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Eukaryotic microbes, species recognition and the geographic limits of species: examples from the kingdom Fungi

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The claim that eukaryotic micro-organisms have global geographic ranges, constituting a significant departure from the situation with macro-organisms, has been supported by studies of morphological species from protistan kingdoms. Here, we examine this claim by reviewing examples from another kingdom of eukaryotic microbes, the Fungi. We show that inferred geographic range of a fungal species depends upon the method of species recognition. While some fungal species defined by morphology show global geographic ranges, when fungal species are defined by phylogenetic species recognition they are typically shown to harbour several to many endemic species. We advance two non-exclusive reasons to explain the perceived difference between the size of geographic ranges of microscopic and macroscopic eukaryotic species when morphological methods of species recognition are used. These reasons are that microbial organisms generally have fewer morphological characters, and that the rate of morphological change will be slower for organisms with less elaborate development and fewer cells. Both of these reasons result in fewer discriminatory morphological differences between recently diverged lineages. The rate of genetic change, moreover, is similar for both large and small organisms, which helps to explain why phylogenetic species of large and small organisms show a more similar distribution of geographic ranges. As a consequence of the different rates in fungi of genetic and morphological changes, genetic isolation precedes a recognizable morphological change. The final step in speciation, reproductive isolation, also follows genetic isolation and may precede morphological change.

Keywords: microbial species recognition; microbial geographic range; phylogenetic species recognition; endemism

1. INTRODUCTION

In a series of publications, Bland Finlay & Thomas Fenchel have demonstrated that a large number of morphologically recognized species of free-living microbial eukaryotes smaller than 1–10 mm have global distributions that show no correlation between geographic and genetic distances (e.g. Finlay 2002; Fenchel & Finlay 2004). They contrast the global distributions of these microbial morphospecies with the more narrow endemic ranges of large eukaryotic plants and animals, and postulate how the global distribution of small organisms affects microbial community structure (Finlay & Fenchel 2004).

Finlay & Fenchel's claim of global ranges for eukaryotic microbes echoes the thoughts of Baas-Becking (Baas-Becking 1934) who famously proclaimed, 'Everything is everywhere, the environment selects'. The logic of this claim is that due to the omnipresence of microbes, where the same environment is created in different locations, the same microbial community will develop with the same microbial species. Baas-Becking developed his hypothesis about cosmopolitan species from studies of bacteria in cultivation, and Finlay & Fenchel have extended it from studies of small aquatic animals and protists at two aquatic sites, one freshwater site in the UK and another marine site in Denmark. They have generalized their claim to include all small eukaryotes, both aquatic and terrestrial. Recently, their hypothesis has been challenged with molecular genetic evidence from a variety of microbes (LaChance 2004), including prokaryotes (Whitaker *et al.* 2003), diatoms (Telford *et al.* 2006) and protists (Katz *et al.* 2005; Foissner 2006). Here, we challenge this 'everything is everywhere' hypothesis by reviewing the evidence from a kingdom of small eukaryotes, the Fungi. The data from the fungi show that reliance on morphological species recognition (MSR) can confound assessment of the range of small eukaryotic species. We consider three reasons to account for the observation that fewer microbial species are recognized by morphology compared to measures of genetic or reproductive

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isolation than is the case for macrobes. First, microbes, although morphologically diverse, may have fewer morphological characters than macrobes. Second, microbial morphology is more difficult to assess than macrobial morphology. Third, the rate of change of morphological characters may be greater in macrobes than in microbes. We formulate a testable hypothesis concerning the rate of taxonomic character evolution that helps to account for the disparity in the geographical ranges of morphological species between small and large organisms.

Finlay & Fenchel distinguish between microbes that are free living and those that are not free living due to their symbiotic relationships with other life, whether parasite or mutualist. They exclude those microbes that associate with larger organisms from their hypothesis of global distribution because symbiotic microbes are likely to follow the distribution of the larger partner. Given the many interactions among organisms, one might fairly ask, is there any organism that could be considered free of entanglements with other organisms and thereby free living? These biological interactions constitute the part of the environment which is most susceptible to rapid change, and their change would create differences in otherwise chemically and physically identical environments and narrow the range of seemingly free-living microbes. Only autotrophic organisms can be considered energetically autonomous, but even microscopic algae have their zooplankton predators (McCauley & Murdoch 1990) and parasites, among them viruses (Dunigan *et al.* 2006) and fungi (e.g. *Polyphagus* species; Sparrow 1960, p. 449), whose presence would influence the distribution of each species.

Finlay & Fenchel use morphological characters to recognize species of the small eukaryotes that they study (Fenchel & Finlay 2006). We may ask, can microbial species be recognized solely by morphology or phenotype? Finlay & Fenchel are aware that MSR in microbes has been challenged by other methods, e.g. species recognition by reproductive isolation (biological species recognition, BSR) or by genetic isolation (phylogenetic species recognition, PSR) and make two arguments defending MSR over BSR or PSR (Finlay 2002). First, they cite an example of PSR finding several genetically isolated species in a morphospecies of foraminifera (de Vargas *et al.* 1999), and note that *a posteriori*, the three phylogenetic species were found to have morphologically diagnosable characters. We would argue the contrary that PSR allows biologists to sort individual organisms into species and then to find which of many variable phenotypic characters correlate with the phylogenetic species. The useful phenotypic characters are those that change on the time-scale of the speciation events which define the species. A fungal example is provided by the morphological species, *Aspergillus flavus*. Only after this fungus was shown to embrace at least four phylogenetic species (Geiser *et al.* 1998) was it possible to find diagnostic phenotypic characters for each phylogenetic species (Geiser *et al.* 2000). Second, Finlay & Fenchel also note that members of different sibling phylogenetic species from different geographic regions may still be able to mate (Coleman *et al.* 1994; Stoeck *et al.* 1998), implying that they are truly one biological species. Here, we would argue that geographic separation is an important means of reproductive isolation and that if such populations can be recognized as genetically diverged, they cannot be used to support the hypothesis that 'everything is everywhere'. The fungus *Schizophyllum commune* provides a good example of this point and will be discussed in the fungal examples featured later. Third, Finlay & Fenchel claim that the morphological richness of the protists that they examine is sufficient for species-level taxonomy. We argue that MSR must be challenged by methods of species recognition that measure genetic or reproductive isolation. Microbial eukaryotes may have many morphological features, but they neither have as many morphological characters as do larger eukaryotes nor are the characters as easily observed. Equally important is the rate of change of morphological characters, which must be appropriate for species recognition. If the rate of change of morphological characters is slow when compared with genetic or reproductive isolation, fewer species will be recognized by morphology and their ranges will appear to be larger.

2. THE FUNGI, A KINGDOM OF MICROBIAL EUKARYOTES

The Fungi, a large and important kingdom, has not yet been considered in this debate over global distribution of microbial eukaryotes. The fungi, however, provide organisms to specifically test the hypothesis of Finlay & Fenchel. First, there are large numbers of saprobic (free living) fungi that inhabit a wide range of environments. Second, almost all fungi have propagules under 1 mm (in fact, almost all spores are two orders of magnitude smaller than this limit), and many exhibit structures adapted to promote wide dissemination of those propagules. Third, although mycologists have traditionally used MSR and BSR to infer the taxonomy of fungi, in no other group of microbial eukaryotes has more effort been devoted to species recognition by phylogenetic methods using nucleic acid variation than in fungi (Taylor *et al.* 2006). Fungi, therefore, provide an ideal group of organisms in which to compare MSR, BSR and PSR. Here, we challenge the hypothesis put forward by Finlay & Fenchel and address the question, are there endemic species of microbial eukaryotes? After a brief introduction to the aspects of fungi germane to the debate over microbial species, we will present case histories of species recognition from five familiar genera of free-living fungi. In four cases, phylogenetically recognized species occupy less than global ranges (*Neurospora* and *Saccharomyces* from the Ascomycota and *Schizophyllum* and *Lentinula* from the Basidiomycota). The fifth example is the only fungus known to have a global distribution, the Ascomycete, *Aspergillus fumigatus*.

(a) *Size of fungi*

Finlay & Fenchel (2004) have proposed that the division between micro- and macrobiota lies within the size range of 1–10 mm, and that organisms smaller than 1 mm are likely to have a global distribution (Finlay 2002). In large animals, gene flow requires

migration of the entire individual. In sedentary macrobes, bryophyte or ferns, for example, gene flow may be accomplished by microscopic spores. In flowering plants, it may occur via pollen and seeds. In fungi, gene flow is accomplished by microscopic spore dispersal. Some individual thalli of mushrooms species are as large as whales or trees (Smith *et al.* 1992), but almost all fungi produce spores (meiotic and mitotic propagules), most of which are *ca* 10 μm in their longest dimension. Even larger spores have been shown to be capable of long-distance dispersal; for example, mitospores of the plant pathogen *Puccinia graminis* (not smaller than 26×16 μm; Cummins 1971) can travel from South Africa to Australia (Watson & De Sousa 1983), and mitospores of another plant pathogen, *Blumeria graminis* (not smaller than 24×12 μm; Braun 1995) can cross the North Sea from continental Europe to Britain (Brown *et al.* 1991). In many fungi, spores function as gametes, so partners in mating need not be in close proximity. The smallest fungi are yeasts (Kurtzman & Fell 1998), e.g. *Saccharomyces cerevisiae*, and yeasts and their spores are single cells with a largest dimension not greater than 10–15 μm. In addition, on the small side for fungi are the monocentric Chytridiomycota, whose swimming spores are not larger than the yeasts, and whose sporangia can have diameters as large as 100 μm; even these thalli are still an order of magnitude smaller than 1 mm (Sparrow 1960). The remaining fungi, filamentous Chytridiomycota, Zygomycota, Glomeromycota, Ascomycota and Basidiomycota, make vegetative colonies larger than 10 mm in diameter (especially in culture), but they again have spores that are all much smaller than 1 mm. These examples highlight the fact that the small size and high dispersability of propagules allow for the possibility of global distributions of fungi. However, as discussed later, this potential is rarely realized.

(b) *Fungal reproduction*

Reproductive mode (clonal or recombining) has been thought to be important to speciation by many authors (Fisher 1930; Mayr 1957; Maynard Smith & Szathmary 1995; Coyne & Orr 1998). Recently, Barraclough *et al.* (2003) considered theoretical aspects of speciation by organisms that can recombine or that are strictly clonal to see if there were any fundamental differences. They conclude that both clonal and recombining organisms will form species, but that recombining organisms may form them faster because they respond more rapidly to selection (Goddard *et al.* 2005). Barraclough *et al.* (2003) note that speciation involves not only divergence, but also maintenance, particularly when newly derived species coexist. Here, the most important factor for species maintenance is the adaptation of newly diverged species to different niches; without this adaptation, they cannot coexist whether they are clonal or recombining. For species maintenance in recombining organisms, differential adaptation of coexisting populations must be accompanied by reproductive isolation.

Although species will form in both recombining and clonal organisms, there are aspects unique to each reproductive mode (Barraclough *et al.* 2003). Sexual reproduction with some outcrossing maintains cohesion within species, whereas reproductive isolation leads to the formation of new species (Coyne & Orr 1998). Recombination has been shown to facilitate more rapid adaptation than clonality (Goddard *et al.* 2005) and recombining organisms may show larger and more phenotypic differences among species. These effects may increase the chance that newly diverged species will have adapted to different niches and be able to coexist, provided that they are reproductively isolated in sympatry (Felsenstein 1981; Barraclough *et al.* 2003). On the other hand, clonal organisms can expand their population rapidly and may be better adapted to unpredictably hospitable habitats, e.g. the apparently asexual bdelloid rotifers occupy habitats more prone to unpredictable drought than do their sexual relatives (Ricci 1987; Barraclough *et al.* 2003). However, clonal organisms should have a higher extinction rate than recombining organisms because they are slower to respond to changing environments (Goddard *et al.* 2005) and because they are less able to purge their genomes of deleterious mutations (Muller 1964).

For many fungi, it is not a question of whether they reproduce by recombination or clonality, because they can do both. Morphological observation, alone, cannot be used to determine reproductive mode. Recombined progeny result from the sexual processes of mating and meiosis, but progeny of uniparental genetic origin (i.e. functionally clonal) can also result from sexual reproduction by self-fertilization, as well as by clonal reproduction. To make determinations of reproductive mode even more difficult, often fungi that never have been observed to undergo sexual reproduction nevertheless have populations consistent with recombination (Taylor *et al.* 1999). In fact, only one fungus that lacks the morphology of sexual reproduction has been shown to be exclusively asexual and clonal by population genetic criteria, *Penicillium marneffei* (Fisher *et al.* 2005; Vanittanakom *et al.* 2006). Many fungi that generally self-fertilize (i.e. homothallic and pseudohomothallic species) also retain the ability to outbreed and, therefore, to produce a combination of clonal and recombined progeny. Additionally, in a single species, clonal and recombining reproduction can be temporally or spatially separated. In some fungi causing disease of crop plants, reproduction is clonal in agricultural environments, but elsewhere populations retain the ancestral capacity for sexual recombination (Taylor *et al.* 1999; Couch *et al.* 2005). Clonal or inbreeding fungi should be able to invade more distant regions more easily because only one individual is needed to establish a population. Thus, in terms of endemism, one might expect that these fungi would have larger distributions than their obligately outbreeding relatives. Two of the fungi that we will examine can produce both meiotic and mitotic spores (*Neurospora* and *Saccharomyces*), two produce only meiotic spores (*Schizophyllum* and *Lentinula*), one makes only mitotic spores (*A. fumigatus*) and two can both self- and cross-fertilize, *S. cerevisiae* and *Neurospora tetrasperma*.

(c) *Fungal species recognition*

Fungal species are recognized by phenotype (MSR), reproductive isolation (BSR) and genetic isolation (PSR;

Taylor *et al.* 2000). MSR is the default means of recognizing fungal species because every properly named fungus is accompanied by a morphological description. The morphological characters have been augmented by other phenotypic characters for fungi of simple morphology (e.g. substrate utilization in yeasts; Kurtzman & Fell 1998) or for those taxa that are industrially important (e.g. water availability of the substrate and growth temperature in *Penicillium*; Pitt 1979). Reproductive isolation is the basis of BSR, which is assessed by mating tests and has been employed with fungi that can be induced to mate in cultivation. However, BSR has not been widely applied because less than 15% of fungi can be cultivated (P. Crous 2004, personal communication), and of these many cannot be induced to mate. PSR depends on evidence of genetic isolation, which is provided by analysis of the congruence of genealogies based on DNA sequence of appropriately polymorphic loci from a sufficient sampling of individuals (Avise & Wollenberg 1997). PSR has been widely used for fungi, including those that cannot be cultivated, e.g. powdery mildews (Adam *et al.* 1999) or rusts (Weber *et al.* 2003).

(d) *Fungal case studies*

(i) Neurospora

Outcrossing species in the genus *Neurospora* are associated with recently burned vegetation, or food or food waste that has been heated. *Neurospora* species have not been shown to prefer particular plant species (Perkins *et al.* 1976; Perkins & Turner 1988; Turner *et al.* 2001; Jacobson *et al.* 2004) and they are considered to be free-living heterotrophs. The early publications on these fungi describe four species, three with eight spores per ascus and one with just four (Shear & Dodge 1927; Tai 1935). Although the pioneering descriptions of the *Neurospora* species were morphological, it is important to note that both Shear and Dodge, and Tai, conducted mating studies of *Neurospora* individuals prior to making their morphological descriptions. The clear results of the mating studies likely influenced their ostensibly morphological and taxonomic decisions.

The morphological features recorded by these early authors from small numbers of individuals were challenged by studies of many more individuals by Perkins *et al.* (1976),

> The conidiating species of Neurospora cannot be distinguished from one another by vegetative appearance… Taxonomic diagnosis of the 8-spored heterothallic species [the one 4-spored pseudohomothallic species, *N. tetrasperma*, is readily recognized] has stressed the size and shape of ascospores, asci and perithecia. Dimensions taken from the literature and from our measurements, however, show that there is a considerable overlap in size between species, and certainly extensive variability within species. In several strains we have noted genotypes that result in completely misleading measurements…

Instead of morphology, Perkins and colleagues, in this and subsequent publications (Perkins & Turner 1988; Turner *et al.* 2001), advocated the use of mating tests to pairs of tester strains (one for each of the two mating types, designated *mat A* and *mat a*) to assign individuals to species. This criterion was used to describe a new outbreeding species, *Neurospora discreta* (Perkins & Raju 1986). Therefore, the eight-spored, outbreeding *Neurospora* species would be considered one circumglobal, tropical and subtropical species if recognized solely by morphology. Under BSR using the tester strains, however, each of the four eight-spored species have their own biogeography, which will be discussed later.

Recently, Dettman *et al.* (2003*a*,*b*) compared methods of fungal species recognition in the five outbreeding *Neurospora* species recognized by mating to tester strains (*Neurospora crassa*, *Neurospora sitophila*, *Neurospora intermedia*, *N. tetrasperma* and *N. discreta*). From a collection of more than 5000 natural isolates, Dettman *et al.* selected *Neurospora* individuals to represent all the five outbreeding species with emphases on two species, *N. crassa* and *N. intermedia*, and four geographic locations, the Caribbean basin, Africa, India and Asia. For PSR, they compared gene genealogies for four highly polymorphic loci that were sequenced from 147 individuals. For BSR, they compared reproductive success in most of the possible matings for 73 of the individuals used for PSR. PSR found not only the five described species, but also three additional genetically isolated species with few members and narrow endemic ranges, e.g. phylogenetic species 1 (PS1) with three individuals from Haiti, PS2 with seven individuals (five from Yucatan and two from Madagascar), and PS3 with five individuals from Congo. For BSR, Dettman *et al.* (2003*b*) focused on just *N. crassa* and *N. intermedia*, and the three new phylogenetic species, PS1–PS3. From matings among the 73 individuals representing these species, they found that PSR and BSR identified almost the same species; the only difference was that PSR found PS3, but with BSR, PS3 was indistinguishable from *N. crassa* (figure 1*a*). In other words, PS3 was genetically isolated from all other *Neurospora* species and was reproductively isolated from all species except *N. crassa*. The comparison among methods of species recognition with *Neurospora* showed that MSR alone found two species (one with eight ascospores per ascus and one with four), BSR found seven species and PSR found eight (consistent with the observation that genetic isolation precedes reproductive isolation in fungi). The amount of genetic variation seen within the seven species was similar, except for *N. discreta*, which was the most basal of the outbreeding *Neurospora* species and which had as much variation as measured by nucleotide polymorphism as the other species combined (Dettman *et al.* 2003*a*).

The exceptional variation found in *N. discreta* stimulated studies of species recognition in this taxon (Dettman *et al.* in press). *Neurospora discreta*, like the other *Neurospora* species, had been considered a tropical and subtropical species until its discovery in temperate western North America ranging from New Mexico to 63° N latitude in Alaska (Jacobson *et al.* 2004). Found beneath the bark, or visible through fissures in the bark, on recently burned woody shrubs and trees, this fungus demonstrates that biologists can fail to perceive even abundant and distinctive microbes. Not only is it common after forest fires in western

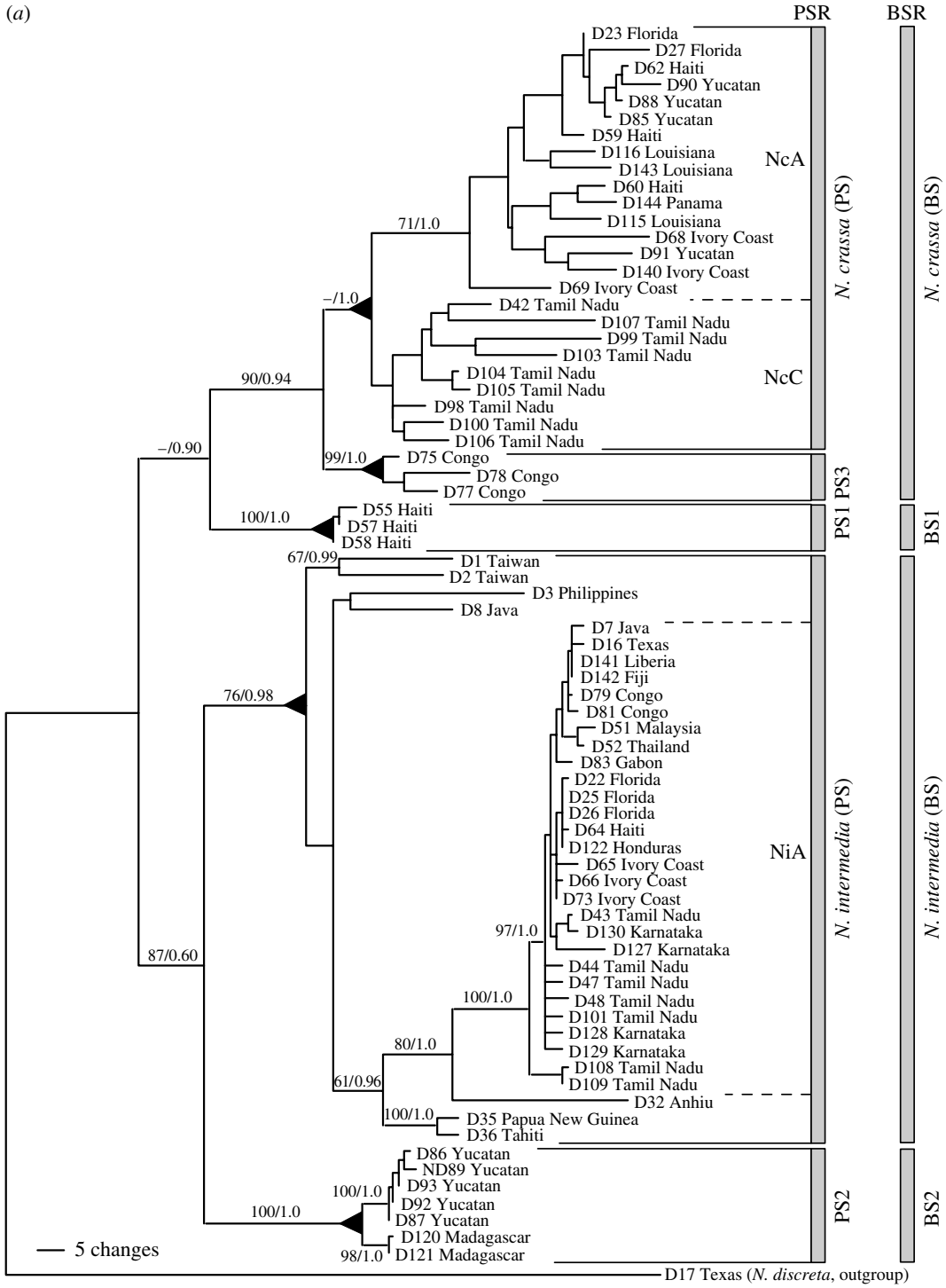

Figure 1. (*a*) Phylogenetic species recognition applied to *Neurospora* and a graphic comparison to biological species recognition applied to the same individuals. Maximum-parsimony (MP) phylogram produced from the combined analysis of DNA sequences from four anonymous nuclear loci (TMI, DMG, TML and QMA loci, a total of 2141 aligned nucleotides). Tree length =916 steps; consistency index =0.651. Labels to the right of the phylogram indicate groups identified by phylogenetic species recognition and biological species recognition. Triangles at nodes indicate that all taxa united by (or distal to) a node belong to the same phylogenetic species. Taxon labels indicate strain number and geographic source. Branch support values for major branches with significant support are indicated by numbers above or below the branches (MP bootstrap proportions/Bayesian posterior probabilities). Figure and legend adapted from Dettman *et al.* (2003*b*) with permission of the authors and publisher. (*b*) Summary of results of phylogenetic species recognition in *Neurospora*. Neighbour-joining phylogram produced from three loci combined (DMG, TMI and TML) using exemplars of described species, and new phylogenetic species of *Neurospora*. Figure and legend adapted from Dettman *et al.* (in press) with permission of the authors and publisher.

North America, but also it is almost the only *Neurospora* species found there; of 500 individuals collected in western North America, 95% were assigned to *N. discreta* by mating tests (Jacobson *et al.* 2004). Subsequent to the discovery in North America, *N. discreta* was found in southern Europe, again associated with recently burned vegetation (Jacobson *et al.* in press). Rather than being the predominant *Neurospora* species, *N. discreta* was found along with similar numbers of individuals of *N. crassa*, *N. sitophila*

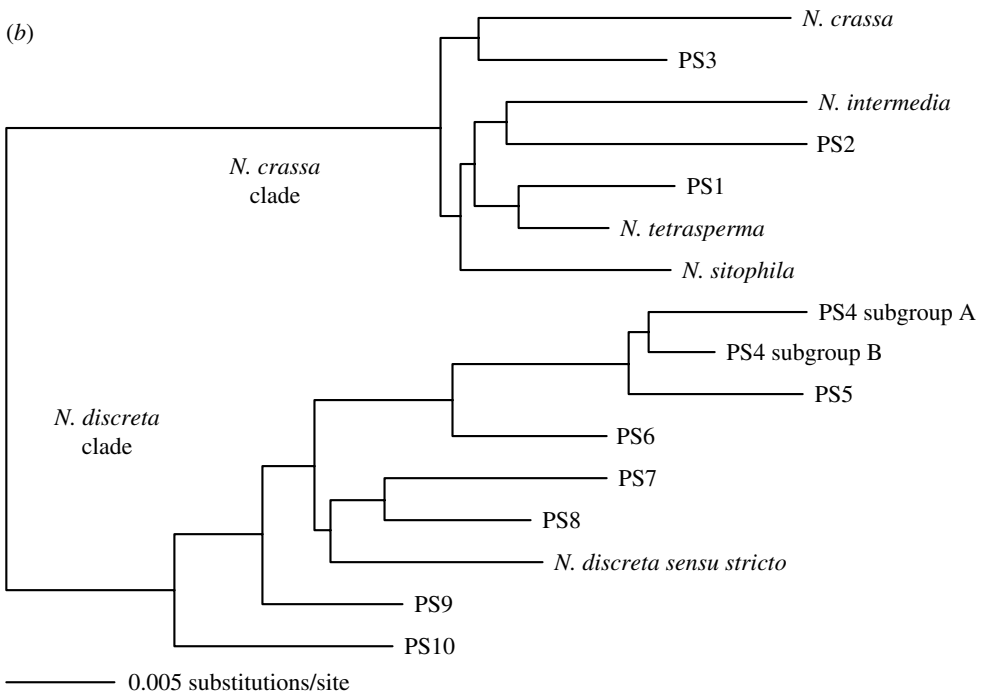

Figure 1. (*Continued.*)

and *N. tetrasperma*, extending the range of these species well into the temperate zone. However, *N. intermedia* was not found in these surveys, and although some historic sources place it in Western Europe (see Jacobson *et al.* in press), it is clearly absent from western North America. PSR was applied to 70 individuals assigned to *N. discreta* by mating tests (BSR), including individuals from the recently discovered temperate regions. These analyses show that this single biological species contains eight phylogenetic species that have intraspecific and interspecific variation similar to the previously recognized phylogenetic species (Dettman *et al.* in press; figure 1*b*).

How has the use of PSR altered our understandings of *Neurospora* biogeography? Remember that by MSR, the eight-spored *Neurospora* species would form a single species with a circumglobal tropical and subtropical distribution. Using BSR based on mating to tester strains, Perkins and colleagues recognized four, eight-spored species with the following distributions (Perkins & Turner 1988; Turner *et al.* 2001). *Neurospora intermedia* had the broadest, being found in tropical and subtropical regions of Asia, the New World, Africa, India and Australia. *Neurospora sitophila* had a similar distribution, but was not as commonly encountered in Asia. *Neurospora crassa* was not found in East Asia or in Australia. *Neurospora discreta*, while encountered much less frequently than the other species, had a distribution most similar to *N. crassa*, but with rare occurrences in Australasia.

The biogeography of the two species that received most attention in the Dettman *et al.* study, *N. intermedia* and *N. crassa*, was consistent with the results of Turner *et al.* (2001) in that both fungi are centred in the tropics and subtropics, but only *N. intermedia* could be found at higher latitudes in Asia (Turner *et al.* 2001) and only *N. crassa* could be found at higher latitudes in North America and Europe (Jacobson *et al.* 2004, in press). In addition, *N. crassa* was missing from Asia (Perkins *et al.* 2001). Although *N. intermedia* and *N. crassa* would be one morphological species with a global distribution, by BSR or PSR, they were shown to be two species, each with a different geographic range. While it is true that inferences about geographic distributions of species are dependent on the quality of the sampling, given that sampling in Asia has been thorough enough to find representatives of four outbreeding species, the absence of *N. crassa* from Asia is difficult to dispute.

Dettman *et al.* (2003*a*) also found three new phylogenetic species, PS1, PS2 and PS3, which have narrow geographic distributions: Haiti, Yucatan and Madagascar, and Congo, respectively. When more individuals of PS2 become available, it is likely that distinct *Neurospora* phylogenetic species will be recognized in Yucatan and Madagascar. Here, again, applying MSR to these fungi would obscure the existence of the three, narrowly endemic, phylogenetic species. Admittedly, ranges inferred from small numbers of individuals are likely to be underestimates. However, given the global scope of individuals in the dataset, the restricted biogeography of the new phylogenetic species is suggestive of narrow endemism.

In the *N. discreta* clade, examination of the biogeography of phylogenetic species shows that no single phylogenetic species has a global distribution; in general, however, sampling is insufficient to make definitive claims about the complete distributions of the eight phylogenetic species. Nevertheless, sampling in western North America is sufficient to argue that PS4, alone, dominates western North America (Jacobson *et al.* 2004; Dettman *et al.* in press) and that none of the seven other species are likely to be found in that region (figure 1*b*). Therefore, the *N. discreta* complex represents another group of phylogenetic species with less than global distributions that would be considered to be part of one global

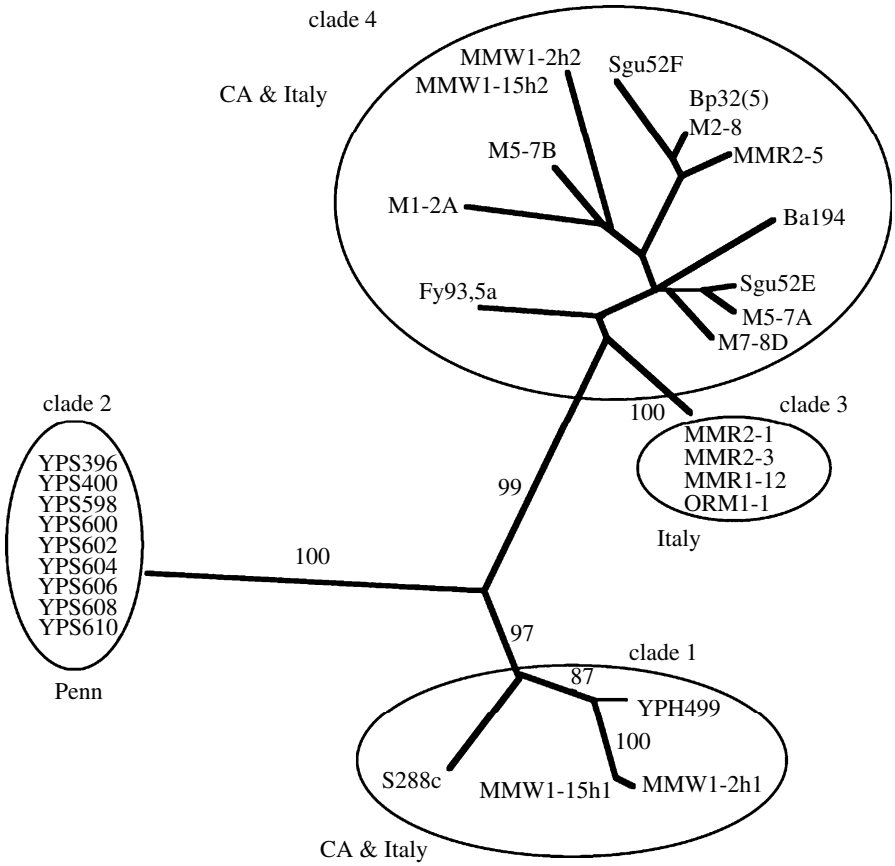

Figure 2. Phylogenetic species recognition applied to *Saccharomyces cerevisiae*. Unrooted distance trees based on four loci combined (*CDC 19*, *FZF1*, *SSU1* and *PHD1*). Support of internal branches given as bootstrap percentages from 10 000 resamplings of the data. Construction of trees from the same data using the parsimony optimality criterion yielded trees with essentially the same topology. Figure and legend adapted from Aa *et al.* (2006) with permission of the authors and publisher.

species under MSR or BSR. Dettman *et al.* not only provide additional evidence that *Neurospora* refutes the 'everything is everywhere' hypothesis, but also show that biogeographical inference is very sensitive to methods of species recognition. In sum, what would be two nearly global morphological *Neurospora* species, one four-spored and one eight-spored, proved to be 15 phylogenetic species, none of them truly global.

(ii) Saccharomyces

The second group of free-living ascomycetes that we will discuss is the yeast of commerce, *S. cerevisiae*, and its closest relative, *Saccharomyces paradoxus*. Unicellular and not larger than 10–15 μm in diameter either as a vegetative cell or a sexual spore (Kurtzman & Fell 1998), these yeasts certainly qualify as microscopic eukaryotes. Morphologically, all species in the group are indistinguishable (Naumova *et al.* 2003), but were shown by BSR to consist of several reproductively isolated species (Naumov 1987, 1996; Naumov *et al.* 2000). Although the geographic distributions of these yeast species are not as well studied as those of *Neurospora*, an examination of the best-known member, *S. cerevisiae*, and its closest relative, *S. paradoxus*, challenges the concept of global fungal species. Mating tests between *S. cerevisiae* and *S. paradoxus* demonstrate reproductive isolation (Sniegowski *et al.* 2002). *Saccharomyces cerevisiae* has been found associated with agriculture, in particular with grapes and wine production, and both *S. cerevisiae* and *S. paradoxus* are found associated with oak trees in natural environments (Sniegowski *et al.* 2002; Johnson *et al.* 2004).

Aa *et al.* (2006) recently applied PSR to a collection of *S. cerevisiae* individuals obtained from laboratories, agriculture, wineries and oaks. The laboratory and winery or vineyard isolates were from Italy and California, the oak isolates were from Pennsylvania. The Pennsylvania *S. cerevisiae* isolates had been shown by BSR to be conspecific with *S. cerevisiae* individuals from Europe (Sniegowski *et al.* 2002). Analysis of DNA sequence from four loci (6600 nucleotides) from 27 individuals showed that the Pennsylvania oak individuals were genetically isolated from the California and the Italian isolates, whereas the latter formed three clades, two of which contained isolates from both wine-producing regions (figure 2). A plausible interpretation of the data is that *S. cerevisiae* isolates involved in commerce have been moved between California and Italy, but that oak individuals have not. The genetic isolation of Pennsylvanian *S. cerevisiae* from populations in Europe or California shows that this small, free-living eukaryote has genetically isolated populations. Whether or not the population structure in *S. cerevisiae* correlates with geographic distance, or with ecological factors such as substrate, is not yet known. It is certain, however, that it is not one global, phylogenetic species, and that again, everything is not everywhere.

Saccharomyces paradoxus provides a similar story. First, what was considered to be one biological species was shown by allozyme analysis to consist of two genetically

differentiated populations, one in Europe and the other in Far East Asia (Naumov *et al.* 1997). Second, collections from North America that had been identified as *S. paradoxus* by mating to tester strains were found to be reproductively isolated from European *S. paradoxus* individuals (Sniegowski *et al.* 2002). Recently, Koufopanou *et al.* (2006) obtained DNA sequence from six genes in 112 individuals from the United Kingdom and Europe, Japan and Canada. The Canadian population showed 5% nucleotide divergence from the European and Asian populations, the latter pair were separated by 1.5% nucleotide divergence (the same as human and chimp). No shared polymorphisms were found among the three populations. For *S. paradoxus*, what would appear to be a single species with a near global distribution under MSR is revealed to be three species under PSR, each occupying a different continent.

(iii) Schizophyllum

One of the best-studied, free-living Basidiomycota in terms of natural variation and the distribution of alleles conferring mating compatibility is the genetic model organism, *S. commune*. This decay fungus has been found to decompose more than 150 different plant species throughout the world and it has basidiospores not larger than 8 μm in any dimension (Cooke 1961). Based on BSR, and also on morphology, *S. commune* has a global distribution (Raper *et al.* 1958).

James *et al.* (1999) characterized 11 polymorphic allozymes for a collection of 136 individuals from North America, Central America, South America, Africa, Australasia and Europe. While alleles at either of the two mating loci showed no subdivision of the global *S. commune* population (James *et al.* 1999), allozyme analysis showed genetic differentiation between populations in the Eastern and Western Hemispheres (James *et al.* 1999). Again, population studies suggest that everything is not everywhere. The most recent examination of *S. commune* employed nucleic acid variation (James *et al.* 2001). In this study, the most variable region of the nuclear ribosomal repeat, the intergenic spacer (IGS) was sequenced for 195 individuals from Africa, Asia, Australasia, Europe and in the New World, North, Central and South America. Phylogenetic analysis of IGS variation showed three well-supported clades, one embracing isolates from North and Central America (NAM), a second with isolates from South America (SAM) and a third with the European and Asian/Australasian individuals (EAS; figure 3). James *et al.* also found evidence of recent migrations, both from South America to the Caribbean, and from Europe to western North America. Based on the fact that *S. commune* lives on cut logs, the authors speculate that migration and population expansion of *S. commune* may be tied to human activity (James *et al.* 2001). Again, what was thought to be one species by MSR or BSR, and perhaps two species by allozyme analysis, proved to be three, geographically distinct species when the rapidly evolving characters underlying nucleic acid polymorphism were used to implement PSR.

James & Vilgalys (2001) then investigated *S. commune* spore dispersal directly by sampling basidiospores in the air at four locations bridging the

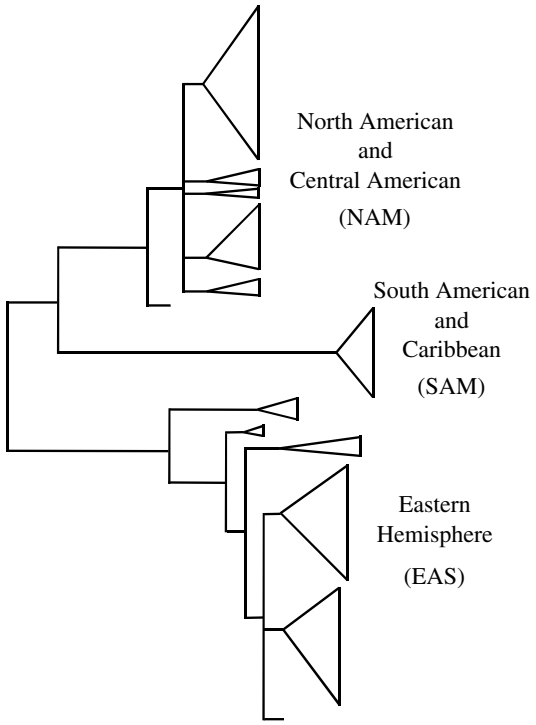

Figure 3. Phylogenetic species recognition applied to *Schizophyllum commune* based on IGS1 sequence data and heuristic parsimony analysis. Heuristic searches found 9700 equally parsimonious trees. Cartoon of one of 9700 equally parsimonious trees is shown (tree length = 285 steps; CI = 0.795). Clades are represented by triangles and large clades, NAM, SAM and EAS, represent phylogenetic species. Detailed trees with branch support are to be found in James *et al.* 2001. Figure and legend modified from those in James *et al.* (2001) with permission of the authors and publisher.

South American–Caribbean and North American–Central American populations: Guyana, Puerto Rico, Bahamas and Florida. To 'trap' basidiospores of *S. commune*, they exposed, overnight, at each location *ca* 25 cultures of an unmated, haploid individual from the North American–Central American species. Next, they examined the cultures for evidence of fertilization by spores settling from the air. The mated colonies were then genotyped using four polymorphic loci and, knowing the genotype of the haploid strain proffered as bait, the haploid genotype of the fertilizing spore was deduced for 199 matings. From these data, they estimate that 18 *S. commune* spores impact each square metre of land per hour. Moreover, the genotype of the spores trapped at each location matched the genotypes of local individuals better than those from distant locations, although the neighbour-joining analysis used to examine this question did not include a test of significance. They did not find evidence of spores traversing the Caribbean Sea from South America to Florida.

These findings dispute the hypothesis that the airborne spore population encompasses all available genotypes across locations and indicate that spores, like individuals on cut logs, show population subdivision (James & Vilgalys 2001). Although more distant genotypes could be present in the airborne spores, perhaps at densities too low to be detected by the spore-trap assay, there is no evidence from resident

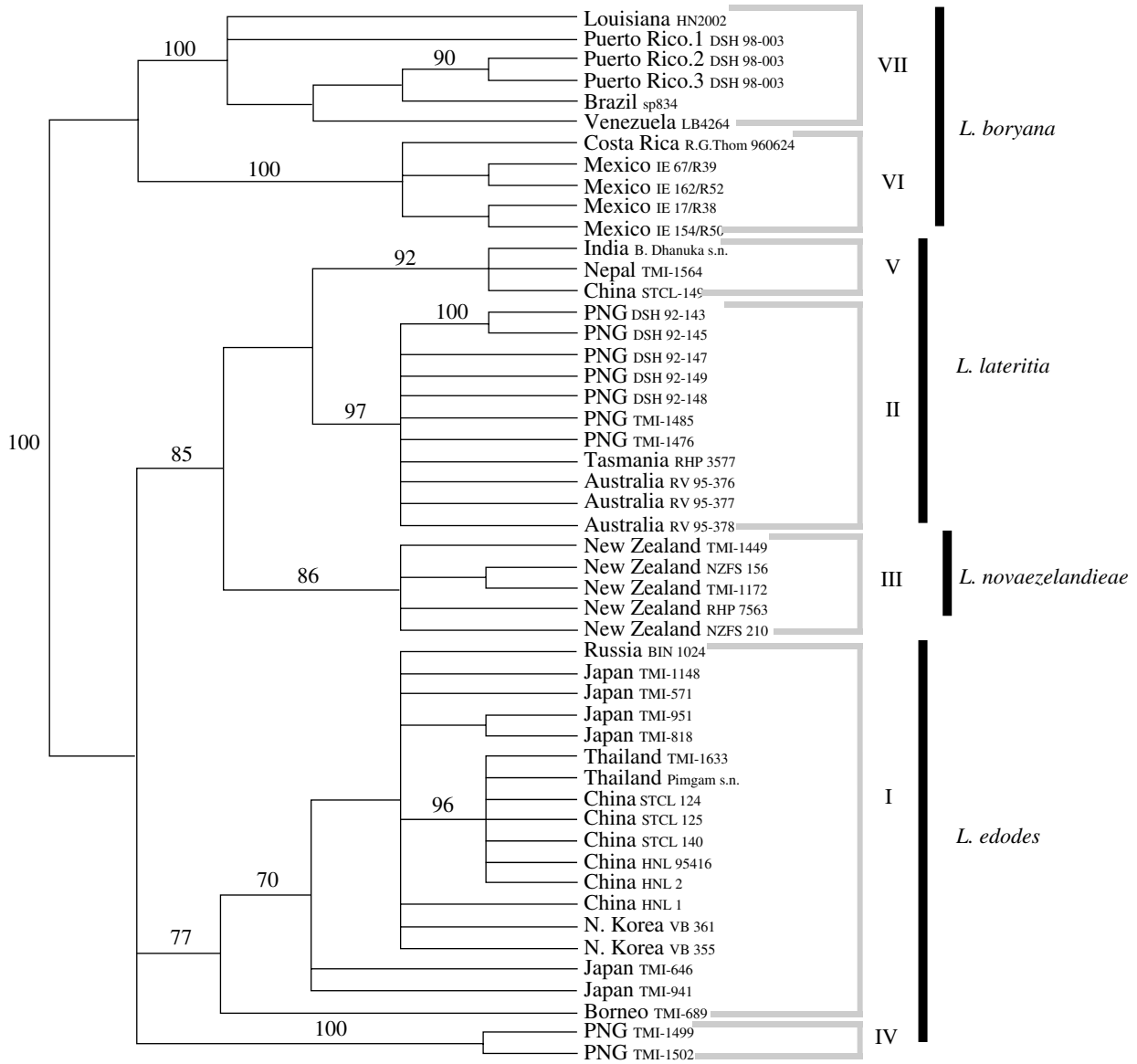

Figure 4. Phylogenetic species recognition applied to *Lentinula* based on ITS sequences. Strict consensus of 3000+ most parsimonious trees (tree length =234 steps; CI=0.861). Bootstrap values greater than 70% are shown above the branches. Numbered groups are phylogenetic species of *Lentinula*. Morphologically recognized species names are at right. PNG=Papua New Guinea. Figure and legend based on those in Hibbett (2001) with permission of the authors and publisher.

individuals that distant genotypes become incorporated into local populations.

The taxonomical history of *S. commune*, initially described as a morphological species of global distribution and then revealed to consist of several biological or phylogenetic species of more narrow distribution, is typical of many mushroom-forming Basidiomycota (Petersen & Hughes 1999). Many of these fungi are symbiotic, either as pathogens (*Armillaria*, Korhonen 1978; Anderson & Ullrich 1979; Anderson *et al.* 1980; *Heterobasidion*, Chase & Ullrich 1990; Johannesson & Stenlid 2003) or as mycorrhizae (*Rhizopogon*; Kretzer *et al.* 2003), but others are decay fungi, and provide additional tests for endemism in free-living fungi.

(iv) Lentinula

Another carefully studied example is provided by the genus *Lentinula*, which contains several species of wood decay fungi including the well-known edible mushroom, *Lentinula edodes*, better known as shiitake. Morphological study of this fungus had resulted in the description of four convincing species of *Lentinula*, three in the Old World and one in the New World (Pegler 1983). After analysing the sequence for rapidly evolving parts of the rDNA repeat from a worldwide collection including members of all four species, Hibbett (2001) concluded that only one of the four morphological species, *Lentinula novaezelandieae*, was truly a single, phylogenetic species (figure 4). Each other morphological species contained two phylogenetic species apiece. The two phylogenetic species found in the New World morphological species, *Lentinula boryana*, were distributed similarly to the phylogenetic species of *S. commune*, one in South America and the Caribbean, the other in Mexico and Central America (Hibbett 2001).

These four examples of fungi that have one broadly distributed morphological species, but two or more biological or phylogenetic species, each with a more narrow distribution, show that small eukaryotes in the

fungal kingdom can be endemic like their larger eukaryotic relatives in the plant or animal kingdoms, but that endemism can be perceived only when fungal species are characterized by BSR or PSR. When compared directly in fungi, the fewest species are recognized by MSR, more by BSR and the most by PSR. In this kingdom, genetic isolation precedes reproductive isolation and both of these precede morphological divergence. As mentioned earlier, the key differences between microbes and macrobes that underlie this difference in species recognition involve the number, ease of observation and rate of change of morphological characters.

(e) *Maintenance of species*

This discussion about the absence or presence of endemism in free-living microbes has focused on the pattern of species and not on the processes of species formation and maintenance. Finlay notes that phylogenetic species may not be reproductively isolated, and therefore might still be considered as one species (Finlay 2002). We would argue that phylogenetic species isolated only by geography (but not by sexual incompatibility) have independent evolutionary trajectories and will inevitably evolve reproductive isolation barriers, if geographic isolation persists (Coyne & Orr 2004). As Kohn (2005) has pointed out, the process of microbial speciation may be best studied experimentally. However, one can make inferences about the roles of geographical and biological isolation barriers in the maintenance of microbial species from studies of natural populations. In the study comparing PSR and BSR in *Neurospora* (Dettman *et al.* 2003*b*), the hybrid matings among *N. crassa*, *N. intermedia*, PS1 and PS2, shed light on the barriers to reproduction that maintain species. These barriers can be intrinsic or extrinsic. Intrinsic barriers lead to no progeny or the inviability or infertility of hybrid progeny. Extrinsic barriers are contingent on geographic or environmental parameters and range from the inability to mate due to geographic separation (a very early barrier to mating) to the inability of a viable hybrid progeny to compete favourably with either parent (a very late-acting barrier).

In the course of their study of BSR in *Neurospora*, Dettman *et al.* (2003*b*) made an interesting observation about reproduction and geography. In addition to the obvious result that reproductive success in heterospecific *Neurospora* matings was significantly lower than in conspecific matings, they found that hybrid matings between geographically separated (allopatric) partners were more successful than those between partners living in the same location (sympatry), and that the allopatric pairings proceeded significantly further in the mating pathway. The appropriate scale of geographic separation at which populations can be considered allopatric is dependent on the biology of the organism; for example, separation sufficient to preclude matings among moles would hardly suffice for gulls. Since the geographic separation needed to achieve allopatry is unclear in fungi, analyses were conducted with sympatry defined at three different scales: the regional scale (i.e. Caribbean, Africa, India or Asia), the sub-regional scale (e.g. Florida, Tamil Nadu or Ivory Coast) and the scale of single collecting sites. At all scales, reproductive barriers were significantly stronger between the sympatric pairs. When the percentages of matings that reached each of the seven stages in the mating pathway were compared for sympatric and allopatric matings (figure 5), it was apparent that the most important barriers were post-fertilization and that far more sympatric pairings than allopatric pairings aborted before significant investment was made in sexual structures. The presence of strengthened barriers to mating between sympatric members of different species provides clear evidence that when extrinsic barriers are absent, stronger intrinsic barriers help to keep *Neurospora* individuals from forming one global panmictic population.

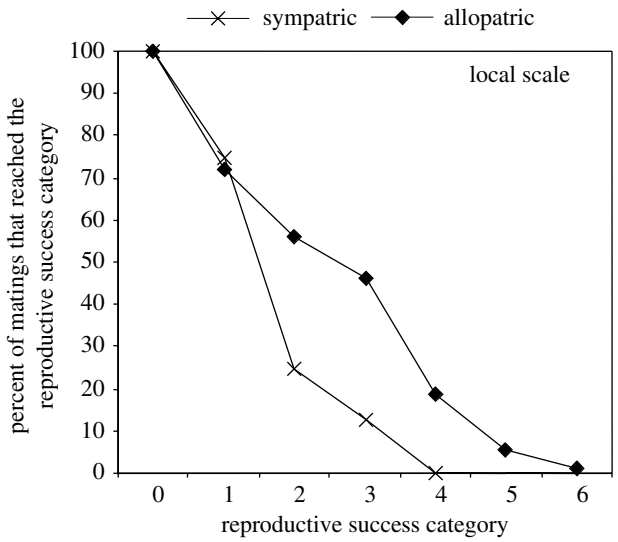

Figure 5. *Neurospora* mating success for sympatric and allopatric heterospecific pairings: the percentage of heterospecific matings between allopatric or sympatric individuals that reached the successive categories of reproductive success. At all geographic scales (regional, sub-regional and local), allopatric matings were significantly more likely than sympatric matings to proceed through the consecutive stages of the sexual cycle. The discrepancy between the allopatric and sympatric curves increases as the scale of sympatry decreases and is most pronounced for local sympatry, shown here. Categories of reproductive success: 0, no response; 1, aborted perithecia; 2, perithecia with no ascospores ejected; 3, perithecia with less than 1% of ejected ascospores having strongly melanized (i.e. black) walls; 4, perithecia with less than 15% black ascospores; 5, perithecia with less than 50% black ascospores; 6, perithecia with more than 50% black ascospores. Figure and legend modified from Dettman *et al.* (2003*b*) with permission of the authors and publisher.

The mixture of extrinsic and intrinsic barriers appears to be very effective; no naturally occurring hybrid *Neurospora* individuals have been found in the collection of *ca* 3000 *N. crassa* and *N. intermedia* individuals. Furthermore, when all matings conducted by Dettman *et al.* (2003*b*) were considered, both conspecific and heterospecific, a negative correlation was detected between geographic distance and mating success. Although the presence of conspecific matings in the analysis confounds the issue, the negative correlation is driven by the effect of geography on heterospecific matings. If microbes truly had global ranges, then no amount of geographic separation could

suffice to make microbial populations allopatric and one would not see a negative correlation between geography and mating success.

Although hybrid individuals of *Neurospora* have not been sampled from nature, hybrids are well known from studies of plant pathogenic fungi (Brasier 2000; Schardl & Craven 2003): *Fusarium* (O'Donnell *et al.* 2000, 2004), *Botrytis* (Nielsen & Yohalem 2001), *Neotyphodium* (Schardl & Craven 2003; Moon *et al.* 2004), *Ophiostoma* (Brasier *et al.* 1998; Konrad *et al.* 2002), *Melampsora* (Newcombe *et al.* 2001) and *Heterobasidion* (Garbelotto *et al.* 2004). The difference between these fungi and *Neurospora* may be explained by the effect of human activity on two key extrinsic factors that separate species, allopatry and ecological inviability in hybrid progeny. Allopatry can be overcome as a barrier to reproduction by the global transport of crop plants and their pathogens, which bring together fungi that would otherwise be geographically separated. Ecological inviability of hybrid progeny due to competition with the parental species can be mitigated by increased access to hosts, as a consequence of farming and forestry or access to new host species by human-facilitated migration. Of course, a fungus does not have to be a symbiont to be affected by human activity. For example, an increase in cut logs associated with forestry may also increase the available habitat for free-living fungi like *S. commune*. Nevertheless, the contrast between *Neurospora* and other free-living fungi as compared to plant pathogens highlights the role of extrinsic barriers to reproduction in maintaining species of eukaryotic microbes, and provides indirect evidence for endemism in fungi.

Comparison of free-living fungi such as *Neurospora* with plant pathogenic fungi can also shed light on mechanisms of speciation (cf. Kohn (2005) for a discussion of the many mechanisms of fungal speciation). Allopatric speciation, where geographic distance provides the early extrinsic mating barrier, is well accepted (at least in microbes). If sibling species are brought together, reproductive barriers developed in allopatry may be strengthened by reinforcement (Noor 1999). Although reinforcement has been controversial, examples have been provided in *Drosophila* (Ortiz-Barrientos *et al.* 2004) and other animals (Coyne & Orr 2004; Hoskin *et al.* 2005) and *Neurospora* (Dettman *et al.* 2003*b*). Sympatric speciation is conceptually more difficult (Coyne & Orr 2004; Kohn 2005). A possible means for parasites to speciate in sympatry postulates that adaptation to different hosts (solving the problem of spatial coexistence of two new species) be coupled with an absence of gene flow between populations on the different hosts (solving the problem of recombination destroying adaptive gene complexes). The coupling could be achieved by one gene that affects both host choice and assortative mating, or by genetically associated pairs of genes that affect each trait separately.

Phytophagous insects provide the most applicable model for speciation of pathogenic fungi (Dres & Mallet 2002), but insects can use behaviour to achieve host preference and assortative mating. With plant pathogenic fungi, there may not be animal-like behaviours to support assortative mating or host preferences, although the activity of insects to facilitate mating in rusts (Craigie 1927) might provide an isolation barrier akin to plant pollination. Giraud *et al.* (2006) have considered the problem of sympatric speciation of pathogenic fungi that broadcast spores and gametes with no means of selecting hosts or mating partners. Using simulations, they find that sympatric speciation can occur in an ancestral population, provided it is polymorphic for host preference and if its gametes do not disperse so that mating is favoured for partners selected for growth on the same host. Conversely, for fungi whose gametes can disperse long distances, e.g. among the many fungi showing host preference, *Armillaria* (Basidiomycota; Anderson & Ullrich 1979), *Heterobasidion* (Basidiomycota; Chase & Ullrich 1990), *Sclerotinia* (Ascomycota; Carbone & Kohn 2001) or *Magnaporthe* (Ascomycota; Couch *et al.* 2005), allopatry would seem to be the most effective premating reproductive barrier. If everything were everywhere, it is difficult to see how new species could form in these fungi, or in fungi without host preference, like *Schizophyllum* or *Neurospora*.

Given the four examples of free-living fungi with less than global distributions noted earlier, and the many other examples of symbiotic pathogens or mutualists with even narrower distributions, one might think that there could be no truly global fungal species. However, just as there are macrofauna with global distributions, e.g. the Arctic tern or Norway rat, there is at least one example of a carefully studied fungus with a global population, *A. fumigatus*.

(f) Aspergillus fumigatus

This ascomycete causes disease in humans, most often in those undergoing immune system suppression in preparation for bone marrow or organ transplants (Latgé 1999). It can be found in almost any soil as well as composting vegetation, owing in part to its ability to grow at 37°C (Raper & Fennell 1965). When medical mycologists first investigated large collections of clinical and environmental isolates of *A. fumigatus* using polymorphisms caused by a mobile repeated element (Debeaupuis *et al.* 1997), they found no correlation between DNA fingerprint and either geography, environment or disease. PSR and population structure were then investigated by two different research groups, each working with different worldwide collections of individuals and each employing different polymorphic DNA regions as population genetic markers (Pringle *et al.* 2005; Rydholm *et al.* 2006). Pringle *et al.* (2005) assembled a collection of 63 *A. fumigatus* individuals from every continent except Antarctica and sequenced five polymorphic loci for each individual. They found two phylogenetic species, one comprising just five of the individuals. Both species had global distributions with no indication of endemism (figure 6). Rydholm *et al.* (2006) analysed DNA sequence from three sequenced intergenic regions for 70 isolates, again from every continent except Antarctica. This study found one global species with no population structure. They also analysed sequence from the three loci for another 33 isolates collected from five sites in North America and Europe to search for any

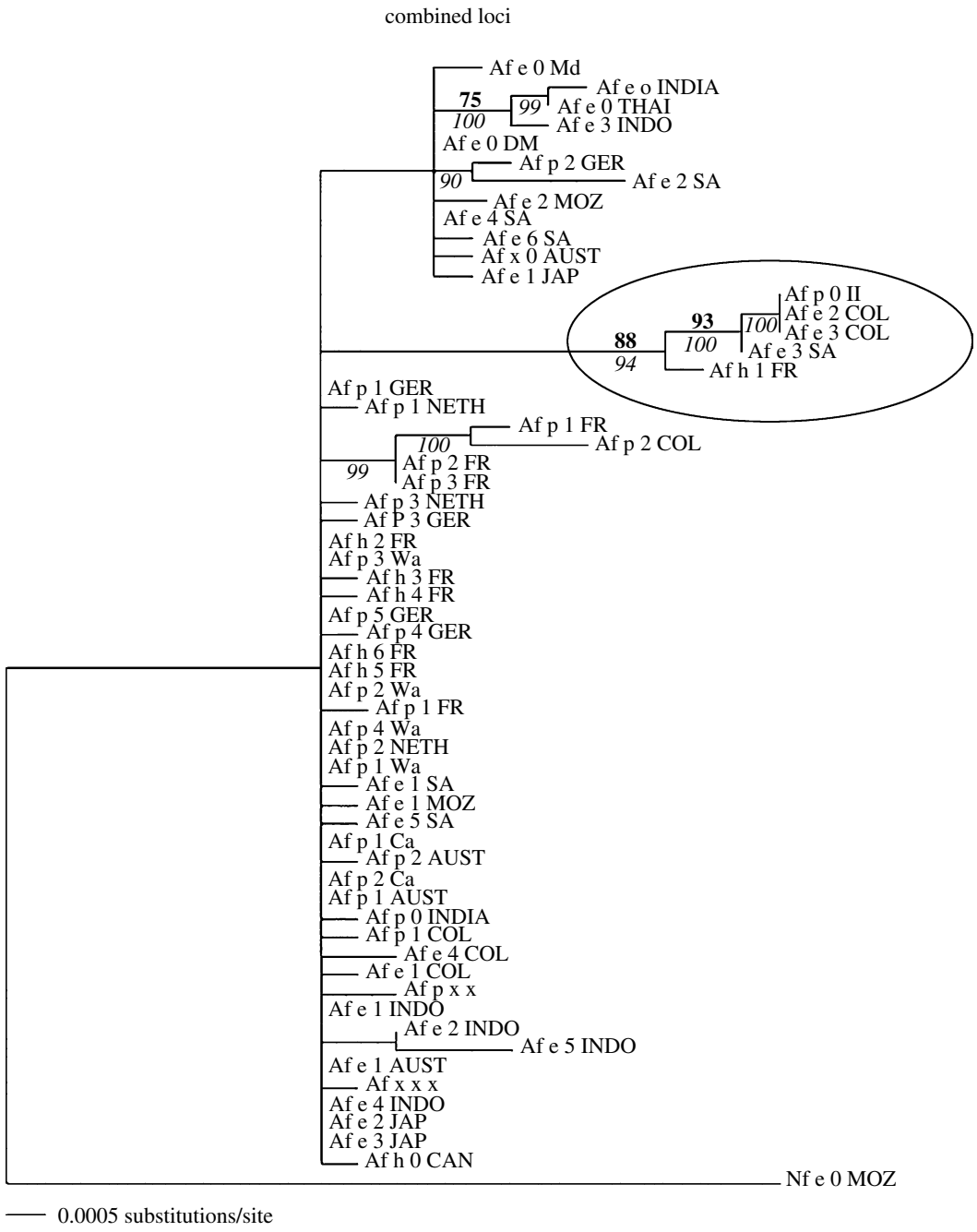

Figure 6. Phylogenetic species recognition applied to *Aspergillus fumigatus*. Bayesian analysis of five loci combined with separate substitution models for each partition. Parsimony bootstrap support above 70% is given in bold. Bayesian posterior probability above 90 is italicized. The phylogeny is rooted using *Neosartorya fischeri* as an outgroup. Figure and legend modified from Pringle *et al.* (2005) with permission of the authors and publisher.

correlation between genetic and geographic distances; none was found. *Aspergillus fumigatus*, therefore, has a global distribution.

These two research groups also investigated reproductive mode in *A. fumigatus* by population genetic means and neither of them were able to reject the null hypothesis of recombination (Pringle *et al.* 2005; Rydholm *et al.* 2006). In addition, Pöggeler (2002), Dyer *et al.* (2003) and Paoletti *et al.* (2005) found that the genes known to be required for mating in fungi that can reproduce sexually in cultivation are also found in *A. fumigatus*. Furthermore, Paoletti *et al.* (2005) showed that *A. fumigatus* individuals of each of the two mating types were found in equal proportion throughout the range of the species. Therefore, not only is *A. fumigatus* a single global species, but it also appears to reproduce by recombination as well as clonal means.

How does *A. fumigatus* maintain a global population when other fungi do not? One hypothesis postulates that *A. fumigatus* can maintain a global reach due to the small size of its mitospores, 2–3 μm in their largest dimension (Latgé 1999). However, as noted at the outset, far larger fungal spores can move from South Africa to Australia (Watson & De Sousa 1983), weakening the argument that the unique distribution of *A. fumigatus* is a consequence of its very small spores. The other hypothesis is that the environment

favoured by *A. fumigatus*, composting vegetation, has been greatly expanded by human activity (Pringle *et al.* 2005). If this hypothesis is correct, then *A. fumigatus* should show evidence of a recent population expansion. The analysis of Rydholm *et al.* (2006) supports an expansion by showing low genetic diversity among, and a recent common ancestor for, *A. fumigatus* individuals as compared to the closely related species, *Neosartorya fischeri*. A completely satisfying explanation for the global distribution of *A. fumigatus* awaits further study. However, the expansion of suitable environment, aided by human activity, appears to provide a part of the answer.

These few examples of free-living fungi show that small eukaryotes exhibit species-level geographic distributions similar to those of their large relatives. Some fungi have narrowly endemic ranges, some have continental ranges, and at least one has a global range. In all the cases, species recognition based on morphology is more inclusive than that based on reproductive or genetic isolation. Therefore, hypotheses of global ranges for small eukaryotes based on studies of morphospecies must be viewed with caution. Until these hypotheses have been challenged with studies employing BSR and PSR, any conclusions about microbial community structure are premature.

(g) *Reconciling Finlay & Fenchel's MSR observations with fungal endemism*

How then, may we reconcile the results of fungal studies using PSR or BSR with Finlay & Fenchel's demonstration of a dramatic decrease in the percentage of endemic protist morphospecies when organismal size drops below 1–10 mm (figure 7*a*)? We believe that there are two reasons that explain why MSR, BSR and PSR find the same species (or nearly the same species) in macrobes but not in microbes. The number of morphological characters is the most potentially obvious reason. At one extreme, no matter how many morphological characters one might find in a single-celled organism, a two-celled organism might have twice as many. As the number of cells increases to the point that macroscopic morphology emerges, the number of characters would increase even faster, for the possible permutations of cell organization are enormous. A corollary of the relationship of organismal size and the number of morphological characters is that such characters are easier to observe in larger organisms. Doubtless, there are microscopic characters that could be used to distinguish between, for instance, black and grizzly bears, but they are not needed. There are more than enough macroscopic characters to accomplish the task.

The second reason for the discrepancy between microbe and macrobe in the level of species recognition among MSR, BSR and PSR is evident from the case histories of fungi. Endemism may be demonstrated only when the characters used to make taxonomic assessment evolve at a rate that is fast enough to yield evolved differences among species (figure 7*a*). Such characters may be molecular evidence of genetic isolation, reproductive isolating barriers or morphological characters. Evidence from the fungi discussed earlier shows that genetic isolation precedes reproductive isolation and that morphological differentiation comes last. The rate of nucleotide substitution in fungi (10^{-8}–10^{-9} nucleotide substitutions per nucleotide position per year), a key factor in phylogenetics, has been shown to be similar to that in bacteria and macroscopic eukaryotes (Kasuga *et al.* 2002). If the rate of phylogenetic character evolution is similar in microbes and macrobes, it must be that the rate of morphological character evolution is higher in larger organisms, where canalized but modular and highly evolvable systems of development permit rapid morphological innovation (Kirshner & Gerhart 1998). From the perspective of biological function, the cells of unicellular organisms must be able to perform every function required for

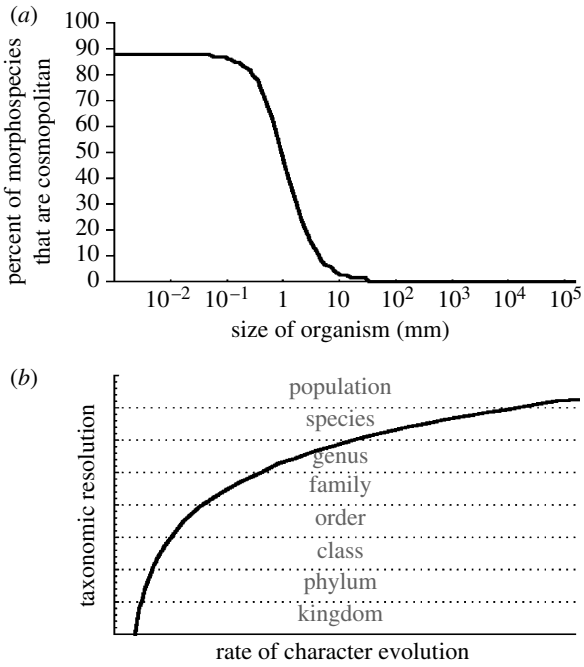

Figure 7. A graphical depiction of a plausible explanation for the differences observed between studies of microbial endemism using PSR, BSR and MSR: morphological characters evolve more slowly than those used for PSR or BSA, thereby recognizing taxonomic groups broader than species and masking endemism. (*a*) The observation of Finlay & Fenchel that the percent of morphospecies that are cosmopolitan is much higher in smaller organisms, with a sharp point of inflexion *ca* 1–10 mm. Data from fungi suggest that morphological characters evolve more slowly in small organisms, thereby recognizing coarser taxonomic groups and obscuring endemism. (*b*) Relationship between rate of character evolution and taxonomic resolution. Faster evolving characters yield greater resolution for more refined taxonomic distinctions. Slower evolving characters yield greater resolution for coarser taxonomic distinctions. PSR is usually performed on characters that are polymorphic among isolates, and thus it is appropriate for resolution of species. BSR is implicitly performed on reproductive isolating factors, which generally evolve rapidly and are naturally appropriate for the resolution of biological species. MSR, like PSR, is ideally performed on characters that are polymorphic among isolates. However, if no or few morphological characters are polymorphic among isolates, morphospecies will map to coarser taxonomic groups than the species observed by BSR and PSR.

life, and this requirement imposes a constraint on the evolution of those cells.

We would not expect the remarkable morphological diversity found among the specialized cells composing the various organs of macrobes to occur in unicellular species. From the perspective of biological form, if we try to imagine all of the unique ways to arrange very small numbers of identical spherical cells, we see that the number of possible arrangements increases much faster than the number of cells. Considering only those arrangements where the imaginary edges connecting the cell vertices are collinear or form a right angle, we find that two cells have only one arrangement (linear) and three cells have two possible arrangements (one linear and one planar), but four cells have at least nine (one linear, four planar and four three-dimensional). Simply, organismal shape can be changed faster by moving cells than by changing cell shape. If there is a correlation between the rate of morphological character evolution and the size of the organism, then the observation in figure 7*a* is a natural consequence of the decreasing resolution of morphological characters for species-level taxonomy, due to their diminished rates of evolutionary change in smaller organisms.

We submit that the fungal comparisons of species recognition based on morphology, genetic isolation and reproductive isolation show that these microbes can be narrowly endemic or demonstrate a global population, and everything in between. Finlay & Fenchel and others who claim that morphology, alone, can be used to recognize species in other microbial eukaryotes must challenge their hypotheses with studies of genetic and reproductive isolations. This type of research already has been initiated, but, as yet, with too few loci that may evolve too slowly (Katz *et al*. 2005; Foissner 2006). When PSR benefits from multiple genealogies of genes evolving at appropriate rates, we predict that all types of eukaryotic microbes will be found to have the diversity of geographic ranges found in fungi as well as large plants and animals.

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