Genetic variation among isolates of the entomopathogenic fungus, Beauveria bassiana (Bals.) Vuill. (Ascomycota: Hypocreales): Use of rRNA internal transcribed spacers and group I introns, and a novel polymorphic minisatellite locus for the identification of strains.

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# TABLE OF CONTENTS

**LIST OF FIGURES**

**LIST OF TABLES**

**ABSTRACT**

**CHAPTER 1. GENERAL INTRODUCTION**

- Introduction 1
- Thesis Organization 2
- Literature Review 3

**CHAPTER 2. *BEAUVERIA BASSIANA* HAPLOTYPE DETERMINATION BASED ON NUCLEAR rDNA INTERNAL TRANSCRIBED SPACER PCR-RFLP**

- Abstract 21
- Introduction 22
- Materials and Methods 24
- Results 28
- Discussion 29
- References 33

**CHAPTER 3. EVOLUTIONARY MOBILITY, SEQUENCE VARIATION, AND AN OPEN READING FRAME IN NUCLEAR LARGE SUBUNIT rRNA GROUP I INTRONS OF THE ENTOMOPATHOGENIC FUNGUS, *BEAUVERIA BASSIANA*.**

- Abstract 46
- Introduction 47
- Materials and Methods 49
- Results 54
- Discussion 58
- References 73

**CHAPTER 4. ALLELIC VARIATION OF A *BEAUVERIA BASSIANA* MINISATELLITE IS INDEPENDENT OF HOST RANGE AND GEOGRAPHIC ORIGIN.**

- Abstract 78
- Introduction 79
- Materials and Methods 83
- Results 86
- Discussion 87
- References 92
LIST OF FIGURES

FIGURE 2.1. 506 base pair *Beauveria bassiana* ITS region sequence alignment. 37

FIGURE 2.2. Phylogeny of *B. bassiana* ITS region PCR-RFLP haplotypes. 44

FIGURE 3.1. PAGE separation of PCR products detecting group I introns indels. 67

FIGURE 3.2. *Beauveria bassiana* group I introns 1 PCR-RFLP products. 68

FIGURE 3.3. Group I introns internal guide sequence (IGS) structures. 69

FIGURE 3.4. DNA sequence of loop2 insertion and ORF from group I introns 2. 70

FIGURE 3.5. Phylogenetic relationships between *B. bassiana* group I introns. 72

FIGURE 4.1. 483 bp insert DNA sequence from clone pGEM-BbMSGA-07. 97

FIGURE 4.2. PAGE separation of eight *BbMin1* minisatellite alleles. 98
LIST OF TABLES

TABLE 2.1. Origin, ecotype, and ITS region haplotype of *B. bassiana* isolates. 39
TABLE 2.2. ITS region PCR-RFLP restriction fragment sizes. 43
TABLE 2.3. AMOVA table comparing *B. bassiana* ecotypes define by insect 45
TABLE 3.1. Distribution of intron subtypes among 101 *B. bassiana* isolates 65
TABLE 3.2. Feature map showing group-I introns insertion and PCR primers. 66
TABLE 4.1. Distribution *BbMin1* minisatellite alleles among *B. bassiana* isolates. 99
TABLE 4.2. AMOVA table comparing *B. bassiana* ecotypes define by insect. 100
ABSTRACT

The filamentous ascomycete fungus *Beauveria bassiana* is a pathogen of certain crop insects and is used in insect biological control regimes, yet little investigation has been devoted to the description of reliable genetic fingerprinting techniques or the analysis of population genetic diversity. An allele-specific molecular genetic approach was devised for the differentiation of 120 isolates of the entomopathogenic fungus *B. bassiana* using nuclear ribosomal RNA (rRNA) mutation and allelic variation at a single minisatellite locus. High resolution PCR-RFLP and direct DNA sequence analysis was used to describe the level of variation in two nuclear rRNA internal transcribed spacer (ITS) regions and four rRNA large subunit (LSU) group I introns. Differential cleavage at seven restriction sites within the ITS region identified 31 unique genotypes among 111 *B. bassiana* isolates. Inter- and intraspecific PCR-RFLP variation in four group I introns inserted into the *B. bassiana* LSU rRNA identified thirty seven different fungal strains. A second locus was isolated from a partial *B. bassiana* genomic library and shown to contain a novel polymorphic minisatellite repeat, *BbMin1*. Size variation in PCR amplified fragments identified eight allelic types within the sample set that varied in the number of complete repeat units. In conjunction with rRNA loci and allelic variation at the *BbMin1* locus, 89 genetic groups were delineated. Fixation indices (Fst) were calculated separately for each genetic marker data set, and neither indicated statistical correlation between genotype and geographical origin or pathogenic phenotype. All genetic markers were suggested to be selectively neutral since similar genotypes were randomly distributed between arbitrary subpopulations of *Beauveria*. 
CHAPTER 1. GENERAL INTRODUCTION

Introduction

Entomopathogenic fungi cause common and widespread diseases in insects, and have shown large epizootics events that decimate local insect populations. Effective utilization of such fungi as biological control agents require a better understanding of their biology, physiology, and genetics. Additionally, core knowledge of taxonomy and population diversity with respect to pathogenic action promises to facilitate the selection of appropriate strains. The use of transgenic maize expressing the Δ-endotoxin from *Bacillus thuringiensis* (Bt) has exposed the need for examination of the environmental impact on non-target insect species. Without detailed knowledge of genetic diversity, isolation of insect-specific strains and virulence factors from entomopathogenic fungi may prove difficult.

The species *Beauveria bassiana* is an obligate parasite of most insect orders, and also resides in soil-borne habitats and endophytic relationships. Basic understanding of pathology has been gathered from field isolates obtained from specific insect hosts. Despite the potential use of *B. bassiana* for control of insects that cause crop plant damage, little knowledge exists of inter- and intraspecific genetic diversity, or relationship among strains. This thesis research describes methods to define, characterize, and compare distinct genetic groups within the species *B. bassiana*. Statistical analysis of molecular data was conducted to define relationships among genetic types that may express unique and insect species-specific virulence factors. The strains identified through this research may assist in the isolation of genes with desired traits, be useful for the genetic improvement of the entomopathogen, and applied to tracking of released isolates in field trials.
Thesis Organization

This thesis is organized into five chapters. Chapter 1 presents background information on genome organization and population genetic variation of *B. bassiana* or related species. Later subsections of chapter 1 also were devoted to brief descriptions of the nuclear ribosomal RNA genes, group I intron structure and function, mutation mechanisms involved in generation and duplication minisatellite sequences not included within chapters 2, 3, or 4. References for chapter 1 are presented in a cumulative list following and including those from chapter 5.

Chapters 2, 3, and 4 present research articles either accepted or submitted to peer review journals. The first two chapters describe the detection of mutation within monocistroninc nuclear rRNA repeats of *B. bassiana*. Specifically, polymerase chain reaction restriction fragment length polymorphism (PCR-RFLP) of two internal transcribed spacer (ITS) regions that flank the 5.8S rRNA gene, and four group I introns inserted within nuclear large subunit rRNA (nuLSU rRNA) are described. The novel locus *B. bassiana* minisatellite 1, *BbMin1*, is characterized in chapter 4. All loci were considered to be regions of reduced function, that, in turn reduced nucleotide constraint compared to other regions of the genome (see literature review).

Through the duration of this research project, samples were continually added to increase the geographic and phenotypic range of *B. bassiana* isolates under analysis. Therefore, the number of isolates increased from 95 in chapter 2 to a maximum of 101 in chapter 3. Complete analysis of rRNA and *BbMin1* was performed on 111 isolates. A large percentage of the disparity represented omission of related *Beauveria* species from manuscript submissions.
Lastly, chapter 5 provides a cumulative description of genetic diversity among 111 isolates (Appendix B, Table 2). Particular emphasis was placed on categorizing isolates based on genetic marker data, and the determination of relationship among different genotypes within context of a population. All statistical analysis determining possible correlation between fungal genotype, and insect host range and geographical origin was performed individually upon genetic marker data in appropriate chapters.

Literature Review

At least 750 species of entomopathogenic fungi from 85 genera have been identified (Carruthers and Hural, 1990). The fungal species Beauveria bassiana (Bals.) Vuill. (Ascomycota: Hypocreales) is an imperfect filamentous entomopathogen belonging to the Ascomycete subclass Euascomycetidae and the form subclass Hyphomycetidae in the form class Deuteromycetes (Griffin, 1994). The fungus has been recognized as a potent fungal pathogen of insects (Vuillemin, 1912; Petch, 1926). Beauveria bassiana-mediated biocontrol of insect pests has been shown (Hajek et al, 1987; Bing and Lewis, 1991; Bing and Lewis, 1992; Harrison, 1992; Johnson and Goettel, 1993; James et al. 1995; Labotte et al., 1996; Inflis et al, 1997; Lacy et al., 1999; Godonou et al., 2000).

Historical Classification of Beauveria spp.

Beauveria bassiana, and related species of the genus, initially were characterized by Vuillemin (1912), and subsequently described biologically and morphologically by Petch (1926). The genus Beauveria initially was divided into two species, B. bassiana and B. tenella (McLeod, 1954), but de Hoog (1972) later identified three species; B. bassiana, B.
brongniartii, and B. alba. Additionally, two infectious types (white and yellow muscardine) were reported with B. bassiana pathogenic towards Bombyx mori (Aoki et al., 1975). Recent classification has included four additional species; B. vermiconia (de Hoog and Rao, 1975; Glare et al., 1993), B. velata and B. amorpha (Samson and Evans, 1982), and B. caledonica (Bisset and Widden, 1988). The most recent taxonomy survey of Beauveria spp. presented eight species; B. alba, B. amorpha, B. bassiana, B. brongniartii, B. densa, B. stephanoderia, B. velata, and B. vermiconia (Magnai et al., 1989).

Attempts to classify isolates into species groups found that cultural characters were variable and overlapped between species (Petch, 1926; Mugnai et al., 1989). Petch (1926) and MacLeod (1954) used spore shape to identify two related species groups; B. bassiana and B. brongniartii. MacLeod (1954) also sighted misleading spore morphologies on slide cultures, which produced ellipsoidal to cylindrical secondary spores, called blastospores (Thomas et al., 1987). More recent comparison of morphological characters among species of Beauveria has been conducted. Mugnai et al. (1989) indicated that spore form was the most reliable character for differentiation of species despite inability to separate B. bassiana and B. alba. Glare and Inwood (1998) found conidial measurements to be highly variable within five species of Beauveria, and surmised that prolonged culture on artificial medium resulted in altered spore morphology (Townsend et al., 1995).

Early attempts at taxonomic classification of B. bassiana within the genus Beauveria indeed was wrought with multiple difficulties, and even the boundary of the genus was question by Arx (1986) where synonymy with Tolypocladium was proposed. Classification of the Beauveria complex has resulted in different interpretations (de Hoog 1972; Von Arx, 1986). Placement of species within the genus also has been variable, i.e. B. densa and B.
caledonica were suggested to be distinct classes within a highly variant population of B. bassiana, and thus merely subspecies of the latter (Magnai et al., 1989; St Leger et al., 1992).

Molecular Characterization of B. bassiana Chromosomal and Mitochondrial DNA

Characteristics of melting point (Tm) and buoyant density (ρ) were used to estimate the percentage of guanine and cytosine (%G+C) base pairs in Beauveria bassiana DNA (Pfeifer and Khachatourians, 1989). Hypochromicity is the phenomena where double-stranded helical DNA has a lower UV absorbance than it does in single stranded form (Blackburn and Gait, 1996). The guanine-cytosine base pair chromatic shifts are measured at 280 nm (Mandel and Marmur, 1968), and the transition of a double stranded helix to two single stranded chains is temperature dependent where the midpoint of the denaturation curve is referred to as the melting point (Tm). Equilibrium density gradient ultracentrifugation in cesium chloride (CsCl) constitutes a method for determination of nucleic acid buoyant densities, ρ, and separation of satellite bands consisting of mitochondrial and chloroplast DNA from the major band of chromosomal DNA. From both melting point and buoyancy data, the percentage of G-C base pairs within the genome of an organism can be calculated (DeLey, 1970).

The melting curve for B. bassiana chromosomal DNA indicated a Tm of 92.6 ±0.8°C, that corresponded to a %G+C of 56.9 ±1.9%. The buoyant density of B. bassiana chromosomal DNA, 1.7130 g/ml, indicated a %G+C of 53.4% ±0.3 (Pfeifer and Khachatourians, 1989). Earlier work by Storck and Alexopoulos (1970) based on buoyant density data from B. tenella, indicated a %G+C of 53.0%, and suggested that a similar base composition may be present among members of the genus Beauveria. The genus has a high
%G+C compared to the mean percentage from 13 Ascomycete fungi (45%) (Griffin, 1994), but *B. bassiana* was within the range of 38 to 54%. Mitochondrial DNA analysis indicated that two Tm's were present, 76.2 ±0.5 and 93.1 ±0.7°C, which resulted in estimations of %G+C calculations of 16.8 ±1.2 and 58.0 ±1.7%. Also mitochondrial DNA buoyant density, 1.7118 g/ml, corresponded to a %G+C of 52.1 ±0.3 %. Mitochondrial DNAs of fungi have typically been shown to have buoyant densities equal to or less than that calculated for the chromosomal DNA component (Villa and Storck, 1968). *Beauveria bassiana* did not provide any exception, but the close percentages were suggested to result from large AT rich mitochondrial segments that was partly confirmed by a small hypochromatic shift that resulting in a minor peak in the melting curve.

The mitochondrial DNA (MtDNA) from *B. bassiana* was further analyzed by Pfeifer and Khachatourians (1989; 1993), and Hegedus et al. (1991; 1998). Scanning electron microscopy (SEM) was used to visualize a quasi-spherical mitochondrial DNA molecule with a diameter between 0.21 to 0.68µm, with an average of 0.38µm. Three mitochondrial DNA molecules were circular whereas eight other were linear when view under the SEM (Pfeifer and Khachatourians, 1989), but were suspected to have been broken during isolation. Linear forms also have been described from *Physarum polycephaleum* and *Hansenula markii* (Taylor, 1986), but only circular forms were observed from *Podospora anserina* (Cummings et al., 1979). Size of the *B. bassiana* MtDNA was determined to be 28.5 kilobases (kb), which was smaller than most fungal MtDNA (Pfeifer and Khachatourians 1989). A physical map was created with enzymes *Bst*EII, *Clal*, *Eco*RI, *Sal*I, and *Xho*I, where no *Pst*I, *Kpn*I, *Bam*HI, or *Sma*I sites were identified (Pfeifer et al. 1993a). The first *B. bassiana* MtDNA sequence data were obtained from five mitochondrial tRNA
genes, tRNA\textsuperscript{Ile}, tRNA\textsuperscript{Pro}, tRNA\textsuperscript{Ser}, tRNA\textsuperscript{Trp}, and tRNA\textsuperscript{Val} located in a region 5' to the large subunit rRNA (Mt LSU rRNA) gene (Hegedus et al., 1991). A unique anticodon, UCA, for tRNA\textsuperscript{Trp} was predicted from a secondary structure model as opposed to the CCA found in prokaryotic and eukaryotic nuclear tryptophan tRNAs. The UCA anticodon was predicted to bind UGG Trp codons as well as the UGA stop codon. Results further suggested that, like \textit{Neurospora crassa}, yeast, and mammalian mtDNAs (Heckmann et al., 1980), the UGA codon is not used as a stop codon in the \textit{B. bassiana} mtDNA coding sequences. Partial DNA sequence was later generated from six MtDNA encoded genes (Pfeifer et al. 1993a). The only full length DNA sequence to be provided was for the mitochondrial small rRNA gene (Hegedus et al., 1998) that used for phylogenetic comparison to similar DNA sequences from \textit{P. anserina} (GenBank X14734; Cummings et al, 1990), \textit{N. crassa} (GenBank L33367), \textit{Aspergillus niusiens} (GenBank V00653; Kochel and Kuntzel, 1981), \textit{Penicillum chrysogenum} (GenBank L01493; Sekiguchi et al, 1990).

The \textit{B. bassiana} genome size, and chromosome number and size was determined (Pfeifer and Khachatourians, 1993c; Viaud et al, 1996), and aneuploidy and chromosome length polymorphism (CLP) was shown to exist within the species (Viaud et al, 1996). The first pulsed field gel electrophoresis (Chu et al., 1986) karyotype of \textit{B. bassiana} chromosomes was prepared by Pfeifer and Khachatourians (1993c), where eight chromosomes were identified from isolate GK2016. Size range of the chromosomes was between 2.9 and 8.1 mega-base pairs (Mbp), and individual chromosomes were sized as 2.9 ±0.1, 2.9 ±0.1, 3.9 ±0.2, 4.6 ±0.2, 5.2 ±0.2, 5.9 ±0.4, 7.1 ±0.6, and 8.1 ±0.8 Mbp.

Aneuploidy and CLP was documented among \textit{B. bassiana} isolates via pulse field gel electrophoresis karyotyping (Viaud et al., 1996). Results indicated that five to eight
chromosomes were present among isolates, and sizes ranged from 1.2 to 7.7 Mbp. The resultant total genome size estimates ranged from 34.3 to 44.1 Mbp, which was inclusive of the size estimated from isolate GK2016 by Pfeifer and Khachatourians (1993c). The B. bassiana chromosome sizes were within the size range characterized from other filamentous fungi, 0.3 Mbp from Acremonium chrysogenum (Smith et al., 1991) to 12.6 Mbp from N. crassa (Orbach et al., 1991). Viaud et al. (1996) indicated a single isolate of B. sulfurescens, Bs2, had a genome size of 31.8 Mbp, and a chromosome size range of 2.4 to 7.7 Mbp. The entire B. bassiana genome was subsequently estimated to be 40.6 ±1.1 Mbp, and was similar in size to the genomes of N. crassa (47.7 Mbp) (Orbach et al., 1988), Penicillium janthinellum (42.9 Mbp) (Kayser and Schulz, 1991), and Trichoderma reesi (37.9 Mbp) (Gilly and Sands, 1991).

Viaud et al. (1996) used four genomic DNA probes to map chromosomal location of β-tublin, histone 4, the 28s rRNA, and protease 1 (Joshi et al., 1995) genes. All nine B. bassiana isolates that were evaluated had the protease 1 gene located on 7.2 Mbp chromosomes, whereas location of the other three genes were not conserved among isolates (Viaud et al., 1996). Chromosomal deletions and translocations likely have resulted in observed CLP among isolates, and variable chromosomal location of genes within the Beauveria genome. Recombination between non-sister chromatids at regions of repetitive sequence, such as the transposable elements hupfer from B. bassiana (Maurer et al., 1997) and Restless from B. nivea (Kempken et al., 1998), may have facilitated translocation. The high level of observed B. bassiana CLP, also may suggest that repetitive elements are common and the fungus tolerates resultant chromosomal aberrations.
Two Class II insertion elements, the 3.3 kilobase (kb) *hupfer* from *B. bassiana* (Maurer et al., 1997) and 4.1 kb *Restless* from *B. nivea* and *B. rosariensis* (Kempken et al., 1998), have been characterized from the *Beauveria* genome. The *B. bassiana* element, *hupfer*, inserted into a palindromic TA dinucleotide sequence at +27 of the nitrate reductase (*nia*) gene, contained inverted 30 bp imperfect terminal repeats (ITRs) that were flanked internally by CA dinucleotides (Randice et al., 1994; Polard and Chandler, 1995). The *hupfer* element was described as a composite transposon, in that besides the encoded transposase an additional 2.0 kb of DNA sequence was present that was similar in structure to Tec1 and Tec2 elements from ciliate protozoans (Jahn et al., 1993). Like other IS- and mariner-*Tcl1*-like transposons, non-coding *hupfer* DNA sequence was suggested to consist of foreign sequence sequestered by “trapping” (Capy et al., 1996). Southern hybridization of *BgIII* or *BamHI* digested total genomic DNA revealed that 4 or 5 *hupfer* elements were present per genome, and great variation in location among 11 isolates was evident (Maurer et al., 1997).

A second Class II transposon, *Restless*, was originally characterized from *Tolypocladium inflatum* (Kempken et al., 1996) and later described from two isolates of *B. nivea* (Kempken et al., 1998). The element was shown to be similar to members of the *hAT* family of transposons that have short inverted repeats, an 8 bp target site duplication that remains after excision, and a highly conserved putative transposase coding sequence (Essers and Kunze, 1995). Copy number of the *Restless* elements in the genome of four *B. nivea* and a single *B. rosariensis* isolate was determined by Southern hybridization analysis (Kempken et al., 1998). The *B. nivea* strain ATCC 34921 (syn. *T. inflatum*) had 15 insertions, as did a single strain of *B. rosariensis*. Two *B. nivea* strains had a single copy within their genome,
and a third *B. nivea* strain was devoid of *Restless* elements. Also, *Restless* was found to have three variant forms, two full length copies (*Restless* and *Restless*-2) that deviated from one another by 0.12%, and a truncated version (*Restless-di*) that lacked the entire 5-prime region of the element. The *Restless-di* variant of *B. nivea* isolate ATCC 42437 was characterized as having a truncated transposase coding region that also possessed a frameshift mutation, which suggested that it was incapable of excision (transposition). The penetrance of *Restless* into the *Beauveria* genome appeared to be variable, with zero to 15 copies per genome.

Similar copy number variance was observed from the *Fotl* element of *F. oxysporum* (Daboussi and Langin, 1994), *Flipper* from *Botrytis cinerea* (Levis et al., 1997), and *Nhtl* from *Nectria hematococca* (Enkerli et al., 1997). Low copy number may either indicate a recent introduction of *Restless* into the species by horizontal transfer whereby the element has not had sufficient time to replicate itself, or defects in transposase expression may have occurred. Evidence presented by Daboussi and Langin (1997) indicated that variation in *Fotl* transposable elements had occurred within the genus *Fusarium*, and suggested that horizontal transfer was responsible. Genetic exchange of extra-chromosomal material during hyphal anastomosis was shown to occur (Collins and Saville, 1990), and *hAT* transposon likely move horizontally via free circular DNA derivatives formed during transposition (Kempken and Kuck 1998b).

*Beauveria* Molecular Genetic Variation

Evolution of the vertebrate mtDNA molecule has been shown to be between 5 and 10-fold faster than in nuclear DNA (Vawter and Brown, 1986), whereas that from fungal mitochondrial small rRNA evolves 16-fold faster than nuclear counterparts (Bruns and Szaro,
1992). Mitochondrial DNA is not methylated and has a high copy number (Bruns et al., 1991). Since the *B. bassiana* mtDNA does not house genes related to entomopathogenicity, evaluation of neutral gene mutations is possible (Pfeifer and Khachatourians, 1993a; Hegedus and Khachatourians, 1993a). Hegedus and Khachatourians (1993a) used five mtDNA probes to identify 5 RFLP types from *EcoRI* digested DNA from 18 *Beauveria* spp. isolates. Two *EcoRI* RFLP variants were found only among *B. bassiana*, one from 4 of 15, and the other from 1 of 15 isolates. *EcoRI* RFLP variants unique from those from *B. bassiana* were shown to exist in single isolates of *B. brongniartii* and *B. densa*, and the most common genetic types from characterized from 10 of 18 isolates that included both *B. bassiana* and *B. caledonica*. Probing of *HindIII* digested DNA with RFLP probe pBbmtE3 differentiated all *B. bassiana* isolates from a single *B. caledonica* sample. DNA sequencing located the polymorphic *HindIII* site between the terminus of the mtATP6 gene and start of the small rRNA gene. Based upon MtDNA RFLP similarities between *B. bassiana*, *B. densa*, and *B. caledonica*, the two later species were suggested to be subspecies or sister species of the first (Hegedus and Khachatourians, 1993a), and that the *B. brongniartii* MtDNA type was significantly different from that of other three species. Lastly, no correlation between geographic location or host species and MtDNA RFLP patterns were observed.

Genetic markers have been used for the identification and differentiation of strains, and analysis of population genetic structure. Randomly amplified polymorphic DNA (RAPD)/arbitrarily primed PCR (Williams et al., 1990; Welch and McClelland 1990) were used in studies by Bidochka et al. (1994), Cravanzola et al. (1997), Maurer et al. (1997), Urtz and Rice (1997), Piatti et al (1998), Castrillo and Brooks (1998), Berretta et al. (1998), and
Glare and Inwood (1998). Additionally, markers from the rRNA (Neuveglise et al. 1994a; Neuveglise et al. 1994b; Neuveglise et al. 1997; Glare and Inwood, 1997) and isozyme loci (Poprawski et al. 1988; St. Leger et al. 1992; Rakotonirainy et al. 1994) were used. Assessment of population differentiation based on geographic origin and insect pathogenic capacity often has been the main focus of studies on *B. bassiana* molecular variation.

The first RAPD-PCR study was performed by Bidochka et al. (1994) whom evaluated three *B. bassiana* isolates and indicated that no evidence for geographical or host range clustering of genotype was evident, in that the three genetically similar *B. bassiana* isolates were derived from either the USA and Crete. Bidochka et al. (1994) further proposed that the genetic similarity of *B. bassiana* isolates may result from clonal propagation or recent speciation. Urtz and Rice (1997) evaluated 18 *B. bassiana* isolates from the rice water weevil, *Lissorhoptrus oryzophilus*, and found no relationship between genotype and geographic origin. In fact RAPD-PCR results indicated that two distinct groups had a common geographic distribution. Urtz and Rice (1997) further suggested that the groups may represent different populations that exist sympatrically in the same geographic area. An automated RAPD-PCR fragment detection protocol was instituted by Berretta et al. (1998) for DNA fingerprinting of *B. bassiana* isolated from *Diatraea saccharalis*, the sugar cane borer, larvae collected in Argentina, and eight other isolates pathogenic against other insects. Phylogenetic analysis clustered ten *B. bassiana* isolates from *D. saccharalis* into four groups, and indicated that geographic location had no detectable influence on genetic makeup.

Twenty seven *Beauveria brongniartii* isolates from two species of *Melolontha*, the European cockchafer, were analyzed by RAPD-PCR analysis by Cravanzola et al. (1997). Results showed that *B. brongniartii* isolates were closely related regardless of geographical origin or
insect host. A follow-up RAPD-PCR study by Piatti et al. (1998) on 50 additional *B. brongniartii* isolates collected from *M. melolontha* larvae in Valle d’ Aosta France. As was found by Cravanzola et al. (1997), a high degree of genetic similarity was shared among isolates. From UPGMA-based phylogenetic analysis Piatti et al. (1998) suggested that five highly related (similarity >0.745) groups were present, of which only one group was significantly different from the others. Also, Piatti et al. (1998) indicated a mild correlation between site of isolation and phylogenetic grouping of *B. brongniartii* in the study, but an overlapping range of isolates from the three groups suggested that those isolates were merely variants present within a single population.

While the aforementioned studies did not find a definitive correlation between population structure and insect host species, results by Tigano et al. (1990), Powrawski et al. (1988), and Maurer et al. (1997) did. All isolates originally obtained from the host *Ostrinia nubilalis* were shown to be monomorphic (Poprawski et al., 1988; Tigano et al., 1990), and were suggested to reproduce by clonal propagation (St. Leger et al., 1992). Maurer et al. (1997) indicated that similarity was found among isolates from *O. nubilalis*, as well as those from the weevil genus *Sitona*. In contrast, Maurer et al. (1997) also found lack of genetic similarity between isolates from the insect order Chrysomelidae and the pyralid genus Maliarpha, which suggested that, in some instances, a correlation between population structure and host species may exist.

Nuclear Ribosomal RNA (rRNA): Structure and Population Genetic Variation

The nuclear ribosomal RNA (rRNA) is repeated up to several hundreds of times per genome. All eukaryotic rRNA gene clusters contain the small subunit (SSU, 18S), large
subunit (LSU, 28S), and 5.8S rRNA genes, and are transcribed as a monocistronic unit. The 5.8S rRNA gene is separated from the 18S by the internal transcribed spacer I (ITS1) and from the 28S by ITS2. The entire gene cluster is flanked by a nontranscribed spacer (NTS). The 5S rRNA gene is variable in position and orientation to the remainder of the rRNA cluster. In ascomycete fungi the 5S rRNA gene is located at a locus independent of the cluster described above. In Basidiomycetes the 5S is located within the NTS and in the same orientation as the other genes, whereas Oomycetes also have the 5S within the NTS but with an opposite orientation (Griffin, 1994). The ITS and NTS regions have limited function and incur mutation at a rate higher than structural rRNA genes do, although portions of the ITS1 may be involved in maturation of SSU rRNA precursors (van Nues et al., 1994). Ribosomal loci undergo concerted evolution, in that all clusters mutate as a single unit. Since rRNA evolves as a unit and does not experience recombination, inheritance pattern haplotypes are likened to that of mitochondrial loci.

Ribosomal RNA gene sequences have been determined from several eukaryotic organisms, and oligonucleotide primers from conserved regions have been developed (White et al., 1990). Cloning and sequencing of PCR amplified fragments often are used to establish evolutionary relationships among fungi (Rakotonirainy et al., 1991; Bruns et al., 1992; Rakotonirainy et al., 1994). Portions of *B. bassiana* SSU and LSU rDNA sequences have been published (Nikoh and Fukatsu, 2000; Suh et al., 2001) or submitted to GenBank (Accessions AF373871, BBU35418, BBU35303, BBU35282, BBU35281, BB28SD2, BB28SD1).

The ITS region sequences are less constrained than ribosomal gene sequences, but still must retain ability to excise from pre-rRNA transcripts (van der Sande et al., 1992). The
level of genetic variation within ITS regions has been used for the identification and taxonomic classification of fungi (Carbone and Kohn, 1993; LoBuglio et al., 1993; Hopple and Vilgalys, 1994; Neuveglise et al., 1994a; Rehner and Uecker, 1994; Vilgalys and Sun, 1994; Berbee et al., 1995; Liu et al., 1995; Yan et al., 1997; Shinohara and LoBuglio, 1999; Aanen et al., 2001; Jensen and Eilenberg, 2001). In several instances efforts were made to correlate genetic differentiation and common ancestry with fungal ecotypes.

Isolates of B. brongniartii were differentiated by PCR-RFLP analysis of a 930 bp fragment that contained ITS1, the 5.8 S rRNA gene, and the ITSII (Neuveglise et al., 1994a). Seven restriction endonucleases showed that twenty-two different fragment patterns were possible, and patterns were used to identify six groups among 28 B. brongniartii isolates. Two B. brongniartii genotypes housed 22 of 28 (78.6%) isolates, and an eight group contained two B. bassiana isolates. DNA sequence alignment indicated that 0.7 to 14.7% and 1.8 to 16.7% nucleotide variation existed in the B. brongniartii ITS1 and ITSII, respectively. Neuveglise et al. (1994a) noted that B. brongniartii was a heterogeneous group that contained a homogeneous genotype infecting H. marginalis from Madagascar and Le Reunion, France. Evidence for separation of isolates based on host preference was shown in the presence of a nucleotide insertion from Bt39 and Bt102 isolated from Lepidoptera, whereas all isolates without the insertion were from Coleoptera, and the presence of a unique EcoRI site from Bt102.

Glare and Inwood (1998) used PCR-RFLP of the ITS regions, RAPD-PCR, and morphological characteristics to compare native New Zealand and exotic strains of Beauveria spp. Intraspecific B. bassiana variation was detected by MseI, ThaI, and Tsp509I assays, and defined three genotypic groups. In combination with two other unique restriction
assays that produced polymorphic banding patterns, a total of six genetic groups were identified among 23 isolates of *B. amorpha*, *B. bassiana*, *B. brongniartii*, *B. caledonica*, and *B. vermiconia*. PCR-RAPD analysis by Glare and Inwood (1998) generated 330 bands that were used for dendogram construction. The dendogram separated four subgroups; 1) *B. bassiana* and *B. brongniartii*, 2) *B. bassiana* from New Zealand, 3) *B. brongniartii* (3 of 5 native), and 4) single isolates of *B. amorpha*, *B. caledonica*, and *B. vermiconia*. Glare and Inwood (1998) analyzed PCR-RFLP and RAPD data, and concluded that two genetically distinct groups were present in New Zealand; 2) New Zealand *B. bassiana* plus *B. amorpha*, *B. caledonica*, and *B. vermiconia*, and 2) all other *B. bassiana* and *B. brongniartii*. It also was postulated that the two genetic groups represented native and introduced isolates.

Group I Introns: Structure, and Molecular Variation

Group I introns are intervening sequences present in some pre-mitochondrial, pre-chloroplast, and pre-nuclear rRNA gene transcripts from plants, fungi, and protists. Group I introns form characteristic secondary structures facilitating ribozyme function during a self-splicing reaction from primary transcripts prior to translation or RNA maturation. A minimum of ten stem loop structure, P1 to P10, are present, and P4, P4, and P7 directly involved in formation of the catalytic core made up of P,Q, R, and S domains. Proper splice site determination is mediated by formation of loop P9.0. Intricacies of group I ribozyme structure and splicing mechanism were reviewed by Burke et al. (1987), Cech (1988), Michel and Westhof (1990), Cech (1990), and Cech et al. (1992) and should be referred to for additional background information. Additionally, introductory information in chapter 3
refers to subsequent research that refined ribozyme functional requirements and splicing mechanisms.

The term 'optional' is used to describe the irregular distribution of group I introns among individuals of a genus of species at a given locus. The phenomenon of intron mobility has resulted in a sporadic history of intron acquisition and loss at homologous loci, whereby the opposing mechanisms have reached equilibrium in extant species. Mechanism of group I intron excision has been attributed to the presence of homing endonucleases, that function to recognize cognate insertion sites in intronless genes such that an intron copy subsequently will be incorporated by a double stranded break repair mechanism. Highly similar group I introns are observed among some distantly related taxa, and mobility of the intervening sequence element has been hypothesized to facilitate horizontal transfer between species.

Introns will sometime contain open reading frames (ORFs) that encoded proteins (inteins). Translation products either function as maturases that promote ribozyme splicing activity, or endonucleases that contribute to intron mobility and homing (reviews: Dujon, 1989; Lambowitz and Belfort, 1993). Mitochondrial and chloroplast group I intein coding sequences (CDS) are inframe with plastid codon regions, and processed out of the exon-intein fusion protein (Weiss-Brummer et al., 1982; Carignani et al., 1983). Group I intron encoded polypeptides inserted into rRNA are expressed by different mechanisms. An ORF encoded protein, I-PpoI, from the nuclear rRNAs gene intron 3 of *Physarum polycephalum* was described (Muscarella and Vogt, 1989) and demonstrated to be expressed from an RNAPI-type promoter (Lin and Vogt, 1998).
Allelic Variation at Minisatellite Loci

Minisatellites loci contain 6 or more nucleotides long DNA sequences in tandem repeated array, whereas microsatellites house repeat units of 2 to 5 nucleotides (Tautz 1993). Distribution of human (CA) microsatellite regions is random, and on average found every 25 kb (Stallings et al., 1991). The initial polymorphic minisatellite was described from a human genomic clone (Wyman and White, 1980), and minisatellites have since been identified at other human loci (Higgs et al., 1981; Bell, 1982; Capon et al., 1983; Giraudeau et al., 1999). Minisatellite arrays range from 0.1 to 20 kb in length (Jeffreys et al., 1985). A conserved GC-rich minisatellite core was described which was proposed to be responsible for cross hybridization of minisatellite probes used in multilocus RFLP analysis (Jeffreys and Wells, 1989). Due to inherent minisatellite repeat lengths the probability of finding such repeats is $10^{-4}$ to $10^{-100}$ (Haber and Louis, 1997), and a nonrandom distribution of minisatellites at telomeric and centromeric regions was described (Royle et al., 1988; Amarger et al., 1998). Rate of new minisatellite allele generation by mutation has been estimated at 10-4 to 10-2 per kb per generation (Jeffreys and Wells, 1985) and $7.34 \times 10^{-5}$ per cell division (Ayres Sia et al., 1997). Maintenance of heterogeneity at polymorphic loci has been attributed to unequal crossover (Jeffreys et al. 1985; Jeffreys et al. 1988), gene conversion (Bishop et al. 2000; Buard and Vergnaud 1994; Jeffreys et al. 1994), or strand slippage during replication (Levinson and Gutman 1987).

Three minisatellite repeats were described from budding yeasts Saccharomyces cerevisiae (Horowitz and Haber, 1984; Louis et al., 1994), and Saccharomyces carlsbergensis (Anderson and Nilsson-Tilgren, 1997). Two hypervariable minisatellites previously were described from the filamentous fungi, Botrytis cinerea (Giraud et al., 1998),
and *Podospora anserina* (Hamann and Osiewacz, 1998). Minisatellites from the genus *Beauveria* have not been reported.

The *S. cerevisiae* minisatellite, the Y' element (Horowitz and Haber, 1984), consists of 9 to 26 repeated copies of a 36 bp sequence located in subtelomeric regions. The element was found to have a copy number of 8 and 20 per genome. The second *S. cerevisiae* minisatellite, STR-B, consisted of a 56 bp repeat located adjacent to a Y' element (Louis et al., 1994). STR-B was single copy, and repeated up to 8 times at the locus. A single 12 repeat unit minisatellite from the yeast *S. carlsbergensis* was isolated from the *S. cerevisiae* YCL010c homolog (Anderson and Nilsson-Tilgren, 1997). The locus contained 13 tandem repeats, and the presence of intraspecific repeat unit mutation. Later analysis of all yeast minisatelites by Haber and Louis (1997) identified the presence of conserved 5 nucleotide sequences flanking each minisatellite repeat; TAGTG, GGACA, and GAAAG for the Y' element, STR-B, and YCL010c, respectively. Haber and Louis (1997) also cited examples of five human minisatellites that had repeated 5-prime and 3-prime flanking sequences, and suggested that those short direct repeats provided the initial duplication whereby replication slippage, unequal crossover, or gene conversion caused repeat expansion.

The AT-rich minisatellite, MSB1, was isolated from the *B. cinerea* ATP synthase intron (Giraud et al., 1998), and shown to vary in the number of thirty seven nucleotide repeats. Forty seven isolates showed that repeat number fluctuated between 5 and 11, and that intraspecific repeat unit point mutations were present. Southern hybridization indicated that MSB1 was a single copy locus, and unique to *B. cinerea* since no signal was detected from *Fusarium oxysporum, Leptospheria maculans, Magnaporthe grisea*, or *P. anserina*. Giraud et al. (1998) identified a lack of recombination between MSB1 flanking regions and
unaltered physical order of repeat unit mutants as evidence for elimination of unequal
crossover as a potential mechanism for repeat expansion. Replication strand slippage or gene
conversion was subsequently suggested as mechanisms for repeat unit mutations.

Hamann and Osiewacz (1998) described a polymorphic minisatellite locus from *P. anserina*, *PaMinl*. The *PaMinl* repeat unit sequence was sixteen nucleotide long (GTGTGTGTGTGAAGGA), and was considered unique in that it contained a contiguous stretch of GT repeats. A second repetitive element was identified from the original cloned sequence, and consisted of seven repeats of AGAGGAAGAGGG. Screening of eighteen *P. anserina* strains identified six alleles at the *PaMinl* locus that varied between 280 and 360 bp in length. Fluctuation between 5 to 10 perfect repeat units was determined to cause length variation between allele sizes. Because only perfect repeat units were found Hamann and Osiewacz (1998) suggested that unequal crossover of intact repeat units was the mechanism by which allelic variation was generated.
CHAPTER 2. BEAUVERIA BASSIANA HAPLOTYPEx DETERMINATION BASED ON NUCLEAR RDNA INTERNAL TRANSCRIBED SPACER PCR-RFLP.

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Abstract

DNA sequence alignment of the nuclear 5.8S ribosomal RNA (rRNA) gene and internal transcribed spacers (ITS) from Beauveria bassiana demonstrated that 6.62% sequence variation existed between nine isolates. A higher level of mutation was observed within the ITS regions, where 8.39% divergence occurred. Polymerase chain reaction restriction fragment length polymorphism, PCR-RFLP, and DNA sequence alignment of the ITS1 and ITS2 regions identified seven polymorphic restriction endonuclease sites, Alu I, Hha I, Hinf I, Sin I, Tru 9AI and two Tha I restriction sites. The allelic frequency of each genetic marker was determined from 96 isolates. PCR-RFLP defined 24 B. bassiana genotypes within the sample set, from which eight phylogenetic clusters were predicted to exist. AMOVA and Fst (θ) indicated that no significant correlation existed between B. bassiana haplotype and insect host range as defined by insect order from which each isolate was derived.

Introduction

The entomopathogenic fungus Beauveria bassiana (Bals.) Vuill. (Ascomycota, Hypocreales) has shown use within insect biocontrol regimes for suppression of the crop pests Ostrinia nubilalis (Hübner) (Bing & Lewis, 1991; Bing & Lewis, 1992), Diabrotica unidecimpunctata (Krueger & Roberts, 1997) and Diabrotica virgifera virgifera (Mulock &
Chandler, 2000). Success within biological control practices has spearheaded an interest in documentation of molecular differences, and the genetic identification ("fingerprinting") of particular strains. Variable morphological characteristics, such as conidia size and shape, have been defined between *B. bassiana* (Bradley, 1979a), and *Beauveria brongniartii* (Sacc.) Petch (Bradley, 1979b). The presence of phenotypic diversity with respect to host specificity implies that genetic differences are present within the species (St. Leger, Allee, May, Staples & Roberts, 1992). Mutation detection techniques have proven useful in the differentiation of species of *Beauveria* (Neuveglise, Brygoo, Vercanbre & Riba, 1994; Glare & Inwood, 1998), yet, insufficient data exists regarding molecular differences among *B. bassiana* isolates.

Nuclear ribosomal RNA gene sequences are well described, with degrees of conservation observed throughout evolution (White, Bruns, Lee & Taylor, 1990) that have allowed for phylogenetic comparison among species (Gaudet, Julien, Lafay & Brygoo, 1989; Rakotonirainy, Cariou, Brygoo & Riba, 1994). Defined regions within the initial transcripts have demonstrated differential rates of change at the nucleotide level, and intraspecies comparison of fungi has shown a preponderance of sequence polymorphism within two internal transcribed spacer (ITS) regions (Neuveglise *et al.*, 1994; Buscot, Wipf, Di Battista, Munch, Botton & Martin, 1996; Chew, Strongman & MacKay, 1997). A moderate level of mutation was reported in the first internal transcribed spacers (ITS1) of *B. bassiana*, where two DNA sequences showed 91% similarity (Shih, Lin, Liou & Tzean, 1995). The second *B. bassiana* internal transcribed spacer (ITS2) sequence was determined to be invariant, and the 5.8S rRNA gene showed 98% similarity (Shih *et al.*, 1995). Additional nucleotide data have been provided from *B. bassiana* rRNA coding and ITS regions (Rakotonirainy, Dutertre,
Brygoo & Riba, 1991; Neuveglise et al., 1994; Rakotonirainy et al., 1994), but no intraspecies comparisons were made.

Mutation detection within DNA sequences can be facilitated by several means. Restriction endonuclease cleavage of PCR-amplified DNA fragments, PCR-RFLP, has proven useful in the intraspecies differentiation of *B. bassiana* (Glare & Inwood, 1998) and *B. brongniartii* (Neuveglise et al., 1994). Based on a DNA fragment that contained the ITS1, 5.8S rRNA gene, and ITS2, Glare & Inwood (1998) reported five RFLPs that separated *Beauveria* species into two groups. The first contained *B. bassiana* and *B. brongniartii*, and the other *B. amorpha* (Hohnel), *B. caledonica* (Bisset & Widden), and *B. vermiconia* (de Hoog & V.G. Rao). Of the ten enzymes used, *Mse* I, *Tha* I, and *Tsp* 509 were polymorphic within eleven *B. bassiana* isolates, but 100% linkage between *Mse* I and *Tsp* 509 mutations was shown to exist. Glare & Inwood (1998) also reported two genetically distinct subgroups of *B. bassiana* in New Zealand, that were hypothesized to represent native and introduced European genotypes. Sequence and PCR-RFLP analysis of the *B. brongniartii* ITS region from 28 isolates indicated that 0.70 to 14.67% and 1.80 to 16.67% sequence variation occurred within ITS1 and ITS2, respectively (Neuveglise et al., 1994). Seven polymorphic *B. brongniartii* restriction sites identified six unique genotypic groups within the species, and uniform *B. brongniartii* genotypes were described within all isolates derived from the white grub *Hoplochelus marginalis* (Fairmaire). Within the same study Neuveglise et al. (1994) found that two different *B. bassiana* isolate ITS region sequences were 100% identical.

This report describes the characterization of molecular polymorphism among *B. bassiana* isolates. We wished to investigate the level of neutral nucleotide mutation within
nuclear rRNA ITS regions of *B. bassiana*. In doing so we wanted to identify unique genetic
types, and recognize the evolutionary relationship among isolates infecting various insects.

**Materials and Methods**

**Samples**

In total, 96 isolates were received from a variety of sources (Table 1). Eighty-five *Beauveria*
*bassiana* isolates with diverse geographical origin and pathogenic capacity were obtained
from the ARSEF Collection of Entomopathogenic Fungi maintained by the US Department
of Agriculture, Agricultural Research Service, Plant Protection Research Unit, U.S. Plant,
Soil, and Nutrition Laboratory, Ithaca, NY (Humber, 1992). Isolate Bb6715 was originally
isolated from an adult western corn rootworm, *Diabrotica virgifera virgifera* (LeConte), and
received from Barbra Mulock, USDA-ARS, Brookings, SD. Isolate Bb726 was obtained
from Stephan Jaronski, Myotech Corporation, Butte, MT. Field isolates EL03 and EL12 to
EL19 were derived from European corn borer larvae, *Ostrinia nubilalis* (Hübner), and
maintained at the USDA-ARS Corn Insects and Crop Genetics Research Unit (CICGRU),
Ames, IA. Isolate Bb1022 was collected by the USDA-ARS CICGRU from a corn plant
near Champaign, IL. Isolates were propagated on 10 cm Sauborauds dextrose agar plates at
30°C. DNA extractions were performed in accordance to those described by Neuveglise *et
al.* (1994). Resultant nucleic acid pellets were resuspended in sterile deionized water and
stored at −20°C prior to use.
**DNA Sequencing and Alignment**

Oligonucleotide primers, ITS4 and ITS5 (White et al., 1990) were used for PCR amplification of a 25 µl reaction that contained 2.5 mM MgCl2, 150 µM dNTPs, 5.0 pmol of each primer, 0.425 U *Taq* polymerase (Promega, Madison, WI) and 50 ng of DNA template. The PTC-100 thermocycler (MJ Research, Watertown, MA) program included an initial denaturation at 94 °C for 3 min, then 40 cycles of 94 °C for 40 sec, annealing at 55 °C for 50 sec, and 72 °C extension for 50 sec. Successful amplification was confirmed by running 5 µl from each reaction on a 2% agarose gel that contained 0.5 µg/ml ethidium bromide.

Template was prepared from 20-µl of the ITS4 and ITS5 primed PCR product amplified from isolates Bb153, Bb501, Bb726, Bb1022, Bb1149, Bb1155, Bb3113, and Bb3167. Purification was carried out using Qiaquick PCR Purification columns (Qiagen, Valencia, CA) according to directions of the manufacturer. Samples were quantified using UV absorbance at 260 nm on a Molecular Devices SpectraMAX Plus UV spectrophotometer (Molecular Devices, Sunnyvale, CA). Template DNA was submitted to the DNA Sequencing and Synthesis Facility at Iowa State University, Ames, IA at a concentration of 2.5 ng/100-bp/1.0 µl. Oligonucleotide primers ITS1F (5’-ATTACCGAGTATTCTCAACTCCC-3), and ITS2R (5’-ACCTGATTCGAGGTCAACGTTC-3’) were submitted at 5.0 pmol/µl for priming of DNA template in two separate sequencing reactions.

DNA sequence data from isolates Bb153, Bb501, Bb726, Bb1022, Bb1149, Bb1155, Bb3113, and Bb3167 was reconstructed from ABI Prism Sequencer 5010 data output of both primer reactions using Contig Express (Informax, San Francisco, CA). Multiple sequence alignment of reconstructed isolate sequences took place using AlignX software (Informax).
**ITS Region Mutation Detection**

Oligonucleotide primers, ITS1F, ITS1R (5'-TCACTGGATTCTGCAATTCAC-3'), ITS2F (5'-CATGCCTGTTCGAGCGTC-3'), and ITS2R were designed using the Primer3 (Rozen & Skaletsky, 1998) based upon *B. brongniartii* sequence (Neuveglise *et al*., 1994), and synthesized at Integrated DNA Technologies (Coralville, IA). ITS1F is positioned 22 bp from the 3' end of primers ITS5 (White *et al*., 1990), and ITS2R is 21 bp from the 3' end of ITS4 (White *et al*., 1990). The predicted 255 bp ITS1F/ITS1R and 199 bp ITS2F/ITS2R primed PCR products were designed to reduce fragment size such that polyacrylamide gel electrophoresis could be performed and individual PCR-RFLP mutations localized to respective ITS regions. Both ITS1F/ITS1R (ITS1 fragment) and ITS2F/ITS2R (ITS1 fragment) primer pairs were PCR amplified in a 25 µl reaction containing 2.5 mM MgCl₂, 150 µM dNTPs, 3.0 pmol of each primer, 0.425 U Taq polymerase (Promega) and 25 ng of template DNA. The PTC-100 thermocycler (MJ Research) program included an initial denaturation at 94 °C for 3 min, followed by 35 cycles of 94 °C for 30 sec, 57 °C for 40 sec, and 72 °C for 20 sec. Amplified ITS1 fragments were digested with *Hinf*I, *Tru* 9AI (Promega), and *Tha*I (Life Technologies, Rockville, MD) according to directions of the supplier. ITS2 PCR products were digested with *Alu*I, *Ilha*I, *Sin*I (Promega), and *Tha*I (Life Technologies). All PCR-RFLP assays were carried out in individual reactions that contained 3.0 µl of PCR product, 2 µl 10X Buffer, 0.2 µl of 10 mg/µl BSA, and 0.5 U enzyme in a total reaction volume of 20 µl. After incubation at 37 °C or 60 °C for 8 to 14 h, restriction products were separated on a 20 cm 6% polyacrylamide (19:1 acrylamide:bisacrylamide) gel in 1X TBE buffer at 160 V for 4.5 h. Restriction fragments were
visualized using SYBR Green I stain (Molecular Probes, Eugene, OR), and image capture took place on a Fotodyne FOTO/Analyst Investigator PC- FOTO/Eclipse Electronic Documentation System (Fotodyne, Hartland, WI). DNA fragment size estimations were made from the digital images using Gel-Pro Analyzer software (Media Cybernetics, Silver Spring, MD).

**Data Analysis**

Phylogenetic relationships among PCR-RFLP haplotypes were estimated by Wagner parsimony methods (Eck & Dayhoff, 1966) after 1000 bootstrap resampling steps, where calculations were performed using PHYLIP (Felsenstein, 1989). Analysis of molecular variance (AMOVA) and θ (Fst) (Weir & Cockerham, 1983; Excoffier, Smouse & Quattro, 1992; Weir, 1996) calculations were generated from PCR-RFLP data using Arlequin software (Schnieder, Knuefer, Roessli & Excoffier, 1997). Arlequin input defined eight groups according to the insect from which the isolates were derived (Humber, 1992). Groups were placed into four subpopulations; subpopulation Lepidoptera (Lep.), Coleoptera (Col.), Hymenoptera/Diptera (HD), and Hemiptera/Homoptera and others (HHO) (Table 1).

Subpopulation Lepidoptera (Lep.) consisted of two groups; group 1, *O. nubilalis*, and group 2, other Lepidoptera, excluding *O. nubilalis*, and subpopulation Coleoptera (Col.), contained group 3: *Diabrotica* spp., and group 4, other Coleoptera excluding *Diabrotica* spp..

Subpopulation Hymenoptera/Diptera (HD), included group 5, Hymenoptera, and group 6, Diptera, and subpopulation Hemiptera/Homoptera and others (HHO), consisted of group 7, Hemiptera/Homoptera, and group 8 all other insect orders.
Results

**PCR and DNA Sequence Alignment**

The PCR amplified ITS region of *B. bassiana* was approximately 525-bp when primers ITS4 and ITS5 were used (data not shown). Products were obtained from all isolate DNA samples tested (Table 1), and no length differences were detected. Sequence data were generated from nine *B. bassiana* isolates, and deposited in Genbank (Benson, Karsch-Mizrachi, Lipman, Ostell, Rapp & Wheeler, 2000); Bb 153 (AF322924), Bb 501 (AF322925), Bb 726 (AF322926), Bb1022 (AF322927), Bb1149 (AF322928), Bb1155 (AF322929), Bb2515 (AF322930), Bb3113 (AF322931), and Bb3167 (AF322932). Multiple sequence alignment identified a 506 nucleotide consensus sequence (Fig. 1) with slight length discrepancies observed between isolates. The number of aligned nucleotides from Bb1022 totaled 501, whereas Bb726, Bb1149, Bb1155, Bb2515, and Bb3113 fragment lengths were 502 nucleotides, and Bb153, Bb501, and Bb3167 were 504. Internal transcribed spacer regions 1 and 2, and the 5.8s rRNA gene were identified in all DNA sequences via comparison to Genbank accession AB027382. A total of 30 *B. bassiana* intraspecies point mutations were identified, of which 83.3% (25 of 30) resided within the ITS regions. Discounting deletions, *B. bassiana* ITS1 and ITS2 sequences were 0.0 to 7.1%, and 0.0 to 9.2% different, respectively. AlignX software identified nucleotide polymorphism at positions in *Alu* I, *Hinf* I, *Hha* I, *Sin* I, *Tru* 9AI, and two *Tha* I restriction endonuclease recognition sequences.

**Mutation Detection**

Digestion of ITS1F/ITS1R PCR products with *Hinf* I, *Tha* I, and *Tru* 9AI, and ITS2F/ITS2R with *Alu* I, *Hha* I, *Sin* I, and *Tha* I, indicated that molecular differences were
present at locations predicted from DNA sequence alignments (Fig. 1). Twenty-four haplotypes, A to X, were identified from 96 *B. bassiana* isolates (Table 1). Restriction fragment patterns (Table 2) were identical to those predicted from DNA sequence data, except that fragments smaller than 40 bp typically were not visible (data not shown). During analysis, haplotypes were identified as either allelotype 0 or 1 (Table 2).

**Data Analysis**

AMOVA results generated from PCR-RFLP data indicated that 89.12% of the total genetic variation was present within subpopulations defined by similar insect host preference (Humber, 1992), and the fixation index (Fst, 0), 0.1088, suggested that little genetic differentiation had occurred between the same supopulations (Table 3). A consensus phylogeny was generated from PCR-RFLP data using Wagner parsimony methods (Eck & Dayhoff, 1966) after 1000 bootstrap resampling steps and showed the presence of eight clusters that included the 24 ITS region haplotypes, three of which consisted of single genetic types (Fig. 2). No apparent population structure was observed, in that isolates from either *O. nubilalis* or *Diabrotica* spp. were place in the same or adjacent phylogentic clusters (Fig. 2).

**Discussion**

Differences in nucleotide sequence and in PCR-RFLP pattern from *B. bassiana* internal transcribed spacer regions 1 and 2 were characterized. Multiple sequence alignment of nine *B. bassiana* isolate 5.8s rRNA gene and ITS region data indicated that 0.00 to 6.62% difference has evolved between isolates. From ITS1 and ITS2 regions, 0.0 to 7.1%, and 0.0 to 9.2% sequence variation was respectively shown, and represented a level greater than that
observed by Shih et al. (1995). The twenty-four ITS region haplotypes identified from 96 *B. bassiana* isolates increased 12-fold the number of haplotypes reported previously by Glare & Inwood (1998), but was less than the 39 genetic groups identified by St. Leger et al. (1992) using four isozyme markers. The level of ITS region variation corroborated findings by St. Leger et al. (1992) who suggested, due to the overall degree of genetic diversity observed, *B. bassiana* had maintained a large effective population size over a long period of time. St. Leger et al. (1992) also stated that gene diversity and genetic distances were affected by the frequency of recombination among fungal strains (Leung & Williams, 1986; Zambino & Harrington, 1989), and the speed by which reproductive isolation had developed (Ayala, 1979). *Beauveria bassiana* has shown an absence of a sexual phase, and possesses vegetative compatibility groups (VCGs) (Paccola & Meirelles, 1991) that serve as barriers to heterokaryon formation (Couteaudier & Viaud, 1997) and genetic exchange by recombination prior to reestablishment of the haploid state. Couteaudier & Viaud (1997) indicated that genetic exchange did not occur between coexisting isolates of different VCGs, showed that genetic variation was present between VCGs when telomeric RFLPs were used, suggested an absence of genetic transfer in recent evolutionary time, and proposed that isolates from *O. nubilalis* are clonal as a result of heterokaryon incompatibility.

Based on ITS region PCR-RFLP differences, a fixation index (Fst, 0) of 0.10880 suggested that low level genetic divergence of *B. bassiana* has occurred between subpopulations based on original insect host defined by the ARSEF catalog of strains (Humber, 1992). Additionally, AMOVA indicated that 89.12% of the observed molecular variance was present between isolates within each subpopulation, and 5.16% of the total variation occurred between subpopulations. From analysis of PCR-RFLP data we concluded
that a weak association was present between isolate haplotype and insect preference. Genetic investigations also have reported little correlation between insect host range and genotype of isolates from *Aspergillus flavus* (St. Leger et al., 2000), or *Metarhizium* (Cobb & Clarkson, 1993, Bidochka, McDonald, St. Leger & Roberts, 1994). Alternatively, Maurer, Couteaudier, Girard, Bridge & Riba, (1997) reported unique homogenous subpopulations of *B. bassiana* derived from the pyralids, *Ostrinia* and *Diaryctria*, and the curulionid beetle *Sitona*.

Isolates recovered from *O. nubilalis* have been identified as belonging to a distinct group (Couteaudier & Viaud, 1997), or as genetically homogeneous (Viaud, Couteaudier, Levis & Riba, 1996). Three isolates that were originally derived from *O. nubilalis* were separated into two groups distinguished by a polymorphic glutathione reductase (St Leger et al., 1992). Maurer et al. (1997) proposed that host insects were the predominant factor in some population structures of *B. bassiana*, and *O. nubilalis* appeared to be selective against isolates that were not derived from *Ostrinia*. Eighteen isolates derived from *O. nubilalis* were analyzed by PCR-RFLP of the ITS region, and were placed in six haplotype groups (Table 1). Eleven isolates were on the same phylogenetic branch, as haplotypes A and E, whereas the seven remaining isolates were classified as haplotype M, N, P, or X, and located on separate phylogenetic branches (Fig. 2). Fifteen of 20 isolates that were defined as haplotype A infected Lepidoptera, of which 10 were obtained from *O. nubilalis*. Although not statistically significant, the genetic similarities among geographically disperse samples that were isolated from *O. nubilalis* remain intriguing, but may have failed to indicate a strong selective pressure imposed by the host (Maurer et al, 1997). *Beauveria bassiana* isolated from Coleoptera have been hypothesized to display weak host specificity, or be
cross-infective (Glare & Inwood, 1998). Similar data has been collected within this study, in that six haplotypes (C, L, O, Q, S, and T) infected Diabrotica species. Six haplotypes also were found among isolates that were derived from Ostrinia, and suggested that isolates that infected Ostrinia have not been exposed to stronger selection forces than isolates that originally infected Diabrotica species.

Three of four isolates obtained from North American Diabrotica species, D. berberi, D. undecimpunctata, and D. virgifera (haplotype C, S, and Q or T, respectively), were placed on the same branch of the phylogenetic tree. Isolates derived from South American species, D. speciosa, and D paranoense, were identified as either haplotype C, L, or O, of which haplotype C was the only one shared with isolates infective toward the North American species D. berberi. In a rRNA PCR-RFLP study, Glare & Inwood (1998) demonstrated the importance of geographical origin within the context of observed haplotypes and population structure of B. bassiana. Correlation between strain and geographic origin was found when endogenous New Zealand and European isolates, and New Zealand and South American isolates were compared (Glare & Inwood, 1998). Poprawski, Riba, Jones & Aioun (1988) found isozyme monomorphism within isolates infecting Sitona species collected in proximity, but those from different geographical locations were genetically variable.

Four ITS region PCR-RFLP haplotypes were identified among ten isolates recovered from O. nubilalis larvae near Ames, IA, USA, and suggested that geography might not play a strong role in genetic diversity, or is case dependent. Alternatively, the evolutionary history of the chromosomal location analyzed and the level of mutation detected through molecular assay may also influence the level of diversity observed. Internal transcribed spacer regions have shown a high rate of genetic divergence (White et al., 1990), and the seven PCR-RFLP
markers may provide increased resolution. The variation observed among \textit{B. bassiana} ITS regions marker are likely due to selectively neutral spontaneous mutation (Kimura, 1983). Rapid evolution of genetic variants from monokaryotic culture has been shown to result in a virulence change in isolates (Samson, Evans & Latge, 1988), and may occur more rapidly than ITS region mutation detected by PCR-RFLP assays. Therefore, changes that occurred at virulence-associated loci may have been the driving force of genetic diversity among \textit{B. bassiana} isolates. Comparatively, random mutation at neutral loci may have evolved more slowly, and some selective virulence switching could have taken place among genetic types that possessed ITS regions that were identical by descent. Thus, the species \textit{B. bassiana} could represent an opportunistic insect pathogen, where selective virulence switching (Samson et al., 1988) may have facilitated rapid adaptation to available nutrient sources.

Further studies are needed to investigate the relationship among different \textit{B. bassiana} ecotypes. Additional genetic markers are needed to identify individual isolates as unique genetic types, resolve monophyletic groups within the phylogenetic tree, and to investigate correlation between insect host preference and \textit{B. bassiana} haplotype. The development of additional \textit{B. bassiana} genetic markers has begun. The new markers will be applied to the identification of additional \textit{B. bassiana} haplotypes, and investigation of relationships between isolate ecotypes defined by insect host preference.

References


Figure 2.1. 506 base pair *Beauveria bassiana* ITS region sequence alignment. ITS1 and ITS2 regions are italicized in the Bb0153 sequence, primer binding sites are highlighted and labeled showing primer direction, deletion mutations are indicated by asterisks (*), identical bases are shown as dashes (-), and relevant restriction sites are labeled appropriately.
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Table 2.1. Origin, subpopulation designation, and haplotype of 96 *Beauveria bassiana* isolates as determined by PCR-RFLP of the rRNA ITS regions 1 and 2. Four subpopulations, Lepidoptera (Lep.), Coleoptera (Col.), Hymenoptera/Diptera (HD), Hemiptera/Homoptera and others (HHO), based on insect order(s) from which the isolate was originally recovered (Humber, 1992), are separated by horizontal lines. Restriction fragments are labeled 1 or 0, as described in Table 2. \( Hi = Hi\)nfl, \( Tr = Tru\) 9AI, \( Th = Tha\) I, \( Al = Alu\) I, \( Hh = Hha\) I, and \( Si = Sin\) I.

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Table 2.2. Restriction endonuclease fragment sizes observed after separation PAGE on a 0.1 x 20 cm 6% polyacrylamide 19:1 (acrylamide:bisacrylamide) 1X TBE buffered gel at 160 V for 4.5 h. Size estimates are in base pairs (bp) and were made from DNA sequence data. Allele designations, 1 or 0, also were used in Table 1.

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Figure 2.2. Consensus phylogeny of *B. bassiana* ITS region PCR-RFLP haplotypes. Wagner parsimony, using MIX followed by CONSENSE programs in the PHYLIP package after 1000 bootstrap resampling steps, was performed.
Table 2.3. AMOVA table comparing *B. bassiana* isolates from eight groups within four subpopulations (subpops.). Subpopulation Lepidoptera (Lep.) consisted of two groups; group 1, *O. nubilalis*, and group 2, other Lepidoptera, excluding *O. nubilalis*, and subpopulation Coleoptera (Col.), contained group 3: *Diabrotica* spp., and group 4, other Coleoptera excluding *Diabrotica* spp.. Subpopulation Hymenoptera/Diptera (HD), included group 5, Hymenoptera, and group 6, Diptera, and subpopulation Hemiptera/Homoptera and others (HHO), consisted of group 7, Hemiptera/Homoptera, and group 8 all other insect orders.

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Fixation Indices

Fsc (F_{IS}) 0.05473
Fst 0.10880
Fct (F_{IT}) 0.05720
CHAPTER 3. EVOLUTIONARY MOBILITY, SEQUENCE VARIATION, AND AN OPEN READING FRAME IN NUCLEAR LARGE SUBUNIT rRNA GROUP I INTRONS OF THE ENTOMOPATHOGENIC FUNGUS, BEAUVERIA BASSIANA.

A paper submitted to Journal of Molecular Evolution

Brad S. Coates, Richard L. Hellmich, and Leslie C. Lewis

Abstract

The haploid imperfect filamentous fungus, Beauveria bassiana, has demonstrated importance as an insect biological control agent. Four optional group I introns in the nuclear large subunit rDNA of B. bassiana (Bb LSU rRNA) were shown to differ among isolates. Mutation analysis used PCR-RFLP and PAGE of intron specific PCR amplified products, and indicated that intraspecific sequence polymorphism had occurred. Three intron 1 (Bb LSU1) subtypes (Bb LSU1-1, i1-2, and i1-3) were identified, and similarly four, two, and five subtypes were respectively described from introns 2, 3, and 4. DNA sequence data was used to classify group I introns, Bb LSU1, i2, i3, and i4, into subgroups IB3, IA1, IA2, and IC2, respectively, and determine interspecific evolutionary relationships. The secondary structure of each intron was identified, where all LSUi3 regions had conserved stem structure P9.3 deleted, Bb LSUi2 lacked stem P9.1, and two of five Bb LSUi4 subtypes had the P9 region deleted. A 1011 bp insertion was found in the Pl loop (L1) of Bb LSUi2 from isolates Bb2515 and Bsp509, and contained a potential 244 amino acid long ORF, Bbl2EP. Optional group I intron insertion and subtyping was used to identify 37n haplotypes from 101 B. bassiana isolates.

Keywords: Beauveria bassiana, group I intron, sequence evolution, genetic variation, strain identification.
Introduction

The endophytic and entomopathogenic fungus *Beauveria bassiana* has been shown to be an effective insect biocontrol agent (Bing and Lewis 1992; Bing and Lewis 1991; Krueger and Roberts 1997; Mulock and Chandler 2000). Genetic data from *B. bassiana* ecotypes has been gathered. Isozyme alleles identified an absence of genetic differentiation among isolates from the European corn borer, *Ostrinia nubilalis*, whereas profiles from other isolates were variable (St Leger et al. 1992). Evidence of host specialization also was reported by Maurer et al. (1997) using RFLP and PCR-RAPD analysis, showed that isolates recovered from *O. nubilalis* and *Ostrinia furnicalis* were phylogenetically related, as were those that infected the sugarcane borer, *Diatraea saccharalis*. Maurer et al. (1997) also reported that isolates from the genus *Sitona* were distinguishable from isolates derived from other curculionid beetles. Glare and Inwood (1998) identified three polymorphic restriction sites from the internal transcribed spacer (ITS) region of the rRNA, and differentiated two genetic groups that were proposed to represent native New Zealand and introduced European genotypes. For the related species, *Beauveria brongniartii*, Neuveglise et al. (1994a) identified seven haplotypes, of which one was highly correlated with isolates that infected the white grub, *Hoplochelus marginalis*.

Group I introns have been described within the nuclear rRNA large subunit (LSU) of fungi (Tan 1997; Neuveglise et al. 1997; Neuveglise et al. 1994b; Liu et al. 1992; Mercure et al. 1994, Vader et al. 1994). Neuveglise et al. (1997) used 28s rRNA group I intron insertion and PCR-RFLP to distinguish 12 genetic types from 47 isolates, and identified a single haplotype of *B. brongniartii* that was pathogenic against *M. melolontha*. The plant pathogen, *Gaeumannomyces graminis*, was discovered to have three optional group I introns, A, T, and
AT, in the 26s rDNA. Based upon the presence or absence of the rDNA introns, four G. graminis pathogenic ecotypes were distinguished from one another (Tan 1997).

Two consecutive transesterification reactions are involved in the self-splicing mechanism of group-I introns from pre-rRNA (Cech 1987; Zuag et al. 1983). Site-specific autocatalysis was shown to depend upon metal ions (Grosshans and Cech 1984; Gampel et al. 1989, Jaeger et al. 1991, Colmenarejo and Tinoco 1999), and conserved structural elements (Lagault et al. 1992; Setlik et al. 1993). Although little similarity is maintained among group-I intron DNA sequences, ten conserved stem structures, P1 thru P10, were proposed (Michel 1982; Davies et al. 1982), and verified by probing (Inoue and Cech 1985) and mutagenesis (Couture et al. 1990). Structural models were proposed (Burke 1987; Cech 1988; Michel and Westhof 1990), of which Michel and Westof (1990) identified a core ribozyme of P5-P4-P6 and P7-P3-P8-P9.0 stems after the description of the P9.0 interaction (Michel 1989; Burke 1989; Burke et al. 1990). An internal guide sequence (Davies 1982: Michel et al. 1982), and a conserved guanosine in stem P7 (Michel 1989) are important in molecular positioning during cleavage and ligation reactions, and binding of the free guanosine or GTP cofactor, respectively. Additionally, optional splice-assisting endonucleases and maturases have been identified (Chen et al. 2000; Ho and Waring 1999; Ho et al. 1997; Caprara et al. 1996; Saldanha et al. 1995; Matsuoka et al. 1994).

Circularization of excised group I introns was shown to take place (Grabowski et al. 1981), and the mechanism shown to involve a 3-prime terminal G and cyclization sites within the 5-prime region of the intron (Been and Cech 1987).

We investigated the distribution and level of sequence variation among four Beauveria bassiana LSU rRNA group I introns (Bb LSU1, i2, i3, and i4) from 101 isolates
of diverse insect host and geographic origin. Descriptions of group I intron mobility and endogenous structural variation was desired, from which phylogenetic relationships could be determined. The overall research objective was to identify unique genetic types useful for strain identification, and to investigate the evolution of group I introns among *B. bassiana* isolates.

**Materials and Methods**

**Sample preparation**

A total of 101 *Beauveria* isolates were evaluated in the present study (Table 1). Ninety *B. bassiana* two isolates with diverse geographical origin and pathogenic capacity were obtained from the ARSEF Collection of Entomopathogenic Fungi maintained by the US Department of Agriculture, Agricultural Research Service, Plant Protection Research Unit, U.S. Plant, Soil, and Nutrition Laboratory, Ithaca, NY (Humber 1992). Two of the isolates, Bsp509 and Bsp1195, were described as *Beauveria* spp. isolates, and suggested to be either isolates of *B. bassiana* or *B. brongniartii* by Humber (1992). Sample Bb6715 was isolated from an adult western corn rootworm, *Diabrotica virgifera virgifera*, and provided by Barbara Mulock, USDA-ARS, Brookings, SD. The isolate Bb726 was obtained from Stephan Jaronski, Myotech Corporation, Butte, MT. Field isolates EL03 and EL12 to EL19 derived from European corn borer larvae, *Ostrinia nubilalis*, were maintained at the USDA-ARS Corn Insects and Crop Genetics Research Unit (CICGRU), Ames, IA. Isolate Bb1022 was collected by the USDA-ARS CICGRU from a corn plant near Champaign, IL. Propagation was on Sauborauds dextrose agar at 30°C. DNA extractions were performed in
accordance to that described by Neuveglise et al. (1994). Resultant nucleic acid pellets were resuspended in sterile deionized water and stored at -20°C prior to use.

Large subunit rDNA intron amplification

The 3-prime region of the LSU rDNA was polymerase chain reaction (PCR) amplified from all 101 isolates using oligonucleotide primers PN29 and E24 (Table 2). The 50-µl PCR reactions used 2.5 mM MgCl₂, 150 µM dNTPs, 10 pmol of each primer, 1.2 U Taq polymerase (Promega, Madison, WI), and 40 to 50 ng of template DNA. The PTC-100 thermocycler (MJ Research, Watertown, MA) program included an initial denaturation at 95°C for 3 min, then 40 cycles of 94°C for 40 sec, annealing at 60°C for 40 sec, and 72°C extension for 3 min. Amplification was confirmed by electrophoresis of 5 µl from each reaction on 1.5% agarose containing 0.5 µg/ml ethidium bromide, and visualized under UV illumination.

Primer pairs PN29/PN38 and E23/E24 (White et al. 1990; Table 2) were respectively used for the amplification of introns BbLSUi4 and i1. Primer pairs I21/I22, and I31/I32 (Neuveglise et al. 1997) (Table 2) primed reactions amplifying introns Bb LSUi2 and i3, respectively. Introns were detected in a 25 µl nested PCR reaction that used 2.5 mM MgCl₂, 150 µM dNTPs, 5 pmol of each primer, 0.85 U Taq polymerase (Promega), and 1.0 µl of PN29/E24 primed PCR product. The PTC-100 thermocycler (MJ Research) program included an initial denaturation at 95°C for 3 min, then 40 cycles of 94°C for 40 sec, annealing at 60°C for 40 sec, and 72°C extension for 50 sec. Amplification was confirmed
by electrophoresis of 5 µl from each reaction on 2% agarose containing 0.5 µg/ml ethidium bromide, and visualized under UV illumination.

DNA Sequencing and Alignment

Direct DNA sequencing of PCR products was performed as opposed to plasmid cloning procedures. Sequencing template was amplified in separate 50 µl PN29/E24 primed PCR reactions using conditions previously described. PCR product purification was carried out using Qiaquick PCR purification columns (Qiagen, Valencia, CA), according to manufacturer directions. Samples were quantified using UV absorbance at 260-nm wavelength on a Molecular Devices SpectraMAX Plus UV spectrophotometer (Molecular Devices, Sunnyvale, CA). Template was submitted to the DNA Sequencing and Synthesis Facility at Iowa State University, Ames, IA at a concentration of 2.5 ng/100-bp/1.0 µl, and appropriate primers (Table 2) were supplied at 5 pmol/µl. Sequencing of Bb LSUi1 from isolates Bb502, Bb2297, Bb2737, and EL18 used primers E23 and E24. Sequence data for Bb LSUi2 was acquired from Bb1121, Bb1988, and Bb2297 using primers I21 and I22, and the isolate Bb2515 required additional primers I23, I24 and I2EP-F. Intron Bb LSUi3 was sequenced from isolates Bb1001 and Bb2297 and with oligonucleotide primers I31 and I32, and Bb LSUi4 sequence reactions were primed by oligonucleotides PN29 and PN38 and used to collect data from isolates Bb151, Bb726, Bb1962, Bb2297, and Bb2515. The intronless variant isolate Bb3167 was sequenced with primers PN29, I21, I22, and E24.

DNA sequencing reactions were preformed in duplicate with each primer, and full-length intron sequences were reconstructed from overlapping sequence data using Contig Express (Informax, San Francisco, CA). Multiple sequence alignment of reconstructed
sequences was performed individually for introns Bb LSUi1, i2, i3, and i4 with AlignX software (Informax).

*Intron-specific PCR and Mutation analysis*

Intron specific oligonucleotides 112, 113, I14, I15, I33, I34, I41, and I42 (Table 2) were designed to prime PCR amplification reactions at internal polymorphic regions of group I introns BbLSUi1, BbLSUi2, and BbLSUi4 identified in DNA multiple sequence alignments. Oligonucleotide primers were designed with Primer3 (Rozen and Skalentsky 1998) and synthesized at Integrated DNA Technologies (Coralville, IA). Primer pairs E23/I12, I13/I14, and E24/I15 were used for insertion/deletion (indel) mutation detection of BbLSUi1, while I33/I34 and I41/I42 respectively were specific for length variable regions of introns BbLSUi3 and i4. All indel mutations were detected via nested PCR amplification that was performed in 12.5 µl reaction volume, and used 2.0 mM MgCl2, 150 µM dNTPs, 4 pmol of each primer, and 0.425 U *Taq* polymerase (Promega), and 0.5 µl PN29/E24 PCR product. The PTC-100 thermocycler (MJ Research) program had an initial denaturation at 94°C for 3 min, then 40 cycles of 94°C for 40 sec, annealing at 57°C for 40 sec, and 72°C extension for 12 sec. (I33/I34 and I41/I42), 20 sec. (E23/I12, and E24/I15), or 30 sec. (I21/I24). Electrophoresis of E23/I12, E24/I15, I33/I34, and I41/I42 PCR products used 4.0 µl of each sample per lane of a 0.1 x 20 cm 6% polyacrylamide 19:1 (acrylamide: bisacrylamide) 1X TBE gel, and were separated at 150 V for 5 hr. The I21/I24 PCR products that were detected in 2% agarose with 0.5 µg/ml ethidium bromide and separated for 1.5 hr at 90V on a 25 cm BioRad Subcell GT horizontal electrophoresis unit (BioRad, Hercules, CA). All gels, except the 2% agarose with I21/I24 products, were stained with
SYBR Green I (Molecular Probes, Eugene, OR). Digital image capture used a Fotodyne FOTO/Analyst Investigator PC-FOTO/Eclipse Electronic Documentation System. Size estimations were made from the digital images using Gel-Pro Analyzer software (Media Cybernetics, Silver Spring, MD).

PCR-RFLP assays were performed on appropriate intron specific PCR products (Table 2). The AluI, ApaI, BstOI, HaeIII, HinfI,MspI, RsaI, Sau3AI, and Tru9AI restriction enzyme reactions used 4.0 µl of PCR product, 2 µl 10X Buffer, 0.2 µl 10 mg/µl BSA, and 0.5 U enzyme (Promega) in a 20 µl reaction volume. Digestion with enzymes BfaI, BstUI, NlaIII and Tsp509I used 4.0 µl of PCR product, 2 µl 10X buffer, and 0.5 U enzyme (New England Biolabs, Beverly, MA) in a 20 µl reaction volume. After incubation at 37°C for 8 to 14 h, except for BstOI, BstUI, and Tsp509I which were incubated at 65°C, the entire 20 µl reaction was loaded on a 0.1 x 20.0 cm 4% polyacrylamide (19:1 acrylamide: bisacrylamide) 1X TBE buffered gel and run at 160 V for 4.0 h. Restriction fragments were visualized by ethidium bromide staining. 8 µl of each I41/I42 BstOI digestion reactions was separated per lane on a 0.1 x 20.0 cm 8% polyacrylamide (19:1) 1X TBE gel at 160V for 10 h, and detected by SYBR Green I staining (Molecular Probes). Image capture of all restriction fragments took place on a Fotodyne FOTO/Analyst Investigator PC-FOTO/Eclipse Electronic Documentation System. DNA fragment size estimations were made from the digital images using Gel-Pro Analyzer software (Media Cybernetics).
Data Analysis

Intraspécific intron alignment of *B. bassiana* group I sequence data derived from this study was constructed using AlignX (Informax) with gap penalty of one. Secondary structure models, proposed by Michel and Westhof (1990), Cech (1988), and Burke (1988), were used to manually identify stem and loop regions. Open reading frames (ORFs) within the Bb2515 Bb LSUi2 L1 region were identified using Vector-Suite NTI 6.0 (Informax). Properties of the predicted ORF peptide were estimated using Compute pI/MW (Wilkins et al. 1998; Bjellqvist et al. 1993), PHD (Rost 1996) and PHDsec (Rost and Sander 1993) at the PredictProtein server. Nucleic acid and protein sequence databases were searched using BLAST 2.0 (Altschul et al. 1997), and Maxholm (Sander and Schneider 1991). The relationship among BbLSU intron DNA sequence data was estimated from an unrooted consensus phylogeny generated by Wagner parsimony methods (Eck and Dayhoff 1966). Parsimony calculations used GenBank accessions AF293965, AF293966, AF293967, and AF293968 (Wang et al. unpublished data), Bt114 intron 1 (Neuveglise et al. 1994b), as well as 15 original sequences generated from this study. Analysis of aligned intron DNA sequence data used Seqboot of the PHYLIP package (Felsenstein 1989) to perform 10,000 bootstrap iterations. DNAPars (Felsenstein 1989) was then used to estimate a set of unrooted phylogenetic trees, from which Consense (Felsenstein 1989) estimated a consensus tree that contained internal branch support. Treefiles were viewed on TreeView 1.6.5 (Page 1996).

Results

Intron Distribution and Intraspecific Mutation Analysis
Mobility was documented in fourteen combinations of four group I introns from the large subunit rRNA gene of *B. bassiana* (Bb LSU rRNA), and was used to characterize 37 unique genetic types among 101 isolates (Table 1). The presence of Bb LSUi1, i2, i3, and i4 was shown in 29 of 101, 34 of 101, 41 of 101, and 66 of 101 isolates, respectively, and 20 of the 101 isolates were devoid of introns (Table 1). PCR amplification used primers that flanked intron insertion points (Table 2), and suggested *B. bassiana* group I intron location was similar to those for *B. brongniartii* (Neuveglise et al. 1997) and *Candida albicans* (Mercure et al. 1993). *Beauveria bassiana* LSU rDNA PCR products that contained introns were approximately 550, 550, 600, and 900 bp in length for Bb LSUi1, i2, i3, and i4, respectively (data not shown), except for an approximate 700 bp Bb LSUi2 from isolates Bb477, Bsp1195, Bb1988, Bb2564, and Bb2953 (Fig. 1), and a 1400 bp Bb LSUi2 from isolates Bb2515 and Bsp509 (Fig. 1).

Intraspecific variation was detected from introns by PAGE of Bb LSU i1, i3 and i4 specific PCR products and agarose gel separation of Bb LSUi2 products (Fig. 2). PCR-RFLP assay results (Table 3) typically failed to detect fragments smaller than 50 bp, where visible restriction products of representative I13/I14 primed Bb LSUi1 intron fragment PCR-RFLP are shown (Fig. 3). Mutation analysis identified three BbLSUi1 subtypes, as well as four i2, two i3, and five i4 subtypes among 101 *B. bassiana* isolates. From isolates that had Bb LSUi1, subtypes i1-1, i1-2, and i1-3 were identified in 22 of 29, 5 of 29, and 2 of 29, respectively. Similarly, subtypes Bb LSUi2-1, i2-2, i2-3, i2-4, and i2-5 were respectively in 25 of 34, 2 of 34, 2 of 34, and 5 of 34 isolates. The Bb LSUi3-1 subtype was described in 39 of 41 isolates that had Bb LSUi3, and Bb LSUi3-2 was present in only 2 of 41 isolates. *Beauveria bassiana* LSUi4 subtype i4-1 was found in 14 of 66 isolates, and Bb LSUi4-2, i4-
3, i4-4, and i4-5 were present from 31 of 66, 16 of 66, 3 of 66, and 2 of 66 isolates, respectively.

DNA Sequence Analysis

Interspecific structural variation of group I intron was evaluated from alignments constructed from isolate Bb151, Bb726, Bb502, Bb1001, Bb1962, Bb1988, Bb2297, Bb2515, Bb Bb2737, Bb2297, and Bb EL16 DNA sequence data (Fig. 3). GenBank accessions were made for 14 unique intron sequences, and the rDNA region from the intronless variant, Bb3167 (GenBank: AF391119), which was used to determine intron insertion points. Three Bb LSU1 sequence variants were identified from isolates Bb502 and Bb 2737, Bb2297, and field-isolate EL16. A nucleotide length of 416 bp was defined for Bb LSU11 (subtype il-1) from isolates Bb502 (AF322934) and Bb 2737 (AF322935), and second a 426 bp intron 1 subtype (i1-2) was discovered in Bb2297 (AF322936). Field isolate EL16 (AF336302) possessed a Bb LSU1 subtype (i1-3) with a shortened nucleotide length of 387 bp. Slight length variation occurred between the 402 bp Bb LSU12 from isolate Bb1121 (AF391117), subtype i2-1, and the 401 bp subtype i2-2 from isolate Bb2297 (AF322937). Two Bb LSU12 subtypes, i2-3 and i2-4, had enlarged Pl loops (L1) when compared to subtypes i2-1 and i2-2. A 499 bp subtype i2-4 intron was described from isolate Bb1988 (AF391118), and a 1402 bp Bb LSU12-3 subtype was found in isolate Bb2515 (AF322938). The Bb LSU13-1 from isolate Bb1001 (AF322939) was 431 bp in length, whereas a 421 bp variation (i3-2) was present from Bb2297 (AF322940). Lastly, intron Bb LSU14 contained five unique sequences, where isolates Bb2297 (AF322944), Bb151 (AF322942), Bb726 (AF322941), Bb2515 (AF322943), and Bb1962 (AF391116) possessed 413 (i4-4), 427 (i4-2), 443 (i4-1),
445 bp (i4-3), and 445 bp (i4-5) introns, respectively. The subtypes Bb LSUi4-1 and i4-5 were distinguished by BstOI digestion of I41/I42 PCR products (Table 3).

Within all *B. bassiana* group I intron subtypes, the secondary RNA structure elements were manually identified (Fig. 4), based on models by Cech (1988), Michel and Westhof (1990), and Burke et al. (1987). Stem structures, P1 to P10, were described from all *B. bassiana* group I intron sequences, and all Bb LSUi2 sequences lacked stem P9.1 and two of the sequenced Bb LSUi4 regions has stem P9 deleted. Additionally, all Bb LSUi3 regions had stem and loop P9.3 omitted. Ribozyme core regions, P, Q, R, and S, (Cech 1989) (Fig. 3) were identified, as was the internal guide sequence, IGS (Davies 1982; Michel 1989) (Fig. 4). The increased length of intron subtypes Bb LSUi2-3 and i2-4 were respectively attributed on 1011 and 105 bp insertions within loop 1 (Fig. 5). A potential 244 amino acid ORF was described from the Bb LSUi2-3 L1 insert, as were class II promoter elements within its 5-prime UTR (Fig. 6B). The proposed peptide, with a RRKR nuclear localization signal (NLS) (Hicks and Raikhel, 1995), has a theoretical isoelectric point (pI) of 9.23, molecular mass of 27.3 kDa, and low, 16%, helical structure. DNA sequence data also was used to define the Bb LSU introns i1, i2, i3, and i4, as belonging to group I intron subgroups IB3, IA1, IA2, and IC2, respectively (Michel and Westhof 1990).

Evolutionary and Phylogenetic Analysis

The phylogenetic relationship among 18 *B. bassiana* group I intron DNA sequences and one *B. brongniartii* sequence, Bt 114, (Neuveglise et al. 1994b) was determined by Wagner parsimony (Fig. 6). The Bb LSUi2, i3, and i4 intron subtypes were each grouped separate from other introns, and formed distinct lineages. Although graphically difficult to
visualize, Bb LSUi1 subtypes also formed a separate lineage. Unlike others, the Bb LSUi1 sequence from isolate Bb2297 had weak internal branch support (4393 of 10,000) at the node separating it from introns Bb LSUi2, i3, and i4.

**Discussion**

The distribution of fourteen Bb LSU intron subtypes among 101 *B. bassiana* isolates was used to define 37 unique haplotypes (Table 1). These data identified a greater level of genetic diversity is present within the *B. bassiana* population than observed previously by Glare and Inwood (1998), and more polymorphism was detected than was shown from among *B. brongniartii* 28s rRNA group I introns (Neuveglise et al. 1997). DNA sequence alignment indicated that a significant level of sequence diversity was maintained in *B. bassiana* group I introns, and mutation assays determined that 14 Bb LSU intron subtypes were distributed within the population.

Evidence for classification of Bb LSUi1 into subgroup IB resided in a bulged A within P7 that is preceded by a G•C base pair extension, a P6 that began with base pairs other than G•U and C•G, lack of a P11 stem, and no G bulge within the P4 stem. The Bb LSUi1 was placed into subgroup IB3, and based on nucleotides 3, 4, and 5 of the R element being GUA. The R element variant was identified in non-standard group IA introns by Cech (1988), but also represented in subgroup IB3 introns (Michel and Westhof 1990). Overall, Bb LSUi1 was found to be 89% similar to *B. brongniartii* 28s rRNA subgroup IB intron 1 (Neuveglise et al. 1994b), and 75 to 83%, 91%, 100%, and 100% similar within the P, Q, R, and S elements, respectively (data not shown).
All Bb LSUi4 subtypes were placed into group I intron subgroup IC2 due to an observed G residue in the Q element forming a bulge within P4, along with no P11 stem. Also, a bulged C in P7 preceded an A•U base pair, which, although Cech (1988) identified A•U extensions of P7 as residing solely in subgroup IA introns, Michel and Westhof (1990) indicated several group IC2 introns within a similar extension. The first two base pairs of Bb LSUi4 stem P6 had G•U and C•G nucleotide pairs, typical of group IA introns (Cech 1988), but also present in group IC1 (Michel and Westhof 1990), that indicated Bb LSUi1 may be a subgroup IC1/IC2 hybrid.

*Beauveria bassiana* LSUi3 contained a bulged C in stem P7 preceded by an A•U base pair, but the intron was placed into subgroup IA2 since it additionally lacked the indicative IC bulged G in P4. *Beauveria bassiana* LSUi3 showed similar features with four variant group IA introns from *Chlamydomonas eugametos* chloroplast rRNA (Ce LSU) that also lacked several common subgroup IA2 features. Specifically, the P6 stem of both Bb LSUi3 and the Ce LSU chloropast rRNA introns began with two G•C base pairs instead of G•U and C•G, and contained GUA at nucleotides 3 to 5 of the R element, instead of UCA. Unlike CE LSU introns, Bb LSUi3 retained a 5-bp contiguous P7 stem and lacked an extended P6 loop (L6). The intron Bb LSUi3 was thus placed into subgroup IA2, that possessed other introns subgroup IA introns that the stem-loop P11 omitted between R and the 3-prime end of P3 (Michel and Westhof 1990). Subgroup IA2 also was chosen due to Bb LSUi3 reduction in L6.

*Beauveria bassiana* LSUi2 was classified as subgroup IA due to a bulged C in P7 preceded by an A•U base pair, an omitted G bulge in P4, and G•U and C•G base pairs respectively at positions P6-1 and 2. Intron subtypes Bb LSUi2-2, i2-3, i2-4 had A•U and
C•G stem base pairing at homologous P6 positions, and were similar to the other subgroup IA members described by Michel and Westhof (1990). The intron Bb LSU12 also was further classified as group IAl, due to similarity of the P1 stem with Neurospora crassa LSU (Waring and Davies 1984) and P. anserina LSU1,2 (Cummings et al. 1989) IAI intron sequences. Additional evidence was observed in that subgroup IAI members were the only subgroup IA members that had insertions in L1, as was found in subtype Bb LSU12-3 from isolates Bb2515 and Bsp590, and subtype Bb LSU12-4 from Bb1988.

The secondary structure of group I introns involve the base pairing of ten short stems (P1 to P10). The stem loop structures, P1 to P10, have been implicated in the maintenance of proper P, Q, R, and S element orientation (Waring and Davies 1984; Cech 1988) which is important for efficient autocatalysis of group I intron core elements. The core ribozyme of B. bassiana LSU group I introns included the P9.0 region within the S element (Michel et al. 1989; Michel et al. 1990; Burke 1989; Burke et al. 1990), which was reduced to two or fewer residues in all cases. The group I intron subgroup IC2 members Bb LSU14-2, i4-4, and i4-5 had the P9.0 region reduced to a single G residue, which was consistent with other IC2 elements (Michel and Westhof 1990). Base paired stems were identified within B. bassiana introns, with exceptions being that all Bb LSU12 and i3 regions had stem P9.3 deleted, and i2 additionally lacked P9.1. Subtypes Bb LSU14-2 and i4-4 had the P9 region deleted, which, as observed from I41/I42 PAGE assays (Fig. 2, lanes 10 and 12), were present in 34 of 66 isolates (Table 1). Deletion of helices P9.1 and P9.2 has previously been observed in Tetrahymena (Barfod and Cech 1985), where the alteration had no measurable affect core ribozyme activity, but a reduced efficiency was noted in the exon-ligation reaction. The L2 and L9 structures were predicted to contain a consensus GNRA motif that may interact with
P8 and P5, respectively (Michel and Westhof 1990). Salvo and Belfort (1992) were able to show that L2 pseudorevertants of the T4 td group I intron were able to restore partial ribozyme function, and concluded that there must be an alternative intramolecular conformation that facilitates proper intron positioning for ribozyme catalysis. An analogous situation may be shown in the omission of P2 from over one-fourth of known group I introns, including *sunY* from the T4 bacteriophage. None of the L2 regions from *B. bassiana* LSU group I introns contained a GNRA as described by Michel and Westhof (1990), which was confirmed independently for Bb LSU i1, i2, i3, and i4 in GenBank accessions AF293965, AF293968, AF293967, and AF293968, respectively. Michel and Westhof (1990) proposed covariation between P5 regions and a L9 GNRA tetraloop motif, which also was observed from Bb LSU i1 and i3 introns. A consensus GNRA tetraloop from L9 was observed as GAAA (GNAA) and GAGA (GYRA) variations, where the former was present in intron types Bb LSU i1-1, i1-3, and i3-1, and the later within BbLSU i1-2. Only subtype Bb LSU i1-3 additionally had a C•G base pair at P5-2, which occurred in 43 or 46 group I introns reported by Michel and Westhof (1990). Remaining Bb LSU i1 P5-2 base pairs were identified as G •C, along with a constitutive C•G at P5-3. A single configuration occurred in all Bb LSU i3 subtypes, where L9 contained a GNAA consensus motif along with C•G and C•G base pairs at P5-2 and 3, respectively. Altered Bb LSU intron structures that have P9.1 and P9.3 deleted and L2 and L9 GNRA tetraloop motifs omitted may, as implied by Barfod and Cech (1985), and Salvo and Belfort (1992), have a modified three-dimensional conformation that maintains function of the core ribozyme.

Tertiary interaction between joining regions and stem structures also has been proposed through the covariation of primary sequence outside of the stem and loop
interactions previously indicated (Michel and Westhof 1990; Cech 1988; Cech 1990; Burke 1988). The predicted interactions have been identified between the joining region J3/4 and stem P6, and J4/5 and P1/P10 from Bb LSU introns. The Bb LSUi4 region joining stems P3 and P4 (J3/4) was shown to have nucleotides A and U at positions 2 (J3/4-2) and 3 (J3/4-3), respectively. J3/4 respectively interacts with base pairs G•U and C•G at P6-1 and 2, as described by Michel and Westhof (1990) to take place in subgroup IC2 introns. The remaining Bb LSU intron J3/4 interactions with P6 are non-conventional. Nucleotides GA were present at positions 2 and 3 of the P3/P4 joining region (J3/4) from introns Bb LSUi1 and i2, and respectively predicted to covarying with G•C and U•A, and C•G and U•G base pairs of the P6 stem. Nucleotides G and A were previously observed at J3/4-2 and 3 from Physarum polycephalum, Schw., intron Pp LSU2 (Nomiyama et al. 1981), but covaried with A•U and G•C base pairs of the P6 stem. Beauveria bassiana LSUi3 introns had G and U nucleotides at position 2 and 3, respectively, of the J3/4 region and G•C and C•G base pairs at P6-1 and 2. A GU configuration of J3/4-2 and -3 was previously observed in the Chlamydomonas moewusii, Gerloff, Cm psbA,2 intron (Turmel et al. 1989) and the Aspergillus nubilans An OX1,3 intron (Waring and Davies 1984), both of which covared with G•C and G•C base pairs in the P6 stem.

Structure of the internal guide sequence (IGS), involving the P1 and P10 elements, was identified within each Bb LSU intron type (Fig. 4), as was the conserved guanosine binding site in P7 (Michel 1989). Positioning of the IGS relative to the guanosine binding site was proposed to involve nucleotide J4/5-3 that covaried with the UG pair base pair on top P1 (Michel and Westhof 1990). All nucleotides at position P1 bp+1 and P10 bp 1 of Bb LSU introns IGS structures were Watson-Crick base pairs, as were most group I introns.
(Michel and Westhof 1990). The J4/5-3 residues are an A from Bb LSUi2 and i4, whereas a G was present in all Bb LSUi1 and i3 introns. Both of the observed scenarios at *B. bassiana* J4/5-3 residues mirror the situation encountered in 43% of group I introns evaluated by Michel and Westhof (1990).

Group I intron associated proteins, RNA chaperones, nuclear-encoded splicing factors, and intron-encoded factors, are predicted to assist in proper ribozyme folding (Weeks 1997). The L1 region of Bb LSUi2-3 subgroup IA1 intron from contains an open reading frame (ORF) of 732 nucleotides that encodes a 244 residue polypeptide (Fig. 5). The presence of Class II promoter elements provides strong evidence for expression of the ORF. These elements include a putative TATA box (TATAAAA) at -30 to -35, a transcription initiator UUAUCUUC (YYAN(U/A)YY) with A at +1, and an A at -3 from the AUG translation start codon. Upstream elements that included a CCAAT box (-8 to -12) and GC box (+40 to +46) were identified downstream of the TATA box. A predicted nuclear localization of the polypeptide, referred to as Bb intron 2-encoded polypeptide (Bb I2EP), was based on the presence of four basic amino acids, RKKR, located near its N-terminus (Hicks and Raikhel 1995). Database searches failed to find significant similarity between Bb I2EP and known nucleic acid or protein sequences. The peptide, Bb I2EP, was determined not to be a maturase due to its lack of an indicative LAGLIDADG motif (Lambowitz et al. 1999). Additionally, all known maturases are have been shown to occur within intron subgroups IB1, IB4, and ID (Ho and Waring 1999). Since the putative coding sequence of Bb i2ep has been documented within a subgroup IA1 intron and shown not to encode of maturase, the I2EP peptide may encode an optional RNA chaperone or a protein that assists in the maintenance of proper tertiary interaction among intron stem and loop structures. All
Bb LSUi2 sequences are unique from other *B. bassiana* group I introns in that they lacked P9.1, but it remains unknown how the omission has affected ribozyme function. P1 and P10 helices of Bb LSUi2-3 and LSUi2-4 may form an extended IGS region when compared to subtypes Bb LSUi2-1 and i2-2 (Fig. 5). The increased number residues that form the IGS of Bb LSUi2-3 and i2-4 helices may from a more stable interaction, and the presence of Bb I2EP may imply that increased stability invoked by one structure may compensate for deletion of weak interaction at another structure. In future studies it will be interesting to see if Bb I2EP has evolved the function of a Bb LSUi2 ribonucleoprotein that may act to facilitate core ribozyme or exon-ligation reactions.

Wagner parsimony (Eck and Dayhoff 1966) identified four groups among subtypes of the four group I introns from *B. bassiana* (Fig. 6). Felsenstein (1989) suggested that internal branch support greater than 50% may be necessary to assure significance at each node. The 50% threshold level was attained at all nodes, except the one that separated Bb LSUi1 subtypes from those of Bb LSUi2, i3, and i4. Although the placement of Bb LSUi1 within the hierarchy was questioned; all but two other intron sequences were placed within their nodes with frequencies greater than 90.5%.

In future studies it will be interesting to what additional levels of group I intron sequence diversity exist among isolates of the genus *Beauveria*, and how that diversity will integrate into the phylogeny that was presented. Also, investigation of Bb i2ep gene expression and activity potentially will determine its functioning within the splicing mechanism of *B. bassiana* group I introns.
Table 3.1. Intron subtype distribution that defined 37 *Beauveria bassiana* LSU rRNA region haplotypes (HT) identified from 101 isolates. Subtypes are as described in the text.

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Table 3.2. Feature map showing group-I intron insertion points within the 5-prime region of the *Beauveria bassiana* LSU rRNA gene, and relative PCR primer location and sequence.

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<td>3</td>
<td>I33</td>
<td>GCGAGACAACTCTGGACGG</td>
</tr>
<tr>
<td></td>
<td>I41</td>
<td>CCGGTAACGACCACGTTTA</td>
<td>3</td>
<td>I34</td>
<td>GACCGCTGACCGACACCTT</td>
</tr>
<tr>
<td></td>
<td>I42</td>
<td>CTYRCAGAACCCACACCATT</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Fig. 3.1. Electrophoreis of *B. bassiana* 28s (LSU) rDNA group-I intron PCR products that show indel mutations. Whole intron 2 I21/I22 primed PCR of products from BbLSUi2-3, lane 1, i2-4, lane 2, and i2-1/i2-2, lane 3. Lanes 4, 5, and 6 respectively show Bb LSUi2-3, i2-4, and i2-1/i2-2 subtypes PCR amplified with primers I21 and I24. Intron 1 E23/I12 primed products with Bb LSUi1-1 in lane 7, i1-2 in lane 8, and i1-3 shown in lane 9. I15/E24 products from Bb LSUi1-1, i1-2, and i1-3 are respectively in lanes 10, 11, and 12. I33/I34 PCR defined subtypes Bb LSUi3-2, lane 13, and i3-1 lane 14. Intron 4 I41/I42 PCR products from Bb LSUi4-1/i4-5, i4-2, i4-3, and i4-4 are in lanes 15, 16, 17, and 18, respectively. M = 100 bp, and L = 25-bp ladders (Promega Corp.).
Fig. 3.2. *Beauveria bassiana* LSU rRNA intron 1 (Bb LSUi1) I13/I14 primed PCR-RFLP assay results. Product from I13/I14 *ApaI* lane 7, i1-1; lane 8, i1-2; lane 9 i1-3), I13/I14 *HinfI* (lane 10, i1-1; lane 11, i1-2; lane 12, i1-3, I13/I14 *MspI* (lane 13, i1-1; lane 14, i1-2; lane 15, i1-3), I13/I14 *NlaI* (Lane 16, i1-1; lane 17, i1-2; lane 18 i1-3), and I13/I14 *Sau3A1* (lane 19, i1-1; lane 20, i1-2; lane 21, i1-3) assays are shown. \( L = 100 \text{ bp ladder (Promega).} \)
Fig. 3.3. Internal guide sequence (IGS) from A) Bb LSUi1, B) Bb LSUi2-1 and i2-2, C) Bb LSUi2-3, D) Bb LSUi3, and E) Bb LSUi4.

A)

\[
\begin{align*}
\text{P1} & \quad \text{cccacuAAAUUU} \\
& \quad \quad \quad | | | | | o | | \\
& \quad \quad \quad \text{GGGUGGUUGUUUCUUUG} \\
& \quad \quad \quad | | o | o | o | o | \\
& \quad \quad \quad \text{Gaauaggaacu} \\
\end{align*}
\]

B)

\[
\begin{align*}
\text{P1} & \quad \text{gggauAA} \\
& \quad \quad \quad | | o | | \\
& \quad \quad \quad \text{CCC-GUUUGUC-A} \\
& \quad \quad \quad | | o | | \\
& \quad \quad \quad \text{Gaacuggcu} \\
\end{align*}
\]

C)

\[
\begin{align*}
\text{P1} & \quad \text{gggau-AAAGGGGAUGAAC} \\
& \quad \quad \quad | | | | | o | | o | o | o | \\
& \quad \quad \quad \text{CCCUGCUUUCU-GU-CG} \\
& \quad \quad \quad | | o | | | o | | o | \\
& \quad \quad \quad \text{GGaacugg-cuugug} \\
\end{align*}
\]

D)

\[
\begin{align*}
\text{P1} & \quad \text{gacccuGAA} \\
& \quad \quad \quad | | | | | o | | \\
& \quad \quad \quad \text{CUGGGGYAACUACGAAUAG} \\
& \quad \quad \quad | | o | | | o | | | o | | | o | | | o | | | o | | | o | | | o | \\
& \quad \quad \quad \text{Gguuga-gcuguag} \\
\end{align*}
\]

E)

\[
\begin{align*}
\text{P1} & \quad \text{augacuCG} \\
& \quad \quad \quad | | o | | \\
& \quad \quad \quad \text{AC-GGGCAGG} \\
& \quad \quad \quad | | o | | \\
& \quad \quad \quad \text{Gc-uyu} \\
\end{align*}
\]
Fig. 3.4. A) The 105 bp L1 insertion sequence from Bb LSUi2-4 of isolate Bb1988, and B) the 1011-bp L1 sequence of Bb LSUi2-3 from isolate *B. bassiana* Bb2515, housing a 244 amino acid ORF. Class II promoter features and a putative nuclear localization signal (NLS) are labeled appropriately.

A)

```
01 ACUGUNAUAA AAACAUGAGC GGGUCCAAAC CAAUCGAAUU ACUUCAUCGC CGNCGCUGCG
061 AAUAUUAGCU UUGAAGUUUU UUUUUUANGU UUUUAGGACU CGUCC
```

B)

```
P10 TATA box CCAAT box +1 →
001 AGGGAGUAGAC UGUUAUAAA ACAAUGACGGG GUCCAAACCA AUCGAAUUUA CUUCAGCGCC

GC box M R G
062 GCCGCUCAC CAGAGUUUGG CACCAAGGCG GGCGGCUCCC UUACAGCCCA AACAGGCGCC

R L P H S H N F Y L V V L S G V R L G R
121 GCCGCUCCC UCACAGGGCC ACCUUUUACC UAGUCGUACU CUCGGAGUUC CGCGGGAC

R R L R L N P L G R G T V R V Y S S P
181 GCCGGAAGAC GCUUCGCGCG ACCUCUGCCUG GGCGGGGGAAC GGGAAGAGUUC UACUCUCAC

T Q M P L D L S T V A G E S Q S S G T A
241 CAACCCAAAU GCCCCUCGGA CUAAGCACUG UUGACGGAGU CUCCGACGUU UCCGGACAG

Y V R A T S P S N Q D E C S Q P C P D L
301 CCAUAGUGAAC AGGCAGCGCC CCCAGCAACC AGGACGAGUG CUCUGAGCGG UGGCCGAC

S V E R Q R E L N L E A L R Y S V Q E
361 UUUGGCGAGGA GCCGAAGAGG CGUUCGGAGG UUGAAGAGAC UCUGGGAGAG AGUGGCAGG

K P D E A R R I L D G M A S E T L G W C
421 AGAAGGCCGA CGAGGCCAGG CGUAAUCUGU UGGCAUGGAC UCUGAGCGGU UCCGGCGAGG

R S S V L K P N K Q D G Y I Q V S F K G
481 GUCGCUCAG UGUCGUGAAA CCCUAUAACAC AAGAUGGCGU CAUUCGGGUU UCGUUUAAGG

A N K I A L L H E L V L W A D G L Q C N
541 GCGCGAACAA AAAGGUCUIUCUU CUAACGACGC UUGUGUGUUG GGGGGCGGGA CUCAGGCGCA

P I R N G Q H I S H L C A N P R C M T L
601 AUCCUAAGAC CAACGGUCAG CAUAUAAGAC ACUAUAUGAC GAACCCCGCGA UGUAUGACAC

G H L V V E D A A I N Q S K G C G K I
661 UGGGCACCU UGUCGCUGCAGA GGCGUGCUGCA UCAACCAACC UCGUGAGGGA UGGGGGAAA
```
K A C R H A G C S K W V Y F C D H K P R

UCAAGGCAUG UAGGCAAGGC GCCUGUUCAU AGUGGGUUGA CUUUUGUGAU CAUAAGCCGC

C I R F V E G F A S W K E F Q A K G E C

GUUGCAUUCG AUUCCUGGAA GCCUUCGCCU CUUGGAAGGA AUUCCAAGCG AAGGGCGAGU

C *

GUUGGCAAAGG UGCGUUCACA UUAUAGGCGG UUAGGUUCUG GACUUUAGAU UAUUGGGGAG

AGUUUAGGAC GACUCCCCACA GUCAGCUUGGU GUGCAUUCGG GCACUGAAUA UUAGCUUUGA

AGUUUUUUUG GUUUUUUGGA CGCUUCUCUC UCGUCCCCAG GACUACCGU G
Fig. 3.5. Phylogenetic relationships between *B. bassiana* group I introns based on a 483 bp consensus DNA sequence, and determined by Wagner parsimony (Eck and Dayhoff, 1966) after 10,000 bootstrap iterations. Internal branch support at each node is indicated.
References


Bing LA, Lewis LC (1991) Suppression of *Ostrinia nubilalis* (Hübner) (Lepidoptera: Pyralidae) by endophytic *Beauveria bassiana* (Balsamo) Vuillemin. Environ Ent 20: 1207-1211


Gampel A, Tzagoloff A (1987) In vitro splicing of the terminal intervening sequence of Saccharomyces cerevisiae cytochrome b pre-mRNA. Mol Cell Biol 7:2545-2551


Matsuoka M, Matsubara M, Kakehi M, Imanaka T (1994) Homologous maturase-like proteins are encoded within the group I introns in different mitochondrial genes specifying *Yarrowia lipolytica* cytochrome c oxidase subunit 3 and *Saccharomyces cerevisiae* apocytochrome b. *Curr Genet* 26:377-381


Sander C, Schneider R (1991) Database of homology derived structures and the structural meaning of sequence alignments. Proteins 9:56-68


Schneider S, Kueffer JM, Roessli D, Excoffier L (1997) Arlequin ver.1.1: A software for population genetic data analysis. Genetics and Biometry Laboratory, University of Geneva, Switzerland.


CHAPTER 4. ALLELIC VARIATION OF A *BEAUVERIA BASSIANA* MINISATELLITE IS INDEPENDENT OF HOST RANGE AND GEOGRAPHIC ORIGIN.

A paper accepted into Genome

Brad S. Coates, Richard L. Hellmich, and Leslie C. Lewis

Abstract

The minisatellite locus, *BbMin1*, was isolated from a partial *Beauveria bassiana* genomic library that consisted of poly-GA flanked inserts. Polymerase Chain Reaction (PCR) of the *BbMin1* repeat demonstrated allele size variation among 95 *B. bassiana* isolates. Amplification also was observed from single isolates of *Beauveria amorpha*, *Beauveria brongniartii*, and *Beauveria calcedonia*. Eight alleles were identified at the haploid locus, where repeat number fluctuated between one and fourteen. AMOVA and θ (Fst) indicated that fixation of repeat number has not occurred within pathogenic ecotypes or geographically isolated samples of *B. bassiana*. Selective neutrality of allele size and rate of *BbMin1* mutation, and age of the species may contribute to host and geographic independence of the marker. Presence of alleles with large repeat unit number may be attributed to rare the occurrence of somatic recombination or DNA replication error. The molecular genetic marker was useful for identification of genetic types of *B. bassiana* and related species.

Keywords: *Beauveria bassiana*, strain identification, minisatellite variation
Introduction

The haploid imperfect filamentous fungus *Beauveria bassiana* (Bals.) Vuill. (Ascomycota: Hypocreales) has both endophytic and entomopathogenic characteristics. Agricultural biocontrol of *Ostrinina nubilalis*, (Hübner) (Lepidoptera; Crambidae) (Bing and Lewis 1992; Bing and Lewis 1991), and *Diabrotica* sp. (Coleoptera; Chrysomelidae) (Krueger and Roberts 1997; Mulock and Chandler, 2000) has been documented. Ambiguous results have been provided in regard to host specialization and geographic distribution of genetic variants of *Beauveria* spp. Specifically, Viaud et al. (1996) and Neuveglise et al. (1994) indicated that molecular variation among *Beauveria* isolates was related to insect host range, by use of RFLP and internal transcribed spacer region analysis, respectively. Similar correlation was found based on isozyme marker data (Poprawski et al. 1989; Mugnai et al. 1989). PCR-RAPD (Williams et al. 1990; Welch and McClelland 1990) genotyping of *B. bassiana* indicated that isolates from the sugar can borer, *Diatraea saccharalis*, shared ≥80% of 276 bands (Berretta et al. 1998). Regional variation in PCR-RAPD marker data of *B. brongniartii* isolates isolated from the European cockchafer, *Melolontha* spp., in France indicated that a high degree of similarity was present (Cravanzola et al. 1997; Piaatti et al 1998). Cravanzola et al. (1997) further indicated that differences between most strains represent minor variations of a common genotype, yet it was stated that the similarities in genotype failed to show a correlation between genotype and pathogenicity (Cravanzola et al. 1997; Piaatti et al 1998). The high degree of relatedness among *Beauveria* isolates was suggested to result from clonal propagation or recent speciation within the genus (St Leger et al. 1992; Bidochka et al. 1994; Viaud et al. 1996).
In contrast, Urtz and Rice (1997) used PCR-RAPD analysis to distinguish two separate genetic groups of *B. bassiana* that infected the rice water weevil, *Lissorhoptrus oryzophilus*, in Louisiana, USA, that were 45% divergent at 172 polymorphic bands. Urtz and Rice (1997) also suggested that the two groups represented different populations that existed sympatrically. Based on PCR-RAPD and RFLP Maurer et al. (1997) showed that *B. bassiana* isolates derived from coleopteran insect species showed a high level of genetic differentiation. Also, no evidence for host range clustering was shown for the entomopathogenic fungi *Metarhizium anisopliae* and *Metarhizium flavoviride* (Bidochka et al. 1994) when PCR-RAPD bands were analyzed. More recently, microsatellite data from *Aspergillus flavus* reported a lack of significant genetic similarity of infective types (St. Leger et al. 2000). Geographic component of isolate variation also was found not to contribute to isolate differentiation. In several instances *B. bassiana* isolates from the same region and collected from the same insect species were genetically dissimilar (Berretta et al. 1998, Urtz and Rice 1997), or similar genetic types were described from widely separated geographic locations (St. Leger et al. 1992; Bidochka et al. 1994; Poprawski et al. 1989).

Microsatellite loci are described as having 2 to 6 tandemly repeated nucleotide units, while minisatellites are comprised of a variety of larger repeat units (Tautz 1993). Polymorphic minisatellite alleles could arise via unequal crossover (Jeffreys et al. 1985; Jeffreys et al. 1988), gene conversion (Bishop et al. 2000; Buard and Vergnaud 1994; Jeffreys et al. 1994) or strand slippage (Levinson and Gutman 1987). Most minisatellites have been mapped to telomeric and centromeric regions (Royle et al. 1988), and were proposed to constitute recombination hot spots (Chakravarti et al. 1986; Steinmetz et al. 1987) or fragile sites (Oliva et al. 2000).
Minisatellites are destabilized through strand slippage (Levinson and Guttman 1987) and have been observed from DNA replication component mutants in yeast. Deletion of the *Saccharomyces cerevisiae* (Ascomycota; Saccharomycetales) rad27 nuclease involved in Okazaki fragment maturation resulted in an 11-fold increase in the rate of minisatellite mutation (Koskoska et al. 1998). A temperature sensitive mutant pol3-t allele from yeast (Tran et al. 1995; Tran et al. 1996) increased the rate of minisatellite instability 13-fold via an altered catalytic subunit of DNA polymerase (Kokoska et al. 1998). Mutation of the yeast DNA replication processivity factor, proliferating cell nuclear antigen (PCNA), encoded by the POL30 gene was characterized to have defects in DNA replication. Specifically, the cold sensitive pol30-52 mutation caused a 6-fold increase in observed minisatellite mutations (Kokoska et al. 1999).

Experiments with yeast estimated the rate of GT/CA microsatellite mutation at $6.7 \times 10^{-6}$, and a 20 nucleotide repeat unit minisatellite at $7.4 \times 10^{-5}$ (Ayres Sia et al. 1997). Microsatellite repeat unit changes were shown to arise as neutral mutations in accordance with the hypothesis of random drift (Jeffreys et al. 1988). Multiple allelic types at each locus have been used in the estimation of fungal genetic diversity (Bart-Delabesse et al. 1998; Bart-Delabesse et al. 1999; St. Leger et al. 2000). Minisatellite motifs discovered within fungi have included a 12 bp repeat from the unicellular brewing yeast *Saccharomyces carlsbergensis* (Ascomycota; Saccharomycetales) (Andersen and Nilsson-Tillgren 1997), that was found within a homolog of the *S. cerevisiae* ORF, YCL010c. Two subtelomeric minisatellites, STR-B (Louis et al. 1994) and the Y element (Horowitz and Haber 1984) were found to consist of 36 bp and 56 bp repeat elements, respectively. A minisatellite from the filamentous ascomycete *Podospora anserina* (Ascomycota; Sordariales) has been
characterized (Hamann and Osiewacz 1998). The \textit{P. anserina} locus, \textit{PaMin1}, consisted of a GT-rich 16 bp repeat element, and intraspecies variation defined six allelic types. The second known minisatellite from a filamentous ascomycete fungus, \textit{MSBl}, was discovered in the \textit{Botrytis cinerea} (Ascomycete; Leotiale, Sclerotiniaceae) ATP synthase intron, and contained seven allelic types that varied in the number of an AT-rich 37 bp repeat motif (Giraud et al. 1998).

We report \textit{B. bassiana} isolates that were differentiated on the basis of allelic types present at the newly described minisatellite locus, \textit{BbMin1}, which varied in the number of 16 nucleotide repeats. The minisatellite was only the third such motif to be isolated from a filamentous ascomycete fungus. Interspecific amplification of the locus from four related species, \textit{Beauveria amorpha}, \textit{Beauveria brongniartii}, \textit{Beauveria caledonica}, and \textit{Beauveria vermiconia} was desired in order for evolutionary conservation of the locus to be determined. We wished to use \textit{BbMin1} allele variation to compare isolates with respect to geography and insect host preference displayed by entomopathogenic fungus \textit{B. bassiana}, which may resolve ambiguity between previous studies.
Materials and Methods

Beauveria Isolates and Sample Preparation

Sixty six *B. bassiana* (Bb) isolates and one isolate each of *B. amorpha* (Ba), *B. brongniartii* (Bt), *B. caledonica* (Bc), and *B. vermiconia* (Bv) were obtained from the US Department of Agriculture, Agricultural Research Service, Plant Protection Research Unit, U.S. Plant, Soil, and Nutrition Laboratory, Ithaca, NY (Humber 1992). Isolate Bb6715 was originally isolated from an adult western corn rootworm, *Diabrotica virgifera virgifera*, and received from Barbra Mulock, USDA-ARS, Brookings, SD. Isolate Bb726 was isolated from a grasshopper, by Stephan Jaronski, Myotech Corp., Butte, MT. Field isolates EL03 and EL12 to EL19 derived from European corn borer larvae, *Ostrinia nubilalis* (Lepidoptera; Crambidae), were maintained at the USDA-ARS Corn Insects and Crop Genetics Research Unit (CICGRU), Ames, IA. Isolates NR1 to NR5 were from northern corn rootworm, *D. barberi* (Coleoptera; Chrysomelidae) adults, and WR1 to 15 from *D. virgifera virgifera* (Coleoptera; Chrysomelidae) adults collected from two fields in Jackson Co., IA, USA, approximately 2 km apart. Bb1022 was isolated by the USDA-ARS CICGRU from a corn plant near Champaign, IL. Propagation was on Saubordauds dextrose agar incubated at 30°C. DNA extractions were performed as described by Neuveglise et al. (1997). Resultant nucleic acid pellets were diluted with sterile deionized water and stored at –20 °C prior to use.

Satellite DNA Isolation

Degenerate (GA)$_8$RY primers were used to amplify total genomic DNA from isolate Bb1022 in a touchdown PCR reaction (Don et al. 1991). Products were separated on a 1 mm x 20
cm 6% polyacrylamide 29:1 (acrylamide:bisacrylamide) 1X TBE gel with 20 µl PCR product per lane. Allele fragments were visualized by ethidium bromide staining, and image capture took place on a Fotodyne FOTO/Analyst Investigator PC-FOTO/Eclipse Electronic Documentation System (Fotodyne, Hartland, WI). PCR product ligation into the pGEM-T easy cloning vector (Promega) took place according to manufacturers instructions. E. coli SURE® (Stratagene, La Jolla, CA) was transformed by electroporation on a MicroPulsar apparatus (BioRad, Hercules, CA). Clone selection and blue white screening was performed (Maniatis et al. 1989). Eleven clones with unique insert sizes were propagated in 25 ml terrific broth that contained ampicillin (Maniatis et al. 1989). Plasmid DNA was isolated with the QIAprep spin miniprep kit (Qiagen, Valencia, CA) according to manufacturer directions. Template was submitted to the DNA Sequencing and Synthesis Facility at Iowa State University at 50 ng/1.0µl. Individual plasmids were sequenced in separate reactions with primers T7 and SP6. Each insert DNA sequence was reconstructed from T7 and SP6 reaction electropherogram data using Contig Express (Informax, San Francisco, CA).

**PCR Amplification and Screening**

Primers BbMin1-F (5’-CATGTTGGTGACGAAGTGAGC-3’), and BbMin1-R (5’-GAGAGAGAGCCCTCGTCTGATAT-3’) were designed based on insert DNA sequence data from clone pGEM-BbMS-07, and used the Primer3 website (Rozen and Skaletsky 1998). Both oligonucleotides were synthesized at Integrated DNA Technologies (Coralville, IA). PCR amplification took place using 6 pmol of each primer, 20-25 ng of sample DNA, 0.425 U Taq polymerase (Promega), 1.25 µl 10X thermal polymerase buffer (Promega), 2.5 mM MgCl₂, and 150 µM dNTPs in a 12.5 µl reaction volume. Thermocycler reaction took place at 94°C for 3 min,
followed by 40 cycles of 94°C for 30 sec, 52°C for 30 sec, and 72°C for 30 sec. PCR products were separated at 150V for 5 hr on a 20 cm x 1 mm 6% polyacrylamide 19:1 (acrylamide:bisacrylamide) 1X TBE gel with a 25 bp ladder (Promega) for size comparison. Bands were visualized by ethidium bromide staining, and image capture took place on a Fotodyne FOTO/Analyst Investigator PC-FOTO/ Eclipse Electronic Documentation System (Fotodyne, Hartland, WI). DNA fragment size estimations were made from the digital images using Gel-Pro Analyzer software (Media Cybernetics, Silver Spring, MD).

Estimated repeat number of each allele size was confirmed by sequencing BbMin1 PCR products from isolates Bb3543, Bb730, Bb1022, Bb938, and Bb201. PCR reactions described previous were scaled up to 50 µl, and were purified using the QIAprep spin miniprep kit (Qiagen, Valencia, CA) according to manufacturer directions. Due to difficulty in sequencing small PCR products, BbMin1 alleles from isolates Bb3167 (87 bp), Bb1155 (103 bp), and Bb726 (119 bp) were cloned into pGEM-T easy cloning vectors (Promega). Alleles inserted into each plasmid clone were identified using colony PCR with primers BbMin1-F and BbMin1R, followed by electrophoresis as indicated above. Plasmid DNA was prepared for sequencing as described previously.

**Data Analysis**

*Beauveria bassiana* population dynamics were separately analyzed on the basis of pathogenic capacity and geographic distribution. Four ecotypes (subpopulations) were defined on the basis of which insect order isolates were derived (Humber 1992). Each ecotype consisted of two groups that further defined isolate phenotype; Ecotype 1 consisted of two groups *Ostrinia nubilalis* and other Lepidopteran insects, Ecotype 2 with groups
Diabrotica sp. and other Coleoptera, Ecotype 3 with groups Hemiptera/Homoptera and Hymenoptera, and Ecotype 4 containing groups Orthoptera and all other insects. Five geographic subpopulations were defined from Eastern Asia and Australia (A), Eurasia (E), Africa (F), North America (N), and South America (S). All calculations were performed using Arlequin (Schnieder et al. 1997). Analysis of molecular variance (AMOVA) and \( \theta \) (Fst) were determined by methods described by Excoffier et al. (1992), Weir and Cockerham (1983), and Weir (1996).

**Results**

A 483 bp plasmid insert DNA sequence from clone pGEM-BbMS-07 contained a 16 bp minisatellite motif with seven repeats of 5'-GAGAATATCAGACGGG-3' (Figure 1; GenBank accession: AF387913), and was subsequently named *B. bassiana* minisatellite 1, *BbMin1*. The pGEM-BbMS-07 insert sequence that contained *BbMin1* also had a short internal (GT)_4 microsatellite, and by nature of its construction, had two flanking (GA)_8 microsatellites. Initial PCR amplification of the locus *BbMin1* with primers *BbMin1*-F and *BbMin1*-R took place from isolate Bb1022 DNA, and resulted in a 199 bp product as predicted from the cloned sequence. Ninety five *B. bassiana* isolates were similarly PCR amplified and showed that *BbMin1* was mono-allelic in each haploid isolate, and size variable among isolates, with eight alleles from 87 to 295 bp in length (Table 1; Figure 2). The 95 isolates were divided into four ecotypes based on insect host range and five groups according to geographic location of original isolation (Table 1). The frequency of each allele was calculated for the entire population (Table 1). DNA sequence data from isolates Bb3543, Bb730, Bb1022, Bb938, Bb201, Bb1155, Bb726, and Bb3167 (data not shown), representing all observed *BbMin1* allele size variants, identified full repeat units as the
basis for each allelic size variant. The locus was also amplified from related species *B. amorpha, B. brongniartii, and B. caledonica* (Table 1), but amplification from a single *B. vermiconia* sample failed despite repeated optimization attempts. Population structure and relation among defined *B. bassiana* ecotypes and geographic divisions were evaluated by AMOVA and fixation indices (Table 2).

**Discussion**

Allele size differences at the *B. bassiana* minisatellite locus, *BbMin1*, have been characterized, and used for identification of isolates. The locus is the third such repeat element reported from a filamentous fungus, where the first was a GT-rich 16 bp repeat at the *PaMin1* locus of the ascomycete *Podospora anserina* (Hamann and Osiewacz 1998). Like *PaMin1*, *BbMin1* was isolated from a genomic clone that contained a microsatellite. Linkage between microsatellite and minisatellite elements also was reported from human genomic clones (Giraudeau et al. 1999). Each *BbMin1* repeat unit (5'-GAGAATATCA GACGGG-3') has 37.5% G/C-content and a reduced core region (underlined) that is similar to a majority of G-rich minisatellites (Dover 1989).

Electrophoresis of *BbMin1* PCR products indicated that allelic polymorphism existed among isolates of *B. bassiana*, with eight alleles that contained 1 to 14 repeat units. Based on *BbMin1* allele differences, a fixation index (Fst, 0) of 0.08204 suggested that little genetic divergence had occurred between pathogenic ecotypes. AMOVA indicated that 91.8% of *B. bassiana* population variation was present between individuals within each pathogenic ecotype, and 0.4% occurred between ecotypes. Similarly, AMOVA results predicted 70.83% of population variation was within groups separated by geographic location, but greater genetic separation was present compared to
pathogenic ecotypes \( (F_{st}, \theta = 0.29175) \). Therefore we concluded that little statistical evidence existed to correlate \( BbMin1 \) allele size with either insect host preference or geographic location.

The number of alleles maintained among individuals in a population depends upon rate of generation by mutation and loss by genetic drift. Rate of minisatellite allele loss is assumed to be constant, and polymorphism is dependent upon the rate of allele generation (Jarman and Wells 1989). The distribution of minisatellite alleles is skewed favoring those of decreased repeat number, and implies greater stability with decreased allele length (Wong et al. 1986). Hamann and Osiewacz (1998) suggested that unequal crossover of complete \( PaMin1 \) repeat units during nuclear division (Jarman and Wells 1989; Tautz and Schlotterer 1994) was responsible for minisatellite generation in \( P. anserina \). Rarity of somatic recombination (Buard et al. 2000) would imply that generation of new minisatellite alleles has been an infrequent event. Range of \( BbMin1 \) allele sizes among \( B. bassiana \) isolates and presence of alleles with large repeat number (isolates Bb3543 and Bb710) may suggest an alternate mechanism has functioned in \( BbMin1 \) repeat expansion. Giraud et al. (1998) proposed strand slippage (Levinson and Guttman 1987) as the mechanism by which minisatellite mutation occurred in the ascomycete \( B. cinerea \). Mutation in \( S. cerevisiae \) DNA replication and repair elements RAD27 (Koskoska et al. 1998), POL3 (Koskoska et al. 1998), and POL30 (Kokoska et al. 1999) have also been implicated in minisatellite allele generation. The mechanism by which repeat number has expanded and contracted at \( BbMin1 \) is yet to be determined, but may have involved one or all processes of somatic recombination, strand slippage, or DNA replication and repair errors.

In total, 62 of 95 \( B. bassiana \) isolates showed \( BbMin1 \) alleles with 1 to 4 repeat units, and 31 of 95 isolates showed alleles with 6 or 8 repeat units. The largest allele sizes contained repeat unit numbers of 10 and 14, and each were present in 1 of 95 of isolates. Population variation at the
PaMin locus demonstrated that *P. anserina* isolates differed by up to four repeat units (Hamann and Osiewacz 1998), and seven alleles at the MSBJ locus of *B. cinerea* varied between 5 and 11 repeat units (Giraud et al. 1998). The frequency of *BbMin1* alleles with increased repeat number could be evaluated in two ways. First, the polymorphic state of *BbMin1* among *B. bassiana* isolates may be explained if a relatively high rate of new minisatellite allele generation is assumed. Second, the original *BbMin1* repeat unit may have underwent duplication early in evolutionary history and the rate of new *BbMin1* allele generation may have been low due to the lack of a meiotic process in the *B. bassiana* genome. Therefore the presence of multiple allelic forms would be attributable to time since original repeat unit duplication. The second hypothesis may be supported by proposed mechanisms of mutation at other ascomycete minisatellite loci (Hamann and Osiewacz 1998; Giraud et al. 1998), and multiple repeats characterized from related *Beauveria* species (see below).

The *BbMin1* minisatellite locus was PCR amplified from the *Beauveria* species, *B. amorpha*, *B. bassiana*, *B. brongniartii*, and *B. caledonica*. Results indicated that flanking DNA sequence and the repeat unit has been evolutionarily conserved. Tandem 16 bp repeat units were characterized from single *B. amorpha* and *B. brongniartii* isolates, whereas a *B. caledonica* isolate retained a single copy of the repeat. Presence of greater than one repeat unit in the genome of three *Beauveria* species suggested that repeat unit duplication occurred prior to speciation. Loci with variable numbers of tandem repeat units sometimes have gone undetected among close taxonomic relatives (Angers and Bernatchez 1997; Taylor et al. 1999), and failed amplification of *BbMin1* from *B. vermiconia* isolate, Bv2922, was another example. *Beauveria vermiconia* was identified as a primitive species due to lack of an entomopathogenic phenotype (Mugnai et al. 1989). During time since common ancestry with other *Beauveria* species, point mutation at BbMin1-F or BbMin1-R primer binding sites *B. vermiconia* may have occurred.
Previous data indicated that *B. bassiana* isolates were similar among those obtained from the same host insect or same geographic region (Magnai et al. 1989; Poprawski et al. 1989; Neuveglise et al. 1994; Cravanzola et al. 1997; Berretta et al. 1998; Piatti et al. 1998). Berretta et al. (1998) suggested that a shared genetic character was associated with isolates that were most virulent toward *D. saccharlais* larvae. Berretta et al. (1998) also indicated that similar PCR-RAPD patterns among isolates of Argentina and Brazil provided evidence for clonal reproduction (St. Leger et al. 1992). Results from *B. brongniartii* rRNA ITS region PCR-RFLP assays indicated isolates from the insect *Hoplochelus marginalis* were genetically identical regardless of their point of origin (Neuveglise et al. 1994), which was interpreted to suggest linkage between genotype and pathogenic phenotype.

Few minisatellite or microsatellite studies have been conducted on pathogenic fungi. An investigation of *Aspergillus flavus* reported that no significant genetic similarity was present among infective types (St. Leger et al. 2000). We suggested that mutation rate of *BbMin1* was low, and implicated the time since original repeat duplication as the basis for high allelic variability. We further hypothesize that the independent distribution of *BbMin1* allele among pathogenic types and geographically distant isolates of *B. bassiana* (Table 2) is due to neutral mutation and subsequent random genetic drift (Jeffreys et al. 1988). We identified three subsets isolates that occupied common ecological niches and were likely to share a recent common ancestry; those isolated from *O. nubilalis* larvae 1) near Ames, IA, USA and 2) in China, and 3) those from *Diabrotica* spp. adults from North America. Eight *B. bassiana* isolates from *O. nubilalis* larvae from Iowa, USA were collected from a 15 square kilometer area, and had two *BbMin1* alleles that differed by four repeat units (four mutation steps) (Table 1). Four isolates that infected *O. nubilalis* in China had two separate
alleles that were separated by a difference of five repeat units. Twenty-three isolates collected from closely related members of the insect genus *Diabrotica* in North America possessed three different *BbMin1* alleles separated by as many as five repeats. Allelic differentiation between isolates that share similar ecological niches may indicate that *B. bassiana* is more genetically heterogeneous than previously reported (Cravanzola et al. 1997; Berretta et al. 1998; Piatti et al. 1998), and could support the existence of multiple sympatric lineages (Urtz and Rice 1997).

The locus *BbMin1* contained the first minisatellite described from the genus *Beauveria*. The molecular genetic marker was used for identification of isolates from *B. bassiana*, and related species *B. amorpha*, *B. brongniartii*, and *B. caledonica*. AMOVA and fixation indices suggested no relation between *BbMin1* allelic component and insect host preference or geographic origin. Increased rate of mutation at the minisatellite locus *BbMin1* may account for the dissimilarity of alleles among isolates that occupy the same ecological niche and geographical location, or those that share a recent common ancestry. Allelic variation at the *BbMin1* locus suggested that it is a neutral genetic marker. Additional satellite DNA markers are being developed to continue investigation of satellite region mutation in ascomycete fungi.
References:


Figure 4.1. 483 bp insert DNA sequence from clone pGEM-BbMSGA-07 (GenBank accession: AF387913). Alternate 16 bp GAGAATATCAGACGGG repeat units and four GT repeats are underlined, and primer binding sites are underscored by arrows indicating direction.
Figure 4.2. PAGE separation of eight observed alleles at the *BbMin1* minisatellite locus, performed on 20 cm x 1.0 mm 6% polyacrylamide (19:1 acrylamide:bisacrylamide) 1X TBE gels at 150V for 5 h. Alleles with 14 (lane 1), 10 (lane 2), 8 (lane 3), 6 (lane 4), 4 (lane 5), 3 (lane 6), 2 (lane 7), and 1 (lane 8) repeat units are shown. L = Promega 100 bp ladder.
Table 4.1. The distribution *BbMin1* minisatellite alleles among 95 *Beauveria bassiana* divided into four ecotypes: Ecotype 1: Lepidoptera, Ecotype 2: Coleoptera, Ecotype 3: Hemiptera/Homoptera and Hymenoptera, and Ecotype 4: Acrididae and other insects. Within each ecotypes isolates are bracketed to indicate geographic location; A = Eastern Asia and Australia, E = Eurasia, F = Africa, N = North America, S = South America, and * = unknown. R indicates repeat number, and size is given in base pairs. Isolates of *B. amorpha* (Ba), *B. brongniartii* (Bt), and *B. calcedonia* (Be) are underlined.

<table>
<thead>
<tr>
<th>Ecotype 1</th>
<th>Ecotype 2</th>
<th>Ecotype 3</th>
<th>Ecotype 4</th>
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<tr>
<td><strong>R</strong></td>
<td><strong>Size</strong></td>
<td><strong>Freq.</strong></td>
<td><strong>Ecotype 1</strong></td>
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<tr>
<td>14</td>
<td>295</td>
<td>0.0105</td>
<td>[3543] = N</td>
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<tr>
<td>10</td>
<td>231</td>
<td>0.0105</td>
<td></td>
</tr>
<tr>
<td>08</td>
<td>199</td>
<td>0.2526</td>
<td>[501, 502, 533] = [1060, 1454, 2520]</td>
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<td></td>
<td></td>
<td></td>
<td>[1022, 1038, 311] = [3037, NR5] = N</td>
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<td></td>
<td></td>
<td>EL03, EL13-EL1</td>
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<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td>[959] = S</td>
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<tr>
<td>06</td>
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<td>0.3474</td>
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<td>WR01-WR15 ] =</td>
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<tr>
<td>03</td>
<td>119</td>
<td>0.0526</td>
<td>[151] = E</td>
</tr>
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<td>[2685] = F</td>
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<td>01</td>
<td>87</td>
<td>0.0316</td>
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Table 4.2. AMOVA table comparing *B. bassiana* from eight groups within four ecotypes that define the insect order from which isolates were derived. Ecotype 1 consisted of two groups, *Ostrinia nubilalis* and other Lepidopteran insects, Ecotypes 2 with groups *Diabrotica* sp. and other Coleoptera, Ecotype 3 with groups Hemiptera/Homoptera and Hymenoptera, and Ecotype 4 contained groups Acrididae and all other insects.

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<td>---</td>
<td>---------------</td>
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<tr>
<td>Among groups</td>
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Fixation Indices

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<th>95% Confidence intervals</th>
<th>Fixation Indices</th>
<th>95% Confidence intervals</th>
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<td>Fct (FR)</td>
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CHAPTER 5. GENERAL CONCLUSIONS

General Discussion

Genetic Variation

Genetic variation was observed among isolates of *B. bassiana* and related species, *B. amorpha, B. brongniartii, B. caledonica*, and *B. vermiconia*. Polymorphic regions of the nuclear rRNA repeat, the internal transcribed spacers and group I introns, were used for the characterization of genetic point mutation and insertion/deletion events. PCR-RFLP of the ITS regions from 96 isolates resolved 24 different genetic types (chapter 2), but an additional 6 haplotypes were resolved upon analysis the full 111 isolates. The level of *Beauveria* ITS region diversity presented was greater than that described by Neuveglise et al. (1994a) or Glare and Inwood (1997). Optional presence of group I intron insertion at four positions of the *B. bassiana* nuclear large subunit rRNA (Bb LSU rRNA) prevented ubiquitous application group I intron mutation detection assays, in that 18 isolates were devoid of introns (Chapter 3). Although a high level of sequence heterogeneity was observed, and 12 different intron sequence variants were characterized, incomplete resolution of strains was encountered. Previously 9 intron types were described among 28 isolates of *B. brongniartii* (Neuveglise et al., 1997). Considering that 101 *B. bassiana* isolates were screened in chapter 3, a limited amount of genetic variation may be present within group I introns since a linear increase in genetic types was not observed as the number of isolates was increased and no additional sequence variants were detected within the complete set of 111.

The novel *B. bassiana* minisatellite marker, *BbMin1*, showed PCR amplified allele length variation. The locus represented only the third such repeat obtained from a filamentous fungus (chapter 5). Repeat unit number variation at the *BbMin1* locus ranged
from one to fourteen and defined eight Beauveria genotypes. Considered in combination with polymorphic ITS and LSU rRNA group I intron markers, BbMin1 successfully differentiated ten additional genotypic groups. The final number of unique genetic strains identified by single locus assays presented in chapters 2, 3, and 4 was 89 (Appendix B, Table 2). Seventy-six genotypes were identified that had single samples confined to each, and may represent uniquely identifiable strains. With eight isolates, genotype 3 was the largest collection of unresolved isolates that remained after all genotyping measures were enacted (Appendix B, Table 2). All but one isolate from genotype 3, Bb654, was derived from a Lepidoptera and six were from O. nubilalis larvae. Viaud et al. (1996) and St. Leger et al (1992) identified similar genotypes among Beauveria isolated from O. nubilalis larvae, and implicated a clonal mode of inheritance. Statistical analysis presented in both chapters 2 and 4 contradict the aforementioned clonal hypothesis, along with the observation that isolates infecting O. nubilalis also were classified into nine other genotypic classes; 7 (EL12), 24 (EL18, 25 (EL16), 26 (EL17), 42 (Bb1121), 56 (Bb501, Bb 560, Bb 3113), 57 (Bb652), and 62 (Bb 1038). The fact that the genotypic class 56 (Appendix B, Table 2) was the second largest unresolved cluster of isolates and that most inclusive isolates also were derived from O. nubilalis, may legitimize results observed by Viaud et al. (1996) and St. Leger et al. (1992) considering sampling bias and restricted number of genetic markers could generate erroneous results.

The final level of resolution achieved by the aforementioned set of genetic markers was approximately 80% (89 +111) (Appendix B, Table 2). The low percentage of identical strains (20%) may have resulted from the analysis of a diverse collection of isolates, whereby contribution of clustered isolates did not contribute substantially to overall population
similarity. As eluded to above, resolution of isolates may strongly be affected by sampling practices, and also by the type or variation detected by genetic markers. Evaluation of multiple samples obtained from the same insect species in proximity did not appear to skew results when highly polymorphic ITS, group I intron, or $BbMin1$ markers were used individually. Although not statistically evaluated after separation based upon all three markers, the population can easily be assumed to behave similarly.

Although all isolates were not fully resolved by unique combinations genetic markers, the aforementioned assays provided reliable means for the identification of $B. bassiana$ strains. The described level of diversity has not been described within the literature. Techniques may make possible tracking of individual isolates in field trials and evaluation of a host of biological questions. It is clear that additional $B. bassiana$ genetic markers are necessary before current methods can be used for DNA fingerprinting and identification of all $B. bassiana$ strains.

Recommendations for Future Research

This thesis described methods for genetic differentiation of $Beauveria bassiana$ isolates, and was used to identify 89 genotypes from 111 individual samples. Techniques for identification and genetic fingerprinting are used in forensics, paternity tests, and marker assisted selection of agronomic crops. Tracking artificially introduced species or isolates for biocontrol is another, yet mostly unrealized, area of application. $Beauveria bassiana$ have been characterized in laboratory bioassay, and isolates with desired pathogenic characteristics often are applied to production fields. Indirect evidence has suggested a correlation between application of the entomopathogen and increased host species mortality.
More direct means of evaluating isolate efficiency and impact upon insect infestations may be possible by releasing defined strains that are phenotypically virulent to an insect of choice. If the field regional *B. bassiana* can be shown to be devoid of genotypes that match that of the released isolate, detailed tracking experiments could be conducted.

Environmental impact of genetically defined fungal entomopathogens has yet to be performed. Contribution and persistence of applied the isolates to overall host mortality and crop protection need be addressed. Effects from natural epizootics likely are addresses by comparison of replicates of treated and untreated plots within randomized blocks. Uncertainty arises when long-term effectiveness and local population impact is evaluated. For instance, will a sufficient population of the applied isolate persist in following years, or will yearly application be required. Genetic markers also could be applied to quantification of given strain either in endophytic or entomopathogenic relationships. Techniques in quantitative PCR have been devised, and are highly sensitive to target DNA. Markers from the rRNA likely would not be good candidates for such analysis, since primers likely would amplify other related fungi. The minisatellite, *BbMin1*, may be a locus unique to *Beauveria* spp. and not be subject to cross genus amplification.
REFERENCES CITED


Bing LA, Lewis LC (1991) Suppression of *Ostrinia nubilalis* (Hubner) (Lepidoptera: Pyralidae) by endophytic *Beauveria bassiana* (Balsamo) Vuillemin. Environ Entomol 20:1207-1211


Hajek AE, Soper RS, Roberts DW, Anderson TE, Biever KD, Ferro DN, LeBrun RA, Storch RH (1987) Foliar application of Beauveria bassiana (Balsamo) Vuillemin for control of the Colorado potato beetle, Leptinotarsa decemlineata (Say) (Coleoptera; Chrysomelidae): an overview of pilot tests from the northern United States. Can Entomol 119:959-974


Hopple JS, Vilgalys R (1994) Phylogenetic relationships among coprinoid taxa and allies based on data from restriction-site mapping of nuclear rRNA. Mycolgia 86:96-107


Kempken F, Kuck U (1998b) Evidence for circular transposition derivatives from the fungal *hAT*-transposon *Restless*.


Lacey LA, Horton DR, Chauvin RL, Stocker JM (1999) Comparative efficacy of *Beauveria bassiana*, *Bacillus thuringiensis*, and aldicarb for control of Colorado potato beetle in an irrigated desert agroecosystem and their effects on biodiversity. Entomol Exp Appl 93:189-200


Schneider S, Kueffer JM, Roessli D, Excoffier L (1997) Arlequin ver. 1.1: A software for population genetic data analysis. Genetics and Biometry Laboratory, University of Geneva, Switzerland


APPENDIX A. GENBANK ACCESSIONS

LOCUS AP322933 499 bp DNA
DEFINITION Beauveria amorpha internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence.
ACCESSION AP322933
VERSION AP322933.1 GI:12007457
KEYWORDS .
SOURCE Beauveria amorpha.
ORGANISM Beauveria amorpha
Eukaryota; Fungi; Ascomycota; Pezizomycotina; Sordariomycetes; Hypocreales; Clavicipitaceae; mitosporic Clavicipitaceae; Beauveria.
REFERENCE 1 (bases 1 to 499)
AUTHORS Coates,B.S., Hellmich,R.L. and Lewis,L.C.
TITLE Determination of Beauveria bassiana haplotypes based on nuclear rDNA internal transcribed spacer PCR-RFLP
JOURNAL Unpublished
REFERENCE 2 (bases 1 to 499)
AUTHORS Coates,B.S., Hellmich,R.L. and Lewis,L.C.
TITLE Direct Submission
JOURNAL Submitted (22-NOV-2000) Corn Insects Lab, USDA-ARS, 111 Genetics Lab, Ames, IA 50011, USA
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121 ttaccagcat gttctgaata cgccgcaagg caaaacaaaa ttaaaacttt
181 tctcttggct ctggcatcga tgaagaacgc agcgaaacgc gataagtaat
241 gaatccagtg aatcatcgaa tctttgaacg cacattgcgc ccgccagcat
301 atgccctgttc gagcgtcatt acaacactcg acctcccggg ccagcccgtg
361 ttggggatcg gcagcacacc gccggccctg aaatggagtg gcggcccgtc
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481 ttcctgaacct gtcgcccttg

LOCU5 AF322932  495 bp DNA  PLN  02-JAN-2001

DEFINITION Beauveria bassiana isolate Bb3167 internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence.

Accession AF322932

Version AF322932.1 GI:12007456

KEYWORDS

SOURCE Beauveria bassiana.

ORGANISM Beauveria bassiana
Eukaryota; Fungi; Ascomycota; Pezizomycotina; Sordariomycetes; Hypocreales; Clavicipitaceae; mitosporic Clavicipitaceae; Beauveria.

REFERENCE 1 (bases 1 to 495)
AUTHORS Coates,B.S., Hellmich,R.L. and Lewis,L.C.
TITLE Determination of Beauveria bassiana haplotypes based on nuclear rDNA internal transcribed spacer PCR-RFLP
JOURNAL Unpublished

REFERENCE 2 (bases 1 to 495)
AUTHORS Coates,B.S., Hellmich,R.L. and Lewis,L.C.
TITLE Direct Submission
JOURNAL Submitted (22-NOV-2000) Corn Insects Lab, USDA-ARS, 111 Genetics Lab, Ames, IA 50011, USA

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121 tatcagcatc ttctgaatac gccgcaaggc aaaacaaata aattaaaact
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VERSION AF322931.1 GI:12007455

KEYWORDS

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Eukaryota; Fungi; Ascomycota; Pezizomycotina; Sordariomycetes; Hypocreales; Clavicipitaceae; mitosporic Clavicipitaceae; Beauveria.

REFERENCE 1 (bases 1 to 494)
AUTHORS Coates,B.S., Hellmich,R.L. and Lewis,L.C.
TITLE Determination of Beauveria bassiana haplotypes based on nuclear rDNA internal transcribed spacer PCR-RFLP
JOURNAL Unpublished

REFERENCE 2 (bases 1 to 494)
AUTHORS Coates,B.S., Hellmich,R.L. and Lewis,L.C.
TITLE Direct Submission
JOURNAL Submitted (22-NOV-2000) Corn Insects Lab, USDA-ARS, 111 Genetics Lab, Ames, IA 50011, USA

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REFERENCE 1 (bases 1 to 494)
  AUTHORS Coates,B.S., Hellmich,R.L. and Lewis,L.C.
  TITLE Determination of Beauveria bassiana haplotypes based on nuclear rDNA internal transcribed spacer PCR-RFLP
  JOURNAL Unpublished
REFERENCE 2 (bases 1 to 494)
  AUTHORS Coates,B.S., Hellmich,R.L. and Lewis,L.C.
  TITLE Direct Submission
  JOURNAL Submitted (22-NOV-2000) Corn Insects Lab, USDA-ARS, 111 Genetics Lab, Ames, IA 50011, USA
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421 gtacataacct agctggcacc gcgaacccgga cggagggcacc gcgttaaaca ccacacactc
ttcg ctttcggcgga aactcttgta ttcaacaacgc aactctttgta ctgaatcttc gcggcgttcg
481 gaacgttgcc cttg
//
LOCUS AF322929 494 bp DNA PLN 02-JAN-2001
DEFINITION Beauveria bassiana isolate Bb1155 internal transcribed spacer 1. 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence.
ACCESSION AF322929
VERSION AF322929.1 GI:12007453
KEYWORDS
SOURCE Beauveria bassiana.
ORGANISM Beauveria bassiana
Eukaryota; Fungi; Ascomycota; Pezizomycotina; Sordariomycetes; Hypocreales; Clavicipitaceae; mitosporic Clavicipitaceae; Beauveria.
REFERENCE 1 (bases 1 to 494)
AUTHORS Coates,B.S., Hellmich,R.L. and Lewis,L.C.
TITLE Determination of Beauveria bassiana haplotypes based on nuclear rDNA internal transcribed spacer PCR-RFLP
JOURNAL Unpublished
REFERENCE 2 (bases 1 to 494)
AUTHORS Coates,B.S., Hellmich,R.L. and Lewis,L.C.
TITLE Direct Submission
JOURNAL Submitted (22-NOV-2000) Corn Insects Lab, USDA-ARS, 111 Genetics Lab, Ames, IA 50011, USA
FEATURES Location/Qualifiers
source 1..494
/organism="Beauveria bassiana" /isolate="Bb1155" /db_xref="taxon:5582"
misc_RNA 1..159
/r.product="internal transcribed spacer 1"
rRNA 160..316
/product="5.8S ribosomal RNA"
misc_RNA 317..494
/r.product="internal transcribed spacer 2"
BASE COUNT 114 a 161 c 122 g 97 t
ORIGIN 1 attaccqagt ttgcaacctc ccacacccttc tgtgacaccta cctatcgttg cttcgccgga
61 ctcggcccaag cccgagcagc gacttgacaca ggtaggcccgc ggggacccca aactcctgta
121 ttccagcctc tttcgaacctc gcgcagcaacg aacaaaaatg aacaaactc ttcacaaacg
181 gatctcttggt ctctggcagca tgtcgaaggac gcacgcaaacc ggctgataagtc atgtgatagtt
241 cagacacccag tgacagcctgct cctcttgaac cgcacattgc gcccggccagc attctggcgcg
301 gcagcgcctgt cggccgctcat ttcacccctc gagccctcctcg ccctgcggggcttgggccttggg
361 gaccgctgca aacccggccg cccctgaaatg gctctggcgcg ccctgccgccc gcgcctctgc
421 gatactgttc acgtgctgcc cgaacccccca gcccggccagc ccggccagcc gcgttaaatcg cccaaacctct
481 gaacgctgctc cttg
//
LOCUS AF322928 494 bp DNA PLN 02-JAN-2001
DEFINITION Beauveria bassiana isolate Bb1149 internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence.
ACCESSION AF322928
VERSION AF322928.1 GI:12007452
KEYWORDS
SOURCE Beauveria bassiana.
ORGANISM Beauveria bassiana
Eukaryota; Fungi; Ascomycota; Pezizomycotina; Sordariomycetes; Hypocreales; Clavicipitaceae; mitosporic Clavicipitaceae; Beauveria.
REFERENCE 1 (bases 1 to 494)
AUTHORS Coates, B.S., Hellmich, R.L. and Lewis, L.C.
TITLE Determination of Beauveria bassiana haplotypes based on nuclear rDNA internal transcribed spacer PCR-RFLP
JOURNAL Unpublished
REFERENCE 2 (bases 1 to 494)
AUTHORS Coates, B.S., Hellmich, R.L. and Lewis, L.C.
TITLE Direct Submission
JOURNAL Submitted (22-NOV-2000) Corn Insects Lab, USDA-ARS, 111 Genetics Lab, Ames, IA 50011, USA
FEATURES
   source 1..494
      /organism="Beauveria bassiana"
      /isolate="Bb1149"
      /db_xref="taxon:5582"
   misc_RNA 1..159
      /product="internal transcribed spacer 1"
   rRNA 160..316
      /product="5.8S ribosomal RNA"
   misc_RNA 317..494
      /product="internal transcribed spacer 2"
BASE COUNT 113 a 160 c 124 g 97 t
ORIGIN
1 attaccgagt ttccacactc ctaacccttc tgtgaaccta cctatcgttg cttcggccga
 61 ctcgcccccag cccgaccccg gacgtgacca cccgccccgcc gggacaccca aacctcttga
121 ttccagcatc ttctgaatac gccgcaaggc aaaacaaatg aatcaaaact
181 gatctcttgg ctctggcatc gatgaagaac gcagcgaaac gcgataagta
241 cagaatccag tgaatcatcg aatctttgaa cgcacattgc gcccgccagc
gcatgcctgt tcgagcgtca tttcaaccct cgacctcccc ttggggaggt
361 gaccggcagc acaccgccgg ccctgaaatg gagtggcggc ccgtccgcgg
gcagtaatac agctcgcacc gggaccccga cgcggccacg ccgtaaaaca
gacgttgcc ctgg
481 gaacgttgcc cttg
LOCUS       AF322927   493 bp DNA  PLN  02-JAN-2001
DEFINITION  Beauveria bassiana isolate Bb1022 internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence.
ACCESSION   AF322927
VERSION     AF322927.1 GI:12007451
KEYWORDS    .
SOURCE      Beauveria bassiana.
ORGANISM    Beauveria bassiana
            Eukaryota; Fungi; Ascomycota; Pezizomycotina; Sordariomycetes; Hypocreales; Clavicipitaceae; mitosporic Clavicipitaceae; Beauveria.
REFERENCE   1 (bases 1 to 493)
            Coates, B.S., Hellmich, R.L. and Lewis, L.C.
            TITLE Determination of Beauveria bassiana haplotypes based on nuclear rDNA internal transcribed spacer PCR-RFLP
            JOURNAL Unpublished
REFERENCE   2 (bases 1 to 493)
            Coates, B.S., Hellmich, R.L. and Lewis, L.C.
            TITLE Direct Submission
            JOURNAL Submitted (22-NOV-2000) Corn Insects Lab, USDA-ARS, 111 Genetics Lab, Ames, IA 50011, USA
FEATURES    source 1..493
            /organism="Beauveria bassiana"
            /isolate="Bb1022"
            /db_xref="taxon:5582"
            misc_RNA 1..158
            /product="internal transcribed spacer 1"
            rRNA 159..315
            /product="5.8S ribosomal RNA"
            misc_RNA 316..493
            /product="internal transcribed spacer 2"
BASE COUNT 113 a 158 c 125 g 97 t
ORIGIN 1 attaccgagt tttcaactcc ctaccctctc tggagaacctac ctatcgttgc ttcggcggac
        61 ttcggcggac ccggagcccg actggaacccag cgggccgccc gcggacctca aacctttgta
        121 tccagcatct tctgaatacg ccgaaaggca aaacaaatga atcaaaactt atcggattgct
        181 atctctttgctc tctggcatcg atgaagaacg cagcgaaacg cgataaactt tcaaaactcttgag
        241 cagctgacgtc gatcatcga atctttgaac gcacggtttgc cccgccagca
        301 cagtaataac gctacgcaccg gacccgagac gcgggccagc
        361 acgcggagca caccggcggt cctgaaatgtt cgggccgccc gcgtgagcttc gcggcgaggtg
        421 cgtgactgagcc gcggcgcagc
ggacccgacc cttccctctt tgtgaattgc ttgctctttgctg atcacaaggtg
        481 aacgctgactgagcc ggacccgacc cttccctctgtatctctttgctg
LOCUS AF322926 494 bp DNA PLN 02-JAN-2001
DEFINITION Beauveria bassiana isolate Bb726 internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence.
ACCESSION AF322926
VERSION AF322926.1 GI:12007450
KEYWORDS .
SOURCE Beauveria bassiana.
ORGANISM Beauveria bassiana; Eukaryota; Fungi; Ascomycota; Pezizomycotina; Sordariomycetes; Hypocreales; Clavicipitaceae; mitosporic Clavicipitaceae; Beauveria.
REFERENCE 1 (bases 1 to 494)
AUTHORS Coates,B.S., Hellmich,R.L. and Lewis,L.C.
TITLE Determination of Beauveria bassiana haplotypes based on nuclear rDNA internal transcribed spacer PCR-RFLP
JOURNAL Unpublished
REFERENCE 2 (bases 1 to 494)
AUTHORS Coates,B.S., Hellmich,R.L. and Lewis,L.C.
TITLE Direct Submission
JOURNAL Submitted (22-NOV-2000) Corn Insects Lab, USDA-ARS, 111 Genetics Lab, Ames, IA 50011, USA

FEATURES Location/Qualifiers
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    /organism="Beauveria bassiana"
    /isolate="Bb726"
    /db_xref="taxon:5582"
  misc_RNA 1..159
    /product="internal transcribed spacer 1"
  rRNA 160..316
    /product="5.8S ribosomal RNA"
  misc_RNA 317..494
    /product="internal transcribed spacer 2"

BASE COUNT 114 a 158 c 125 g 97 t

ORIGIN
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  61 ctcgccccag cccggacgcg gactggacca gcggcccgcc ggggacctca
  121 ttccagcatc ttctgaatac gccgaaaggc aaaacaaatg aatcaaaact
  181 gatctcttgg ctctggcatc gatgaagaac gcagcgaaac gcgataagta
  241 gagaatccag tgaatcatcg aatctttgaa cgcacattgc gcccgccagc
  301 gcatgcctgt tcgagggtca ttctgaaccct cgacctcccc ttgggggggt
  361 gaccggcagc acaccgccgg ccctgaaatg gagtggcggc ccgtccgcgg
  421 gcagtaatac agctcgcacc ggaaccccga cgcggccacg ccgtaaaaca
  481 gaacgtgacc cttg
LOCUS AF322925 495 bp DNA PLN 02-JAN-2001
DEFINITION Beauveria bassiana isolate Bb501 internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence.

ACCESSION AF322925
VERSION AF322925.1 GI:12007449
KEYWORDS

SOURCE Beauveria bassiana.

ORGANISM
Eukaryota; Fungi; Ascomycota; Pezizomycotina; Sordariomycetes; Hypocreales; Clavicipitaceae; mitosporic Clavicipitaceae; Beauveria.

REFERENCE 1 (bases 1 to 495)
AUTHORS Coates,B.S., Hellmich,R.L. and Lewis,L.C.
TITLE Determination of Beauveria bassiana haplotypes based on nuclear rDNA internal transcribed spacer PCR-RFLP
JOURNAL Unpublished

REFERENCE 2 (bases 1 to 495)
AUTHORS Coates,B.S., Hellmich,R.L. and Lewis,L.C.
TITLE Direct Submission
JOURNAL Submitted (22-NOV-2000) Corn Insects Lab, USDA-ARS, 111 Genetics Lab, Ames, IA 50011, USA

FEATURES Location/Qualifiers
source 1..495
/misc_RNA 1..159
/rRNA 160..317
/misc_RNA 318..495

BASE COUNT 113 a 160 c 124 g 98 t

ORIGIN
1 attaccqagt ttccaaatcc ctaacccttc tgtgaaccta cctacgttg ccttgacgga
61 ctggcccaag cccgcaccg gacttgacca ggcgccggcc gggacgccca acctcttgta
121 ttccagcatc ttctgaatcc ggcgcaagcc aaacaaatgc aactcttgta
181 ctgtcttttg gcgtgccgacg gatgacgtaa gacagctgaaacc gcaatgcaatgctcgagtc
241 catgtaatcga tggaatcggc gacacatgta cacaccgacg cagcacacatgta cattctggcc
301 ggcatgcctg ttcgagcgtc atttcaaccc tgggacgcag cctgggccatct cgggaccgcc
361 ggcgacgac ccacggccgg gcctgaatgcc gactgaccgacg cagacagccg cctgacacgac
421 ggcagctata caagcttgac gcggaccgag cctgggccag cctgacaccac accaacacgtc
481 tgtgacgtgac ctcgg
//
LOCUS AF322924 494 bp DNA PLN 02-JAN-2001
DEFINITION Beauveria bassiana isolate Bb153 internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence.
ACCESSION AF322924
VERSION AF322924.1 GI:12007448
KEYWORDS .
SOURCE Beauveria bassiana.
ORGANISM Eukaryota; Fungi; Ascomycota; Pezizomycotina; Sordariomycetes; Hypocreales; Clavicipitaceae; mitosporic Clavicipitaceae; Beauveria.
REFERENCE 1 (bases 1 to 494)
AUTHORS Coates,B.S., Hellmich,R.L. and Lewis,L.C.
TITLE Determination of Beauveria bassiana haplotypes based on nuclear rDNA internal transcribed spacer PCR-RFLP
JOURNAL Unpublished
REFERENCE 2 (bases 1 to 494)
AUTHORS Coates,B.S., Hellmich,R.L. and Lewis,L.C.
TITLE Direct Submission
JOURNAL Submitted (22-NOV-2000) Corn Insects Lab, USDA-ARS, 111 Genetics Lab, Ames, IA 50011, USA
FEATURES Location/Qualifiers
source
1..494
/organism="Beauveria bassiana"
/isolate="Bb153"
/db_xref="taxon:5582"
misc_RNA
1..158
/product="internal transcribed spacer 1"
rRNA
159..316
/product="5.8S ribosomal RNA"
misc_RNA
317..494
/product="internal transcribed spacer 2"
BASE COUNT 121 a 152 c 120 g 101 t
ORIGIN
1 attaccgagt tttcaactcc caaaccctta tgtgaacctg cttcggccga
61 ctcgcccccg ccggacgcgg actggaccag cggccgccgg ggacatcaa
121 tatcagcatc tttctgaatac gccgcaaggc aaaaacaaata aattaaaact ttcacaacag
181 gatctcttgag ctctggcatc gatgaagaac gcagcgaaat gcgataagta
241 cagaatccag tgaatcatcg aatctttgaa cgcaacattgc gcgcagcacc gcgggcccagc attctggccgg
301 gcatgcctgt tcgagcgtca tttcaactcc cgacctccct ttggggaagt
361 gaccggcagc acaccgccgg ccctgaaatg gagtggcggc ccgtccgcgg
421 gtagtaaacc aactcgcacc ggaaccccga cgtggccacg ccgtttaaaaca cccaacttct
481 gcacgttcgccttgg
//
**LOCUS** AF391119 1027 bp DNA PLN 19-JUL-2001
**DEFINITION** Beauveria bassiana isolate Bb3167 28S ribosomal RNA gene, partial sequence.
**ACCESSION** AF391119
**VERSION** AF391119.1 GI:14915821
**KEYWORDS** .
**SOURCE** Beauveria bassiana.
**ORGANISM** Beauveria bassiana
Eukaryota; Fungi; Ascomycota; Pezizomycotina; Sordariomycetes; Hypocreales; Clavicipitaceae; mitosporic Clavicipitaceae; Beauveria.

**REFERENCE** 1 (bases 1 to 1027)
**AUTHORS** Coates,B.S., Hellmich,R.L. and Lewis,L.C.
**TITLE** Diversity among nuclear large subunit rRNA group I introns of the entomopathogenic fungus, Beauveria bassiana
**JOURNAL** Unpublished

**REFERENCE** 2 (bases 1 to 1027)
**AUTHORS** Coates,B.S., Hellmich,R.L. and Lewis,L.C.
**TITLE** Direct Submission
**JOURNAL** Submitted (13-JUN-2001) Corn Insects and Crop Genetics

**FEATURES**
- **source**
  - Location/Qualifiers
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    - /organism="Beauveria bassiana"
    - /isolate="Bb3167"
    - /db_xref="taxon:5582"
    - /note="lacking group I introns"
    - /product="28S ribosomal RNA"

**BASE COUNT** 257 a 223 c 291 g 256 t

**ORIGIN**

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1  agggttgccg acgtttggtc ttggaaggac gcctcgggag cagcgccgca ctagccggcg
61 acacggccgg cgcagccag ccagaaagtg tggccggag ccagaaagtg
121 gcgtctggct aacaacctgg ctgacctggt ggaaggggct gccgctgggt
181 aaacactagc attgcatgg ccagaaagtg tggccggag ccagaaagtg
241 ctctgtaatgt caaagtggag taattcagcc ggcttgagg caccgcgttg
301 actctcttaaa ggtatcggaa tgcctctgta tctaatattcg cagcgcagatc aggtggtgag
361 cggatccccc acctgctccct tctactatct cggcggcggc cggcggcggc
ggatatcggctgg gcggtggtc cggcgccggg cggcgccggg
cggatatcggctgg gcggtggtc cggcgccggg cggcgccggg
cggatatcggctgg gcggtggtc cggcgccggg cggcgccggg
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cggatatcggctgg gcggtggtc cggcgccggg cggcgccggg
cggatatcggctgg gcggtggtc cggcgccggg cggcgccggg

961 ccactaatag ggaacgtgag ccagtctgtc ctggttgagg cggcggcggg cggcggcggg
cggatatcggctgg gcggtggtc cggcgccggg cggcgccggg
cggatatcggctgg gcggtggtc cggcgccggg cggcgccggg
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cggatatcggctgg gcggtggtc cggcgccggg cggcgccggg
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cggatatcggctgg gcggtggtc cggcgccggg cggcgccggg
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LOCUS AF322934 416 bp DNA PLN 02-FEB-2001

DEFINITION Beauveria bassiana isolate Bb502 28S ribosomal RNA gene, intron

ACCESSION AF322934

VERSION AF322934.1 GI:12656619

KEYWORDS .

SOURCE Beauveria bassiana.

ORGANISM Beauveria bassiana;

Eukaryota; Fungi; Ascomycota; Pezizomycotina; Sordariomycetes;

Hypocreales; Clavicipitaceae; mitosporic Clavicipitaceae;

Beauveria.

REFERENCE 1 (bases 1 to 416)

AUTHORS Coates,B.S., Hellmich,R.L. and Lewis,L.C.

TITLE Nucleotide Sequence Variation Beauveria bassiana 28S rDNA Group-I Intron Insertion and JOURNAL Unpublished

REFERENCE 2 (bases 1 to 416)

AUTHORS Coates,B.S.

TITLE Direct Submission

JOURNAL Submitted (22-NOV-2000) Corn Insects & Crop Genetics, USDA-ARS,

111 Genetics Lab, Ames, IA 50011, USA

FEATURES Location/Qualifiers

source 1..416

/organism="Beauveria bassiana"
/isolate="Bb502"
/db_xref="taxon:5582"
/clone="l-1"

intron 1..416

(note="28S ribosomal RNA group I intron"
/number=1

BASE COUNT 92 a 95 c 141 g 88 t

ORIGIN

1 aaatttgtttt cttgttgttg gggatagtgc ggtctcttgta gcaggattac gcgggctagt

61 gcagcatgaca tagctgtcag ggtgctatgcg gcgacacacac tgggtacgag gggagtattaat

121 ggcctgcagg ctcttgtaacc tgcagtctgc gcccgcacac aagctatggc cgcgtggtgta cgacgcgtcct

181 tgtagacgcc ggaagggtgt ggggtgactct tctaggtacg cctaggaaggt cgttaaggg

241 acgtgccaga ccccgcggga acgcgtgctcg atgcgaaggg cctgagcagc agatctagct

301 ggtggctttttaggccccgagg gaaatgccccg gaagagcctgg tataaccaca tagttaaggg

361 acggacgccc tgtcagtagt gacgaggcag gtcatttat atcatgtag tattcg
LOCUS AF322935 416 bp DNA PLN 02-FEB-2001

DEFINITION Beauveria bassiana isolate Bb2737 28S ribosomal RNA gene, intron 1.

ACCESSION AF322935

VERSION AF322935.1 GI:12656620

KEYWORDS .

SOURCE Beauveria bassiana.

ORGANISM Beauveria bassiana

  Eukaryota; Fungi; Ascomycota; Pezizomycotina; Sordariomycetes; Hypocreales; Clavicipitaceae; mitosporic Clavicipitaceae; Beauveria.

REFERENCE 1 (bases 1 to 416)

  AUTHORS Coates,B.S., Hellmich,R.L. and Lewis,L.C.

  TITLE Beauveria bassiana 28S rDNA Group-I Intron Insertion and Nucleotide Sequence Variation

  JOURNAL Unpublished

REFERENCE 2 (bases 1 to 416)

  AUTHORS Coates,B.S.

  TITLE Direct Submission

  JOURNAL Submitted (22-NOV-2000) Corn Insects & Crop Genetics, USDA-ARS,

  111 Genetics Lab, Ames, IA 50011, USA

FEATURES Location/Qualifiers

  source 1..416

  /organism="Beauveria bassiana"

  /isolate="Bb2737"

  /db_xref="taxon:5582"

  /clone="l-1"

  intron 1..416

  /note="28S ribosomal RNA group I intron"

  /number=1

BASE COUNT 92 a 95 c 141 g 88 t

ORIGIN

  1 aaatttgggcttctgtgttg ggtatgatgtcg ggtcctgtttg gcaggattac gcgggtcagtt
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  121 gcccgcgcag ccatgtgcaac tccgagtgctc ggttgggtgtgg gagtctgatgt atagagatc
  181 tgtggtggcct ggaaggtggt gttgtgactct tccaggtcag cctagaggtg cgcgggttgt
  241 agcggcgagc cccccgggag acgcgggtg gacgcttggt ctgtagccag ccgggtgtgag
  301 tgtggtggctt gggcgcgggc ggaatgggct cggagcgtg gtataccaca tagataaggg
  361 acggagcggac gtcaggtgta gacgggtgacg cgtattggc atctatgttg atttc
LOCUS AF322936  426 bp DNA  PLN  02-FEB-2001  
DEFINITION Beauveria bassiana isolate Bb2297 28S ribosomal RNA gene, intron 1.  
ACCESSION AF322936  
VERSION AF322936.1 GI:12656621  
KEYWORDS .  
SOURCE Beauveria bassiana.  
ORGANISM Beauveria bassiana  
Eukaryota; Fungi; Ascomycota; Pezizomycotina; Sordariomycetes; Hypocreales; Clavicipitaceae; mitosporic Clavicipitaceae; Beauveria.  

REFERENCE 1 (bases 1 to 426)  
AUTHORS Coates,B.S., Hellmich,R.L. and Lewis,L.C.  
TITLE Beauveria bassiana 28S rDNA Group-I Intron Insertion and Nucleotide Sequence Variation  
JOURNAL Unpublished  

REFERENCE 2 (bases 1 to 426)  
AUTHORS Coates,B.S.  
TITLE Direct Submission  
JOURNAL Submitted (22-NOV-2000) Corn Insects & Crop Genetics, USDA-ARS,  
111 Genetics Lab, Ames, IA 50011, USA  

FEATURES  
source  
1..426  
/organism="Beauveria bassiana"  
/isolate="Bb2297"  
/db_xref="taxon:5582"  
/clone="l-2"  
intron 1..426  

BASE COUNT  
94 a 98 c 149 g 85 t  

ORIGIN  
1 agaccttttaa cgctcgagatg atgtttctta ttggtgtgtaa ttagctgggtc ctgctggcag  
61 gattactacc ggctgagatg atgtttctta ttggtggtaa tagtcgggtc  
121 gatcggggga ggctacgggc cctacaaggt ctatgctaat cccgagtgta  
181 agagatcttc cgggacgcac gtagagcgcg gaaaggtgtg ggtgacccta  
241 ctggagggtt gcttaaggga cgtgccagac ccacgggaga  
301 cctgcagtct ggatcatcta ggtggcgaag aggccgggag gaaatgcccg  
361 gtatatcaca tggtttaggg acgagacggg cgccggccgc gacggtcgcac tatacgtgt  
421 gttgctg

//
LOCUS AF336302 387 bp DNA PLN 05-FEB-2001
DEFINITION Beauveria bassiana isolate ECB L16 28S ribosomal RNA gene, intron 1.
ACCESSION AF336302
VERSION AF336302.1 GI:12659337
KEYWORDS .
SOURCE Beauveria bassiana.
ORGANISM Beauveria bassiana
  Eukaryota; Fungi; Ascomycota; Pezizomycotina; Sordariomycetes; Hypocreales; Clavicipitaceae; mitosporic Clavicipitaceae; Beauveria.
REFERENCE 1 (bases 1 to 387)
  AUTHORS Coates,B.S., Hellmich,R.L. and Lewis,L.C.
  TITLE Beauveria bassiana 28s rDNA Group-I Intron Insertion and Nucleotide Sequence Variation
  JOURNAL Unpublished
REFERENCE 2 (bases 1 to 387)
  AUTHORS Coates,B.S., Hellmich,R.L. and Lewis,L.C.
  TITLE Direct Submission
  JOURNAL Submitted (12-JAN-2001) Corn Insects Lab, USDA-ARS, 111 Genetics Lab, Ames, IA 50011, USA
FEATURES
  source Location/Qualifiers
  1..387
    /organism="Beauveria bassiana"
    /isolate="ECB L16"
    /db_xref="taxon:5582"
    /clone="l-3"
    /note="28S ribosomal RNA group I intron"
    /number=1
  intron 1..387
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ACCESSION AF391118
VERSION AF391118.1 GI:14915820
KEYWORDS
SOURCE Beauveria bassiana.
ORGANISM Beauveria bassiana
  Eukaryota; Fungi; Ascomycota; Pezizomycotina; Sordariomycetes; Hypocreales; Clavicipitaceae; mitosporic Clavicipitaceae; Beauveria.
REFERENCE 1 (bases 1 to 499)
  AUTHORS Coates,B.S., Hellmich,R.L. and Lewis,L.C.
  TITLE Diversity among nuclear large subunit rRNA group I introns of the entomopathogenic fungus, Beauveria bassiana
  JOURNAL Unpublished
REFERENCE 2 (bases 1 to 499)
  AUTHORS Coates,B.S., Hellmich,R.L. and Lewis,L.C.
  TITLE Direct Submission
  JOURNAL Submitted (13-JUN-2001) Corn Insects and Crop Genetics Research Unit, USDA ARS, 111 Genetics Lab/Iowa State University, Ames, IA 50011, USA
FEATURES
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181 ctacacacct ggcacctgngg acatcttcgg taccagccg taagctcactt gttgggtgtgt
241 actcactcgt tccaatgaca ggggtacggt aataatctgt gatgatagagga gattgttattc
301 ctggtttgagg tctttggaaaa gaggtgctgct gctctggctaa gcggtcttttt tgggtagtggt
361 gtaaattggc actcactcgttt tattgaggtc gctctggcta gcttggttggt
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481 tcctgcaggg gtatcaggg

LOCUS AF391117 401 bp DNA PLN 19-JUL-2001
DEFINITION Beauveria bassiana isolate Bb1121 28S ribosomal RNA gene, intron 2.
ACCESSION AF391117
VERSION AF391117.1 GI:14915819
KEYWORDS .
SOURCE Beauveria bassiana.
ORGANISM Beauveria bassiana
Eukaryota; Fungi; Ascomycota; Pezizomycotina; Sordariomycetes; Hypocreales; Clavicipitaceae; mitosporic Clavicipitaceae; Beauveria.
REFERENCE 1 (bases 1 to 401)
AUTHORS Coates,B.S., Hellmich,R.L. and Lewis,L.C.
TITLE Diversity among nuclear large subunit rRNA group I introns of the entomopathogenic fungus, Beauveria bassiana
JOURNAL Unpublished
REFERENCE 2 (bases 1 to 401)
AUTHORS Coates,B.S., Hellmich,R.L. and Lewis,L.C.
TITLE Direct Submission
JOURNAL Submitted (13-JUN-2001) Corn Insects and Crop Genetics Research

FEATURES Location/Qualifiers
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361 agtatgcgcg gggagtcttc actcctcgca gggtatcag
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DEFINITION Beauveria bassiana isolate Bb2515 28S ribosomal RNA gene, intron 2; and I2EP (I2EP) gene, complete cds.
ACCESSION AF322938
KEYWORDS .
SOURCE Beauveria bassiana.
ORGANISM Eukaryota; Fungi; Ascomycota; Pezizomycotina; Sordariomycetes; Hypocreales; Clavicipitaceae; mitosporic Clavicipitaceae; Beauveria.
REFERENCE 1 (bases 1 to 1402)
AUTHORS Coates, B.S., Hellmich, R.L. and Lewis, L.C.
TITLE Beauveria bassiana 28S rDNA Group-I Intron Insertion and Nucleotide Sequence Variation
JOURNAL Unpublished
REFERENCE 2 (bases 1 to 1402)
AUTHORS Coates, B.S.
TITLE Direct Submission
JOURNAL Submitted (22-NOV-2000) Corn Insects & Crop Genetics, USDA-ARS, 111 Genetics Lab, Ames, IA 50011, USA
FEATURES Location/Qualifiers
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    /number=2
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DEFINITION Beauveria bassiana isolate Bb151 28S ribosomal RNA gene, intron 2.
ACCESSION AF322937
KEYWORDS .
SOURCE Beauveria bassiana.
ORGANISM Beauveria bassiana
     Eukaryota; Fungi; Ascomycota; anamorphic Ascomycota; Beauveria.
REFERENCE 1 (bases 1 to 500)
AUTHORS Coates,B.S., Hellmich,R.L. and Lewis,L.C.
TITLE Beauveria bassiana 28S rDNA Group-I Intron Insertion and Nucleotide Sequence Variation
JOURNAL Unpublished
REFERENCE 2 (bases 1 to 500)
AUTHORS Coates,B.S.
TITLE Direct Submission
JOURNAL Submitted (22-NOV-2000) Corn Insects & Crop Genetics, USDA-ARS, 111 Genetics Lab, Ames, IA 50011, USA
FEATURES Location/Qualifiers
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DEFINITION  Beauveria bassiana isolate Bb2297 28S ribosomal RNA gene, intron 3.
ACCESSION  AF322940
VERSION     AF322940.1   GI:12656623
KEYWORDS    .
SOURCE      Beauveria bassiana.
ORGANISM    Beauveria bassiana
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REFERENCE   1 (bases 1 to 443)
            AUTHORS Coates,B.S., Hellmich,R.L. and Lewis,L.C.
            TITLE  Beauveria bassiana 28S rDNA Group-I Intron Insertion and Nucleotide Sequence Variation
            JOURNAL Unpublished
REFERENCE   2 (bases 1 to 443)
            AUTHORS Coates,B.S.
            TITLE  Direct Submission
            JOURNAL Submitted (22-NOV-2000) Corn Insects & Crop Genetics, USDA-ARS,
                  111 Genetics Lab, Ames, IA 50011, USA
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            361 ggtttctgtga ggcggcggtgc tgccaactct tacagggtct ctactccac
            421 tctgaaagga tgggtactga gttg
LOCUS AF322939 431 bp DNA PLN 02-FEB-2001
DEFINITION Beauveria bassiana isolate Bbl00l 28S ribosomal RNA gene, intron 3.
ACCESSION AF322939
VERSION AF322939.1 GI:12656622
KEYWORDS
SOURCE Beauveria bassiana.
ORGANISM Beauveria bassiana
  Eukaryota; Fungi; Ascomycota; Pezizomycotina; Sordariomycetes; Hypocreales; Clavicipitaceae; mitosporic Clavicipitaceae; Beauveria.
REFERENCE 1 (bases 1 to 431)
  AUTHORS Coates,B.S., Hellmich,R.L. and Lewis,L.C.
  TITLE beauveria bassiana 28S rDNA Group-I Intron Insertion and Nucleotide Sequence Variation
  JOURNAL Unpublished
REFERENCE 2 (bases 1 to 431)
  AUTHORS Coates,B.S.
  TITLE Direct Submission
  JOURNAL Submitted (22-NOV-2000) Corn Insects & Crop Genetics, USDA-ARS,
  111 Genetics Lab, Ames, IA 50011, USA
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ACCESSION AF391116
VERSION AF391116.1 GI:14915818
KEYWORDS .
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ORGANISM Beauveria bassiana
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   Beauveria.
REFERENCE 1 (bases 1 to 445)
AUTHORS Coates,B.S., Hellmich,R.L. and Lewis,L.C.
TITLE Diversity among nuclear large subunit rRNA group I introns of the entomopathogenic fungus, Beauveria bassiana
JOURNAL Unpublished
REFERENCE 2 (bases 1 to 445)
AUTHORS Coates,B.S., Hellmich,R.L. and Lewis,L.C.
TITLE Direct Submission
JOURNAL Submitted (13-JUN-2001) Corn Insects and Crop Genetics Research
Unit, USDA ARS, 111 Genetics Lab/Iowa State University, Ames, IA 50011, USA
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DEFINITION Beauveria bassiana isolate Bb2297 28S ribosomal RNA gene, intron 4.
ACCESSION AF322944
VERSION AF322944.1 GI:12656627
KEYWORDS .
SOURCE Beauveria bassiana.
ORGANISM Beauveria bassiana
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  Hypocreales; Clavicipitaceae; mitosporic Clavicipitaceae;
  Beauveria.
REFERENCE 1 (bases 1 to 413)
  AUTHORS Coates, B.S., Hellmich, R.L. and Lewis, L.C.
  TITLE Beauveria bassiana 28S rDNA Group-I Intron Insertion and Nucleotide Sequence Variation
  JOURNAL Unpublished
REFERENCE 2 (bases 1 to 413)
  AUTHORS Coates, B.S.
  TITLE Direct Submission
  JOURNAL Submitted (22-NOV-2000) Corn Insects & Crop Genetics, USDA-ARS,
  111 Genetics Lab, Ames, IA 50011, USA
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LOCUS AF322943  445 bp DNA  PLN  02-FEB-2001
DEFINITION Beauveria bassiana isolate Bb2515 28S ribosomal RNA gene, intron 4.
ACCESSION AF322943
VERSION AF322943.1 GI:12656626
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ORGANISM Beauveria bassiana
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REFERENCE 1 (bases 1 to 445)
  AUTHORS Coates,B.S., Hellmich,R.L. and Lewis,L.C.
  TITLE Beauveria bassiana 28S rDNA Group-I Intron Insertion and Nucleotide Sequence Variation
  JOURNAL Unpublished
REFERENCE 2 (bases 1 to 445)
  AUTHORS Coates,B.S.
  TITLE Direct Submission
  JOURNAL Submitted (22-NOV-2000) Corn Insects & Crop Genetics, USDA-ARS,
           111 Genetics Lab, Ames, IA 50011, USA
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    /db_xref="taxon:5582"
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LOCUS     AF322942  427 bp DNA PLN 02-FEB-2001
DEFINITION Beauveria bassiana isolate Bb151 28S ribosomal RNA gene, intron

ACCESSION AF322942
VERSION    AF322942.1 GI:12656625
KEYWORDS   .
SOURCE     Beauveria bassiana.
ORGANISM   Beauveria bassiana
            Eukaryota; Fungi; Ascomycota; Pezizomycotina; Sordariomycetes; Hypocreales; Clavicipitaceae; mitosporic Clavicipitaceae; Beauveria.

REFERENCE  1 (bases 1 to 427)
            AUTHORS Coates,B.S., Hellmich,R.L. and Lewis,L.C.
            TITLE Beauveria bassiana 28S rDNA Group-I Intron Insertion and Nucleotide Sequence Variation
            JOURNAL Unpublished

REFERENCE  2 (bases 1 to 427)
            AUTHORS Coates,B.S.
            TITLE Direct Submission
            JOURNAL Submitted (22-NOV-2000) Corn Insects & Crop Genetics, USDA-ARS, 111 Genetics Lab, Ames, IA 50011, USA

FEATURES             source  1..427
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                      intron 1..427
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                      /number=4

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            121 gttggctgta aactcactcc ccagcgagcc caactgctca a tcggcgggaa cattgtaaaag
            181 ccaaccaact ccagcgggac tgtaaactcc g ggttaatggc tctgggttaa
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            301 gacgcgccc tgaattgctt ggtcagggcc cagaaacctg gaaatggcagt gggttcagc
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            421 ctgagtt

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DEFINITION Beauveria bassiana isolate Bb726 28S ribosomal RNA gene, intron
ACCESSION AF322941
VERSION AF322941.1 GI:12656624
KEYWORDS .
SOURCE Beauveria bassiana.
ORGANISM Beauveria bassiana
Eukaryota; Fungi; Ascomycota; Pezizomycotina; Sordariomycetes; Hypocreales; Clavicipitaceae; mitosporic Clavicipitaceae; Beauveria.
REFERENCE 1 (bases 1 to 443)
AUTHORS Coates,B.S., Helmlich,R.L. and Lewis,L.C.
TITLE Beauveria bassiana 28S rDNA Group-I Intron Insertion and Nucleotide Sequence Variation
JOURNAL Unpublished
REFERENCE 2 (bases 1 to 443)
AUTHORS Coates,B.S.
TITLE Direct Submission
JOURNAL Submitted (22-NOV-2000) Corn Insects & Crop Genetics, USDA-ARS,
111 Genetics Lab, Ames, IA 50011, USA
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DEFINITION Beauveria bassiana BbMin1 minisatellite sequence.
ACCESSION AF387913
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REFERENCE 1 (bases 1 to 483)
AUTHORS Coates, B.S., Hellmich, R.L. and Lewis, L.C.
TITLE A minisatellite from the filamentous ascomycete, Beauveria bassiana, shows allelic variability independent of host range and geographic origin
JOURNAL Unpublished
REFERENCE 2 (bases 1 to 483)
AUTHORS Coates, B.S., Hellmich, R.L. and Lewis, L.C.
TITLE Direct Submission
JOURNAL Submitted (02-JUN-2001) Corn Insects & Crop Genetics Research Unit, USDA-ARS, 111 Genetics Lab/Iowa State University, Ames, IA 50011, USA
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481 ctc
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VERSION AF322930.1 GI:12007454
KEYWORDS .
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ORGANISM Beauveria bassiana
Eukaryota; Fungi; Ascomycota; Sordariomycetes; Hypocreales; Clavicipitaceae; Cordyceps.
REFERENCE 1 (bases 1 to 494)
AUTHORS Coates,B.S., Hellmich,R.L. and Lewis,L.C.
TITLE Determination of Beauveria bassiana haplotypes based on nuclear rDNA internal transcribed spacer PCR-RFLP
JOURNAL Unpublished
REFERENCE 2 (bases 1 to 494)
AUTHORS Coates,B.S., Hellmich,R.L. and Lewis,L.C.
TITLE Direct Submission
JOURNAL Submitted (22-NOV-2000) Corn Insects Lab, USDA-ARS, 111 Genetics Lab, Ames, IA 50011, USA
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### APPENDIX B. ADDITIONAL TABLES

Table B1. Additional *Beauveria* spp. isolates used in genotype analysis, but not listed in Table 1 of chapter 1.

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Table B3. Arlequin analysis for *Beauveria bassiana* rRNA ITS region PCR-RFLP data.

A) Arelequin input file used in statistical analysis in Chapter 2.

```plaintext
[Profile]
Title="B. bassiana rRNA ITS region PCR-RFLP Fst"
NbSamples=8
GenotypicData=0
DataType=RFLP
LocusSeparator=WHITESPACE
CompDistMatrix=1
MissingData='?'

[Data]
[HaplotypeDefinition]
HaplListName="Bb ITS region RFLP"
HaplList= {
    A    1011111
    B    1011110
    C    1011101
    D    1011100
    E    1011011
    F    1011010
    G    1011001
    H    1011000
    I    1010000
    J    1010001
    K    1001101
    L    1001001
    M    1001000
    N    1000011
    O    1000011
    P    1000001
    Q    0111101
    R    0111011
    S    0111001
    T    0110101
    U    0110001
    V    0110000
    W    0100001
    X    0100000
}
```
# [Samples]

## #1
- **SampleName** = "Ostrinia nubilalis"
- **SampleSize** = 18
- **SampleData** = 
  - A: 10
  - E: 1
  - L: 1
  - N: 1
  - P: 1
  - R: 3
  - X: 1

## #2
- **SampleName** = "Other Lepidoptera"
- **SampleSize** = 13
- **SampleData** = 
  - A: 5
  - C: 2
  - G: 1
  - I: 1
  - L: 4

## #3
- **SampleName** = "Diabrotica spp."
- **SampleSize** = 16
- **SampleData** = 
  - C: 4
  - L: 8
  - O: 1
  - Q: 1
  - S: 1
  - T: 1

## #4
- **SampleName** = "Other Coleoptera"
- **SampleSize** = 13
- **SampleData** = 
  - F: 1
  - J: 1
  - L: 4
  - M: 3
  - Q: 2
  - V: 1
  - W: 1
sampleName="Hymenoptera"  
sampleSize=9  
sampleData=  
  {  
    B 1  
    H 2  
    K 1  
    L 1  
    M 2  
    O 1  
    U 1  
  }  

sampleName="Diptera"  
sampleSize=5  
sampleData=  
  {  
    A 3  
    F 1  
    P 1  
  }  

sampleName="Heteroptera"  
sampleSize=15  
sampleData=  
  {  
    A 1  
    B 1  
    D 1  
    F 1  
    L 5  
    M 3  
    W 2  
    X 1  
  }  

sampleName="Other Insect Orders"  
sampleSize=7  
sampleData=  
  {  
    A 1  
    C 2  
    L 1  
    M 1  
    W 1  
    X 1  
  }
[[Structure]]
StructureName="By Insect Host Range"
NbGroups=4
IndividualLevel=0
DistMatLabel=""
Group={
  "Ostrinia nubilalis"
  "Other Lepidoptera"
}
Group={
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  "Other Coleoptera"
}
Group={
  "Hymenoptera"
  "Diptera"
}
Group={
  "Heteroptera"
  "Other Insects"
}

B) Significance tests (1023 permutations)

Vc and Fst : P(rand. value < obs. value) = 0.00000
P(rand. value = obs. value) = 0.00000
P(rand. value <= obs. value) = 0.00000+-0.00000

Vb and Fsc : P(rand. value > obs. value) = 0.02639
P(rand. Value = obs. value) = 0.00000
P(rand. value >= obs. value) = 0.02639+-0.00415

Va and Fct : P(rand. value > obs. value) = 0.20528
P(rand. value = obs. value) = 0.00782
P(rand. value >= obs. value) = 0.21310+-0.01188
Table B4. Arlequin (Schneider, 1997) analysis of *Beauveria bassiana BbMinl* allelic variation based on insect host range.

A) Arlequin input file.

```plaintext
[Profile]
  Title="Fst analysis of BbMinl allelic variation based on insect host range"
  NbSamples=8
  GenotypicData=0
  #No gametic phase of haploid fungus
  GameticPhase=0
  DataType=MICROSAT
  LocusSeparator=WHITESPACE

[Data]
[[Samples]]
  SampleName="Hymenoptera"
  SampleSize=6
  SampleData= {  
    Genot11 10
    Genot21 8
    Genot31 7
    Genot43 2
  }
  SampleName="Ostrinia nubilalis (ECB)"
  SampleSize=17
  SampleData= {  
    Genot510 8
    Genot66 4
    Genot71 2
  }
  SampleName="Other Lepidoptera"
  SampleSize=12
  SampleData= {  
    Genot81 15
    Genot94 8
    Genot10 1 7
    Genot11 1 4
    Genot12 1 3
    Genot13 3 2
    Genot14 1 1
  }
```
SampleName="Diabrotica spp. (CRW)"
SampleSize=36
SampleData= {
    Genot15  4  8
    Genot16  5  7
    Genot17 23  4
    Genot18  4  2
}

SampleName="Other Coleoptera"
SampleSize=7
SampleData= {
    Genot19  1  8
    Genot20  2  3
    Genot21  4  2
}

SampleName="Heteroptera"
SampleSize=8
SampleData= {
    Genot22  3  8
    Genot24  1  4
    Genot25  4  2
}

SampleName="Orthoptera"
SampleSize=3
SampleData= {
    Genot26  1  4
    Genot27  1  3
    Genot28  1  2
}

SampleName="Other Insect Orders"
SampleSize=5
SampleData= {
    Genot29  1  8
    Genot30  1  4
    Genot31  1  3
    Genot32  1  2
    Genot33  1  1
}
B) Significance tests (1023 permutations)

\[
V_c \text{ and } F_{st}: P(\text{rand. value} < \text{obs. value}) = 0.08309 \\
P(\text{rand. value} = \text{obs. value}) = 0.00000 \\
P(\text{rand. value} \leq \text{obs. value}) = 0.08309 \pm 0.00899
\]

\[
V_b \text{ and } F_{sc}: P(\text{rand. value} > \text{obs. value}) = 0.98143 \\
P(\text{rand. value} = \text{obs. value}) = 0.00000 \\
P(\text{rand. value} \geq \text{obs. value}) = 0.98143 \pm 0.00414
\]

\[
V_a \text{ and } F_{ct}: P(\text{rand. value} > \text{obs. value}) = 0.20723 \\
P(\text{rand. value} = \text{obs. value}) = 0.01173 \\
P(\text{rand. value} \geq \text{obs. value}) = 0.21896 \pm 0.01215
\]
C) Pairwise Fst values between subpopulations based on *BbMin1* allelic variation. Distance method: Sum of squared size difference (RST).

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D) Pairwise matrix of migration (M) values between subpopulations (M=Nm for haploid data).

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Table B5. Arlequin(Schneider, 1997) Analysis of *Beauveria bassiana* *BbMin1* locus variation based on geographic origin

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SampleName="E3S"
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Table B6. PHYLIP (Felsenstein, 1989) input

A) *Beauveria* rRNA ITS region PCR-RFLP data used for Wagner Parsimony (Eck and Dayhoff, 1968) analysis.

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Table B7. Cumulative summary of *B. bassiana* ITS region haplotypes described from 120 isolate samples. In order to facilitate haplotype (HT) abbreviation a two letter systems was adopted, where AA to AX are identical to A to X in presented in chapter 2.

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