Sexual structures in *Aspergillus* -- morphology, importance and genomics

David M. Geiser
Department of Plant Pathology
Penn State University
University Park, PA

PENNSTATE

Geiser mini-CV

- 1989-95: PhD at University of Georgia (Bill Timberlake and Mike Arnold): *Aspergillus* molecular evolutionary genetics (*A. nidulans*)
- 1995-98: postdoc at UC Berkeley (John Taylor): (*A. flavus/oryzae/parasiticus*, *A. fumigatus, A. sydowii*)
- 1998-: Faculty at Penn State; Director of Fusarium Research Center -- molecular evolution of *Fusarium* and other fungi

Chaetosartorya

Petromyces

Hemicarpenteles

Neosartorya

Fennellia

Aspergillus

Neocarpenteles

Eurotium

Warcupiella

Neopetromyces

Emericella

Sexual structures in *Aspergillus* -- morphology, importance and genomics

- Sexual stages associated with *Aspergillus*
- The impact (and lack thereof) of the sexual stage on population biology
- What does it mean?

PENNSTATE

Characteristics of clinically important *Aspergillus* spp.

- Ability to grow at 37C
- Commonly encountered by humans
- Prolific sporulators

- *Nothing here about sexual stages*

PENNSTATE

Approx. 1/3 *Aspergillus* species has a known sexual stage

Aspergillus (427 names)

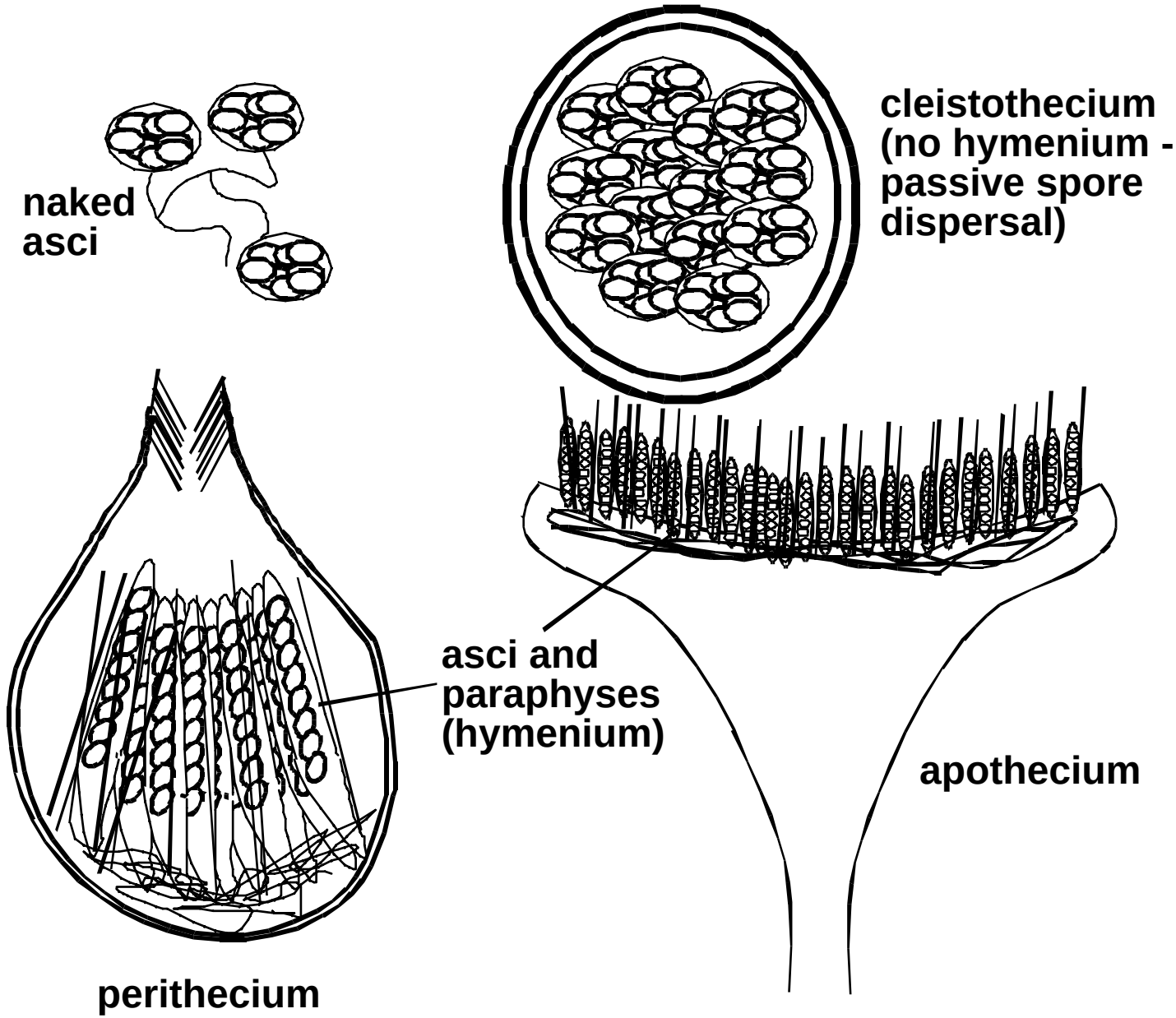
- *Petromyces* (3)
- *Neopetromyces* (1)
- *Neosartorya* (32, 3 heterothallic)
- *Chaetosartorya* (4)
- *Emericella* (34, 1 heterothallic)
- *Fennellia* (3)
- *Eurotium* (69)
- *Warcupiella* (1)
- *Hemicarpenteles* (4)
- *Neocarpenteles* (1)

148 homothallic
4 heterothallic

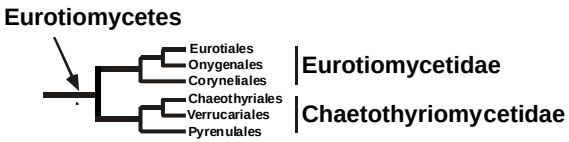
Heterothallics rare; virtually all have a conidial stage

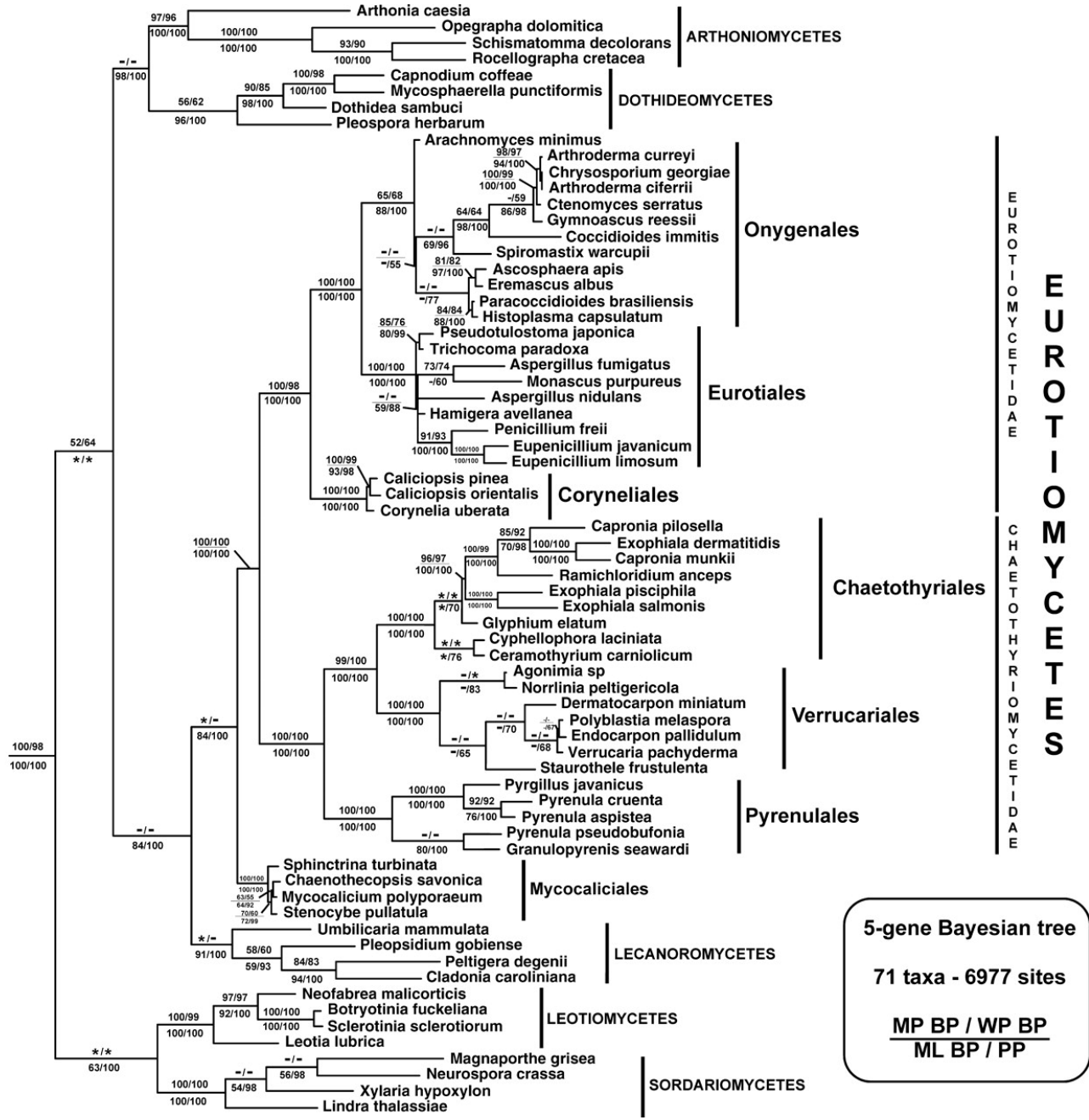
PENNSTATE

Types of ascomata



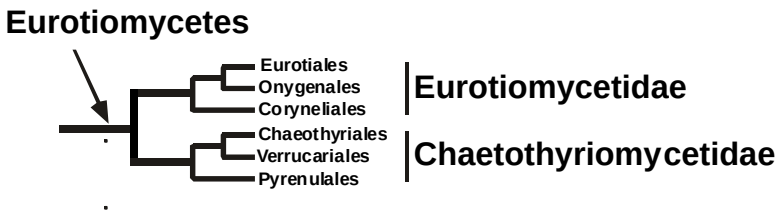
Eurotiomycetes





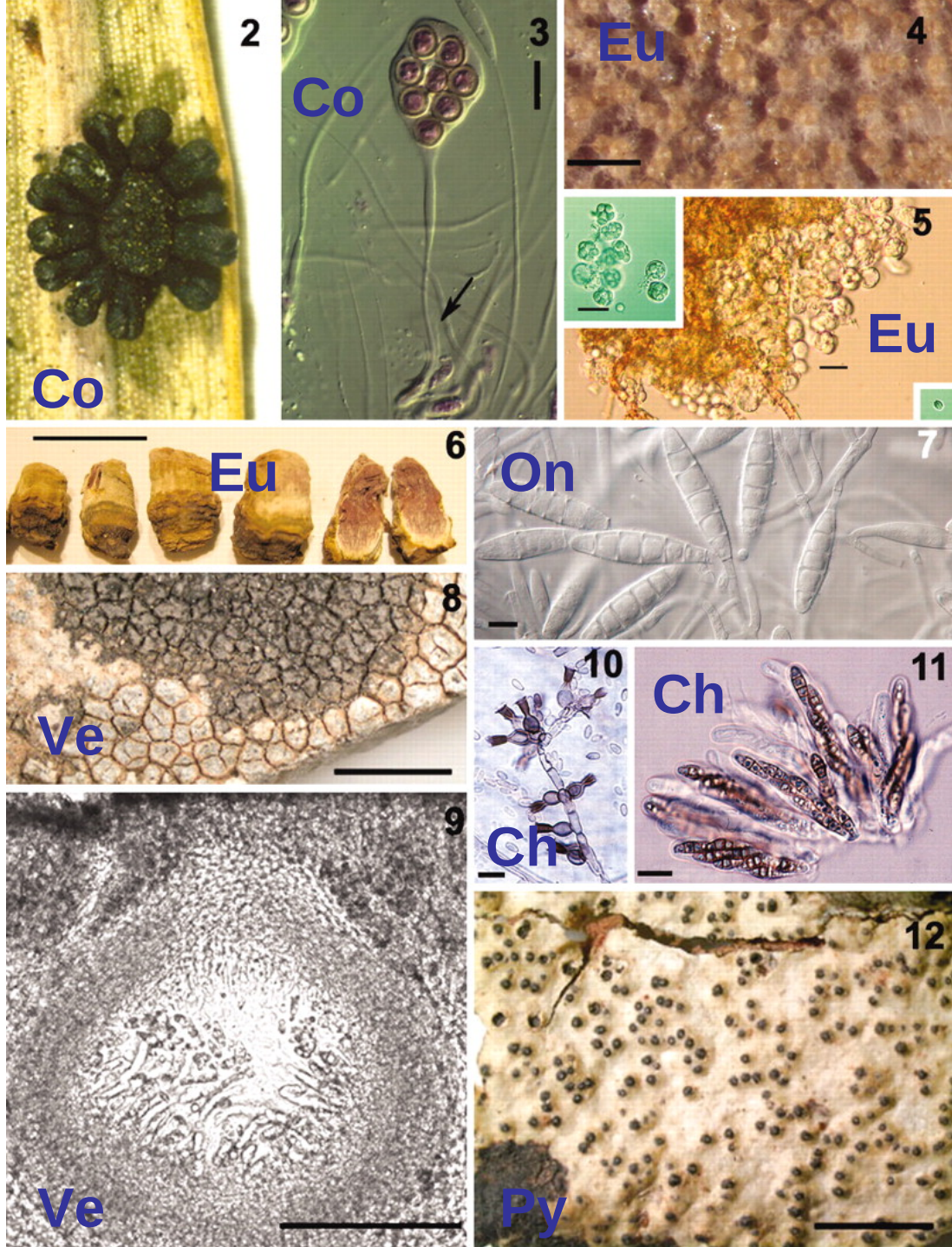
Geiser, D.M. et al. 2006. Eurotiomycetes: Eurotiomycetidae and Chaetothyriomycetidae. Mycologia 98: 1053-1064.

Eurotiomycetes



Geiser, D.M. et al. 2006. Eurotiomycetes: Eurotiomycetidae and Chaetothyriomycetidae. Mycologia 98: 1053-1064.

PENNSTATE

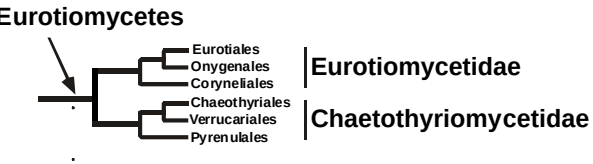


Aspergillus species have typical Eurotialean sexual characters

- Gametangia: Usually undifferentiated, composed of simple coiled hyphae
- Ascospores: Lens-shaped, single-celled, usually with equatorial rings
- Asci: Round, prototunicate (i.e., breaking down at maturity to release ascospores)
- Ascomata: Asci scattered within a cleistothecium (closed ascoma), with no hymenium and no interascal elements (i.e., paraphyses and periphyses). Cleistothecial walls composed of a variety of different tissue types depending on the genus
- Stroma: Cleistothecia may or may not be borne in developmentally distinct stromatic tissue (e.g., hülle cells, “sclerotia”)

PENNSTATE

Coryneliales: The Missing Link to Eurotiomycetes?



Geiser, D.M. et al. 2006. Eurotiomycetes: Eurotiomycetidae and Chaetothyriomycetidae. Mycologia 98: 1053-1064.

PENNSTATE

Peter Johnston

Giant Eurotialean Ascomata!

Trichocoma **-- Has biverticillate *Penicillium* anamorph**

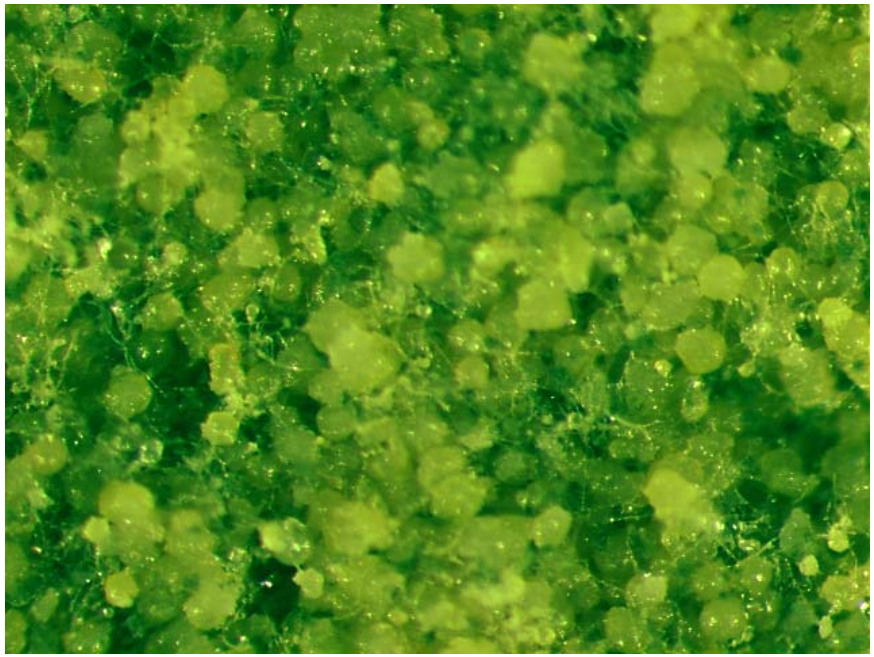
Geiser, D.M. et al. 2006. Eurotiomycetes: Eurotiomycetidae and Chaetothyriomycetidae. Mycologia 98: 1053-1064.

Elaphomyces **-- "Hart's truffle"**
Giant, stromatic cleistothecia.
Ectomycorrhizal

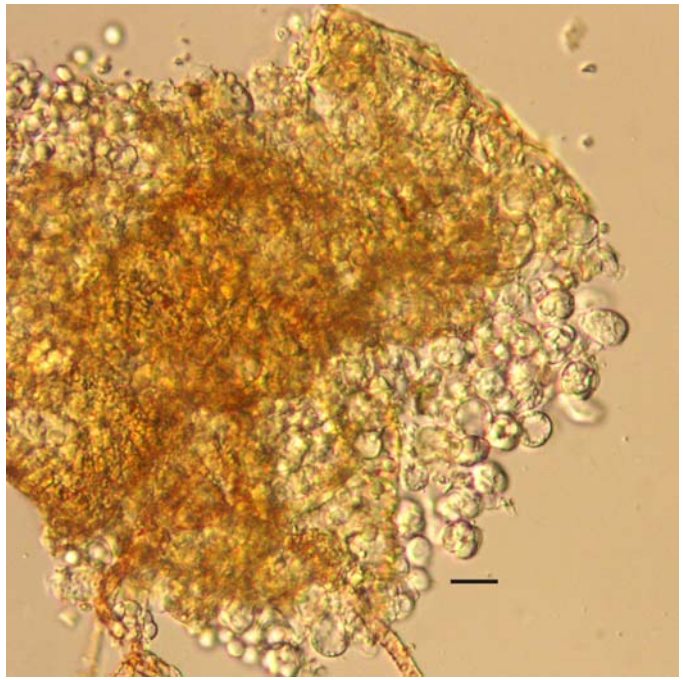
QuickTime™ and a
decompressor
are needed to see this picture.

Pseudotulostoma
Terry Henkel

Sexual characters associated with *Aspergillus*



Eurotium cleistothecia under dissecting scope



Ruptured *Eurotium* cleistothecium; Bar = 10 µm

Globose *Eurotium* asci

Lenticular *Eurotium* ascospore with two equatorial rings

PENNSTATE

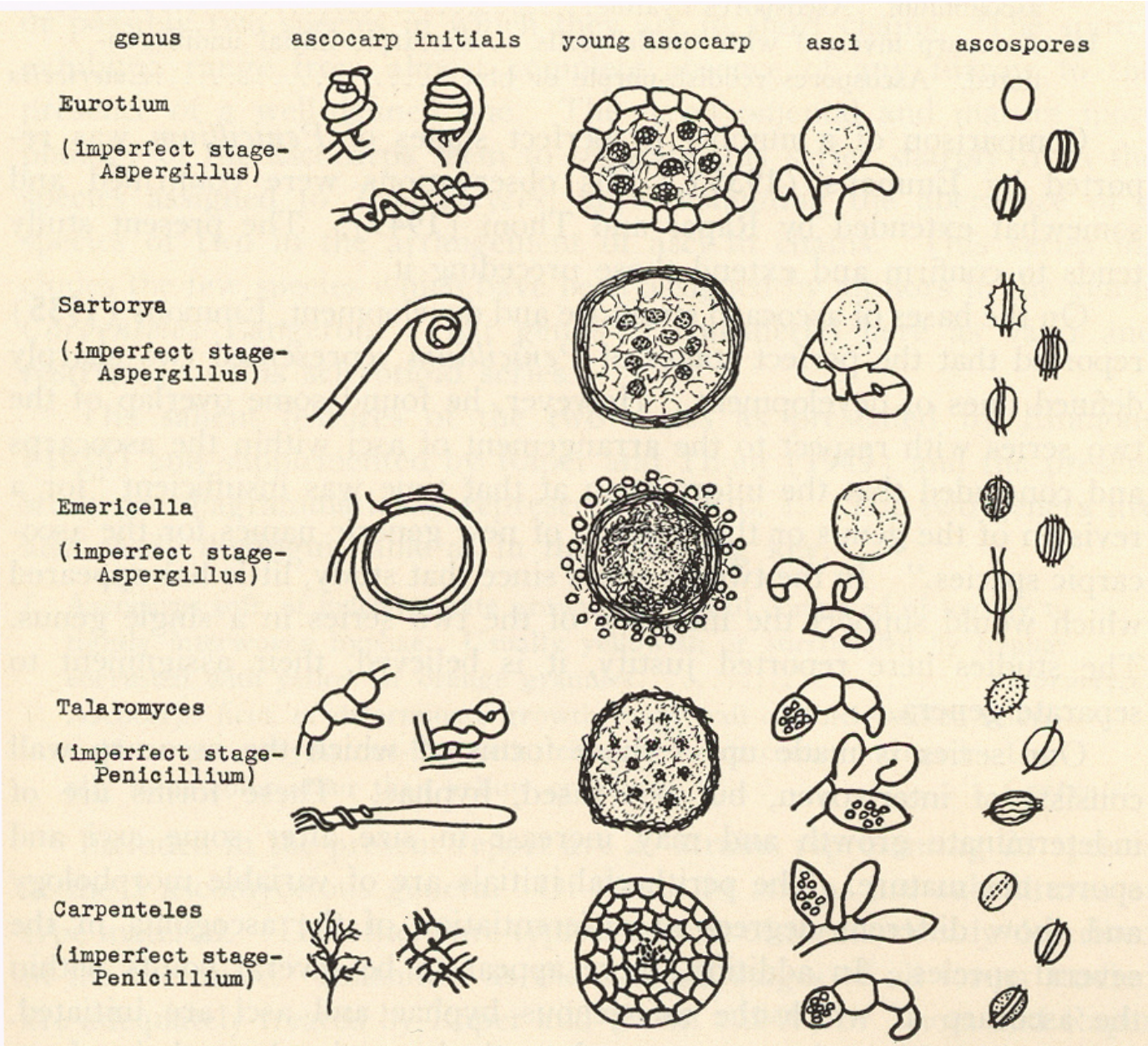
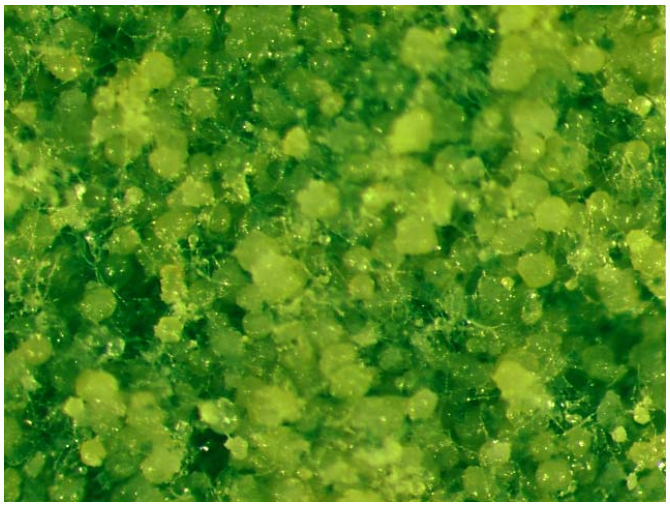


FIG. 1. Diagrammatic representation of the ascocarpic structures of the genera possessing *Aspergillus* and *Penicillium* conidial stages.

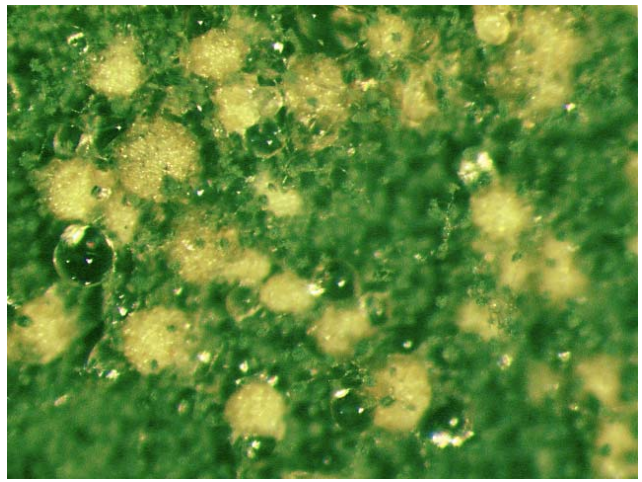
Benjamin, C.R. 1955. Ascocarps of *Aspergillus* and *Penicillium*. Mycologia 47: 669-687

PENNSTATE

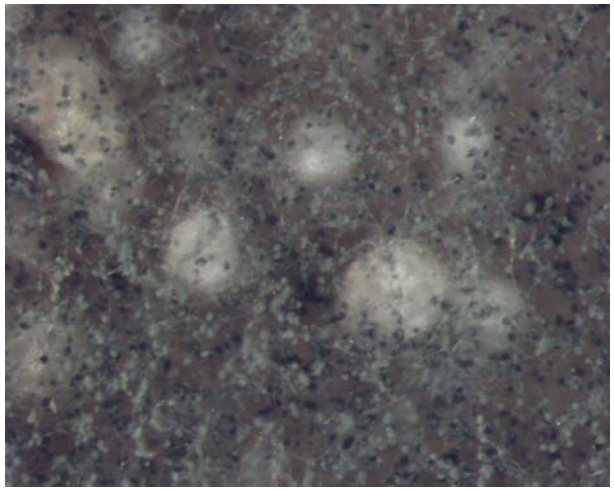
Some sexual genera associated with *Aspergillus*



Eurotium sp. Single-layered cleistothecium composed of flattened cells



Emericella nidulans: Cleistothecium surrounded by hülle cells

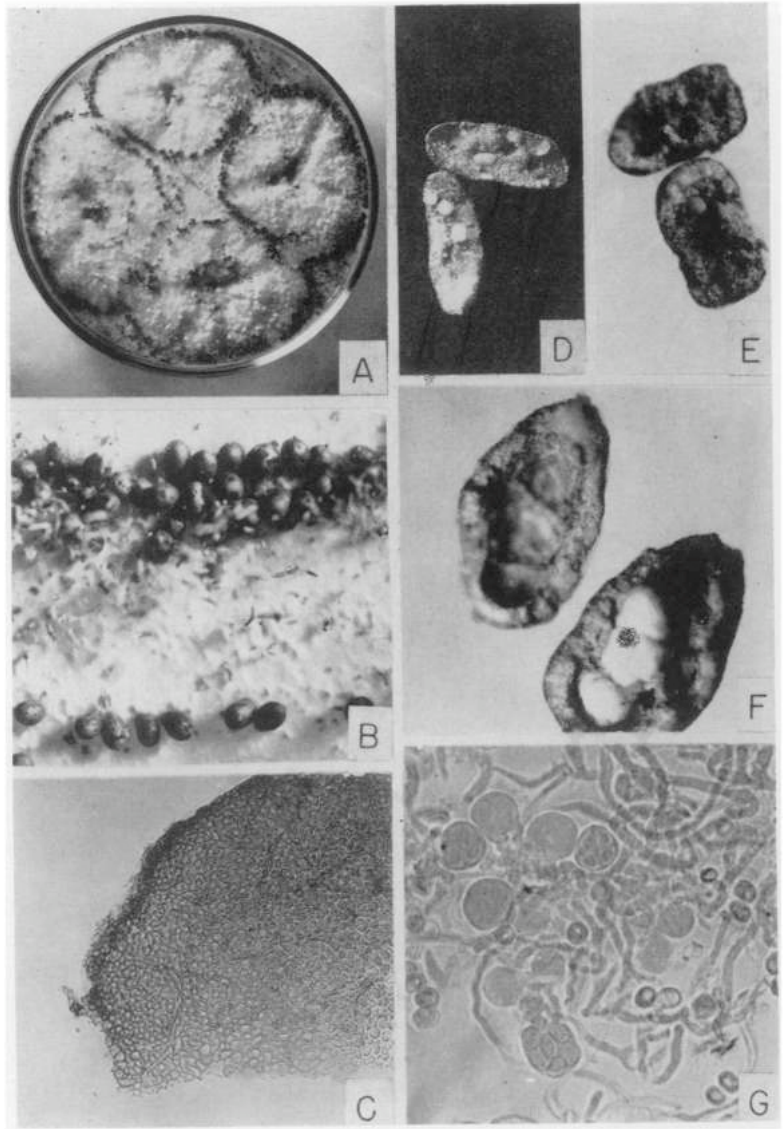


Neosartorya fischeri: Cleistothecial wall composed of flattened hyphae

PENNSTATE

Petromyces: cryptic sexual stage of *Aspergillus alliaceus*

‘Sclerotia’ turn out to be ascomata!



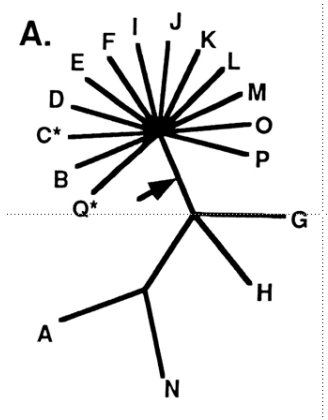
Fennell, D.I. and Warcup, J.H. 1959. Ascocarps of *Aspergillus alliaceus*. Mycologia 51: 409-415

PENNSTATE

Sclerotial species of *Aspergillus* with no known sexual stage

- *Aspergillus niger*
- *Aspergillus ochraceus*
- *Aspergillus flavus* (population genetic evidence for recombination exists)

Geiser et al. 1998. PNAS USA 95: 388-393



QuickTime™ and a decompressor are needed to see this picture.

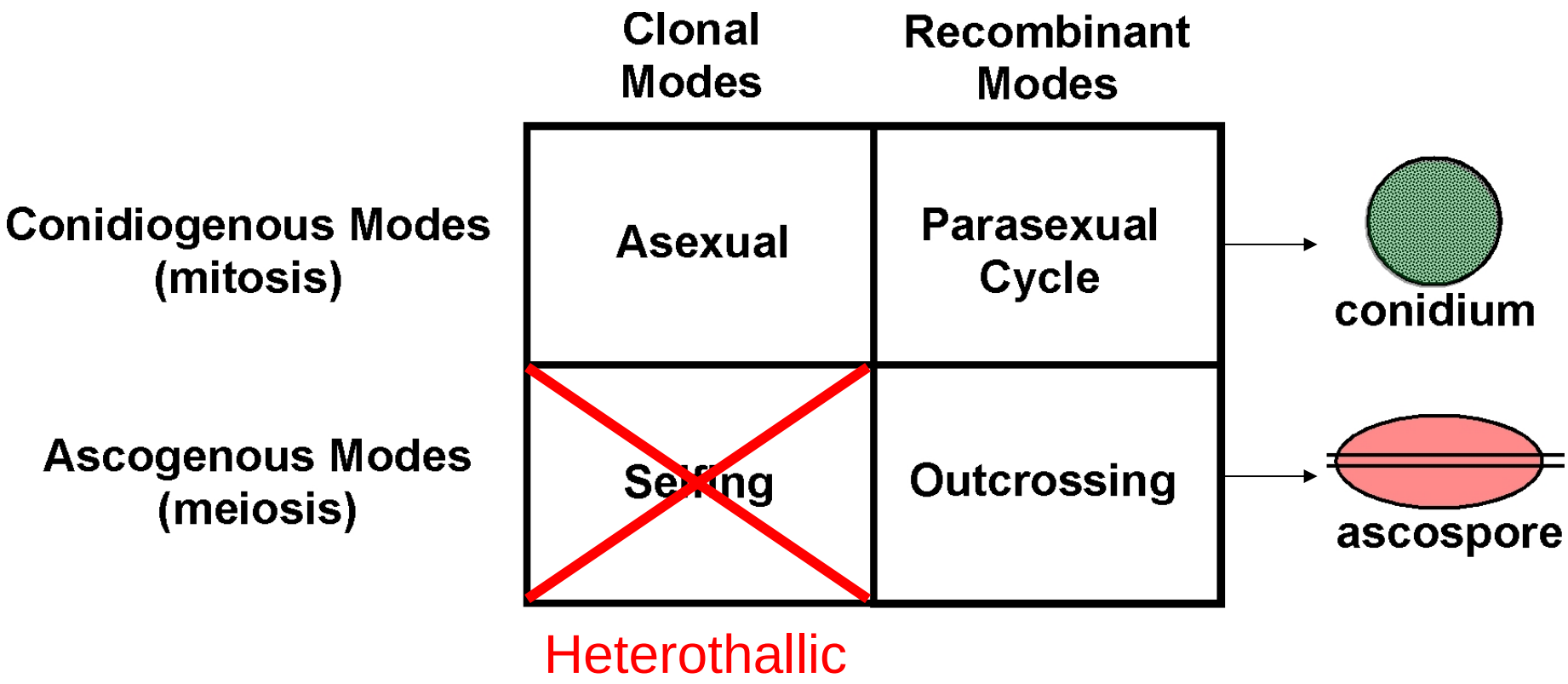
PENNSTATE

Why is there so much homothallism in *Aspergillus*?

- Heterothallism: forces outcrossing
- Homothallism: allows for selfing (which is apomictic in *Aspergillus* since it is haploid)

PENNSTATE

Genetic Consequences of Reproductive Mode



Geiser et al. 1994. PNAS USA 91: 2349-2352.

PENNSTATE

Are homothallics more clonal/less recombinant than heterothallics?

- As a rule, NO!
- *Aspergillus* (*Emericella*) *nidulans*: homothallic; low levels of linkage disequilibrium among loci (Geiser et al. 1994)
- *Fusarium graminearum*: homothallic; *highly* recombinant populations
- Homothallics may be *more* recombinant in populations than heterothallics!
- Ecological advantage of homothallism: gain benefits of ascospore with no need to find a mate
  - Ascospores are often resistant to harsh treatment
  - *Neosartorya, Eurotium*

PENNSTATE

Why is there so much homothallism in *Aspergillus*?

- The ascospore probably tends to have a major ecological role
- Homothallism allows constitutive ascospore production

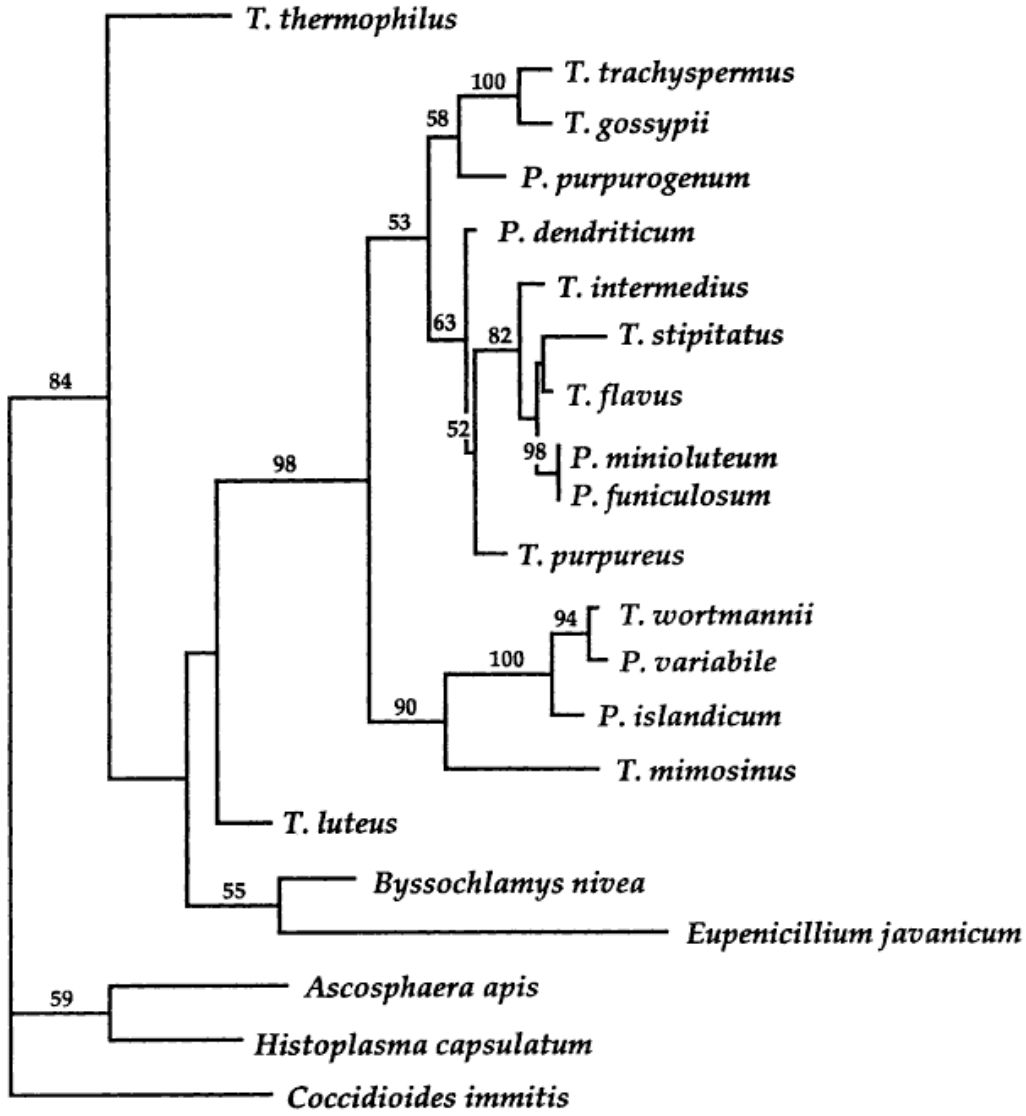
- *But maybe there is more heterothallism than people thought!*

PENNSTATE

Loss of sexual stages?

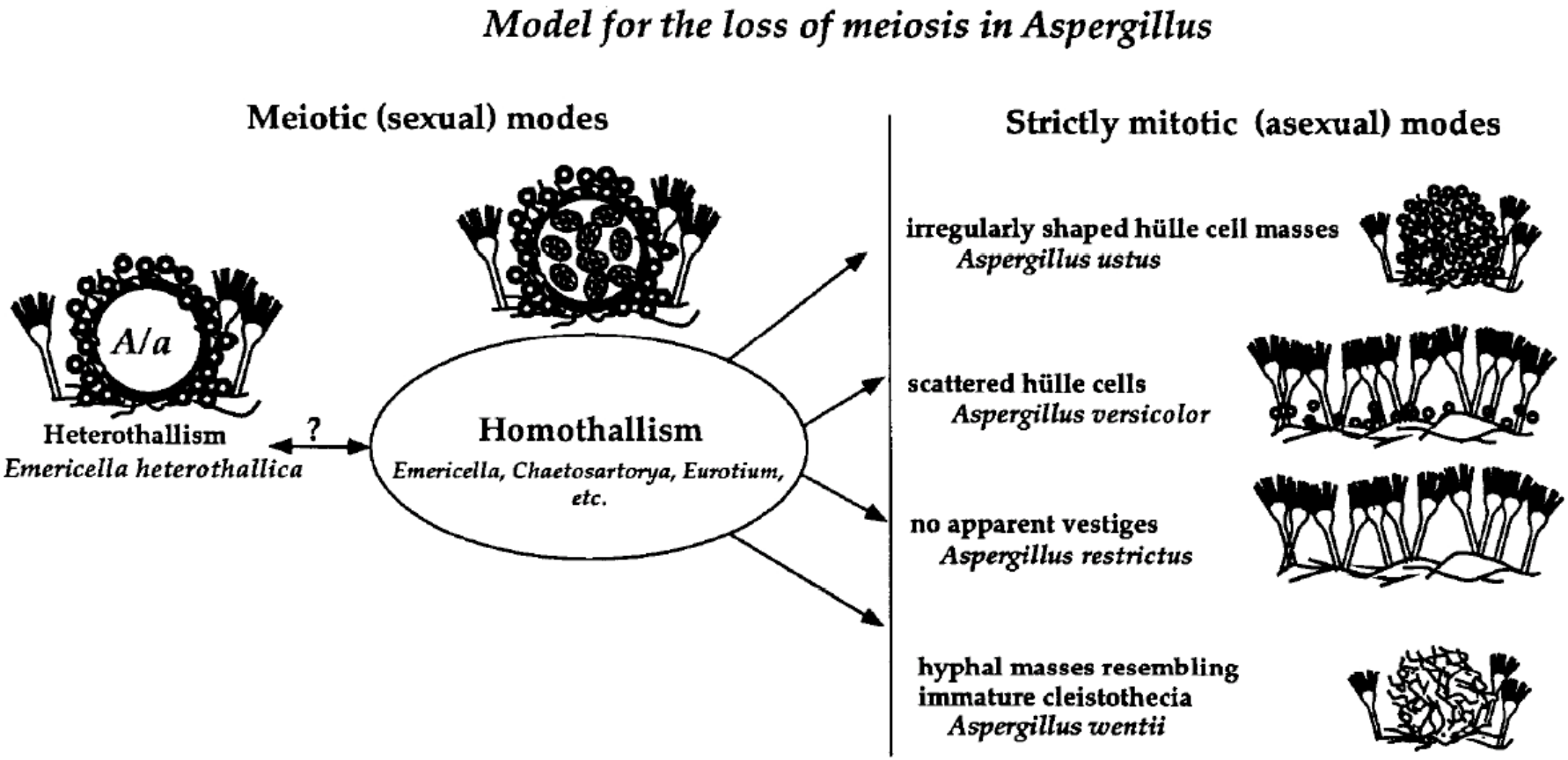
LoBuglio, K.F. et al. 1993. Phylogenetic analysis of two ribosomal DNA regions indicates multiple independent losses of the sexual *Talaromyces* state among asexual *Penicillium* species in subgenus *Biverticillium*. Mycologia 85: 592-604.

Are these asexual lineages that go extinct due to Mueller's Ratchet?



PENNSTATE

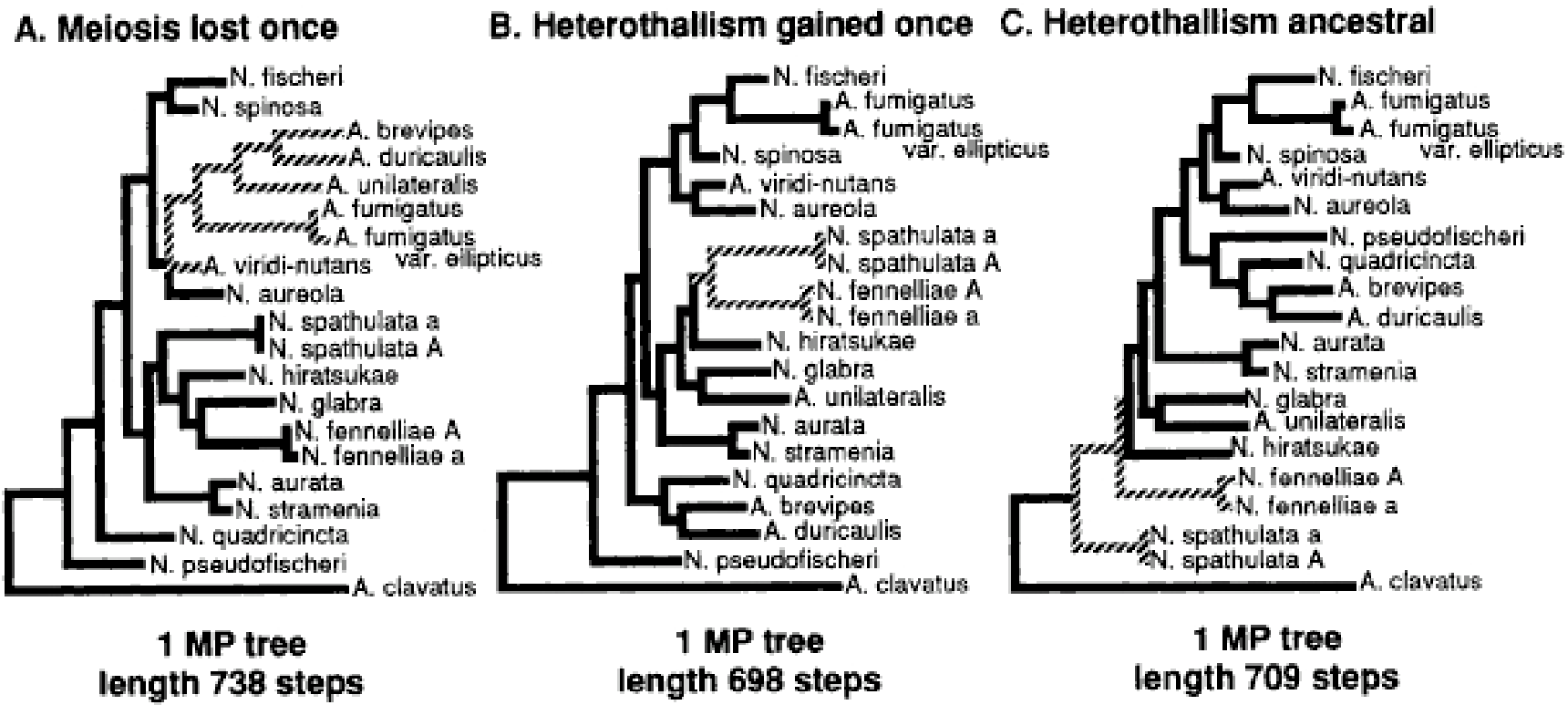
Loss of sexual stages?



Geiser et al. 1996. Loss of meiosis in *Aspergillus*. Mol. Biol. Evol. 13: 809-817.

PENNSTATE

Was heterothallism derived from homothallism in *Aspergillus*?

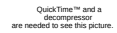


Geiser et al. 1998. Evolutionary relationships in *Aspergillus* section *Fumigati* inferred from partial beta-tubulin and hydrophobin DNA sequences. Mycologia 90: 831-845.

PENNSTATE

Molecular basis of homothallism in *Cochliobolus*

Homothallism is clearly derived from heterothallic ancestors -- is *Aspergillus* different?



Yun et al. 1999. Evolution of the fungal self-fertile reproductive lifestyle from self-sterile ancestors. PNAS USA 96: 5592-5597.

PENNSTATE
1855

Countless postdocs wasted countless hours trying to isolate MAT idiomorphs in *Aspergillus* via PCR and hybridization

What we really needed were some complete *Aspergillus* genome sequences!

PENNSTATE

Aspergillus fumigatus: appears to be heterothallic!

QuickTime™ and a
decompressor
are needed to see this picture.

Paoletti, M. et al. 2005. Evidence for sexuality in the opportunistic fungal pathogen *Aspergillus fumigatus*. Current Biology 15: 1242-1248.
Pringle, A. et al. 2005. Cryptic speciation in the cosmopolitan and clonal human pathogenic fungus *Aspergillus fumigatus*. Evolution 59: 1886-1899.

PENNSTATE

If *A. fumigatus* is heterothallic, then why has no one seen a sexual stage?

- Isolates of opposite mating-type must be paired, and the correct conditions for sexual development must be employed
- This takes patience and careful observation (it isn't a coincidence that two of the known heterothallic *Aspergillus* species were described by the same person (J. Kwon-Chung)

PENNSTATE

Hypotheses about sexual and asexual modes in *Aspergillus*

- Perhaps most or even all *Aspergillus* species with no known sexual stage are actually heterothallic. Paul Dyer's lab is pursuing this.
- Evidence for historical recombination (random association of loci) is generally found in 'asexual' fungi. Never assume a fungus is clonal without population genetic evidence.
- As a general rule, homothallism is probably derived from heterothallism in *Aspergillus*, as it is in other ascomycetes.
- The relative abundance of homothallic *Aspergilli* is probably more about ecology than genetics.

So who cares about *Aspergillus* sex?

- *Aspergillus* species known to be meiotic (homothallic) tend to be minor players clinically
  - As a general rule, there is a tradeoff: more ascospores means fewer conidia
- Clinicians should be aware of (or more realistically, have access to information about) sexual stages, but the sexual stage itself probably doesn't have much clinical relevance, other than as a morphological character useful in identification

Recombination is important

- Recombinant organisms are a tougher target
  - Fungicide resistance
  - Novel virulence
- The recombinant/clonal population dynamics of pathogenic fungi are important to consider…but one cannot assume relative levels of recombination/clonality based on the presence/absence of a sexual stage

Thanks to…

- Bill Timberlake, Mike Arnold, John Taylor
- NSF-sponsored All Fungal Tree of Life and Deep Hypha grants: esp. Meredith Blackwell, Joey Spatafora, Cécile Guiedan, Conrad Schoch
- NSF, USDA/NRI, Bausch & Lomb for their support

Sexual stages and names

- A special provision in the International Code of Botanical Nomenclature allows pleiomorphic fungi to have two names:
  - Anamorph (Phylum Deuteromycota)
  - Teleomorph (Phylum Ascomycota)
- The teleomorph name takes precedence over the anamorph as assigned to the whole fungus (i.e., the Holomorph)
- As strictly anamorphic fungi (e.g., *Aspergillus fumigatus*) are connected to their correct teleomorphs (*Neosartorya*), they can retain both names, but the teleomorph name takes precedence.