Mycologia, 101(3), 2009, pp. 423–429. DOI: 10.3852/09-011

Sexual reproduction in *Aspergillus flavus*

Bruce W. Horn[1]

National Peanut Research Laboratory, Agricultural Research Service, U.S. Department of Agriculture, Dawson, Georgia 39842

Geromy G. Moore
Ignazio Carbone

Center for Integrated Fungal Research, Department of Plant Pathology, North Carolina State University, Raleigh, North Carolina 27695

Abstract: *Aspergillus flavus* is the major producer of carcinogenic aflatoxins in crops worldwide and is also an important opportunistic human pathogen in aspergillosis. The sexual state of this heterothallic fungus is described from crosses between strains of the opposite mating type. Sexual reproduction occurred between sexually compatible strains belonging to different vegetative compatibility groups. Multiple, indehiscent ascocarps containing asci and ascospores formed within the pseudoparenchymatous matrix of stromata, which places the fungus in genus *Petromyces*. The teleomorph of *P. flavus* could not be distinguished from that of *P. parasiticus* (anamorph = *A. parasiticus*), another aflatoxin-producing species, based on morphology of the sexual structures. The two species can be separated by anamorph morphology, mycotoxin profile and molecular characters.

Key words: aflatoxin, *Aspergillus alliaceus*, *Aspergillus parasiticus*, heterothallism, *Petromyces alliaceus*, *Petromyces flavus*, *Petromyces parasiticus*, Trichocomaceae

INTRODUCTION

Aspergillus flavus Link is the major producer of aflatoxins worldwide in corn, peanuts, tree nuts, cottonseed, spices and other crops. These polyketide-derived mycotoxins are among the most carcinogenic compounds known from nature and are also acutely hepatotoxic as well as immunosuppressive (Eaton and Groopman 1994, Turner et al 2003). Aflatoxigenic strains of *A. flavus* generally produce aflatoxin B_1, the most toxic of the naturally occurring aflatoxins (Cullen and Newberne 1994), and lesser amounts of B_2 (Horn et al 1996). Aflatoxins are highly regulated in human and animal food in more than 100 countries (van Edmond and Jonker 2005), and commodities with aflatoxin concentrations that exceed established limits either must be reprocessed or destroyed. In regions of the world where aflatoxins are not regulated, outbreaks of aflatoxicosis and associated deaths in human populations occur periodically (Krishnamachari et al 1975, Lye et al 1995, Azziz-Baumgartner et al 2005).

In addition to aflatoxins *A. flavus* produces another unrelated mycotoxin, cyclopiazonic acid (CPA), an indol-tetramic acid that targets the liver, kidneys and gastrointestinal tract in animals (Burdock and Flamm 2000). Aflatoxins and CPA often cocontaminate agricultural products, and several of the symptoms associated with turkey ''X'' disease in poults that led to the discovery of aflatoxins in the early 1960s can be attributed to CPA (Cole 1986). *Aspergillus flavus* is also an important opportunistic human pathogen in aspergillosis. The species is the most common cause of aspergillosis involving skin, oral mucosa and subcutaneous tissue and is second only to *A. fumigatus* Fresen. in invasive aspergillosis that includes the systemic infection of immunocompromised patients (Hedayati et al 2007).

Aspergillus flavus belongs to section *Flavi*, which contains an assemblage of phylogenetically related aflatoxin- and nonaflatoxin-producing species (Peterson 2008). One of the hallmarks of *A. flavus* populations is the extreme genetic diversity, as reflected by differences in morphology and mycotoxin production (Bayman and Cotty 1993, Horn et al 1996) and by the large number of DNA fingerprint groups and vegetative compatibility groups (VCG) (Bayman and Cotty 1991, Horn and Greene 1995, Wicklow et al 1998). The vegetative compatibility system in fungi is determined by a series of *het* loci whose alleles all must be identical for stable hyphal fusions to occur (Leslie 1993). Vegetatively compatible individuals often are grouped into VCG and in aflatoxigenic fungi, most variation in morphology and mycotoxin production occurs among VCG, with little variation occurring within a VCG (Bayman and Cotty 1993, Horn et al 1996). Considerable genetic diversity is also present in populations of *A. parasiticus* Speare (Horn and Greene 1995, Horn et al 1996, McAlpin et al 1998, Carbone et al 2007a), a closely related aflatoxin-producing species from section *Flavi* that is most prevalent in peanuts. Both *A. flavus* and *A. parasiticus* are heterothallic, with individuals containing either a *MAT1-1* or *MAT1-2* mating-type gene (Ramirez-Prado et al 2008). In *A.*

Accepted for publication 29 January 2009.
[1] Corresponding author. E-mail: bruce.horn@ars.usda.gov

TABLE I. Incidences of sexual state in *Aspergillus flavus* crosses between opposite mating types

MAT1-1 [1]		*MAT1-2* [1]					
NRRL strain[2]	VCG[3]	NRRL strain[2]	VCG[3]	Number of sclerotia/ stromata per slant[4]	Number of sclerotia/ stromata examined	% with ascocarps[4,5]	% with ascospore-bearing ascocarps[4,6]
29532	58	29506	32	601 ± 15	300	10.0 ± 2.6	0
29512	38	29506	32	707 ± 81	300	5.0 ± 1.0	0.3 ± 0.6
29534	60	29506	32	633 ± 57	300	4.3 ± 3.2	0.7 ± 0.6
29473	17	29506	32	604 ± 34	440	8.2 ± 7.0	6.5 ± 4.9
29518	44	29519	45	1 ± 2	7	29.1 ± 5.9	12.5 ± 17.7
29537	63	29474	23	97 ± 15	386	33.5 ± 5.9	22.8 ± 2.6
29507	33	29506	32	489 ± 85	300	33.0 ± 4.0	28.7 ± 4.6
29530	56	29519	45	117 ± 42	308	49.2 ± 11.9	37.7 ± 13.3
29537	63	29536	62	342 ± 2	300	72.7 ± 4.0	50.3 ± 14.0
29507	33	29487	25	392 ± 40	300	52.3 ± 9.8	51.0 ± 10.4
29526	52	29519	45	9 ± 9	47	67.5 ± 26.2	66.2 ± 25.5
29473	17	29487	25	445 ± 35	300	79.3 ± 5.1	78.3 ± 5.8

[1] Mating-type designations from Ramirez-Prado et al (2008).
[2] Strain numbers (NRRL) from Agricultural Research Service Culture Collection, Peoria, Illinois.
[3] Vegetative compatibility groups based on Horn and Greene (1995).
[4] Means ± s.d. (n = 3–5 culture slants).
[5] Percentage of total number of sclerotia/stromata examined containing one of more ascocarps irrespective of the presence of ascospores.
[6] Percentage of total number of sclerotia/stromata examined containing one or more ascospore-bearing ascocarps.

parasiticus, crosses between opposite mating types led to the discovery of sexual reproduction and genetic recombination (Horn et al 2009a). The sexual state of *A. parasiticus* was similar to that of *Petromyces alliaceus* Malloch & Cain (anamorph = *A. alliaceus* Thom & Church), a nonaflatoxin-producing species from section *Flavi*, and therefore was assigned to the same genus and described as *P. parasiticus* B.W. Horn et al (2009b).

Because of the close relationship between *A. flavus* and *P. parasiticus*, *A. flavus* strains of the opposite mating type were crossed in an attempt to induce sexual reproduction. The teleomorph associated with *A. flavus* is formally described in this paper.

MATERIALS AND METHODS

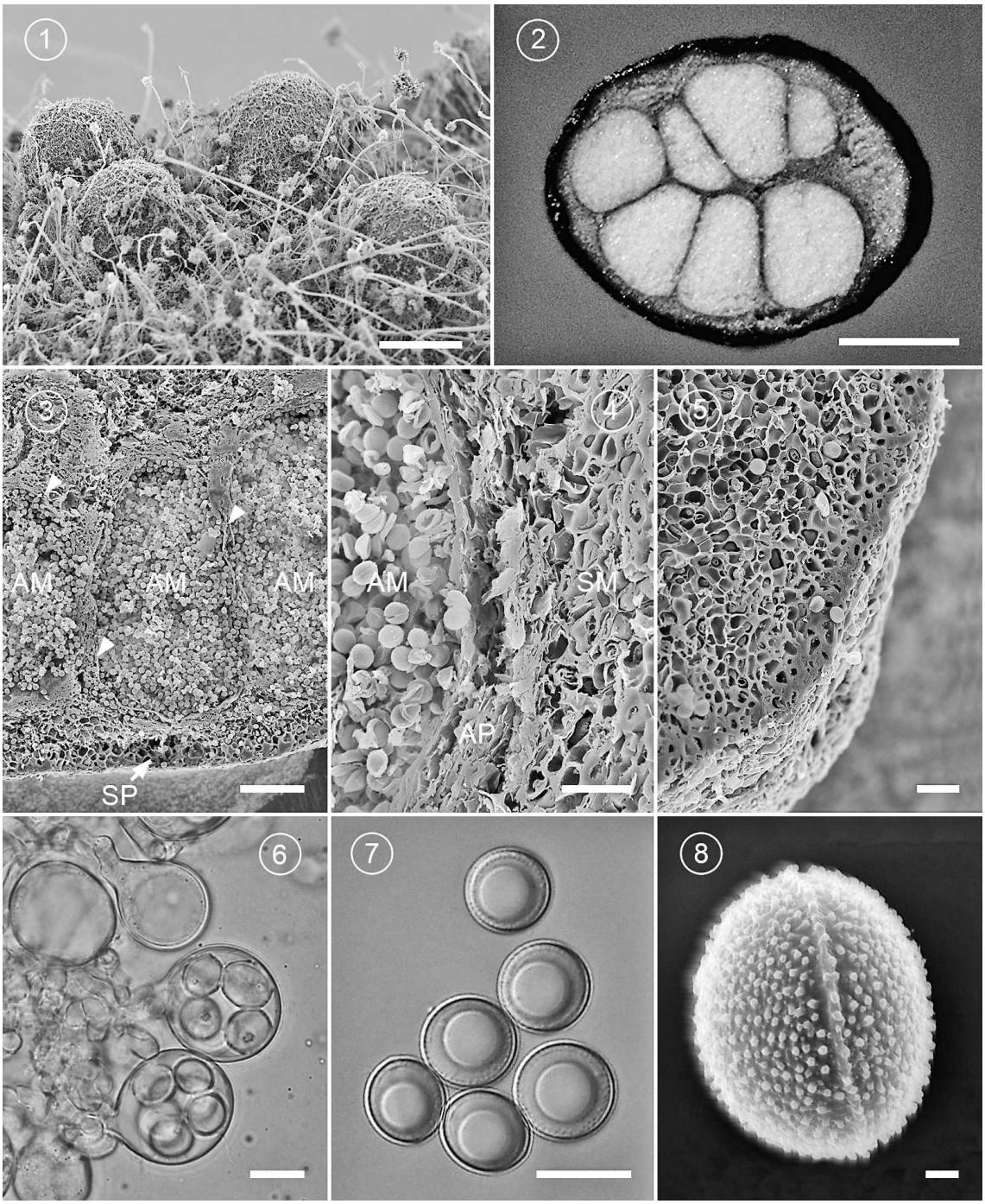
Strains of *A. flavus* were obtained from soil and peanut seeds in a single peanut field in Terrell County, Georgia, USA (Horn and Greene 1995). Mating-type genes *MAT1-1* and *MAT1-2* were identified by Ramirez-Prado et al (2008) and VCG were determined by Horn and Greene (1995) (TABLE I). *Aspergillus flavus* strains of the opposite mating type were paired on slants of mixed cereal agar (McAlpin and Wicklow 2005) and slants were incubated 6–11 mo at 30 C in sealed plastic bags (Horn et al 2009a). Sclerotia/stromata were harvested from slants and examined for ascocarp formation according to Horn et al (2009a). Specimens were sectioned and prepared for bright field, differential interference contrast and scanning electron microscopy as described by Horn et al (2009a, b). The *Aspergillus* anamorphic state was examined from cultures grown on Czapek agar and malt extract agar (Raper and Fennell 1965).

TAXONOMY

Petromyces flavus B.W. Horn, I. Carbone et G.G. Moore, sp. nov.

MycoBank MB512910 FIGS. 1–8

Coloniae in agaro Czapekii crescentes post 7 dies in temperatura 25 C diam 3.5–5.5 cm atque in temperatura 37 C 5.5–7.0 cm attingentes, velutinae vel leniter floccosae, cinereo-virides, saepe per sclerotia dominatae. Sclerotia stromataque globosa vel ellipsoidalia, 300–1250 μm, atro-brunnea vel atra, contextum pseudoparenchymaticum continentia. Ascocarpi 1–7, intra stromata geniti, globosi vel subglobosi vel irregulariter formati, maturitate 150–630 μm, non ostiolati, peridio 9.8–27.0 μm crasso circumdati. Asci globosi vel subglobosi, 19.0–30.0 × 16.5–26.5 μm, plerumque ascosporas 8 continentes. Ascosporae ubique oblatae, sed in aspectu frontali globosae vel late ellipsoidales 8.0–12.5 × 7.5–12.0 μm, leniter tuberculatae, crista tenui aequatoriali praeditae, hyalinae vel pallide brunneae, gutta olei unica magna continentes. Capitula conidialia uniseriata vel biseriata, radiata vel columnaria, diametro usque ad 600 μm. Stipites proxime sub vesicula 10–23 μm lati, longitudine 300–1200 μm, hyalini vel pallide brunnei, echinulati. Vesiculae globosae vel subglobosae, 15.0–70.0 μm diam. Metulae 6.0–10.0 × 4.0–7.5 μm. Phialides 6.0–12.0 × 3.0–4.5 μm. Conidia globosa vel subglobosa, 3.0–6.0 μm, laevia vel leniter asperata.



FIGS. 1–8. *Petromyces flavus*. 1. Sclerotia formed in culture. 2. Sectioned stroma containing seven ascocarps. 3. Cross section of stroma with three ascocarps containing ascospores; arrowheads show peridia of ascocarps. 4. Ascocarp peridium separating stromal matrix from ascocarp matrix containing ascospores. 5. Section of stroma showing outer peridium and pseudoparenchymatous tissue of matrix. 6. Asci containing ascospores. 7. Ascospores containing single oil droplets. 8. Ascospore showing finely tuberculate ornamentation and an equatorial ridge. 1, 3–5, 8 = SEM; 6 = bright field microscopy; 7 = differential interference contrast microscopy. Bars: 1, 2 = 400 μm; 3 = 100 μm; 4, 5 = 20 μm; 6, 7 = 10 μm; 8 = 1 μm. Abbreviations: SP, stromal peridium; SM, stromal matrix; AP, ascocarp peridium; AM, ascocarp matrix.

Colonies on Czapek agar attaining 3.5–5.5 cm diam in 7 d at 25 C; growth at 37 C in 7 d reaching 5.5–7.0 cm diam. Colony surface velvety to occasionally floccose, often dominated by sclerotia. Conidial heads en masse grayish green (29–30E4–6; Kornerup and Wanscher 1978) at 14 d. Reverse light yellow brown. *Sclerotia* (FIG. 1) and *stromata* similar in external appearance, globose to ellipsoidal, (250–)300–1250

(–1400) μm, white becoming pink brown and finally dark brown to black; inner matrix light to dark brown, consisting of pseudoparenchymatous tissue (FIG. 5). *Ascocarps* (FIGS. 2, 3) produced within stromata, globose to subglobose or irregularly shaped, nonostiolate, with white to light brown interior; each stroma containing 1–7(–10) fertile ascocarps, 1–6(–12) infertile ascocarps, or a combination of the two; *fertile ascocarps* (110–)150–630(–660) μm (mean = 328 ± 105 μm, *n* = 151) × (80–)100–540(–570) μm (mean = 253 ± 91 μm); *infertile ascocarps* (40–)60–210(–230) μm (mean = 118 ± 32 μm, *n* = 86) × (35–)50–175(–190) μm (mean = 95 ± 27 μm); *ascocarp peridium* (FIG. 4) 9.8–27.0 μm thick, yellow brown to red brown, consisting of compact layers of irregular flattened cells. *Asci* (FIG. 6) globose to subglobose, often containing eight inordinately arranged ascospores but irregular numbers (1–6) not uncommon, (17.5–)19.0–30.0(–32.0) μm (mean = 24.1 ± 3.3 μm, *n* = 48) × (15.0–)16.5–26.5(–28.0) μm (mean = 21.2 ± 2.9 μm). *Ascospores* (FIGS. 7, 8) oblate, finely tuberculate with a thin equatorial ridge, hyaline to pale brown, generally containing a single large oil droplet, globose to broadly ellipsoidal in face view, variable in size, (7.5–)8.0–12.5(–14.0) μm (mean = 10.0 ± 0.9 μm, *n* = 100) × (7.0–)7.5–12.0(–13.0) μm (mean = 9.3 ± 0.9 μm). *Conidial heads* uniseriate or biseriate, radiate to columnar, up to 600 μm diam. *Stipes* (100–)300–1200(–2000) μm long, 10–23 μm wide immediately below vesicle, hyaline to pale brown, echinulate. *Vesicles* globose to subglobose, 15–70 μm diam. *Metulae* 6.0–10.0 × 4.0–7.5 μm. *Phialides* 6.0–12.0 × 3.0–4.5 μm. *Conidia* globose to subglobose, 3.0–6.0(–7.5) μm, smooth to finely roughened.

Holotype. Dried slant culture with ascocarp-bearing stromata consisting of *A. flavus* NRRL 29473 (*MAT1-1*) crossed with *A. flavus* NRRL 29487 (*MAT1-2*); deposited with the National Fungus Collections, US Department of Agriculture, Beltsville, Maryland (BPI 878851). NRRL 29473 was isolated from a peanut seed harvested 14 Oct 1992 from a field in Terrell County, Georgia, USA, and NRRL 29487 was isolated from soil collected 8 Jun 1992 from the same peanut field (Horn and Greene 1995). Living cultures of both strains have been deposited in the ARS Culture Collection, Peoria, Illinois, USA.

Additional sexual crosses examined: All *A. flavus* strains were obtained from soil and peanut seeds as described for the holotype. Additional crosses (in order of *MAT1-1* × *MAT1-2*) were: NRRL 29532 × 29506; NRRL 29512 × 29506; NRRL 29534 × 29506; NRRL 29473 × 29506; NRRL 29518 × 29519; NRRL 29537 × 29474; NRRL 29507 × 29506; NRRL 29530 × 29519; NRRL 29537 × 29536; NRRL 29507 × 29487; NRRL 29526 × 29519.

DISCUSSION

The sexual state of *A. flavus* with its formation of multiple, nonostiolate ascocarps within the pseudoparenchymatous matrix of stromata clearly places the fungus in genus *Petromyces* Malloch & Cain (1972). *Petromyces flavus* shares *Aspergillus* section *Flavi* with two other sexually reproducing species, *P. parasiticus* and *P. alliaceus*. Morphological characters of the teleomorph in this study were insufficient to distinguish *P. flavus* from *P. parasiticus*. Dimensions of sexual structures in *P. flavus* were similar to those of *P. parasiticus* (Horn et al 2009b) respectively for stromata (300–1250 μm vs. 300–1200 μm), fertile ascocarps (150–630 × 100–540 μm vs. 160–530 × 140–420 μm), asci (19.0–30.0 × 16.5–26.5 μm vs. 19.0–29.0 × 16.0–27.0 μm) and ascospores (8.0–12.5 × 7.5–12.0 μm vs. 7.1–13.0 × 6.5–12.0 μm). Ascospores of both *P. flavus* and *P. parasiticus* are oblate and finely tuberculate and encircled by a thin equatorial ridge. Although ascospore ornamentation in *P. flavus* was often finer than that of *P. parasiticus* under SEM, there was considerable overlap in the coarseness of ornamentation and the character could not unequivocally separate the two species. In contrast the smooth and small (5.5–9.0 × 5.0–7.0 μm) ascospores of *P. alliaceus* (Fennell and Warcup 1959) distinguish this species from both *P. flavus* and *P. parasiticus*.

The *Aspergillus* anamorphic states of *P. flavus* and *P. parasiticus* are easily separated morphologically. Conidial heads of *P. flavus* when cultured on Czapek agar are grayish green, whereas those of *P. parasiticus* are dark green (Raper and Fennell 1965, Horn et al 1996, Klich 2002). Furthermore conidia of *P. flavus* under the light microscope are smooth to finely roughened and those of *P. parasiticus* are coarsely roughened (Klich and Pitt 1988). The anamorphic state of *P. alliaceus* differs markedly from both *P. flavus* and *P. parasiticus*. Conidial heads of *P. alliaceus* are yellow orange to cinnamon buff, and conidia are smooth and subglobose to oval and are smaller (2.5–4.0 × 2.0–3.5 μm) than those of *P. flavus* (3.0–6.0 μm) and *P. parasiticus* (4.0–6.0 μm) (Raper and Fennell 1965, Klich 2002).

Petromyces species also differ in their mycotoxin profiles. *Petromyces flavus* produces aflatoxins B_1 and B_2 and CPA, whereas *P. parasiticus* produces aflatoxins B_1, B_2, G_1 and G_2 but not CPA (Horn et al 1996). However mycotoxin profiles are not entirely diagnostic for species identification because many strains of *P. flavus* do not produce aflatoxins or CPA (Horn and Dorner 1999). Nonaflatoxigenic strains of *P. parasiticus* are rare, and strains that do not produce aflatoxins usually accumulate *O*-methylsterigmatocys-

tin, the immediate precursor to aflatoxin B_1 (Horn et al 1996). *Petromyces alliaceus* does not produce aflatoxins but instead accumulates the nephrotoxic and carcinogenic mycotoxin, ochratoxin A (Bayman et al 2002).

Molecular studies have verified the close relationship between *P. flavus* and *P. parasiticus*. Kurtzman et al (1986) first compared *A. flavus* and *A. parasiticus* at the molecular level with nuclear DNA complementarity. Because of the relatively high DNA homology (70%) they considered *A. flavus* and *A. parasiticus* to be conspecific and designated *A. parasiticus* as a subspecies of *A. flavus*. Subsequent RFLP, AFLP fingerprint and DNA sequence analyses clearly differentiate *A. flavus* from *A. parasiticus*, suggesting they are distinct species (Moody and Tyler 1990a, b; Lee et al 2006; Barros et al 2007; Peterson 2008). Phylogeny within section *Flavi* based on DNA sequences from four loci (Peterson 2008) is correlated with morphological differences among *Petromyces* species. *Petromyces alliaceus* is well separated from the *A. flavus/A. parasiticus* clade and differs markedly from this clade in both teleomorphic and anamorphic states. In contrast the teleomorphs of sibling species *P. flavus* and *P. parasiticus* are nearly identical and the anamorphs differ in few characters.

In summary *P. flavus* as a holomorph is best distinguished from *P. parasiticus* by anamorph morphology, mycotoxin profile and molecular characters. Field and laboratory observations also suggest that *P. flavus* and *P. parasiticus* differ ecologically in several respects. *Petromyces flavus* is the dominant aflatoxin-producing species in the majority of crops (Horn 2005a). In contrast *P. parasiticus* is more restricted in its crop specificity and is most prevalent in peanuts and uncommon in aerial crops such as corn and cottonseed. Peanut pods develop underground and are relatively cool compared to aerial crops that often are exposed to direct sunlight; hence the occurrence of *P. parasiticus* in peanuts might be due to its lower temperature optimum for invading crops (Horn 2005b). In addition *P. flavus* is a common agent for human aspergillosis, whereas clinical cases involving *P. parasiticus* have never been reported (Hedayati et al 2007).

Small infertile ascocarps frequently were observed in stromata of *P. flavus* and also are present in *P. parasiticus* (Horn et al 2009a). Furthermore both species show large differences among crosses in the frequency of ascocarp and ascospore formation (Horn et al 2009a, TABLE I). Differences in fertility might be due to various prezygotic and postzygotic genetic barriers that override the sexual compatibility system (Horn et al 2009a, b). In the present study all *A. flavus* crosses involved pairs of sexually compatible strains belonging to different VCG (TABLE I). Fertile ascocarps also developed in *P. parasiticus* stromata when sexually compatible strains from different VCG were crossed (Horn et al 2009a). Therefore the vegetative compatibility system is not a barrier to sexual reproduction in either species.

The involvement of different VCG in *P. flavus* crosses indicates that sexual reproduction is occurring between strains that often differ in their capacity to produce mycotoxins. Populations of *P. flavus* show a high level of variation in mycotoxin production, with individuals producing both aflatoxins and CPA, aflatoxins alone, CPA alone or neither mycotoxin (Horn et al 1996, Horn and Dorner 1999). Aflatoxins are synthesized by a well characterized gene cluster containing approximately 25 genes located in a 70 kb telomeric region on chromosome 3 (Yu et al 2004, Carbone et al 2007b), and CPA is thought to originate from a gene minicluster next to the aflatoxin gene cluster (Chang et al 2009). The inability to produce aflatoxins or CPA in *P. flavus* is often due to various deletions in these gene clusters (Chang et al 2005, 2009). In closely related *P. parasiticus*, distinct recombination blocks have been identified in the aflatoxin gene cluster (Carbone et al 2007a) and genetic recombination has been demonstrated (Horn et al 2009a). Therefore recombination during sexual reproduction in *P. flavus* might account for much of the variation in mycotoxin production observed in populations.

ACKNOWLEDGMENTS

We appreciate the help of Patricia Eckel who prepared the Latin diagnosis, Travis Walk and Michael Munster for technical assistance and Valerie Knowlton at the Center for Electron Microscopy (NC State University) for assistance with the SEM. This work was supported in part by the National Research Initiative of the USDA Cooperative State Research, Education and Extension Service to IC (grant number 2005-35319-16126).

LITERATURE CITED

Azziz-Baumgartner E, Lindblade K, Gieseker K, Rogers HS, Kieszak S, Njapau H, Schleicher R, McCoy LF, Misore A, DeCock K, Rubin C, Slutsker L, Aflatoxin Investigative Group. 2005. Case-control study of an acute aflatoxicosis outbreak, Kenya, 2004. Environ Health Perspect 113:1779–1783.

Barros GG, Chiotta ML, Reynoso MM, Torres AM, Chulze SN. 2007. Molecular characterization of *Aspergillus* section *Flavi* isolates collected from peanut fields in Argentina using AFLPs. J Appl Microbiol 103:900–909.

Bayman P, Baker JL, Doster MA, Michailides TJ, Mahoney NE. 2002. Ochratoxin production by the *Aspergillus*

ochraceus group and *Aspergillus alliaceus*. Appl Environ Microbiol 68:2326–2329.

———, Cotty PJ. 1991. Vegetative compatibility and genetic diversity in the *Aspergillus flavus* population of a single field. Can J Bot 69:1707–1711.

———, ———. 1993. Genetic diversity in *Aspergillus flavus*: association with aflatoxin production and morphology. Can J Bot 71:23–31.

Burdock GA, Flamm WG. 2000. Safety assessment of the mycotoxin cyclopiazonic acid. Int J Toxicol 19:195–218.

Carbone I, Jakobek JL, Ramirez-Prado JH, Horn BW. 2007a. Recombination, balancing selection and adaptive evolution in the aflatoxin gene cluster of *Aspergillus parasiticus*. Molec Ecol 16:4401–4417.

———, Ramirez-Prado JH, Jakobek JL, Horn BW. 2007b. Gene duplication, modularity and adaptation in the evolution of the aflatoxin gene cluster. BMC Evol Biol 7:111.

Chang P-K, Horn BW, Dorner JW. 2005. Sequence breakpoints in the aflatoxin biosynthesis gene cluster and flanking regions in nonaflatoxigenic *Aspergillus flavus* isolates. Fungal Genet Biol 42:914–923.

———, ———, ———. 2009. Clustered genes involved in cyclopiazonic acid production are next to the aflatoxin biosynthesis gene cluster in *Aspergillus flavus*. Fungal Genet Biol 46:176–182.

Cole RJ. 1986. Etiology of turkey ''X'' disease in retrospect: a case for the involvement of cyclopiazonic acid. Mycotoxin Res 2:3–7.

Cullen JM, Newberne PM. 1994. Acute hepatotoxicity of aflatoxins. In: Eaton DL, Groopman JD, eds. The toxicology of aflatoxins: human health, veterinary, and agricultural significance. San Diego: Academic Press. p 3–26.

Eaton DL, Groopman JD, eds. 1994. The toxicology of aflatoxins: human health, veterinary, and agricultural significance. San Diego: Academic Press. 544 p.

Fennell DI, Warcup JH. 1959. The ascocarps of *Aspergillus alliaceus*. Mycologia 51:409–415.

Hedayati MT, Pasqualotto AC, Warn PA, Bowyer P, Denning DW. 2007. *Aspergillus flavus*: human pathogen, allergen and mycotoxin producer. Microbiology 153:1677–1692.

Horn BW. 2005a. Ecology and population biology of aflatoxigenic fungi in soil. In: Abbas HK, ed. Aflatoxin and food safety. Boca Raton, Florida: Taylor & Francis. p 95–116.

———. 2005b. Colonization of wounded peanut seeds by soil fungi: selectivity for species from *Aspergillus* section *Flavi*. Mycologia 97:202–217.

———, Dorner JW. 1999. Regional differences in production of aflatoxin B_1 and cyclopiazonic acid by soil isolates of *Aspergillus flavus* along a transect within the United States. Appl Environ Microbiol 65:1444–1449.

———, Greene RL. 1995. Vegetative compatibility within populations of *Aspergillus flavus*, *A. parasiticus*, and *A. tamarii* from a peanut field. Mycologia 87:324–332.

———, ———, Sobolev VS, Dorner JW, Powell JH, Layton RC. 1996. Association of morphology and mycotoxin production with vegetative compatibility groups in *Aspergillus flavus*, *A. parasiticus*, and *A. tamarii*. Mycologia 88:574–587.

———, Ramirez-Prado JH, Carbone I. 2009a. Sexual reproduction and recombination in the aflatoxin-producing fungus *Aspergillus parasiticus*. Fungal Genet Biol 46:169–175.

———, ———, ———. 2009b. The sexual state of *Aspergillus parasiticus*. Mycologia 101:275–280.

Klich MA. 2002. Identification of common *Aspergillus* species. Utrecht, the Netherlands: Centraalbureau voor Schimmelcultures. 116 p.

———, Pitt JI. 1988. Differentiation of *Aspergillus flavus* from *A. parasiticus* and other closely related species. Trans Brit Mycol Soc 91:99–108.

Kornerup A, Wanscher JH. 1978. Methuen handbook of colour. 3rd ed. London: Eyre Methuen. 252 p.

Krishnamachari KAVR, Bhat RV, Nagarajan V, Tilak TBG. 1975. Hepatitis due to aflatoxicosis: an outbreak in western India. Lancet 1:1061–1063.

Kurtzman CP, Smiley MJ, Robnett CJ, Wicklow DT. 1986. DNA relatedness among wild and domesticated species in the *Aspergillus flavus* group. Mycologia 78:955–959.

Lee C-Z, Liou G-Y, Yuan G-F. 2006. Comparison of the *aflR* gene sequences of strains in *Aspergillus* section *Flavi*. Microbiology 152:161–170.

Leslie JF. 1993. Fungal vegetative compatibility. Ann Rev Phytopathol 31:127–150.

Lye MS, Ghazali AA, Mohan J, Alwin N, Nair RC. 1995. An outbreak of acute hepatic encephalopathy due to severe aflatoxicosis in Malaysia. Am J Trop Med Hyg 53:68–72.

Malloch D, Cain RF. 1972. The Trichocomataceae: ascomycetes with *Aspergillus*, *Paecilomyces*, and *Penicillium* imperfect states. Can J Bot 50:2613–2628.

McAlpin CE, Wicklow DT. 2005. Culture media and sources of nitrogen promoting the formation of stromata and ascocarps in *Petromyces alliaceus* (*Aspergillus* section *Flavi*). Can J Microbiol 51:765–771.

———, ———, Platis CE. 1998. Genotypic diversity of *Aspergillus parasiticus* in an Illinois corn field. Plant Dis 82:1132–1136.

Moody SF, Tyler BM. 1990a. Restriction enzyme analysis of mitochondrial DNA of the *Aspergillus flavus* group: *A. flavus*, *A. parasiticus*, and *A. nomius*. Appl Environ Microbiol 56:2441–2452.

———, ———. 1990b. Use of nuclear DNA restriction fragment length polymorphisms to analyze the diversity of the *Aspergillus flavus* group: *A. flavus*, *A. parasiticus*, and *A. nomius*. Appl Environ Microbiol 56:2453–2461.

Peterson SW. 2008. Phylogenetic analysis of *Aspergillus* species using DNA sequences from four loci. Mycologia 100:205–226.

Ramirez-Prado JH, Moore GG, Horn BW, Carbone I. 2008. Characterization and population analysis of the mating-type genes in *Aspergillus flavus* and *Aspergillus parasiticus*. Fungal Genet Biol 45:1292–1299.

Raper KB, Fennell DI. 1965. The genus *Aspergillus*. Baltimore, Maryland: Williams & Wilkins. 686 p.

Turner PC, Moore SE, Hall AJ, Prentice AM, Wild CP. 2003. Modification of immune function through exposure to

dietary aflatoxin in Gambian children. Environ Health Perspect 111:217–220.

van Edmond HP, Jonker MA. 2005. Worldwide regulations on aflatoxins. In: Abbas HK, ed. Aflatoxin and food safety. Boca Raton, Florida: Taylor & Francis. p 77–93.

Wicklow DT, McAlpin CE, Platis CE. 1998. Characterization of the *Aspergillus flavus* population within an Illinois maize field. Mycol Res 102:263–268.

Yu J, Chang P-K, Ehrlich KC, Cary JW, Bhatnagar D, Cleveland TE, Payne GA, Linz JE, Woloshuk CP, Bennett JW. 2004. Clustered pathway genes in aflatoxin biosyntheis. Appl Environ Microbiol 70:1253–1262.