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Genetic diversity of *Ganoderma* in oil palm plantings

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Isolates of *Ganoderma* spp. associated with basal stem rot of oil palm were obtained by sampling from two palm plantings in Malaysia. Members of a subset of these were compared using a combination of somatic incompatibility and molecular genetic analyses. Incompatibility interactions between heterokaryons in culture indicated that *Ganoderma* generally occurred as numerous distinct strains, with compatible strains usually confined to individual palms. Analysis of restriction fragment length polymorphisms (RFLPs) in presumptive mitochondrial DNA (mtDNA) revealed heterogeneity among isolates from neighbouring palms, and from within individual palms. The combination of mtDNA variability and somatic incompatibility suggests that *Ganoderma* BSR does not spread by direct root-to-root contact between palms. The results may indicate infection spread through basidiospore dispersal or through contact with a heterogeneous long-term residual inoculum in debris.

Keywords: basal stem rot (BSR), *Ganoderma*, mitochondrial variation, oil palm, somatic incompatibility

Introduction

Root and stem rots caused by *Ganoderma* spp. result in extensive worldwide losses of many tropical perennial crops, including oil palm, coconut, betel palm, rubber and tea. *Ganoderma* basal stem rot (BSR) of oil palm is of particular economic importance in south-east Asia because it shortens the productive life of plantations, an effect which tends to become cumulative over successive planting cycles of this monoculture. *Ganoderma* BSR is now recognized as a significant constraint to sustainable production in Asia, and development of techniques for disease management has been highlighted as a key research priority (Anon, 1997).

Taxonomic divisions within the genus *Ganoderma* are currently chaotic, due to the presence of heterogeneic forms, dubious nomenclature and inconsistencies in application of the numerous criteria by which the genus has been subdivided (Bazzalo & Wright, 1982; Gilbertson & Ryvarden, 1986). The species concepts for the BSR-associated *Ganoderma* isolates are very confused; 15 species of *Ganoderma* have been recorded worldwide as probable causal agents of basal stem rot in oil palm (Turner, 1981), and six of these have been recorded on oil palm in Malaysia and Sumatra (Steyaert, 1967). Ho & Nawawi (1985) considered that those associated with BSR all conformed to *G. boninense*, as did Miller *et al.* (1995b), who also confirmed the pathogenicity of isolates from diseased and symptomless palms following seedling inoculation tests. In view of the uncertain species concepts in this genus, *Ganoderma* populations on oil palm are herein referred to by the generic name alone.

Little is known of the mechanisms of infection and spread within oil palm plantings. Traditionally, initial establishment of *Ganoderma* BSR in an oil palm field has been considered to occur by mycelial contact, through growth of living oil palm roots into an inoculum source comprising saprophytically colonized debris within the soil and largely remaining from the previous planting. Entry has also been postulated to occur through wounded tissues or dead roots (Turner, 1965b). As the roots of an oil palm can extend across up to four planting rows (Lambourne, 1935), root-to-root contact might enable the subsequent spread of *Ganoderma* between living palms. The observation that patches of BSR infection appear to enlarge over time (Singh, 1991) has also led to the assumption that most spread of infection in the field occurs by root contact between healthy and diseased palms.

In an attempt to eliminate initial inoculum, sanitation prior to replanting often involves 'windrowing', i.e. the uprooting of previous bole and trunk tissues, which are then stacked along the inter-rows. In some cases, the

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stem tissues are also chopped up mechanically to hasten breakdown. Although disease incidence after windrowing is generally lower in subsequent plantings than in stands replanted without bole removal, the process is both labour-intensive and costly, and often fails to prevent the recurrence or spread of BSR.

Despite the dubious value of current replanting strategies and the general failure of control strategies (curative surgery, fungicide treatment, cultural methods) in existing oil palm stands, few studies have been conducted to test the validity of current assumptions about the spread of the pathogen in oil palm plantings. This is largely because morphology-based characterization approaches have not allowed the differentiation of subpopulations or individuals required for pathogen population studies. The goals of the study were thus to determine the genetic relationships within *Ganoderma* populations in two oil palm plantings, through mtDNA RFLPs and somatic incompatibility studies, in order to elucidate possible mechanisms of disease establishment and spread.

Materials and methods

Isolate sources

Two 16-palm × 16-palm plots were randomly selected within each of two oil palm plantations, in Selangor, Malaysia, known to be affected by BSR. Plot SB was located at Sungei Buloh Estate, Sime Darby Plantations, Carnavon Division (3°14′ N 101°19′ E). This field was planted between 1969 and 1972, on a Bernam series soil (fine clay, isohyperthermic, typic tropaquepts). Plot BC was located 6 km away from Sungei Buloh at Bukit Cloh Estate, Sime Darby Plantations, Braunston Division. This field was planted in 1974, on a mixed soil type of Bernam and Tongkang (clay, isohypothermic, sulphic tropaquepts) series. Both plots had been previously cropped with rubber, itself planted from cleared forest.

All living and dead (fallen and decayed) palms within each plot were examined for the presence of *Ganoderma*, either as basidiomata occurring on the trunk or as mycelium present in internal tissue cores (recovery from roots into semiselective agar is variable and prone to errors in determining the root origin, and so was not used here). Internal tissue cores were extracted with chainsaw drills from four equidistant points around each palm stem, 0·5 m above the ground. All *Ganoderma* basidiomata were collected and their position on each infected palm noted. *Ganoderma* was isolated onto a semiselective medium (GSM; Ariffin & Seman, 1991). Isolation plates were incubated in darkness at 25–30°C and examined after 5 days. Subcultures were transferred to 9-cm Petri plates containing 3% malt extract agar (MEA; Smith & Onions, 1983); colony characteristics were observed after 14 days of incubation at 25°C and stocks of each *Ganoderma* isolate were maintained in agar under mineral oil and liquid nitrogen. Isolate information is presented in Table 1. A subsampling strategy was used to select representative isolates among those associated with disease to give a total experimental subsample of around one-third of all isolates recovered (39 from plot SB and 18 from plot BC).

Somatic incompatibility studies

Isolates were examined under the microscope to confirm the presence of clamp connections, and each somatic incompatibility group (SIG) determined by pairing heterokaryotic isolates in agar culture, using procedures similar to those described previously (Adams, 1974). Inocula (1 mm^3), taken from actively growing cultures, were paired in all combinations in a 10-mm-square grid pattern arrangement in 120-mm-square plastic Petri plates containing 1% MEA. Plates were then incubated inverted in darkness at 25°C for up to 28 days and reactions observed every 7 days. All combinations were evaluated in triplicate.

Incompatibility reactions between paired isolates, evident as either a zone of sparse mycelial growth or barrage formation with or without dark pigment deposition, indicated different genetic constitution, whilst a compatible reaction, evident as a coalescence of paired isolates, indicated identical genetic constitution (Rayner & Todd, 1979; Rayner *et al*., 1984).

Mitochondrial DNA RFLPs

Isolates were grown on 3% MEA at 25°C for 7 days. Macerated mycelial plugs were transferred to 250-mL conical flasks of 60 mL glucose yeast medium (GYM, Paterson & Bridge, 1994) and incubated at 25°C, on a shaker at 120 r.p.m., for 4 days. Mycelia were washed with sterile distilled water, harvested by vacuum filtration, frozen at −20°C and freeze-dried. Total DNA was extracted using an adaptation (Paterson & Bridge, 1994) of a cetyltrimethyl ammonium bromide (CTAB)-based method (Zolan & Pukilla, 1986). DNA (3–8 μg) was digested with '4-base cutter' restriction endonucleases *Hae*III or *Msp*I (Gibco BRL, UK) to produce a small number of high molecular weight fragments presumed to correspond to mitochondrial DNA (Marriot *et al*., 1984; Spitzer *et al*., 1989). Fragments were separated in 1% agarose gels by electrophoresis at 5 $V\,cm^{-1}$ and stained with ethidium bromide (0·5 $\mu g\,ml^{-1}$). Presumptive mtDNA RFLPs were viewed on a UV transilluminator at 254 nm. Fragment sizes were calculated on the basis of migration distance within each gel, against a 1-Kb ladder size marker (Gibco BRL, UK). Commonality of mtDNA restriction sites between different isolates were determined by numerical analyses (MVSP software; Kovach Computing Services, Anglesey, UK). RFLP data was coded in binary form, with 1 representing the presence of a particular band and 0 representing its absence. Similarity matrices of data were generated using Sorenson's coefficient (Sneath & Sokal, 1973), and cluster analyses conducted by unweighted pair group analysis using arithmetic means (UPGMA; Sneath & Sokal, 1973).

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Table 1 Sources and characteristics of *Ganoderma* isolates

Isolate Number	Palm/plot	Source of material	External palm symptoms	Internal core tissue appearance	SIG[a]	*Msp*I profile	*Hae*III profile
1·1	9/SB	Basidioma	Foliar[b], BSR lesion[c]	No discoloration[d]	1	–[e]	–
1·2	9/SB	Basidioma	Foliar, BSR lesion	No discoloration	1	–	–
2	10/SB	Basidioma	Asymptomatic	No discoloration	1	–	–
3	35/SB	Basidioma	Foliar	No discoloration	2	–	–
4	47/SB	Basidioma	Asymptomatic	No discoloration	3	1	–
5	73/SB	Basidioma	BSR lesion	No discoloration	4	2	–
6	75/SB	Basidioma	Asymptomatic	Diseased[f]	5	–	–
7	92/SB	Basidioma	BSR lesion	Diseased	6	4	–
8	82/SB	Basidioma	Foliar	No discoloration	7	3	–
9	100/SB	Basidioma	Foliar	Diseased	8	–	–
10·1	102/SB	Basidioma	Foliar, BSR lesion	No discoloration	9	5	1
10·2	102/SB	Basidioma	Foliar, BSR lesion	No discoloration	10	5	1
10·3	102/SB	Basidioma	Foliar, BSR lesion	No discoloration	11	–	–
10·4	102/SB	Basidioma	Foliar, BSR lesion	No discoloration	11	–	–
11·1	103/SB	Basidioma	BSR lesion	No discoloration	12	5	–
11·2	103/SB	Basidioma	BSR lesion	No discoloration	12	6	–
12	177/SB	Basidioma	Foliar, BSR lesion	No discoloration	13	–	–
13	199/SB	Basidioma	BSR lesion	Diseased	14	–	–
14	155/SB	Mycelium	Foliar	Diseased	15	7	–
15·1	173/SB	Basidioma	Dead	Diseased	16	8	2
15·2	173/SB	Basidioma	Dead	Diseased	17	8	2
16·1	188/SB	Basidioma	Foliar, BSR lesion	Diseased	18	9	3
16·2	188/SB	Basidioma	Foliar, BSR lesion	Diseased	19	–	4
16·3	188/SB	Basidioma	Foliar, BSR lesion	No discoloration	20	–	5
16·4	188/SB	Basidioma	Foliar, BSR lesion	Diseased	21	–	–
17	205/SB	Basidioma	Dead, BSR lesion	None	22	–	–
18·1	221/SB	Basidioma	Asymptomatic	No discoloration	23	–	–
18·2	221/SB	Basidioma	Asymptomatic	Diseased	24	13	9
18·3	221/SB	Basidioma	Asymptomatic	Diseased	25	–	–
18·4	221/SB	Basidioma	Asymptomatic	Diseased	26	13	9
19·1	219/SB	Basidioma	Dead, BSR lesion	None	27	–	7
19·2	219/SB	Basidioma	Dead, BSR lesion	None	27	11	7
19·3	219/SB	Basidioma	Dead, BSR lesion	None	28	12	8
20·1	207/SB	Basidioma	Asymptomatic	Diseased	29	10	6
20·2	207/SB	Basidioma	Asymptomatic	Diseased	30	–	–
21	224/SB	Basidioma	BSR lesion	Diseased	31	14	10
22	239/SB	Basidioma	Asymptomatic	No discoloration	32	15	11
23	229/SB	Basidioma	Asymptomatic	No discoloration	33	–	–
24	241/SB	Basidioma	BSR lesion	Diseased	34	16	12
25	2/BC	Basidioma	Asymptomatic	Diseased	35	17	13
26	9/BC	Basidioma	Asymptomatic	Diseased	48	–	–
27	11/BC	Basidioma	Foliar	Diseased	49	–	–
28	41/BC	Basidioma	Foliar, BSR lesion	Diseased	50	18	–
29·1	47/BC	Basidioma	Foliar	Diseased	36	19	14
29·2	47/BC	Basidioma	Foliar	Diseased	37	–	–
30	58/BC	Basidioma	Decayed	None	51	–	–
31	73/BC	Basidioma	Foliar	Diseased	52	20	15
32	153/BC	Mycelium	Dead, BSR lesion	Diseased	38	21	16
33	179/BC	Basidioma	Foliar	No discoloration	39	–	17
34	211/BC	Basidioma	Asymptomatic	No discoloration	46	–	–
35	216/BC	Basidioma	Foliar	No discoloration	47	22	18
36	230/BC	Basidioma	Foliar, BSR lesion	No discoloration	40	23	–
37·1	232/BC	Basidioma	Foliar, BSR lesion	No discoloration	41	24	–
37·2	232/BC	Basidioma	Foliar, BSR lesion	No discoloration	42	–	–
37·3	232/BC	Basidioma	Foliar, BSR lesion	No discoloration	43	–	–
38	254/BC	Basidioma	Decayed	None	44	25	–
39	256/BC	Mycelium	Asymptomatic	No discoloration	45	26	19

[a] SIG: somatic incompatibility group.
[b] Foliar symptoms: multiple unopened spear leaves and chlorosis leading to general paleness of leaf canopy.
[c] BSR lesion: dry rot of internal tissues at stem base, extending to periphery.
[d] No discoloration of internal tissue at sample point.
[e] Not determined.
[f] Diseased: light to dark brown discoloration of internal tissue.

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Results

Somatic incompatibility studies

All isolates of *Ganoderma* possessed clamp connections, indicating that they were heterokaryons. Reactions between incompatible strains, macroscopically visible after 7 days, became progressively more distinct over 14 days. All isolates completely intermingled when paired against themselves, indicating self-compatibility. This phenotype was used as a standard; when pairs of isolates from different origins gave a similar reaction, they were judged to be compatible. Evidence of incompatible reactions (zone of sparse mycelia, barrage and/or pigment deposition) was interpreted as representing different genetic constitutions. All three replicates of pairings of isolates consistently revealed the same distinctions between compatible and incompatible groups.

In Sungei Buloh Estate, 34 somatic incompatibility groups were identified among the 39 isolates selected from 24 infected palms (Fig. 1a). In only two out of eight cases studied were all isolates from a single palm found to belong to the same somatic incompatibility group (SIG group 1 isolates and group 12 isolates; see Table 1). In the remainder, incompatibility reactions were observed (even among isolates from the same sampled area of the palm) indicating the presence of several individuals within the stem (SIG groups 9–11, 16 & 17, 18–21, 23–26, 27 & 28, 29 & 30). In only one instance were isolates of the same compatibility group recovered from immediately neighbouring palms (SIG group 1 isolates). In all other cases, each infected palm contained a population genetically distinct from those in all other palms studied.

Similar results were found for the pairings of 18 isolates from Bukit Cloh Estate (Fig. 1b). All of the representative isolates paired were genetically distinct from one another. Somatic incompatibility was observed between paired isolates through all levels of comparison: over distances which could permit root-to-root contact and mycelial spread (e.g. SIG groups 48–52), between neighbouring palms (e.g. groups 46 & 47, 50–52) and within individual palms (groups 36 & 37, 41–43).

Mitochondrial DNA RFLPs

Examination of *Msp*I-generated RFLP profiles from individual isolates from the two plots showed widespread heterogeneity, with 26 distinct DNA profiles among the 30 isolates examined (Fig. 2). The sum of the fragment sizes for each isolate varied between 39 and 45 Kb. In a number of cases, multiple isolates from the same palm displayed identical RFLP profiles (profiles 5, 8 and 13); this was not observed throughout, with two distinct profiles being obtained from isolates from palm 103/SB (profiles 5 and 6) and palm 219/SB (profiles 11 and 12). Isolates examined from immediately neighbouring palms were nearly always genetically distinct (see Table 1) and were found to give the same DNA profile in only one case (palms 102/SB & 103/SB; profile 5). Cluster analysis of band patterns showed that, with the exception of DNA profiles 5 (shown by three isolates), 8 (two isolates) and 13 (two isolates), each isolate gave a distinct pattern with little common similarity (Fig. 3). Profiles did not

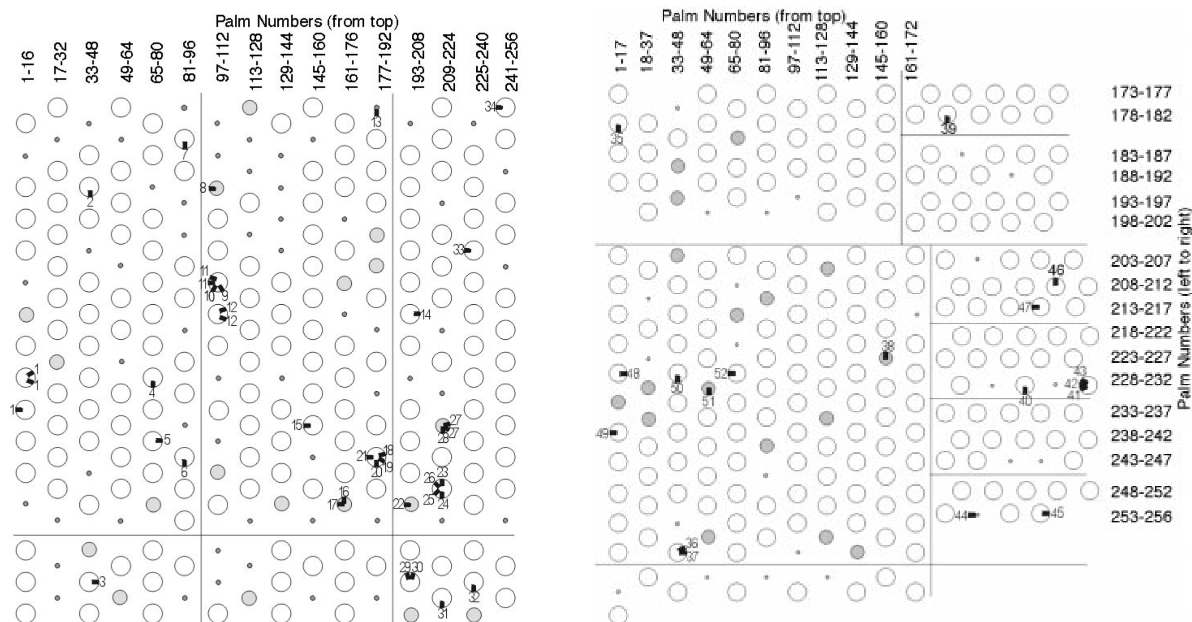

Figure 1 Palm layout and distribution of *Ganoderma* SIGs for selected isolates from oil palms in a plot at (a) Sungei Buloh Estate and (b) Bukit Cloh Estate. Numbered squares within circles show positions of the different SIGs. Large open circles represent living oil palms, medium-sized shaded circles indicate palms which died recently, and small shaded circles represent palms dead for a longer time period, which usually constitute vacancies within the plot. Thin straight lines indicate the presence of drainage channels.

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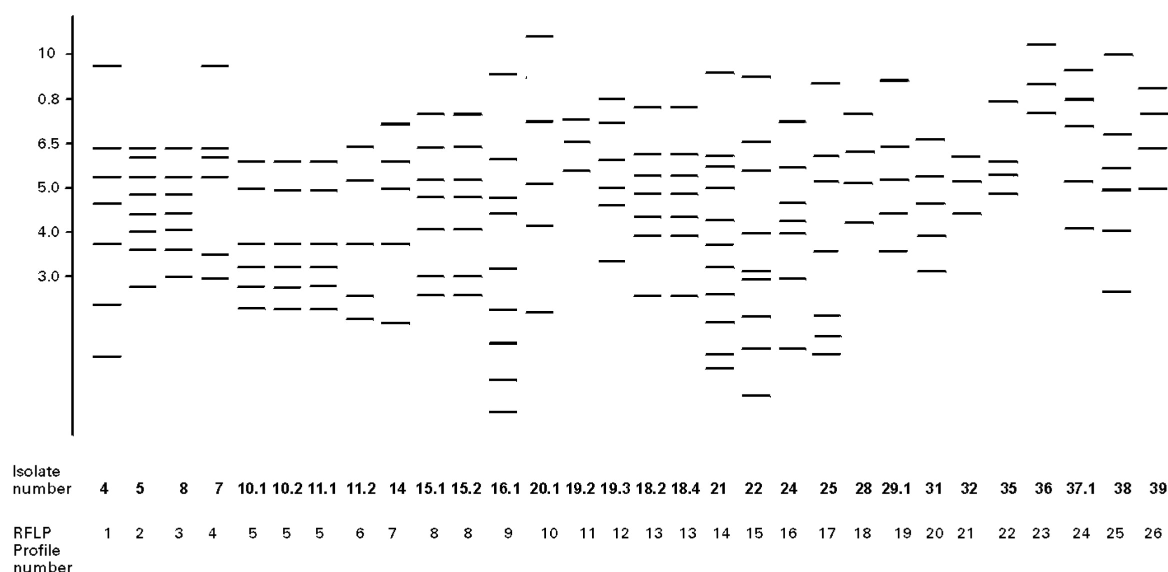

Figure 2 Schematic representation of all restriction patterns from *Msp*I RFLP analysis of mtDNA for the representative isolates. The scale on the left of the figure indicates fragment sizes (in kilobase pairs).

group according to location when sites were analysed either together (Fig. 3) or separately (results not shown).

Restriction digest fragments generated using the restriction endonuclease *Hae*III gave a distribution of profiles similar to that obtained from *Msp*I (result not shown), with an additional example of the occurrence of more than one mitochondrial DNA group in isolates from a single palm (*Hae*III profiles 3, 4 and 5).

Relationship between mtDNA RFLP and SIG groupings

Comparison of the 30 isolates for which both RFLP (*Msp*I-derived) and SIG data were obtained showed that, in general, single RFLP profiles were associated with single SIGs (Table 1). However, in three cases single common RFLP profiles were obtained from isolates of more than one SIG. RFLP profile 5 was shown by isolates of SIGs 9, 10 and 12, profile 8 by isolates of SIGs 16 and 17, and profile 13 by isolates of SIGs 24 and 26. In one instance, two different RFLP profiles occurred within a single SIG (SIG group 12, which showed RFLP profiles 5 and 6). With RFLP data derived from *Hae*III, RFLP profiles were associated with single SIG groups in 16 cases (mitochondrial DNA groups 3, 4, 5, 6, 7, 8, 10–19). Mitochondrial DNA groups were associated with more than one SIG group in 3 instances (profile 1 associated with SIG groups 9 and 10, profile 2 with SIG groups 16 and 17, and profile 9 with SIG groups 24 and 26).

Discussion

Although understanding of the mechanisms determining somatic incompatibility in *Ganoderma* remains incomplete, the use of incompatibility reactions in the study of disease development in populations is well-documented within basidiomycete tree pathogens (e.g. Guillaumin *et al.*, 1994; Morrison *et al.*, 1994). Somatic incompatibility reactions determined within this study indicated that the sampled *Ganoderma* populations within oil palm systems occurred as numerous distinct individuals ('genets' *sensu* Rayner), contrasting with typically clonal distribution patterns for other basidiomycetes, where single clones can spread over large areas of forests (Shaw & Roth, 1976; Stenlid, 1985). Numerous separate genets were detected in the sampled populations, with a total of 34 detected in plot SB (out of 39 isolates tested) and 18 (out of 18) within plot BC. In both cases, incompatibility between paired isolates was observed over distances that could theoretically permit root-to-root contact, and hence mycelial spread, between neighbouring palms (9 m apart), between isolates, or between nonadjacent palms (up to 36 m apart). Incompatibility was also found between isolates colonizing the same infected palm. Only in one instance were two isolates from neighbouring palms compatible. Similar variability has been found in other oil palm blocks (Ariffin & Seman, 1991).

Mitochondrial DNA RFLPs have been used to compare genetic relatedness in a number of fungi and were reported to be rich in polymorphisms at the intraspecific level (Smith & Anderson, 1989; Forster *et al.*, 1990; Gardes *et al.*, 1991). Mitochondrial genomes of many organisms are also believed to evolve at a relatively fast rate (Brown *et al.*, 1979), and the molecule is not normally recombined during sexual reproduction. Within *Phytophthora*, for example, recombination of nuclear genomes occurred in oospore progeny of crosses between different strains, without segregation, elimination or

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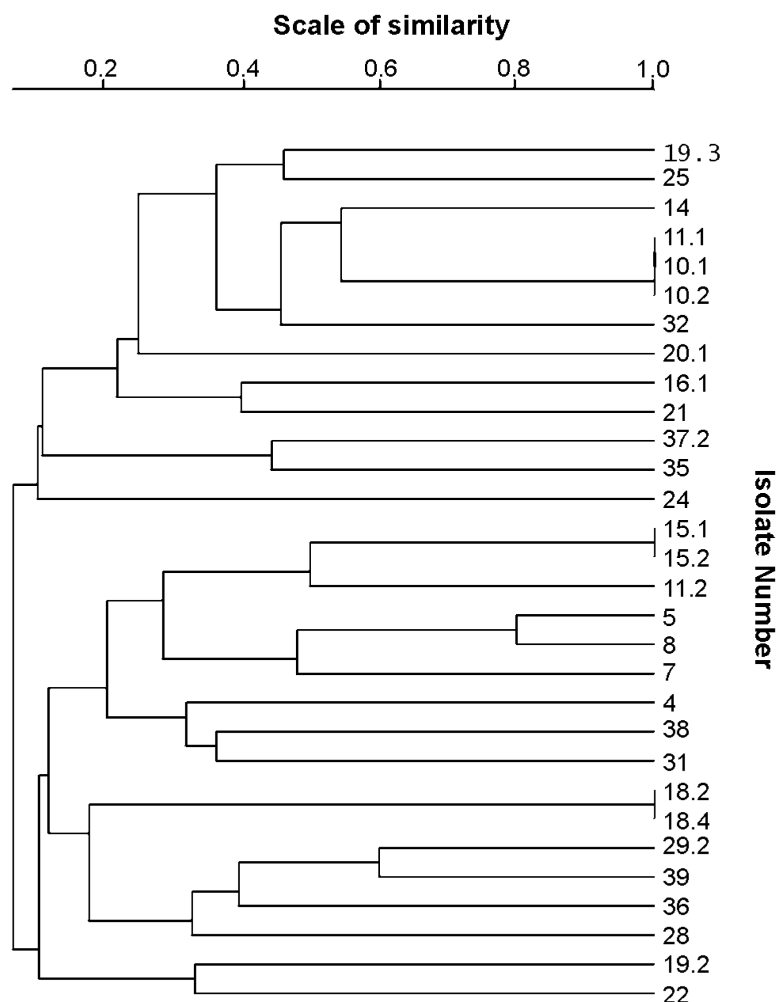

Figure 3 UPGMA constructed dendrogram of binary coded *Msp*I RFLP data of *Ganoderma* isolates. Similarities were derived using Sorenson's (Dice) Coefficient.

recombination of mtDNA types, indicating uniparental inheritance of mtDNA (Forster & Coffey, 1990). MtDNA has also been used to follow lines of inheritance in *Phytophthora*, with RFLPs revealing that it may code for antibiotic resistance (Whittaker *et al*., 1996). MtDNA is therefore considered a useful marker for the study of population genetics and close evolutionary relationships.

Digestion of total genomic DNA with restriction enzymes that cleave short G/C sequences results in a large number of small DNA fragments and a few larger A/T-rich fragments. Fungal mtDNA is typically rich in A/T sequences compared with nuclear DNA of the same species (Bendich, 1993). These large fragments have been widely presumed to approximate to mtDNA, and this was demonstrated by comparison with banding patterns obtained from purified mitochondrial DNA for a number of species (e.g. Spitzer *et al*., 1989; Varga *et al*., 1993; Freeman & Shabi, 1996). Although direct comparisons between the *Msp*I and *Hae*III total genomic digests and purified mitochondrial DNA have seldom been undertaken with basidiomycete fungi, it would seem reasonable to assume that the majority of the bands obtained in these profiles are of mitochondrial origin, as they are in ascomycetes and oomycetes (Marriot *et al*., 1984; Whittaker *et al*., 1996).

In concordance with the SIG data, mtDNA RFLPs revealed considerable heterogeneity between isolates, including those from the same (e.g. 219/SB) and adjacent (e.g. 207/SB and 224/SB) palms. Of the 26 lines identified by *Msp*I-derived RFLPs among the isolates studied, only two isolates from neighbouring palms had the same mtDNA RFLP profile (102/SB and 103/SB; 9 m apart). The majority of isolates obtained from within individual palms gave a single mitochondrial DNA profile, and only two palms (103/SB and 219/SB) gave isolates with different RFLP profiles.

In previous studies on other fungi, the relationship between RFLP and SIG groupings was reported to be complex (e.g. Manicom *et al*., 1990), ranging between equivalent RFLP and SIG groupings, more than one RFLP grouping within a SIG, and more than one SIG within an RFLP grouping. Within this study, results from mitochondrial DNA analyses and somatic incompatibility tests were not always in accord. More than one SIG frequently occurred within a single mitochondrial DNA group, as previously reported in *Armillaria*

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(Guillaumin *et al*., 1994; Smith *et al*., 1994). This could be interpreted as variability arising at the compatibility loci as a result of sexual recombination, while mitochondrial DNA is maintained through unilinear inheritance. Each SIG would therefore represent a nuclear genomic variant, with different genets originating from locally dispersed basidiospores. This interpretation is further supported by comparison of relationships by cluster analysis of the mtDNA RFLP profiles (Fig. 3); isolates from the same or nearby palms did not cluster together. These isolates showed few bands in common, implying that recombination (whereby progeny could be expected to contain a proportion of bands identical to parents) had not occurred. In this case, therefore, different RFLP profiles would indicate isolates derived from different lines, presumably arising from different dikaryotic basidiomata and mycelium (although isolates with identical mtDNA RFLP profiles could still represent different lines). However, in one instance a single SIG group was found to have two RFLP lines. This may indicate either that more than one mitochondrial type can exist (possibly through recombination) within a single population, or that self-incompatibility is controlled by nonmitochondrial markers. Clearly, mtDNA should not be recommended in isolation for differentiation of lines within *Ganoderma*.

The frequency of different SIG genets within the two oil palm plantings indicates numerous separate infection incidents, rather than mycelial spread of *Ganoderma*. The numerous genets must have arisen through sexual recombination and subsequent dispersal of recombinants via basidiospores. However, the role of basidiospores in the infection process remains unresolved. New inoculum sources could be formed by saprobic colonization of substrates such as stumps or felled palm trunks and debris. Such mechanisms have been widely reported for other root and butt rot pathogens (Turner, 1976, 1981; Stenlid, 1985). Despite the release of huge numbers of airborne spores from each basidioma, the majority of palms remain uninfected, indicating that basidiospores either may not be able to initiate a basal stem rot infection or may require very specific conditions to establish infection. Previous studies with spore inoculum did not result in direct infection of living palms (Turner, 1965a; Yeong, 1972). Nonetheless, spores are a likely infection mechanism in upper stem rot of oil palm (Thompson, 1931), often in association with *Phellinus* spp. Although *Ganoderma* basidiospores are most likely to be windborne, additional mechanisms suggested for their dispersal have included insect vectors (Genty *et al*., 1976). However, to date, no conclusive link has been made between insects and basal stem rot incidence and development. Alternatively, the numerous SIG clones may indicate the presence of many spatially separated populations, each originating from a unique mycelial inoculum source.

Separate sources of inoculum may have originated from infected debris left over from previous stands or colonized by spores. Both plots were replanted from rubber, which in turn replaced primary forest. Although either vegetation could have supported *Ganoderma* populations, variation in strains adapted to palms is more likely to have originated in native palm infections. Evidence of such an origin was found previously (Miller *et al*., 1995a), with isolates from palmaceous and nonpalmaceous hosts separating on the basis of extracellular pectinase zymograms. Oil palm is propagated as seed from crosses between cultivars Dura and Pisifera, so that an oil palm stand is not a genetically homogeneous host. This may create additional selection pressure for variation in the pathogen.

Mitochondrial DNA RFLP studies also provide evidence against previous assumptions of the significance of secondary mycelial spread of *Ganoderma* from palm to palm. MtDNA is maintained through unilinear inheritance in *Ganoderma* (C. Pilotti, personal communication). The presence of numerous mitochondrial DNA groups therefore indicates spatially separated populations originating from a diverse initial inoculum.

These studies thus did not support the current assumption that spread of *Ganoderma* occurs through radial mycelial growth from individual inoculum sources to neighbouring palms via root-to-root contact. Within the two plantings, both SIG and RFLP data indicated that *Ganoderma* populations were highly heterogenous over localized areas. Nonetheless, the actual extent of some lines identified by SIG and mtDNA RFLP profiles may be larger than shown here, given the sampling constraints involved. It is anticipated that, through exhaustive studies on populations from oil palm plantings, using additional nuclear DNA markers, and through the determination of mtDNA stability in *Ganoderma*, mechanisms of spread of *Ganoderma* in oil palm may be further clarified.

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