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Molecular diversity of arbuscular mycorrhizal fungi colonising *Hyacinthoides non-scripta* (bluebell) in a seminatural woodland

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Abstract

Arbuscular mycorrhizal (AM) fungi form symbiotic associations with plant roots. Around 150 species have been described and it is becoming clear that many of these species have different functional properties. The species diversity of AM fungi actively growing in roots is therefore an important component of ecosystem diversity. However, it is difficult to identify AM fungi below the genus level from morphology *in planta*, as they possess few informative characters. We present here a molecular method for identifying infrageneric sequence types that estimate the taxonomic diversity of AM fungi present in actively growing roots. Bluebell roots were sampled from beneath two different canopy types, oak and sycamore, and DNA sequences were amplified from roots by the polymerase chain reaction with fungal-specific primers for part of the small subunit ribosomal RNA gene. Restriction fragment length polymorphism among 141 clones was assessed and 62 clones were sequenced. When aligned, discrete sequence groups emerged that cluster into the three families of AM fungi: Acaulosporaceae, Gigasporaceae and Glomaceae. The sequence variation is consistent with rRNA secondary structure. The same sequence types were found at both sampling times. Frequencies of *Scutellospora* increased in December, and *Acaulospora* increased in abundance in July. Sites with a sycamore canopy show a reduced abundance of *Acaulospora*, and those with oak showed a reduced abundance of *Glomus*. These distribution patterns are consistent with previous morphological studies carried out in this woodland. The molecular method provides an alternative method of estimating the distribution and abundance of AM fungi, and has the potential to provide greater resolution at the infrageneric level.

Keywords: arbuscular mycorrhizas, diversity, Glomales, *Hyacinthoides non-scripta*, seminatural woodland, SSU rRNA

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Introduction

Arbuscular mycorrhizal (AM) fungi are ubiquitous in terrestrial ecosystems and play an important role in nutrient cycling in the soil (Smith & Read 1996). Although early work on AM associations concentrated on their role in increasing plant phosphorus uptake, their multifunctional nature is now better understood (Newsham *et al*. 1995a). About 150 species of AM fungi have been described (Morton & Benny 1990) and there is increasing evidence that these species may be functionally distinct, for example in phosphorus uptake (Jakobsen *et al*. 1992), or overall benefit to the host plant (Francis & Read 1995; see review by Sanders *et al*. 1996). However, the taxonomy is based on spore morphology, and many studies have shown that spore counts do not reflect changes in species composition or abundance (Clapp *et al*. 1995; Morton *et al*. 1995; Merryweather & Fitter 1998a). In addition, spores cannot readily be related to individual colonies within roots in field soils, as they are formed on hyphae in the surrounding soil and are easily detached. It has hitherto been difficult to identify each individual fungal species that inhabits the roots of a plant, mainly because on the basis of intraradical hyphal morphology alone they are almost indistinguishable below the genus level. The main advantage of being able to identify the

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fungi *in planta* is that it is likely that the actively growing fungi are being sampled.

As an alternative, molecular studies have been used to investigate phylogenetic relationships and genetic variation among the glomalean fungi. The most commonly used sequence for these studies are the ribosomal RNA (rRNA) genes. This multicopy gene cluster consists of three genes coding for structural RNAs (small subunit (SSU), 5.8S and large subunit) separated by internal transcribed spacers. The SSU has predominantly been used for phylogenetic analysis (Simon *et al*. 1992, 1993; Gehrig *et al*. 1996; Simon 1996) and for identifying the presence of the three families of the Glomales (Clapp *et al*. 1995).

In this study, we describe a method that identifies different sequence types present in the roots of the host plant. This allows comparison of morphological and molecular methods and provides more information on changes in species composition in time and space. Using this approach we have sampled the bluebell (*Hyacinthoides non-scripta* (L.) Chouard ex. Rothm.), a plant that has been shown to be dependent on AM fungi for adequate phosphorus uptake (Merryweather & Fitter 1995), in two different habitat types, at two times of the year.

Materials and methods

Sampling

The sample sites were located in Pretty Wood, Castle Howard, North Yorkshire (Ordnance Survey grid reference SE730692). Root samples were taken from single bulbs within a clump in July 1996 and December 1996 from two sites beneath each of two canopy types: oak (*Quercus petraea* Leib.) colonized by ectomycorrhizal fungi, with a species-poor ground flora; and sycamore (*Acer pseudoplatanus* L.) colonized by endomycorrhizal fungi, which is associated with a species-rich flora. A site with each canopy type was selected from the eastern and the western side of the woodland (Table 1). Up to five plants were sampled from each site, each bulb being taken from a separate clump. Half of the roots were retained as voucher specimens for staining.

Molecular analysis

DNA was extracted from plant roots using a potassium ethyl xanthate (PEX) extraction method (Edwards *et al*. 1997) and, where necessary, samples were diluted and reconcentrated using a 100 kDa microfilter (Microcon, Millipore) to remove low-molecular-weight compounds that inhibit PCR. Partial SSU DNA fragments (≈ 550 bp) were amplified using *Pfu* DNA polymerase (a proof-reading enzyme) (Stratagene) using a universal eukaryotic primer NS31 (Simon *et al*. 1992) and a general fungal primer AM1 (Helgason *et al*. 1998) designed to exclude plant DNA sequences. The reaction was performed in the presence of 0.2 mM dNTPs, 10 pmols of each primer and the manufacturer's reaction buffer. PCR was carried out for 30 cycles (10 cycles at 95 °C for 1 min, 58 °C for 1 min and 72 °C for 2 min, 19 cycles at 95 °C for 30 s, 58 °C for 1 min and 72 °C for 3 min, and 1 cycle at 95 °C for 30 s, 58 °C for 1 min and 72 °C for 10 min) on a Gradient96 Robocycler (Stratagene). The resulting blunt-ended products were cloned into pCR-Script Amp SK(+) (Stratagene) and transformed into *Escherichia coli* (XL1-blue MRF'). Putative positive transformants were selected and screened using standard T3/T7 amplification. Up to 10 positives from each individual were digested with the restriction enzymes *Alu*I and *Hin*fI, according to the manufacturer's instructions (Promega). One clone of each restriction fragment length polymorphism (RFLP) type present in each individual was sequenced on an ABI377 automated sequencer. The T3/T7-amplified PCR products were cleaned using Qiaquick PCR purification spin columns (Qiagen) and sequenced according to the manufacturer's instructions using the Dye terminator cycle sequencing kit with AmpliTaqFS DNA polymerase (ABI Perkin-Elmer) using T3 and T7 as the sequencing primers.

Data analysis

Forward and reverse sequences were aligned using Autoassembler (ABI Perkin-Elmer). CLUSTALW (Thompson *et al*. 1994) was used for multiple alignment and neighbour-joining phylogeny (Saitou & Nei 1987), using

Table 1 Site and sampling descriptions. Figures in parentheses show the number of roots from which PCR products were obtained

Site name	Canopy	Location	No. of roots sampled (amplified)	
			July 1996	December 1996
A	Sycamore	Western	5 (4)	3 (3)
B	Oak	Western	5 (5)	4 (4)
C	Oak	Eastern	5 (4)	4 (4)
D	Sycamore	Eastern	5 (5)	4 (4)
Total clones			58	83
Mean clones per root			3.22	5.53

Geosiphon pyriforme as an outgroup (Gehrig *et al*. 1996). General loglinear analysis using a multinomial model was carried out using SPSS version 8.0.

Results

The sequences amplified using NS31–AM1 are from the order Glomales

Clones were selected for sequencing on the basis of the *Alu*I and *Hin*fI RFLP typing. One clone from each RFLP type found in each root sample was sequenced. A total of 62 clones from the order Glomales was sequenced, and a further 79 classified by RFLP typing. These came from a total of 33 roots (out of 35 sampled) that yielded enough PCR product to clone (Table 1). Seven sequences that showed low similarity to AM fungi were excluded from the analysis. One was closely related to the SSU of Pyrenomycetes, a group of Ascomycetes. Another showed its closest similarity to *Glomus* spp., but only for part of the sequence, suggesting that this is a chimeric sequence (see below). Other sequences showed no significant similarity to any database sequences, but these were usually of a slightly different length from the fungal sequences and were probably the result of nonspecific binding at the PCR stage.

Alignment of the partial SSU fragments generated using the fungal-specific primers produces a tree in which the majority of the sequences cluster within the Glomales, and largely within well-supported groups corresponding to the three families, Acaulosporaceae, Gigasporaceae and Glomaceae (Fig. 1). This is consistent with previously published phylogenies (Simon *et al*. 1993; Gehrig *et al*. 1996) Two roots sampled in December produced clones that were most similar to *Geosiphon* and the AM fungi, but rather distant from them (Other1). They have been included in Fig. 1, but in the absence of information on the identity of this sequence group, it has been excluded from subsequent analysis.

Discrete sequence groups are identifiable

Alignment of the sequences reveals discrete sequence groups. In many cases sequences from different habitat types and different times are identical. In other groups the sequences are more variable. Within each cluster, the number of nucleotide differences is relatively small, with pairwise similarities ranging from 97 to 100%. In two clusters (Acau1 and Glo2) there appear to be two separate sequence types within the cluster, but these are not well supported by the bootstrap values, nor are they distinguishable by the *Hin*fI and *Alu*I RFLP patterns. For this reason they are classified as single types (Fig. 1). Differences due to PCR error are a potential source of variation, given that the sequences are from cloned PCR products. The error rate of *Pfu*, however, has been estimated to be only 1.3×10^{-6} mutations per base pair per cycle (Stratagene technical information), which for a product of ≈ 550 bp amplified for 30 cycles would lead to 2.1% of products with a single PCR error. This corresponds to only about three of the 141 clones analysed here, so the PCR reaction contributes no spurious phylogenetic information.

Only one of the observed sequence types aligns clearly with any of the reference sequences available. The sequences in group Scut1 cluster around three clones

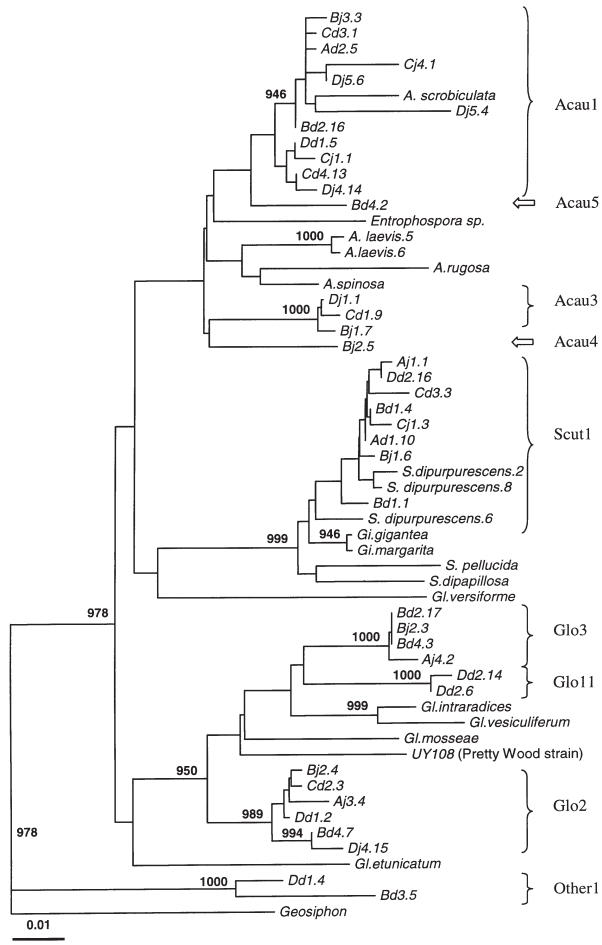

Fig. 1 Neighbour-joining tree showing all sequence types identified in bluebell roots. The sequence types identify groups whose sequences have > 97% similarity. All bootstrap values > 90% are shown (1000 replicates). Named sequences are from Simon (1996), Gehrig *et al*. (1996), Helgason *et al*. (1998) or from library cultures sequenced in this laboratory. Individual clones are identified by site (A,B,C,D see Table 1), sample date (j = July 1996, d = December 1996), root sample number and clone identifier. Sequence type identifiers are consistent with data previously published in Helgason *et al*. (1998). All new sequences have been submitted to the GenBank database (Accession nos AS131020-AS131055).

sequenced from an accession of *Scutellospora dipurpurescens* (International Collection of Arbuscular and Vesicular-Arbuscular Mycorrhizas, INVAM ref. WV109A). The *Scutellospora* spores isolated from Pretty Wood have also been identified as *S. dipurpurescens* (Clapp *et al*. 1995; Merryweather & Fitter 1998a) and, although this has not been conclusively confirmed with data from cultures obtained from Pretty Wood spores, this is strong circumstantial evidence that the Scut1 sequence group is closely related to *S. dipurpurescens*. A reference sequence of *Acaulospora scrobiculata* [Bank of European Glomales (BEG) 33] clusters within the Acau1 group, but differs considerably from all the field sequences and, without further confirmation, Acau1 cannot be confidently named as *A. scrobiculata*. The *Glomus* sequences from roots are distinct from a *Glomus* taxon cultured from Pretty Wood (UY108 in Fig. 1)

The variation is consistent with rRNA secondary structure

The variation is largely base pair substitution with few insertions and deletions, although the sequences vary in length from 504 to 512 bp (excluding primers). The most variable region within the PCR product lies 80–300 bp from the 5′ of the PCR product (excluding primers) (bases 600–820 bp of the SSU gene). This confirms previous observations by Simon (1996). This variable region corresponds to the V4 region of the SSU rRNA gene, which is the largest and most complex variable region in this gene (Nickrent & Sargent 1991). In fungi, this region has a length of ≈ 325 bp. The variation present in this part of the sequence is typical of rRNA sequences in that it is compensatory. If the sequences in this study are compared to published structural analyses of fungal SSU sequences (from Hendriks *et al*. 1991; Van de Peer *et al*. 1998), mutations in one part of a stem structure show compensatory mutations in the opposite strand.

One clone (Bd4.2) has a 9-bp deletion in the V4 region. Another near-identical sequence with the same deletion was isolated from another host plant at a different sampling time, suggesting that this is not a PCR artefact (T. Helgason *et al*. unpublished). Structural analysis of this deletion shows that a loop within stem 24 (numbering after van de Peer *et al*. 1998) has been lost.

Chimeric and recombinant sequences

A number of the clones sequenced that do not cluster closely with sequences of the same RFLP type were found to have portions of sequence that match other taxa. There are two possible reasons for this: (i) they may be chimeric PCR products that may be formed when more than one target sequence is present in the template (Bradley & Hillis 1997); or (ii) they may be genotypes formed by recombination.

Chimeric PCR products have been shown to represent nearly half of the clones from a 30-cycle PCR reaction where a heterozygote individual is analysed, although the error rate was found to be only 8% for the proof-reading enzyme Vent polymerase, a similar enzyme to the one used in this study (Bradley & Hillis 1997). In root samples where both sequence types are present, it is reasonable to assume, in the absence of any evidence to the contrary, that a mixed sequence is the product of recombination during the PCR reaction (e.g. Bd1.1).

It is more difficult to determine the origin of a mixed sequence when the two putative components are not also cloned individually from a root (e.g. Cd3.3 and Dj5.4). However, two sequences similar to Dj5.4 have also been cloned from roots of other host plants and from distant sites, suggesting that this is not a PCR artefact, as the probability of such a similar recombination event occurring is small (T. Helgason *et al*. unpublished).

The same sequences appear at both sampling times

Using the taxa defined above, the remaining clones were classified by RFLP type (Table 2). The dominant sequences

Table 2 Number of clones of each *Hin*fI/*Alu*I restriction type. Names as in Fig. 1, and consistent with Helgason *et al*. (1998)

Sample time	Site	Acau1	Acau3	Acau4	Acau5	Glo3	Glo2	Glo11	Scut1	Other1	Total
July 1996	A					4	5		2		11
	B	10	2	1		4	2		2		21
	C	12							1		13
	D	9	2			1	1				13
Total		31	4	1	0	9	8	0	5	0	58
Dec. 1996	A	1					6		4		11
	B	3			2	6	1		8	8	28
	C	4	4				3		8		19
	D	3					6	5	10	1	25
Total		11	4	0	2	6	16	5	30	9	83

present in bluebell roots are the same at each sampling time, suggesting continuity from season to season. Unlike many of the plants in this habitat, bluebells have no roots from July to September, and are actively growing throughout the winter. Colonization is rapid when the roots begin to emerge from the bulbs, and it may be that these plants are important hosts for the fungi while few other species are actively growing (Merryweather & Fitter 1998b). The three dominant sequences (Acau1, Glo2 & Scut1) represent the three genera *Acaulospora*, *Glomus*, and *Scutellospora*. Of the three most common taxa, Acau1 is dominant in July, towards the end of the season, while Scut1 and Glo2 are found more frequently in the December samples, about a third of the way through the subsequent growing season. The diversity of fungi in bluebell roots, measured using the Shannon–Weiner diversity index, is higher in the winter ($H = 1.622$) than it is in July ($H = 1.363$). A rank abundance plot (Fig. 2) shows the difference in diversity in another form; the steeper slope of the July sample reflects the abundance of a single sequence type (Acau1) at that sample time. In plant community analysis this type of pattern is typical of communities where the habitat is ephemeral and contains a large number of ruderal species, which might be analagous to a population inhabiting roots, which are also ephemeral. We do not know enough about resource utilization in AM fungi to look at niche separation, but this analysis shows how we may compare the allocation of resources by the fungi to growth, rather than reproduction, as has been attempted previously using spore counts (Morton *et al*. 1995).

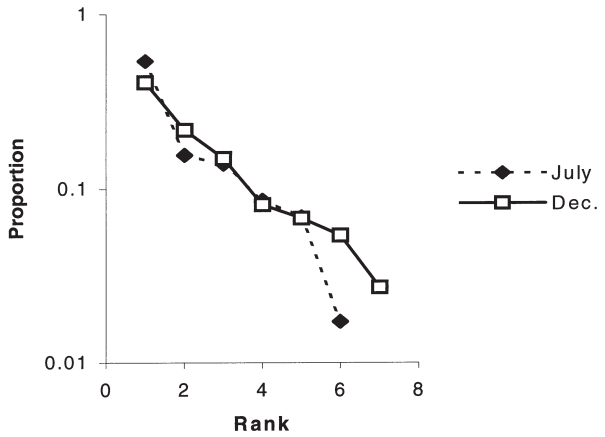

Fig. 2 Rank abundance curves for each sampling time. The figure is discussed in the text.

Loglinear analysis reveals differences in time and space

As the properties of each AM fungal family varied with sampling date, canopy type and location, loglinear modelling was used to assess the relative importance of these factors. The data matrix produced by this experiment (Table 3) can be defined by a loglinear model that includes interaction terms for all the possible combinations, termed the saturated model (eqn 1). By definition, this model produces a perfect fit to the observed data. By removing interaction terms and comparing the fit to that of the saturated model, we can determine the minimal model that generates expected frequencies close to the observed values. Terms in the model that are fixed by the design, in this case those that involve only the independent variables of canopy, sampling date and location, must be included in the minimal model. The remaining terms are all those that include the response variable, AM fungal family count.

$$\log_{m_{ijkl}^{ACDL}} = \mu + \alpha_i^A + \alpha_j^C + \alpha_k^D + \alpha_l^L + \alpha_{ij}^{AC} + \alpha_{ik}^{AD} + \alpha_{il}^{AL} + \alpha_{jk}^{CD} + \alpha_{jl}^{CL} + \alpha_{kl}^{DL} + \alpha_{ijk}^{ACD} + \alpha_{ijl}^{ACL} + \alpha_{ikl}^{ADL} + \alpha_{jkl}^{DCL} + \alpha_{ijkl}^{ACDL} \quad (1)$$

Equation 1 shows a saturated loglinear model, where i = AM fungal family, j = Canopy, k = sampling Date, and l = Location. Interaction terms in bold are fixed by the experimental design. The model is used to generate expected values for the data matrix.

Removal of the four-way interaction among canopy, location, sampling date and AM fungal family produces a

Table 3 Data from Table 2 arranged by family and sampling to illustrate structure used for loglinear analysis. All marginal totals can be reconstructed from this table

Date	Canopy	Location	Acau	Glo	Scut	Totals
July	Sycamore	East (D)	11	2	0	13
		West (A)	0	9	2	11
	Oak	East (C)	12	0	1	13
		West (B)	13	6	2	21
Total			36	17	5	58
December	Sycamore	East (D)	3	11	10	24
		West (A)	1	6	4	11
	Oak	East (C)	8	3	8	19
		West (B)	5	7	8	20
Total			17	27	30	74

significantly worse fit (Table 4), suggesting that all three explanatory factors are interacting and that there is no single factor that explains the distribution of AM fungal families. This result notwithstanding, we can show that some factors exert a greater influence, particularly the interaction between sampling date and location (model 1b). The other three-way interactions do not significantly raise the likelihood ratio (models 1c, 1d). Each of the factors exerts a significant main effect, a result that is consistent with a significant higher-order interaction (models 1e, 1f, 1 g). The interaction among all the explanatory variables is largely due to the *Scutellospora* data, reflecting the low observed values for *Scutellospora* in July. Removal of the counts for this taxon results in a model without the four-way interaction that has a very low likelihood ratio and hence a close fit to the saturated model (model 2c). There is no interaction between canopy and location (model 3c) for this data set but, as before, each of the factors has a significant main effect (models 3d, 3e, 3f).

These results show that there is a significant association of *Acaulospora* with oak and *Glomus* with sycamore canopies, and that the seasonal shifts are different at the eastern and the western sites (the populations are much more similar in December than they are in July). The eastern and western samples were intended to introduce replication into the experimental design, but it is clear that they have not done so. It may be that a habitat that appears to be similar, at least on the basis of canopy type and ground cover, is not homogeneous from the perspective of the fungi, and only further replication will determine if this is the case.

Analysing these data at the family level allows the comparison of the data with previous morphological analysis. Merryweather & Fitter (1998b) have shown that, while *Scutellospora* dominates from the beginning of the season, colonization by other species gradually increases to a peak around February–March which is followed by an eventual decrease in overall colonization. The mean number of clones amplified from each root is greater in December samples than in July (Table 1), suggesting an overall decline in colonization. *Scutellospora* dominates in bluebell in December and *Acaulospora* dominates in July. Both these patterns reflect the data from morphological analyses.

Table 4 Likelihood ratios (LR) produced by general loglinear modelling according to the model specified in eqn 1. The table shows the LR produced when the interaction term shown is removed from the saturated model. These results are explained in the text. All models are hierarchical

	Model	LR	d.f.
1	Complete data set (Table 3)		
a	– ACDL	5.37	2
b	– ACDL, -ADL	9.22	4
c	– ACDL, -ACD	6.02	4
d	– ACDL, -ACL	6.67	4
e	– ACDL, -ACD, -ACL, -AC	28.20	8
f	– ACDL, -ACD, -ADL, -AD	42.52	8
g	– ACDL, -ACL, -ADL, -AL	32.03	8
2	Removing each AM fungus family in turn		
a	– ACDL -Acau	2.18	1
b	– ACDL -Glo	5.13	1
c	– ACDL -Scut	0.001	1
3	Model without Scut (2c)		
a	– ACDL, -ADL	7.70	2
b	– ACDL, -ACD	3.31	2
c	– ACDL, -ACL	0.27	2
d	– ACDL, -ACD, -ACL, -AC	26.26	4
e	– ACDL, -ACD, -ADL, -AD	20.51	4
f	– ACDL, -ACL, -ADL, -AL	28.56	4

Discussion

Morphological vs. molecular data

The molecular data described here show the same pattern of species composition and abundance as the extensive morphological analysis described in Merryweather & Fitter (1998a, 1998b), so we can say with some confidence that, for the well-sampled sequence types at least, it is possible to use the abundance of clones as a simple quantitative measure of species composition and abundance.

Merryweather & Fitter (1998a) were able to discriminate three morphotypes within each of the genera *Acaulospora* and *Glomus*, the same as the number of sequence groups within each genus defined in this study. Two types within each genus, however, were not present in sufficient quantity to be included in the morphological analysis because a large number of characters are required for reliable identification. The improved sensitivity of the molecular method allows the detection of taxa where there is not enough root colonization present for the taxa to be identified visually in roots. The nature of the sequence data is such that the analysis provides as much information about the rare types as the common types, providing the sampling is intensive enough to reveal them. The dominant taxa are detected at nearly every sampling point, allowing a quantitative assessment.

Whereas morphological techniques demonstrated the presence of three genera of AM fungi in the roots of a single species at this site, molecular techniques revealed that there are at least eight AM fungal taxa involved. These fungi have been shown to exhibit complex spatial and temporal patterns, which emphasizes an ignorance of their functional behaviour. It is implausible that all eight taxa function identically, and fungi are known to differ in

their ability to transport phosphorus to plants (Jakobsen *et al*. 1992). The key question therefore is whether the fungi differ simply in symbiotic effectiveness, with some taxa being more effective partners than others, or whether they differ in function as well, either through habitat or seasonal preference, or through the ability to perform other functions in the symbiosis, such as defence against pathogens (Newsham *et al*. 1995b).

Estimating abundance from the molecular data

The clones derived from a root will reflect the relative abundance of the corresponding fungi in the root provided that three assumptions are valid: (i) from plant roots with multiple infections, the quantity of fungal DNA template extracted is proportional to the amount of colonization; (ii) each type amplifies in direct proportion to its template concentration; and (iii) each sequence type is cloned equally efficiently.

Competitive PCR with a heterologous internal standard has been found to correlate well with the percentage root length colonized in *Glomus mosseae* (Edwards *et al*. 1997), and PCR with mixed templates has been used successfully to estimate diversity of other viable nonculturables (VNCs) (Liu *et al*. 1997). With field samples, however, it is likely that each AM fungus in the root does not have an equal proportion of hyphae, arbuscules and other structures present. Any differences in cell wall properties among these structures or among species may result in variable extraction efficiency.

The AM fungal sequences amplified from roots differ in length by only 8 bp (545–553 bp) and range in GC content from 38 to 41%. As these sequences code for the same type of structural RNA, we can assume that the structural properties of the sequences are similar, and indeed the sequences are highly conserved over 60% of their length. Preferential amplification of one sequence over another is therefore unlikely, provided that the primer matches the sequence precisely (Zimmerman & Mannhalter 1996). The AM1 primer was designed to match AM fungi precisely at the 3′ end, but being based on only 12 available reference sequences, may not be a precise match for all AM fungi.

As the sequences are of such similar length, the cloning reaction is also unlikely to result in significant preferential cloning of one sequence over another. Despite the problems listed above, this nevertheless represents a reproducible estimate of the AM fungal population *in planta*.

The most likely problem with this data set is that inadequate sampling, especially where some plants are poorly colonized and produce weak amplification, has led to poor estimation of AM fungus type abundance. The relative abundance of the dominant sequence groups estimated by this method is a reasonable estimate of the amount of colonization of each type in the host plant. It is important to note, however, that we are not assuming that the fungi with the greatest level of colonization are those that are contributing the most to the plant–fungal symbiosis. The contribution of each strain growing in a mixed colonization to plant phosphorus uptake is not known, nor are the effects of competition among strains well understood, although strains of AM fungi in competition have been shown to vary in their response to the presence of another fungus (Hepper *et al*. 1988; Pearson *et al*. 1994)

Bridging the gap between roots and spores

This study shows that it is possible to use molecular methods to measure the diversity of AM fungi present in the roots of plants taken from the field. The resolution of that diversity is limited, in theory, only by the variability of the gene used to identify the taxa present. In this study, partial fragments of the ribosomal SSU resolved distinct sequence groups at the infrageneric level. Studies on spores trapped and cultured from this site are now being analysed in order that names may be put to these taxa. Using this information, and the voucher specimens from the samples analysed here, we hope to be able to bridge the gap between spore-based taxonomy and what is present *in planta*. In this way the behaviour of AM fungi in the field, both as individual strains and as a community, can be investigated in a detail not hitherto possible.

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This work forms part of the extensive interdisciplinary work being carried out at the University of York on symbiotic microorganisms. Thorunn Helgason is a postdoctoral researcher working on molecular identification of AM fungi in woodland soils. The groups led by Profs Peter Young and Alastair Fitter also study AM diversity in agricultural crops and the ecophysiology of mycorrhizal associations, as well as other aspects of root ecology and the evolution and molecular ecology of the legume–rhizobium symbiosis.
