Status of the *Botrytis cinerea* species complex and microsatellite analysis of transposon types in South Asia and Australia

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Botrytis cinerea has been reported as a species complex containing two cryptic species, Groups I and II. The potential existence of these cryptic species was tested in newly sampled populations from South Asia and Australia using molecular markers. With 169 unique microsatellite haplotypes from both regions, cleaved amplified polymorphic sequence (CAPS) profiles of the Bc-*hch* locus were consistent only with Group II. The absence of Group I suggests there is restricted global migration of *B. cinerea* isolates from Europe to South Asia and Australia. Based on the presence and absence of two transposons, *Boty* and *Flipper*, four transposon types were detected. In Bangladesh the most prevalent transposon types were *flipper*-only and *transposa* but most haplotypes from India and Nepal being *boty*-only. In contrast, the most prevalent transposon types in the Australian populations were *transposa* and *boty*-only, and no *flipper*-only isolates were detected. Matrix correlation tests (MCT) based on genetic distance were used to evaluate the extent of genetic differentiation among transposon types and geographic origins. MCT showed a stronger correlation with geographic origin than with transposon type assignment. This was supported by Bayesian inference cluster analysis which, assigned haplotypes into clusters corresponding to geographic origin rather than transposon type. Although, frequencies of transposon types indicated qualitative differences between geographic regions, microsatellite markers did not show genetic differentiation that was concordant with transposon types, rather it was consistent with the revised phylogenetic classification of *B. cinerea* into two cryptic groups.

Key words: *Botrytis cinerea*, cryptic species, genetic differentiation, genetic structure, microsatellite analysis, transposons

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Introduction

The Ascomycete, *Botrytis cinerea* Pers., is a haploid necrotrophic fungal pathogen of over 200 plant species of economic importance, including grape and chickpea (Pande *et al.,* 2006). However, *B. cinerea* was proposed to be a species complex (Giraud *et al.,* 1997, 1999; Albertini *et al.,* 2002, Munoz *et al.,* 2002; Fournier *et al.,* 2003). Initially, two sympatric sibling species or transposon types were described: 1) *transposa* that contained two transposons *Boty* and *Flipper* and 2) *vacuma* which contained no transposons (Diolez *et al.,* 1995; Levis *et al.,* 1997; Giraud *et al.,* 1997). Recently, Fournier *et al.,* (2005) showed that genetic differentiation determined from multiple gene sequences was not concordant with either of the previously described transposon types (*transposa* or *vacuma*) and revised partitioning of *B. cinerea* into Group I and Group II phylogenetic cryptic species. These cryptic species have also been shown to coincide with resistance to the fungicide fenhexamid, and synonymously known as FenR (resistant) = Group I and FenS (sensitive)

= Group II (Albertini *et al.,* 2002). Diagnostic molecular markers for these groups have been developed based on cleaved amplified polymorphic sequence (CAPS) profiles of the Bc-*Hch* gene, a homologue of the *Neurospora crassa* vegetative incompa-tibility *hch* locus (Albertini *et al.,* 2002; Fournier *et al.,* 2003).

To date, *vacuma*, *flipper*-only, and *boty*-only transposon types have been detected with no *transposa* types in Group I and all transposon types have been detected in Group II (Giraud *et al.,* 1999; Albertini *et al.,* 2002; Fournier *et al.,* 2003; Ma and Michailides 2005). In grapevine pathology studies, *transposa* isolates were shown to be more virulent than *vacuma* isolates and changes in transposon type frequencies during crop development were possibly due to differences in their saprotrophic and pathogenic fitness (Martinez *et al.,* 2003, 2005). Thus, these observations supported the possibility of genetic differentiation between transposon types (Martinez *et al.,* 2003, 2005).

Botrytis cinerea Group II has shown to be the predominant cryptic species however, it was still unclear whether transposon types were genetically differentiated within this group (Fournier *et al.,* 2005). Ma and Michailides (2005) examined isolates from a range of field crops in California and found no differentiation between transposon types using microsatellite-primed or inter simple sequence repeat (ISSR) PCR markers. However, their study detected a small number of Group I isolates of the *boty*-only type (Ma and Michailides, 2005).

The presence of *B. cinerea* Group I and II cryptic species in Asia and Australia is unknown and knowledge of this status may be useful in determining global migration patterns. Furthermore, it has not been determined if frequencies of transposon types differ in other populations, if transposons *Boty* and *Flipper* were associated with one another or if transposon types were genetically differentiated within *B. cinerea* Group I or II complex. This could be due to molecular markers that lack high levels of polymorphism such as in sequence analyses or studies that have had insufficient sample size (Fournier *et al.,* 2005). Since *Boty* and *Flipper* transposons can be found together within genomes or separately between genomes, the frequencies and association between transposon markers may elucidate genetic differentiation between populations from different geographic origins (Munoz *et al.*, 2002).

The correlation of genetic differentiation with geographic origin or transposon type could indicate allopatric or sympatric mechanisms of speciation in *B. cinerea* (Giraud *et al.,* 1997, 1999; Kohn 2005). Genetic differentiation between transposon types have not been studied using highly polymorphic microsatellite markers with a substantial sample from regions outside Europe and North America, such as South Asia and Australia (Fournier *et al.,* 2002, 2005; Ma and Michailides 2005). Microsatellites may be highly suitable for detecting subtle genetic structure using recently developed clustering analysis (Pritchard *et al.,* 2000; Falush *et al.,* 2003) and these methods have not been investigated with *B. cinerea*.

The status of cryptic speciation in *B. cinerea* could have important implications in concurrent population structure analysis and thus integrated disease management strategies in South Asia and Australia (McDonald and Linde 2002; Pande *et al.*, 2006). In this paper, the primary objective was to determine the frequency and distribution of cryptic species Group I and II and transposon types between *B. cinerea* populations from South Asia (Bangladesh, India and Nepal) and Australia. The second objective was to use microsatellite allele frequency data to determine if genetic differentiation corresponded between transposon types or between populations denoted by their geographic region.

Materials and methods

Isolates and DNA extraction

Botrytis cinerea isolates from South Asia (particularly the Indo-Gangetic plain) were obtained from naturally infected chickpea and opportunistically from other hosts such as lentil, dahlia and marigold within chickpea growing regions (Total 194 isolates). Isolates from Australia were obtained from field collections of chickpea, lentil, grape and other horticultural species (Total 72 isolates). Isolates were subcultured onto Potato Dextrose Agar (PDA) and selective medium described by Burgess *et al.,* (1997) and incubated (10-14

days) at 20°C with 12 hr photoperiod with light/black UV light. Microscopic examinations to identify *B. cinerea* isolates were based on the arrangement of conidia and conidiophores, and conidium size and shape descriptors (Morgan 1971; Burgess *et al.,* 1997). Germinated, single spores were excised from water agar plates and transferred to PDA.

For DNA extraction, 1 mm^2 agar plugs were transferred to 5 mL liquid nutrient medium (Czapek Dox) and grown for 10-14 days at 20°C under 12 hr photoperiod, until a mycelial mat formed. DNA was extracted from mycelium (100-200 mg fresh weight) by grinding it in liquid nitrogen in a mortar and pestle, and processing with the Qiagen Plant-mini kit (Qiagen) according to the manufacturer's instructions. DNA quality and quantity was assessed using agarose gel (1%) electrophoresis.

Characterisation of Bc-Hch locus

The Bc-*hch* homologue in *B. cinerea* was amplified using primers 262 and 520L and thermocycling conditions described by Fournier *et al.,* (2003). CAP profiles were produced by the digestion of the Bc-*hch* PCR product with *Hha* I enzyme (New England Biolabs) for 1.5 hr at 37°C and resolved on 1.5% agarose gels stained with ethidium bromide. The restriction fragment at 601 bp and 517 bp determined diagnosis of Group I and Group II isolates respectively (Fournier *et al.,* 2003).

PCR detection of transposons Boty and Flipper

PCR of transposon markers *Flipper* and *Boty* was conducted using PCR primers for *Flipper* described by Levis *et al.,* (1997) and a *Boty* 764 bp amplicon (F-GAC CGC TTT CAA AAC AAG ATAC, R-GAC CTT CCA AAT ATA CTC GCC) based on Genbank sequence X81791 designed using the PRIME program (GCG). PCR was conducted separately in triplicate for *Flipper* and *Boty* markers and included intergenic transcribed spacer (ITS) region primers 1 and 4 (White *et al.,* 1990) for the internal PCR control. Each PCR reaction (25 µL) contained 10-20 ng DNA, 0.1 µM ITS primer, 0.8 µM Flipper or 0.2 µM Boty of each forward and reverse primer, 100 µM each of dATP, dGTP, dTTP and dCTP, 1.5 mM $MgCl_2$, buffer (20 mM Tris-HCl pH 8.4, 50 mM KCl) and 1 U of *Taq* DNA polymerase (Invitrogen). Thermocycling conditions consisted of 35 cycles of 94ºC for 30 sec, 55ºC for 30 sec and 72ºC for 1 min, with an initial denaturation of 94ºC for 2 min and a final extension of 72ºC for 5 min. PCR reactions were repeated three times and resolved on 1.5% agarose gels stained with ethidium bromide. The markers for transposons *Boty* and *Flipper* were scored for their presence and absence in isolates or haplotypes. Isolates that contained both transposon markers were named *transposa,* isolates in which these markers were absent were named *vacuma* types. Isolates containing *Boty* only were named *boty*-only and those containing *Flipper* only were named *flipper*-only types. Fishers exact tests were used to evaluate the association of each of the transposons with the observed frequencies from each geographic region. The null hypothesis, (H_0) assumed no association between *Boty* and *Flipper* transposons.

Microsatellite genotyping and data analysis

Microsatellite amplicons were produced from isolates with the nine primer pairs described by Fournier *et al.,* (2002, Table 1). Each PCR reaction (25 µL) contained 10-20 ng DNA, 0.2 µM of each primer, 100 µM each of dATP, dGTP, dTTP and dCTP, 1.5 mM $MgCl_2$, buffer (20 mM Tris-HCl pH 8.4, 50 mM KCl) and 1 U of *Taq* DNA polymerase (Invitrogen). Amplification was conducted in a MJ Research PTC-200 thermocycler with cycling described by Fournier *et al.,* (2002). Amplified fragments were resolved by sequencing polyacrylamide gel (5%) electrophoresis, stained with silver (Promega). Allele identities were assigned and approximate allele sizes were determined by comparisons to a 50 bp step ladder (Promega). PCR reactions were repeated at least three times on gel runs to distinguish closely sized alleles and to minimise scoring error. Allele identities rather than size were analysed with programs and

Table 1. Estimated allele size range, number of alleles (Na) and gene diversity (*H*) for each microsatellite locus per population.

Locus	Allele size range (bp)	Bangladesh		Nepal/India		Australia	
		Na	*H*	Na	*H*	Na	*H*
Bc1	220-280	10	0.83	5	0.71	24	0.94
Bc2	180-250	9	0.81	5	0.68	17	0.90
Bc3	200-230	5	0.39	3	0.54	14	0.88
Bc4	98-130	2	0.04	2	0.44	5	0.47
Bc5	150-170	9	0.69	5	0.52	19	0.93
Bc6	80-260	13	0.78	6	0.68	27	0.94
Bc7	115-140	5	0.65	5	0.77	10	0.79
Bc9	150-180	5	0.55	6	0.58	10	0.63
Bc10	160-210	6	0.65	4	0.52	16	0.90
Total Na / mean *H*		64	0.60	41	0.61	142	0.82

algorithms that suited this data format and therefore the analysis was not affected by inaccurate allele size estimates (see below).

Microsatellite haplotypes were tagged with their respective transposon type for further analysis. Differentiation between geographic or transposon types were tested by analysis of inter-haplotype distances by matrix correlation and clustering methods. Pairwise genetic distances, D_{AS} (Jin and Chakraborty, 1993), were calculated between haplotypes using the POPULATIONS v.1.2.28 program (Langella 1999, http://www.cnrs-gif.fr/pge/bioinfo/ populations/). Genetic distance measures based on simple allele sharing frequency rather than measures based on step-wise mutation models have been shown to be reliable in deriving phylogenies from microsatellite data in fungi (Fisher *et al.,* 2000).

Matrix correlation tests (MCT) were used to test the relationship between these genetic distance matrices and two matrices corresponding to grouped transposon type and geographic origin both independently (two-way) and sequentially (three-way). In the three-way MCT, three matrices were compared in two orders: 1) genetic distance and transposon type or 2) genetic distance and geographic origin, the residuals were then fitted by the third matrix, either 1) geographic origin or 2) transposon type. The significance of the partial regression coefficient "r" was based on the Z statistic (Smouse *et al.,* 1986). In essence, MCT evaluated the distribution of genetic variation in response to two variables since both could have independent or correlated effects. MCTs were conducted using the MXCOMP module in NTSYS v2.1 program with 10,000 permutations (Rohlf, 2000). Geographic origin was classified into two regions: South Asia (containing Bangladesh, India/ Nepal) and Australia; or three regions in which Bangladesh and India/Nepal were considered different, to produce three geographic regions. Isolates from India and Nepal were treated as being from a single region due to the small sample size and because they were sampled from a region overlapping the countries' border.

Bayesian inference cluster analysis of haplotypes was implemented using the STRUCTURE v 2.1 program (Pritchard *et al.,* 2000; Falush *et al.,* 2003). This procedure can identify clusters based on distinctive allele frequencies. The admixed model using the correlated allele frequencies option was implemented with a 50,000 burn-in period and 500,000 Markov chain Monte Carlo (MCMC) iterations. Each data set of 5 runs was used to determine the level of variation of the posterior probability, LnP(D), of each assuming the true number of populations or clusters (*K*) was from a range of 1-10. The best estimation of *K* was that associated with highest LnP(D) or log $P(X/K)$ of STRUCTURE runs expressed as a modal value or magnitude of ΔK (Evanno *et al.,* 2005). This approach is less ambiguous than using $P(K/X)$ alone and reduces the risk of over estimating *K* (Evanno *et al.,* 2005).

Results

Microsatellite analysis at nine SSR loci produced 170 alleles and 169 unique

Table 2. Number of haplotypes (n) detected and frequency of transposon type in South Asia and Australia.

Population	n	*Trans-posa*	*boty*-only	*flipper*-only	*Vacu-ma*
Bangladesh	86	23	2	60	1
India/Nepal	18	1	13	4	0
Australia	65	38	20	0	7
Total	169	62	35	64	8

Table 3. Two-way and three-way matrix correlation test with genetic distance (D_{AS}) and variables geography and transposon types of South Asia and Australia (*transposa*, *flipper*-only, *boty*-only and *vacuma*).[1]

Matrix correlation test	r
Two-way geography A [2]	0.46
Two-way geography B [3]	0.50
Two-way transposon type	0.38
Adjusted geography A - transposon type	0.30
Adjusted geography B - transposon type	0.26
Adjusted transposon type – geography A	0.40
Adjusted transposon type – geography B	0.42

[1]Significance at $P < 0.01$

[2]Geographic origin assigned to South Asia (includes Bangladesh, India/Nepal) and Australia

[3]Splitting regions within South Asia into Bangladesh, India/Nepal and Australia

Table 4. Two-way and three-way matrix correlation test with genetic distance (D_{AS}) and variables geography and transposon types common to South Asia and Australia (*transposa*, *boty* and *vacuma*).[1]

Matrix correlation test	r
Two-way geography	0.25
Two-way transposon type	0.17
Adjusted geography - transposon type	0.18
Adjusted transposon type - geography	0.26

[1] Significance at $P < 0.01$

haplotypes among the 243 isolates screened (Table 1). A proportion of missing data or null alleles occurred at three loci: 0.6% with *Bc2*, 4.1% with *Bc7* and 0.3% with *Bc10*, confirmed by at least three PCR assays. The number of alleles and gene diversity varied with population and loci. The highest number of alleles (142) and gene diversity (0.82) was shown in Australia (Table 1).

All haplotypes revealed the 517 bp fragment of the Bc-*hch* locus consistent with *B. cinerea* Group II (Fig. 1). Based on the presence and absence of *Boty* and *Flipper* transposon amplicons, all four possible types of *B. cinerea* transposon types were distinguished. These were: *transposa* (containing *Boty* and *Flipper*), *boty*-only, *flipper*-only and *vacuma* (lacking both *Boty* and *Flipper*). The frequency and distribution of transposon types varied markedly between geographic regions (Table 2). Among the 86 haplotypes from Bangladesh, the frequencies of transposon types ranked from highest to lowest were: 69% *flipper*-only, 24% *transposa*, 2% *boty*-only and 1% *vacuma*. Among the 18 haplotypes from India/Nepal, the frequencies of transposon types ranked from highest to lowest were: 72% *boty*-only, 22% *flipper*-only, 6% *transposa* and 0% *vacuma*. Among the 65 haplotypes from Australia, the frequencies of transposon types from highest to lowest were: 58% *transposa*, 31% *boty*-only, 11% *vacuma* and 0% *flipper*-only (Table 2).

The heterogeneity tests for association between haplotypes containing transposons *Boty* and *Flipper* were not significantly associated thus the H_0 for Bangladesh haplotypes was not rejected ($P < 0.001$). However, a significant association was shown among haplotypes from India/Nepal and Australia (both $P < 0.001$).

Two-way matrix correlation tests (MCT) showed a slightly greater association of genetic distance with geographical region at either two or three regions (r = 0.46, 0.50, $P < 0.01$) than with transposon type (r = 0.38, $P < 0.01$, Table 3). Three-way MCT showed a stronger association with geographical origin after adjustment for transposon type than when transposon type was fitted first (r = 0.40, 0.42, $P < 0.01$, Table 3).

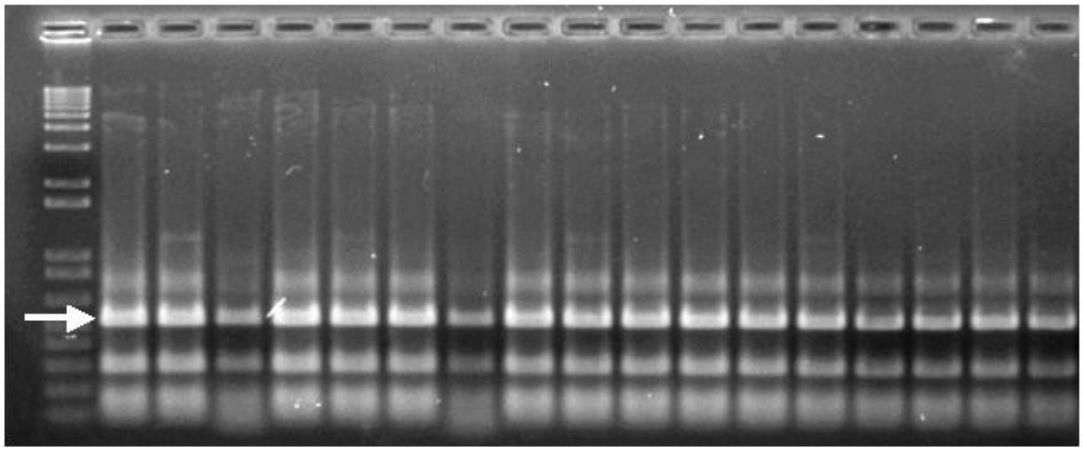

Fig. 1. Example of gel electrophoretic profile of the Bc-*hch* locus digested with *Hha I* from Group II *B. cinerea*. The 517 bp diagnostic fragment is arrowed.

MCTs were conducted with grouped transposon types common to both populations, thus omitting *flipper*-only as they were not found in Australia and would strongly influence the result due to over representation from one geographic region. Two-way MCT produced a higher and significant association to geographical region ($r = 0.25$, $P < 0.01$) than did transposon type ($r = 0.17$, $P < 0.01$, Table 4). Again the, three-way MCT showed a higher association with geographical region after adjustment for transposon type ($r = 0.26$, $P < 0.01$) than when transposon type was fitted first ($r = 0.18$, $P < 0.01$, Table 4).

With Structure analysis, the highest magnitude of ΔK was found with $K = 4$ and therefore four genetic clusters were detected (Fig. 2). At $K = 4$, the Structure bar plot showed the assignment of haplotypes to generally cluster to each geographical region and splitting the Australian population into two distinct clusters (Fig. 3). Clustering did not clearly correspond to transposon type however, the predominant transposon type in Bangladesh, *flipper*-only, did assign into one distinct cluster which also included the only *vacuma* and 16 (70%) of *transposa* types from Bangladesh (Fig. 3). The *boty*-only types were split between two main clusters according to geography, one corresponding to South Asia and the other to Australia, which indicated differentiation between *boty*-only isolates from different geographic region (Fig. 3). Within the Australian population, transposon types *Boty*-only, *transposa* and *vacuma* did not differentiate or split into distinct clusters. Two clusters were revealed within Australia with several haplotypes that showed considerable proportion of membership to each cluster indicative of admixture (Fig. 3).

Discussion

The presence of 517 bp and absence of 601 bp fragments of the Bc-*hch* locus CAPS profile (Fig. 1), showed all 169 microsatellite haplotypes to be consistent with Group II *B. cinerea* (Fournier *et al.,* 2003) the most common cryptic species reported in *B. cinerea*. In contrast Group I *B. cinerea*, has so far only been found at low frequencies in Europe and America (Fournier *et al.,* 2003; Ma and Michailides 2005). The implications for future disease management strategies are important, as Group I isolates have been shown to be phenotypically associated with resistance to the fungicide fenhexamid (FenR) but Group II are sensitive (FenS) (Albertini *et al.,* 2002; Fournier *et al.,* 2003). Genetic differentiation at microsatellite loci between Group I and II could not be elucidated since no Group I isolates were identified. Recently, substantial differentiation, and therefore genetic isolation has been reported between Group I and II populations (Fournier *et al.,* 2005).

Group I isolates have not been detected in Australia and South Asia and may indicate that gene flow between South Asia/Australia and Europe/America has been restricted. The presence of two *B. cinerea* cryptic species in

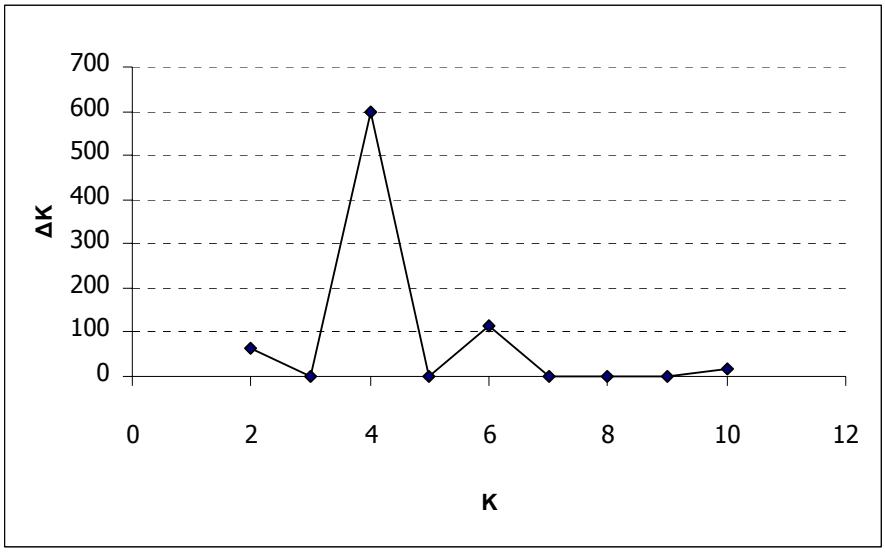

Fig. 2. The magnitude of Δ*K* at each level of *K* (1-10) used to determine the most probable number of genetic clusters (*K* = 4) with *B. cinerea* haplotypes from South Asia and Australia determined by Structure.

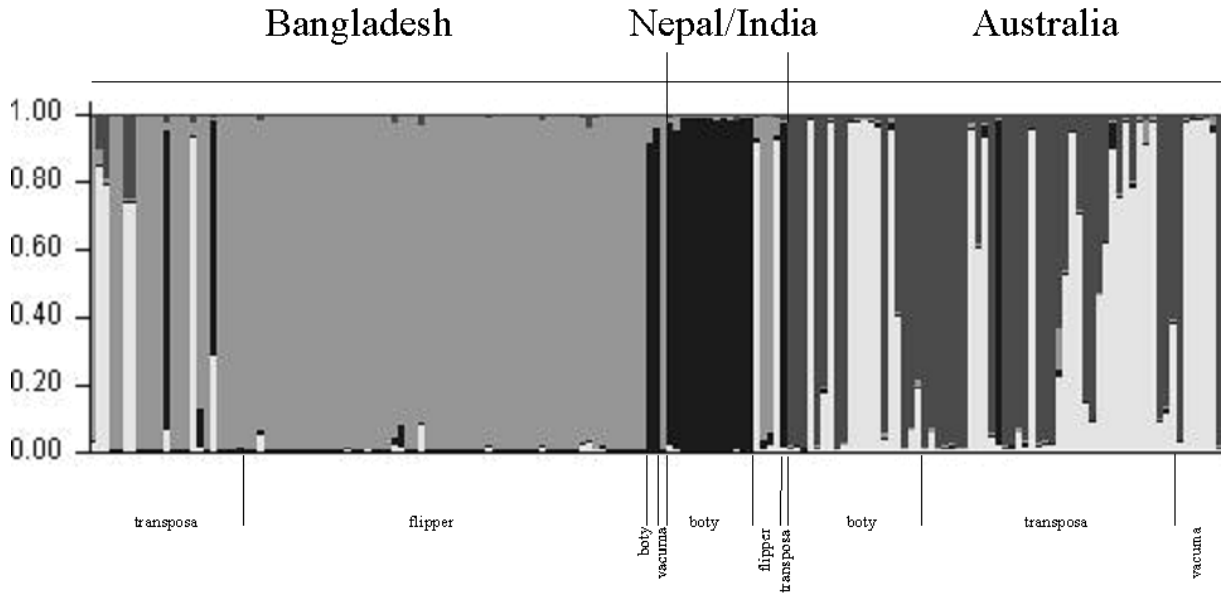

Fig. 3. Structure bar-plot that reveals the assignment of haplotypes at *K* = 4 clusters from Bangladesh, Nepal, India and Australia. Transposon types are indicted on the bottom. Scale (x-axis) indicates the membership coefficient of individual haplotypes to cluster *K* indicated by shaded bar area.

Europe and America indicated that this speciation event and recent migration has occurred between these regions (Fournier *et al.,* 2005; Ma and Michailides 2005). *Botrytis cinerea* is thought to have originated from temperate hosts in the northern hemisphere, and long distance migration was probably the result of human trade and colonisation (Beever and Weeds, 2004). Australian quarantine barriers have increased remarkably since its initial European colonisation over 200 years ago and therefore, have likely restricted more recent introductions of *B. cinerea*. In regions of South Asia such as Bangladesh, trade activity may have led to the initial introduction of *B. cinerea* however, *B. cinerea* was not a problem to crops such as chickpea until it was able to build sufficient inoculum to this host under a tropical and warmer climate (Pande *et al.*, 2006).

Various transposon types *transposa*, *vacuma*, *flipper*-only and *boty*-only were found (Table 2). *Flipper*-only and *transposa* were found to be the most prevalent in South Asia. This was the first report of *flipper*-only in such high frequency and could imply these isolates were better adapted to this environment or had arisen by drift. In contrast to the Australian *B. cinerea* population, no *flipper*-only transposon types were detected, with *transposa* and *boty*-only isolates being the most prevalent. Heterogeneity tests showed a significant association between the transposons *Flipper* and *Boty* in India/Nepal and Australia but not in Bangladesh. This may indicate that the transposons could be used as qualitative markers in the elucidation of genetic differenttiation. Limited dispersal of the pathogen is supported by the differences in both allele and transposon frequencies and the association between transposons between the India/Nepal and Bangladesh isolates.

The genetic distances between microsatellite haplotypes were not concordant with the transposon type groupings but were associated with geographic origin (Tables 3 and 4). The detection of all four possible transposon types in South Asia and three transposon types in Australia may indicate the complexity and potential of subdivided genetic groups within and among these populations. However, structure analysis supported the geographic grouping from three distinct locations and showed that the same transposon types from different geographic origin did not cluster together (Figs. 2 and 3). The same transposon types, such as *transposa*, were more genetically differentiated between geographic regions than to other transposon types within regions. Therefore, genetic differentiation between transposon types within *B. cinerea* Group II initially proposed by Giraud *et al*., (1997) was not supported, due to the lack of genetic differentiation between transposon types irrespective of geographic origin.

Previously, it was proposed that *transposa* and *vacuma* were genetically isolated and they were found in sympatry on various hosts irrespective of geography (Giraud *et al.,* 1997, 1999). However, these results were confused by inadvertent pooling of Group I and II *vacuma* isolates (Giraud *et al.,* 1997; Fournier *et al.,* 2005). Therefore, due to geographical barriers, allopatric rather than sympatric speciation seems to be a more plausible mechanism for speciation in *B. cinerea* (Kohn 2005). This was not the first time that the genetic structure analysis of *B. cinerea* failed to cluster isolates according to transposon types (Ma and Michailides, 2005). ISSR markers have shown that there was no clear relationship between transposon types and Group I (FenR phenotypes) isolates (Ma and Michailides, 2005). Phylogenetic studies by Albertini *et al.,* (2002) and Fournier *et al.,* (2003, 2005) also indicated only weak evidence of genetic differentiation between transposon types. These studies were consistent with the present study and suggest that transposon types are not substantially differentiated and should not be classified as cryptic species.

The new broad geographic samplings that extended to inter-continental regions indicated that genetic differentiation, possibly as a result of geographic isolation, was likely to have occurred recently. Only a small proportion of haplotypes showed assignment to clusters shared between geographical regions which could be a result of recent divergence between populations or errors due to sample size and microsatellite scoring and analysis. The lowest gene diversity was found in Bangladesh despite the highest number of isolates screened. The lower gene diversity and genetic differentiation

found between Bangladesh and other regions could indicate genetic drift induced by a founder effect or restricted sampling within these regions (Nei *et al.*, 1975; McDermott and McDonald, 1993). A founder effect may be quite possible since *B. cinerea* infecting chickpea in Bangladesh has only been recorded since 1981 (Pande *et al.*, 2006). Moreover, the Bangladesh population may have experienced more extinction and recolonisation events that can also induce genetic drift which are not uncommon to plant pathogen populations (Wade and McCauley, 1988; Whitlock and McCauley, 1990; McDermott and McDonald, 1993).

The Australian population produced two distinct clusters with several haplotypes that showed considerable membership to both clusters. Mixed membership to clusters indicated admixture likely through recombination. Genetic recombination may have been a major source of genetic diversity and could account for the higher levels of gene diversity and allele number found within the Australian population (Milgroom, 1996; McDonald and Linde, 2002). The role of location, host and thus gene flow between *B. cinerea* isolates from within South Asia and Australia warrants further investigation (Pande *et al*., 2006).

In conclusion, Group II *B. cinerea* was the only group identified in the South Asian and Australian isolate collection and Group I isolates were not detected in these populations. Transposons *Boty* and *Flipper* were ubiquitous in *B. cinerea* Group II however, transposon types were not genetically differentiated as determined by microsatellite analysis and should not be considered genetic sub-groups or species (Fournier *et al.,* 2005; Ma and Michailides, 2005). Therefore, these findings supported the revised structure of *B. cinerea* described by Fournier *et al.,* (2005). Genetic differentiation between regions from South Asia and Australia has indicated that geographical barriers and possible founder or bottleneck effects have had a role in the genetic differentiation between *B. cinerea* populations from these regions. This could have implications for disease management strategies based on host resistance between these regions (McDonald and Linde, 2002; Pande *et al.*, 2006).

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