

Commentary

Producing patterns in plants

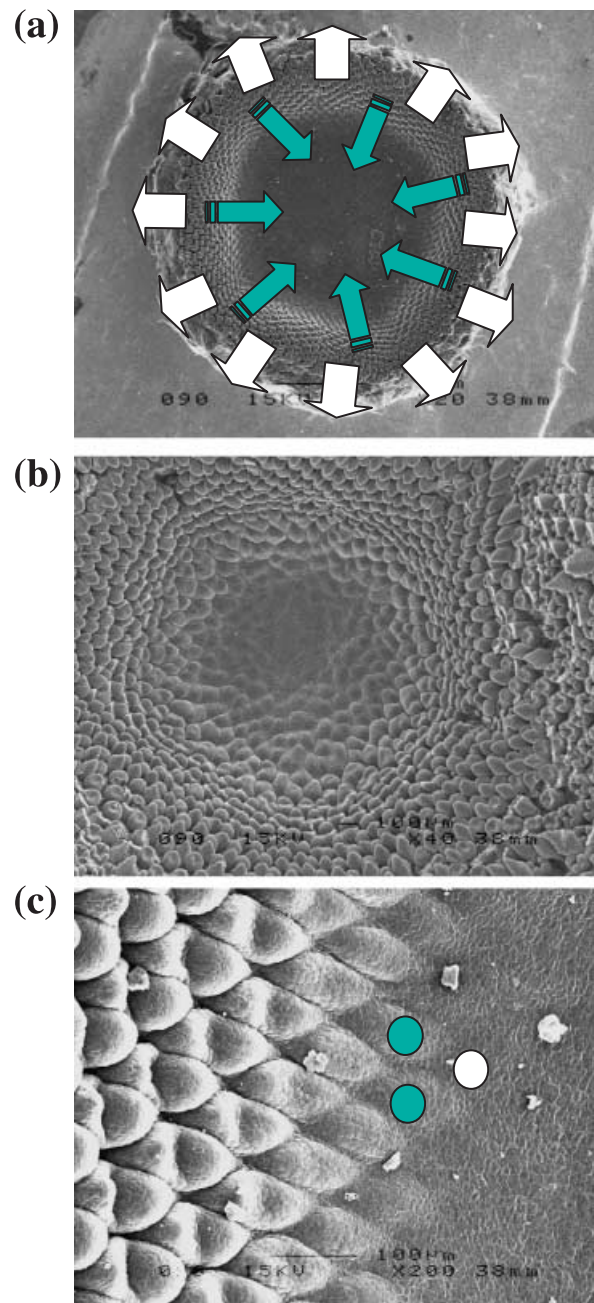
The beauty of plants lies in their ability to form complex, yet precise and reproducible, geometrical forms. This ability reaches its peak in the mechanism of flower formation. For example, the head of a sunflower consists of many hundred individual, tiny flowers generated in spirals encroaching from the circumference of an expanding apical structure (capitulum) to form a gigantic but geometrically perfectly patterned flower (Fig. 1). In this issue, Dosio *et al.* (pp. 711–722) provide a quantitative analysis of the process of capitulum growth, and the gradual but controlled process by which elements within the capitulum become destined to form individual flowers. These data provide a solid foundation for the modelling of flower formation, and for linking the observed parameters of floral morphogenesis with the wealth of molecular data on organogenesis in plants. The combination of careful quantitative analysis to create models of plant morphogenesis with molecular tools to test those models is an emerging and exciting area of research in plant developmental biology.

‘... can the recently published models developed in Arabidopsis be extended to a field of tissue which is orders of magnitude larger in size?’

Auxin flux and organ formation

With respect to the underlying principles of organ-pattern formation in plants, the most significant insight has come from recent data on the analysis of auxin flux and distribution during leaf initiation. Auxin is a small indole-related compound, which has long been recognized as a key plant growth regulator. It has also long been known that the flux of auxin through tissue can be mediated via a specific transport process, the

Fig. 1 (a) The sunflower capitulum consists of a disc of tissue that is expanding (white arrows). At the same time, elements within an annulus of tissue become determined to form small, discrete flowers. This annulus is constricting (shaded arrows), so tending to fill the expanding field of tissue with individual flowers that form the head of the sunflower. (b) A later stage of development, in which the many flowers formed are encroaching into the centre



of the capitulum. (c) The flowers formed on the capitulum arise in a highly ordered and specific pattern. Thus the position of a presumptive flower (white circle) is geometrically related to the position of the adjacent flowers just visible in this image (shaded circles). What is the nature of the signalling process by which this precise pattern formation occurs? (Adapted from Fig. 1 of Dosio *et al.*, 2006.)

polar auxin-transport system (Rubery & Sheldrake, 1974). A flood of significant papers over the past few years have identified key components of this system that mark the cells through which auxin flux occurs, enable the direction of auxin flux to be interpreted and, via molecular genetic manipulation, enable auxin flux within the plant to be modified (Benková *et al.*, 2003; Friml *et al.*, 2003; Blilou *et al.*, 2005). One important conclusion from this work is that, in the shoot apical meristem, the position of the presumptive leaf initiation is marked by a local accumulation of auxin as a result of directed auxin flux within the tissue (Reinhardt *et al.*, 2003). Moreover, it appears that the system transporting auxin is itself responsive to auxin (Paciorek *et al.*, 2005), creating a dynamic system in which fluxes of auxin are integrated across a growing sheet of tissue to create patterns of auxin that dictate the pattern of organogenesis. As organogenesis itself disrupts or alters the pattern of auxin flux, an iterative system is set in place. Recent papers have described mathematical models that can mimic this iterative process and predict the outcome of alteration in particular components of the transport process (Barbier de Reuille *et al.*, 2006; Jönsson *et al.*, 2006; Smith *et al.*, 2006). Although there are some differences in the modelling techniques used and the interpretations made, a key experimental approach has been to obtain *in vivo* quantitative analysis of growth in the shoot apical meristem, as also exemplified by other recent publications (Kwiatkowska & Dumais, 2003; Reddy *et al.*, 2004; Reddy & Meyerowitz, 2005).

Floral modelling

The models that have been developed can be used to describe alternative patterns of leaf formation (phyllotaxis), but as yet have not been extended to patterns of floral initiation. Does a similar system underpin floral patterning? It seems likely, yet there are distinct differences between, for example, leaf initiation in an *Arabidopsis* shoot apical meristem and floral patterning on a sunflower capitulum. One of the most obvious is scale – can the recently published models developed in *Arabidopsis* be extended to a field of tissue that is orders of magnitude larger in size? In floral organogenesis, it is common for several (indeed, many) organs to be initiated at different points around the circumference of a meristem essentially simultaneously, in contrast to the generally sequential formation of leaf primordia. Can patterns of auxin flux respond in an appropriate frame of time and space to allow this to happen in such large fields of tissue? Or is the apparently large responding field of a sunflower capitulum in reality made up of many much smaller interacting subdomains? The quantitative data provided by Dosio *et al.* provide important physiological parameters for the modellers to work with. An interesting conclusion of the three recent phyllotaxis modelling papers is that they all predict or require that there is ‘something special’ about the central region of the meristem, so that auxin either does not act there to induce

organogenesis, or is somehow excluded. The sunflower data are consistent with a similar phenomenon, but show clearly that this special feature of the central region can gradually be lost, so that eventually all tissue is consumed in flower formation. The situation in the capitulum may be linked with the spatial and temporal control of factors specific to flower formation, but it might also provide an insight into what is so special about the central zone of the meristem. Irrespective of this particular point, the conservation of molecular machinery involved in auxin transport should allow future investigation of whether the polar auxin-transport system underlies the patterning process so carefully analysed in the present study in the sunflower meristem, and whether the emerging models of phyllotaxis are applicable to the process of floral patterning.

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Key words: auxin, development, flower, meristem, modelling, organogenesis.

Letters

Common symbiosis genes of *Lotus japonicus* are not required for intracellular accommodation of the rust fungus *Uromyces loti*

Obligate biotrophic fungi are typically unable to grow in the absence of a host plant, and comprise a taxonomic diversity of basidio-, asco-, zygo- and oomycetes (Voegelé & Mendgen, 2003; Schulze-Lefert, 2004). Although many biotrophic pathogens (such as rust fungi, powdery mildew fungi and oomycetes) cause devastating diseases of crop plants, other biotrophs (such as arbuscular mycorrhizal fungi) engage in beneficial mutualisms that actually improve plant health (Parniske, 2004). Considering their impact on a global ecology (Vance, 2001), this symbiosis is probably the most important plant–fungus interaction.

One common feature of many biotrophic interactions is the formation of elaborate intracellular accommodation structures that maintain an intimate coexistence of both fungal and plant cells over a prolonged period (Panstruga, 2003). These structures, called haustoria in pathogenic interactions, or arbuscules in the arbuscular mycorrhiza symbiosis, have superficially similar structural and functional properties, and are believed to be the main site of nutrient flow between interacting cells (Mendgen & Hahn, 2002).

The commonalities between these intracellular biotrophic interactions have kindled speculation about their evolutionary and genetic relationship (Parniske, 2000). Genetic analyses of biotrophy are most advanced for the arbuscular mycorrhiza symbiosis, in which seven plant genes have been identified that are required to support infection (Kistner *et al.*, 2005). These plant genes, which are also required for nitrogen-fixing root nodule symbiosis with bacteria, are referred to as the common

symbiosis (*SYM*) genes, and encode proteins that carry the hallmarks of signalling molecules (Oldroyd & Downie, 2004). This, together with the phenotype of the corresponding plant mutants, indicates that the common *SYM* genes are involved in a signalling process that is required for successful infection of plant cells by symbiotic fungi and bacteria.

The AM symbiosis is believed to be at least as old as the earliest land plants (Remy *et al.*, 1994; Redecker *et al.*, 2000), and the radiation of the basidiomycetes and ascomycetes that cause plant diseases occurred later (Berbee & Taylor, 1993; Taylor *et al.*, 1999). One of our major unanswered questions about biotrophic interactions is whether the plant uses a similar or the same genetic program for the intracellular hosting of pathogenic and symbiotic fungi (Parniske, 2000). Although it might seem unlikely that plants would maintain genes to assist infection by pathogenic biotrophs, it is entirely possible that parasites may exploit some aspects of the mutualistic plant program that evolved much earlier, but that still provide a strong selective advantage for the plant. Research on biotrophic pathogenic interactions has mostly focused on resistance processes, therefore plant genetics of compatibility in these systems is still in its infancy (Schulze-Lefert & Panstruga, 2003; Schulze-Lefert, 2004). In the current study we used a genetic approach to unravel the overlap between mutualistic and parasitic biotrophy. In order to determine whether pathogenic rust fungal infection is dependent on plant genes required for mutualistic symbioses, we inoculated *Lotus japonicus* symbiosis-defective mutants representing six different common *SYM* genes with a compatible isolate of the *Lotus* rust fungus *Uromyces loti*.

The symbiosis receptor-like kinase SYMRK (DMI2 in *Medicago truncatula*) has an extracellular domain that is potentially involved in the perception of a fungal or bacterial signalling molecule (Endre *et al.*, 2002; Stracke *et al.*, 2002). Because of this predicted structure and mutant phenotype, SYMRK has been placed conceptually as one of the most upstream components in a symbiotic signalling pathway (Parniske, 2004). Although mutant plants are somewhat

Table 1 Compatible growth of the rust fungus *Uromyces loti* on common symbiosis mutants of *Lotus japonicus*

Mutant allele	Line designation	References	Identity (if known)
<i>symRK-2</i>	EMS61	Stracke <i>et al.</i> (2002); Szczyglowski <i>et al.</i> (1998)	Receptor-like kinase
<i>castor-2</i>	EMS1749	Bonfante <i>et al.</i> (2000); Imaizumi-Anraku <i>et al.</i> (2005)	Plastid-localized cation transporter
<i>pollux-2</i>	EMS167	Imaizumi-Anraku <i>et al.</i> (2005); Szczyglowski <i>et al.</i> (1998)	Plastid-localized cation transporter
<i>ccamk-2</i>	cac57.3	Schauser <i>et al.</i> (1998); Tirichine <i>et al.</i> (2006)	Calcium and calmodulin-dependent protein kinase
<i>nup133-3</i>	EMS247	Kanamori <i>et al.</i> (2006); Szczyglowski <i>et al.</i> (1998)	Nucleoporin
<i>sym6-3</i>	EMS126	Szczyglowski <i>et al.</i> (1998)	?

responsive to bacterial symbiotic signalling molecules (lipochitin oligosaccharides) called Nod factors, inoculated root hairs do not respond to mutualistic bacteria by producing microbe-accommodating infection threads or with the rapid transcriptional upregulation of symbiotic marker genes (Stracke *et al.*, 2002; Kistner *et al.*, 2005). Although *SYMRK* is also required for mycorrhizal infection, it was not important for infection by the phytopathogenic *Lotus* rust fungus. We observed hyphal proliferation and sporulation on *symRK* mutant plants. Both in timing and appearance, fungal growth on *symRK* mutants was indistinguishable from that on wild-type leaves (Table 1; Fig. 1).

Perception of bacterial Nod factors is quickly followed (within 10 min) by intracellular calcium oscillations known as 'calcium spiking' (Oldroyd & Downie, 2004). Two closely related putative cation transporters called *CASTOR* and *POLLUX* (Imaizumi-Anraku *et al.*, 2005) are required for this calcium response and for the arbuscular mycorrhizal symbiosis. Importantly, these plastid-localized transporters are not required for infection by the *Lotus* rust fungus (Table 1). However, measurements of calcium levels in single susceptible rust-infected cowpea vein epidermal cells revealed no increased calcium levels at early stages of wall penetration and intracellular infection (Xu & Heath, 1998). This, together with pharmacological data, has led to the suggestion that changes in intracellular calcium concentration are not likely to have an influence on the outcome of compatible interactions with phytopathogenic rust fungi. It will be interesting to determine whether infection by powdery mildew fungi that appears to be dependent on calcium/calmodulin signalling through the susceptibility determinant *MLO* (Kim *et al.*, 2002) has a comparable or different response on the *castor* and *pollux* mutants.

Physical infection by rhizobia and arbuscular mycorrhizal fungi involves the active participation of the plant, which probably includes the localized release of cell wall-degrading enzymes such as pectinases that allow the microbe successfully to colonize the inter- and intracellular spaces of the root. For arbuscular mycorrhizal interactions, one such response is the epidermal opening response that is dependent on the presence of the *Lotus* gene *CCamK* (Demchenko *et al.*, 2004; Tirichine *et al.*, 2006). We clearly demonstrate that the presence of *CCamK* is not required for infection by the rust *U. loti* (Table 1; Fig. 1), a fungus that is more likely to enter plant cells

through a combination of physical force and secretion of cell wall-degrading enzymes (Tucker & Talbot, 2001).

In brief, *Lotus* mutants defective for *SYMRK*, *CCamK*, *CASTOR*, *POLLUX*, *NUP133* and *SYM6*, which are dysfunctional in establishment of symbiotic infection by mutualistic mycorrhizal fungi and mutualistic bacteria, were all successfully colonized by the *Lotus* rust fungus (Table 1). More importantly, there was no evidence of fungal cell death or fungal encasement in mesophyll cells of mutant plants, and there were no obvious signs of any adverse plant responses, such as cell death or browning (Fig. 1). Together, our cytological investigation revealed nothing to distinguish rust fungal interactions with symbiosis-defective mutant plants from that seen in the wild-type *Lotus* accession *Gifu*.

Our data suggest there is little, if any, significant overlap in the initial signalling processes between root mutualistic vs leaf phytopathogenic biotrophic interactions. One caveat of our study is that the response of different tissues (root vs leaf) was compared. It is therefore still possible that genes paralogous to those involved in root-associated mutualisms may affect infection by foliar leaf pathogens, which would not have been identified in the current study. Given that pathogen species may exhibit different infection mechanisms in roots vs leaves (Sesma & Osbourn, 2004), it is possible that different plant genes are involved in accommodating infection by biotrophs in different plant organs.

That rust fungi, powdery mildew fungi and oomycetes all successfully infect the model plant *Arabidopsis thaliana* in the absence of clear orthologues of, for example, *SYMRK* (Kevei *et al.*, 2005) or *CCaMK* (Levy *et al.*, 2004; Mitra *et al.*, 2004) in the completely sequenced *Arabidopsis* genome appears to support this view. Recent data in rust fungal systems suggest that successful establishment of compatible infection is determined at a very early stage of infection, when the fungus is just beginning to penetrate the plant cell wall (Mould & Heath, 1999), but that success or failure of infection depends more on the presence or absence of plant defence responses (Mellersh & Heath, 2001, 2003) than on any genetically predetermined plant accommodation program. It will perhaps be of greater future interest to focus on those later stages of mutualistic and phytopathogenic biotrophic interactions (during or after formation of intracellular infection structures),

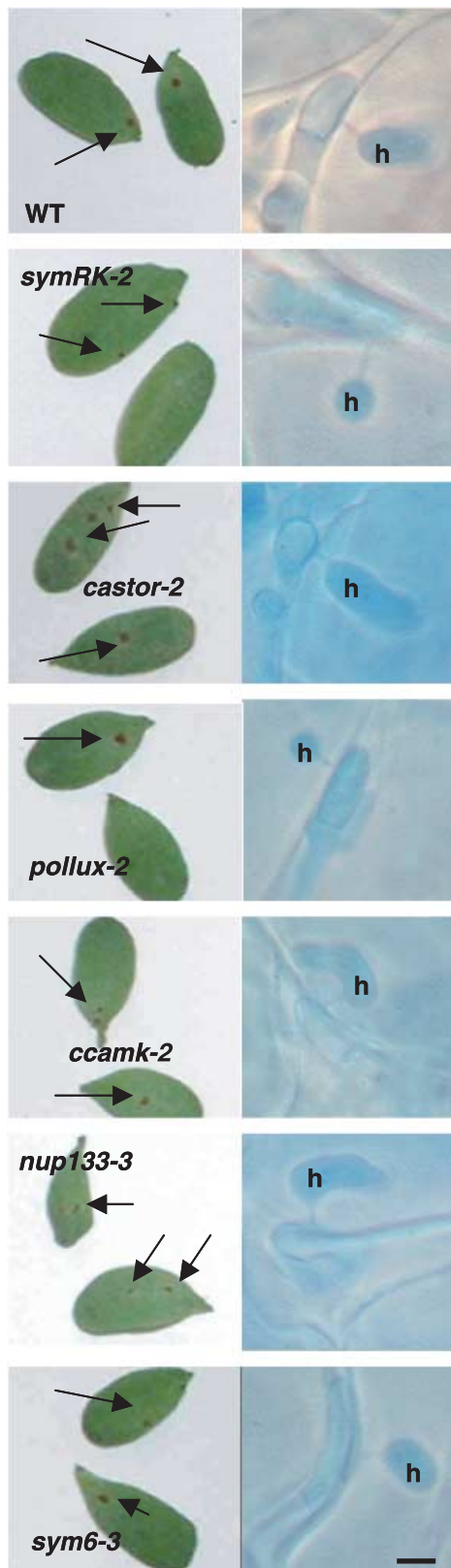


Fig. 1 Leaves of *Lotus japonicus* wild-type Gifu and several common *sym* mutants infected with the *Lotus* rust fungus *Uromyces loti*. Successful colonization of *Lotus* cotyledons results

where changes in plant cell metabolism affecting the exchange (either uni- or bidirectional) of nutrients and signals between host and microbe could shed more light on shared elements involved in the establishment of biotrophic compatibility across a variety of microbial taxa.

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in the formation of rust pustules (arrows, left panels) 9 d after inoculation. Note the formation of healthy, non-encased, fungal haustoria (h) in the light micrographs of fixed, cleared rust-infected *Lotus* cotyledons (right panels, bar = 10 μ m). Leaves were fixed and decolorized in 95% (v/v) ethanol, stained with trypan blue-lactophenol to visualize fungal structures, and cleared in saturated chloral hydrate before being mounted in modified Hoyer's medium, as described previously (Stumpf & Heath, 1985).

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Key words: symbiosis, *Lotus japonicus*, rust, biotroph, arbuscular mycorrhiza, intracellular, SYMRK.

Unravelling phenotypic plasticity – why should we bother?

Summary

The ability of a genotype to change its phenotype was once considered rather a nuisance – making it difficult to define a genotype. This led to the idea that there was a problem called ‘instability’. But quite early it was recognized that stability was under genetic control, and was a character like other attributes of an individual. From this realization came the idea that there were two sides to the character of ‘instability’, and that the ability to change could be important. This ability was thus given the title of ‘plasticity’. Once recognized, it became clear from surveys of different species and populations that plasticity can (i) be a complex character, and (ii) be selected to fit species to the particular demands of different environments. For plants, which cannot meet variations in environment like animals by behavioural responses, phenotypic plasticity can be very important. Plants should therefore be valuable tools for unravelling the mechanisms of plasticity whilst also demonstrating its contribution to fitness experimentally. We ought also to be

able to demonstrate that appropriate genetic variability is available through which complex responses can be built up by selection. Genes must exist not only to determine character means, but also to determine character response, which adds interesting complexity to our ideas about evolution.

Introduction

Upon receiving an invite to open the 14th *New Phytologist* Symposium on 'Plant ecological development' (see accompanying meeting report; Ackerly & Sultan, this issue, pp. 648–653) it seemed plain to me that the last time I considered the topic of phenotypic plasticity properly was a long time ago. Yet this topic has never really left me since the time when I was studying adaptive evolutionary differentiation in the 1950s, and kept finding that about half of what I saw was phenotypic – which few people seemed to be bothering about. Today, of course, the study of phenotypic plasticity is considered central to ecology and evolution. So to have been in amongst a lot of people at the cutting edge of a revitalized topic at this meeting was a great delight; here I outline some of my thoughts on this topic as presented in my keynote address.

Early ideas – on stability

In my early days as a scientist, the ability of a genotype to change its phenotype was considered just an annoyance – a confusion that made it difficult to know what a genotype 'really' was – in other words how its characteristics could be defined. From this came the idea that there was a phenomenon that could be called 'instability' – a source of error, as when we refer to an unstable person as not always to be relied upon.

But this instability can be a serious matter. At its simplest, it can imply lack of adaptation – indicating that a genotype or a character can easily be pushed around. This is nowhere more important than in crop varieties, where variation in yield can be a source of great trouble – scarcities leading to serious supply difficulties, and gluts leading to collapse of market prices. Because of the postwar concern to produce enough food, a lot of work was being done on this issue of stability, leading to sophisticated systems of cultivar assessment (e.g. Finlay & Wilkinson, 1963; Eberhart & Russell, 1966). The wheat cultivar Capelle, for instance, was a very popular cultivar of the 1960s. It was not as high yielding as some of its contemporaries, but was popular because its yield was very reliable. Quite early, geneticists working with both *Drosophila* (Mather, 1953; Waddington, 1960) and crop plants (Paxman, 1956; Williams, 1960) started to show that stability was under genetic control like other attributes of an individual. Thereafter, in the 1960s, plant breeders started to pay attention to stability as a character that could be selected for, and academics began deedy discussions about

fitness, and how stability might be made up (e.g. Waddington, 1957). Stability in functional performance and fitness is often given the grander Greek term 'homeostasis', while the developmental processes that cause trait stability are termed 'canalization'. It remains an important topic for anyone interested in adaptation.

Later ideas – on plasticity

With this work on stability came the realization that there were two sides to this important character, and that the ability to change – (which I termed 'plasticity') could be important as well as fascinating, especially in plants. This plasticity, of course, has a very significant relationship to stability. It can be a simple sign of weakness – of lack of fitness – mentioned by many authors, but it can also be a sign of strength, reflecting mechanisms maintaining fitness. If a plant grows smaller in a windy habitat it might be about to die; but it could be demonstrating that it has a special mechanism for avoiding wind damage. Interestingly, botanists had recognized the phenomenon and its evolutionary significance quite a long time previously (e.g. Kerner, 1895; Nilsson-Ehle, 1914). Even Darwin had thoughts on it which he described in a letter to Karl Semper (in 1881). Because plants cannot move to avoid stressful conditions, as can animals, they have to put up with what comes. Phenotypic changes that minimize stress therefore seem understandable.

Indeed, once the significance of plasticity was recognized, it was realized that it should be a character capable of contributing to evolutionary adaptation like any other character. To investigate it further, comparative studies are a good place to start. These involve looking at related species and populations occupying different environments. Related populations are perhaps best, because they will have close genetic affinities, and any observable differences will be more likely to be related to present environments. Differences between species may have evolved in past environments experienced by the species, and may not be easily related to present conditions. Nevertheless, so long as this is appreciated, species differences can be very valuable.

The outcome of comparative surveys has been the revelation of a wealth of examples, involving all sorts of different species and different mechanisms. These have been reviewed by many authors (such as Kerner, 1895; Salisbury, 1940; Bradshaw, 1965; Sultan, 2000). The wealth and diversity of examples are still worth contemplating – they have a lot to teach us. Perhaps one of the best examples to make one think is the phenomenon of etiolation. We all know that most plants get long and thin in shade – and it may seem pretty obvious that they should. But is it obvious? With the production of less photosynthates as a result of shading, surely it would be more likely that plants would grow less. But they do not – at least not in length, and we now know this reaction

is controlled by a fairly subtle bit of machinery (Smith, 1990; Schmitt, 2003). Equally importantly, not all species etiolate to the same extent (Morgan & Smith, 1979). Some woodland species, such as dog's mercury (*Mercurialis perennis*), hardly etiolate at all – which, when you come to think of it, is highly sensible for a woodland plant. Then, when we examine the machinery involved, it is interesting to discover that both the receptor and the responding organ can be in surprising and different places in different species. You will all know the plastic response to shading of the petioles of *Trifolium repens*, recognized first by Kerner. In other legumes, stems and not petioles are the place of response. All this may seem curious. But in fact it is logical, relating to the morphology of the species and the environment in which it grows.

I once worked on the taxonomy of the two British species of hawthorn, *Crataegus monogyna* Jacq, the common species found in hedgerows and scrub, and *Crataegus laevigata* (Poir.) DC, the closely related Midland hawthorn (with which it hybridizes) typically found in fairly dense woodland. In scrub conditions they look the same. But sometimes they can be found together, caught up in an area of young woodland or coppice. Under these circumstances *C. monogyna* grows up vertically with its green branches high up in the woodland canopy and all its lower branches shed, with its leaves in a higgledy-piggledy arrangement. So it looks rather like a lavatory brush chasing the light. In the same conditions, *C. laevigata* produces a series of spreading branches near the ground with its leaves in a more or less perfect mosaic. Evidently these closely related species have evolved to express quite different architectural plasticity in shaded conditions.

The plasticity of growth form is particularly fascinating in water plants, where it can be related to the fact that, if a species that roots in bottom muds is to exploit the complete range of environments available to it, it has to cope with three environments – underwater, water surface, and aerial conditions – for which different leaves are necessary. Trout fishermen can see this necessity if they watch the ways in which the surface leaves and the underwater fronds of water crowfoot, *Ranunculus peltatus*, cope (or fail to cope) with the current of a chalk stream. It is equally interesting to contemplate that the switch mechanism for these distinct leaf types depends on the species. It can be the presence of the water surface or a fixed switch related to the time of the year.

A plethora of such observations is possible. More generally, we can conclude that plasticity entails the following characteristics:

- 1 specificity for a particular character;
- 2 specificity in relation to particular environmental influences, including patterns of environmental variation;
- 3 specificity in pattern and direction;
- 4 having specific adaptive values or maladaptive effects;
- 5 being under the control of quite elaborate mechanisms of perception and expression;

6 being under genetic control; and therefore
7 being able to be radically altered by selection to fit the demands of different environments.

All this is what we might expect of a character or property that contributes to evolutionary adaptation. But it is always possible to think that plasticity is an accident – just a by-product of the way plants are made up, and just an incidental part of other more important characters of an organism. Geneticists and others, such as Via (1993), suggest that this nonadaptive plasticity is a correlated response resulting from pleiotropy, with the implication that it is some of the froth of evolution. Some perhaps is. However, other aspects of plasticity, such as the etiolation response, are not the predicted outcome of, for example, low light but rather something more positive and organized. Greater understanding of the mechanisms of plasticity may allow us to better interpret individual cases as either adaptive or accidental.

Some thoughts on future directions

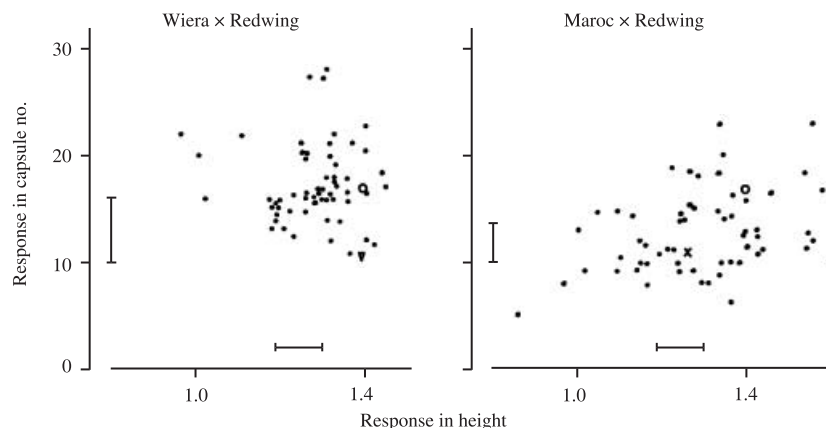
It seems to me that we should be thinking/working on the occurrence and properties of plasticity in different situations to see what more we can discover and unravel. This unravelling will involve many different approaches, as this plant eco-devo symposium has amply shown (Ackerly & Sultan, this issue, pp. 648–653).

Our starting point must be the realization that knowing the mean expression of a character is no longer good enough. Firstly we need to know its variance, its eco-devo, which means we will need to know its response to environmental conditions. But these conditions can be of different sorts – light, temperature, drought, etc. This should lead to some complicated multifactorial experiments, the value of which has been shown by Pigliucci *et al.* (1995), and we have to stop thinking that there is a single condition called 'stress' (Bradshaw & Hardwick, 1989). To understand how organisms cope with various demands, responses to each element or aspect of stress must be separately investigated.

Then there is the need to relate the plasticity, the eco-devo response, to details of the environment (the type of variation, its spatial scale, its timing, the cues available and their reliability, the speed and cost of the response, and its reversibility), and to its effects on fitness, as has been well argued by Dudley (2004).

The need to know more about the nature of the mechanisms involved will, to many investigators, be the essence of work on plasticity. Such work is crucial to our understanding. But I hope that it can put plasticity in perspective in terms of adaptation so as to reveal the subtleties of this remarkable plant character. There are a number of fascinating early examples of such work, such as that on *Capsella* (Sørensen, 1954) and *Solidago* (Bjørkman & Holmgren, 1963), as well as more recent studies on the whole matter of

Fig. 1 The correlation of height and capsule response of a random set of 60 F3 lines, from crosses between cultivars of *Linum usitatissimum*, to high (1-inch spacing) and low (6-inch spacing) density. The distribution of responses and the relationship between the responses of the two characters are shown, where response was measured as the ratio of the value at wide spacing to that at close spacing. Cultivars: Redwing O, Maroc X and Weira V. The error is indicated as the least significant difference at 5%. For more detail, refer to Khan *et al.* (1976), from which the figure was adapted.



response to shading (Schmitt *et al.*, 2003). But when looking for explanations, we must beware of falling into an *a posteriori* trap, and just look critically at what is involved.

The genetic underpinnings of plasticity remain a poorly understood subject. The surveys involving species or population comparisons already mentioned provide crude evidence of genetic control. Specific mechanisms, however, can only be revealed by proper genetic experiments. One approach is to look for 'plasticity' genes and QTLs, using molecular techniques. A more old-fashioned yet still extremely informative method is traditional crossing experiments from which both the genetic components and the heritability of plastic responses can be determined. For instance, in the 1970s Khan did some elegant work on flax density response which has not received the attention it deserved (Khan *et al.*, 1976). He examined 60 F3 families from two different crosses between flax and linseed. These he subjected to two different environments (6-inch and 1-inch spacing). Figure 1 shows the results of one of his experiments. If you follow these and the further details in the published paper, this single experiment shows that plastic responses can (i) vary between genotypes, (ii) be highly heritable, (iii) have different unconnected components, and (iv) that these can be unconnected across environments. It also shows (v) that in a cross, transgressive segregants can turn up. Such a Mendelian approach allows the components of the plastic response as well as the genes involved to be sorted out.

The last property of plasticity that is crucial is whether or not it can be selected. In many ways, if we knew enough about what I have already discussed, we could happily conclude that plasticity was a character that must be able to play a significant part in evolution. Differences found between populations are *a fortiori* evidence that the character can play its part in evolution by natural selection. The wide range of variability that can arise in crosses is even better. But the best evidence is the progress that can be achieved by direct selection over several generations, such as in the work by Scheiner & Lyman (1991). More such experiments are needed.

Similarly, studying cultivars produced by plant breeding can reveal the potential for selective differentiation within a species. For example, the two types of *Linum usitatissimum* – flax and linseed – have extraordinary differences in density response, which must have been brought about by selection out of the ancestral *Linum* material (Khan & Bradshaw, 1976). The differences between wild and cultivated sunflowers are an excellent similar example (M. I. Khan, in Bradshaw, 1973).

Conclusion

Clearly, plants can have different and complex patterns of behaviour, of ecological development or 'eco-devo'. What still needs to be done, however, is to disentangle more of the underlying mechanisms and the genes responsible. If genes exist that not only determine character means, but also determine character response, we have something very interesting to contribute to ideas about evolution and adaptation. Perhaps, as suggested by Trewavas (2005), we shall have to be talking about plants having intelligence.

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Key words: epistatic gene systems, genetic control of plasticity, homeostasis, instability, phenotypic plasticity.

Meetings

Mind the gap: the emerging synthesis of plant 'eco-devo'

New directions in plant ecological development: the 14th New Phytologist Symposium, London, UK, January 2006

The past 10 years have witnessed great advances in developmental genetics and molecular techniques, as well as new conceptual insights into organisms as dynamic systems interacting with their environments. While researchers in the field of plant phenotypic plasticity have largely aimed to characterize pheno-

typic variation in response to environment, those in the field of ecological development, or 'eco-devo', aim for a more mechanistic understanding of this response process at all levels: the perception of environmental signals; their transduction by the organism at molecular, cellular, and hormonal levels; the phenotypic expression that results from these multiple, interacting regulatory processes; and the consequences of those phenotypic outcomes for functioning individuals, ecological communities, and evolving plant populations. The broad goal of eco-devo, then, is to close the gap between mechanisms and ecology, and provide a genuinely integrated view of plant development in its real environmental context. The 14th New Phytologist Symposium (www.newphytologist.org/eco-devo), held recently at the Royal Society in London,

addressed this exciting goal under the theme of 'New directions in plant ecological development' (see also the recent feature on this topic; Sultan, 2005). The symposium was attended by participants from 11 countries and a diversity of research disciplines, at all levels from graduate students and postdocs to eminent senior researchers, notably Professors Anthony D. Bradshaw and Harry Smith. Here we highlight the presentations of the speakers under two broad themes that emerged through the presentations and ensuing discussions.

Anthony Bradshaw opened the symposium with a brief and stimulating plenary, providing an historical perspective on the study of plasticity and raising some of the issues that led to his pioneering 1965 paper (Bradshaw, 1965; see accompanying article in this issue, Bradshaw, pp. 644–648). He argued the fundamental point that to understand organisms 'knowing a character's mean isn't good enough, we must know how it is expressed in actual environments.' In this light, Professor Bradshaw reminded the participants of the continuing importance of understanding the mechanisms and significance of phenotypic stability as well as plasticity, and the relationship between the two. His remarks set the stage for the ensuing presentations, as the dissection of mechanisms underlying plant development is rapidly shedding new light on these questions.

'... the complementary notion of "ecological annotation": how much of the observed phenotypic variation among life history stages, individuals, populations, or species can be attributed to allelic or regulatory variation in a particular gene'

Genes and gene function in ecological context

Developmental genetics examines the regulatory pathways that transform environmental or ontogenetic signals into cascades of gene transcription, translation, and phenotypic outcomes from the molecular to morphological levels. In an ecological context, there has been great progress in understanding the genetic architecture underlying 'model traits' such as photomorphogenesis (Chen *et al.*, 2004) and flowering time (Boss *et al.*, 2004). Eco-devo research critically extends our understanding of these mechanistic models by studying them in the context of naturally occurring genetic and environmental variation. For example, J. Schmitt (Brown University, Providence, RI, USA) and colleagues have shown that geographically widespread natural populations of *Arabidopsis thaliana* show genetic variation for the *FRIGIDA* locus and its repressor flowering locus *c* (*FLC*), key flowering

genes that interact epistatically (Caicedo *et al.*, 2004). Under common environments, these genetic variants exhibit different flowering times mirroring their latitude of origin (Stinchcombe *et al.*, 2004). Allelic variation at these loci is maintained within populations in part through antagonistic fitness effects in winter vs spring annual cohorts. A central theme of the symposium was the question 'How can detailed knowledge of developmental genetics provide essential insights into ecological phenomena?' One answer that emerged is that the genetic architecture of a trait imposes constraints (at least in the short term) on phenotypic responses to the environment, analogous to the constraints imposed by antagonistic genetic correlations on microevolutionary responses to selection. In the flowering time example, understanding variation in interacting signaling pathways may inform predictions about the responses of different populations to climate change. Short-term responses will be shaped by current developmental mechanisms. The variation among populations in these mechanisms demonstrates that they may be rapidly adjusted by evolutionary change, so the time-scale of these constraints will be linked to generation times and the strength of selection on the resulting traits.

Knowledge of genetic mechanisms may also be essential to understand the degree of correlation or independence of plant responses to different environmental factors. There are several signal perception, transduction, and gene regulatory pathways (such as ethylene and abscisic acid (ABA) concentrations and phytochrome activation) that appear repeatedly in eco-devo studies of different species, traits, and environmental factors. Given these shared pathways, one intriguing research avenue is the degree to which plants have evolved to respond appropriately to distinct environmental signals. L. Voesenek (Utrecht University, Utrecht, the Netherlands) discussed his elegant studies of the mechanisms underlying stem elongation in response to flooding in the aquatic species *Rumex palustris* (Voesenek *et al.*, 2004). Ethylene plays a key role, as endogenous production in plant cells leads to high ethylene concentrations in cells of submerged plants, as a result of the reduction in diffusion coefficients underwater. The build-up of ethylene inhibits ABA synthesis, and reduced ABA leads to higher gibberellic acid (GA) concentrations, promoting stem elongation (of course the full regulatory network is more complicated than this). Comparative studies of the closely related terrestrial species *Rumex acetosa* illuminate critical control points in the pathway that distinguish species with and without this submergence response (Benschop *et al.*, 2005). Surprisingly, components of this same pathway are also involved in the elongation response to shade, although in this case phytochrome is employed in the mechanism of environmental perception. Voesenek asked whether a few 'master switch genes' are involved in diverse environmental responses, and if knowledge of these mechanisms would allow us to better understand and predict cross-responses and interactions among different signals.

The multifaceted role of phytochromes in plant development (Sullivan & Deng, 2003) continues to offer novel challenges to our understanding of signal transduction in plant responses to environment. Harry Smith (University of Nottingham, Nottingham, UK) synthesized his lifelong interest in the remarkable role of phytochromes as a 'precise, graded, and unambiguous signal' of neighbor density. Indeed, the evolution of phytochromes B, D and E, which are critical to shade avoidance responses such as shoot elongation, may have been a key step in the evolution of angiosperms and their dominance in highly productive vegetation (see Mathews, 2005). Recent studies demonstrate that woody plants differing in shade tolerance exhibit variable, phytochrome-mediated growth responses to the red:far-red ratio (R:FR), similar to those of herbaceous taxa (Gilbert *et al.*, 2001). Microarray data suggest that the elongation response to far-red may involve a rapid transcription event inducing expression of numerous genes involved in growth within 10 min of the plant's perception of a far-red signal. These precise events as well as the localization of phytochromes on the plant body, the functional interactions among the different phytochromes, and the extent to which plants in certain habitats may grow toward light rather than away from shade remain to be determined.

C. Galen (University of Missouri, Columbia, MI, USA) offered a novel perspective on the role of blue-light perception and negative phototropism in root growth, in relation to the attenuation of light through the very top layers of the soil. Experiments with phototropin-disabled mutants (Galen *et al.*, 2004) show that loss of function of these blue-light photoreceptors can lead to less downward-directed root growth, resulting in lower seedling establishment and total biomass in plants grown in dry soil. Concomitant effects on water use efficiency, which is higher in plants unable to sense blue light (an inducer of stomatal opening), raise the important question of how plants integrate changes in multiple functional traits in response to one or many environmental signals. Galen's research also exemplifies how a characteristic that may be perceived as 'normal development' (the downward growth of roots) may be dependent on active mechanisms of signal perception and transduction.

The theory of niche construction (Odling-Smee *et al.*, 2003) emphasizes ecological and evolutionary feedbacks between organisms and their environments. K. Donohue (Harvard University, Cambridge, MA, USA) has applied this theory to the study of gene action and plasticity in plants (Donohue, 2005). The action of a gene early in the life history of a plant alters the phenotype, and thus effectively alters the environment experienced by that plant (or by parts of the plant) later in life. Thus, plant responses to the environment modify not only the phenotype, but also the future environment. These feedbacks can have important consequences for the mechanisms, impacts, and evolution of plasticity. For example, experiments with *Arabidopsis* ecotypes reveal that the timing of germination in spring vs fall is influenced in part by the action of phytochromes, via effects on light

perception and temperature responsiveness. Germination timing will dramatically alter the growth environment for the rest of the life cycle, and this can be seen as an extended phenotypic effect of the genes controlling germination. These indirect pathways provide the mechanism to generate epistatic interactions among genes operating in different plant organs or at different times during the life history of the plant, and are thus critical to understanding the integrated effects of gene action on lifetime performance and fitness.

Ultimately, the study of plants in their natural context is essential to obtain a meaningful answer to the question: what is the function of a gene? The traditional approach to functional annotation of the genome is based on knockout or overexpression of genes, preferably in an isogenic background, to determine the phenotypic effect of each gene. However, the dependence of the observed function on the particular background and laboratory environment is not always recognized. C. Weinig (University of Minnesota, St. Paul, MN, USA) introduced the complementary notion of 'ecological annotation': how much of the observed phenotypic variation among life-history stages, individuals, populations, or species can be attributed to allelic or regulatory variation in a particular gene (Weinig & Schmitt, 2004). In studies of *Arabidopsis* grown in the field, she has shown that different quantitative trait loci (QTLs) contribute to variation in growth and fitness in the presence and absence of competition. The contrast of functional vs ecological annotation presents an interesting analogy to the methods of prospective and retrospective sensitivity analysis in population biology (Caswell, 2001). These two scenarios contrast the sensitivity analysis of the engineer ('how much will the system change in response to each component?') with the decomposition of variance of ecology and evolution ('how much of the observed variance is attributable to variation in each component?').

A deeper understanding of phenotypic plasticity, and stability, will emerge from the synthesis of developmental, physiological and evolutionary approaches. F. Valladares (CCMA-CSIC, Madrid, Spain) discussed a broad range of mechanistic and adaptive factors that may contribute to species diversity in phenotypic plasticity. These approaches focus, respectively, on costs and constraints that may limit plasticity, such as energetic costs of signal perception and growth responses, vs environmental heterogeneity and 'adversity' as selective forces acting on plasticity (Valladares *et al.*, 2002; van Kleunen & Fischer, 2005). Understanding how plants integrate and respond to contrasting environmental signals (Niinemets & Valladares, 2004) and novel biotic environments (Hobbs *et al.*, 2006) is an immediate priority in the context of global environmental change.

Eco-devo and community-level interactions

Scaling up from molecules to organs, individuals, communities and ecosystems, the direct effects of gene action tend to become

more diffuse and increasingly difficult to detect. Interactions among species, especially plants and their symbionts or herbivores, present one ecological arena in which eco-devo is providing fascinating insights. Below-ground, the infection of roots by nitrogen-fixing symbiotic bacteria involves a highly co-ordinated sequence of morphogenetic events, with reciprocal triggering of gene expression between the two partners leading to the production of nodules and their occupation by the bacterium (Miklashevichs *et al.*, 2001). The genes involved in nodulation were apparently recruited, in evolutionary terms, from the ancestral mycorrhizal association, where the same genes participate in the process of fungal colonization of roots (Stracke *et al.*, 2002). A. Hodge (University of York, York, UK) presented the results of her meticulous growth studies, demonstrating that reciprocal interactions between roots and arbuscular mycorrhizas influence the well-known proliferation response of roots in nutrient-rich soil patches. In fact, mycorrhizas also proliferate, enhancing decomposition and nitrogen mineralization in organic soil patches (Hodge *et al.*, 2001). Importantly, roots that are colonized by mycorrhizas exhibit enhanced proliferation in response to nutrient-rich patches when they are grown in competition, and this leads to pre-emptive uptake of mobile ions, including nitrogen (Hodge, 2003). Much remains to be learned about the fascinating dynamics of these widespread plant–mycorrhiza interactions, including whether there are direct interactions among genetic regulatory networks and whether each partner contributes to proliferation in the other.

Moving above-ground, one of the key aspects of the plant environment (often overlooked by both developmental geneticists and functional ecologists) is the activity and phenotypic impact of insects and other herbivores. J. Schultz (Pennsylvania State University, University Park, PA, USA) discussed the transcriptional and regulatory overlap between self-regulated plant development and insect-induced responses. In plant galls induced by insects such as grape phylloxera (*Daktulosphaira vitifoliae*), the insect manipulates its host by altering the expression of floral induction loci such as *LEAFY* and *APETALAI* (which are required for MADS box gene activity), possibly by direct injection of uric acid (which is known to have up-regulatory cytokinin-like effects). These same pathways are critically important for normal development of flower and fruit development, and the selective constraints imposed in that context create an opportunity for insects to co-opt the developmental program of the plant to their own advantage. A. Kessler (Cornell University, Ithaca, NY, USA) presented a very different aspect of the complex plastic responses of plants to insect attack, by showing how specific transcriptional changes induced by caterpillar attack create both direct and indirect plant defenses in *Nicotiana*. Interestingly, plants can maintain fitness despite such attack as a result of wound-induced up-regulation of genes important to primary metabolism. ‘Taking molecular tools out into nature’

can be particularly revealing. Kessler’s experiments with a lipoxygenase (*lox-3*) *Nicotiana* knockout mutant (causing reduced induction of defensive nictotines, proteinase inhibitors, and volatile organics) explanted into a natural garden community showed that the loss of function of this gene actually attracted a new species of herbivore to the plants, a vivid demonstration of the importance of induced individual responses to the larger ecological community (Kessler *et al.*, 2004).

A. Novoplansky (Ben-Gurion University of the Negev, Midreshet Ben-Gurion, Israel) gave a provocative presentation examining the ability of individual plants to distinguish self from nonself tissues in competitive arrays. He argued that environmental cues and physiological signals may provide plants with information about whether nearby leaves or roots, which are competing for limited resources, are part of the same plant (i.e. a physiologically integrated individual, of the same or different genotype). Roots that are spaced equidistant from self and nonself neighbors exhibit enhanced growth towards the nonself neighbors, presumably enhancing resource uptake relative to competitors (Falik *et al.*, 2003; Gruntman & Novoplansky, 2004). The mechanisms of these responses are essentially unknown, although the available data suggest that they are dependent primarily on physiological integration within the plant, rather than biochemical mechanisms of allorecognition (as in many animals systems). This topic will certainly receive continued interest and scrutiny.

‘Fifty years ago, Bradshaw recalled, at a time when biologists were captivated by the New Synthesis approach, phenotypic responses to the environment were viewed as “an annoyance, a confusion that made it difficult to know what a genotype was ... The froth of evolution”.’

Conclusions and prospects

Closing the gap between the study of mechanism and the study of ecological and evolutionary diversity is one of the great challenges of 21st century biology: indeed the interface of these domains can be seen as the major ‘new frontier’ in biology (Kafatos & Eisner, 2004). As plant biologists facing this challenge, we seek to know what developmental genetics brings to ecology, and vice versa. The use of ‘molecular tools in nature’ is growing steadily and will continue to bring fascinating new possibilities to the field. The performance of

single gene mutants (gene alleles, knockout, overexpression, etc.) and recombinant inbred lines under field conditions provides insight into the ecological and evolutionary significance of specific genetic changes; luminescent reporter constructs linked to genes with dynamic expression across time and space allow us to 'see' gene expression in field settings, as in Weinig's studies of circadian transcription cycles; sampling of RNA constructs can be used as a physiological markers of plant responses, as in Voeselek's studies of ethylene production patterns; microarray analysis is just scratching the surface to reveal how thousands of genes may be involved in plant responses to abiotic and biotic factors. Ecology also has much to offer developmental genetics. More than anything, as Weinig cogently argued, the study of gene function must be carried out in nature as well in as the laboratory: the 'function' of a gene in natural environments varies depending on the environment and the genetic makeup of the population (Weinig & Schmitt, 2004). Genetic variation in natural populations also provides an important source of material for experimental study by developmental geneticists. Naturally occurring alleles may point the way towards functionally significant nucleotide substitutions, insertion–deletion (indel) events, or gene rearrangements that might not be anticipated by analysis of sequences or protein structures. More broadly, comparative studies incorporating interspecific diversity are a critical component of eco-devo research (e.g. Benschop *et al.*, 2005; Griffith & Sultan, 2005), to reveal aspects of morphological and functional response variation as well as important phenomena that may be absent in our favored model taxa. Ecologists should take a proactive role in promoting the development of new model organisms that exhibit an ecologically interesting diversity of form and function, paralleling the efforts by phylogeneticists to select taxa spanning the tree of life.

Fifty years ago, Bradshaw recalled, at a time when biologists were captivated by the New Synthesis approach, phenotypic responses to the environment were viewed as 'an annoyance, a confusion that made it difficult to know what a genotype was ... The froth of evolution'. Following his pivotal 1965 paper, plasticity became a central focus of ecological and evolutionary research, bringing enormous insights into the realized phenotypic variation that shapes ecological interactions and selective change (Sultan, 2003). In many ways, we hope this symposium marks another turning point, as a newer mechanistic approach emerges linking advances in developmental genetics to the study of environmental responses. This newer approach views the genotype as a dynamic, integrated developmental system with flexible expression, and seeks to understand that system all the way from its molecular basis to its ecological and evolutionary roles. This ambitious undertaking will require collaborative, synthetic research to identify connections from molecular to higher levels of organization and among interacting regulatory pathways and traits.

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Key words: ecological development, environmental cues, induced defense, mycorrhiza, phenotypic plasticity, phototropins, phytochromes, signal transduction.

Plant–microbe interactions in Yucatán: hurricanes didn't curb the whirlwind of discovery

XII International Congress on Molecular Plant–Microbe Interactions, Mérida, Yucatán, Mexico, December 2005

Uxmal, the beautiful Mayan site near Mérida, means *three times rebuilt*. A similar fate had befallen the XII International Congress on Molecular Plant–Microbe

Interactions, said Federico Sanchez, chair of the organization, in his opening address. He and colleagues of the local Scientific Committee and the Board of the International Society for Molecular Plant–Microbe Interactions (IS-MPMI) had needed to re-organise the meeting three times after two major hurricanes had hit Yucatán in the last season. Thanks to their perseverance, the meeting was eventually held in Mérida (<http://www.ismpminet.org/meetings>), with excellent attendance and a full breakthrough-packed programme from the first lecture to the last.

The aim of IS-MPMI meetings is to present data at the forefront of research, over a wide field of interactions between plants, fungi, bacteria, viruses and nematodes. Their emphasis is on the genetic and molecular analysis of the processes under study. While viruses and pathogens in general represented the leading topics of earlier meetings, this year mutualistic interactions, such as those of legumes with *Rhizobium*, helped to balance and broaden the meeting's overall orientation.

'The increasing evidence of crosstalk in signalling pathways suggests that a few key steps could be shared among a range of different interactions'

Symbioses, plant responses and methods

A glance at the scientific program revealed that lectures on symbioses and plant cellular responses to microbes were amongst the major topics, together with an emerging theme of innate immunity being closely associated with nonhost resistance. Besides this global impression, attendees are likely to have left the XII IS-MPMI meeting with their bags full of novel methods, ideas and perspectives, impatient to go back to work and apply them to their own study. Among these ideas was the use of fluorescent proteins to report gene activation and protein localisation within a cell, giving our approach to plant science a very powerful instrument. Many communications in Mérida reflected this, and most major lectures included microscopy data as key evidence (Adrienne Hardham, Australian National University, Canberra, Australia), providing support for the molecular biology approach and unveiling new opportunities from live cell imaging (Takemoto & Hardham, 2005). Another exciting breakthrough was a demonstration of gene transfer from non-*Agrobacterium* Rhizobiaceae to plants (Broothaerts *et al.*, 2005), which will open an unexpected potential for easier, and more widely applicable, plant transformation (Richard Jefferson, CAMBIA Canberra, Australia). Whether this will mark a return of plant biotechnologies to the arena of field application and food production remains to be seen.

Pathogen–host control and nonhost resistance

The modulation of plant–host defences by pathogens via the secretion of polypeptide effector molecules is receiving increased attention as several new fungal examples have been discovered. For example, examination of *Phytophthora infestans* secretome identified proteins – essential for pathogenicity – that contain an RXLR amino-acid motif (Birch *et al.*, 2006). The intriguing fact in this case is that RXLR is similar to the RXLK(E/Q) motif required for targeting proteins from malaria parasites (*Plasmodium* species) into the red blood cells of their mammalian host, and represents an unexpected case of evolutionary convergence or conservation in host–pathogen interactions (Sophien Kamoun, Ohio State University, Wooster, OH, USA). In true fungi the idea that secreted proteins are involved in determining pathogenicity is receiving further support, for example in the corn smut agent *Ustilago maydis* whole gene clusters encoding secreted proteins have been seen to be required for full pathogenicity (unpublished results, Regine Kahmann, Max Planck Institute, Marburg, Germany). We await with interest the precise functional definition of these gene products to see if any might be classed as ‘effectors’ that control the plant.

Several of the speakers at the meeting focussed on nonhost resistance and the importance of PAMP (pathogen-associated molecular pattern) perception and transduction (Paul Schulze-Lefert, Max Planck Institute, Cologne, Germany; Shauna Somerville, Carnegie Institution, Stanford, CA, USA). Essential elements of the genetic and molecular basis for resistance (e.g. the *pen* genes) in *Arabidopsis* against usually nonpathogenic powdery mildews are now known. In this area recent advances have shown how plant receptors are poised to alert their hosts of potential attacks (Lipka *et al.*, 2005). The role of bacterial flagellin and its perception (Zipfel *et al.*, 2005) was also mentioned repeatedly by speakers (Georg Felix, Botanisches Institut der Universität, Basel, Switzerland). Perhaps one of the most impressive of these talks was that which convincingly demonstrated the internalisation of the *Arabidopsis* flagellin receptor in response to ligand binding (Silke Robatzek, Botanisches Institut der Universität, Basel, Switzerland). Another advance was shown in understanding how plants and microbes adhere to surfaces, for example, by studying how bacteria adhere to the plant surface (Jan Kijne, Leiden University, Leiden, the Netherlands; Laus *et al.*, 2005) or organise into complex biofilms on a substrate (Ann Hirsch, University of California, Los Angeles, CA, USA). In the case of the latter, several symbiotic genes were shown to be important for biofilm formation (Fujishige *et al.*, 2006).

Scientific legacy and the future

Finally, there is mounting evidence that a surprisingly wide variety of interactions, ranging from nematodes to

endosymbionts to rhizospheric bacteria, involve, directly or indirectly, a common set of genes (Sanchez *et al.*, 2005), proteins and cell mechanisms (Vivienne Gianinazzi-Pearson, CMSE-INRA, Dijon, France). A central role has been very clearly attributed to calcium-dependent signalling pathways, with CCaM-dependent kinases representing a key step in the molecular interaction (Kanamori *et al.*, 2006) whose constitutive activation can even lead to root nodule formation in the absence of any exogenous signal (Allan Downie, JIC, Norwich, UK). Between expositions of such novel results the meeting also evoked its recent past in the form of a moving commemoration of Georges Truchet, who has made an outstanding contribution to the field of plant–microbe interactions. His most significant legacy was probably his involvement in the discovery of the nodulation (Nod) factors (Truchet *et al.*, 1991), which demonstrated the concept of a molecular dialogue between plant and microbes, something which we are still unravelling today.

The jesters amongst us speculated that Chac, the Mayan rain god, had cast a curse on this conference. If so, let us only hope that Vulcan will spare the next XIII IS-MPMI congress that will be held in Sorrento – not a million miles away from Pompeii!

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Key words: nonhost resistance, plant pathogens, polypeptide secretion, signalling pathways, symbiotic genes, symbiotic microbes.



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