CHAPTER 3

TAXONOMY AND GENETIC VARIATION OF *BOTRYTIS* AND *BOTRYOTINIA*

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Abstract. The species of the anamorphic genus *Botrytis* and its associated *Botryotinia* teleomorphs are briefly assessed. Recent progress in understanding the genetics of variation in the polyphagous *B. cinerea* (teleomorph *Bt. fuckeliana*) is summarised, with emphasis on chromosome complement and extrachromosomal elements. Sexual and vegetative compatibility studies are reviewed in relation to the limited evidence of clonality revealed by DNA population markers. It is concluded that in contrast to the traditional view of this species, sexual reproduction plays a major role in determining variation whereas heterokaryosis plays only a limited role. Evidence supporting the existence of a second polyphagous species within *B. cinerea sensu lato* is discussed. The limited knowledge of the genetics of the host-restricted species is briefly described.

1. Introduction

Botrytis, especially the species *Botrytis cinerea,* has a reputation for unusual variability, although the underlying mechanisms involved are not well understood. Jarvis (1977, 1980) and Lorbeer (1980) provide comprehensive reviews of both the taxonomy and genetics, but these treatments are now over 20 years-old. This review examines recent advances with emphasis on evidence of variability and the mechanisms that give rise to it.

The generalised life cycle of *Botrytis* comprises various stages: a somatic (vegetative), mycelial system that produces asexual conidia (strictly macroconidia – the *Botrytis* anamorph stage), sclerotia and microconidia (spermatia). Sclerotia usually germinate to produce mycelium or conidia, but after appropriate pre-conditioning and fertilization, they may germinate to produce apothecia (the *Botryotinia* teleomorph stage), containing ascospores resulting from meiosis. For convenience, and because not all *Botrytis* species are known to have teleomorphs, we use *Botrytis* in the generic sense to include both *Botrytis* (abbreviated *B*.) and *Botryotinia* (abbreviated *Bt*.).

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2. Taxonomy

The genus *Botrytis* comprises over 20 species, with a proportion linked to sexual stages (Table 1). Broadly speaking the group forms a coherent grouping, although Hennebert (1973), for example, segregated *B. ricini* into the genus *Arthrobotrys*. As well as the species listed in Table 1, additional host-specific species undoubtedly remain to be described, and a number of described species are poorly characterised. For example, a globose-spored species was described in the USA from *Vicia villosa* (not *Linaria* as listed by Farr et al., 1989) under the name *B. viciae* Green (an illegitimate homonym of *B. viciae* Berk. 1846). This fungus has also been reported from other *Vicia* species in Australia (Stovold and Walker, 1980; Backhouse et al., 1984) but further studies are needed. Furthermore, some species presently in *Sclerotinia*, such as *S. veratri*, may be species of *Botryotinia* (Kohn, 1979a).

Table 1. Some Species of *Botrytis* and *Botryotinia*

Botrytis sp. (anamorph)	*Botryotinia* sp. (teleomorph)	Mating system	Major Hosts	Refs[1]
B. aclada Fresen.	-	-	*Allium*	4, 7, 8, 16
B. allii Munn	-	-	*Allium*	4, 8, 16
B. anthophila Bondartsev	?[2]	-	*Trifolium*	4, 8, 12
B. byssoidea J.C. Walker	?[3]	-	*Allium*	4, 7, 8, 16
B. calthae Hennebert	*Bt. calthae* Hennebert & M.E. Elliott	-	*Caltha*	4, 6, 7, 8, 9
B. cinerea Pers.:Fr.	*Bt. fuckeliana* (de Bary) Whetzel	Heterothallic	Polyphagous	4, 7, 8, 9
B. convallariae (Kleb.) Ondřej	-	-	*Convallaria*	8
B. convoluta Whetzel & Drayton	*Bt. convoluta* (Drayton) Whetzel	-	*Iris*	4, 7, 8, 9
B. croci Cooke & Massee	-	-	*Crocus*	7, 8, 11
B. elliptica (Berk.) Cooke	?*Botryotinia* sp.	Heterothallic	*Lilium*	4, 7, 8, 13, 14
B. fabae Sardiña	*Bt. fabae* J.Y. Lu & T.H. Wu	-	*Vicia*	7, 8, 15
B. ficariarum Hennebert	*Bt. ficariarum* Hennebert	-	*Ficaria*	6, 7, 8, 9
B. galanthina (Berk. & Broome) Sacc.	-	-	*Galanthus*	4, 7, 8
B. gladiolorum Timmerm.	*Bt. draytonii* (Buddin & Wakef.) Seaver	-	*Gladiolus*	4, 7, 8, 9
B. globosa A. Raabe	*Bt. globosa* N.F. Buchw.	Homothallic	*Allium*	1, 7, 8, 9
B. hyacinthi Westerd. & J.F.H. Beyma	-	-	*Hyacinthus*	4, 7, 8

Botrytis sp. (anamorph)	*Botryotinia* sp. (teleomorph)	Mating system	Major Hosts	Refs[1]
B. narcissicola Kleb. ex Westerd. & J.F.H. Beyma	*Bt. narcissicola* (P.H. Greg.) N.F. Buchw.	-	*Narcissus*	4, 7, 8, 9
B. paeoniae Oudem.	-	-	*Paeonia*, *Allium*	4, 7, 8, 9
B. pelargonii Røed	*Bt. pelargonii* Røed	-	*Pelargonium*	7, 8, 9
B. polyblastis Dowson	*Bt. polyblastis* (P.H. Greg.) N.F. Buchw.	-	*Narcissus*	4, 7, 8, 9
B. porri N.F. Buchw.	*Bt. porri* (J.F.H. Beyma) Whetzel	Homothallic	*Allium*	3, 7, 8, 9
B. ranunculi Hennebert	*Bt. ranunculi* Hennebert & W.H. Groves	Heterothallic	*Ranunculus*	4, 6, 7, 8, 9
B. ricini N.F. Buchw.	*Bt. ricini* (G.H. Godfrey) Whetzel	Homothallic	*Ricinus*	4, 5, 7, 8, 9
Botrytis sp.	*Bt. fritillarii-pallidiflori* Q.T. Chen & J.L. Li	-	-	10
Botrytis sp.	*Sclerotinia spermophila* Noble[2]	Homothallic	*Trifolium*	4, 8, 9, 12
B. sphaerosperma N.F. Buchw.	*Bt. sphaerosperma* (P.H. Greg.) N.F. Buchw.	-	*Allium*	7, 8, 9
B. squamosa J.C. Walker	*Bt. squamosa* Vienn.-Bourg.	Heterothallic	*Allium*	2, 4, 7, 8, 9
B. tulipae Lind	-	-	*Tulipa*, *Lilium*, *Allium*	4, 7, 8, 9

[1]1=Buchwald, 1953; 2=Bergquist and Lorbeer, 1972; 3=Elliott, 1964; 4=Farr et al., 1989; 5=Godfrey, 1923; 6=Hennebert and Groves, 1963; 7=Hennebert, 1973; 8=Jarvis, 1980; 9=Kohn, 1979a; 10=Li and Chen, 1987; 11=Moore, 1959; 12=Noble, 1948; 13=Van den Ende and Pennock, 1996; 14=Van den Ende and Pennock-Vos, 1997; 15=Wu and Lu, 1991; 16=Yohalem et al., 2003.

[2]It has been assumed (e.g. Farr et al., 1989) that *Sclerotinia spermophila* is the teleomorph of *B. anthophila* but Noble (1948), although discussing this possibility, concluded that the linkage needed confirmation. Although *S. spermophila* may belong to *Botryotinia* (Kohn, 1979a), the combination of *Bt. spermophila* used by Jarvis (1977) has not been formalised.

[3] It has been assumed (e.g. Jarvis, 1980) that *Bt. allii* (Sawada) Yamam. is the teleomorph of *B. byssoidea*. However, Kohn (1979b) provided evidence that Yamamoto was in error in concluding Sawada's species produces a *Botrytis* anamorph and she recombined *Sclerotinia allii* (Sawada) as *Ciborina allii* (Sawada) Kohn. Nevertheless, Yamamoto (1959), who worked on Japanese isolates, determined the anamorph as *B. byssoidea*, in which case the teleomorph he describes is that of *B. byssoidea*, albeit as yet unnamed. However, the relationships of the Japanese fungus needs further study (Hennebert, 1963; Neilsen et al., 2001).

Botryotinia is closely related to *Sclerotinia* and indeed findings with the rDNA internal transcribed spacer (ITS) region challenge the generic delimitation and provide support for a broad concept for *Sclerotinia* (Kohn, 1979a; Holst-Jensen et al., 1998). The association of host-specific taxa with primarily northern hemisphere temperate hosts indicates *Botrytis* is a northern hemisphere genus that has probably been widely distributed by human activity including movement to southern temperate regions. Knowledge of sexual breeding systems is critical to understanding variation, but the rarity of apothecia of most species in the field, and

the patience needed to produce the sexual stage in the laboratory, mean information is incomplete.

Three species concepts have been emphasised in fungi, albeit with different shades of interpretation: the morphological based on appearance, the biological based on interbreeding, and the phylogenetic based on common descent, although with plant pathogens host specificity is also emphasised (Brasier, 1997; Harrington and Rizzo, 1999; Taylor et al., 2000). Species of *Botrytis* have to date been delimited primarily on the basis of morphological and cultural characteristics coupled with host specificity (Hennebert, 1973; Jarvis, 1977, 1980). Features such as sclerotial size and form and conidium size are useful in delimiting some species, but many species are morphologically similar and growing conditions significantly influence variation. No key to all recognised species is available and identification of species based on traditional criteria can be fraught (Nielsen et al., 2001). Use of the biological species concept based on conducting sexual crosses *in vitro* is limited and sexual crosses have been largely confined to elucidation of the sexual system, although Bergquist and Lorbeer (1972) reported crosses were unsuccessful between *B. squamosa* and *B. cinerea*. Most field isolates of *B. cinerea* can be readily crossed in the laboratory producing highly viable ascospores, suggesting the existence of one inter-breeding population (Sect. 3.2). Nevertheless, recent evidence suggests the existence of a group of *B. cinerea*-like isolates unable to cross with *B. cinerea* tester strains, at least in parts of Europe (Sect. 3.6).

Another approach to defining biological species involves determining the distribution of allelic variation to define populations that are inter-breeding. This approach has been applied to *B. cinerea* using DNA markers in particular, and supports the proposal that *B. cinerea,* as currently recognised, does indeed comprise two distinct inter-breeding populations (Sect. 3.6). The phylogenetic approach to systematics has been boosted by the advances in DNA sequencing, but for *Botrytis* this approach is in its infancy. The ITS rDNA region has been widely used for species-level discrimination of fungal species, but variation in the ITS region within *Botrytis* is low, limiting its use in this genus (Nielsen et al., 2001). The intergenic spacer region (IGS) rDNA region may offer better prospects (Giraud et al., 1997), although its usefulness may be limited by recombination. The value of using multiple genes to recognise species on the basis of concordance between independent gene phylogenies has been emphasised by Taylor et al. (2000). A preliminary phylogenetic analysis for four nuclear genes (Leroux et al., 2002b) demonstrates the existence of two phylogenetically distinct groups in *B. cinerea sensu lato* and also confirms a close relationship between these species and *B. calthae*, *B. convoluta* and *B. fabae*. Indeed, the analysis suggests *B. calthae* and *B. convoluta* are segregates within the *B. cinerea* 'phylogenetic' species. These latter species are all in the *B. cinerea* complex recognised by Hennebert and Groves (1963), and have been distinguished primarily by host range coupled with subtle differences in morphology.

An exemplar for the application of modern methods to species recognition in *Botrytis* has been provided for the neck-rotting species of onion. Universal-primed polymerase chain reaction (UP-PCR) fingerprinting, coupled with restriction analysis of ITS rDNA regions, allowed five groups to be distinguished: *B. cinerea*,

B. squamosa, *B. byssoidea*, and two groups in *B. aclada* (AI and AII) (Nielsen et al., 2001). The two groups of *B. aclada* could also be distinguished on spore size and chromosome number (AII has 32 compared with 16 in the other species), and comparison of sequence-characterised DNA fragments provided strong evidence that AII arose as an interspecific hybrid between AI and *B. byssoidea* (Nielsen and Yohalem, 2001). Subsequently, Yohalem et al. (2003) proposed that the name *B. aclada* be reserved for the small-spored subgroup (A1) and the previously synonymised *B. allii* name be applied to the larger-spored group (AII). The species concept resulting from this study is essentially the operational species unit (OSU) of Brasier (1997), defined as population units "sharing a common gene pool and exhibiting a common set of physiological, ecological and morphological attributes".

3. *Botrytis cinerea*

Most genetic studies in *Botrytis* have been carried out on *B. cinerea*. The two single ascospore mating type tester strains SAS56 and SAS405 provide a common focus in many studies and are referred to where appropriate. A recurrent theme of variation in *B. cinerea*, indeed perhaps the main reason for its reputation as an unusually variable fungus, is the existence of morphologically distinct cultural types. Usually they are grouped as mycelial, sporulating (conidial) and sclerotial (Jarvis, 1977; Lorbeer, 1980), but sub-types can be recognised (Faretra et al., 1988; Martinez et al., 2003). Typically, such morphotypes are essentially stable when subcultured using mass inoculum, but subcultures from single conidia often differ from the parents and each other (Sect. 3.4.2). It is obvious that mycelia, sclerotia and conidia have different abilities for survival and dispersal, and the relative roles of these structures will vary greatly depending on ecosystem and season. Perhaps their dual occurrence reflects a major role of disruptive selection with, for example, sclerotial types being favoured on perennial hosts over winter and conidial types on annual hosts with abundant susceptible flowers.

3.1. Nuclear number and chromosomes

It has been long established that both hyphal cells and conidia are multinucleate with numbers for conidia usually in the range 3-6 (Grindle, 1979; Lorenz and Eichorn, 1983; Shirane et al., 1988). Microconidia are, on the other hand, uninucleate, seldom germinate on laboratory media, and apparently function primarily as male gametes in sexual crosses. Asci initially contain one diploid nucleus that undergoes meiosis generating a tetrad of nuclei that divide again producing nuclei around which the eight ascospores are formed; subsequently, mitotic divisions occur resulting in about four nuclei in the mature single-celled ascospores (Lorenz and Eichorn, 1983; Faretra and Antonacci, 1987).

The small size of fungal chromosomes makes their cytological karyotyping difficult. Nevertheless, Shirane et al. (1988), using an elegant method to release chromosomes from the tips of germlings, provided clear evidence for the presence in mitotic metaphase of 16 chromosomes, and 16 chromosomes have also been reported in developing asci (Faretra and Grindle, 1992).

The small size of fungal chromosomes is an advantage for pulsed-field gel electrophoresis, which has revealed that fungi in general exhibit a high degree of chromosome-length polymorphism with homologous chromosomes differing in length (Zolan, 1995), and in addition supernumerary chromosomes ("B" chromosomes) are common (Covert, 1998). Studies with *B. cinerea* provide evidence for up to 13 major bands (in the range 1.8-4.6 Mbp), corresponding to one or more large chromosomes, along with up to three minor bands (in the range 220-580 Kbp), corresponding to small chromosomes and mitochondrial DNA (Van Kan et al., 1993; Faretra et al., 1996; Vallejo et al., 1996, 2002). Vallejo et al. (2002) reported seven different profiles amongst 22 field strains with from five to eight bands per strain, and found individual karyotypes were highly reproducible following repeated subculturing. Estimates of minimal genome size based on the assumption of only one chromosome per band, range from 13.19-22.64 Mbp (Vallejo et al., 2002). Higher estimates of 33.9-39.7 Mbp were calculated by Van Kan et al. (1993), assuming intense bands correspond to two chromosomes. These latter estimates equate to a chromosome complement of 12-14 chromosomes that, given the cytological evidence of 16 chromosomes, indicates some bands may correspond to more than two chromosomes. The chromosome profiles of ascospore progeny in some instances match one or other parent, but other siblings may differ from either parent and show novel profiles (Vallejo et al., 2002). These authors conclude that generation of new chromosomal bands, and loss of others, is a result of meiotic crossing-over between homologous chromosomes having heterologous regions, thus generating homologous chromosomes of different length. Use of an rDNA probe showed the rDNA gene cluster was located in a single high molecular weight band, which varied in size depending on the strain. Faretra et al. (1996) reported some small chromosomes showed anomalous segregation, indicating they are supernumerary.

The ploidy level of various strains was examined by Büttner et al. (1994) using fluorescent microscopy to estimate DNA content of individual nuclei relative to the single ascospore strain SAS56. They treated SAS56 with benomyl under conditions that induce haploidisation of diploid strains in 'model' fungi, and recovered a series of strains that showed reduced DNA content, including one with only one-third of that of the parental strain. They suggested strain SAS56 is polyploid, perhaps triploid, with the derived strains representing euploid, or more likely aneuploid, derivatives. Single ascospore strain SAS405 showed a DNA content of 0.69x that of SAS56, consistent with it perhaps being diploid, and a series of field strains showed values both lower and higher than strain SAS56. Büttner and Tudzynski (1996), in a preliminary cytological report, detected differences in chromosome number both within and between strains. In addition, individual nuclei in a given strain showed a spectrum of values over a 3-fold range some of which were ‘haploid’ (P. Tudzynski, Institut für Botanik, Münster, Germany, pers. comm.). Various forms of heteroploidy have been suggested for a range of fungi (Tolmsoff, 1983), and a variety of mechanisms including failure of mitosis or fusion of nuclei, coupled with chromosome loss or even gain may be involved. A small amount of the variation in DNA content of the benomyl-induced variants may be a result of the elimination of supernumerary chromosomes during vegetative growth (Covert, 1998). These

findings must be considered in light of the knowledge that strains SAS405 and SAS56 in particular, and most strains in general, are able to cross sexually producing viable progeny (Sect. 3.2). It may be that the sexual crossing process acts to restrict participation to haploid nuclei, whereas somatic cell function encourages heteroploidy. If this is so, ascospores may initially be strict haploids, but give rise to heteroploid colonies as they grow.

3.2. The sexual cycle in nature and in the laboratory

B. cinerea apothecia (Figure 1) are seldom found in nature (Lorbeer, 1980), although it is salutary to reflect that Anton de Bary described *Peziza* (*Botryotinia*) *fuckeliana* and *B. cinerea* from grapevine in Switzerland well over a century ago (Gregory, 1949). While lack of searching and confusion with apothecia of *Sclerotinia* and *Monilinia* may account in part for their apparent rarity, we suspect apothecia are genuinely uncommon, at least when compared with these other genera. Sclerotia have not been found, or are rare in the field in regions with warm dry summers, including Almeria, Spain (Raposo et al., 2001) and Israel (Yunis and Elad, 1989), and it is thus unlikely apothecia will be found in these regions.

Figure 1. Apothecia of *Botryotinia fuckeliana* the teleomorph of *Botrytis cinerea* on a peach mummy collected in the field in Hawke's Bay, New Zealand (left and centre) along with a cluster of apothecia arising from a sclerotium produced in the laboratory (right)

Despite the rarity of apothecia in nature, they can be readily obtained in the laboratory following protocols refined by Faretra and Antonacci (1987) and Faretra et al. (1988). Critically, they confirmed that most strains are heterothallic, carrying one or other allele of the mating type gene, and designated standard single ascospore tester strains for each mating type; SAS56 for *MAT1-1* and SAS405 for *MAT1-2*. No DNA sequence information is available for these genes in *B. cinerea*, but it is likely they will comprise alleles of very different sequence (idiomorphs) as has been found with other filamentous ascomycetes (Coppin et al., 1997). Crossing involves preconditioning sclerotia of the female (sclerotial) parent in the cold, before fertilizing them with a suspension of microconidia and vegetative cells derived from the male (fertilizing or spermatial) parent. Strains are usually able to act as female or

male parents, although strains incapable of sclerotial production cannot act as female parents.

In studies involving numerous isolates sourced from around the world most strains from the field were heterothallic, crossing successfully with one or other tester strain (Lorenz and Eichorn, 1983; Faretra et al., 1988; Beever and Parkes, 1993; Faretra and Pollastro, 1993; Van der Vlugt-Bergmans et al., 1993; Delcán and Melgarejo, 2002). The percentage of *MAT1-1* strains was slightly higher than *MAT1-2* strains in most populations. In addition some strains behaved in homothallic fashion producing fertile apothecia without spermatization and/or with both tester strains. Faretra et al. (1988) concluded such *MAT1-1/2* strains (16% in their study) are heterokaryotic for the mating type genes, that is, they are pseudohomothallic. Some single ascospore progeny were also homothallic, but as the ascospore nuclei derive ultimately from one haploid nucleus, such strains cannot readily be explained by heterokaryosis. Reports of the incidence of homothallic single ascospore strains vary from 4-6% (Faretra et al*.,* 1988; Faretra and Pollastro, 1991, 1996), although Lorenz and Eichorn (1983) reported five of six strains behaved in this manner. Faretra and Pollastro (1996) further showed some pairs of ascospores dissected in order from eight-spored asci behaved in homothallic fashion, and these 'homothallic' ascospores occupied the positions where *MAT1-2* would have been expected, suggesting a process of unidirectional mating type switching similar to that reported for some other ascomycetes including *Sclerotinia trifoliorum* (Uhm and Fujii, 1983). The molecular basis of this instability is not yet understood, but may result from the presence of both mating type idiomorphs in 'switching' strains and the deletion of one of these during switching (Raju and Perkins, 2000). The *B. cinerea* data are consistent with the proposal that in a few asci, one or two of the four meiotic nuclei are epigenetically modified as a result of which switching occurs in the following mitotic divisions and a mycelium heterokaryotic for mating type is produced. The observation that *MAT1-1* strains typically exceed *MAT1-2* strains in field samples is consistent with switching during meiosis, although switching during mitotic growth cannot be excluded.

3.3. Extrachromosomal elements

Fungi in general, and *B. cinerea* in particular, possess a variety of extrachromosomal genetic elements including the chromosomes of mitochondria, viruses, plasmids and transposable elements (Rosewich and Kistler, 2000). While mitochondria are clearly essential to cell growth, the others are generally considered dispensable and to behave as 'selfish genetic elements', although transposons integrate into chromosomes and may play an important long-term role in evolution (Kidwell and Lisch, 2001). Apart from encoding genes involved in their own existence, such 'genomic parasites' may influence their hosts in various ways. One critical question is the nature of the inheritance and loss of such elements. It is probable such elements are readily transmitted via conidia, but transmission into ascospore progeny may in some cases be limited or occurs only via the maternal parent. Transmission via somatic cell fusion may be restricted to a greater or lesser extent by vegetative incompatibility (Sect. 3.4.1). In *Neurospora crassa* it is suggested the

primary role of various gene 'silencing' processes such as the meiosis-associated processes of 'repeat-induced point' mutation (RIP) and 'meiotic silencing by unpaired DNA' (MSUD), as well as the somatic cell process of quelling, may act to restrict the spread of 'genomic parasites' (Shiu et al., 2001). It is not known whether such processes are active in *B. cinerea.*

3.3.1. Mitochondria and mitochondrial plasmids

Mitochondrial DNA (mtDNA) provides a distinct non-nuclear source of genetic variation especially suitable for the study of intraspecies variation in fungi (Typas et al., 1998). The ascomycete mitochondrial genome is typically circular and the full sequences of *N. crassa* (Griffiths et al., 1995) and some other species are available, providing a rich resource for future investigation in *B. cinerea.* Preliminary studies indicate that *B. cinerea* has a circular genome of about 25.8 Kbp (Vallejo et al., 1996). Holst-Jensen and Schumacher (1994) found no polymorphisms amongst seven isolates using a *Neurospora* mitochondrial rDNA probe, whereas all were distinguishable by RFLP using a nuclear rDNA probe. Both linear and circular mitochondrial plasmids are known in filamentous ascomycetes (Griffiths, 1995), and linear plasmids of 2-3 Kb have been found in *B. cinerea* (Hiratsuka et al., 1987). Such plasmids are generally considered to confer no selective advantage or disadvantage on their host, merely encoding genes needed for their own replication, although some may be associated with mitochondrion-induced 'senescence' (Griffiths, 1995). Gene transfer via cytoplasmic contact between both compatible and incompatible strains has been invoked to explain patterns of distribution of plasmids within other fungal species, and direct horizontal transfer has been demonstrated (Rosewich and Kistler, 2000).

3.3.2. Transposable elements

Transposable elements in general can be divided into Class I and Class II types (Daboussi, 1996; Kidwell and Lisch, 2001). Class I elements transpose by reverse transcription of an RNA intermediate, whereas Class II elements transpose at the DNA level by excising from one site and reintegrating at another site. Two transposable elements have been found in some strains of *B. cinerea*. *Boty* is a 6-kb putative Class I retrotransposon, characterised by a long terminal repeat (LTR), present in multiple copies in different regions of the genome (Diolez et al., 1995; Giraud et al., 1999). *Flipper* is a 1842 bp Class II element, present in up to 20 copies per genome, and is known to be mobile by its insertion into nitrate reductase during spontaneous mutant selection (Levis et al., 1997). Initially dot blot methods were used to detect these elements, but more recently PCR methods have been developed (Muñoz et al., 2002). When first reported, both elements were found in the same strain (*transposa* strains), whereas *vacuma* strains lack both elements. However, strains containing only the *Boty* element have now been detected in Europe (Giraud et al., 1999; De Miccolis et al., 2004) and Chile (Muñoz et al., 2002), and strains containing only the *Flipper* element have been detected in Europe (Albertini et al.,

2002; De Miccolis et al., 2004). The nine copies of *Flipper* in strain SAS405 each segregated in Mendelian fashion in a cross with SAS56, which lacks the element (Levis et al., 1997). The copies segregated independently, indicating they were unlinked, and copy number did not change during the cross. This observation suggests *Flipper* at least will spread rapidly through a population if sexual crossing is occurring.

3.3.3. Mycoviruses

Mycoviruses, including those characterised by encapsulated genomes and others that lack protein capsids, are common in fungi but unlike viruses in other organisms, are not infectious *per se* (Buck, 1998; Ghabrial, 1994). They are typically readily transmitted into asexual progeny, but transmission to sexual progeny is often inefficient or absent (Coenen et al., 1997; Chun and Lee, 1997). Most mycovirus genomes are double-stranded RNA (dsRNA) and their presence can be readily detected by gel electrophoresis. Such dsRNAs are common in *B. cinerea.* Howitt et al. (1995) reported that over 70% of 200 isolates were infected. The dsRNA profiles observed in this survey varied widely in number (1-8 bands) and size (c. 0.8-1.5 Kbp) and few of the 143 profiles were identical. This complexity may reflect mixed infections, the presence of satellite viruses, or defective dsRNAs derived by deletion. Isometric, bacilliform and filamentous virus-like particles have been found in *B. cinerea* (Howitt et al., 1995), and two isometric dsRNA mycoviruses have been partially characterised (Vilches and Castillo, 1997; Castro et al., 1999). In both cases, the viruses were located in the cytoplasm and associated with some cellular degeneration. Apart from this observation, there is no evidence that dsRNA viruses have any major phenotypic effect (Howitt et al., 1995). Transmission studies indicate they are not passed to ascospore progeny (F. Faretra, Università di Bari, Bari, Italy, pers. comm.).

As well as dsRNA mycoviruses, two single-stranded RNA (ssRNA) mycoviruses have been characterised and indeed fully sequenced from *B. cinerea* (Howitt, 1998; Howitt et al., 2001). Both were associated in the same isolate with flexuous rod-shaped particles resembling plant 'potex-like' viruses. Botrytis virus F (BVF) contains a genome of 6827 nucleotides, and Botrytis virus X (BVX) a genome of 6966 nucleotides both with poly(A) tracts. They each differ sufficiently from existing viruses, and from each other, to warrant recognition as new viral genera. The similarity between these mycoviruses and plant viruses, including, a remarkable 73% amino acid identity between the putative RNA-dependent RNA polymerase of BVX and that of the allexivirus *Garlic virus A*, suggests some form of horizontal gene transfer between plants (specifically *Allium*) and *B. cinerea.*

Hypovirulence-associated dsRNA mycoviruses, which reduce the pathogenicity of their host fungi, have attracted particular interest because of their possible use in biocontrol (Buck, 1998; Dawe and Nuss, 2001). Such viruses are known from *Sclerotinia* spp. (Boland, 1992; Deng et al., 2002) and have recently been identified in *Botrytis* (Castro et al., 2003).

3.4. Somatic compatibility and heterokaryosis

3.4.1. Somatic compatibility

Characterisation of groups of somatically compatible individuals provides a powerful approach to subdividing fungal species (Correll and Gordon, 1999; Glass et al., 2000). Evidence for the existence of vegetative compatibility groups (VCGs) has usually been obtained using auxotrophic mutants derived from the strains being tested. Different classes of chlorate resistant, nitrate non-utilising (Nit) mutants have been widely used for this purpose because spontaneous mutants are often readily selected in target fungi, they can be easily classified into different nitrogen usage phenotypes on minimal medium amended with various nitrogen sources, and complementation is easily scored on minimal medium. Typically, a range of mutants are recovered, but the most reliable are usually those deficient in nitrate reductase apoenzyme (*nit1*) and those defective in synthesis of the molybdenum containing cofactor needed for nitrate reductase and xanthine dehydrogenase activity (NitM).

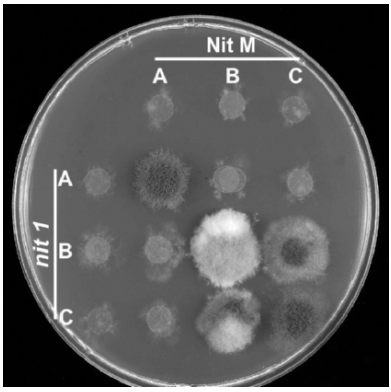

Figure 2. Complementation matrix demonstrating existence of VCGs in *B. cinerea*. Spore suspensions of nit1 (rows) and NitM (columns) mutants derived from three field strains (A,B,C) have been superimposed on nitrate medium + Triton (Beever and Parkes, 2003). Mutant pairs from the same parent all complement as do the mutants from strains B and C, indicating they are in the same VCG

Beever and Parkes (2003) devised a method facilitating recovery of both *nit1* and NitM pairs of *B. cinerea* mutants and confirmed the existence of multiple VCGs, with all six field strains examined being in different groups. These strains all crossed successfully with SAS56 or SAS405 tester strains, which were themselves in different VCGs. Subsequent studies have shown a large number of VCGs exist within the *B. cinerea* population, and to date we have recognised 45 amongst 57 field strains, with few isolates being in the same group (Figure 2) (P. Weeds and R. Beever, unpubl.). Selenate resistant mutants have also been used to demonstrate complementation (Korolev et al., 2003).

The genetic basis of vegetative incompatibility in *B. cinerea* is not known, but evidence to date suggests it conforms to the system found in other ascomycetous fungi (Glass et al., 2000). In these, vegetative incompatibility is typically determined by a series of vegetative incompatibility (*vic* or *het*) genes that exist in two or occasionally more allelic states. Strains that carry identical alleles at all loci are compatible, those that differ at one or more loci are incompatible. VCGs are determined by unique combinations of *vic* genes, such that if six *vic* loci with two alleles per locus are segregating in a population, 64 (2^6) groups are theoretically possible. Sexual crossing has been shown to generate new VCGs in *B. cinerea* (Beever and Parkes, 2003). Considering both field and single ascospore isolates we have identified over 66 distinct VCGs consistent with the presence of at least seven *vic* genes in the *B. cinerea* population (P. Weeds and R. Beever, unpubl.). The existence of homothallic strains of *B. cinerea* heterokaryotic for mating type (Faretra et al., 1988) indicates *MAT1* does not act as a *vic* gene in this species, as it does in *N. crassa*. The large number of VCGs, and the limited occurrence of isolates in the same VCG, suggests sexual recombination plays an important role in the field in *B. cinerea.* The diversity of dsRNA profiles seen in field strains (Howitt et al., 1995) suggests hyphal fusion is uncommon, a proposal consistent with the existence of multiple VCGs. The homologue (Bc-*hch*) of the *N. crassa het-c* gene has been cloned and sequenced in a number of *B. cinerea* strains, but it does not appear to act as a *vic* gene in this species (Fournier et al., 2003).

Numerous fungi, including *Sclerotinia* and *Monilinia*, form distinctive interaction lines when paired on agar media (Kohn et al., 1990; Free et al., 1996). While such "barrage" lines indicate the existence of incompatibility, caution should be exercised in assuming that mycelial compatibility groups (MCGs) recognised in this manner match VCGs described above. All tester isolates for the nine VCGs recognised by Beever and Parkes (2003) formed strong interaction zones with other tester strains, an observation consistent with the congruence of these two systems. However, classifying field strains into distinct groups using the mycelial compatibility test is often difficult, with the number and intensity of dark lines varying depending on strain combination. Delcán and Melgarejo (2002) examined the interactions between numerous strains and found few were compatible, although a mycelial-free space, rather than a dark interaction line, was observed in some interactions indicating that more than one phenomenon may be involved. R. Beever and S.L. Parkes (unpubl.) obtained single ascospore strains after a series of backcrosses that complemented using Nit mutants but nevertheless still produced a dark interaction line. Ford et al. (1995) likewise reported the lack of a direct correlation between these two systems in *S. sclerotiorum*, and Micali and Smith (2003) concluded the two phenomena are distinct in *N. crassa*. We conclude it is premature to equate mycelial and vegetative incompatibility in *B. cinerea*.

3.4.2. Heterokaryosis

Despite the longstanding interest in heterokaryosis in *B. cinerea* (Hansen, 1938; Menzinger, 1966; Jarvis, 1977; Grindle, 1979; Lorbeer, 1980), evidence for its

existence is rather limited, although the formation of 'laboratory' heterokaryons between mutants from the same parent is clearly established (Weeds et al., 1998; Beever and Parkes, 2003). Broadly speaking early work showed monoconidial isolates derived from a single parent often differ morphologically. For example, Hansen (1938) recognised so-called mycelial (M), conidial (C) and intermediate (M/C) types, and found M and C types ('homotypes') generated only M and C types respectively, whereas the M/C types ('heterotypes') gave rise to all three types. He concluded the M/C types were natural heterokaryons, comprising a mixture of C and M nuclei. Menzinger (1966) obtained broadly similar results, although in some instances finding more than two 'homotypes', and concluded *B. cinerea* isolates are frequently heterokaryotic (18 of 29 isolates examined). Grindle (1979) also studied variation of monoconidial progeny, and found that while five strains showed little variation, one strain generated a diversity of morphotypes and in addition some monoconidia from this isolate were non-viable, perhaps carrying a lethal gene. He concluded that while heterokaryosis as interpreted by Hansen (1938) might account for his findings, the possible role of cytoplasmic elements needed consideration.

The major question about heterokaryosis is to what extent it accounts for biologically significant variation of field strains. The most direct evidence for significant heterokaryosis in field strains comes from mating type gene studies. Faretra et al. (1988) found that monoconidial isolates of homothallic field isolates either remained homothallic (i.e. were presumably heterokaryotic *MAT1-1* + *MAT1-2*) or behaved as heterothallic (i.e. were presumably either *MAT1-1* or *MAT1-2* homokaryons). Some evidence on heterokaryosis has come from the study of fungicide resistance. Summers et al. (1984) demonstrated that a dicarboximide resistant field isolate was a heterokaryon and they were able to resolve it using monoconidial isolates into homokaryotic sensitive and resistant types. Furthermore, they showed the relative balance of fungicide-resistant and fungicide-sensitive nuclei in the heterokaryon responded to the presence of fungicide in the medium. Faretra and Pollastro (1993), working with field and laboratory dicarboximide-resistant isolates, and Pollastro et al. (1996), working with field dichlofluanid-resistant isolates, found some isolates did not always transmit their resistant character to sexual progeny, and resistance was also lost from some asexual progeny, indicating the parent isolates were heterokaryons between resistant and sensitive nucleotypes. Low-level anilinopyrimidine-resistant strains from the field behave as heterokaryons in which the resistant nucleotype is lethal in the homokaryotic state (De Miccolis Angelini et al., 2002).

Findings on population structure (Sect. 3.6), sexual crossing (Sect 3.2) and somatic compatibility (Sect. 3.4.1) allow an explicit interpretation of how heterokaryosis may operate in *B. cinerea*. Population and VCG studies indicate sexual reproduction plays a major role in determining population variation, and sexual crossing studies indicate most strains are heterothallic and capable of crossing. It is reasonable to propose that ascospore progeny will be initially

homokaryotic, carrying one or other mating type gene, and mostly be in different VCGs. The large number of VCGs observed in the field, and the diversity of their dsRNA profiles, suggest fusion of genetically different strains in the same VCG is uncommon. Thus the question can be asked as to how heterokaryons comprising genetically different nuclei do arise in the field. As for mating type, it is reasonable to suggest that it arises by 'switching' after meiosis within a single ascospore lineage (Sect. 3.2), and thus the heterokaryotic nuclei will be homozygous for other loci including the *vic* loci, a prediction that can be tested. This special case apart, we suggest the main mechanism for heterokaryon generation is likely to be mutation within somatic lineages. The apparently infrequent occurrence of apothecia in the field implies such lineages may be long-lived, in which case it is axiomatic that mutations will occur and, if recessive in particular, might persist for long periods. It is probable that many such mutations will affect morphological properties such as conidiation and sclerotium production and be essentially recessive in the heterokaryotic state. And of course long-lived lineages would be expected to produce multiple nuclear types within the same lineage; the recognition of 'dual' heterokaryons comprising just two nucleotypes may be an over-simplification. Such an explanation might account, for example, for highly variable strains such as Strain 9 studied by Grindle (1979) and Strain 16 studied by Menzinger (1966).

It is possible that a form of the parasexual cycle (Debets, 1998) implying at least transient diploid nucleus formation, aneuploid formation and gene recombination, may operate in *B. cinerea*. The heteroploidy observed by Büttner et al. (1994) is consistent with, but by no means evidence for, the existence of such a process. Parasexual recombination could at least in theory provide an alternative to sexual recombination in generating diversity (Sect. 3.6). However, the scarcity of VCGs with multiple members suggests it is unlikely to play a significant role.

3.5. Linkage studies

Although a method to routinely cross *B. cinerea* has been available for over a decade, few studies involving sexual crossing have been published. Nevertheless, these are sufficient to establish that the essential features conform to those of model fungi such as *N. crassa*. Single gene markers encoding resistance to benzimidazole fungicides (*Mbc1*) and dicarboximide fungicides (*Daf1*) have been identified and shown to be loosely linked to each other, but not to *Mat1*, using random ascospore analysis (Faretra and Pollastro, 1991; Beever and Parkes, 1993). Tetrad analysis demonstrated *MAT1* is located about 12 map units from its centromere, and *Mbc1* may be loosely linked to its centromere (Faretra and Pollastro, 1996). Two single genes (*Dic1* and *Dic*2) encoding resistance to the fungicide dichlofluanid have been identified, showing loose linkage to *Daf1* in some crosses (Pollastro et al., 1996). Weeds et al. (1998) showed neither *Sel1* (encoding resistance to selenate) nor *nit1* was linked to *Daf1* or *Mbc1*. As well as their intrinsic value in beginning a framework for a genetic map of *B. cinerea*, a major use of these various marker genes is to confirm whether crossing has indeed occurred in laboratory crosses.

3.6. Population studies using molecular markers

The development of molecular techniques has revolutionised and energised fungal population genetics by providing numerous readily available genetic markers (Chapter 4; Bridge et al., 1998). Multilocus techniques such as RAPDs and AFLPs are convenient and allow ready scoring of numerous polymorphic loci, but are limited by features including difficulties of reproducibility and the assumption that co-migrating bands are identical. Single-locus techniques such as RFLPs are highly reproducible and allow greater precision for estimating genetic parameters, but are more labour intensive (McDonald, 1997). Microsatellite markers, which offer numerous polymorphisms coupled with high reproducibility and convenience, have been developed for *B. cinerea* (Fournier et al., 2002), but as yet not widely applied.

Studies using RAPDs and AFLPs with *B. cinerea* have usually recognised 50 or more polymorphic markers. Van de Vlugt-Bergmans et al. (1993) studied eight Dutch field isolates, as well as SAS56 and SAS405, and found all could be differentiated, although two of the eight field isolates, recovered 4 years apart from different hosts, differed by only one marker. Crossing studies showed most markers segregated independently in Mendelian fashion and were unlinked. Kerssies et al. (1997) studied 29 isolates collected from inside and outside Dutch glasshouses and found that for all 70 markers scored, only two were identical, although a few others showed high similarity. Cluster analysis recognised three groups, but no pattern relating to biology of the groups was detected. Alfonso et al. (2000) studied 40 strains from Spain and found the population as a whole was highly heterogeneous, with little differentiation of the subpopulations between different greenhouses or regions; nor was significant differentiation detected when isolates from other countries (Israel, Italy, Holland) were included in the analysis. Moyano et al. (2003), working with 44 Spanish isolates from six greenhouses, found only two haplotypes (multilocus genotypes) had more than one member, one comprised of three isolates, the other of two. Six isolates showing the benzimidazole-sensitive/procymidone-resistant fungicide resistance phenotype clustered together, but no other biological correlations were detected. Thompson and Latorre (1999), studying isolates from various hosts in Chile, also found high genotypic diversity and, based on only 15 isolates, speculated that there was some clustering based on host. Yourman et al. (2000) found all 56 isolates from greenhouses in South Carolina (USA) were different, but found some clustering in relation to fungicide sensitivity. Muñoz et al. (2002) found all of 69 isolates from Chile, including two from the same kiwifruit, had different haplotypes indicating the absence of clonal lineages; however, the data indicated some clustering of isolates by host. In summary, these findings all indicate the *B. cinerea* population is genetically very diverse with no indication of widespread clonal lineages, even in relation to fungicide resistance that might have imposed a genetic bottleneck on some populations.

The most comprehensive population studies of *B. cinerea* have been conducted by Y. Brygoo and colleagues (Brygoo et al., 1998; Chapters 4 and 12) using a range of markers including presence or absence of the transposable elements *Boty* and *Flipper*, a suite of PCR-RFLP markers (based on the rDNA intergenic spacer region, nitrate reductase, ATP synthase and ADP-ATP translocase, and some unidentified

sequences) as well as single gene-encoded fungicide resistance to benzimidazole and dicarboximide fungicides. Giraud et al. (1997), using 16 such markers, identified 134 haplotypes in a sample 259 isolates collected from grape in Champagne, France, with the most common accounting for only 5% of isolates. Isolates collected from the same plant always had different haplotypes and up to five different haplotypes were found in spores isolated from a single berry. Even isolates identical for all 16 markers could be further differentiated on the number and location of copies of the transposable elements. This extensive genotypic diversity indicates limited clonal propagation and a significant role for recombination. Additionally they found highly significant differences in allelic frequencies between *transposa* isolates, carrying the transposable elements *Flipper* and *Boty*, and *vacuma* isolates that lack them, and four alleles were restricted to one or other population. Linkage disequilibrium estimates suggested 17.9% of pairs of loci for *vacuma*, and 5% for *transposa*, were in linkage disequilibrium, low values consistent with limited clonal reproduction.

In a subsequent study, Giraud et al. (1999) used the previous markers as well as some additional ones, including resistance to the fungicide fenhexamid, to examine 107 field isolates from various host plants growing around the vineyards where the grape isolates were recovered. Their findings mirrored those of the grape study, with 74 haplotypes being found – with the most common constituting only 8% of strains. Genotypic diversity (number of haplotypes/ number of isolates x 100) was similar in both studies, with *transposa* values of 55% (56% in grapes) and *vacuma* of 57% (70% in grapes). Giraud et al. (1997, 1999) concluded their data indicated the existence of two 'sibling species' *vacuma* and *transposa*, a conclusion consistent with the restriction of transposons to one population. The two groups recognised in this way correlated with a slight but statistically significant difference in spore size, spores of *vacuma* being slightly larger than *transposa* isolates. Subsequent studies have extended and modified these conclusions (Albertini et al., 2002; Fournier et al., 2003). While two distinct populations are still recognised, they are not fully coincident with those previously recognised. The new groups are differentiated unequivocally by fixed amino acid level polymorphisms in two genes, *Cyp51* (14α-demethylase gene) and Bc-*hch* (*B. cinerea* het-c homolog), as well as by their response to the fungicide fenhexamid: Group I isolates are resistant to fenhexamid, whereas Group II isolates are sensitive to fenhexamid. Group I isolates were all *vacuma* type, whereas Group II isolates included both *vacuma* and *transposa* types. Fournier et al. (2003) re-analysed part of the data of Giraud et al. (1997) and confirmed the genetic distinctiveness of Groups I and II. Leroux et al. (2002a, b) provide additional data on the nature of Groups I and II. Fertile crosses have been obtained between strains within each group (including both *vacuma* and *transposa* types in the case of Group II), but not between the groups, indicating they are reproductively isolated.

Mycelium compatibility tests suggest that Group I isolates comprise one MCG, as distinct from Group II isolates, which encompass numerous MCGs. Group I strains also have longer conidia and faster mycelium growth rate than Group II strains. Furthermore, combined phylogenetic analysis of four nuclear genes, including Bc-*hch*, and *Cyp51*, clearly support the distinction between Group I and II

strains. In summary, while some data are still to be published in full, these findings provide sound support for recognition of a second polyphagous *Botrytis* species reproductively and genetically distinct from *B. cinerea*. Leroux et al. (2002a, b) name but do not formally describe Group I isolates as *B. 'pseudocinerea'*. This species is so far known only from Europe (France, Germany, UK), where it occurs on a number of hosts (Leroux et al., 2002a; Fournier et al., 2003). In many ways the situation here resembles that of *B. fabae*, which also closely resembles *B. cinerea* but can be differentiated morphologically by its slightly larger conidia (Harrison, 1988).

Excluding the *B.* '*pseudocinerea' vacuma* group from consideration leaves questions about the relationship of *vacuma* and *transposa* subpopulations within *B. cinerea sensu stricto*. In particular, if sexual reproduction is common, why have the transposons not infected all strains in the population? One possibility is that they are still spreading through the populations (Muñoz et al., 2002), a possibility supported by the rarity of apothecia in the field. Another is that *B. cinerea* possesses a mechanism to resist infection or to remove transposons from their genome. Martinez et al. (2003) report on phenotypic differences between *transposa* and *vacuma* groups in a sample of 121 isolates from near Bordeaux (France). Only two were *B.* '*pseudocinerea',* and thus the findings apply essentially to *B. cinerea sensu stricto*. Pathogenicity of both groups on grape and tobacco leaves was similar, but mycelial growth rate of *transposa* strains was slightly lower than *vacuma* strains at favourable temperatures.

3.7. *Botrytis cinerea* - a synthesis

A changing picture is emerging of the genetic structure of *B. cinerea*. Traditionally, sexual reproduction has been considered to play a minor role, but most strains retain the ability to intercross, producing fully fertile progeny (Sect. 3.2). On the other hand, heterokaryosis has traditionally been considered very important, but the numerous VCGs in the population, presumably resulting from sexual recombination, suggest this process plays only a limited role (Sect. 3.4.2). Molecular markers indicate high levels of recombination (Sect. 3.6) which, given the limitations imposed on heterokaryosis by vegetative incompatibility, probably results from sexual rather than parasexual processes. The rarity of identical haplotypes, even when hundreds are examined, suggests the total number of variants in populations is very large. The effective size of such populations is unknown, but *B. cinerea* is recognised as present in appreciable numbers in the air spora (Jarvis, 1977). It is possible that migration over considerable distances is more important than generally appreciated, especially in increasing variation in dry climates where apothecia are unlikely to occur.

The infrequent discovery of apothecia in the field, the large proportion of field strains infected by mycoviruses and the observation that many strains do not carry transposons, all suggest that, despite its critical role, sexual reproduction is nevertheless infrequent and lineages may be relatively old. It is probable that mutations conferring morphological variation and fungicide resistance will arise in such lineages and, depending on selection, increase. The extent mutation plays in

generating haplotype variation within ascospore lineages needs clarification. Recent findings indicate that other processes such as heteroploidy (including aneuploidy) may be important but exactly how is still unclear (Sect. 3.1), as is the role of extrachromosomal determinants (Sect. 3.3). The impact of agricultural practice on *B. cinerea* genetics has yet to be clarified, but has likely been very significant both in providing large susceptible monocultures and protected environments encouraging proliferation in otherwise non-conducive climates. It remains to be seen whether a 'natural' population of *B. cinerea* can be located, but if so it would likely provide fresh insights into its genetics. In summary, we conclude the *B. cinerea* population comprises a very large number of different ascospore lineages that are both widespread and highly mobile. The ability of most strains to intercross with standard tester strains suggests *B. cinerea*, excluding *B.* 'pseudocinerea', comprises a single species.

4. Genetics of other species of *Botrytis*

In contrast to *B. cinerea*, relatively little is known about the genetics of other species of *Botrytis*. Shirane et al. (1989) reported chromosome numbers of 16 for *B. byssoidea*, *B. squamosa* and *B. tulipae,* while two chromosome numbers were found in *B. allii sensu lato*; 16 for what is now recognised as *B. allii sensu stricto* and 32 for the slightly larger spored *B. aclada sensu stricto* (Sect. 2). Additionally, they noted significant differences in nuclear number per conidium with mean values for *B. allii* and *B. aclada* of 1.3-1.5, of *B. byssoidea* of 5.0-5.1 (similar to *B. cinerea* with values of 4.0-5.1) and *B. squamosa* of 18.4.

Horizontal gene transfer may play a greater role in fungal evolution than in the evolution of other eukaryotes, nevertheless remaining 'difficult to prove beyond reasonable doubt' (Rosewich and Kistler, 2000). The possibility of such transfer has been explored in relation to *B. porri* and *B. elliptica*, as part of a study on possible horizontal gene transfer between species of Sclerotiniaceae (Holst-Jensen et al., 1999). Specifically, these authors proposed that a nuclear rDNA intron might have been transferred from a hypothetical *Myriosclerotinia*-like ancestor to these *Botrytis* species, as well as to species in other genera, including *S. sclerotiorum*. The intron is not present in *B. cinerea*, *B. calthae* or *B. convoluta*. They suggest that the transfer may have been mediated by an RNA or DNA mycovirus moving between the species, and speculate that such gene transfer may be on-going.

4.1. *Botrytis elliptica* and *Botrytis tulipae*

The population genetics of *B. elliptica* has been studied by examining 69 isolates from the USA and Taiwan using 22 polymorphic RAPD markers (Huang et al., 2001). A total of 43 unique haplotypes were identified divided into two clusters, one restricted to Taiwan, the other to the USA. Only a few haplotypes were represented by more than one isolate, mostly but not exclusively from samples collected within one nursery block. Provided that these presumptive clones were excluded, there was little indication of gametic disequilibrium. The findings suggest that sexual reproduction plays a significant role in determining the population structure in this

species, although apothecia have never been observed in the field in the USA or Taiwan. Nevertheless, apothecia have been reported from the field in The Netherlands, albeit with scant details available (Van den Ende and Pennock, 1996; Van den Ende and Pennock-Vos, 1997). Unpublished results with Dutch isolates using multiple gene sequence information and AFLP data confirm *B. elliptica* is highly variable (M. Staats and J. van Kan, Wageningen University, The Netherlands, pers. comm.). In contrast, Dutch isolates of *B. tulipae* showed little variation, suggesting this species is primarily clonal, consistent with the lack of a sexual stage.

4.2. *Botrytis* species from onion

Morphological mutants have been recovered in *B. squamosa* using chemical mutagenesis as well as mutants resistant to the fungicide botran, the latter segregating as a single gene (Bergquist and Lorbeer, 1973). The existence of heterokaryosis in this species, based on the morphology and stability of single-spored conidial cultures (Sun, 1989) has been suggested but, as with *B. cinerea* (Sect. 3.4.2), other interpretations are possible.

Variation of a number of onion-associated species has been investigated using UP-PCR (Nielsen et al., 2001). *B. squamosa* showed high diversity with 10 of 11 isolates showing unique haplotypes, consistent with its known heterothallic sexual reproduction. In contrast, *B. aclada* and *B. allii* (*B. aclada* subgroup AII) showed little variation consistent with a high degree of clonality and the absence of known teleomorphs for these species. While only three isolates of *B. byssoidea* were examined, they were all identical, despite being from the USA, The Netherlands and the UK, suggesting the population may be mainly clonal. Its presumed teleomorph, *Bt. allii,* is only known from Japan where it is associated with a leaf blight rather than neck rot. The precise relationship between these entities remains to be elucidated (Table 1).

4.3. *Botrytis fabae*

This species closely resembles *B. cinerea* but is a specialised pathogen of *Vicia* bean, distinguished by higher pathogenicity, somewhat larger spore size, tendency to produce small sclerotia in culture and protein electrophoresis patterns (Backhouse et al., 1984; Harrison, 1988). Hutson and Mansfield (1980) explored the pathogenicity of 15 different macrocondial lineages from one parent, and found a two-fold difference in lesion diameter hinting at the possibility the original strain was a heterokaryon or heteroplasmon.

5. The future

Botrytis genetics is poised for rapid advances in the next few years, following elucidation of the full nuclear genome of at least one selected strain of *B. cinerea*. While this information will help resolve outstanding questions such as the number of chromosomes, it will leave unanswered many questions in taxonomy, population genetics and ecological and host specialisation. Progress in both taxonomy and genetics of *Botrytis* depends on availability of characterised strains and we make a

plea for workers to deposit strains in recognised culture collections, and where appropriate to include well-studied strains in their work.

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