Gross N, Robson TM, Lavorel S, Albert C, Le Bagousse-Pinguet Y, Guillemin R. 2008. Plant response traits mediate the effects of subalpine grasslands on soil moisture. *New Phytologist* **180**: 652–662.

Jenny H. 1941. *Factors of soil formation*. New York, NY, USA: McGraw-Hill.

Lavorel S, Garnier E. 2002. Predicting changes in community composition and ecosystem functioning from plant traits: revisiting the Holy Grail. *Functional Ecology* **16**: 545–556.

Pontes LDS, Soussana JF, Louault F, Andueza D, Carrere P. 2007. Leaf traits affect the above-ground productivity and quality of pasture grasses. *Functional Ecology* **21**: 844–853.

Quetier F, Thebault A, Lavorel S. 2007. Plant traits in a state and transition framework as markers of ecosystem response to land-use change. *Ecological Monographs* **77**: 33–52.

Raunkiaer C. 1937. *Plant life forms*. Oxford, UK: Clarendon Press.

Suding KN, Lavorel S, Chapin FS, Cornelissen JHC, Diaz S, Garnier E, Goldberg D, Hooper DU, Jackson ST, Navas ML. 2008. Scaling environmental change through the community-level: a trait-based response-and-effect framework for plants. *Global Change Biology* **14**: 1125–1140.

Vile D, Shipley B, Garnier E. 2006. Ecosystem productivity can be predicted from potential relative growth rate and species abundance. *Ecology Letters* **9**: 1061–1067.

Key words: community dynamics, ecosystem change, effect traits, effect–response framework, functional traits, response traits, vegetation models, water availability.

Tree root architecture – form and function

Over 25 years ago, Fitter (1982) recognized that the form (architecture) of the branching root system was almost certainly related to the acquisition of essential soil resources (function). Since then, there have been many studies that have advanced our understanding of how plasticity in the birth and death of lateral root branches might confer a competitive advantage on individual plants and may structure plant communities, and the topic continues to generate significant scientific interest and debate to this day (e.g. Kembel & Cahill, 2005; de Kroon & Mommer, 2006; Hodge, 2006; Grime, 2007; Kembel *et al.*, 2008). The primary focus to this point in the ecological literature has been on understanding rates of root length proliferation and we know that root length can sometimes respond dramatically to increased availability of 'patchy' soil resources. However, missing from many studies of root proliferation and acquisition of essential soil resources is a detailed understanding of how plants have altered their form (morphology and especially anatomy). Guo *et al.* (this issue; pp. 673–683) make an important contribution by comparing the lateral root branch anatomy of 23 species of temperate trees. They address the following questions. How are multitasking temperate tree root systems designed? Is the branching root system anatomically similar across species? Are lateral branches constructed so that only the most distal roots are responsible for absorption of nutrients?

> *'The questions of how plant root systems are constructed and how their form is related to the capture of essential soil resources have intrigued ecologists and plant biologists for decades ...'*

Multitasking root systems in patchy soil

Trees, like all plants growing in the wild, must solve a host of problems using their root systems. Root systems anchor the plant, sometimes from gale-force winds. Perennial plants have a growth rhythm designed to help the plant survive periods of cold, drought and defoliation, and roots (as well as shoots) store nonstructural carbohydrates to provide the plant with the energy it needs to survive changes in climate and periodic disturbances that alter whole-plant source–sink relationships. Roots are a part of the plant vascular system that takes up soil solution and transports water and other compounds (Pratt *et al.*, 2008). Finally, roots and their associated symbiotic bacteria and fungi are responsible for fixing atmospheric nitrogen and acquiring the essential nutrients required for growth.

The questions of how plant root systems are constructed, and how their form is related to the capture of essential soil resources, have intrigued ecologists and plant biologists for decades because we have learned that there can be as much variation in the availability of essential nutrients across 20 cm of soil as there is across an entire field or across a significant ecological gradient (Gross *et al.*, 1995; Farley & Fitter, 1999). An interesting example of how microsite mineralization of soil nitrogen can be influenced by plant roots and their associated mycorrhizas is presented by Schimel & Bennett (2004). Plants are basically sessile organisms, so the only way that they can 'forage' for 'patchy' essential soil resources is to dynamically change their form or their physiology, or both.

Lateral roots – dynamic plant modules

Lateral roots are the plant modules that grow and die on small spatial scales in the soil and they, along with their associated mycorrhizas, are primarily responsible for nutrient acquisition. Variations in the size, shape, surface area and demography of lateral branches and associated mycorrhizas, along with concomitant changes in root physiology, are the way in which a plant can 'forage' for water and nutrients in the soil. Lateral root branches arise in the pericycle of the parent root, grow through the cortex and form lateral branches, which are sometimes complex in their architecture (Pregitzer, 2002; Pregitzer *et al.*, 2002). The plant root system can sense a

© The Author (2008). Journal compilation © *New Phytologist* (2008)

change in resource availability in the soil and initiate new lateral roots in 'hot spots', places in the soil where essential resources are more available (Walch-Liu *et al.*, 2006; Nibau *et al.*, 2008).

Some years ago, Grime (1965) argued that comparative patterns in trait variation could tell us something about functional specialization. This is essentially the approach that Guo *et al.* have taken. They systematically dissected the lateral branches of 23 species of temperate trees growing in China following the protocol of Pregitzer *et al.* (2002). They also quantified and used the anatomy of the distal root branches as a surrogate for distinguishing root branches involved in active metabolic uptake of nutrients vs transport and storage. In addition to quantifying the anatomy of branching root segments, they quantified which of the lateral branch orders were colonized by mycorrhizal fungi.

The results of Guo *et al.* suggest that most of the active absorption of nutrients occurs in first-order roots, the tiny lateral branches at the very distal end of the root system (Pregitzer *et al.*, 2002). Mycorrhizas were associated with the first three orders of roots, although the degree of activity of mycorrhizas in the second-order and third-order roots is not clear from their results. Based on the results of Guo *et al.* and what we know about the relationship between root nitrogen concentration and rates of root respiration (Reich *et al.*, 2008), I suspect that the first-order roots are the primary carbon depot for mycorrhizal hyphae, which ramify away from the root tip to forage widely in the spatially and temporally heterogeneous soil. Guo *et al.* also found that the morphology and anatomy of the root system seems to be conserved within a species, an observation increasingly reported in the literature (Pregitzer *et al.*, 2002; Kembel & Cahill, 2005; Grime, 2007). The implication is that different species have evolved specialized mechanisms to sense changes in the availability of essential soil resources and to alter their lateral branch architecture and demography to compete effectively for limiting water or nutrients (Walch-Liu *et al.*, 2006; Nibau *et al.*, 2008). However, the evolutionary costs and benefits of lateral root plasticity have not yet received the attention they deserve (de Kroon & Mommer, 2006).

Unanswered questions

The results of Guo *et al.* raise several unanswered questions. To start with, the systematic dissection of lateral root branches into orders is an arbitrary approach. Granted, this systematic approach has led us to understand that most of the absorptive length and metabolic activity in tree roots is correlated with the distal ends of the branching root system (Pregitzer *et al.*, 1998; Pregitzer *et al.*, 2002; Reich *et al.*, 2008). However, we still do not really understand the variation in morphology and anatomy of lateral branches that arise in the pericycle. We know that the plant can sense external changes in essential soil resources, alter endogenous factors that regulate lateral root development and produce a plastic response – but how plastic? Do trees primarily produce new first-order roots and alter mycorrhizal fungi associations in response to changing soil conditions or are new lateral branches more complex in architecture? Is lateral root branch architecture highly conserved within a species, or highly plastic? How heritable is lateral root branch architecture?

One of the dangers in trying to make global generalizations about root form and function is the comparison of roots that differ significantly in how they are constructed and how active they are metabolically. Guo *et al.* demonstrate that root order predicts root anatomy fairly consistently across 23 species of temperate trees. However, if we were to compare trees with perennial forbs and annual grasses we would find a different outcome. Studies of root relative growth rate in 'patchy' soil mostly ignore the fact that lateral roots can differ dramatically in both form and function (Pregitzer, 2002). Cumulative evidence now strongly suggests that the lateral roots of trees vary in their construction and maintenance costs depending on their position on the branching root system. As time progresses and our understanding of tree root systems improves, it increasingly seems as if 'fine roots' – roots actively involved in the uptake of nutrients and roots that have short life expectancies – consist primarily of first-order to third-order lateral roots that arise dynamically from the pericycle in response to changes in soil resource availability. In reality, this generalization is a gross over-simplification. The distal branches of the perennial roots system are the hub of mycorrhizal activity and the host to root hairs (Fig. 1). A more detailed understanding of the dynamic and integrated 'root–fungal module' awaits the attention of innovative new studies. Clearly, the

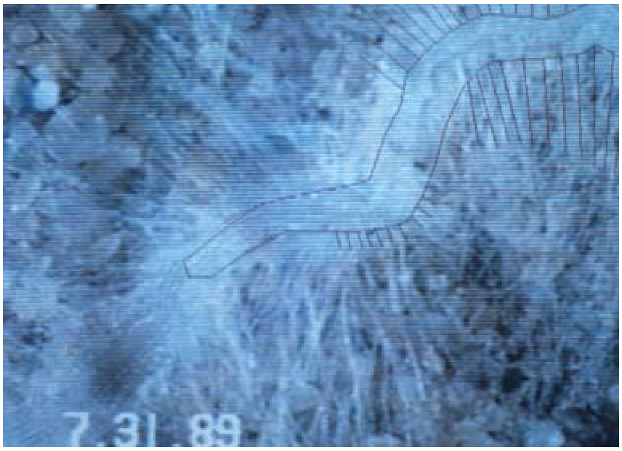

Fig. 1 *Prunus pennslvanica* L. (pin cherry) lateral root tip and associated roots hairs growing ('foraging') in a patch of nitrogen-enriched sandy soil at the University of Michigan Biological Station in 1989. Individual grains of sand and scores of root hairs are visible in the image. The outline shows the position of the root and root hairs 12 h previously. Lateral root architecture, root hairs and mycorrhizal hyphae are all responsive to changes in soil resource availability, but relationships between root form and function remain relatively poorly understood. (See Pregitzer *et al.*, 1993 for details of this particular study.)

© The Author (2008). Journal compilation © *New Phytologist* (2008)

metabolic action in woody plants with complex lateral branching root systems is at the tips of the lateral root branches. It seems that the functional architecture of plant roots is as diverse and interesting as the functional architecture of shoots. Perhaps we should not be surprised by this because biotic diversity is high, competition is keen and resource capture is spatially and temporally complex in the patchy soil.

Acknowledgements

This work was supported by the Division of Environmental Biology (Ecosystem Studies) of the National Science Foundation, the Office of Biological and Environmental Research (BER) of the Department of Energy, and the Nevada Agricultural Experiment Station. I am grateful for this support.

Kurt S. Pregitzer

Professor and Chair
Department of Natural Resources & Environmental Science
University of Nevada
1000 Valley Road, Mail Stop 186
Reno, NV 89512, USA
(tel +775-784-4000; fax +775-784-4583;
email ksp@cabnr.unr.edu)

References

Farley RA, Fitter AH. 1999. Temporal and spatial variation in soil resources in a deciduous woodland. *Journal of Ecology* **87**: 688–696.

Fitter AH. 1982. Morphometric analysis of root systems: application of the technique and influence of soil fertility on root system development in two herbaceous species. *Plant, Cell & Environment* **5**: 313–322.

Grime JP. 1965. Comparative experiments as a key to the ecology of flowering plants. *Ecology* **46**: 513–515.

Grime JP. 2007. The scale – precision trade-off in spatial resources foraging by plants: restoring perspective. *Annals of Botany* **99**: 1017–1021.

Gross KL, Pregitzer KS, Burton AJ. 1995. Spatial variation in nitrogen availability in three successional plant communities. *Journal of Ecology* **83**: 357–368.

Guo D, Xia M, Wei X, Chang W, Liu Y, Wang Z. 2008. Anatomical traits associated with absorption and mycorrhizal colonization are linked to root branch order in twenty-three Chinese temperate tree species. *New Phytologist* **180**: 673–683.

Hodge A. 2006. Plastic plants and patchy soils. *Journal of Experimental Botany* **57**: 401–411.

Kembel SW, Cahill JF Jr. 2005. Plant phenotype plasticity belowground: a phylogenetic perspective on root foraging tradeoffs. *The American Naturalist* **166**: 216–230.

Kembel SW, De Kroon H, Cahill JF Jr, Mommer L. 2008. Improving the scale and precision of hypotheses to explain root foraging ability. *Annals of Botany* **101**: 1295–1301.

de Kroon H, Mommer L. 2006. Root foraging theory put to the test. *Trends in Ecology and Evolution* **21**: 113–116.

Nibau C, Gibbs DJ, Coates JC. 2008. Branching out in new directions: the control of root architecture by lateral root formation. *New Phytologist* **179**: 595–614.

Pratt RB, Jacobsen AL, North GB, Sack L, Schenk HJ. 2008. Plant hydraulics: new discoveries in the pipeline. *New Phytologist* **179**: 590–593.

Pregitzer KS. 2002. Fine roots of trees – a new perspective. *New Phytologist* **154**: 267–273.

Pregitzer KS, DeForest JL, Burton AJ, Allen MF, Ruess RW, Hendrick RL. 2002. Fine root architecture of nine North American trees. *Ecological Monographs* **72**: 293–309.

Pregitzer KS, Hendrick RL, Fogel R. 1993. The demography of fine roots in response to patches of water and nitrogen. *New Phytologist* **125**: 575–580.

Pregitzer KS, Laskowski MJ, Burton AJ, Lessard VC, Zak DR. 1998. Variation in sugar maple root respiration with root diameter and soil depth. *Tree Physiology* **18**: 665–670.

Reich PB, Tjoelker MG, Pregitzer KS, Wright IJ, Oleksyn J, Machado J-L. 2008. Scaling of respiration to nitrogen in leaves, stems and roots of higher land plants. *Ecology Letters* **11**: 793–801.

Schimel JP, Bennett J. 2004. Nitrogen mineralization: challenges of a changing paradigm. *Ecology* **85**: 591–602.

Walch-Liu P, Ivanov II, Filleur S, Gan Y, Remans T, Forder B. 2006. Nitrogen regulation of root branching. *Annals of Botany* **97**: 875–881.

Key words: lateral roots, root anatomy, root demography, root turnover, root update.

Letters

Multilocus genotyping of arbuscular mycorrhizal fungi and marker suitability for population genetics

Arbuscular mycorrhizal fungi (AMF) are an ecologically important group of plant symbionts and their species richness has been shown to influence plant diversity and productivity (Van der Heijden *et al.*, 1998). Genetic diversity within AMF species is important as genetically different isolates have been shown to differentially affect plant growth and nutrition (Munkvold *et al.*, 2004; Koch *et al.*, 2006). The study of AMF diversity in ecosystems, particularly identifying which AMF species associate with different host plants, requires reliable identification of different AMF. It has long been recognized that identifying AMF across broad geographical ranges requires molecular tools for fast and reliable genotyping directly from soil material.

© The Authors (2008). Journal compilation © *New Phytologist* (2008)

Previously, genotyping methods for distinguishing AMF species have mostly been restricted to ribosomal DNA (rDNA) sequences. The advantage of these loci is the potential for cross-species amplification using universal primers, and the relative ease of amplification from different material (e.g. colonized root pieces, single spores, etc.). A large body of studies have identified the species composition of AMF communities in many different ecosystems (Öpik *et al.*, 2006; Rosendahl, 2008). However, studies of genetic variability within AMF species are important for understanding the basic biology, genetics and ecology of AMF fungi, which cannot be addressed at the community level. For example, a hierarchical study of genetic variability from the local scale within populations right up to an inter-continental scale is lacking. Such hierarchically designed studies could lay the foundation that will allow us to answer fundamental questions about the biology of AMF, their genetics, whether they form recombinant populations, the amount of genetic exchange among populations, the importance of drift and selection in AMF species, and the distribution of genetic and functional diversity in AMF over different geographic scales, and allow us to examine the co-evolutionary relationships between AMF genotypes and their host plant genotypes.

For most of these applications ribosomal markers are unsuitable because of a lack of sufficient within-species variability and are potentially problematic because of confounding intra-sporal variability (Sanders *et al.*, 1995) and copy number polymorphism (Corradi *et al.*, 2007). A population genetics approach to the study of AMF requires multilocus genotyping of nonribosomal loci. Stukenbrock & Rosendahl (2005a,b) first developed and applied this approach by amplifying three different loci in a large set of spores of three *Glomus* species harvested from the field. However, ideally, multilocus genotyping should comprise a much larger number of loci. Two simultaneously published studies (Croll *et al.*, 2008; Mathimaran *et al.*, 2008), describing genetic markers for AMF, should now make this possible. Both studies identified multiple loci that were variable among isolates of a commonly studied AMF, *Glomus intraradices*. Length differences among the alleles were used to identify genetic differences. Part, but not all, of the variation was found in repeat regions, and both studies referred to the markers as either microsatellites or simple sequence repeat markers. The simultaneous publication of the two studies might lead to some confusion for researchers who may now want to use these markers. Here, our aim is to clarify how many new and different loci have actually been identified and which loci are likely to be suitable for population genetics studies, to highlight potential problems with the genotyping techniques used, and to discuss future approaches to their use in AMF population biology.

The study by Mathimaran *et al.* (2008) identified 18 loci and Croll *et al.* (2008) showed polymorphism in 13 loci, of which two had previously been identified by Raab *et al.* (2005). The two studies used similar, but not identical, strategies to identify repetitive DNA stretches by searching publicly available databases (Table 1). Candidate sequences were then amplified in a set of isolates and potential length polymorphism was scored. In both studies, loci were amplified in a number of isolates from different geographic locations. It should be noted that one locus described by Mathimaran *et al.* (2008) is the same as one polymorphic locus identified by Croll *et al.* (2008) but has been given two different designations. The variation in two more loci reported by Mathimaran *et al.* (2008) is documented in previously published work. We hope that Table 1 will help researchers who intend to use these markers to identify the different loci for which primers have been developed and prevent unintentional studies of the same locus under two different names.

Locus *Glint08* identified by Mathimaran *et al.* (2008) is identical to locus Bg348 from Croll *et al.* (2008), even though the primers are located at different distances from the repetitive sequence region. Loci *Glint09* and *Glint18* identified by Mathimaran *et al.* (2008) were previously published by Corradi & Sanders (2006) and described as genes encoding P-type IID ATPases. Corradi & Sanders (2006) reported polymorphism in a population of *G. intraradices* based on a comparison of different alleles at the same locus. Furthermore, the gene was found to exist in two variants in each of several isolates and in three variants within one isolate (Corradi & Sanders, 2006). Locus *Glint09* is based on the sequence of the third variant; however, the primers designed by Mathimaran *et al.* (2008) are not specific for this particular variant. As a consequence, the primers based on locus *Glint09* potentially amplify up to three different locations in the genome within a single isolate. Locus *Glint18* was identified in an assembled sequence (contig) that matches the P-type IID ATPase variants. However, the resulting consensus sequence does not exactly match any of the original P-type IID ATPase variants, probably as a consequence of the contig being assembled from several different variants (i.e. a chimaeric contig). Consequently, primers for locus *Glint18* do not specifically amplify one of the several variants. Loci *Glint09* and *Glint18* are separated by approx. 500 bp. In our opinion, these two loci are unsuitable for most population genetic studies because of the multi-copy nature of the gene they are located in, unless primer sequences are chosen that restrict the amplification to one variant.

The studies of Mathimaran *et al.* (2008) and Croll *et al.* (2008) both describe polymorphic loci exhibiting size differences of 1 or 2 bp among some alleles. Scoring such a polymorphism is potentially problematic even if PCR products are separated on a capillary sequencer, Spreadex polymer or polyacrylamide gels. These methods offer a high resolution of allele length differences, but the amplification of repeat motifs often leads to the presence of stutter peaks (or shadow bands) as a result of DNA polymerase error. Where small length differences are observed among alleles, it is advisable to

Table 1 Summary of markers developed for *Glomus intraradices*

Locus	Accession no.	Database	Function	Type	Length polymorphism	Reference
Bg32	CG431930	GSS	Unknown	Probably noncoding	Indels	Croll *et al*. (2008)
Bg42	CG431913	GSS	Unknown	Probably noncoding	(TA) repeat + other indels	Croll *et al*. (2008)
Bg62	CG431880	GSS	RNA polymerase II large subunit	Proximate coding region	(TAAAA) repeat + other indels	Croll *et al*. (2008)
Bg196	CG431972	GSS	Unknown	Probably noncoding	Several repeat motifs + other indels	Croll *et al*. (2008)
Bg235	CG432041	GSS	Unknown	Probably noncoding	Several indels	Croll *et al*. (2008)
Bg273	CG432137	GSS	Unknown	Probably noncoding	(T) + (A) repeats + other indels	Croll *et al*. (2008)
Bg276	CG432062	GSS	Unknown	Probably noncoding	Several indels	Croll *et al*. (2008)
Bg303	CG432175	GSS	Unknown	Probably noncoding	Several indels	Croll *et al*. (2008)
Bg348	CG432294	GSS	Predicted protein of unknown function	Proximate coding region	(TAA) + (TAAA) repeats + other indels	Croll *et al*. (2008)
Bg355	CG432269	GSS	Unknown	Probably noncoding	Several indels	Croll *et al*. (2008)
Nuclear intron	BE603853	EST	Intron in gene of unknown function	Proximate coding region	(T), (A) + (TAA) repeats	Croll *et al*. (2008)
mtLSU int1	AJ973189-193	Standard	Intron in mitochondrial LSU gene	Proximate coding region	Several indels	Raab *et al*. (2005); Croll *et al*. (2008)
mtLSU int2	AJ973189-193	Standard	Intron in mitochondrial LSU gene	Proximate coding region	Indel	Raab *et al*. (2005); Croll *et al*. (2008)
Glint01	CG432086+113*	GSS	Unknown	Coding	(AAAT) repeat + other indels	Mathimaran *et al*. (2008)
Glint02	DT883628	EST	Unknown	Coding	(GAA) repeat only?	Mathimaran *et al*. (2008)
Glint03	BI452162	EST	Unknown	Coding	(TTAT) repeat? + other indels	Mathimaran *et al*. (2008)
Glint04	BM959176*	EST	Unknown	Coding	(TTA) repeat? + other indels	Mathimaran *et al*. (2008)
Glint05	BE603957*	EST	Putative cell wall protein	Coding	(TAT) repeat? + other indels	Mathimaran *et al*. (2008)
Glint06	BM959329	EST	Unknown	Coding	(CAT) repeat? + other indels	Mathimaran *et al*. (2008)
Glint07	BE603778*	EST	Unknown	Coding	(TTA) repeat? + other indels	Mathimaran *et al*. (2008)
Glint08 (same asBg348)	CG432294	GSS	Predicted protein of unknown function	Proximate coding region	(AATA) repeat? but see Bg348 above	Mathimaran *et al*. (2008)
Glint09	AM118108	Standard	P-Type IID ATPase	Coding	(AATG) repeat? + other indels	Corradi & Sanders (2006); Mathimaran *et al*. (2008)
Glint10	BM027318	EST	Unknown	Coding	(AATGGT) repeat? + other indels	Mathimaran *et al*. (2008)
Glint11	BI452145	EST	Unknown	Coding	(CAA) repeat only?	Mathimaran *et al*. (2008)
Glint12	BM959214	EST	Unknown	Coding	(CAA) repeat + other indels	Mathimaran *et al*. (2008)
Glint13	BM959443*	EST	Unknown	Coding	(AAT) repeat? + other indels	Mathimaran *et al*. (2008)
Glint14	BM027461*	EST	Unknown	Coding	(T) repeat only?	Mathimaran *et al*. (2008)
Glint15	BM959581*	EST	Unknown	Coding	(T) repeat only?	Mathimaran *et al*. (2008)
Glint16	CG431704+705*	GSS	Unknown	Probably noncoding	(A) repeat only?	Mathimaran *et al*. (2008)
Glint17	CG431789+901*	GSS	Unknown	Probably noncoding	(T) repeat only?	Mathimaran *et al*. (2008)
Glint18 (same as *Glint09*)	AM118108	Standard	P-Type IID ATPase	Coding	(A) repeat only?	Corradi & Sanders (2006); Mathimaran *et al*. (2008)

Loci are named according to the original publications (Raab *et al*., 2005; Corradi & Sanders, 2006; Croll *et al*., 2008; Mathimaran *et al*., 2008). The putative functions of loci are noted if known from previously published work or if a BLASTX database search on National Center for Biotechnology Information (NCBI) revealed a highly significant match with a known fungal protein (alignment score > 50). Accession numbers show the original sequence of the repeat motif. * denotes accession numbers of loci where highly similar sequences from the database were assembled to make a contig covering the repeat motif. In these cases, the accession number indicates one of the original sequences covering the complete repeat locus. Databases are either the standard nucleotide collection, the genome survey sequences (GSS) or the expressed sequence tag (EST) databases from NCBI. All loci were classified accordingly to their likelihood of being coding or noncoding, depending on whether they are located in an expressed sequence or not. The length polymorphisms among the alleles at each locus were described according to the available sequence data (Croll *et al*., 2008; Mathimaran *et al*., 2008); a question mark has been added to the proposed repeat motif if no sequence data were available. For loci where sequence data were not available for all alleles, the length differences among the alleles were used to determine whether the predicted repeat motif alone can explain the observed length polymorphism or whether other indels must be present among the alleles.

obtain sequences that verify that the differences are real and not an artifact of the electrophoresis. This was not done for all loci showing 1- or 2-bp differences in the study by Mathimaran *et al.* (2008) and we suggest more rigorous testing of these differences before using these markers in genotyping studies. If large sets of isolates need to be analysed, the risk of artifacts in the allele identification may be dramatically reduced by using only loci with 3-bp or longer repeat motifs.

Assuming that the length differences are accurate, most of the markers identified by Croll *et al.* (2008) and Mathimaran *et al.* (2008) are useful for demonstrating genetic differences among *G. intraradices* isolates. This does not, however, mean that they are suitable for studying all aspects of AMF population biology. Mutation rates vary across the genome and it is generally assumed that noncoding regions evolve at a higher rate than coding regions, as a result of selective constraints on proteins encoded by the genes. Therefore, it is important to identify the location of the loci in the genome to predict their suitability for particular studies. Mathimaran *et al.* (2008) mostly identified length polymorphism in expressed sequence tags (ESTs). Repeat motifs identified in ESTs are likely to be under selective pressure to maintain functional integrity of the protein. However, most of the markers reported by Croll *et al.* (2008) and some of those reported by Mathimaran *et al.* (2008) originate from sequences obtained in a genome survey, where regions throughout the genome were randomly sequenced. Because of their random location in the genome, these sequences are likely to be outside of coding regions. However, *G. intraradices* was shown to have a relatively small genome of approx. 15 Mb (Hijri & Sanders, 2004) and, therefore, gene density could be relatively high. Neutral loci are preferable for population genetic studies, as the polymorphism more likely reflects random genetic processes such as mutation, migration or drift. As expected, a majority of the loci from both studies show length polymorphism in the repeat motif. However, a large number of indels and substitutions were also found outside the repeat motif (Table 1). Therefore, the markers do not represent pure simple sequence repeats (or microsatellites) and length differences among alleles should be considered carefully. However, the presence of a large number of substitutions enables researchers to use these markers for a variety of applications such as single nucleotide polymorphism (SNP) genotyping.

Genotyping on a large scale requires amplification of DNA from single spores directly collected from the field, instead of passing through the laborious process of *in vitro* cultivation. However, the small size of *G. intraradices* spores poses a challenge for the amplification of genetic markers because of the very low amount of DNA. Stukenbrock & Rosendahl (2005b) and Mathimaran *et al.* (2008) propose two different approaches to solve this problem. In the first study, a nested PCR was performed and up to five different loci could be amplified. However, it is not known whether this method would perform well with the comparatively small spores of *G. intraradices*. One additional concern is the number of loci that can be amplified simultaneously. Mathimaran *et al.* (2008) chose a promising method called whole-genome amplification (WGA), providing a higher number of template copies of each locus. This method is increasingly used for amplification of DNA from single cells (Spits *et al.*, 2006), unculturable bacteria (Stepanauskas & Sieracki, 2007) or filamentous fungi (Foster & Monahan, 2005), including AMF (Gadkar & Rillig, 2005a,b). While the potential exists to create many template loci from minute samples of cells or spores, several factors may bias the WGA. Notably, WGA is very sensitive to template contamination by other microorganisms as a result of the indiscriminate DNA amplification; a very real concern for spores from pot cultures or the soil (Hijri *et al.*, 2002; Corradi *et al.*, 2004). Furthermore, some parts of the genome tend to be better amplified than others, creating a representation bias in the final product and potentially null alleles (Pinard *et al.*, 2006). In order to apply whole-genome amplification to field-collected spores, the method should be rigorously tested by using well-defined *in vitro* cultivated material as a comparison to whole-genome amplification from single spores of the same culture.

If successfully applied, highly discriminatory markers combined with large-scale hierarchical sampling could elucidate the extent of clonal networks within field sites and resolve patterns of genetic diversity at larger geographic scales. Furthermore, the co-evolution between AMF and their host plants could be studied in detail by identifying spatial distributions of particular genotypes. These areas of investigation have become even more relevant in the context of globally applied inoculum in the absence of data on ecological competitiveness and the potential to persist in the field among native AMF (Schwartz *et al.*, 2006). While the global population genetics of plant pathogenic fungi has received much attention in recent years, studies on plant symbionts will hopefully catch up soon.

Daniel Croll[1], Nicolas Corradi[1], Hannes A. Gamper[2] and Ian R. Sanders[1]*

[1]Department of Ecology & Evolution, Biophore Building,
University of Lausanne, CH-1015 Lausanne, Switzerland;
[2]Netherlands Institute of Ecology, Center for Terrestrial
Ecology, Boterhoeksestraat 48, PO Box 40, NL-6666 ZG,
Heteren, The Netherlands
(*Author for correspondence: tel +41 21 692 4261;
fax +41 21 692 4265; email ian.sanders@unil.ch)

References

Corradi N, Croll D, Colard A, Kuhn G, Ehinger M, Sanders IR. 2007. Gene copy number polymorphisms in an arbuscular mycorrhizal fungal population. *Applied and Environmental Microbiology* 73: 366–369.

Corradi N, Kuhn G, Sanders IR. 2004. Monophyly of beta-tubulin and H^+-ATPase gene variants in *Glomus intraradices*: consequences for molecular evolutionary studies of AM fungal genes. *Fungal Genetics and Biology* **41**: 262–273.

Corradi N, Sanders IR. 2006. Evolution of the P-type II ATPase gene family in the fungi and presence of structural genomic changes among isolates of *Glomus intraradices*. *BMC Evolutionary Biology* **6**: 21.

Croll D, Wille L, Gamper HA, Mathimaran N, Lammers PJ, Corradi N, Sanders IR. 2008. Genetic diversity and host plant preferences revealed by simple sequence repeat and mitochondrial markers in a population of the arbuscular mycorrhizal fungus *Glomus intraradices*. *New Phytologist* **178**: 672–687.

Foster SJ, Monahan BJ. 2005. Whole genome amplification from filamentous fungi using Phi29-mediated multiple displacement amplification. *Fungal Genetics and Biology* **42**: 367–375.

Gadkar V, Rillig MC. 2005a. Application of Phi29 DNA polymerase mediated whole genome amplification on single spores of arbuscular mycorrhizal (AM) fungi. *FEMS Microbiology Letters* **242**: 65–71.

Gadkar V, Rillig MC. 2005b. Suitability of genomic DNA synthesized by strand displacement amplification (SDA) for AFLP analysis: genotyping single spores of arbuscular mycorrhizal (AM) fungi. *Journal of Microbiological Methods* **63**: 157–164.

Hijri M, Redecker D, Petetot JA, Voigt K, Wostemeyer J, Sanders IR. 2002. Identification and isolation of two ascomycete fungi from spores of the arbuscular mycorrhizal fungus *Scutellospora castanea*. *Applied and Environmental Microbiology* **68**: 4567–4573.

Hijri M, Sanders IR. 2004. The arbuscular mycorrhizal fungus *Glomus intraradices* is haploid and has a small genome size in the lower limit of eukaryotes. *Fungal Genetics and Biology* **41**: 253–261.

Koch AM, Croll D, Sanders IR. 2006. Genetic variability in a population of arbuscular mycorrhizal fungi causes variation in plant growth. *Ecology Letters* **9**: 103–110.

Mathimaran N, Falquet L, Ineichen K, Picard C, Redecker D, Boller T, Wiemken A. 2008. Microsatellites for disentangling underground networks: strain-specific identification of *Glomus intraradices*, an arbuscular mycorrhizal fungus. *Fungal Genetics and Biology* **45**: 812–817.

Munkvold L, Kjoller R, Vestberg M, Rosendahl S, Jakobsen I. 2004. High functional diversity within species of arbuscular mycorrhizal fungi. *New Phytologist* **164**: 357–364.

Öpik M, Moora M, Liira J, Zobel M. 2006. Composition of root-colonizing arbuscular mycorrhizal fungal communities in different ecosystems around the globe. *Journal of Ecology* **94**: 778–790.

Pinard R, de Winter A, Sarkis GJ, Gerstein MB, Tartaro KR, Plant RN, Egholm M, Rothberg JM, Leamon JH. 2006. Assessment of whole genome amplification-induced bias through high-throughput, massively parallel whole genome sequencing. *BMC Genomics* **7**: 216.

Raab PA, Brennwald A, Redecker D. 2005. Mitochondrial large ribosomal subunit sequences are homogeneous within isolates of *Glomus* (arbuscular mycorrhizal fungi, Glomeromycota). *Mycological Research* **109**: 1315–1322.

Rosendahl S. 2008. Communities, populations and individuals of arbuscular mycorrhizal fungi. *New Phytologist* **178**: 253–266.

Sanders IR, Alt M, Groppe K, Boller T, Wiemken A. 1995. Identification of ribosomal DNA polymorphisms among and within spores of the Glomales – application to studies on the genetic diversity of arbuscular mycorrhizal fungal communities. *New Phytologist* **130**: 419–427.

Schwartz MW, Hoeksema JD, Gehring CA, Johnson NC, Klironomos JN, Abbott LK, Pringle A. 2006. The promise and the potential consequences of the global transport of mycorrhizal fungal inoculum. *Ecology Letters* **9**: 501–515.

Spits C, Le Caignec C, De Rycke M, Van Haute L, Van Steirteghem A, Liebaers I, Sermon K. 2006. Whole-genome multiple displacement amplification from single cells. *Nature Protocols* **1**: 1965–1970.

Stepanauskas R, Sieracki ME. 2007. Matching phylogeny and metabolism in the uncultured marine bacteria, one cell at a time. *Proceedings of the National Academy of Sciences, USA* **104**: 9052–9057.

Stukenbrock EH, Rosendahl S. 2005a. Clonal diversity and population genetic structure of arbuscular mycorrhizal fungi (*Glomus* spp.) studied by multilocus genotyping of single spores. *Molecular Ecology* **14**: 743–752.

Stukenbrock EH, Rosendahl S. 2005b. Development and amplification of multiple co-dominant genetic markers from single spores of arbuscular mycorrhizal fungi by nested multiplex PCR. *Fungal Genetics and Biology* **42**: 73–80.

Van der Heijden MGA, Klironomos JN, Ursic M, Moutoglis P, Streitwolf-Engel R, Boller T, Wiemken A, Sanders IR. 1998. Mycorrhizal fungal diversity determines plant biodiversity, ecosystem variability and productivity. *Nature* **396**: 69–72.

Key words: arbuscular mycorrhizal fungi (AMF), *Glomus intraradices*, microsatellites, multilocus genotyping, population genetics, simple sequence repeats (SSR).

Unexpected vagaries of microsatellite loci in *Glomus intraradices*: length polymorphisms are rarely caused by variation in repeat number only

Microsatellite markers, or simple sequence repeats (SSRs), are widely used as tools to distinguish genotypes or individuals in paternity analyses, forensics and population genetics (Ellegren, 2004). Microsatellites have been studied extensively in many fungal genomes (Lim *et al.*, 2004) but, surprisingly, have not been exploited to study the population genetics of arbuscular mycorrhizal fungi (AMF), a class of important plant symbionts, until two publications independently claimed the utility of these markers for a specific species, *Glomus intraradices* (Croll *et al.*, 2008a; Mathimaran *et al.*, 2008). In a letter contributed to this forum (Croll *et al.*, 2008b), these 'microsatellite markers' were tabulated with the aim of clarifying possible confusions about their suitability in population genetics. The authors of this letter concluded that 'as expected, a majority of the loci from both studies show length polymorphism in the repeat motif' (cited from Croll *et al.*, 2008b). However, only 10% of the length polymorphisms they observed (Croll *et al.*, 2008a) were caused, at least partially, by changes in the repeat motif (Table 1). The vast majority of length polymorphisms (> 90%) were caused by insertions–deletions (indels) in the flanking regions; some of the so-called SSR loci did not contain any repeat longer than two triplets and were not polymorphic in these areas.

© The Authors (2008). Journal compilation © *New Phytologist* (2008)

Table 1 Nature of polymorphisms observed at simple sequence repeat (SSR) loci in Croll *et al.* (2008a) and this study

Study	Alleles sequenced	Total number of length polymorphisms	Only caused by SSR	Partly caused by SSR	Not at all caused by SSR
Croll *et al.* (2008a)	40	29	2 (7%)	1 (3%)	26 (90%)
Present	36	28[a]	5 (18%)	10 (36%)	13 (46%)

[a]Including length polymorphisms compared with the original sequence obtained from public databases (see Supporting Information Fig. S1).

In our own study, we identified microsatellite loci, using well-defined criteria, in our database screen (at least five identical repeats of two, three or four nucleotides, or a stretch of at least 10 identical single nucleotides). We found clear length polymorphisms in 18 loci selected in this way, examining eight different strains of *G. intraradices*. The target repeat sequence was present in each case, and it would have been logical to assume that the length polymorphisms would have been caused by changes in the numbers of repeat lengths. However, when we sequenced two alleles of different size for each of the 18 loci (see Supporting Information Fig. S1), we found that the length difference was based exclusively on repeat length polymorphism in only 18% of the alleles studied, and at least partially in 36%. For almost half of the alleles studied (46%), the repeat was not affected and the length polymorphism was caused by adjacent indels (Table 1).

The frequency of length polymorphisms in the targeted microsatellites was only marginally higher than in the nontargeted flanking regions (i.e. 5.1% per base pair in the microsatellite region compared with 2.7% per base pair in the flanking region for our study) (Fig. S1).

We conclude that microsatellites of short length ($n \sim 5$ for di-, tri- and tetranucleotides, and $n \sim 10$ for mononucleotides), as investigated in the studies (Croll *et al.*, 2008a; Mathimaran *et al.*, 2008), seem not to enhance significantly the probability to find length polymorphisms of value for population genetic analysis. Nevertheless, as also stated in the accompanying letter (Croll *et al.*, 2008b), length polymorphisms that happen to occur within and around such short microsatellites may still be highly useful in genotyping.

Length polymorphisms such as those analysed here are useful to demonstrate genetic differences among *G. intraradices* isolates in general; it remains an ongoing debate whether markers in expressed sequences or in noncoding regions are of greater interest. Mutation rates vary across the genome, and it is generally assumed that noncoding regions evolve at a higher rate than coding regions, as a result of selective constraints on the transcripts and proteins encoded by the genes. On the other hand, markers in expressed parts of the genome, such as expressed sequence tag (EST)-derived markers, have advantages over nonexpressed markers as they could be both used for gene mapping as well as for population genetics. Moreover, EST-derived markers are believed to be more suitable for cross-species transferability (Varshney *et al.*, 2005; Ellis & Burke, 2007; Hisano *et al.*, 2007). For population genetics, 'neutral' markers not subject to selection are of particular interest, and markers derived from ESTs (Mathimaran *et al.*, 2008) may be less favourable in this respect. However, markers derived from a genome survey (Croll *et al.*, 2008a) may also be expressed. Moreover, nonexpressed parts of the genome can be under equally strong selection as expressed parts and we therefore suggest that 'neutrality', if required, has to be tested for each locus instead of relying on global assumptions.

Accidentally, two of the *bona fide* microsatellites selected in our study, namely *Glint09* and *Glint18* (Mathimaran *et al.*, 2008), were in a sequence previously studied, encoding a P-type II ATPase D (Corradi *et al.*, 2007). Analysis of each of these two loci displayed a clear single band in all our single-spore DNA preparations, indicating that it was represented by an allele (or alleles) of a single size in an individual spore of each strain analyzed. With respect to the locus of *Glint09*, this corresponded to a band of 107 bp, 115/116 bp or 121/122 bp (Mathimaran *et al.*, 2008). Experiments with DNA from mixed spores showed that two alleles of different size showed up as clear doublets with the appropriate size difference (data not shown). The size of the alleles found in individual spores matched the length variants (105/106, 114/115 and 121 bp) found combined either as two or three alleles in DNA preparations of mycelium from root-organ cultures of single strains in the previous study (Corradi *et al.*, 2007). We do not have an explanation for this difference, but we point out that different single spores of a given strain, subjected to whole-genome amplification (WGA), always yielded a unique band of constant length for a given polymorphic locus (Mathimaran *et al.*, 2008).

The ability to detect single alleles at a polymorphic locus in single spores is a clear advantage of the WGA method. Whole-genome amplification is particularly useful for detecting low-copy-number sequences from environmental samples (Gonzalez *et al.*, 2005) where standard polymerase chain reaction (PCR) methods are insufficient, and it has successfully been used to genotype powdery mildew (Fernandez-Ortuno *et al.*, 2007). Owing to the high-fidelity proof-reading function of Phi29 DNA polymerase, the WGA product is a highly accurate copy of the original genome (Dean *et al.*, 2002). Indeed, using this technique with four separate amplifications from single spores of two different isolates of *G. intraradices*, there was faithful amplification for all of the three loci tested (Mathimaran *et al.*, 2008). Thus, the WGA procedure greatly enhances opportunities to detect size polymorphisms at multiple loci in single spores.

The potential SSR markers identified by Mathimaran *et al.* (2008) have been deposited in a newly developed database for Glomus (http://glomus.vital-it.ch/), which is maintained by the Swiss Institute of Bioinformatics and is now accessible to scientists worldwide. In the future, this database will be upgraded to allow users to retrieve as well as to deposit useful length-polymorphic markers for tracing AMF. This is particularly important because large numbers of markers may soon be available from various AMF species, which need to be consolidated into a relational database for easy access of a particular marker locus, as in the case of databases for other eukaryotes (see e.g. the Swiss Vitis Microsatellite Database).

Interestingly, both studies reviewed here and in the accompanying letter (Croll *et al.*, 2008b) clearly show that all the loci characterized by length polymorphisms have a single size within a given isolate and thus are not heterogeneous in descendants of a single spore. This means that such length polymorphisms – whether caused by indels or microsatellite repeat polymorphisms – can be used to genotype AMF strains. This will be a great asset for future population genetic and ecological studies as well as for the re-identification and tracing of AMF strains of particular value used as biofertilizers in agriculture. Moreover, the absence of multiple alleles in a given strain suggests that AMF are essentially homokaryotic with a haploid genome rather than having an unusual heterokaryotic lifestyle, two contrasting hypotheses discussed recently in *New Phytologist* (Rosendahl, 2008; Young, 2008).

Natarajan Mathimaran[1], Laurent Falquet[2], Kurt Ineichen[1], Cyril Picard[2], Dirk Redecker[1], Andres Wiemken[1] and Thomas Boller[1]*

[1]Zurich-Basel Plant Science Center, Botanisches Institut der Universität Basel, Hebelstrasse 1, CH-4056 Basel, Switzerland; [2]Swiss Institute of Bioinformatics, University of Lausanne, Quartier Sorge – Bâtiment Génopode, 1015 Lausanne, Switzerland
(*Author for correspondence: tel +41 61 267 23 20; fax +41 61 267 23 30; email thomas.boller@unibas.ch)

References

Corradi N, Croll D, Colard A, Kuhn G, Ehinger M, Sanders IR. 2007. Gene copy number polymorphisms in an arbuscular mycorrhizal fungal population. *Applied and Environmental Microbiology* 73: 366–369.

Croll D, Corradi N, Gamper HA, Sanders IR. 2008b. Multilocus genotyping of arbuscular mycorrhizal fungi and marker suitability for population genetics. *New Phytologist* **180**: 564–568.

Croll D, Wille L, Gamper HA, Mathimaran N, Lammers PJ, Corradi N, Sanders IR. 2008a. Genetic diversity and host plant preferences revealed by simple sequence repeat and mitochondrial markers in a population of the arbuscular mycorrhizal fungus *Glomus intraradices*. *New Phytologist* **178**: 672–687.

Dean FB, Hosono S, Fang LH, Wu XH, Faruqi AF, Bray-Ward P, Sun ZY, Zong QL, Du YF, Du J *et al.* 2002. Comprehensive human genome amplification using multiple displacement amplification. *Proceedings of the National Academy of Sciences, USA* **99**: 5261–5266.

Ellegren H. 2004. Microsatellites: simple sequences with complex evolution. *Nature Reviews Genetics* **5**: 435–445.

Ellis JR, Burke JM. 2007. EST-SSRs as a resource for population genetic analyses. *Heredity* **99**: 125–132.

Fernandez-Ortuno D, Tores JA, de Vicente A, Perez-Garcia A. 2007. Multiple displacement amplification, a powerful tool for molecular genetic analysis of powdery mildew fungi. *Current Genetics* **51**: 209–219.

Gonzalez JM, Portillo MC, Saiz-Jimenez C. 2005. Multiple displacement amplification as a pre-polymerase chain reaction (pre-PCR) to process difficult to amplify samples and low copy number sequences from natural environments. *Environmental Microbiology* **7**: 1024–1028.

Hisano H, Sato S, Isobe S, Sasamoto S, Wada T, Matsuno A, Fujishiro T, Yamada M, Nakayama S, Nakamura Y *et al.* 2007. Characterization of the soybean genome using EST-derived microsatellite markers. *DNA Research* **14**: 271–281.

Lim S, Notley-McRobb L, Lim M, Carter DA. 2004. A comparison of the nature and abundance of microsatellites in 14 fungal genomes. *Fungal Genetics and Biology* **41**: 1025–1036.

Mathimaran N, Falquet L, Ineichen K, Picard C, Redecker D, Boller T, Wiemken A. 2008. Microsatellites for disentangling underground networks: strain-specific identification of *Glomus intraradices*, an arbuscular mycorrhizal fungus. *Fungal Genetics and Biology* **45**: 812–817.

Rosendahl S. 2008. Communities, populations and individuals of arbuscular mycorrhizal fungi. *New Phytologist* **178**: 253–266.

Varshney RK, Graner A, Sorrells ME. 2005. Genic microsatellite markers in plants: features and applications. *Trends in Biotechnology* **23**: 48–55.

Young JPW. 2008. The genetic diversity of intraterrestrial aliens. *New Phytologist* **178**: 465–468.

Key words: arbuscular mycorrhizal fungi (AMF), *Glomus intraradices*, microsatellites, simple sequence repeats (SSRs), strain identification.

Supporting Information

Additional supporting information may be found in the online version of this article.

Fig. S1 Sequence alignments for all 18 loci described by Mathimaran *et al.* (2008).

Please note: Wiley-Blackwell are not responsible for the content or functionality of any supporting information supplied by the authors. Any queries (other than missing material) should be directed to the *New Phytologist* Central Office.

© The Authors (2008). Journal compilation © *New Phytologist* (2008)