

The giant Madagascar hissing-cockroach (*Gromphadorhina portentosa*) as a source of antagonistic moulds: concerns arising from its use in a public setting

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Summary

Cockroaches and their excreta have been linked to allergies and childhood asthma. The giant Madagascar hissing-cockroach (*Gromphadorhina portentosa*), heralded as an educational tool in classrooms, museums, zoos, is now available to the public as a children's pet raising health concerns. We provide a catalogue of potentially antagonistic moulds associated with this insect. Specimens were obtained from laboratories, classrooms, pet stores and private homes. Three different agar media were used to culture moulds from both internal and external cockroach surfaces. Pure cultures were obtained from hyphal tips excised by scalpel. A total of 14 mould taxa were obtained, mostly from external surfaces. The mycoflora was dominated by species of *Rhizopus*, *Penicillium*, *Mucor*, *Trichoderma* and *Alternaria*, and differed little among nymphs, adults, cast skins (exuviae) and faeces. A two-fold increase of *Aspergillus niger* isolates, however, was detected in exuviae and faeces. The mycoflora appeared to be equally distributed on the body regions in nymphs and adults alike. Most of the moulds recovered are common, well-known saprophytes with a prolific ability to produce asexual spores (e.g. conidia) when supplied with adequate moisture and an organic substrate (e.g. vegetable matter, pet food and exuviae). Cockroach rearing conditions thus serve as an ideal environment for mould growth and proliferation, and the subsequent use (handling) of these insects in a public forum increases the risk of inducing mould-related allergies in humans. Of special concern are moulds also capable of causing secondary infections (e.g. *Rhizopus*, *Mucor*, *Aspergillus*), gaining entry via open wounds and inhalation. This is mainly a point of public awareness aimed at individuals (especially children) prone to infections and allergies that might be exposed to this insect and/or its rearing conditions.

Key words: Cockroaches (*Gromphadorhina portentosa*), fungi, allergens.

Introduction

During the past decade, the giant Madagascar hissing-cockroach, *Gromphadorhina portentosa*, has been the subject of interest in classrooms, zoos, pet stores, trade

shows and museums aimed at the general public, especially children. Although harmless in itself, this cockroach is suspected of triggering allergic reactions in sensitive individuals upon exposure to the species' exuviae (cast skins from molts) and faeces.¹ Direct handling of these insects can result in contact dermatitis, swollen itchy watery eyes, sneezing and occasionally laboured breathing. These symptoms may persist for several days postexposure, but can re-occur when sensitive individuals are re-exposed to the insects and/or their cages. In light of the well-documented increase in cockroach-associated

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allergies,^{2,3} the use of this cockroach species in a public forum without proper precaution is no longer encouraged.¹

Recently, though not surprisingly, two pestiferous cockroach species have also been linked to childhood asthma – the American cockroach (*Periplaneta americana*⁴) and the German cockroach (*Blattella germanica*⁵). Antigens extracted from the midgut and faeces of these species have been shown to cross-react, raising concerns that the presence of these cockroaches in the home setting may exacerbate allergic reactions in humans. Compounding matters, both *P. americana* and *B. germanica* are known carriers of medically important fungi and pose an additional threat to mould-sensitive and/or immunocompromised individuals.^{2,5} The presence of mould spores (conidia) on the exoskeletons of these insects may induce allergic reactions upon inhalation, and can spread potentially-harmful fungi (e.g. *Aspergillus flavus*) to human food sources. Though *G. portentosa* has not been implicated as a household pest, it is conceivable that this cockroach species could also serve as a carrier of moulds given that the conditions in cages often are conducive to mould proliferation (e.g. moist rotting pet food, fruits and vegetables). If true, children that handle *G. portentosa* would likely spread conidia to the eyes, faces, and food sources if their hands are not thoroughly washed prior. The purpose of this study was to determine if, and to what extent, *G. portentosa* harbours moulds both internally and externally.

Materials and methods

Living specimens of *G. portentosa* were obtained from an active colony maintained at The Ohio State University (Columbus, OH) that were reared on commercial dry dog chow and tap water at 25 ± 1 °C, $45 \pm 5\%$ RH, 15 h light: 9 h dark (LD). Whole body analysis focused on adult females, whereas exuviae and faecal pellet analysis spanned mixed life stages collected from the floor of the colony. Additional material was obtained in Clark, Franklin and Miami Cos., OH from local pet stores ($n = 11$), science classrooms (elementary, middle and high schools; $n = 8$) and private home (pet) collections ($n = 6$). Diet and rearing conditions varied among these locations, but generally consisted of animal chow, rotting/fresh fruit and vegetables held at ambient temperature, relative humidity and lighting levels consistent with human comfort standards. Colony size ranged from eight to 10 individuals to as many as >300, but averaged approximately 20 individuals of mixed stages. All cockroaches appeared healthy upon inspection with no evidence of debris or external fungal

hyphae visible to the unaided eye. Collected cockroaches were inspected by light microscopy (40×) and also showed no signs of debris or hyphae. Cockroach handling, transport and storage was carried out under aseptic conditions assisted by utensils, gloves, contact solutions and aspirators subjected to autoclaving (15 min at 121 °C, 19 psi). Presterilized Petri dishes (100 × 15 mm; Fisher Scientific, Pittsburgh, PA, USA) were also employed. Culture work was conducted within 12 h of collection under a laminar flow hood previously disinfected with both bleach and UV light exposure.

Fungal isolations follow the methods outlined by Zettler [6], Yoder *et al.* [7] and Benoit *et al.* [8]. To ensure that a diverse assemblage of cultures were obtained, three different media were utilized: potato dextrose agar (PDA; Difco, Fisher, Philadelphia, PA, USA), modified PDA (MPDA = PDA adjusted with lactic acid, lowering pH to 5.5) and modified Melin-Norkrans agar (MMN⁹). To recover fungi from external cockroach surfaces, each insect was placed intact within a Petri dish and immersed with molten agar. Upon cooling (embedding), plates were incubated at 25 °C in total darkness (0 h : 24 h LD) and inspected every 12 h by light microscopy (40×) for signs of emerging hyphae. Subcultures were prepared only from hyphae that originated from the exoskeleton after 5–7 days, but some cultures required at least 2 weeks. Under a stereomicroscope, hyphal tips were carefully excised using a sterile scalpel, transferred to new Petri plates containing the same medium (one hypha per plate), and incubated under the same experimental conditions. This resulted in pure cultures that typically yielded morphological structures (e.g. phialides and conidia) within 2 weeks. Identification of anamorphic stages to the genus level was carried out using the keys and classification system outlined by Barnett and Hunter [10]. Cultures that failed to yield discernable structures were classified as *Mycelia sterilia*. To determine if *G. portentosa* harboured fungi internally, consistent with previous reports involving ticks and crickets,^{7,8} a slightly modified fungal isolation technique was employed. Briefly, cockroaches were disinfested in a 5 ml (5%) bleach solution (DI water-absolute ethanol – 5.25% NaOCl; 18-1-1, v/v/v) twice, each lasting 1 min. During each wash, cockroaches were vigorously shaken in this solution to further remove external debris (conidia) from the exoskeleton. The insects were then transferred to sterile DI water and rinsed twice (1 min each). Cockroaches were subsequently removed from water and placed within an empty Petri plate where they were then dissected using a sterile scalpel. All three

body regions (head, thorax and abdomen) were disconnected from each specimen and then sectioned into quarters (one sample portion per plate); exuviae were quartered and faecal pellets were halved. As described above, molten agar was poured over each body section and incubated. Subcultures were prepared only from hyphae that could be traced back to internal contents. A total of 120 individual cockroaches were plated on agar (60 external and 60 internal). Within each subset of 60, 20 specimens were placed on each of the three media (PDA, MPDA and MMN). Statistical analysis (ANOVA) was computed using SPSS 14.0 for Windows (SPSS, Inc., Chicago, IL, USA).

Results

Gromphadorhina portentosa yielded a total of 14 common mould genera, mostly from external surfaces (Table 1). The most prevalent taxa on the three media (pooled) were *Rhizopus stolonifer* (c. 40%), *Penicillium* spp. (c. 20%), *Mucor racemosus* (c. 9%), *Cladosporium cladosporioides* (c. 8%), *Trichoderma* spp. (c. 8%) and *Alternaria* spp. (c. 6%); other fungal genera were less abundant (c. <3%). No significant differences were detected among the three media in revealing mould diversity or abundance ($P < 0.05$). Nymphs ($n = 50$ consisting of six different instars) and adult cockroaches alike yielded a

Table 1 Mycoflora of female adult Madagascar hissing-cockroach, *Gromphadorhina portentosa*

Fungi	No. isolates from <i>G. portentosa</i> ($n = 120$ cockroaches)			
	External		Internal	
	Lab	Field	Lab	Field
<i>Absidia corymbifera</i>	4	6	0	1
<i>Alternaria</i> spp.	7	4	2	0
<i>Aspergillus flavus</i>	2	4	0	0
<i>Aspergillus niger</i>	3	7	0	1
<i>Cladosporium cladosporioides</i>	9	10	0	0
<i>Cunninghamella</i> spp.	2	1	0	0
<i>Curvularia</i> spp.	0	2	0	0
<i>Geotrichum</i> spp.	3	1	1	2
<i>Mucor racemosus</i>	10	7	0	0
<i>Mycelia sterilia</i>	2	4	0	0
<i>Penicillium</i> spp.	20	24	0	1
<i>Rhizopus stolonifer</i>	42	50	1	1
<i>Scopulariopsis brevicaulis</i>	0	3	0	0
<i>Trichoderma</i> spp.	9	4	0	0
Total	113	127	4	6

Lab, from laboratory colonies at The Ohio State University (Columbus, OH); Field, field (not wild)-collected specimens from homes, schools, pet stores (Clark, Franklin and Miami Cos., OH). Number of isolates combined from PDA, MPDA and MMN media.

Table 2 Fungi recovered from exuviae and faeces (combined internal and external) of Madagascar hissing-cockroach, *Gromphadorhina portentosa*

Fungi	No. isolates from <i>G. portentosa</i> ($n = 120$ cockroaches)			
	Exuviae		Faeces	
	Lab	Field	Lab	Field
<i>Absidia corymbifera</i>	1	1	0	3
<i>Acremonium</i> spp.	2	1	0	1
<i>Alternaria</i> spp.	4	2	1	2
<i>Aspergillus flavus</i>	0	3	1	0
<i>Aspergillus niger</i>	2	5	2	1
<i>Cladosporium cladosporioides</i>	6	10	5	1
<i>Mucor racemosus</i>	5	4	3	5
<i>Mycelia sterilia</i>	1	0	0	2
<i>Penicillium</i> spp.	8	12	7	8
<i>Rhizopus stolonifer</i>	11	9	7	12
<i>Scopulariopsis brevicaulis</i>	0	0	1	0
<i>Trichoderma</i> spp.	3	4	1	1
Total	43	51	28	36

Lab, from laboratory colonies at The Ohio State University (Columbus, OH); Field, field (not wild)-collected specimens from homes, schools, pet stores in Clark, Franklin and Miami Cos., OH. Number of isolates combined from PDA, MPDA and MMN media.

similar mycoflora profile which might be expected considering the clustering behaviour exhibited by this species.¹¹ Few fungi were isolated internally (Table 1) and without any regularity, suggesting that none of the external moulds were pathogenic or served as endosymbionts. No significant differences ($P < 0.05$) were detected in the mycoflora spanning all three body regions nor their associated parts (antennae and legs). The mycoflora associated with exuviae and faeces was nearly identical to that found on the external surfaces (Table 2); however, a significantly higher ($P < 0.05$) number of *Aspergillus niger* colonies were isolated from exuviae and faeces (c. 8%) compared with external body surfaces (c. 3%). This nearly twofold prevalence of an allergic mould species may explain, in part, why exuviae and faeces are potent allergens. Because *G. portentosa* is regarded as a social cockroach species,^{11,12} the higher prevalence of *A. niger* in faeces was unexpected considering that termites – also social insects and closely related with cockroaches^{13,14} – produce frass that discourages fungal growth.¹⁵

Discussion

All of the fungi recovered from *G. portentosa* normally produce copious quantities of spores (conidia). They are typically saprophytic, functioning as agents of decay except for *Trichoderma* spp. which is mycoparasitic.^{10,16}

Thus, the prevalence of these moulds on exoskeletons of cockroaches was expected considering that cockroach rearing conditions (e.g. moisture, food) are often conducive to conidia germination and fungal proliferation. Our results also agree with similar work linking moulds of medical significance (namely *Rhizopus*, *Mucor*, *Alternaria*, *Penicillium*, *Aspergillus*) to pestiferous cockroach species,^{3,4} but the occurrence of these moulds in a cockroach coveted by the general public is of special concern. The prevalence of *Aspergillus*, *Mucor* and *Rhizopus* on *G. portentosa*, in particular, is troublesome considering that these genera are regarded as 'fungal opportunists' capable of causing secondary infections in humans with a suppressed immune system (e.g. AIDS and leukaemia patients).¹⁷ Approximately eight species of *Aspergillus*, for example, are linked to aspergillosis in humans, gaining entry via open wounds and inhalation of conidia.¹⁷ *Mucor* and *Rhizopus* (Order Mucorales) induce diseases in both birds and humans, and may even exploit the central nervous system.¹⁷ We urge that individuals with a known track record of allergic reactions to moulds, as well as those prone to infections (e.g. children, the elderly) minimize direct contact with this insect and its rearing conditions. Similarly, institutions that utilize this insect for public display (e.g. zoos, museums, schools) should be aware of the health-related consequences for harbouring this species, and take action accordingly.

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