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Identification of the molecular origin and development of a panzootic caused by *Beauveria bassiana* in praying mantis populations in eastern China

Fenggang Luan, Shengli Zhang, Yue Cai, Zhaohong Sun, Bin Wang, Bo Huang, Zengzhi Li *

Provincial Key Laboratory of Microbial Control, Anhui Agricultural University, Hefei, Anhui 230036, China

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ABSTRACT

A panzootic in praying mantid species *Tenodera sinensis* and *Statilia maculate*, caused by *Beauveria bassiana*, occurred in north, southwest and southeast regions of Anhui Province, eastern China in Autumn, 2009. A 3-d principal component analysis (PCA) of 123 isolates from three sites revealed that the *B. bassiana* populations were heterogeneous with obvious dominance. Furthermore, the causal source of the panzootic in Anhui was shown to be polyphyletic. The populations were homogenized into homogenous subunits for investigation of genetic structure by inter-simple sequence repeat (ISSR) markers. Variance was greater than 70%, largely due to genetic differences within populations and subpopulations. Genetic distances and genetic differentiation were negatively associated with geographic distances and it was speculated that this was due to the effects of monsoons and topography. Mantid isolates were divided into five pathotypes based on a two-way cluster analysis of genetic distance. Pathotype I consisted of the predominant subpopulations of Huangcangyu and Chashui populations, with a genetic distance of 0.120 and gene flow up to 1.833. This pathotype caused a widespread epizootic in north and southwest Anhui, and Pathotype III caused enzootic at Site A in September and then epizootic in October, while the other three pathotypes caused enzootics at all three investigation sites. The widespread epizootics and isolated enzootics composed the polyphyletic panzootic in Anhui. A strong gene flow between isolates from the two mantid species was identified, resulting in negligible gene differentiation. This indicated a lack of host specificity in mantid isolates of *B. bassiana*.

1. Introduction

Praying mantids are generalist predators in gardens, forests or other vegetated areas. Young mantids eat many types of insects of their own size or smaller. The Chinese mantid, *Tenodera aridifolia sinensis*, is the largest of the species and was imported as a highly beneficial insect into the USA in 1896. It can consume up to 2000 insects during its lifetime, encouraging its use as a form of biological pest control (Lavies, 1990; Svenson and Whiting, 2004). However, praying mantids are not primary consumers within the food web and have their own natural enemies (Rosenheim, 1998), including spiders, insect eating snakes, bats, larger birds, and presumably, various insect pathogens, although *Beauveria bassiana*, an entomopathogenic fungus, is the only recorded example (Dicuzeide, 1925).

Mantid cadavers infected by *B. bassiana* have rarely been discovered, suggesting that the fungal disease prevails as an enzootic disease. However, in the autumn of 2009, dramatic epizootics caused by *B. bassiana* in mantid populations were detected in wide areas of north and southwest Anhui Province, eastern China. Furthermore, an enzootic caused by *B. bassiana* was detected in southeast Anhui. The highest cadaver density reached 2–4 capita per 10 m^2 and live individuals were rarely detected, displaying a widespread panzootic of praying mantids in the epizootic area. There have been no reports of a natural epizootic of predatory mantids.

A panzootic denotes a disease affecting all, or a large proportion of the animals of a region (Onstad et al., 2006). This type of epizootic caused by entomopathogenic fungi has been reported only once in the case of *Entomophaga maimaiga* infection of gypsy moth populations across the northeastern regions of the US in 1989. An indirect enzyme-linked immunosorbent assay (ELISA) was used for species identification (Hajek et al., 1990, 1991). To date, there are no records of a fungal epizootic associated with either praying mantids as a host of an epizootic or with the cosmopolitan fungus *B. bassiana* as the causal agent of a panzootic.

B. bassiana is a globally ubiquitous insect pathogenic fungus. Based on worldwide data, its insect hosts include 707 species from 521 genera of 149 families in 15 orders (Li, 1988). It has been used for worldwide insecticide development against crop and forest pests for more than 100 years (Zimmermann, 2007) and, as a natural mortality factor of insect populations, can also cause natural epizootics in some ecosystems. To date, however, surveys of

* Corresponding author. Fax: +86 551 5786589.
E-mail address: zzli@ahau.edu.cn (Z. Li).

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natural epizootics have been restricted to macroscopic investigation of the relationship between host, pathogen and the environment and the dynamics of the epizootics (Jankevica, 2004; Kessler et al., 2004; Devi et al., 2006; Ge et al., 2009; Wang et al., 2009). Epizootic studies of entomopathogenic fungi have been made mainly at the species level, although recent research has focused on genetic diversity (Nielsen et al., 2005).

During the last decade, a number of molecular markers have become widely used to study the genetic diversity of entomopathogenic fungi (Wang et al., 2005; Devi et al., 2006, 2007; Gauthier et al., 2007; Takatsuka, 2007; Carvalho et al., 2009; Fernandes et al., 2009). Studies have revealed rich genetic diversity in *B. bassiana* populations both in large areas like North America (Castrillo et al., 2003, 2004), Brazil (Fernandes et al., 2009) and Japan (Takatsuka, 2007), and small local sites (Wang et al., 2005; Takatsuka, 2007; Fernandes et al., 2009), but diversity studies have rarely been related to natural epizootics. Among the molecular markers, inter-simple sequence repeats (ISSR) are easily used and reliable one for genetic diversity that has been used in studies of plants, animals and fungi (Gupta et al., 1994; Zietkiewicz et al., 1994; Kerrigan et al., 2003; Wang et al., 2005; Takatsuka, 2007; Lihme et al., 2009).

Recently the study of genetic diversity in entomopathogenic fungi has been used to investigate epizootic populations following reports of multilocus sequence typing in the study of epizootics (Taylor and Fisher, 2003). A phylogenetic epizootiological study using two nuclear intergenic regions, Efutr and Bloc, was carried out for inference of the phylogenetic origin of *B. bassiana* as African and Neotropical pathogens of the coffee berry borer, *Hypothenemus hampei* (Rehner et al., 2006). Results inferred that the disjunction in monophyletic *B. bassiana* clades predated the world trade in coffee. Meyling and Eilenberg (2006) used a leaf imprinting technique combined with a selective medium to document the natural occurrence of *B. bassiana* on phylloplanes of typical hedgerow plants and used Universally Primed (UP) PCR to study the genetic diversity of the isolates. It was observed that four of the thirteen distinguishable banding patterns were shared between the field sites and all plant species harbored isolates of *B. bassiana* with at least two different banding patterns. They also reviewed the ecology of *B. bassiana* and *Metarhizium anisopliae* in temperate agroecosystems and evaluated their potential for conservation biological control (Meyling and Eilenberg, 2007). Noticeably, they highlighted that the evaluation of biodiversity contribution of *B. bassiana* and *M. anisopliae* in agroecosystems must be based on an assessment of the genetic diversity. Liu et al. (2008) studied the 28S rDNA group I intron marker as a marker of genetic diversity in 40 *B. bassiana* strains involved in a natural epizootic in Mt. Huangshan region. The genotype frequencies in the population of *B. bassiana* indicated that genetic stability and ecological adaptability differed with the genetic backgrounds of the strains of *B. bassiana*, and that the genotype with widest host range predominated. Furthermore, it was revealed that natural heterokaryons occurred at high frequency (up to 17.5%) in the natural broad-leaf forest ecosystem (Liu et al., 2008). Zhou et al. (2010) used ISSR to investigate the genetic diversity of *Nomuraea atypicola* isolates in natural epizootics among spider populations and demonstrated that the genetic similarity of *N. atypicola* isolates was more closely associated with the geographical origin than the host origin. A similar study of 40 isolates of *Entomophthora muscae* sampled at five sites in New Zealand during an epizootic in autumn, 2005, revealed the involvement of several *E. muscae* genotypes and proposed the possibility that the population genetic structure underlying *E. muscae* epidemics could be panmictic consisting of several lineages with a high level of reciprocal migration (Lihme et al., 2009).

Recently, a study of ISSR was used to trace the molecular origin of white muscardine disease in silkworms caused by *B. bassiana*. It was observed that the muscardine was enzootic and was not associated either with the release of a fungal insecticide developed from a strain of *B. bassiana* against the Masson's pine caterpillar, *Dendrolimus punctatus*, or natural prevalence of indigenous strains of the fungus in pine caterpillar populations and an epizootic of praying mantid populations in the area surrounding the site of silkworm rearing (Li et al., 2010, 2011).

In this study, ISSR markers were used to investigate the genetic structure of populations of *B. bassiana* isolates involved in the 2009 panzootic in Anhui, eastern China, in order to understand the nature of the very unusual panzootic, and to provide a more comprehensive knowledge of mantid population dynamics.

2. Materials and methods

2.1. Epizootic sites and fungal isolates

A total of 123 isolates of *B. bassiana* were characterized by ISSR–PCR. Among them, 110 were obtained from mantid cadavers collected at three sites in Anhui Province (Table 1): 62 were collected around shrubs in a farm situated between a mulberry plantation and a pine plantation at Chashui (Site A), Qianshan, southwest Anhui (45 collected on 22-09-2009 and 17 on 16-10-2009); 44 were collected at a deciduous broadleaved forest, National Huangcangyu Forest Park (Site B), Xiaoxian, North Anhui (17-10-2009) and four were collected from the evergreen broad-leaved forest in the National Maren Qifeng Forest Park (Site C) (Fig. 1), Fanchang, southeast Anhui (04-11-2009). Two species of host praying mantids were identified: 66 Chinese mantids, *Tenodera sinensis* and 44 maculated mantids, *Statilia maculate*. A further 13 isolates from other insect cadavers (Coleotera, Homoptera, Hymenoptera, Lepidoptera and Diptera) at Site B (17-10-2009) were collected and analyzed. All 123 samples belonged to three populations based on the location of collection (Table 1), although the Chashui population (Pop 1) and Huangcangyu population (Pop 2) were further divided into two subpopulations based on collection time and host, respectively.

2.2. Extraction of mycelial genomic DNA

DNA extraction was performed using benzyl chloride for chemical disintegration of cell wall according to previously described methods (Wang et al., 2005; Zhu et al., 1994) with the following modifications: the air-dried pellet was resuspended in 500 μl of Tris–EDTA (TE) buffer (10 mM Tris–HCl, pH 8.0; 1 mM EDTA, pH 8.0) and 2 μl RNase (10 mg/ml) was added and incubated at 37 °C for 1 h. The concentration and quality of the purified DNA was evaluated by 0.8% agarose gel electrophoresis and spectrophotometry.

2.3. ISSR amplification

A total of eight ISSR primers were evaluated for their capacity to produce polymorphic, easy-to-score and reproducible banding patterns from *B. bassiana* isolates (Table 2). ISSR–PCR amplifications and analyses were conducted as previously described (Wang et al., 2005) with the following modifications: the number of amplification cycles was increased to 38, and the template DNA was increased to 20 ng. PCR was performed in triplicate using a TPersonal Thermocycler (Biometra®, Germany) and the gel image was recorded by using a Gel Documentation System (Tanon Gis-2008). Each PCR reaction contained 1 × PCR buffer, 2.5 mM $MgCl_2$, 100 μM dNTPs, 0.4 μM primers, 0.75 U DNA Taq polymerase (Dream Taq™, Fermentas Life Science, as above) and 20 ng template DNA. The PCR reaction mix was adjusted to a final volume of 15 μl with DEPC-treated water (Sangon®, Sangon Biotech).