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Phylogenetic origins of African and Neotropical *Beauveria bassiana s.l.* pathogens of the coffee berry borer, *Hypothenemus hampei*

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Abstract

A phylogenetic epidemiological study of *Beauveria bassiana* s.l. was conducted for African and Neotropical pathogens of the coffee berry borer (CBB), *Hypothenemus hampei*, based on inferences from two nuclear intergenic regions, EFutr and Bloc. CBB pathogens were distributed among four terminal clades, however, the majority of African and Neotropical isolates cluster in a well-supported monophyletic group, informally designated AFNEO_1. Although the relationship between African and Neotropical AFNEO_1 is unresolved, the majority of alleles detected were exclusive to either the African or the Neotropical populations. These fixed genetic differences suggest that their disjunction predates the world trade in coffee. Neotropical AFNEO_1 have a broad host range and CBB pathogens are intermixed phylogenetically with isolates from diverse indigenous insects. Several Neotropical AFNEO_1 isolates were isolated from coffee plants as epiphytes or endophytes, thus plants themselves may potentially serve as reservoirs of pathogens against their insect pests. Topological incongruence between the EFutr and Bloc phylogenies of Neotropical AFNEO_1 may signify that individuals within this population are recombining.
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1. Introduction

The coffee berry borer (CBB), *Hypothenemus hampei* (Ferrari) (Coleoptera: Curculionidae), is the most devastating pest of coffee beans worldwide. Endemic to Central Africa (Le Pelley, 1968), the range of CBB has expanded greatly over the last 100 years as a result of the world trade in coffee. *H. hampei* was first reported in Gabon in 1901 (Beille, 1925), in Java in 1908 (Hagedorn, 1910), and in Brazil in 1913 (de Oliveria Filho, 1927). The pest has continued to spread and now occurs in the majority of coffee producing regions throughout the world, and threatens the economic viability of coffee growing in many of these areas.

The biology of CBB presents formidable challenges for the implementation of pest management programs. Inseminated adult females bore into a coffee berry and lay their eggs in the endosperm, on which the larvae feed. Prior to emergence from their natal berry, adult females mate with male siblings and are thereby inseminated and ready to colonize another berry. In contrast, males are apterous and never leave the berry (Le Pelley, 1968; Bustillo et al., 1998). The insects therefore spend the majority of their life cycles cloistered within coffee berries, presenting a very limited time window during which females are exposed to the environment. AFLP fingerprinting of DNA from coffee berry borers collected in 17 countries has revealed an extremely low genetic variability in this species, most likely due to inbreeding and possible genetic bottlenecks occurring during the course of its geographic dispersion (Benavides et al., 2005).

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The use of conventional insecticides for the control of coffee berry borers is a very limited option due to the economic situation of small holder coffee growers, the potential adverse effects of pesticides to human health and the environment, and the development of pesticide resistance by the coffee berry borer to endosulfan (Brun et al., 1989; ffrench-Constant et al., 1994). These constraints have prompted research on biological control, including the introduction of parasitoids and fungal entomopathogens (see Bustillo et al., 1998; and reviews by Moore and Prior, 1988; Waterhouse and Norris, 1989; Barrera et al., 1990; Moore et al., 1990; Murphy and Moore, 1990; Damon, 2000), and the search for new pathogens (Vega and Mercadier, 1998; Vega et al., 1999, 2000).

Forty different fungal species in 22 genera have been found associated with CBB (Pérez et al., 2003) and various entomopathogens are known to infect the insect (Fernãndez et al., 1985; Balakrishnan et al., 1995; Bustillo et al., 1998; Posada-Flórez et al., 1998). Among entomopathogenic fungi associated with CBB, *Beauveria bassiana* (Bals.) Vuill. (Ascomycota: Hypocreales) is by far the most widely reported: Brazil (Averna-Saccá, 1930), Cameroon (Pascalet, 1939), Colombia (Vélez-Arango and Benavides-Gómez, 1990), Democratic Republic of the Congo (Steyaert, 1936), Ecuador (Klein-Koch et al., 1988), Guatemala (Monterroso, 1981), Honduras (Lazo, 1990), India (Balakrishnan et al., 1994), Indonesia (Friederichs and Bally, 1923), Mexico (Méndez-López, 1990), Nicaragua (Barrios, 1992), and Venezuela (Bautista, 2000).

As the principal fungal pathogen of CBB in all coffee growing regions, *B. bassiana* is a leading candidate for its biological control (Bustillo et al., 1998). However, little is known about the geographic or genetic origins of *B. bassiana* that are pathogenic to CBB. It is not known whether ancestral African *B. bassiana* pathogens co-dispersed from Africa with CBB during the global expansion of coffee agriculture or whether these insects have acquired *B. bassiana* pathogens indigenous to regions where CBB has been introduced. According to Bridge et al. (1990), allozyme data support a close relationship among the majority of 16 isolates of *B. bassiana* from CBB from ten countries (Brazil, Ecuador, Guatemala, Indonesia, Jamaica, Kenya, Mexico, New Caledonia, Sri Lanka, and Togo). Although it is possible that the inferred similarity among these *B. bassiana* isolates is due to a common, possibly African origin, the sensitivity and utility of isozymes as molecular markers in *Beauveria* is not well developed and has been superceded by nucleic acid characters. In a geographic survey of *B. bassiana* isolates from CBB and other insects, Gaitan et al. (2002) reported high variability in RAPD banding patterns, low polymorphism in ITS RFLP but no congruence between the groupings resolved with either type of marker. Genetic surveys of *B. bassiana s.l.* from other insects and geographic regions employing dominant markers (Maurer et al., 1997; Glare and Inwood, 1998; Aquino de Muro et al., 2003, 2005) or ITS-RFLP (Coates et al., 2002) also perform poorly in resolving genetic groupings informative to hypotheses of host selection or geographic speciation. Thus, neither RAPD, AFLP nor ITS has proven effective for delineating genetic boundaries or for inferring population genetic or epidemiological processes within *B. bassiana*.

In a recent molecular phylogenetic analysis of *Beauveria*, Rehner and Buckley (2005) showed that the morphospecies *B. bassiana* consists of two unrelated and morphologically indistinguishable clades. These clades are not yet recognized taxonomically, and we refer to them as the "*B. bassiana s.l.*" and the "pseudobassiana" clades. The former is a globally distributed group, believed to include the authentic *B. bassiana*, and the latter is an ad hoc designation for the alternate clade, which remains to be described. This investigation is concerned only with isolates derived within the *B. bassiana s.l.* clade.

In this study, we investigated the phylogenetic diversity of *B. bassiana s.l.* pathogens of CBB from Africa (Cameroon, Côte d'Ivoire, Kenya, Togo) and the Neotropics (Brazil, Colombia, Costa Rica, Mexico, Nicaragua). Phylogenetic analyses presented here are based on nucleotide variation at two intergenic regions, EFutr and Bloc, genetic markers developed expressly for analysis within the *B. bassiana s.l.* complex (SAR, unpublished). A broad geographic sampling of *B. bassiana s.l.* isolates was used to provide a genealogical context for inference of the phylogenetic origins and relationships of *B. bassiana s.l.* pathogens of CBB. Additionally, the relationships of Neotropical *B. bassiana s.l.* CBB pathogens to other co-occurring *B. bassiana s.l.* pathogens of indigenous insect species, and to isolates associated with coffee plants as epiphytes and endophytes, were also determined.

2. Materials and methods

2.1. Source of fungal isolates

Fungal isolates were obtained from the ARS Collection of Entomopathogenic Fungal Cultures (ARSEF; Ithaca, NY), the European Biological Control Laboratory (EBCL; Montpelier, France) or are maintained in the laboratory of FEV in the Insect Biocontrol Laboratory (IBL; Beltsville, MD). Collection data for the 75 *B. bassiana s.l.* isolates included in the phylogenetic analyses conducted in this study are listed in Table 1. The African isolates, all of which were isolated from CBB, included 57 isolates from Cameroon (9), Côte d'Ivoire (17), Togo (28) and Kenya (3). However, only six of these isolates, which encompass the allelic diversity detected in Africa, were included in the present analysis. The 45 Neotropical isolates originated from Brazil (13), Colombia (10), Costa Rica (1), Nicaragua (3), Mexico (18), and included isolates from CBB (29), indigenous Neotropical insects (13) and endophytes (3) and epiphytes of coffee (1). An additional 22 isolates from Asia (9), Australia (2), Europe (4), North America (5) and North Africa (2) representing lineages other than those associated with CBB were included to provide phylogenetic context. An isolate of *Beauveria brongniartii* (Sacc.) Petch, ARSEF 1041, was used as outgroup for some analyses.