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Discovery, measurement, and interpretation of diversity in arbuscular endomycorrhizal fungi (Glomales, Zygomycetes)

Joseph B. Morton, Stephen P. Bentivenga, and James D. Bever

Abstract: Measures of diversity depend on an eclectic taxonomy now being developed from comparisons of morphology, developmental programs, carbohydrate chemistry, fatty acids, and nucleotide sequences in a wide range of arbuscular fungal taxa obtained from living culture collections. Developmental patterns in character origin and transformation are providing clues of intrinsic causation in evolution of diversity. Extrinsic causation is being identified from population-level dynamics, as well as data on species numbers, abundance, composition, and distribution. Detection of species is based solely on sporulation, so that a combination of field sampling and various trap culture methods provide a more comprehensive estimate of fungal community organization. Species distributions rarely correlate with ecological gradients or hypothesized phylogenetic relationships, suggesting that an important causal factor of present-day distributions is dispersal over geologic time. Global distribution of both derived and ancestral species and representation of all genera in most plant root systems further indicate that local diversity has a strong historical component, with ecological processes of subordinate consequence. Ecological dynamics play a crucial role at the local level. They are governed by multilevel diversity among and within organisms of a species assemblage, such as differences in life history traits and heterogeneity of genetic and physiological properties, respectively.

Key words: ecology, phylogeny, systematics, vesicular–arbuscular mycorrhizae.

Résumé : Les mesures de la diversité dépendent d'une taxonomie éclectique présentement développée à partir de comparaisons morphologiques, de programmes de développement, de la chimie des glucides, des acides gras et des séquences nucléotidiques, chez un large ensemble de taxons fongiques arbusculaires obtenus de collections de cultures vivantes. Les patrons de développement, de l'origine à la transformation des caractères, fournissent des indications sur la causalité intrinsèque de l'évolution de la diversité. La causalité extrinsèque est identifiée à partir de la dynamique des populations, ainsi que des données sur les nombres, l'abondance, la composition, et la distribution des espèces. La détection des espèces est basée uniquement sur la sporulation, de sortes qu'une combinaison d'échantillonnages aux champs et de diverses méthodes de trappage, offrent une meilleure évaluation de l'organisation des communautés fongiques. La distribution des espèces est rarement corrélée avec des gradients écologiques ou des relation phylogénétiques hypothétiques, ce qui suggère qu'un important facteur causal des distributions modernes est constitué par la dispersion au cours des époques géologiques. La distribution globale des espèces dérivées ainsi qu'ancestrales, et la représentation de tous les genres sur la plupart des systèmes racinaires végétaux, indiquent que la diversité locale comporte une forte composante historique, les processus écologiques lui étant subordonnés. La dynamique écologique joue un rôle crucial au niveau local. Cette dynamique est gouvernée par la diversité à différents niveaux entre et à l'intérieur des organismes de l'assemblage d'une espèce, tel que les différences dans les cycles vitaux et l'hétérogénéité des propriétés génétiques et physiologiques respectivement.

Mots clés : écologie, phylogénie, systématique, mycorhizes vésiculaires arbusculaires.
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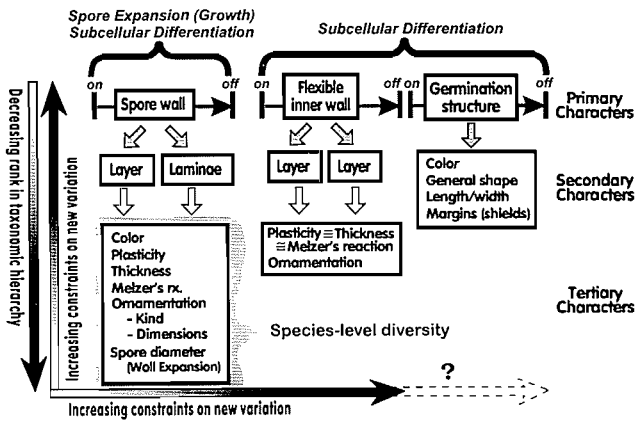
Introduction

Arbuscule-forming endomycorrhizal fungi in Glomales, Zygomycetes (Morton and Benny 1990) are the dominant fungal association in agricultural and horticultural cropping systems

Fig. 1. Hypothesized developmental model depicting morphological character growth (expansion) and hierarchical differentiation in asexual spores of endomycorrhizal fungi in Glomales. On and off refers to discrete timing of origin and cessation of differentiation. Increasing constraints are indicated by less observed variation of a character relative to other characters. This model is derived from comparisons in the family Gigasporaceae (Franke and Morton 1994; Morton 1995), but extended provisionally to all Glomales.



(Robson et al. 1994). Combined evidence from the fossil record (Pirozynski and Dalpé 1989), extant morphologies (Morton 1990*a*), and rDNA nucleotide sequences (Simon et al. 1993) suggest an ancient origin. Thus, issues of origin and maintenance of diversity in arbuscular fungi involve temporal scales of days to over 250 million years and spatial scales of one root system to global distributions.

Patterns of fungal diversity at all organizational levels have been identified and tentative explanations of process have been advanced (Morton and Bentivenga 1994; Walker 1992). However, testing these and newer hypotheses depends on a clear understanding of both the limits and the promise of experimental methods and design. In this paper, we examine the nature and information content of methods now being used to carry out systematic analyses and construct an eclectic taxonomy, to measure and interpret species diversity and distribution, and to determine genotypic and phenotypic variability within and among organisms of species assemblages.

An eclectic taxonomy

A formal taxonomy of arbuscular endomycorrhizal fungi is only 20 years old, the result of which is inevitable growing pains. Numerous obstacles exist, such as (*i*) the necessity of growing the fungi on whole plants or explants, (*ii*) reliance on subcellular morphological characters of spores that are difficult to see and interpret, and (*iii*) lack of training of the majority of working scientists (Morton 1993). Progress is being made through improved and varied culture regimes (Bécard and Piché 1989; Hung and Sylvia 1988; Millner and Kitt 1992; Morton et al. 1993), source material from living culture collections such as the International Collection of Arbuscular and Vesicular–arbuscular Mycorrhizal Fungi (INVAM) (Morton et al. 1993) and the Bank of European Glomales (BEG) (Dodd et al. 1994), and comparative approaches involving a wider range of character data sets.

Morphological characters

Morphology can be defined empirically from developmental patterns and processes for application to taxonomic problems. Comparative studies in *Gigaspora* (S.P. Bentivenga and J.B. Morton, unpublished) and *Scutellospora* (Franke and Morton 1994; Morton 1995) provide the basis for a developmental model (Fig. 1) to test against a wider range of taxa in other genera. This model delineates onset and termination (offset) of character origins as well as a hierarchically ordered sequence in differentiation and transformation of subcellular characters. Preliminary evidence suggests that the character hierarchy (primary, secondary, tertiary) is universal within Glomales, but specific characters and the manner in which they differentiate are unique to each family.

A developmental hierarchy implies constraints on morphological character variation. These constraints are measured by a narrower range of variation in characters of a target group compared with homologous characters in a closely related group (McKitrick 1993). Explanatory power of the model in Fig. 1 relies on comparisons of taxa in closely related (sister) groups relative to an ancestral outgroup. As an example, we compare known characters and their states in two related groups in Gigasporaceae (*Gigaspora* and a subgroup of *Scutellospora* united by an identical bilayered flexible inner wall) relative to a hypothesized outgroup, *Glomus*.

In all three groups, species-level variation is confined mainly to characters of the spore wall (Fig. 1). However, different degrees of constraint on those characters lead to different estimates of the number of possible species in each group, with the greatest constraints in *Gigaspora* and the least in *Glomus* (Table 1). Characters of the spore wall are directly comparable in spores of *Gigaspora* and the *Scutellospora* subgroup because differentiation sequences are identical. Constraints on these characters appear to be coupled with germination events in both groups. Germ tubes arise directly from the innermost papillate layer of that spore wall in *Gigaspora*. In the spore wall of these species, laminae color of healthy spores is white to yellow and ornamentations on the outer layer are absent. Germ tubes arise from a germination shield associated with a flexible inner wall that differentiates separately from the spore wall in the *Scutellospora* subgroup. In spores of these species, laminae color ranges from white to reddish black and the outer layer has many kinds of ornamentations. Constraints in this group shift to the flexible inner wall, which is invariant in all species (Morton 1995).

The lack of constraints on variation in spore wall characters in *Glomus* may be partly explained by the dissociation of germination events from spore wall differentiation, although development in this group has not yet been studied in detail. Other genealogical factors also are likely to be important. *Glomus* may have an evolutionary origin more distant from *Gigaspora* and *Scutellospora* than has been previously hypothesized (Morton 1990*b*; Simon et al. 1993), based on developmentally defined characters (Morton and Bentivenga 1994) and β-1,3-glucan distribution in fungal cell walls (Gianinazzi-Pearson et al. 1994).

The disparity between estimated and realized diversity calculated in Table 1 is partly due to unavoidable sampling

Table 1. Number of observed states in morphological characters in the spore wall of two closely related groups (*Gigaspora* and *Scutellospora*) and a more distantly related outgroup (*Glomus*), and the number of potential versus realized species calculated from these states.

Characters	Number of states[a]		
	Gigaspora	*Scutellospora*[b]	*Glomus*[c]
Spore size	2	2	3
No. of spore wall laminae	1	1	2
Color of spore wall laminae	4	5	4
Thickness of spore wall laminae	Continuum	Continuum	2
Ornamentation of spore wall laminae	1	1	3
No. of outer layers	1	1	3
Permanency of outer layer	1	1	3
Ornamentation of outer layer	1	5	2
No. of potential species[d]	8	50	2592
No. of described species	7	7	84

[a]Estimated from observed variation in known species. Size is partitioned by nonoverlapping median values; laminae is a collective term of layers of the same phenotype, with different phenotypes counted; outer layer is in positional reference to laminae; permanency indicates with or without sloughing in developmentally mature spores.

[b]A subgroup of *Scutellospora* sharing an identical bilayered flexible inner wall and exhibiting species-level variation only in layers of the spore wall (Morton 1995).

[c]As revised by Almeida and Schenck (1990) to include most *Sclerocystis* species.

[d]Calculated from the product of all observed states in spores of each group.

error, but we suspect additional unidentified constraints also are active. All comparative evidence suggests that intrinsic constraining forces override extrinsic ecological factors implicated by Law (1985) and Morton (1990*a*) in channeling species diversity and maintaining species stability at the morphological level. These patterns begin to provide concepts of species based on processes of evolutionary change (or lack of it). They also begin to provide empirical criteria for the grouping and ranking of taxa (Fig. 1).

Molecular characters

Constraints on morphological variation may allow delineation of asexual species, but they then are likely to underrepresent potential total variation in genetic, biochemical, and physiological traits evolved over long time periods in isolated individual organisms. Molecular comparisons are needed to define this variation and map the relationship between it and the patterns of morphological/developmental divergence. Nucleotide sequence variation has been measured directly in amplified rDNA fragments (Simon et al. 1993) and indirectly via random amplified polymorphic DNA (RAPD) assays (Wyss and Bonfante 1993). The former comparisons have been made only at the species level or higher because of the conserved properties of the target rDNA region. In the latter, banding patterns appear to be reasonably stable among disjunct geographic isolates of *Glomus mosseae* (Nicol. & Gerd.) Gerd. & Trappe and *Acaulospora laevis* Gerd. & Trappe.

Fatty acids in spores are cumulative secondary metabolites synthesized and stored in actively growing organisms. Their profiles also afford a closer look at physiological divergence among taxa (Graham et al. 1995), but they have limited value in phylogenetic analysis because of the low number of characters and convergent evolution. Fatty acid libraries still show considerable promise for multiple users in species diagnosis, because profiles are highly stable in different host–environment situations (Bentivenga and Morton 1994).

Phylogenies hypothesized to date measure character divergence in particular components of the whole organism: either selected regions of one gene that are highly conserved (Simon et al. 1993) or subcellular structures of spores and mycorrhizae organized sufficiently to exhibit some divergence and remain stable (Morton 1990*b*). These choices may reflect different phylogenies, especially if the uncoupling of morphological differentiation from mycorrhizal function and possibly other life history traits contributed to differential rates and magnitudes of evolutionary change. Both phylogenies also suffer from nebulous outgroups. Reconstruction of cladograms or trees from nucleotide sequences often references morphology to narrow the field of possible outgroup taxa, but arbuscular fungi possess many taxonomically important morphological characters that have no counterparts in other fungal groups (Morton and Bentivenga 1994). Many of the ambiguities and conflicts are likely to be resolved with a broader sampling of taxa, both within Glomales and in other Zygomycetes.

Species diversity

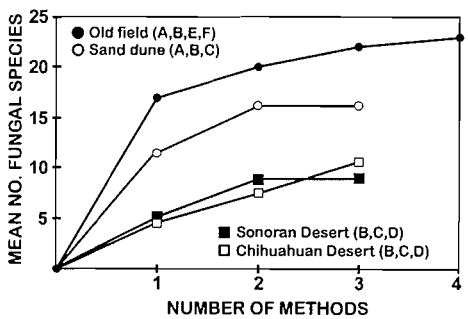
The role of extrinsic ecological factors on macroevolution (speciation) can only be determined by combining biogeographic analyses, measurements of local and regional species diversity, and the study of population-level dynamics. In virtually all studies, spores have been relied on as the experimental units because they are extractable, quantifiable as discrete units, and identifiable.

Table 2. Species richness, as measured in areas ranging from those defined by geopolitical boundaries to those in part of a monospecific plant community.

Location (estimated area)	Sample method[a]	No. of species	Genera in Glomales[b]					Ref.
			G	A	E	Gi	S	
18 states, United States (across country)	F18, T2	43	27	4	0	3	9	Miller et al. 1985
Poland (country boundaries)	F62	21	14	3	1	1	2	Blaszkowski 1989
Wisconsin Great Lake dunes (600-km transect)	F10	14	8	2	2	0	2	Koske and Tews 1987
Eastern U.S. coastal dunes (355-km transect)	F19	26	10	2	1	3	10	Koske 1987
Konza Prairie, Kansas (35 km^2)	F6	21	14	1	1	3	2	Hetrick and Bloom 1983
Cedar Creek Natural History Area, Minnesota (18 km^2)	F15	25	11	6	1	4	3	Johnson et al. 1991*a*
Central Iowa grassland (14 336 m^2)	F32	12	7	3	0	0	2	Walker et al. 1982
Assateague Island dunes, Virginia (850 m^2)	F2, T2	17	4	2	1	2	8	Koske 1987; J.B. Morton, unpublished
Joaquina dunes, Brazil (600 m^2)	F1	12	4	2	0	1	5	Stürmer and Bellei 1994
North Carolina old field (75 m^2)	F1, T3	24	10	7	0	3	4	J.D. Bever, unpublished
West Virginia acid mines soil (60 m^2)	F1, T2	5	1	3	0	0	1	Morton 1986

[a]Collection of spores from: F*n*, field samples, where *n* is the number of sites and T*n*, trap cultures, where *n* = number of variations in culture conditions (e.g., different host species, successive culture generations, exotic versus native host, etc.).
[b]G, *Glomus*; A, *Acaulospora*; E, *Entrophospora*; Gi, *Gigaspora*; S, *Scutellospora*.

Fig. 2. Changes in species richness with successive approaches to recover sporulating fungi from different sites: an old field, Durham, N.C. (●); a sand dune, Assateague, Va. (○); a sonoran desert scrub community, Florence Junction, Ariz. (■); a chihuahuan desert scrub community, Padre Canyon, Tex. (□). Methods of estimating diversity (in parentheses) were identifying spores from the following: A, direct field counts; B, first generation trap culture; C, second generation trap culture; D, third generation trap culture; E, host plant from field site transplanted to sterile soil in pots; and F, trap cultures seeded with host species from field site. Number of species per method was partitioned by measuring total species richness using all methods at a given site and then averaging species richness in all possible combinations of one to four methods.



Species number

Measurement of species richness is the simplest index of taxonomic organization in communities (Magurran 1988). Despite equivocal species identifications in the literature, numbers of observed species are presumed to be accurate because they represent the sum of distinct spore morphotypes present in a sample. The problem is detecting organisms of all species present in roots of a plant community. Niche partitioning is important in spore formation, especially because a threshold level of colonization appears to be required (Franke and Morton 1994; Gazey et al. 1992; Pearson and Schweiger 1993). In the absence of sporulation, hyphal morphology, architecture, and differential histochemical staining provide some measure of taxonomic structure in roots, but only at the family level and above (Abbott and Gazey 1994; Morton and Bentivenga 1994).

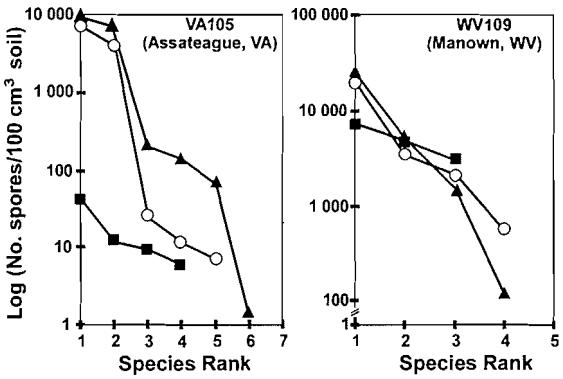
Species richness is assumed to increase with geographic range for any group of organisms (Ricklefs 1989). The lack of such a correlation in Table 2 likely is a result of local sampling error or plant–environment interactions, which affect the proportion of sporulating and nonsporulating fungi in plant roots. Tews and Koske (1986) report that a minimum of 30 field samples are needed to estimate species diversity in sand dune plant communities. Alternatively, species richness in a 75-m^2 undisturbed old field near Durham, N.C., required an exhaustive combination of field collections and culture strategies (J.D. Bever, unpublished; Fig. 2) to recover a majority of the mycorrhizal colonizers. These results suggest that no single method can be standardized for all habitats.

On a local scale, the question of species saturation in a root system has not been resolved, probably because the result depends so much on stochastic dispersal events and contingent host–soil–environmental conditions. A safe generalization is that more than one fungus is usually (if not always) present. Four or more can be present in extreme environments with monospecific plant communities (Morton 1986; Fig. 2) and eight or more sporulating fungi are recovered from pot microcosms (J.B. Morton, unpublished) or widely spaced plants in the field (Koske 1987).

Species abundance

Abundance of each fungal species in a mycorrhizal plant community is enumerated from spore counts, but there is no evidence of a cause and effect relationship (let alone consistent correlation) between sporulation and niche occupation (Brundrett 1991; Johnson et al. 1991*a*; Pearson and Schweiger 1993). Spore numbers provide indirect evidence of increased carbon partitioning to reproduction of an organism after a threshold level is reached (Gazey et al. 1992; Pearson and

Fig. 3. Relationship between rank and abundance of glomalean fungal species in two INVAM accessions, based on spore counts in field soil (■), first generation trap cultures (○), and second generation trap cultures (▲).



Schweiger 1993). Spore biovolume (e.g., Koske 1987) probably is a more accurate measure of resource allocation to sporulation when spores of a species assemblage are of unequal size (as often is the case). At the very least, the various models incorporating richness and abundance into single indices or rank-abundance plots (Magurran 1988) establish some measure of the dominant fungi in a mycorrhizal community at all scales (Table 2). Interpretations so far have taken these limitations into account (Koske 1987; Johnson et al. 1991*a*, 1991*b*; Stürmer and Bellei 1994).

Traditional rank-abundance models have, as part of their assumptions, niche preemption (e.g., geometric and log series plots) or equitability among species in sharing resources (e.g., broken stick model) (Magurran 1988). In a comparison of fungal communities in the field and in associated trap cultures (Fig. 3), spore abundance appeared to be more equitably distributed among species in the former than in the latter. However, greater number of species were recovered in the traps than in the field samples. These data suggest that niche preemption may be as intense in field as in pot culture environments when nonsporulating fungi are taken into account. Rank-abundance models of glomalean fungi, therefore, do not have the same meaning as they do for plant and animal community ecologists. They indirectly reflect carbon allocation patterns of the fungal community to sporulation (Stürmer and Bellei 1994).

Species composition and distribution

Accurate identification of species is needed to define species composition on local or regional scales. Most spores from field soil extractions, especially those of fungi in *Glomus* and *Gigaspora*, often are difficult or impossible to identify unless sporulation is high and parasitism is minimal (Morton 1993). Trap cultures can yield larger numbers of healthy spores and a better quantitative representation of local diversity, especially when they are established from rhizosphere soils containing few spores or infectivity of indigenous fungi is low (Figs. 2 and 3).

It currently is fashionable to view community structure as caused by local ecological processes. Larger scale historical processes, such as long-distance dispersal, vicariance, and speciation, are largely ignored or avoided (Ricklefs 1989). Historical processes may be as important, if not more so, than local ecological processes in the study of arbuscular fungi for a number of reasons. First, species composition in communities on both local versus regional scales often overlap and includes unrelated taxa representative of all families in Glomales (Table 2). Second, the distributions of individual species rarely have been correlated with ecological factors (Morton and Bentivenga 1994). Only in one report was temperature and soil particle composition considered to constrain the distribution range of *Scutellospora weresubiae* Koske & Walker (Koske and Walker 1986). Third, endemism is rationalized on the absence of taxa, whereas the presence of mostly unrelated taxa in speciose localities suggests broad dispersal. As a corollary, available data indicate both ancestral and derived species in all genera (e.g., *Acaulospora gerdemannii* Schenck & Nicol. versus *Acaulospora scrobiculata* Trappe; *Gigaspora gigantea* (Nicol. & Gerd.) Gerd. & Trappe versus *Scutellospora calospora* (Nicol. & Gerd.) Walker & Sanders; *Glomus occultum* Walker versus *Glomus intraradices* Schenck & Smith) have equally broad distributions on a minimum of four continents (INVAM Biogeographic Database, unpublished). Last, as many as eight species can coexist and even sporulate in pot microcosms where root growth is limited and the potential for niche preemption is theoretically high (Fig. 3; J.B. Morton, unpublished). Any study that seeks to make strong ecological interpretations of community dynamics exclusive of historical processes, therefore, must have a reasonable estimate of species diversity at sites prior to application or comparison of treatments. Most of the current literature, especially those involving chronosequences (e.g., Johnson et al. 1991*a*), can be interpreted in different ways depending on the extent to which local versus historical processes are acknowledged.

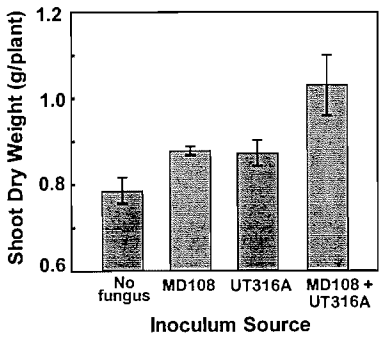
While local species composition has a strong historical component, broader temporal and spatial scales together with stochastic dispersal of fungal propagules reduces the phylogenetic value of biogeographic data. For example, the hypothesis that *Scutellospora* is ancestral to *Gigaspora* is much weaker from data on biogeographic distribution (Walker 1992) than that from rDNA sequence analysis (Simon et al. 1993).

Organisms of species assemblages

Plants in natural settings are colonized by fungal organisms of different species that constitute taxonomic components of a mycorrhizal community (see above). Coexistence of species representing most, if not all, genera in the same root system presupposes the lack of host specialization, but it does not exclude the possibility of preferential host effects directly on an organism's life history traits or indirectly on competition among coexisting fungal organisms.

The dynamics among organisms within or among root systems has been inferred primarily from sporulation measurements. For example, management practices such as grazing (Bethlenfalvay and Dakessian 1984) and cropping history (Johnson et al. 1991*b*; Schenck and Kinloch 1980) affect sporulation of individual fungi. Organisms of different fungal species at a site can sporulate differentially with time and with host species (Bentivenga and Hetrick 1992). While

Fig. 4. Effect of mycorrhizal inoculation on the shoot growth of 6-week-old *Glycine max* (L.) Merr. cultivar Arkansas Centennial. MD108, *G. etunicatum* (100-cm^3 whole inoculum); UT316A, *G. etunicatum*/*G. intraradices* mix (100-cm^3 whole inoculum); MD108 + UT316A, 50-cm^3 whole inoculum of each culture. Bars represent the mean of four replicates; error bars = SE.



sporulation is not an accurate indicator of shifts in species composition or abundance in mycorrhizae, it provides an indirect measure of carbon partitioning for reproduction (see above).

Many traits alter the ability of a fungal genotype to enhance plant growth, such as rate and extent of hyphal exploration of soil, efficiency of nutrient uptake from soil, efficiency of nutrient transfer to the host, and the magnitude and timing of carbon drain/partitioning with the host (Smith and Gianinazzi-Pearson 1988). The physical contribution of each member organism of the mycorrhizal community in one root system requires some direct estimate of the proportion of root length colonized. McGonigle and Fitter (1990) were able to do this by comparing fine and coarse endophytes, whereupon they affirmed that some ecological specificity exists and preferential colonization of a host occurs. Testing the universality of this phenomenon will require other detection and monitoring probes. The problem is that mycorrhizal morphology is not fungal species specific (Abbott and Gazey 1994; Morton and Bentivenga 1994) and monoclonal antibodies (Wright and Morton 1989) and other molecular probes currently lack either specificity, sensitivity, or analytical capability.

Differential effects of individual fungi in a mycorrhizal community at all scales has not been well established, partly because of their complexity with changing host–environment conditions and partly because of difficulties in culturing all organisms from each sample site. Hetrick and Wilson (1990) showed that plant responses can be equivalent between an individual fungus and an assemblage of seven fungal genotypes. However, the assemblage represents a combination of organisms that could be interacting additively, synergistically, or antagonistically. In one preliminary experiment, two fungal organisms of different species increased shoot growth of *Glycine max* L. additively (Fig. 4) (S.P. Bentivenga and McCarthy, unpublished). In another, where fungal assemblages were the experimental unit, management practices appeared to reduce effectiveness by selecting for less beneficial fungal genotypes (e.g., Johnson 1993). This hypothesis is testable by partitioning each organism and defining its life history traits and mycorrhizal phenotype (Abbott and Gazey 1994). Unfortunately, test conditions may not be equivalent to the original environment of the species assemblage, so that results could still be ecologically misleading. The results of field experiments on mycorrhizal growth benefits from single organisms or species assemblages are contradictory (McGonigle 1988).

Population dynamics of arbuscular fungi may best be described by the metapopulation concept currently applied to many plant pathogens (Burdon 1993; Antonovics et al. 1994). This concept is based on recognition that many intraspecific populations are patchily distributed and composed of subpopulations, each with their own set of microenvironmental conditions (Hanski and Gilpin 1993). Local fecundity and extinction are influenced by these microenvironments. The isolated subpopulations interact only when dispersal events bring individuals from disjunct subpopulations together. Mathematical models of metapopulation dynamics may prove useful in describing competition, reaction to disturbance, dispersal, and survival. They may also provide insight into a link between α (within site) and β (between site) diversity.

Suborganismal assemblages

All current ecological models are based on delineation of individuals, the experimental units of a population. Spores or discrete hyphal fragments have operational individuality in that they can be extracted singly and used to initiate a new mycorrhiza. However, the mycelium of a fungal organism is a potential reservoir of different genotypes, depending on the degree of nuclear heterogeneity and migration, histocompatability, and nuclear exchange (Rayner 1991). Arbuscular fungi in Glomales are no exception. They are multinucleate and the nuclei are capable of migration in coenocyctic hyphae. The extent of homogeneity among nuclei of single cells has yet to be empirically defined, so the rate and magnitude of intraorganismal variation remains unknown. Anastomosis appears to occur to at least a limited extent among sympatric organisms of a species (Tommerup 1988), although the potential for genetic exchange among individuals still is poorly defined. The only tools available to study these phenomena are biochemical or genetic markers of individual nuclei or of organisms started from carefully delineated inocula, such as single spores or individual hyphal fragments.

Diversity in the balance

Patterns of diversity at all organizational levels suggest an equilibrium between positive and negative intrinsic developmental and extrinsic ecological forces on coevolution of fungal symbionts and their plant partners. Obligate biotrophy by the fungal partner is balanced by a broad host range. Numerous migratory nuclei in a potentially chimeric mycelium provides genetic versatility in the absence of a sexual cycle; independent assortment of nuclei may substitute for independent assortment of genes. Growth habit and ecological versatility in conjunction with wide host range confer potential immortality and hence a greater probability for widespread distribution through geologic time and space. Historical processes, then, become important in interpreting community and ecosystem dynamics. They are likely to intergrade with local-scale ecological processes, requiring

new approaches to partition the relative contributions of each to measures of diversity at all levels.

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References

Abbott, L.K., and Gazey, C. 1994. An ecological view of the formation of VA mycorrhizas. Plant Soil, **159**: 69–78.

Almeida, R.T., and Schenck, N.C. 1990. A revision of the genus *Sclerocystis* (Glomaceae, Glomales). Mycologia, **82**: 703–714.

Antonovics, J., Thrall, P., Jarosz, A., and Stratton, D. 1994. Ecological genetics of metapopulations: the *Silene–Ustilago* plant pathogen system. *In* Ecological genetics. *Edited by* L. Real. Princeton University Press, Princeton, N.J. pp. 146–170.

Bécard, G., and Piché, Y. 1989. New aspects on the acquisition of biotrophic status by a vesicular–arbuscular mycorrhizal fungus, *Gigaspora margarita*. New Phytol. **112**: 77–83.

Bentivenga, S.P., and Hetrick, B.A.D. 1992. Seasonal and temperature effects on mycorrhizal activity and dependence of cool- and warm-season tallgrass prairie grasses. Can. J. Bot. **70**: 1596–1602.

Bentivenga, S.P., and Morton, J.B. 1994. Stability and heritability of fatty acid methyl ester profiles of glomalean endomycorrhizal fungi. Mycol. Res. **98**: 1419–1426.

Bethlenfalvay, G.J., and Dakessian, S. 1984. Grazing effects on mycorrhizal colonization and floristic composition of the vegetation on a semiarid range in northern Nevada. J. Range Manage. **37**: 312–316.

Blaszkowski, J. 1989. The occurrence of the Endogonaceae in Poland. Agric. Ecosyst. Environ. **29**: 45–50.

Brundrett, M. 1991. Mycorrhizas in natural ecosystems. Adv. Ecol. Res. **21**: 171–313.

Burdon, J.J. 1993. The structure of pathogen populations in natural plant communities. Annu. Rev. Phytopathol. **31**: 305–323.

Dodd, J.C., Gianinazzi-Pearson, V., Rosendahl, S., and Walker, C. 1994. European Bank of Glomales—an essential tool for efficient international and interdisciplinary collaboration. *In* Impact of arbuscular mycorrhizas on sustainable agriculture and natural ecosystems. *Edited by* S. Gianinazzi and H. Schüepp. Birhaüser-Verlag, Basel, Switzerland. pp. 41–45.

Franke, M., and Morton, J.B. 1994. Ontogenetic comparisons of the endomycorrhizal fungi *Scutellospora heterogama* and *Scutellospora pellucida*: revision of taxonomic character concepts, species descriptions, and phylogenetic hypotheses. Can. J. Bot. **72**: 122–134.

Gazey, C., Abbott, L.K., and Robson, A.D. 1992. The rate of development of mycorrhizas affects the onset of sporulation and production of external hyphae by two species of *Acaulospora*. Mycol. Res. **96**: 643–650.

Gianinazzi-Pearson, V., Lemaine, M.C., Arnould, C., Gollotte, A., and Morton, J.B. 1994. Localization of $\beta(1 \rightarrow 3)$ glucans in spore and hyphal walls of fungi in the Glomales. Mycologia, **86**: 478–485.

Graham, J.H., Hodge, N.C., and Morton, J.B. 1995. Fatty acid methyl ester profiles for characterization of glomalean fungi and their endomycorrhizas. Appl. Environ. Microbiol. In press.

Hanski, I., and Gilpin, M. 1993. Metapopulation dynamics: brief history and conceptual domain. Biol. J. Linn. Soc. **42**: 3–16.

Hetrick, B.A.D., and Bloom, J. 1983. Vesicular–arbuscular mycorrhizal fungi associated with native tall grass prairie and cultivated winter wheat. Can. J. Bot. **61**: 2140–2146.

Hetrick, B.A.D., and Wilson, G.W.T. 1990. Relationship of native and introduced mycorrhizal fungi to mycorrhizal dependence of *Andropogon gerardii* and *Koeleria pyranidata*. Mycologia, **82**: 779–782.

Hung, L.L., and Sylvia, D.M. 1988. Production of vesicular–arbuscular mycorrhizal fungus inoculum in aeroponic culture. Appl. Environ. Microbiol. **54**: 353–357.

Johnson, N.C. 1993. Can fertilization of soil select less mutualistic mycorrhizae? Ecol. Appl. **3**: 749–757.

Johnson, N.C., Zak, D.R., Tilman, D., and Pfleger, F.L. 1991*a*. Dynamics of vesicular–arbuscular mycorrhizae during old field succession. Oecologia, **86**: 349–358.

Johnson, N.C., Pfleger, F.L., Crookston, R.K., Simmons, S.R., and Copeland, P.J. 1991*b*. Vesicular–arbuscular mycorrhizas respond to corn and soybean cropping history. New Phytol. **117**: 657–663.

Koske, R.E. 1987. Distribution of VA mycorrhizal fungi along a latitudinal temperature gradient. Mycologia, **79**: 55–68.

Koske, R.E., and Tews, L.L. 1987. Vesicular–arbuscular mycorrhizal fungi of Wisconsin sandy soils. Mycologia, **79**: 901–905.

Koske, R.E., and Walker C. 1986. Species of *Scutellospora* (Endogonaceae) with smooth-walled spores from maritime sand dunes: two new species and a redescription of the spores of *Scutellospora pellucida* and *Scutellospora calospora*. Mycotaxon, **27**: 219–235.

Law, R. 1985. Evolution in a mutualistic environment. *In* The biology of mutualism. *Edited by* D.H. Boucher. Oxford University Press, New York. pp. 29–39.

Magurran, A.E. 1988. Ecological diversity and its measurement. Princeton University Press, Princeton, N.J.

McGonigle, T.P. 1988. A numerical analysis of published field trials with vesicular–arbuscular mycorrhizal fungi. Funct. Ecol. **2**: 473–478.

McGonigle, T.P., and Fitter, A.H. 1990. Ecological specificity of vesicular–arbuscular mycorrhizal associations. Mycol. Res. **94**: 120–122.

McKitrick, M.C. 1993. Phylogenetic constraint in evolutionary theory: has it any explanatory power? Annu. Rev. Ecol. Syst. **24**: 307–330.

Miller, D.D., Domoto, P.A., and Walker, C. 1985. Mycorrhizal fungi at eighteen apple rootstock plantings in the United States. New Phytol. **100**: 379–391.

Millner, P.D., and Kitt, D.G. 1992. The Beltsville method for soilless production of vesicular–arbuscular mycorrhizal fungi. Mycorrhiza, **2**: 9–15.

Morton, J.B. 1986. Three new species of *Acaulospora* (Endogonaceae) from high aluminum, low pH soils in West Virginia. Mycologia, **78**: 641–648.

Morton, J.B. 1990*a*. Species and clones of arbuscular mycorrhizal fungi (Glomales, Zygomycetes): their role in macro- and microevolutionary processes. Mycotaxon, **37**: 493–515.

Morton, J.B. 1990*b*. Evolutionary relationships among arbuscular mycorrhizal fungi in the Endogonaceae. Mycologia, **82**: 192–207.

Morton, J.B. 1993. Problems and solutions for the integration of glomalean taxonomy, systematic biology, and the study of mycorrhizal phenomena. Mycorrhiza, **2**: 97–109.

Morton, J.B. 1995. Taxonomic and phylogenetic divergence among five *Scutellospora* species (Glomales, Zygomycetes)

based on comparative developmental sequences. Mycologia, **87**: 127–137.

Morton, J.B., and Benny, G.L. 1990. Revised classification of arbuscular mycorrhizal fungi (Zygomycetes): a new order, Glomales, two new suborders, Glomineae and Gigasporineae, and two new families, Acaulosporaceae and Gigasporaceae, with an emendation of Glomaceae. Mycotaxon, **37**: 471–491.

Morton, J.B., and Bentivenga, S.P. 1994. Levels of diversity in endomycorrhizal fungi (Glomales, Zygomycetes) and their role in defining taxonomic and nontaxonomic groups. Plant Soil, **159**: 47–59.

Morton, J.B., Bentivenga, S.P., and Wheeler, W.W. 1993. Germ plasm in the International Collection of Arbuscular and Vesicular–Arbuscular Mycorrhizal Fungi (INVAM) and procedures for culture development, documentation, and storage. Mycotaxon, **48**: 91–528.

Pearson, J.N., and Schweiger, P. 1993. *Scutellospora calospora* (Nicol. & Gerd.) Walker & Sanders associated with subterranean clover: dynamics of colonization, sporulation, and soluble carbohydrates. New Phytol. **124**: 215–219.

Pirozynski, K.A., and Dalpé, Y. 1989. Geological history of the Glomaceae, with particular reference to mycorrhizal symbiosis. Symbiosis, **7**: 1–36.

Rayner, A. 1991. The challenge of the individualistic mycelium. Mycologia, **83**: 48–71.

Ricklefs, R.E. 1989. Speciation and diversity: the integration of local and regional processes. *In* Speciation and its consequences. *Edited by* D. Otte and J.A. Endler. Sinauer Associates, Inc., Sunderland, Mass. pp. 599–621.

Robson, A.D., Abbott, L.K., and Malajczuk, N. (*Editors*). 1994. Management of mycorrhizas in agriculture, horticulture, and forestry. Kluwer Academic Publishers, Dordrecht, the Netherlands.

Schenck, N.C., and Kinloch, R.A. 1980. Incidence of mycorrhizal fungi on six field crops in monoculture on a newly cleared woodland site. Mycologia, **72**: 445–456.

Simon, L., Bousquet, J., Lévesque, R.C., and Lalonde, M. 1993. Origin and diversification of endomycorrhizal fungi and coincidence with vascular land plants. Nature (London), **363**: 67–69.

Smith, S.E., and Gianinazzi-Pearson, V. 1988. Physiological interactions between symbionts in vesicular–arbuscular mycorrhizal plants. Annu. Rev. Plant Physiol. Plant Mol. Biol. **39**: 221–244.

Stürmer, S.L., and Bellei, M.M. 1994. Composition and seasonal variation of spore populations of arbuscular mycorrhizal fungi in dune soils on the island of Santa Catarina, Brazil. Can. J. Bot. **72**: 359–363.

Tews, L.A., and Koske, R.E. 1986. Toward a sampling strategy for vesicular–arbuscular mycorrhizas. Trans. Br. Mycol. Soc. **87**: 353–358.

Tommerup, I.C. 1988. The vesicular–arbuscular mycorrhizas. *In* Advances in plant pathology. Vol. 6. Academic Press, New York. pp. 81–91.

Walker, C. 1992. Systematics and taxonomy of the arbuscular endomycorrhizal fungi (Glomales)—a possible way forward. Agronomie, **12**: 887–897.

Walker, C., Mize, C.W., and McNabb, H.S. 1982. Populations of endogonaceous fungi at two locations in central Iowa. Can. J. Bot. **60**: 2518–2529.

Wright, S.F., and Morton, J.B. 1989. Detection of sporulation by *Glomus occultum* in mycorrhizal roots using a dot-immunoblot assay. Appl. Environ. Microbiol. **55**: 761–763.

Wyss, P., and Bonfante, P. 1993. Amplification of genomic DNA of arbuscular–mycorrhizal (AM) fungi by PCR using short arbitrary primers. Mycol. Res. **97**: 1351–1357.