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Distribution and genetic diversity among *Aspergillus flavus* isolates across three agro-ecologies essential for maize cultivation in Ghana

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Fungal mycotoxins are important contaminants of agricultural commodities that pose serious concerns to producers, consumers and exporters. Aflatoxin is a carcinogenic metabolite produced by the fungi *Aspergillus flavus* and *A. parasiticus*. These fungi and other mould species living in the soil contaminate several crops including maize. This study was undertaken with the following objectives: (i) to assess the presence of *A. flavus* and other moulds in Ghanaian soil; (ii) to determine the distribution and density of *A. flavus* under three agro-ecologies noted for major maize production; and (iii) to assess the effect of percentage of carbon (C) and nitrogen (N) and soil pH on *A. flavus* population densities and diversity in the isolates found across the agro-ecologies. The data showed seven moulds that were common across the agro-ecologies. Significant differences ($P < 0.05$) were observed in the *A. flavus* density and distribution within and across these agro-ecologies. Fumesua soils recorded the highest levels of *A. flavus* (1.185×10^3 cfu g^{-1}) while Akomadan recorded the least (9.76×10^2 cfu g^{-1}). Percentage available C, N and soil pH did not significantly influence *A. flavus* density. The *A. flavus* isolates identified in this study varied in genetic sequence within the aflatoxin gene cluster, but these differences were not distinguishable by origin.

Keywords: aflatoxin, *Aspergillus flavus*, contamination, diversity

Introduction

Maize (*Zea mays*) is an important cereal crop widely cultivated in the tropics for human consumption. In addition, it has several industrial uses as well as the formulation of animal diet. In Ghana, bulk production of maize occurs in the middle growing belt and northern regions where the agro-ecologies are suitable for the cultivation of the crop. The current production is estimated to be around 1.96 million tonnes per annum on an approximate land area of 1 000 000 ha (FAOSTAT, 2017). This translates into average yields of 1.96 t ha^{-1} in most farmers' fields. Despite the overwhelming importance of maize, its production is challenged by several factors, of which drought and low soil fertility are of paramount concern.

Drought and levels of some specific elements (C and N) have been implicated as factors worsening pre- and post-infection of maize by *Aspergillus flavus*, and subsequent production of aflatoxin in the kernel (Widstrom *et al.*, 1990; Payne, 1992; Moreno & Kang, 1999). Aflatoxins contaminate soils and negatively affect microbial activities; they can also be taken up by plants through the roots when leached into the soil (Angle & Wagner, 1980; Mertz *et al.*, 1981). Aflatoxins are hepatotoxic, carcinogenic secondary metabolites produced by two major species of *Aspergillus* fungi (*A. flavus* and *A. parasiticus*) and are classified among the most toxic and carcinogenic compounds found in nature. Aflatoxin content in maize is highly regulated internationally, with the most advanced countries imposing high restrictions on the use of maize contaminated with the toxin. Presently some countries including Japan and the USA have set limits of not more than 20 ppb in commercial food (Šošo *et al.*, 2014). However, in most developing countries such as Ghana, regulatory infrastructure does not enable inspection and enforcement. Due to these lapses in inspection, infected maize is often traded and consumed within communities and the household of the producers, exposing people to potential danger. Reports from some West African countries including Benin, Togo and Ghana indicate levels ranging from 0.4 to 490.6 ppb (James *et al.*, 2007), which is far above permissible limits.

Aflasafe, a product made up of atoxigenic *Aspergillus* isolates (those lacking the genetic mechanism of producing aflatoxin) has been explored to control the colonization and infection of toxigenic strains (Callicott &

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Cotty, 2015). These isolates were found by monitoring the pattern of gene deletions within a 70 kb region in the aflatoxin gene cluster on chromosome 3 to determine the inability of the isolates to create aflatoxin. When these strains are applied to maize early in the season, they block the toxigenic strains from effectively colonizing the growing kernels. This approach has yielded some significant control over the toxigenic isolates in Nigeria and the USA (Cotty, 1997; Horn & Dorner, 1998). It would therefore be very informative to study the pattern of distribution and genetic diversity among *A. flavus* isolates in Ghanaian soils in order to identify potential atoxigenic isolates suitable for the control of toxigenic isolates within the three agro-ecologies in Ghana.

In order to identify native atoxigenic *A. flavus* strains from Ghana, and to measure the effect of some selected elements (% C and % N) on their distribution within farms across three agro-ecologies in Ghana, the following objectives were set: (i) to assess the distribution of *A. flavus* and other mould fungi found in soils across the rain forest, forest transition and savanna transition zones in Ghana; (ii) to assess the genetic diversity among the isolates found in the major maize-growing areas in Ghana; and (iii) to assess the relationship between percentage available carbon and nitrogen and pH on *A. flavus* distribution across the 44 towns and villages within the agro-ecologies.

Materials and methods

Field survey locations

Field surveys were carried out in three main agro-ecological zones in Ghana where maize is widely cultivated. The agro-ecological zones fall within four districts (Table 1). In each of the four districts, farms (corresponding to the towns and villages visited) were sampled, totalling 44 soil samples. The sampling sites were radially separated by at least 10–20 km from each other within a district. Average farm area sampled was >10 acres.

Soil sample collection and preparation

Soil samples collected from the 44 locations were processed as described by Dorner *et al.* (2003), where 5–10 scoops of soil were randomly collected in each location from the top soil, at a depth of 4–6 cm, using a sterile trowel. Samples were transported in sterile containers to the soil science laboratory of the CSIR – Crops Research Institute of Ghana (CSIR-CRI) where they were sorted. The soil samples were pooled from each field and oven-dried at 40 °C for 5 days, and then stored in sealed plastic bags at room temperature (22–26 °C) prior to use. The percentage of available nitrogen and carbon were determined for each sample using the protocols described by Bremner (1965) and Walkley & Black (1934), respectively. A modified protocol of Kalra (1995) was followed for pH assessment. Briefly, 10 g sieved soil per sample was combined with 10 mL distilled water, thoroughly mixed to ensure uniformity, and then allowed to stand for 1 h prior to reading. A pH/mV Conductivity S470 Mettler Toledo pH meter (Fisher Scientific) was used to take the pH readings of each sample in two replicates and the mean value determined.

Table 1 List of districts, geographical locations and average maximum and minimum temperatures across the agro-ecological zones sampled in Ghana.

District	Location	Average min. & max. temperature (°C)	Agro-ecological zone
Fumesua	06°42′39″N, 01°31′22″W	21–31	Rain forest zone (RFR)
Wenchi	07°34′38″N, 01°55′45″W	20–35	Savanna transitional zone (SVT)
Akomadam	07°24′00″N, 01°57′00″W	21–33	Forest transitional zone (FRT)
Ejura	07°23′00″N, 01°22′00″W	24–33	Forest transitional zone (FRT)

Identification and determination of *A. flavus* concentration in soil

One gram of soil from each sample was weighed under aseptic conditions and subsequently transferred into a test tube containing 9 mL distilled water. The test tube was placed on a shaker for 15 min. Further dilutions were made by transferring 1 mL of the initial suspension into a new glass flask containing 9 mL distilled water. This procedure was repeated five times with a dilution factor of 10^{-5} using two replications per sample. After the fifth dilution, 1 mL of each final dilution was spread onto the surface of Petri plates containing potato dextrose agar (PDA; Sigma-Aldrich) and incubated for 3 days at 31 °C in the dark to enable the growth of all fungi present in the soil. An inoculation needle was used to transfer single spores onto a slide 3 days after culture growth in the dark for identification and observation using a microscope (Leica Inc.) and then subsequently characterized based on Pitt & Hocking (1997) classification. This included examining the colony characteristics such as colour, spore size and shape and sclerotia diameter. After spore confirmation, the total number of distinct colonies were counted in the two replicates to enable proper estimation of colony-forming units (CFU) following the standard colony count procedure (AOAC, 1984).

The population of *A. flavus* per gram of soil sample was estimated as CFU per gram of sample as described by Miles & Misra (1938) as:

$$\mathrm{CFU/g} = \frac{\text{number of colonies} \times \text{reciprocal of the dilution factor}}{\text{Plating volume (1 mL)}}$$

Preparation of pure cultures

Identified *A. flavus* isolates were transferred onto freshly prepared PDA plates with an inoculation needle and incubated at 31 °C for 3–5 days. Mycelium plugs 5 mm in diameter were transferred into 15 mL test tubes each containing 9 mL distilled water under a laminar flow hood. Serial dilutions were made from the harvested agar plugs carrying mycelia by transferring 1 mL of the initial suspension into a new glass flask containing 9 mL distilled water. This procedure was repeated five times with a dilution factor of 10^{-5}. After the fifth dilution, 1 mL of each final suspension was smeared onto the surface of water agar (2%) and incubated at 31 °C in the dark to enable slow growth of single spores. Each single spore was subsequently transferred onto freshly prepared

agar and reincubated in the dark at 31 °C to produce pure single spore cultures for DNA extraction.

DNA isolation from pure cultures

A modified CTAB-based protocol as described by Seppala *et al.* (1994) was used for the extraction of genomic DNA from pure cultures of individual samples (fungal strains/isolates). The DNA extraction was carried out at the molecular biology laboratories of the Cocoa Research Institute of Ghana. During the extraction process, 5-day-old pure colonies were ground to fine powder in liquid nitrogen in a 1.5 mL Eppendorf tube. The tissue powder was mixed with 500 μL CTAB extraction buffer [100 mM Tris-HCl (pH 8), 1.4 M NaCl, 20 mM EDTA (pH 8), 2% (w/v) CTAB and 2% w/v PVP] and 0.5 μL (0.1% v/v) β-mercaptoethanol, and homogenized by vortexing. The homogenate was incubated at 65 °C for 30 min with intermittent vortexing.

After cooling to room temperature, an equal volume of chloroform-isoamyl alcohol (24:1 v/v) was added followed by several inversions of the tube to ensure complete emulsification. The extract was centrifuged at 14 400 ***g*** for 15 min. The aqueous phase was recovered and re-extracted with chloroform-isoamyl alcohol (24:1). DNA was precipitated at −20 °C overnight by addition of two-thirds volume of ice-cold isopropanol. The DNA was pelleted by centrifuging at 14 400 ***g*** for 5 min followed by washing in 1 mL washing buffer (76% ethanol, 10 mM ammonium acetate) and 1 mL 80% ethanol. It was then dried at room temperature and resuspended in 100 μL molecular grade water prior to use. One microlitre of each sample was used to prepare 1:20 dilutions.

Aflatoxin analysis

Aflatoxin was extracted using the method described by Sirhan *et al.* (2013) with modifications. Soil samples were homogenized into suspension using a Preethi mixer grinder. Two grams of slurry was weighed into a 15 mL centrifuge tube and topped up with 4 mL of a 60:40 (v/v) methanol:acetronitrile solution, and vortexed for 3 min. Anhydrous $MgSO_4$ (1.32 g) and 0.2 g NaCl were added to the mixture, and vortexed for an additional 1 min. The tube was centrifuged for 5 min at 1200 ***g*** and the upper organic layer filtered through a 0.45 μm nylon syringe prior to injection. A volume of 100 μL of the filtered extract was injected into the HPLC.

A Cecil-Adept Binary Pump HPLC coupled with Shimadzu 10AxL fluorescence detector (Ex.: 360 nm, Em.: 440 nm) with Phenomenex HyperClone BDS C18 Column (150 × 4.60 mm, 5 μm) was used for analysis. The mobile phase used was methanol:water (40:60, v/v) at a flow rate of 1 mL min^{-1} with column temperature maintained at 40 °C. To 1 L of mobile phase were added 119 mg KBr and 350 μL of 4 M nitric acid (required for postcolumn electrochemical derivatization with Kobra Cell; R-Biopharm Rhone). Aflatoxin Mix (G_1, G_2, B_1, B_2) standards (ng g^{-1}) were prepared from Supelco aflatoxin standard of 2.6 ng μL^{-1} in methanol. Concentrations of B_1 and G_1 were 0.5, 1, 2, 8, 16 ng g^{-1} per 100 μL injection of each standard.

Concentrations of B_2 and G_2 were 0.15, 0.3, 0.6, 2.4, 4.8 ng g^{-1} per 100 μL injection of each standard. Limit of detection and limit of quantification of total aflatoxin were established at 0.5 and 1 ng g^{-1}, respectively. The unit (ng g^{-1}) is equivalent to ppb. Aflatoxin concentration was estimated as:

$$\text{Concentration } (\text{ng} \cdot \text{g}^{-1}) = A \times (T/I) \times (1/W).$$

where A = ng of aflatoxin as eluate injected, T = final test solution eluate volume (μL), I = volume eluate injected into LC (μL) and W = mass (g) of commodity represented by final extract.

Validation of HPLC method

Recovery studies were conducted to check for precision and accuracy. Blank samples were spiked at five replicated soil samples at 13, 26 and 104 μg g^{-1} with recoveries of 91 ± 1.75%, 98 ± 1.33% and 102 ± 1.87%, respectively. Blanks that were run periodically contained no detectable amount of target analyte. Trueness was further validated using a certified reference material (TR-A1000) from Triology laboratory, USA. The value obtained of 20.17 ± 1.14 μg kg^{-1} from 10 replicates was within the recommended range of the certified value of 21.0 ± 2.9 μg kg^{-1}. The coefficient of variation was <15% for replicates.

Diversity assessment and molecular characterization of *A. flavus* isolates using gene amplification and deletion patterns

Genetic characterization of *A. flavus* isolates was done using multiplex PCR with a set of 32 cluster amplification pattern (CAP) markers (SC01, IC01, IC02, AC01–AC13, CC01–CC04 and ST01–ST12) and an internal amplification control (Callicott & Cotty, 2015) to monitor gene deletion patterns in four separate regions on chromosome three. Sixty-six out of 108 isolates of *A. flavus* were analysed. Forty-two isolates failed to amplify at least one locus of the gene cluster or the internal amplication control. The markers are spaced *c.* 5 kb along the final 157 kb of the subtelomere region containing the sugar, aflatoxin and CPA clusters, as identified in the reference genome of NRRL 3357 (Payne *et al.*, 2006). Multiplex PCRs were carried out using 0.08 μM each primer, 1 × AccuStart II PCR SuperMix (Quanta Biosciences) and 6 ng genomic DNA in a final reaction volume of 10 μL. Each sample was subjected to 1 min at 94 °C; 30 cycles of 30 s at 94 °C, 90 s at 62 °C and 90 s at 72 °C; followed by 10 min at 72 °C. PCR products were visualized on l.4% agarose gel in 1 × sodium boric acid buffer (Brody & Kern, 2004).

Telomere amplifications were performed using 0.25 μM primers teloR (Chang *et al.*, 2005). Reactions were performed in Accustart II Pre-Mix tubes (Bioneer, Inc.) with 6 ng genomic DNA and l.5 mM $MgC1_2$ in a final reaction volume of 20 μL. Samples were subjected to 5 min at 94 °C; 38 cycles of 20 s at 94 °C, 30 s at 54 °C and 5 min at 72 °C; followed by 10 min at 72 °C. Products were visualized on l% agarose gel in 1 × sodium boric acid buffer. Amplicon size was estimated using GeneTool v. 4.02d (Syngene) using mobility relative to the bands in the GeneRuler 1 kb Plus DNA ladder (Thermo Scientific).

Data analysis

Analysis of variance (ANOVA) was performed on concentration of *A. flavus* per gram of soil using GenStat 12th edition (VSN International Ltd). Data on fungal contamination were $\log_{10}$ transformed to reduce the heterogeneity of variance of contamination levels. The software package DARWIN (Perrier & Jacquemoud-Collet, 2006) was used to calculate Dice genetic distance coefficients to estimate genetic similarities among pairwise comparisons of individuals. Genetic distance was estimated for the

CAP marker data using Dice (1945). A cluster analysis based on the genetic distances was performed using the neighbour-joining method in MEGA v. 5.2 (Tamura *et al.*, 2011).

Results

Incidence and distribution of fungi in soil samples

Aspergillus sp. and *Rhizopus* sp. were the most predominant organisms found in all the fields under study (Table 2). Other fungal organisms identified included *Penicillium* sp., *Trichoderma* sp., and much more rarely, *Fusarium* sp. and *Curvularia* sp. The concentration and distribution of *A. flavus* in soils across the locations can be seen in Figures 1–4. Ejura area revealed concentrations ranging from 0 to over 1700 CFU g^{-1} (Fig. 1), where samples from all sites recorded significantly ($P < 0.05$) higher concentration of *A. flavus* compared to Akyena Akura and Miminaso, where *A. flavus* was absent. Similarly, in the Fumesua area, all sites had significantly ($P < 0.05$) higher CFUs compared to Abenase and Jiachie, which had no *A. flavus* (Fig. 2). Soil samples from Wenchi showed significant differences ($P < 0.05$) in the concentrations of *A. flavus* across the 11 towns and villages sampled (Fig. 3), where all sites registered at least some *A. flavus* (although it was very low in Duaponko). In the Akomadan area significant ($P < 0.05$) variation was found in *A. flavus* concentration across the 11 towns within the area, with Srentiatia and CRI Main Station samples free from *A. flavus* (Fig. 4).

Soil factors affecting *A. flavus* and aflatoxin distribution

Average and ranges of soil properties can be found in Table 3 for the four areas under study. Soils from Ejura, Fumesua and Wenchi displayed similar low levels of carbon and nitrogen, and fairly acidic pH levels. The soil from the Akomadan area contained relatively higher percentages of nitrogen and carbon, and a more basic pH. These soil properties may have accounted in part for the trend in *A. flavus* (CFU g^{-1}) distribution across the communities, where there was lower CFUs in the more fertile and basic Akomadan soils (Table 3). Indeed, the relationship between *A. flavus* population density and some soil traits indicated that there was a moderate (0.63) but nonsignificant ($P < 0.05$) correlation between the percentage soil carbon and number of CFUs per gram. Much weaker associations between *A. flavus* population density and soil nitrogen (0.11) and pH (−0.008) were also seen (data not shown). A larger sample would be required to show the significance of these findings, but it seems possible that percentage of soil carbon may influence the population and distribution of *Aspergillus flavus*.

HPLC analysis on soil samples across the 44 communities to assess aflatoxin contamination levels revealed the presence of aflatoxin B_2 only at one site within the Akomadan area. The general observation suggests that aflatoxin-producing strains of *Aspergillus* sp. probably do not readily synthesize aflatoxin in the soils but may require specific substrate other than soil (Table 4).

Genetic diversity assessment in 66 *A. flavus* isolates

Gene deletions and amplification were monitored on a 70 kb region of chromosome 3 of all the isolates collected from Wenchi, Fumesua, Akumadan and Ejura with the set of 32 CAP markers and the internal amplification control. The 32 CAPs were partitioned into eight within each of the cyclopiazonic acid (CPA) and subtelomere clusters, and seven and nine CAPs in the sugar and aflatoxin clusters, respectively. Out of 108 *A. flavus* isolates for which DNA was extracted, only 66 amplified at least one of the 32 CAP markers and the internal amplification control. All remaining 42 isolates failed to amplify at least one locus assayed within the clusters under consideration.

Of 16 isolates from the Wenchi area, only three successfully amplified all the loci inside the sugar, aflatoxin, CPA and subtelomere gene clusters (Table 5). This implies that these three are potentially toxigenic, and may have the ability to produce both toxins (aflatoxins and CPA). All other isolates failed to amplify most marker sequences. Five isolates did not amplify some or all loci in the sugar cluster, which may cause problems for growth of these strains under some conditions. Ten did not amplify some or all sequences within the aflatoxin gene cluster, and may be atoxigenic; 12 isolates did not amplify some or all of the CPA gene cluster, and nine isolates failed in both clusters and may be unable to synthesize either toxin. Eleven isolates failed to amplify some or all of the subtelomeric region and thus provides information on structural variation.

Observations made in relation to the amplification and deletion patterns among the 18 isolates collected across Fumesua and its surrounding towns are presented in Table 6. Five isolates completely amplified both the aflatoxin gene cluster as well as the CPA region. These five may potentially synthesize both aflatoxin and CPA (Table 6).

In the Akomadan area, 16 isolates were studied (Table 7). Two isolates successfully amplified all the loci constituting the aflatoxin gene cluster whilst the rest failed to amplify any gene in the aflatoxin synthesis cluster (Table 7). This suggests that a few isolates may be able to produce aflatoxin while isolates with limited amplification of the aflatoxin gene cluster may be potentially atoxigenic. In the CPA cluster, two isolates amplified all the genes and may potentially synthesize the CPA (Table 7).

Isolates from the Ejura area also displayed different patterns of amplification and deletions within the clusters (Table 8). The patterns revealed that four isolates which amplified all loci in the aflatoxin gene cluster could be potentially toxigenic. Five other isolates displayed a complete deletion of all the loci within the cluster, suggesting that such isolates could not synthesize aflatoxins. Seven

Table 2 Percentage incidence of *Aspergillus flavus* and other fungal genera identified in soil samples across 44 towns.

	Organisms identified						
Location[a]	*Rhizopus* sp.	*A. flavus*	*A. niger*	*Penicillium* sp.	*Fusarium* sp.	*Trichoderma* sp.	*Curvularia* sp.
Ejura (FRT)							
Ejura State farms	+	+	−	−	−	−	−
Koberiti	+	+	−	−	−	−	−
Main research station	+	+	+	−	−	−	−
Kasei	+	+	−	+	−	−	−
Afromaso	+	+	+	−	+	−	−
Adiembra Nkwanta	+	+	−	−	−	−	−
Dromankuma	+	+	+	−	−	+	−
Samari	+	+	−	−	−	−	−
Miminaso	+	−	−	−	−	+	−
Akyena Akura	+	−	+	−	−	+	−
Ashakoko	+	+	+	+	−	−	−
Contamination (%)	100	81.8	45.5	20.0	9.1	27.3	0.0
Fumesua (RFR)							
Asumen	+	+	−	−	−	−	−
Kwadaso Nzema	+	+	+	+	−	−	−
Krapa	+	+	+	+	−	−	−
Kwamo	+	+	+	−	−	−	−
Ampabame	+	+	+	−	−	−	−
Jiachie	+	−	+	−	−	−	−
Boankra	+	+	+	−	−	−	−
F main station	+	+	+	−	−	+	−
Kwaso	+	+	+	−	−	−	−
Kwadaso roundabout	+	+	+	−	−	+	−
Abenase	+	−	−	+	−	+	−
Contamination (%)	100	81.8	81.8	27.3	0.0	27.3	0.0
Wenchi (SVT)							
Akete	+	+	+	−	−	+	−
Branam	+	+	+	−	−	−	−
Duaponko	+	−	+	−	−	−	−
Subinso 3	+	+	+	−	−	−	−
CRI Station	+	+	+	−	−	+	−
Animal husbandry	+	+	+	+	−	+	−
Akrobi	+	+	+	−	−	−	−
Nchiraa	+	+	+	−	−	−	−
Amponsah K	+	+	+	−	−	−	−
Mframanso	+	+	+	−	−	+	−
Methodist University	−	+	+	−	−	+	−
Contamination (%)	90.1	90.1	100	9.1	0.0	45.5	0.0
Akomadan (FRT)							
Srentiatia	+	−	+	−	−	−	−
Main station	+	−	+	−	−	−	−
Serwia	−	+	+	−	−	−	−
Abrofia	+	+	+	+	−	−	−
Mbredani	+	+	+	−	−	−	−
Apatem	+	+	+	−	−	−	−
Tanokrom	+	+	+	−	−	+	−
Mankrampso	+	+	+	−	−	+	−
Mfanti	+	+	+	−	−	−	−
Nkubesa	−	+	+	−	−	+	+
Mpaepemu	+	+	+	−	+	−	−
Contamination (%)	81.8	81.8	100	9.1	9.1	27.3	9.1

+ or −, present or absent, respectively.

[a]SVT, savana transition; FRT, forest transition; RFR rain forest.

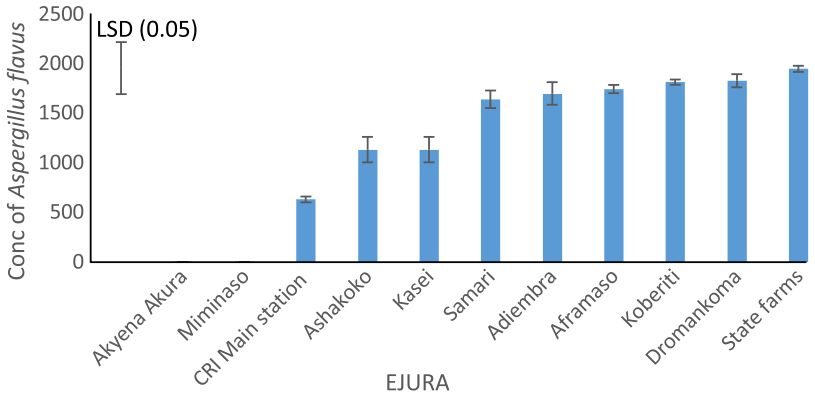

Figure 1 Distribution of *Aspergillus flavus* per gram of soil in Ejura and surrounding communities. Vertical lines represent the standard errors of the mean. [Colour figure can be viewed at wileyonlinelibrary.com].

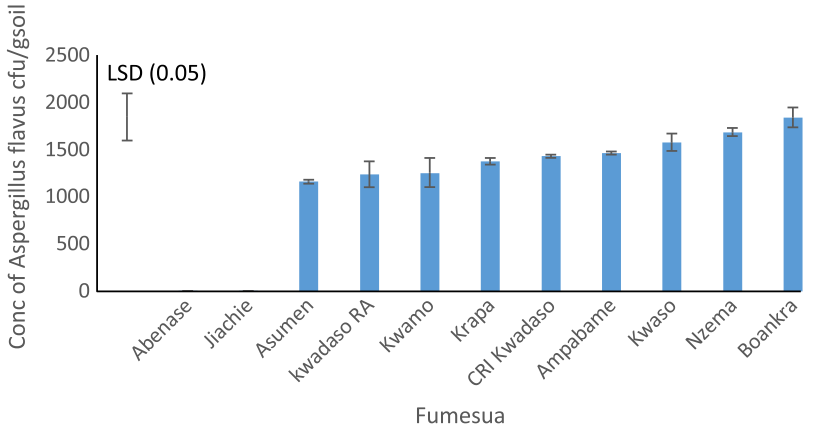

Figure 2 Distribution of *Aspergillus flavus* per gram of soil in Fumesua and surrounding communities. Vertical lines represent the standard errors of the mean. [Colour figure can be viewed at wileyonlinelibrary.com].

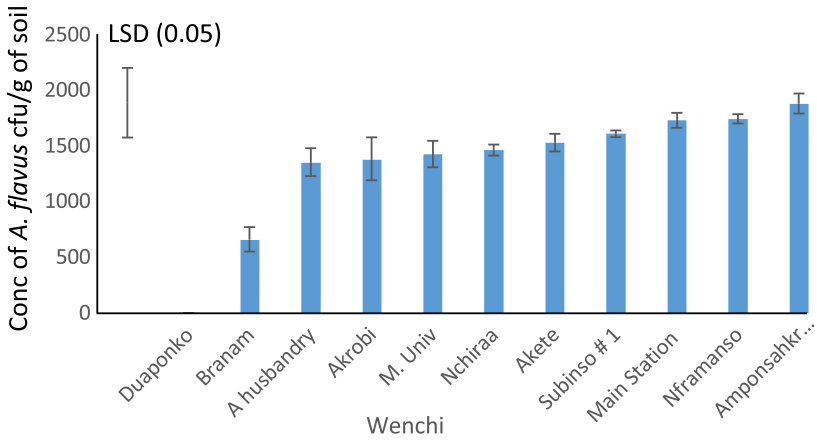

Figure 3 Distribution of *Aspergillus flavus* per gram of soil in Wenchi and surrounding communities. Vertical lines represent the standard errors of the mean. [Colour figure can be viewed at wileyonlinelibrary.com].

isolates showed complete gene deletion of the CPA cluster (Table 8).

Genetic relationship among isolates

The genetic relationship among the 66 isolates was determined using Dice genetic distance generated from the CAP markers (Fig. 5). The dendrogram shows the relatedness and dissimilarity among isolates collected across the four areas, and clusters the isolates into three major groups. Group 1 consists of 27 highly similar isolates from all four areas, although more (*c.* 41%) were from Fumesua than other areas. Group 2 consists of 38 isolates with approximately equal amounts from all four areas but slightly fewer from Fumesua; this cluster was less homogenous than Group 1. Group 3 consists of only one isolate from Ejura. The clustering pattern of the isolates shows similarity within groups that is not based on origin or environment. Because of the markers used, this classification is expected to be more functional, with the

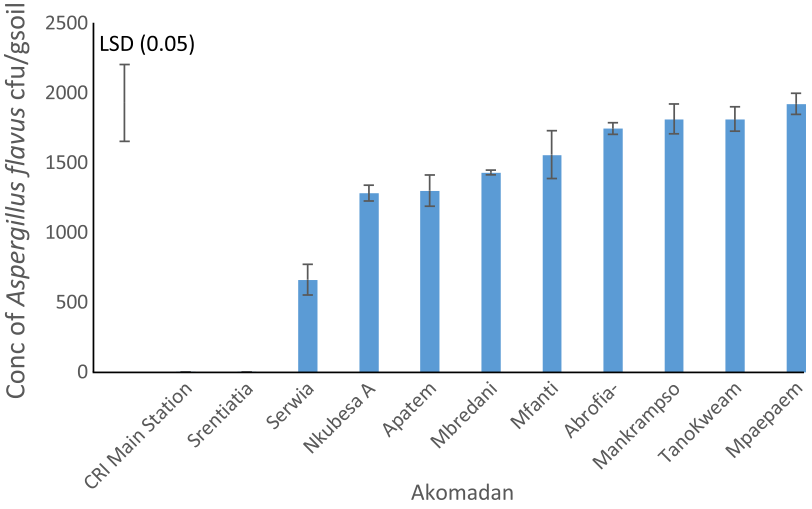

Figure 4 Distribution of *Aspergillus flavus* per gram of soil in Akomadan and surrounding communities. Vertical lines represent the standard errors of the mean. [Colour figure can be viewed at wileyonlinelibrary.com].

Table 3 Environmental factors potentially affecting relative distribution and levels of *Aspergillus flavus* in four major communities in Ghana.

	Colony-forming units per g soil		N (%)		C (%)		pH	
Location	Average	Range	Average	Range	Average	Range	Average	Range
Ejura	1.10×10^3	0.1×10^1–1.83×10^3	0.09	0.03–0.13	0.88	0.41–1.26	5.1	4.67–5.56
Fumesua	1.18×10^3	0.1×10^1–1.84×10^3	0.11	0.07–0.19	1.04	0.66–1.39	5.6	4.50–6.74
Wenchi	1.38×10^3	0.1×10^1–1.88×10^3	0.09	0.06–0.14	0.94	0.65–1.46	5.5	4.82–6.35
Akomadan	9.80×10^2	0.1×10^1–1.92×10^3	0.15	0.09–0.20	1.41	0.70–2.30	6.3	5.41–7.25

Table 4 Ratio of aflatoxin contamination of soil samples within each major community.

Location	No. towns detected with *Aspergillus flavus*/total no.of towns sampled	Aflatoxin (ppb)			
		G_2	G_1	B_1	B_2
Ejura	10/11	ND	ND	ND	ND
Fumesua	9/11	ND	ND	ND	ND
Wenchi	10/11	ND	ND	ND	ND
Akomadan	9/11	ND	ND	ND	6.4

ND, not detected.

isolates in Group 1 more likely to have the potential to synthesize aflatoxin whilst Groups 2 and 3 appear to lack the ability to produce the toxin due to limited amplification of the aflatoxin gene cluster responsible for the toxin production (Fig. 5).

Discussion

This study is the first to provide in-depth information on the presence of specific mould fungi, together with the distribution of *A. flavus*, across the three agro-ecological zones in Ghana where maize is widely cultivated. The isolation and identification of soil mould revealed the presence of many fungi alongside *A. flavus*, including *A. niger*, *Rhizopus* sp., *Penicillium* sp., *Fusarium* sp., *Trichoderma* sp. and *Curvularia* sp. Except *Fusarium* sp. and *Curvularia* sp. which were present in few farms sampled, the fungi were found across all the agro-ecological zones; this indicates wide adaptation of the fungi across the contrasting environments, in agreement with a previous study in Nigeria (Atehnkeng *et al.*, 2008). Although the fungi identified in the present study are all soilborne, they are very relevant to maize production because they can contaminate grains with spores carried by wind, rain or insects (Abbas *et al.*, 2006; Cotty, 2006; Okun *et al.*, 2015).

Despite the ubiquitous nature of *A. flavus* and its presence in nearly all farms sampled, the soilborne saprophytes were absent in a few soils that had previously been cultivated with maize. *Aspergillus flavus* infection of maize kernels and subsequent contamination with aflatoxin emanates from the soil, but different factors influence the final levels present in the kernel compared to the soil, where *A. flavus* is virtually unavoidable. Hence the need for effective cultural control measures to keep plants from experiencing abiotic stresses (which increase fungal growth and toxin production) and the use of resistant host maize varieties. The use of atoxigenic biocontrol strains reduces final aflatoxin levels in grain, but they will not prevent losses due to ear rots in the field or in storage. The absence of *A. flavus* in Miminaso, Akyena Akura, Jiachie, Abenase, Duaponko, Srentiatia and Akomadan Main Station is an indication that not all soil conditions may be conducive for its presence despite its wide adaptation; however, the possibility that

Table 5 Amplification and deletion patterns of sugar, aflatoxin, cyclopiazonic acid (CPA) and subtelomere clusters among 16 *Aspergillus flavus* isolates from across the Wenchi area.

	Sugar cluster								Aflatoxin cluster										CPA cluster									Subtelomere								
Isolate	SC01	IC01	AC01	AC02	AC03	AC04	AC05	iac	AC06	AC07	AC08	AC09	AC10	AC11	AC12	AC13	IC02	iac	CC01	CC02	CC03	CC04	ST01	ST02	ST03	ST04	iac	ST05	ST06	ST07	ST08	ST09	ST10	ST11	ST12	iac
WM8a	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
WM9	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
WM10	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
WMF7	+	+	+	+	+	+	+	+	–	–	–	–	+	+	+	+	–	+	+	+	+	+	+	+	+	–	+	–	+	–	–	–	–	–	+
WM31	–	–	+	–	–	–	–	+	+	–	–	–	+	+	–	+	+	+	+	+	+	+	+	+	–	+	+	+	+	+	+	+	–	+	+
WAF2a	+	–	–	–	–	–	+	+	–	–	–	–	–	–	–	–	–		–	–	–	–	–	–	–	–	+	–	–	–	–	–	–	–	+
WAS3A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	–	–	+	–	–	–	+	+	+	+	+	+	+	+	+
WAF3	+	+	+	+	+	+	+	+	–	–	–	–	+	–	–	+	–	+	–	–	–	–	–	–	–	–	+	–	–	–	–	–	–	–	+
WAF4	+	+	+	+	+	+	+	+	+	–	–	–	+	–	–	+	–	+	+	+	+	+	+	+	–	–	+	–	–	–	–	–	–	–	+
WAS25	+	+	+	+	+	+	+	+	–	–	+	–	+	+	–	+	–	+	+	+	+	+	+	+	–	–	+	–	–	–	–	–	–	–	+
WAF27	+	+	+	+	+	+	+	+	+	+	+	–	+	+	–	+	–	+	+	+	+	+	+	+	–	–	+	–	–	–	–	–	–	–	+
WAS28	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	–	+	+	+	+	+	–	+	–	+
WS21	+	+	+	+	+	+	+	+	–	–	+	–	+	+	–	+	–	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
WS39	–	–	–	–	–	–	–	+	–	–	–	–	+	+	+	+	–	+	–	–	–	–	–	–	–	–	+	–	–	–	–	–	–	–	+
WS40	+	–	–	–	–	–	–	+	+	+	+	+	+	+	+	+	+	+	–	–	–	–	–	–	–	–	+	–	–	–	–	–	–	–	+
WAF32	+	+	+	+	+	–	+	+	–	–	–	–	+	–	–	+	–	+	–	–	+	+	+	+	–	–	+	–	+	+	–	–	–	–	+

The + and – (shaded cells) represent loci amplification and deletions across the various gene cluster, respectively. iac, internal amplification control.

Table 6 Amplification and deletion patterns of sugar, aflatoxin, cyclopiazonic acid (CPA) and subtelomere clusters among 16 *Aspergillus flavus* isolates from across the Fumesua area.

	Sugar cluster								Aflatoxin cluster										CPA cluster									Subtelomere								
Isolate	SC01	IC01	AC01	AC02	AC03	AC04	AC05	iac	AC06	AC07	AC08	AC09	AC10	AC11	AC12	AC13	IC02	iac	CC01	CC02	CC03	CC04	ST01	ST02	ST03	ST04	iac	ST05	ST06	ST07	ST08	ST09	ST10	ST11	ST12	iac
FM3	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
FM7	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
FM11	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
FS35	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
F44	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
FM1	+	+	+	+	+	+	+	+	–	+	+	+	+	+	+	+	–	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
FM2	+	+	+	+	+	+	+	+	–	–	–	–	+	+	+	+	–	+	+	+	+	+	+	+	+	–	+	–	+	+	+	+	–	+	+
FAS1	–	–	–	–	–	–	–	+	–	–	–	–	+	+	–	+	+	+	–	–	–	–	–	–	–	–	+	–	+	+	+	–	+	–	–
FAF2	+	+	+	+	+	+	+	+	–	–	–	–	+	–	–	+	–	+	–	–	–	+	+	+	–	–	+	–	–	–	–	–	–	–	+
FAS10	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	–	–	+	–	–	–	–	–	–	–	+
FS11	–	–	–	–	–	–	+	+	–	–	–	–	–	–	–	+	–	+	–	–	+	+	+	+	–	–	+	–	–	+	–	+	–	–	+
FS20	+	–	–	–	–	–	–	+	–	–	–	–	+	–	–	+	–	+	–	–	–	–	–	–	–	–	+	–	–	+	+	–	+	–	+
FS23	+	–	–	–	–	–	–	+	–	–	–	–	–	–	–	–	–	+	–	–	–	–	–	–	–	–	+	–	–	–	–	–	–	–	+
FAF29	+	+	+	+	+	+	+	+	–	–	–	–	–	–	–	–	–	+	–	–	–	+	–	–	–		+	+	+	+	–	+	–	–	+	+
FAS30	–	–	–	–	–	–	–	+	+	–	–	–	+	–	–	+	–	+	–	–	–	–	–	–	–	–	+	–	–	–	–	–	–	–	+
FS37	+	+	+	–	+	+	+	+	–	–	–	–	–	–	–	–	–	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
FAF16a	+	+	+	+	+	+	+	+	–	–	–	–	+	–	–	+	–	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
FAF33	+	+	+	+	+	+	+	+	+	–	–	–	+	+	–	+	–	+	+	+	+	+	+	+	–	–	+	–	–	–	–	–	–	–	+

The + and – (shaded cells) represent loci amplification and deletions across the various gene cluster, respectively. iac, internal amplification control.

Table 7 Amplification and deletion patterns of sugar, aflatoxin, cyclopiazonic acid (CPA) and subtelomere clusters among 16 *Aspergillus flavus* isolates from across the Akomadan area.

	Sugar cluster								Aflatoxin cluster										CPA cluster									Subtelomere								
Isolate	SC01	IC01	AC01	AC02	AC03	AC04	AC05	iac	AC06	AC07	AC08	AC09	AC10	AC11	AC12	AC13	IC02	iac	CC01	CC02	CC03	CC04	ST01	ST02	ST03	ST04	iac	ST05	ST06	ST07	ST08	ST09	ST10	ST11	ST12	iac
AAS18	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
AM12	+	+	+	+	+	+	+	+	–	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	–	+	–	+	+	–	–	–	–	–	+
AM17	+	–	–	–	–	–	–	+	–	–	–	–	–	–	–	–	–	+	–	–	–	–	–	–	–	–	+	–	–	–	–	–	–	–	–	+
AM20	–	–	–	–	–	+	+	+	–	–	–	–	–	–	–	–	–	+	–	–	–	–	–	–	–	–	+	–	–	–	–	–	–	–	–	+
AM21	–	–	–	–	–	–	–	+	–	–	–	–	+	–	–	+	–	+	–	–	+	+	+	+	+	+	+	+	+	+	+	–	+	–	+	+
AM22	–	–	–	–	–	–	–	+	–	–	–	–	+	–	–	+	–	+	–	–	+	+	–	+	–	–	+	–	–	–	–	–	–	–	–	+
AM24	–	–	–	–	–	+	+	+	–	–	–	–	–	–	–	–	–	+	–	–	–	–	–	–	–	–	+	–	–	–	–	–	–	–	–	+
AM25	–	–	–	–	–	–	–	+	–	–	–	–	+	–	–	+	–	+	–	–	–	+	+	+	–	–	+	–	–	–	–	–	–	–	–	+
AAF17	+	+	+	+	+	+	+	+	–	–	–	–	+	+	–	+	–	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
AAF30	+	–	–	–	–	–	–	+	–	–	–	–	–	–	–	–	–	+	–	–	–	+	–	–	–	–	+	–	–	–	–	–	–	–	–	+
AS31	–	–	–	–	–	–	–	+	–	–	–	–	+	–	–	+	–	+	–	–	–	–	–	–	–	–	+	–	–	–	–	–	–	–	–	+
AAF31	+	+	–	–	+	–	+	+	–	–	–	–	–	–	–	–	–	+	–	–	+	+	+	+	–	–	+	+	+	+	+	–	+	–	+	+
AS32	–	–	–	–	–	–	–	+	–	–	–	–	+	–	–	+	–	+	–	–	–	–	–	–	–	–	+	–	–	–	–	–	–	–	–	+
AAF32a	+	+	–	–	+	–	+	+	–	–	–	–	–	–	–	–	–	+	–	–	+	+	+	+	–	–	+	+	+	+	+	–	+	–	+	+
A43	+	+	+	+	+	+	+	+	–	–	–	–	+	–	–	–	–	+	–	–	–	–	–	–	–	–	+	–	+	+	–	–	–	–	–	+
A41	+	+	+	–	+	+	+	+	+	+	+	+	+	+	+	+	+	+	–	–	–	–	–	–	–	–	+	+	+	+	+	–	–	–	–	+

The + and – (shaded cells) represent loci amplification and deletions across the various gene cluster, respectively. iac, internal amplification control.

Table 8 Amplification and deletion patterns of sugar, aflatoxin, cyclopiazonic acid (CPA) and subtelomere clusters among 16 *Aspergillus flavus* isolates from across the Ejura area.

	Sugar cluster								Aflatoxin cluster										CPA cluster									Subtelomere								
Isolate	SC01	IC01	AC01	AC02	AC03	AC04	AC05	iac	AC06	AC07	AC08	AC09	AC10	AC11	AC12	AC13	IC02	iac	CC01	CC02	CC03	CC04	ST01	ST02	ST03	ST04	iac	ST05	ST06	ST07	ST08	ST09	ST10	ST11	ST12	iac
EAF19	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
EMF11	–	–	–	–	–	–	+	+	–	–	–	–	+	–	+	+	–	+	–	–	–	–	–	–	–	–	+	–	–	–	–	–	–	–	–	+
EM26	–	+	+	+	–	+	+	+	–	–	–	–	–	–	–	–	–	+	–	–	–	–	–	–	–	–	+	–	–	–	–	–	–	–	–	+
EM27	–	+	+	+	–	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
EM29	–	+	+	+	–	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	–	–	+	–	+	+	–	–	–	–	–	+
EM30	–	+	+	–	–	+	+	+	+	–	–	–	+	+	–	+	+	+	+	+	+	+	+	+	–	+	+	–	–	–	–	–	–	–	–	+
EAS8	+	+	+	+	+	+	+	+	–	–	–	–	+	–	–	+	–	+	+	+	+	+	+	+	–	–	+	–	–	–	–	–	–	–	–	+
EAS12	–	–	–	–	–	–	–	+	–	–	–	–	+	–	–	–	–	+	–	–	+	+	–	–	–	–	+	+	+	–	–	–	–	–	–	+
EAS14	+	–	–	–	+	–	–	+	–	–	–	–	–	–	–	–	–	+	–	–	–	–	–	–	–	–	+	+	–	–	–	–	–	–	–	+
EAF16	+	–	–	–	+	–	–	+	–	–	–	–	–	–	–	–	–	+	–	–	–	–	–	–	–	–	+	+	+	+	–	+	–	+	–	+
EAF22	+	+	+	+	+	+	+	+	–	–	+	–	+	+	–	+	–	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
EAF28	+	+	+	+	+	+	+	+	–	–	+	–	+	+	–	+	–	+	+	+	+	+	+	+	+	–	+	–	+	+	–	–	–	–	–	+
EAS29	+	+	+	+	+	+	+	+	–	–	+	–	+	+	–	+	–	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
ES36	+	–	–	–	–	–	–	+	–	–	–	–	–	–	–	–	–	+	–	–	–	–	–	–	–	–	+	–	–	–	–	–	–	–	–	+
E42	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	–	–	–	–	–	–	–	–	+	+	+	+	+	–	+	–	+	+
EAF19a	+	–	–	–	–	–	–	+	–	–	–	–	–	–	–	–	–	+	–	–	–	–	–	–	–	–	+	–	–	–	–	–	–	–	–	+

The + and – (shaded cells) represent loci amplification and deletions across the various gene cluster, respectively. iac, internal amplification control.

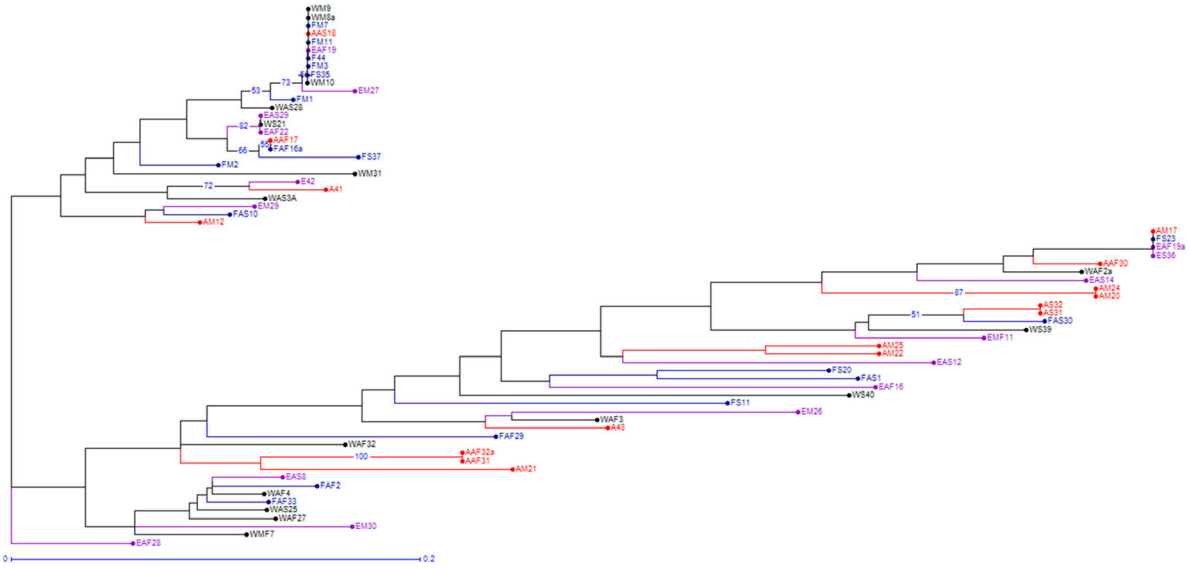

Figure 5 Dendrogram of 66 isolates of *Aspergillus* section Flavi based on cluster analysis with the neighbour-joining method using the Dice genetic distance coefficient on CAPs data obtained with 32 primer pairs generated by MEGA 3 software. The number at each node indicates the percentage bootstrap support (out of 1000) for clusters with >50% support. Isolates labelled in red are from Akomadan, blue are from Fumesua, purple from Ejura and black from Wenchi. [Colour figure can be viewed at wileyonlinelibrary.com].

sampling or measurement error caused the lack of *A. flavus* presence must also not be discounted.

While growth of *A. flavus* in maize kernels thrives under humid conditions, the fungi tend to produce more aflatoxins under arid conditions when the plants experience oxidative damage due to the over-production of reactive oxygen species (Guo *et al.*, 2005; Sobolev *et al.*, 2007). Observations made in this study indicate that *A. flavus* was found to be widespread in high concentrations across all four communities/districts irrespective of the type of agro-ecology. The low concentration of *A. flavus* in Akomadan may have been due to the relatively high alkaline soil pH observed and not due to humidity, which may not be as important for persistence in soil (where it usually forms resistant spores) as it is for growth on maize plants.

The populations of *A. flavus* detected per gram of soil across the three agro-ecologies surveyed were comparable to observations made by Okun *et al.* (2015) on the distribution of both toxigenic and atoxigenic isolates across five agro-ecologies in Kenya. Furthermore, other observations in this study (data not presented) indicated that *A. flavus* concentration did not correlate with aflatoxin biosynthesis as reported in other studies (Shapira *et al.*, 1996; Lund & Frisvad, 2003), possibly due to the many potential atoxigenic strains identified here and general low levels of aflatoxin recorded.

Environmental factors including carbon, nitrogen, pH, temperature, water activity and plant metabolites have been reported to influence *A. flavus* density and aflatoxin production (Proctor, 2000; Bhatnagar *et al.*, 2003). However, results obtained from the present study indicate that pH and percentage of carbon and nitrogen have minimal influence on growth of *A. flavus*. In addition, other studies (Bennett *et al.*, 1979; Niehaus & Jiang, 1989) have established that nitrogen in the form of nitrates impedes *A. flavus* development and toxin production while the ammonium state facilitates it. It could therefore be possible that the samples tested had little amounts of each, which did not influence the density and aflatoxin amounts in the soil.

Aflatoxin was only detected in one location (Apatem) within the Akomadan district where aflatoxin B_2 was detected at a low concentration (6.4 ppb). Because *A. flavus* requires decaying organic matter to grow and produce aflatoxin (Bruehl, 1987; Agrios, 1997; Schomberg & Steiner, 1997; Cookson *et al.*, 1998; Perrone *et al.*, 2014), there may have been too little organic matter in these fields to allow sporulation (typically when aflatoxin is produced). However, this observation contradicts the findings of Okun *et al.* (2015) who detected various concentrations of aflatoxin B and G types in 27 out of 57 soil samples tested in Kenya for aflatoxin. It is unclear whether the fields from which the samples were taken were amended with organic matter or manure prior to the sampling, which could have boosted *A. flavus* growth and sporulation.

The genetic relatedness of the 66 *A. flavus* isolates was further explored by phylogenic tree. Members within each group were more closely related than between groups for the four gene clusters measured. Thus, the groupings were not based on origin but rather aflatoxin and CPA production, which appeared to be more variable than sugar production. This observation is consistent with the findings of Wang *et al.* (2012) and Geiser *et al.* (2000) who reported a lack of distinct geographical

patterns linked to *A. flavus* genotype and aflatoxin production from various sources in the USA. The lack of distinct geographical pattern within *A. flavus* isolates could be due to narrow vegetative compatibility groups (VCG) in circulation in Ghana (Perrone *et al.*, 2014), with similar dominant VCG in particular found within maize-growing areas, resulting in this niche.

Isolates that shared similar genetic sequence clustered together. This is in agreement with the report of Paola *et al.* (2017) who used a similar set of 32 CAP markers to assign *Aspergillus* isolates into toxigenic and atoxigenic groups based on sequence similarity. It is evident from the present study that some isolates from Ejura, Akomadan, Wenchi and Fumesua are closely related although they fall under different agro-ecologies. This confirms the report of Perrone *et al.* (2014) that the *A. flavus* population in some selected communities was narrow. Again, the observation is consistent with the hypothesis that neither sexual reproduction nor mutation has operated at a sufficient level to generate many multilocus genotypes (recombination; Grubisha & Cotty, 2010) or variation.

The pattern of amplification and deletion in the aflatoxin and cyclopiazonic acid synthesis gene cluster implicated a number of isolates that had a complete amplification of the gene cluster as potentially toxigenic for aflatoxin and cyclopiazonic acid. Such isolates include six each from Wenchi and Fumesua, two from Akomadan and four from Ejura. The rest of the isolates displayed large deletions within the aflatoxin gene cluster, which is typically characteristic of atoxigenic isolates. Eleven potentially atoxigenic isolates identified in Wenchi comprise WMF7, WM31, WAF2a, WAF3, WAF4, WAS25, WAF27, WS21, WS39, WS40 and WAF32, while 12 identified within Fumesua were FM1, FM2, FAS1, FAF2, FS11, FS20, FS23, FAF29, FAS30, FS37, FAF16a and FAF33. For the Akomadan area, 14 isolates were identified to be potentially atoxigenic, comprising AM12, AM17, AM20, AM21, AM22, AM24, AM25, AAF17, AAF30, AS31, AAF31, AS32, AAF32a and A43, while 12 identified for the Ejura area were EMF11, EM26, EM30, EAS8, EAS12, EAS14, EAF16, EAF22, EAF28, EAS29, ES36 and EAF19a. These findings need further validation to confirm the toxigenicity or atoxigenicity of the isolates, as the study by Callicott & Cotty (2015) indicated that complete amplification of the entire aflatoxin gene cluster does not guarantee toxin production, because a single nucleotide mutation or substitution could lead to the termination of toxin production. For example, the standard atoxigenic isolate (AF36) used in Aflasafe preparations amplified for all the aflatoxin synthesis cluster but failed to produce aflatoxin because of a nucleotide change in its *pksA* gene that led to early termination of aflatoxin synthesis (Ehrlich & Cotty, 2004). Despite such occurrence, the magnitude of deletions in the biosynthesis cluster may either lead to termination of aflatoxin synthesis or modification of the type of aflatoxin being synthesized (Ehrlich & Cotty, 2004). Such modifications in terms of gene deletions are responsible for the production of the B series of aflatoxin by *A. flavus* isolates, due to a 1.5–2.2 kb sequence deletion in the aflatoxin biosynthetic gene cluster, which resulted in the loss of *cypA*, a gene whose product is required for biosynthesis of aflatoxin G series. Despite these considerations, it is likely that the isolates missing parts of the aflatoxin gene cluster are more likely to be atoxigenic, and should be studied further for their suitability in biocontrol preparations.

In conclusion, major mould fungi found in maize-growing soils across three agro-ecologies in Ghana include *A. flavus*, *A. niger*, *Rhizopus* sp., *Penicillium* sp., *Fusarium* sp., *Trichoderma* sp. and *Curvularia* sp. Among the moulds, three (*A. flavus*, *A. niger* and *Rhizopus* sp.) were ubiquitous in the study area.

Aspergillus flavus was widespread with little or no influence of measured agro-ecological conditions on its density. No evidence of aflatoxin was detected in the soils which were heavily contaminated with *A. flavus*, implying that aflatoxin synthesis requires organic substrate which was absent in these soils. The genetic studies revealed significant diversity between *A. flavus* strains based on the level of deletions in their aflatoxin gene cluster. However, clustering patterns were not linked to origin, but to gene function. Potentially atoxigenic strains were identified that may now be confirmed and could lead to the creation of a biocontrol formula.

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