

## Commentary

# Trait correlations in bryophytes: exploring an alternative world

Much has been written about trait correlations in vascular plants. In this issue of *New Phytologist*, Waite & Sack (pp. 156–172) have turned their attention to bryophytes, with some fascinating conclusions. During our schooldays we learn about ‘plants’ – the obvious plants around us, that is, *vascular* plants. Those of us who pursue the plant sciences further learn about the structure and physiology of these plants in ever-increasing detail, and it is natural for us to come to feel that the vascular-plant way is the *only* proper way for plants to do things! That view is compounded by an over-simplistic, linear view of evolution. However, the evolutionary ‘tree’ is not so much a tree as a thicket, many of whose branchings are hidden deep beneath the canopy of green leaves representing current living species. Every surviving shoot has had to prove its viability and make its own way up to the daylight of the present day. What is seen as ‘basal’ will depend on which shoot you start with at the top – a point repeatedly made by Dawkins (2004) – and may have little relevance to adaptation *now*, in the four-million-and-somethingth century since the origin of archegoniate land plants.

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*‘Vascular plants extended and diversified the niches available to bryophytes, a fact to which the exuberant diversity of forest bryophytes bears eloquent witness.’*

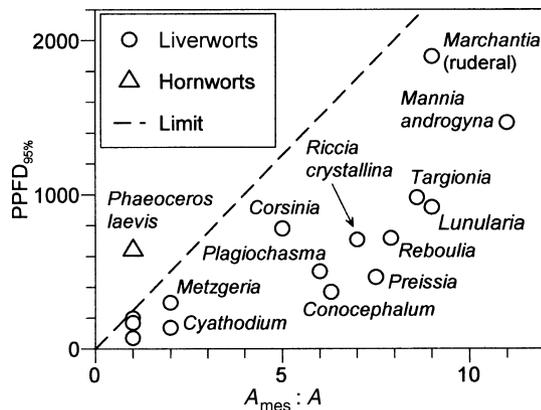
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Waite & Sack have explored an extensive set of morphological and photosynthetic measurements made on 10 Hawaiian moss species, belonging to six families and from different levels, in a forest providing a gradient in light intensity and other environmental factors. They found numerous correlations, both of traits with habitat and between traits –

some were obvious and some were surprising. Their article should set us thinking about three kinds of questions.

As with vascular plants, trait correlations in bryophytes can be seen at various levels: plastic responses of individuals; speciation within genera; or divergences between major clades whose branchings may go far back in time. We see these as ‘phylogenetic’ or ‘environmental’ constraints, but perhaps this distinction is irrelevant to the big picture because the factors driving trait correlations have operated throughout the evolution of plants. Of the six families represented in Waite & Sack’s data, Dicranaceae and Fissidentaceae appear to be phylogenetically rather close, and distant from the others. Dicranaceae typically have long narrow leaves and a long costa, but there is variation in leaf shape, in the relative proportions of costa and lamina, and in cell shape (from elongated to quadrate). By contrast, Fissidentaceae are notably uniform in structure over a range of habitats from submerged aquatic to dry rocks, forest and grassland. Orthotrichaceae are a well-delimited family of tree-bark and rock mosses, are desiccation-tolerant, typically with small isodiametric cells and grow in small cushions. Rhizogoniaceae are one of a group of small, mainly tropical-forest families. Of the very numerous Hypnoid pleurocarpous mosses – which molecular evidence (Goffinet *et al.*, 2004) suggests are among the most advanced of all mosses – Hookeriaceae are plants found in moist shady places and have very large thin-walled cells. Sematophyllaceae, more or less prostrate growing mosses of moist forests, are our sole representative of the numerous families of the Hypnales, sharing their narrow elongated cells with the generality of these, and their lack of a costa with some, but not others. The presence or absence of a costa and the shape of leaf cells – elongated or shortly polygonal – must have functional consequences (Proctor, 1979), but how far these relate to mechanical support, how far to conduction of water or metabolites and how far to chance, are open questions.

It is a trite oversimplification to see bryophytes as simply ‘primitive’. Of the two strategies of adaptation to life on land (Proctor & Tuba, 2002), the poikilohydric strategy must have come first because we can see no way in which natural selection could produce a functional vascular plant *de novo*. How might the vascular-plant leaf have evolved from a nonvascular thallus? Two potentially limiting resources could have driven natural selection, namely water and CO<sub>2</sub>. It is easy to visualize a ventilated photosynthetic tissue evolving in response to CO<sub>2</sub> limitation; modern bryophytes provide models – Marchantiales (Fig. 1), Polytrichaceae (Proctor, 2005) and the sporophytes of mosses



**Fig. 1** Photosynthetic photon flux density (PPFD) at 95% saturation from chlorophyll-fluorescence measurements plotted against the ratio of the estimated area for  $\text{CO}_2$  uptake to the projected thallus area ( $A_{\text{mes}} : A$ ) for 15 thaloid liverworts and one hornwort. The 'limit' (broken line) is calculated assuming an external cell-wall thickness of  $1 \mu\text{m}$  with some tortuosity in the diffusion path, and a (practical) quantum requirement of 16 per molecule of  $\text{CO}_2$  fixed. The three points in the bottom left corner of the graph are (from the lowest symbol upwards) the simple terrestrial thaloid liverworts *Monoclea gottschei*, *Dumortiera hirsuta* and *Pellia epiphylla*. *Metzgeria* is epiphytic and has been assumed to expose two surfaces for  $\text{CO}_2$  uptake. The others are Marchantiales with ventilated thalli.

and Anthocerotae are the most obvious. An 'epidermis', perhaps first evolved as a protection from mechanical damage, would have been a pre-adaptation for further selection to control water loss. However,  $\text{CO}_2$  seems at first sight an unlikely resource to have been limiting in the mid-Palaeozoic, when atmospheric  $\text{CO}_2$  was probably *c.* 10 times the present concentration (Berner, 1998). An alternative scenario might envisage co-evolution of increasing cuticularization of the surface with the development of air spaces within the plant body to compensate for increasing resistance to  $\text{CO}_2$  uptake.

Once evolved, vascular plants radiated rapidly, and quickly (by geological standards) assumed the dominant role in terrestrial vegetation that they have held ever since. But they did not occupy the ecological niche of the bryophytes. True, they relegated bryophytes to a subsidiary role in the world's vegetation, and must have occupied some areas formerly dominated by plants at a 'bryophyte' level of organization. However, vascular plants did not replace bryophytes in situations where the lack of a rooting medium, too-low air temperatures or the lack of accessible nutrients exclude vascular plants – or at the scale at which bryophytes are probably the optimal way to build a (small) green land plant. It is surely significant that not one, but three, groups of 'bryophytes' – mosses, liverworts and hornworts (Anthocerotae) – independently survived from the earliest archegoniate land flora through to the present day (Renzaglia *et al.*, 2007). The only competitor group that natural selection has come up with is the lichens, from quite

different origins but remarkably parallel in ecophysiological adaptation. Vascular plants extended and diversified the niches available to bryophytes, a fact to which the exuberant diversity of forest bryophytes bears eloquent witness.

Trait correlations in bryophytes and vascular plants show both similarities and differences. Many of the latter arise from differences in scale: generally, bryophytes are some two orders of magnitude smaller than vascular plants. Scale has obvious direct effects. Mass is proportional to the cube of linear dimension, so gravity is of paramount importance to trees (as it is to us), but trivial to mosses (or insects). Surface tension works at linear interfaces, so it is much more important to the physiology of bryophytes than to that of vascular plants. And there would simply not be space for the complexity of vascular-plant structures inside a plant body a hundred-times smaller and a millionth of the volume. A second set of scale effects concerns the interaction of the plant with the atmosphere. Exchanges of heat, gases and momentum between the leaves and branches of vascular plants are dominated by rapid eddy diffusion; slow molecular diffusion is only a constraint in the thin laminar boundary-layer of the leaf, through the stomata and in the mesophyll. There are strong selective pressures on leaves for structural strength and to keep momentum-transfer within bounds. At their much smaller scale, these selection pressures scarcely affect bryophytes; in the immediate vicinity of bryophyte leaves, slow molecular diffusion dominates heat and mass transfer. In fluid-dynamic terms, vascular plants and bryophytes operate at very different Reynold's Numbers, just as surely as do the flight of aeroplanes, birds and insects. Most bryophytes have simple leaves or thalli, often only one cell thick. The maximum rate at which  $\text{CO}_2$  can diffuse into a photosynthesizing surface is set by two variables: the difference between the external  $\text{CO}_2$  concentration and the  $\text{CO}_2$  compensation-point; and the resistance of the moist external cell wall to the liquid-phase diffusion of  $\text{CO}_2$ . This, in turn, depends on the thickness of the external cell walls (assuming no superincumbent water). How great a photon flux is needed to fix this  $\text{CO}_2$ ? Fig. 1 shows some data for 95% saturation photosynthetic photon flux density (PPFD) for thaloid liverworts. Large simple thaloid species approximate to a single flat photosynthesizing surface. The epiphyte *Metzgeria* exposes two surfaces for  $\text{CO}_2$  uptake. Marchantiales have more or less complex mesophyll-like systems within the thallus;  $A_{\text{mes}} : A$  is a rough estimate of the ratio of the internal area for  $\text{CO}_2$  uptake to the projected thallus area. The diagonal broken line in the diagram assumes an external wall thickness of  $1 \mu\text{m}$  (which is less than the range that Waite & Sack give for their species), and plausible values for the other parameters. The exception that proves the rule is *Phaeoceros laevis*, standing conspicuously above the limit. However, Anthocerotae, uniquely among bryophytes, have a biochemical carbon-concentrating mechanism. This clearly helps – but, for

a land plant, not nearly as much as a ventilated thallus increasing the area for CO<sub>2</sub> uptake (Meyer *et al.*, 2008)!

It is good to see a paper that is centred around the analysis of a large exploratory data set. Current thinking favours research that is hypothesis-driven; the inductive–deductive model of science is unfashionable. That is based on experience, and allows us to write our papers in much more the way that we actually *do* our science – Medawar (1963) famously wrote an article guying the pretence that the author had, without any preconceptions, reached his conclusions by a pure inductive–deductive process from the data. However, there are occasions when thorough exploration of an extensive block of data produces ideas and insights that no amount of hypothesis and test would have hit upon. The two approaches are complementary. The hypothesis–test approach is effective, satisfying and progressive, as long as you have a good supply of hypotheses. But we also need systematic bodies of data, and the ideas that come from exploring them.

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**Key words:** bryophytes, evolution, morphology, photosynthesis, phylogeny, thallus, trait correlations.

## How can we achieve durable disease resistance in agricultural ecosystems?

Plant pathogens evolve rapidly in agricultural ecosystems. It is likely that this fast pace of evolution is connected to the nature of the agroecosystem, which is dominated by genetically uniform species monocultures grown at a high density and on a vast scale. Mounting evidence indicates that many pathogens emerged over the last 10 000 yr during domestication of the affected crops and that new pathogens emerge regularly in these human-engineered ecosystems (Gibbs *et al.*, 2008; Stukenbrock & McDonald, 2008). While the rapid pace of pathogen evolution presents a major impediment to sustainable food production, it also provides interesting opportunities to better understand the evolutionary biology of host–parasite interactions. Pathogen evolution is usually inferred after resistant crop cultivars deployed in farmers' fields suddenly lose their disease resistance. This 'breakdown' of resistance has been observed many times around the world over the past 100 yr. Less commonly, pathogen evolution is observed in carefully controlled field or glasshouse experiments, that is, through experimental studies of evolution. Two French groups recently used serial passage experiments to show that plant viruses can rapidly evolve virulence against resistant cultivars (Chain *et al.*, 2006; Palloix *et al.*, 2009). A *New Phytologist* article by Brun *et al.* (this issue, pp. 285–299) presents a landmark study in experimental evolution of a fungal plant pathogen that offers a proof of principle for an important concept, the blending of different forms of genetic resistance to provide long-lasting disease resistance.

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*'...it is time to consider optimized deployment strategies for resistance genes in agroecosystems with the aim of achieving sustainable, durable disease resistance.'*

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To control infectious diseases in crops, most plant breeders use resistance genes of major effect (often called *R*-genes) that usually encode NBS-LRR proteins that recognize elicitor molecules produced by the pathogen (reviewed in Bent & Mackey, 2007). Recognition of the pathogen elicitor triggers a host hypersensitive response that leads to the death of

a few infected plant cells very early in the infection process, presenting a disease-free phenotype that is easily recognized and selected by the plant breeder. While *R*-genes have the advantage of an easily selected phenotype and simple Mendelian inheritance, in most cases pathogens rapidly evolve virulence against the *R*-gene by mutating or deleting the pathogen gene encoding the elicitor molecule that was recognized by the *R*-gene. In the parlance of plant pathologists, the resistance mediated by *R*-genes is not 'durable'. Once an *R*-gene has failed, the usual solution is to introduce a new *R*-gene, leading to a boom-and-bust cycle of disease. The 'boom' occurs when the newly deployed *R*-gene provides a high degree of resistance and is quickly adopted by many farmers. The 'bust' occurs when the pathogen population becomes virulent on the *R*-gene and an epidemic ensues.

How can plant breeders break the boom-and-bust cycle and stay ahead in this arms race between crops and their pathogens? Because of the frequent failure of *R*-genes, many breeders and plant pathologists focused their efforts on inherited resistance of smaller effect that has the properties of a quantitative trait. Not surprisingly this is called quantitative resistance (QR). By definition, QR is more difficult to work with because it is based on a phenotype that follows a normal distribution and often exhibits a large genotype  $\times$  environment interaction. The advantage of QR is that it seems to be more durable, that is, less prone to fail suddenly in agroecosystems. Undomesticated plants possess layered defense strategies based on a combination of QR and clusters of *R*-genes. After many years of breeding crop plants for increased yield and quality, sometimes under the protection of fungicides that mask the resistance phenotype in a plant, we have probably lost a significant amount of the 'baseline' QR in our domesticated crops. This may partially explain the boom-and-bust cycle associated with deployment of individual *R*-genes, but it is clearly not the entire explanation. The obvious next step in the breeding process would be to combine the major *R*-gene resistance with QR to reconstitute a multilayered plant resistance. But this is not as easy as it seems, because the major *R*-gene resistance masks the effects of the QR, making it difficult to combine the two forms of resistance using traditional breeding practices.

While plant pathologists have proposed for years that QR can be used to 'protect' *R*-gene resistance, experimental verification of the idea was lacking. The experiment of Brun *et al.* is unique because of its large scale, long duration and thoughtful experimental design. The authors used isogenic lines of *Brassica napus* that differed for two properties, the presence/absence of the Lm6 *R*-gene (also called MX) that provides resistance to the fungus *Leptosphaeria maculans* and the presence/absence of QR originating from the cv Jet Neuf. An advantage of this experiment was that it used natural pathogen inoculum and a natural infection procedure that reflects the process expected to occur in farmers'

fields. An additional strength was that the experimental plots were separated by at least 1000 m, limiting the movement of inoculum among experimental treatments and ensuring that the results were not affected by cross-contamination.

The major outcomes of this field experiment were surprisingly clear. Virulence against the Lm6 *R*-gene emerged after only 3 yr in the Eurol host background lacking QR, while virulence against Lm6 did not emerge in the Darmor host background carrying QR. The pathogen population showed no evidence of becoming adapted to the Darmor host carrying QR. Linkage disequilibrium in the pathogen population emerged rapidly between AvrLm1 and AvrLm6, consistent with the idea that only one, or very few, mutant virulent alleles were amplified in the experimental plots. The authors conclude that QR can be used to extend the life expectancy of *R*-genes. One possible explanation is that the reduction in pathogen effective population size as a result of the action of QR reduced the number of virulent mutants that could be selected by the *R*-gene.

Many resistance gene deployment strategies are easy to draw on paper, but difficult to implement in practice. For example, *R*-genes can be deployed in a temporal or spatial rotation to avoid the typical 'bust' that occurs when a single *R*-gene is deployed over a large area. *R*-genes also can be deployed as clusters (often called 'pyramids') within the same individual or in multilines composed of a mixture of plants that carry different *R*-genes but are otherwise genetically identical. Transgenic and cisgenic technologies can enable *R*-gene deployment strategies that are not practical using traditional breeding methods. Quantitative trait locus (QTL) mapping has made it possible to identify chromosome regions carrying QR and marker-assisted selection (MAS) can be used to transfer these regions into elite crop germplasm possessing high yield and high quality. With all of these tools now in hand, it is time to consider optimized deployment strategies for resistance genes in agroecosystems with the aim of achieving sustainable, durable disease resistance. Why not use molecular plant breeding approaches to create synthetic *R*-gene clusters through genetic engineering and combine them with QTL-based QR using MAS? This approach could be implemented for many important crop diseases over the next 10–20 yr.

However, plant pathogens have evolved successful strategies to overcome layered plant defence strategies over millions of years of coevolution. Even combining QR with genetically engineered pyramids of *R*-genes may provide only a short-term solution, perhaps lasting for 50–100 yr instead of the usual 3–4 yr. The erosion of QR has been documented (Andrivo *et al.*, 2007), so QR is not likely to be the panacea that it was once thought to be. And the nature of QR remains mysterious. What are the underlying determinants of QR? There is some evidence that QR also involves *NBS-LRR* genes. But other recent evidence suggests

an entirely different mechanism (Krattinger *et al.*, 2009). QTL map-based cloning combined with careful experimentation will likely elucidate the mechanisms of QR during the next decade.

Is there a long-term solution to the infectious disease problem that could last us through the next 10 000 yr of agriculture? One idea is to re-engineer the agroecosystem to increase overall host diversity, at the species level as well as at the gene level, to reduce directional selection and present an evolutionary dilemma to the pathogen. This can be accomplished in many ways, both high-tech and low-tech. Hi-tech methods include using genetic engineering and MAS to create multilines and *R*-gene pyramids that can be combined with a particular QR genetic background. Low-tech methods include making greater use of cultivar mixtures, species mixtures (for all who enjoy a multigrain cereal, why not grow some of the cereals together in the same field?), smaller fields planted to a diversity of crops and increased crop rotations. Hi-tech approaches will likely win out in places where industrialized agriculture dominates, but low-tech solutions surely can make significant contributions in the rest of the world. The race is on to double food production by 2050 in order to achieve global food security. About 15% of current global crop production is lost to infectious diseases. What are we waiting for?

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**Key words:** boom-and-bust cycle, durable resistance, experimental evolution, gene deployment strategies, marker-assisted selection, quantitative resistance, *R*-gene.

## Cryptic forest refugia on the 'Roof of the World'

It has long been known that the Quaternary glaciations displaced temperate and boreal forests to lower latitudes or altitudes, where species persisted until warmer climates allowed them to expand again (Reid, 1899). Researchers have assumed for decades that most tree taxa and their associated organisms survived the Last Glacial Maximum ((LGM), 18–20 kyr before present (BP)) only in areas far from the continental ice sheets, such as the southern peninsulas of Europe and the southeastern regions of North America. This long-held belief is now increasingly challenged by palaeoecological and genetic surveys. A rapidly growing body of evidence indicates that numerous forest plant and animal species, in fact, maintained small populations at considerably higher latitudes and altitudes than previously thought (reviewed in Bhagwat & Willis, 2008; Provan & Bennett, 2008; Rull, 2009). A paper by Opgenoorth and co-workers in this issue of *New Phytologist* (pp. 332–342) illustrates this trend with a particularly spectacular and convincing example. Using genetic markers, the authors were able to infer the past population dynamics of a group of *Juniperus* species that are currently growing up to 4900 m above sea level (asl) on the Tibetan Plateau and the northern Himalayas, forming one of the highest known tree lines on earth (Miehe *et al.*, 2007; Fig. 1). Their results represent sound evidence that junipers probably withstood the coldest phases of the Pleistocene at altitudes of > 3500 m asl.

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‘... identification of cryptic refugia has direct consequences on our understanding of the impacts of modern climate change on biodiversity.’

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**Fig. 1** Photograph of the highest known juniper forest stand growing at approx. 4900 m above sea level (asl) in southeastern Tibet (Xizang Autonomous Region, Baxoi County). This stand is also one of the two highest known forests in the world (following a definition widely used in treeline research that considers ‘forests’ groups of individuals > 3 m high; Körner, 2003). The rosaceous tree *Polylepis tarapacana* grows at up to 5100 m asl on the slopes of the volcano Salama in Bolivia. However, stands of this species that fulfill the above definition of a forest occur only up to 4810 m asl (Hoch & Körner, 2005).

At present the Tibetan Plateau is largely covered by alpine pastures and desert-steppes. Juniper forests are mostly restricted to its eastern and southern declivities, although some forest islands occur on the Plateau itself. Pollen records indicate that juniper forests were considerably more widespread in the past and declined as a consequence of human activities during the last few centuries (Miehe *et al.*, 2008). Opgenoorth *et al.* analysed the chloroplast DNA (cpDNA) of almost 600 individuals from 102 *Juniperus* stands. They observed geographic patterns of genetic variation that cannot be explained by a scenario involving complete extinction and subsequent recolonization of the Tibetan Plateau by the investigated species. Hence, they suggest that tree populations must have persisted during the LGM in multiple small refugia situated on the southern slopes of several river gorges that cross the Plateau.

This represents an extreme case of so-called ‘cryptic refugia’ (Provan & Bennett, 2008) or ‘microrefugia’ (Rull, 2009), defined as areas outside the major refugia that allowed species to maintain small populations through the LGM as a result of their favourable local environment and microclimate. The term ‘cryptic’ pinpoints the difficulties in identifying and delimiting such refugia using fossil remains. Inferring the existence of local plant populations from trace amounts of fossil pollen is burdened with uncertainties, whereas macrofossils provide more conclusive evidence but are rare and difficult to find. As a consequence, phylogeographic surveys are increasingly used for this purpose. With adequate population sampling and molecular analyses, they can reveal the presence of otherwise undetectable cryptic forest refugia from which small-scale

expansions took place following postglacial climate warming (Anderson *et al.*, 2006; Provan & Bennett, 2008). The rapidly growing evidence for the existence of such refugia questions the generality of the established paradigm of postglacial recolonization that emphasizes wave-like expansions from distant refugia mediated by repeated long-distance dispersal (Hewitt, 2000). It also suggests that the distances over which species expanded after the LGM have frequently been overestimated, resulting in an overoptimistic appreciation of the ability of species to track future climate changes (Anderson *et al.*, 2006; Svenning & Skov, 2007; Provan & Bennett, 2008). Clearly, the identification of cryptic refugia has direct consequences on our understanding of the impacts of modern climate change on biodiversity.

The study by Opgenoorth and colleagues provides not only convincing evidence for the existence of multiple cryptic refugia but also enables the authors to locate them precisely. Three particular circumstances contribute to this achievement. First, the study is based on extensive and geographically comprehensive population sampling. Second, the topographic structure of the study area is such that suitable sites for juniper survival through the LGM were restricted to the major river gorges that cross the Tibetan Plateau, many of them situated well above 3500 m asl. Third, the analysed junipers contain remarkably high levels of cpDNA diversity, including many haplotypes that are restricted to a single population. Drawing on the generally low mutation rate of cpDNA (especially in trees; Petit & Hampe, 2006), the authors argue that the patterns observed are unlikely to be of postglacial origin but should instead reflect population divisions older than the LGM and, in some cases, possibly even dating back to the late Tertiary (> 1.8 Myr BP). Although this interpretation involves some uncertainty (as cpDNA mutation rates might be unusually high because of strong UV irradiation in the area), it would be in line with other recent studies that found extant tree population structures to reflect surprisingly ancient events (Hampe & Petit, 2007). Interestingly, cases of deep phylogeographic structure have so far only been reported from regions with warm temperate or tropical climates, very different from the cold-dry conditions prevailing on the Tibetan Plateau.

The strong genetic structure observed indicates that Tibetan junipers underwent only localized postglacial range expansions and experienced little admixture between lineages originating from different refugia. This finding is especially remarkable because the paternally inherited cpDNA of junipers is disseminated through wind-dispersed pollen, a priori the most efficient vehicle for long-distance gene flow between populations. However, the Tibetan juniper populations did not actually need to be very mobile in order to track suitable climatic conditions, because the steep terrain permitted them to ascend several hundred metres by expanding only over short distances. In fact, these small

refugia apparently experienced climatic conditions during the LGM that are similar to those prevailing today at the highest known juniper tree lines of approx. 4900 m (Miehe *et al.*, 2007; Opgenoorth *et al.*), suggesting that postglacial population expansions reached areas near the environmentally set growth limits of the species. Such reconstructions extend in time ongoing analyses of tree line responses to climate warming, confirming that these transition zones are particularly temperature sensitive (Harsch *et al.*, 2009). An interesting perspective would be to investigate such rapidly changing tree lines at different spatio-temporal resolutions using an interdisciplinary approach (including population genetic analysis) to characterize their dynamics in greater detail and extrapolate them through time (Holtmeier & Broll, 2005).

Finally, the study of Opgenoorth *et al.* highlights the potential of some tree species to survive over centuries, or even millennia, under extremely cold and dry conditions (Petit & Hampe, 2006). The great longevity of juniper trees probably played a central role for population persistence in view of presumably extremely low rates of reproduction and plant recruitment. Unfortunately, the results also imply that extant Tibetan juniper forests face bleak prospects. The ecological strategy of the species, characterized by great adult longevity and a low dispersal potential (probably exacerbated by low rates of successful plant establishment), renders junipers particularly vulnerable to the major anthropogenic disturbances in the region: uncontrolled logging for firewood, overgrazing, and drought stress resulting from climate change (Miehe *et al.*, 2007, 2008). Water supply on the Tibetan Plateau depends largely on glaciers, which are experiencing a significant melt down (Yao *et al.*, 2007). As a consequence, more water is currently available, but a significant increase in aridity is to be expected soon (Cruz *et al.*, 2007). This increase is likely to exacerbate the already severe effects of logging and overgrazing, leading to continued fragmentation and destruction of the remaining Tibetan juniper forests and the ecological communities which they are sustaining.

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**Key words:** chloroplast DNA, *Juniperus*, Last Glacial Maximum (LGM), microrefugia, phylogeography, Tibetan Plateau, treeline.

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## Meetings

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# Unravelling plant temperature signalling networks

## Plant temperature response networks conference, Durham, UK, September 2009

The North East of England was the setting for the first UK Plant Temperature Response Networks meeting, organized by Heather Knight (Durham University, UK) and Kerry Franklin (University of Leicester, UK) in the Cathedral City of Durham. Delegates congregated at the University College of St Aidan's for a 2-d workshop exploring the impact of temperature on plant function. The aim of the meeting was to bring together UK scientists working on diverse aspects of temperature signalling in plants in the hope of identifying common research goals and developing a strategy for future research direction. Diversity was achieved with a mix of seminars and posters, integrating molecular approaches with whole-plant physiology in both the model plant *Arabidopsis thaliana* and a variety of crop species. The meeting was well supported with a full house of 50 delegates in attendance. Conference costs were kept to a minimum through generous sponsorship from the Society for Experimental Biology and *The Plant Journal*. An important and popular feature of the meeting was the Early Career Scientists' (ECS) forum. In this session, an evening wine reception was combined with informal predinner presentations from PhD students and postdoctoral researchers working on a variety of temperature-regulated responses. Financial support from *New Phytologist* enabled full coverage of all ECS speakers' costs, thereby facilitating greater participation of many groups than would have otherwise been possible.

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*'...plants probably possess multiple thermosensory mechanisms, including membrane fluidity and chromatin remodelling, in addition to direct effects on protein stability and enzyme function.'*

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Topics covered included plant temperature perception, temperature regulation of plant architecture, acclimation to cold and heat stress, interactions between temperature and the circadian clock, and the temperature-regulation of flowering and bud development. Presentations provoked lively discussion, forging new collaborations and strengthening a research area currently displaying considerable expansion within the UK Plant Science community.

### Why temperature?

Plants experience a wide range of temperature fluctuations in natural environments and have therefore evolved mechanisms to minimize cellular damage at temperature extremes. Growth at low temperatures (cold acclimation) enables plants to initiate signalling cascades and metabolic alterations, which enhance tolerance to freezing temperatures (Chinnusumay *et al.*, 2007). When subjected to high-temperature stress, the elevated synthesis of heat shock proteins (HSPs) provides some protection against protein denaturation and misfolding, thereby maintaining cell homeostasis (Wang *et al.*, 2004). Whilst previous research into the effects of temperature on plants may have focussed on extreme heat and cold, an emerging area of interest is the perception of, and response to, more subtle fluctuations in ambient temperature.

Temperature is a key abiotic signal that regulates plant function throughout development (reviewed in Penfield, 2008). Alterations in growth temperature can act as a stimulus to initiate metabolic changes and promote developmental transitions. The regulation of plant development by temperature enables the co-ordination of plant growth with favourable seasons and/or conditions of climatic advantage. Temperature cues are potent regulators of germination and plant architecture through integration with light-signalling pathways (Gray *et al.*, 1998; Penfield *et al.*, 2006; Koini *et al.*, 2009). The transition from vegetative to reproductive development is also tightly regulated by temperature in many species. In winter annuals, a prolonged period of low temperature (vernalization) is often required to initiate flowering the following spring through chromatin remodelling of the floral repressor *FLOWERING LOCUS C* (Henderson & Dean, 2004). Modest elevations in ambient growth temperature have additionally been demonstrated to accelerate *Arabidopsis* flowering time through altered regu-

lation of the floral repressor, *FLOWERING LOCUS M* (Balasubramanian *et al.*, 2006).

Accurate temperature perception also enables plants to buffer their metabolism against environmental fluctuations. An important cellular process requiring such buffering is the plant circadian clock. Recent research has demonstrated that small changes in ambient temperature alter the functional hierarchies between clock components, thereby maintaining rhythmicity across a broad temperature range (Gould *et al.*, 2006). Given the importance of temperature throughout plant development, it is surprising how little we understand about the signalling processes involved in perceiving and transducing this key environmental cue.

### How do plants sense temperature?

A recurrent question emerging from discussions was how plants sense temperature. Is it likely that plants use a common single 'thermosensor' or do multiple thermosensing mechanisms exist? It is well documented that exposure of plants to low temperature results in a rapid elevation of cytosolic calcium (Knight *et al.*, 1991) and that elevated temperatures increase membrane fluidity (Falcone *et al.*, 2004). Alterations in the latter have been demonstrated to initiate temperature-signalling pathways in a variety of organisms, tempting speculation that similar mechanisms may operate in plants (reviewed in Samach & Wigge, 2005). Data from the McWatters laboratory (University of Oxford, UK) supported this possibility, whilst a number of laboratories showed small changes in ambient growth temperature to alter significantly the stabilities of key growth-regulatory proteins.

One of the best characterized plant-temperature responses is the epigenetic regulation of flowering time through vernalization, a research area pioneered by one of the meeting's invited speakers, Caroline Dean (John Innes Centre, Norwich, UK). It is perhaps therefore not surprising that chromatin remodelling formed an active area of debate. The involvement of histone modification in cold acclimation responses was explored in a presentation from the laboratory of Marc Knight (Durham University, UK), whilst exciting data from the Wigge laboratory (John Innes Centre, Norwich, UK) revealed a novel role for chromatin remodelling in ambient temperature perception, thus providing a new plant-thermosensing mechanism. A unifying conclusion from these discussions was that plants probably possess multiple thermosensory mechanisms, including membrane fluidity and chromatin remodelling, in addition to direct effects on protein stability and enzyme function. It was generally agreed that the thermosensors operating at ambient temperature are likely to be distinct from those used by plants to detect temperatures close to freezing.

### What temperatures are 'realistic' for studying plant growth?

A contentious point, reiterated throughout the meeting, was the need to measure plant temperature rather than air temperature when performing experiments and translating research findings into 'natural' scenarios. The majority of plant science laboratories use standard growing protocols with air temperatures maintained above 21°C. Many *Arabidopsis* accessions derive from northern Europe and, as such, would probably experience much lower daily growth temperatures. Furthermore, a plant exposed to direct sunlight would experience radiant heating effects far greater than those achievable in laboratory growth cabinets, requiring caution when predicting 'realistic' plant growth-temperature ranges. Also discussed was the issue of many laboratories using constant temperature through day and night cycles. It was generally agreed that future attempts to improve the relevance of laboratory growth conditions to the field ought to include a reduction in temperature (preferably gradual) during the dark period. Natural genetic variation was a popular subject of discussion, with a number of speakers describing quantitative genetic approaches to investigate temperature-mediated signalling processes. These included vernalization, temperature entrainment of the circadian clock and temperature-dependent shade avoidance strategy.

### Where next?

The study of plant temperature-response networks is clearly of increasing relevance with respect to global climate change. Many phenological shifts can be linked to small changes in ambient temperature, raising concerns over ecosystem sustainability and long-term food security (e.g. Fitter & Fitter, 2002). Future crop production is further threatened by unpredictable weather patterns (e.g. snap frosts) and reduced plant biomass at elevated temperatures. It is therefore imperative that the exciting developments emerging in the *Arabidopsis* model are translated to crop species. The UK effort in this area was predominantly represented by Boulton, Laurie and Wigge, from the John Innes Centre, who presented a range of work using *Brachypodium*, wheat, barley and orchids, and demonstrated the rapidly improving resources now available for genetic study of these species. It is hoped that the newly formed UK Plant Temperature Signalling Network will continue to contribute to global efforts to secure food production in a changing environment.

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**Key words:** chromatin remodelling, circadian clock, cold acclimation, flowering, heat tolerance, plant development, temperature signalling.

## Synthesizing symbiosis, if possible with multiple partners

### 6th International Symbiosis Society (ISS6) Congress, Madison, WI, USA, August, 2009

Over 300 delegates with diverse interests in the evolution, ecology, physiology, genomics, and molecular biology of

symbiosis came together for the 6th International Symbiosis Society (ISS6) Congress <http://people.bu.edu/iss/> held at the University of Wisconsin–Madison. The University of Wisconsin recently hired a number of new faculty members with a common research interest in symbiosis and all of the talks were held in their new Microbial Sciences Building, highlighting the University of Wisconsin's commitment to symbiosis research.

This report provides a brief overview of selected ISS6 talks and posters involving a wide range of research questions and research systems on autotrophic and heterotrophic organisms. In addition to N-fixing and mycorrhizal symbioses, a wide range of systems were discussed in which plants and other photosynthetic organisms serve as microbial hosts, food sources for animals, symbionts of animals and as participants in multitrophic interactions. Cross-cutting themes across all symbiotic systems were the tension between conflict and cooperation, communication between partners, exchange of metabolites, specificity of symbioses, evolution and host defense.

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*‘...plants can preferentially allocate resources to the more mutualistic AMF symbiont, but only when there is spatial separation from less mutualistic, cheater taxa.’*

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### What makes a symbiont?

Barbara Schulz and colleagues (University of Braunschweig, Germany) addressed the question of what circumstances cause a single root endophytic fungus to become a mutualist or a pathogen. In experimental inoculations with the fungus *Phialocephala fortinii*, they showed that the effects of infection depended on the species of host plant and on its health. Thus the outcome of symbiosis may vary with host identity and host environment. Catherine Masson-Boivin (INRA, France) and colleagues addressed the general question of what traits or genes distinguish mutualistic symbionts from free-living or pathogenic relatives. Most rhizobia are alpha-proteobacteria, but several disparate beta-proteobacteria have recently been shown to elicit host nodule formation and nitrogen fixation. Using an experimental evolution approach, they were able to produce strains of a common soil pathogen (*Ralstonia solanacearum*) that were able to eli-

cit nodule formation by the plant although not yet develop nitrogen-fixing bacteroids. Therefore, gene transfer can produce novel symbionts that grow in a common environment with pre-existing symbionts.

Sometimes a symbiont requires a partner in a three-way interaction. Two very different, complex multispecies interactions were described in grass hosts. Christian Hertweck (HKI Jena, Germany) discussed the role of rhizoxin produced by the fungus *Rhizopus microsporus* as the causative agent of rice seedling blight (Partida-Martinez & Hertweck, 2005). Through a series of experiments, he demonstrated conclusively that the rhizoxins were produced by endosymbiotic *Burkholderia*. Further, fungal spore production did not occur in the absence of the endosymbiont, illustrating its complete dependence on the bacterial symbiont. Mustafa Morsy and colleagues (Noble Foundation, OK, USA) presented an analogous interaction where heat tolerance of the grass *Dichanthelium lanuginosum*, which grows in geothermal hot spring soils, is based on infection by the endophytic fungus *Curvularia protuberata*. They showed that the endophytic fungus is infected by a virus, and that curing of the virus eliminates thermotolerance of the host grass (Marquez *et al.*, 2007). Other symbiotic associations should be examined for even smaller, essential partners. A last tripartite symbiosis is the mountain pine beetle and its two fungal symbionts (*Ophiostoma montium* and *Grosmannia clavigera*), which supplement the beetle's nutrient-poor phloem resources. The fungi are, in turn, dispersed and inoculated into new host trees by the beetle. Pine beetle outbreaks have devastating impacts on southern and western pine forests.

### Microbial interactions in the rhizosphere

Plant root systems exist in an extraordinarily diverse soil environment filled with microbial mutualists, pathogens, and saprophytes. Mycorrhizal symbioses are an important component of ISS meetings, although the 2009 meeting conflicted with an international congress in Brazil. In a symposium on the microbial symbiont impacts on communities and ecosystems, Jim Bever (Indiana University, USA) discussed the ecological consequences of diversity of arbuscular mycorrhizal fungi (AMF). In a variety of glasshouse and field experiments, he demonstrated that different AMF species have different effects on plant growth, defense compounds, and soil aggregation. He also posed a solution to a dilemma found in many symbiotic systems – invasion by cheaters that use host resources without benefit in return (Douglas, 2008). His data show that plants can preferentially allocate resources to the more mutualistic AMF symbiont, but only when there is spatial separation from less mutualistic, cheater taxa (Bever *et al.*, 2009). Katayna Turnau (Jagiellonian University, Poland) also discussed the importance of mycorrhizal symbioses in the conservation of

endangered plant species and in the survival of plant species in stressful, human-impacted habitats such as mine spoils.

The symbiotic N-fixing soil actinomycete *Frankia* and its host plants occur worldwide as a primary source of the world's fixed N. Many actinorhizal plants are invasive, far beyond their native ranges, yet readily nodulate and fix N. This raises the question of specificity and whether introduced species bring their symbionts or utilize resident symbionts. Jeff Dawson (University of Illinois, USA) described the spatial distribution of *Frankia* in the soil of a dune/s-wale community with several distinct genera and families of host plants. PCR amplification of 16S rDNA sequences demonstrated clustering of strains by host phylogeny. But, unexpectedly, they found that nodulation was promoted by actinorhizal plants in general, including hosts supporting divergent *Frankia* strains, suggesting that the mechanisms of host specificity and host nodulation are distinct.

The richness of the research presented at the congress on interactions in the rhizosphere was clear, with the two *New Phytologist* poster prizes being awarded to two students working on this topic: Cara Haney (Stanford University, CA, USA), for her work on plant flotillin-like proteins required for symbiosis with N<sub>2</sub>-fixing bacteria; and Allison Schwartz (University of Los Angeles, USA), for her investigations on a strain of plant growth promoting *Bacillus simplex* on *Lotus japonicus*.

### Macroevolutionary patterns

The large- and small-scale distribution patterns of highly diverse symbiotic systems were considered. Francois Lutzoni (Duke University, NC, USA) discussed the global distribution of lichenized fungi to gain insights into the various origins and evolutionary history of lichen symbiosis. David Richardson (St Mary's University, Halifax, Canada) described a Canadian lichen, *Degelia plumbea*, that produces ascospores only and so needs to steal its photosynthetic symbiont from local asexual lichen species. Marc-André Selosse (Centre d'Ecologie Fonctionnelle et Evolutive, Montpellier, France) covered the evolution of ericoid-type mycorrhizas using ITS sequencing and microscopy, but also N and C isotope analyses to infer their functional role for host plants.

### Host defense symbioses

Many symbiotic systems can only be understood in the context of natural enemies of the host. The fungus-farming ants and their actinomycete bacteria that protect the fungal colony from pathogens are an excellent example of the multiple layers of complexity that exist in many symbioses. Michael Poulson (University of Wisconsin, USA) presented evidence that ant colonies rear a single clone in their fungal garden and just a single strain of the antibiotic-producing bacterium. While genetic uniformity of the fungus may not

impart any costs for the ants because of protection by the symbiotic bacteria, one could expect greater diversity of bacterial strains to provide greater antibiotic diversity and better protection to the fungal colony (Currie *et al.*, 2003). In an ant–plant system where ants form domatia on the plant and protect their host from herbivores, Rumsais Blatrix (Centre d'Ecologie Fonctionnelle et Evolutive, Montpellier, France) demonstrated that host defense was based on specific chemical communication from the host plant to ant defenders when active defense is required.

Several presentations focused on the seed-transmitted endophytes of grasses. Grass endophytes represent a tritrophic symbiotic system predicated on protection of host plants. Chris Schardl (University of Kentucky, USA) discussed alkaloid synthesis gene clusters among *Neotyphodium* endophyte strains. Endophytes produce four classes of alkaloids in total but no single strain produces more than three. He suggested that while alkaloids mediate host protection, they are costly and subject to frequency-dependent selection such that a single strain or species encompasses more genetic diversity for alkaloid production than any one isolate. Keith Clay (Indiana University, USA) presented experimental data on the role of endophyte alkaloids in host protection from herbivores and the population dynamic consequences of that protection. Jennifer Rudgers (Rice University, TX, USA) considered the impacts of grass endophytes on community and ecosystem processes. In the globally distributed tall fescue, endophyte infection had dramatic effects on arthropod community composition and abundance. The tremendous diversity of plant–endophyte associations beyond the grasses was highlighted by Elizabeth Arnold (University of Arizona, USA), who described her work on a wide range of nonsystemic fungal endophytes from higher plants and lichens, and their varied phylogenetic origins (Rodriguez *et al.*, 2009).

## Associating to fix N<sub>2</sub>

The genome sequences of three legumes (*Lotus japonicus*, *Glycine max* (soybean) and *Medicago truncatula*) have been obtained recently, promoting rapid progress in understanding the genetics and biochemistry of symbiotic nitrogen fixation. Michael Udvardi (Noble Foundation, OK, USA) explained how a number of mutagenesis approaches, such as tilling, insertion mutagenesis, and fast neutron bombardment, are being developed in *M. truncatula* to understand nutrient exchange between the partners. The scale of genetic resources available for *M. truncatula* means that it is becoming a model not only for symbiotic nitrogen fixation, but also for the AMF symbiosis. Gary Stacey (University of Missouri, USA) reported on efforts to harvest soybean root hairs, which represent single cells, coupled to a broad transcriptomic, proteomic, metabolomic and small RNA analysis, aimed at understanding the earliest stages of interaction between partners. The development of nitrogen-

fixing bacteroids in legume nodules was the focus of several other talks. Recent research in the Poole laboratory (John Innes Centre, Norwich, UK) has revealed that rhizobia in legume nodules shut down aliphatic amino acid biosynthesis and become dependent on the host for their supply, the so-called 'symbiotic auxotrophy' (Prell *et al.*, 2009). In some respects, this resembles the obligate *Buchnera*–aphid symbiosis, where the aphid provides *Buchnera* with many nonessential amino acids in return for essential amino acids. Sandy Macdonald (University of York, UK) also proposed that waste ammonia from the aphid is not excreted, but is instead recycled back into essential amino acids by *Buchnera*. These findings highlight the increasing metabolic interdependence from rhizobia to *Buchnera* and true organelles.

The interaction between plant roots and soil microorganisms is a critical determinant of plant productivity. Many of the key plant–microbe interactions occur in the rhizosphere (the soil region adjacent to plant roots). Jorge Vivanco (Colorado State University, USA) showed that *Arabidopsis thaliana* and *M. truncatula* maintain very different types of soil fungal biodiversity. When each plant was transferred into soil in which the other plant had been grown for many generations, there was a steep decline in fungal biomass, taking at least three generations of planting to reverse. This is very interesting from the perspective of how introduced plants, such as *Centaurea maculosa*, can become weeds because microbial populations in the new environment do not limit plant growth (Callaway *et al.*, 2003). Focusing on one of the earliest steps in colonization, Anne Hirsch (UCLA, USA) reported that the common *nod* genes (*nodDABC*) are essential for biofilm formation of *Sinorhizobium meliloti* on alfalfa roots. Furthermore, these genes are induced during biofilm formation mostly independently of the root flavonoid inducer luteolin. This raises the question of whether *nod* gene induction by flavonoids is more important for maintenance of infection threads than at the first stages of contact with the root. We still know very little about how bacteria grow down infection threads, and what controls this. Graham Walker (University of Illinois, USA) highlighted a number of genes, such as *bacA*, *bluB* and a new RNAase (SMc01113), that are essential for bacteroid development. The role of BacA is still elusive, although it is known to alter very long-chain fatty acid insertion into the lipopolysaccharidic layer, as well as peptide uptake. BluB is required for B12 synthesis, which is essential for ribonucleotide reductase in nodules, but not in free-living bacteria (Campbell *et al.*, 2006). This is likely to be the result of the greater oxidative stress encountered in the nodule as suggested by pioneering work in the Walker laboratory. Again, this protein appears to be absolutely essential in the developing bacteroid, probably because of the greater stress encountered in the nodule. A novel signaling dependent on cAMP synthesis by CyaD1, CyaD2 and CyaR has been discovered by Jacques Batut in

*S. meliloti*. cAMP probably binds to a transcriptional regulator (Csr), which appears to regulate more than a hundred genes. This pathway is induced by nodule or leaf extracts, but not root extracts, and appears to limit the number of infection threads initiated. Overall recent studies of the legume–*Rhizobium* symbiosis have highlighted the importance of the signaling events as bacteria invade the plant and establish N<sub>2</sub>-fixing bacteroids. We understand the first (nod factor synthesis and perception by the plant) and last steps (N<sub>2</sub> fixation biochemistry) best, but the steps in between remain elusive. From the standpoint of symbiosis, though, this is a very interesting transition from free-living bacteria into symbionts.

## Conclusions

Historically, research in symbiosis has been balkanized, with limited cross-communication across system-specific research programs. An important outcome of the ISS6 meeting was the emergence of common themes, tools and research questions applicable across a diversity of symbiotic systems. In a session on how to teach symbiosis, attendees showed each other their own symbiotic models. It was clear that, enriched by these cross-links between all models, research on symbiosis is poised to take a steep upward trajectory. We'll see this at the next meeting in Kraków, Poland, 2012!

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**Key words:** endophytes, evolution, nitrogen, rhizobia, rhizosphere, symbiosis.