergillus conidial states. We cannot be sure that a different sample would not suggest multiple gains rather than losses of meiosis. However, it is biologically more feasible that the meiotic state has been lost multiply than gained multiply. Ascospore production is a complex process that involves the development of a specific growth stage and formation of a specialized structure, the cleistothecium. The genetics of ascospore formation suggests that ascymocytes may harbor a large number of genetic targets that when mutated confer a loss of meiosis. In *Neurospora crassa*, an unrelated ascomycete, over 200 different mutations have been isolated with meiosis-specific effects (Raju 1992). Wild *N. crassa* strains harbor a surprising level of lethal recessive mutations specific to meiotic sporulation. Leslie and Raju (1985) identified 106 lethal recessive diploid phase-specific mutations, most of which were unique, that eliminated or reduced meiotic fertility from 99 wild isolates. In contrast, relatively little is known about the genetics of meiotic sporulation in *Emericella nidulans*, the genetically best-characterized *Aspergillus*. There are many mutations known to eliminate or vastly reduce ascospore formation in this species (Champe, Nagle, and Yager 1994), but few known with phenotypes exclusively related to meiosis, perhaps because no exhaustive search has been made. Most meiotic mutants are considered to be pleiotropic, and were noted as second phenotypes in addition to vegetative or conidial defects. Many of these mutations produce phenotypes similar to the apparent vestiges seen in many asexual species (e.g., the presence of hüle cells without cleistothecium production). For example, *medusa* (*medA*) mutants produce aberrant conidiophores and lack cleistothecia, but do produce hüle cells (Clutterbuck 1969). Likewise, *A. ustus* isolates do not produce cleistothecia but do produce hüle cells. Other mutations, such as *velvet* (*veA*), have a quantitative effect on cleistothecium production (Käfer 1965). Similarly, a great deal of genetic variation in the quantity of cleistothecia produced is observed among *E. nidulans* isolates (Butcher 1968). Overall, these data indicate that there are a large number of potential target genes that could confer losses of meiosis in *Aspergillus*, and that variation in meiosis-specific genes is present in fungal populations.

Multiple independent gains of meiosis are more difficult to imagine. The sexual states associated with *Aspergillus* share a great deal of morphological similarity: all involve the formation of enclosed cleistothecia, produce unordered, evanescent asci, and produce similarly shaped ascospores. It is unlikely that these char-

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**Table 2**

<table>
<thead>
<tr>
<th>Tree Length</th>
<th>Ln Likelihood</th>
<th>Difference from Most Parsimonious Tree</th>
<th>Standard Deviation</th>
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</thead>
<tbody>
<tr>
<td>Nuclear ITS + 5.8S</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unconstrained . . . . . . . . . 139</td>
<td>-1,245.2</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Constrained* . . . . . . . . . 191</td>
<td>-1,535.4</td>
<td>-290.2</td>
<td>36.5</td>
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<tr>
<td>Mitochondrial</td>
<td></td>
<td></td>
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<tr>
<td>Unconstrained . . . . . . . . . 140</td>
<td>-1,639.6</td>
<td>-</td>
<td>-</td>
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<td>-1,902.2</td>
<td>-262.6</td>
<td>44.1</td>
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</tbody>
</table>

*The constrained most-parsimonious tree with the highest log likelihood is given.*
acters evolved together independently several times. It is possible, however, that meiosis is an oscillating character that is lost and regained frequently. The frequency of such oscillation would probably have to be very short, as asexual taxa would likely accrue additional meiosis-specific mutations, reinforcing the loss.

The age of strictly mitotic lineages is difficult to address in fungi, where a paucity of fossil data makes a reasonable calibration of a molecular clock difficult (Berbee and Taylor 1993b). In particular, our data do not eliminate the possibility that A. niger and/or A. flavus represent relatively ancient, asexual lineages. While there are no obvious candidates for closely related meiotic sister taxa to these species other than perhaps Fennellia, we did not sample all of the 72 mitotic taxa in this study.

Of the four strictly mitotic species inferred to be recently derived, three show apparent vestiges of ascospore production (fig. 2). Both A. ustus and A. versicolor produce hülle cells that are similar to those in their inferred meiotic sister taxa. Hülle cells are associated with cleistothecia (meiotic fruit body) production in subgenus Nilukantus, but are also present in many strictly mitotic species. Some isolates of A. wentii produce masses of hyphae that Raper and Fennell (1965) noted were similar to the immature cleistothecia of Chaetosartorya chrysella, although they classified the two species in different groups. A. restrictus does not possess any obvious vestiges of ascospore production, but the fact that it is strongly xerophile may indicate a connection to E. amstelodamensis. The mitochondrial data weakly suggest a connection between A. terreus and Fennellia flavipes. Raper and Fennell (1965) noted similarities between them, and the sclerotium-like bodies present in A. terreus may represent vestigial cleistothecia. Sclerotia, structures resistant to harsh conditions produced in many strictly mitotic species (including A. niger and A. flavus), are proposed to be derived from cleistothecia (Malloch and Cain 1972), and may represent another vestige of ascospore propagation.

The possibility exists that the observed variation in meiotic reproduction does not reflect complete losses of meiosis in nature, but rather losses of our ability to induce meiosis in culture. However, the fact that many single nonlethal mutations can induce losses of meiosis in the same genetic backgrounds under the same cultural conditions strongly suggests that loss of meiosis is a biologically feasible phenomenon.

Heterothallism in Emericella heterothallica is similar to that observed in heterothallic Neurospora species. An isolate must be fertilized by another isolate of opposite mating type for successful ascospore production (Kwon and Raper 1967). Heterothallism is known in only three meiotic species with Aspergillus mitotic states, including E. heterothallica. The other two species, Neosartorya spathulata and Neosartorya fennelliae, are probably closely related to N. fischeri (Kwon-Chung and Kim 1974; Peterson 1993). The question of whether heterothallism is ancestral in fungi has been called “imponderable” (Raper 1966), but its rarity in Aspergillus may suggest that it is derived, perhaps caused by simple mutations that must be complemented to allow ascospore production. Another possibility is that heterothallism is ancestral in the A. ustus/E. heterothallica clade, and A. ustus is asexual because it lost one of the mating types. Mating type genes and genes regulating sexual development have not been isolated in Aspergillus or its sexual relatives, but extinction of one putative mating type is another possible basis for the loss of meiosis, particularly in the lineage leading to A. ustus.

Aspergilli lacking meiosis are capable of efficient propagation via conidium production and may not suffer short-term fitness effects from the loss of the mitotic state. The loss of meiosis may actually provide a short-term benefit, allowing full investment of resources into conidial propagation, which is likely faster and more efficient than ascospore formation (Champe, Nagel, and Yager 1994). However, in addition to recombination, meiosis may also offer typical adaptive benefits associated with sexual reproduction (Bonner 1958), such as dormancy and resistance to dessication (Cook and Whipp 1993). Most Aspergilli can produce mature conidia within 24 h after induction (Timberlake and Clutterbuck 1994), with mature ascospore formation taking from 4 days to several weeks in different Aspergillus-related species (Raper and Fennell 1965). The loss of the resistant qualities of the ascospore may be compensated for by the production of sclerotia in species that produce them, such as A. flavus and A. niger.

The data presented here indicate that asexual Aspergilli are often recently derived from meiotic lineages, and do not give any strong support to the existence of ancient asexual lineages. The patterns of evolution in Aspergillus and its related meiotic relatives, like those seen in Penicillium and its meiotic Talaromyces relatives (LoBuglio, Pitt, and Taylor 1993), are consistent with strictly mitotic lineages being deficient in recombination, and thus susceptible to the accumulation of deleterious mutations through Muller’s ratchet and mutational meltdown. In Aspergillus, although the possibility exists that significant parasexual recombination occurs in natural populations, the general observation is that most isolates capable of undergoing the first step in the parasexual cycle (heterokaryosis) are genetically identical (Croft and Jinks 1977; unpublished data). However, recombination may be occurring in apparently asexual species, and detailed population genetic analyses are still needed to establish this.

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LITERATURE CITED


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