

Need more nitrogen? Nodule numbers not the answer

An obvious route to increasing yield in legumes might, at first sight, be to increase the number of nodules formed in the *Rhizobium*-legume symbiosis. Nodule numbers are regulated by the host (via both root-derived and shoot-derived signals). In the later stages of the growth cycle, no new nodules are formed, even on recently formed roots. Based on this, [Bourion *et al.* \(Dijon, France, pp. 589–598\)](#) have set out to establish whether extending the nodulation phase does in fact improve the N economy of field-grown pea (*Pisum sativum*) plants. They used three normal cultivars, 'Athos', 'Austin' and 'Frisson', the former two having a higher root biomass than 'Frisson', and two hypernodulating mutants, P118 and P121, mutated in the *SYM29* (regulates nodulation via a shoot-derived signal) and *NOD3* (regulates nodulation via a

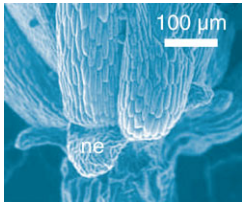
root-derived signal) genes, respectively. Plants were sampled throughout the growth cycle and after seed maturation. The contributions made by soil N and fixed atmospheric N were determined by the isotope dilution technique. The results were clear. Firstly, in hypernodulating mutants, nodulation started earlier and continued later than in the three other lines. The effect of this was that mutant plants possessed between 2.5- and 4-fold more nodules than wild-types and that nodules were maintained for longer. Further, P118 showed higher rates of N-fixation before the seed-filling phase. However, in both mutants, root development was less and overall N accumulation was lower than in the wild-types. Neither did the mutants exhibit a greater percentage of fixed N than the wild-types. Indeed, for P121, this percentage was significantly lower than wild-types. Overall, seed protein content was highest in 'Athos' and 'Austin', the two lines with the greatest root development and therefore the greatest capacity to absorb N from the soil during seed-filling. As the authors are aware, a more 'multi-stranded' approach than simply increasing nodule numbers is needed to improve N absorption and they make several good suggestions as to what that approach should include.



Poppy proves a big turn-off

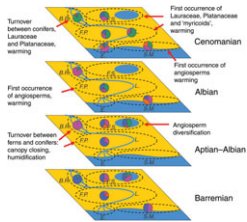
Degradation of double-stranded RNA plays at least two roles in plants. The first is in silencing of endogenous genes via the phenomenon of RNA inhibition. The other is as a defence mechanism against viruses, where the relevant ribonuclease activity is directed against double-stranded viral RNA. By using viral genomes as carriers of particular gene fragments it is possible to induce silencing of those genes in host cells: the plant treats the genes as if they belong to the virus. This technique, known as virus-induced gene silencing (VIGS), pioneered

by David Baulcombe and his colleagues at Norwich, has now been used successfully by [Wege *et al.* \(Bremen and Mainz, Germany, pp. 641–649\)](#) with California Poppy (*Escholzia californica*). The reason for doing the work is that the Papaveraceae is one of the basal angiosperm families and thus of great interest in relation to the evolution of flowering plants. The specific aim was to show whether VIGS would be effective in this species. The authors therefore selected a gene for which silencing would lead to an obvious phenotype, namely *Phytoene Desaturase*, the silenced phenotype of which is photobleaching. A section of this gene, along with the tobacco rattle virus genome, was introduced into *E. californica* using an *Agrobacterium*-based transformation system. Within 2 weeks over 90 % of the plants started to exhibit photobleaching, which at its most extreme resulted in white petals, fruit, shoots and leaves. Evidence that photobleaching was associated with silencing of *Phytoene Desaturase* was obtained by assaying transcripts of the endogenous gene (targetting that part of the sequence not present in the construct used for transformation): strong photobleaching was always correlated with complete or extensive reduction in transcript levels. The authors conclude that this system demonstrates the feasibility of using VIGS for study of gene function in *E. californica*, especially for those genes where the knockout phenotype is readily detectable.



Digging up the past with *CRABS CLAWS*

Increasing knowledge of plant genomes has revealed that 5–10 % of plant genes encode transcription factors. Some of these have key roles in development, as typified by the YABBY gene family in *Arabidopsis thaliana*. These, as discussed by [Fourquin *et al.* at Lyon, France \(pp. 651–657\)](#), are important in floral development and have a general role in specifying the abaxial side of plant organs. One of the YABBY genes is *CRABS CLAW (CRC)* and it is of great interest that it is present in *Amborella trichopoda*, a representative of the most basal angiosperm group. Further, its expression pattern in carpel tissues in *A. trichopoda* is very similar to that in *A. thaliana*, suggesting that the two genes are true functional equivalents (orthologues). The authors tested this directly by transforming *crc-1* mutants of *A. thaliana* with the *A. trichopoda CRC* gene (and, for comparison with a monocot, the putative *CRC* orthologue from rice, the *DROOPY LEAF* or *DL* gene), under the control of the *A. thaliana CRC* promoter. There was some variation in the level of expression of the heterologous genes in different transgenic plants, but in general it was clear that *A. trichopoda CRC* can complement *A. thaliana* mutants in carpel development but not in development of nectaries. The rice *DL* gene, by contrast, partially restored both carpel and nectary functions but a significant proportion of transgenic plants exhibited aberrant leaf development. These data lead the authors to suggest a role for *CRC* in carpel development in the most basal angiosperms with additional roles (nectary development in eudicots, nectary and leaf development in monocots) arising during evolution. The functional similarity of the coding sequences in carpel development in these different *CRC* genes suggests that the additional roles have arisen not via sequence divergence but by other mechanisms, such as gene interactions or the acquisition of additional control elements in their promoters.



Wagnerian approach to invasion of Europe

For the third time this month the topic is angiosperm evolution. Here, however, instead of using the present to dissect the past, a direct analysis of the past is discussed. [Coiffard *et al.* \(Villeurbanne and Rennes, France, pp. 545–553\)](#) note that by the start of the late Cretaceous era angiosperms were already widespread and in many habitats had taken over from the previously dominant gymnosperms. The authors are interested in how this situation arose, especially in relation to the invasion of Western Europe by the angiosperms. Previously, they have demonstrated that a clustering method normally used in molecular phylogenetics (Wagner's Parsimony Method, WPM) may be used to construct relationships between fossil assemblages and palaeoenvironments. For the present paper a new database of European Cretaceous plant fossils was constructed and WPM was used to group the localities according to their species content. This provided a fascinating picture of the changing character of the flora in relation to changing environments through the early and mid-Cretaceous. In the early Cretaceous (Barremian) phase, characterized by warm, dry conditions, matoniaceous ferns (of the order Gleicheniales) dominated the land; some gymnosperms were also present. Angiosperms were mainly confined to freshwater habitats, although fossil pollen from the next phase (the lower Aptian) suggests that some colonization of non-aquatic habitats was under way. Climatic changes in the Aptian led to a dominance of gymnosperms and a decline in the matoniaceous ferns, while a return to warmer conditions in the Albian phase led to some recovery of the ferns. Throughout these phases, angiosperms were gradually becoming established away from aquatic habitats, firstly on the floodplains and then in a wide range of habitats, leading to their presence in all environments by the Cenomanian (mid-Cretaceous) phase. Further, it is possible that the pole-wards migration of the angiosperms, initially from Africa and then through Europe, was driven by global warming.

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