

Commentary

Fruits, frugivores and the evolutionary arms race

The study of plant–animal interactions has been critical in the development of both ecological and evolutionary theory. Studies of plant–pollinator, plant–parasite, and plant–herbivore interactions have spawned broad concepts in ecology and evolution, leading to the development of terms such as coevolution and the ‘evolutionary arms race’ (Ehrlich & Raven, 1965; Dawkins & Krebs, 1979). By contrast, the development of a theoretical framework for the study of interactions between frugivores and the morphological, nutritional, and chemical traits of the fruits they consume has proved more difficult. In this issue, Izhaki *et al.* (2002) (pp. 217–223) report on the first step in understanding the variance and covariance in fruit traits within a species, information that is essential as we attempt to uncover the potential influence of fruit consumers on ripe fruits.

Historical context

Fruits facilitate the dispersal of seeds by providing rewards to seed-dispersers (Van der Pijl, 1972). Theories of interactions between frugivores and fruits originally borrowed heavily from the emerging literature on pollination and herbivory and, in the 1970s and 1980s, the development of a central paradigm posited that coevolved relationships between fruits and frugivores would lead to suites of morphological, behavioral, and chemical characters defining ‘specialized’ and ‘generalized’ dispersal syndromes (Howe & Estabrook, 1977; Howe, 1977). This paradigm led to a series of straightforward predictions regarding variation and covariation in fruit morphology, presentation and nutrient composition in relation to frugivore quality and preference (Herrera, 1986). The existence of testable predictions sparked a flurry of research leading to a general rejection of the theoretical structure behind the initial paradigm (Jordano, 1995).

The importance of frugivores in shaping fruit traits has now been questioned from a number of perspectives. When fruit size, shape, color, nutritive value, manner of presentation, and seed content are examined using broad phylogenies across diverse floras, these traits are typically best explained by phylogeny, not by presumed requirements of dispersers (Herrera, 1998). Coevolution between frugivores and fruiting plants also appears to be constrained by inconsistent selection pressures over space and time (Herrera, 1998) and

unpredictable postdispersal processes may overwhelm any influence of seed dispersers on plant fitness (Herrera *et al.*, 1994; Schupp, 1995; Parciak, 2002).

Exploring the fruit–frugivore landscape

Much of the uncertainty surrounding the evolutionary relationships between fruit traits and frugivores can be traced to three sources:

- A historical failure to integrate fruit-secondary metabolites into studies of fruit traits and theories of fruit–frugivore interactions.
- A focus on broad comparative approaches that ignore variation and covariation at the intragenetic or intraspecific scale.
- A persistently narrow view of frugivory.

The paper by Izhaki *et al.* helps address the first two of these problems, and has implications for the third. Secondary metabolites mediate many of the interactions between fruiting plants and the disparate array of organisms that feed on their fruits. Contrary to popular belief, secondary metabolites are common in the pulp of ripe fruit (Herrera, 1982; Cipollini & Stiles, 1992) and patterns of concentration and identity are not simply a reflection of their presence in unripe fruit (Cipollini *et al.*, 2002). In the same way that recognition of defensive secondary metabolites revolutionized studies of plant–herbivore and plant–pathogen interactions (Rhoades & Cates, 1976), the study of such compounds is beginning to yield a new perspective on fruit–frugivore interactions. Currently, this field is represented only by a small number of theoretical papers (Cipollini & Levey, 1997b; Cipollini, 2000), and a handful of studies reporting patterns or effects of fruit secondary metabolites (Cipollini & Levey, 1997a; Cipollini & Levey, 1997c; Tewksbury & Nabhan, 2001; Cipollini *et al.*, 2002).

Martin Cipollini and colleagues (Cipollini *et al.*, 2002) were the first to use strong phylogenetic controls to examine patterns of variation in fruit morphology, nutrient content and secondary metabolite concentrations at fine taxonomic scales – across the diverse set of species within the genus *Solanum*. Previous studies focused on explaining variation within whole communities of fruiting plants and their dispersers (Herrera, 1992, 1998; Jordano, 1995) rather than looking at variation within a genera, where extensive variation may not be so easily attributed to phylogenetic history (Cipollini *et al.*, 2002).

Izhaki *et al.* focus on variation at even finer scales, providing the first examination of covariation in fruit chemistry, nutritional content, and morphology within a species. Not surprisingly, not all *Rhamnus alaternus* fruits are the same,

but variation in fruit traits appears to have more to do with nutrient content and chemistry than with size. Their finding that small changes in fruit water content are correlated with large shifts in the ratios of the principle organic compounds suggests that shifts in water-stress can cause sizable changes in fruit-quality, potentially affecting frugivore behavior and patterns of seed dispersal (Martinez del Rio & Restrepo, 1993). This possibility becomes even more plausible in light of the strong relationship they found between non-structural carbohydrates and emodin, the primary plant-secondary metabolite found in both leaves and fruit of *Rhamnus* (Izhaki, 2002). Their data suggest that individuals with greater water available for fruits will have higher non-structural carbohydrate concentrations and will also be better protected by higher emodin concentrations.

If we consider nonstructural carbohydrate level as an accurate partial measure of fruit quality (Martinez del Rio & Restrepo, 1993) then this relationship provides evidence for one of three difficult-to-tease-apart hypotheses concerning the relationship between fruit-secondary metabolites, microbial and insect fruit consumers, and vertebrate seed dispersers. Until recently, studies of fruit–frugivore interactions have focused almost exclusively on vertebrate frugivores (Herrera, 1992, 1998; Stiles & Rosselli, 1993; Jordano, 1995). Vertebrates are certainly the most conspicuous consumers, but their effects on plant fitness are often variable spatially and temporally, both between and within species, limiting their ability to influence fruit morphology, nutrition, and chemistry (Cipollini & Levey, 1997b). By contrast, microbial fruit consumers are present in virtually all habitats, they consume fruit, but they do not disperse seeds, and their own survival may depend on making fruits unattractive to frugivores that do disperse seeds (Janzen, 1977). Thus microbial fruit consumers have the potential to create strong, uniform selection pressures on fruit morphology, nutrition and chemistry, as they typically have uniformly negative impacts on plant fitness (Buchholz & Levey, 1990; Cipollini & Stiles, 1993a,b). Of the seven adaptive hypotheses currently proposed to explain the existence of secondary metabolites in ripe fruit, there is one that explicitly considers the role of microbial and insect frugivores: the general deterrence hypothesis. This hypothesis states that secondary metabolites in ripe fruit are directed primarily at insect and microbial pests, and any negative effects these chemicals have on seed dispersers must be somehow offset by the benefits obtained by reducing microbial and insect attack (Cipollini & Levey, 1997b). This hypothesis has generated three sets of mechanistic models linking secondary metabolite load with microbial and insect pressure, fruit quality and morphology, and the preferences of vertebrate seed dispersers (Fig. 1). Izhaki *et al.* provide evidence consistent with both the relative risk and nutrient/toxin titration models. In *Rhamnus*, the nutrient-rich seeds have higher levels of secondary metabolites.

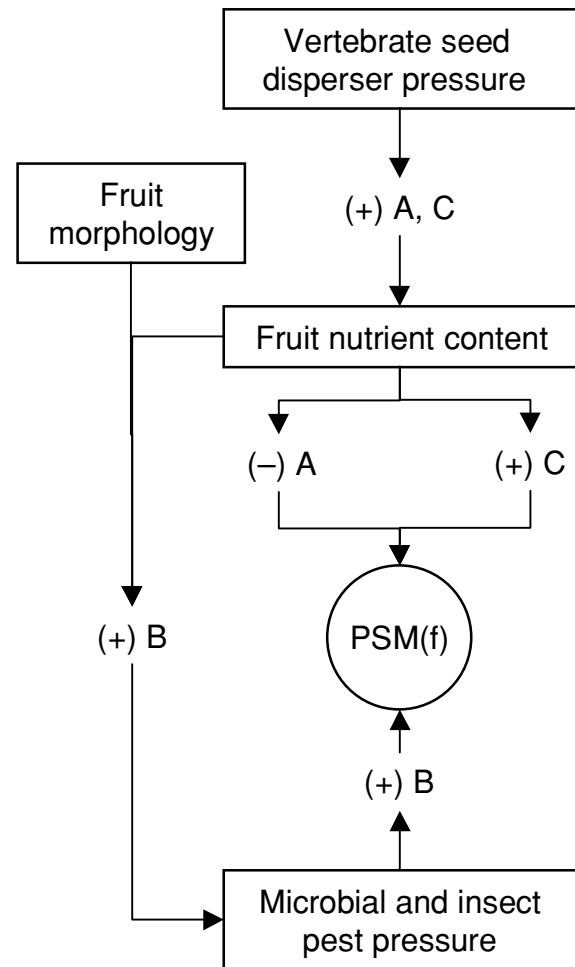


Fig. 1 General deterrence. The general deterrence hypothesis considers three types of relationships between secondary metabolites in ripe fruit ($PSM(f)$), microbial and insect pest pressure, fruit morphology and nutrient content, and seed-disperser preference. In the Removal rate model (A), seed dispersers prefer fruit with high nutrient content, therefore they eat these fruits before microbial frugivores can cause much damage, and this reduces the need to protect the fruit with secondary metabolites. Thus levels of secondary metabolites should be negatively correlated with fruit nutritional quality. In the Relative risk model, fruits that are more susceptible to fast microbial growth or insect pest attack should have higher levels of secondary metabolites, as they are more likely to be attacked by microbial frugivores. Because fruits with higher nutrient contents are typically more susceptible to microbial attack, the Relative risk model predicts a positive relationship between secondary metabolite protection and fruit nutritional quality. The Nutrient/toxin titration model (C) postulates that nutrient rich fruits should be profitable enough to afford the higher levels of chemical defence (Cipollini & Levey, 1997b), as they are worth more to frugivores. The Nutrient/toxin titration model predicts the same positive relationship between fruit secondary metabolites and fruit nutritional quality as does the Relative risk model.

Looking ahead

The current work is a first step into the examination of patterns of secondary metabolite variation within species. As with any first step, it stimulates more questions than it answers. Future studies will need to tease out the genetic and environmental controls on this variation in fruit chemistry, nutrition, and morphology, and determine how tightly linked these traits are. Which of the relationships described represents a physiological trade-off limiting the response to selection and which is flexible under different selection pressures, in different abiotic environments? Finally, *Rhamnus alaternus* in particular appears to be an excellent system in which to investigate the covariation between levels of secondary metabolites in fruits and levels of the same metabolites in leaves.

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Key words: fruits, frugivores, plant–animal interactions, coevolution, variance.

Nitrogen, translocation and *Sphagnum* mosses

The first nitrogen translocation measurements in *Sphagnum*, reported in this issue (Aldous (2002); pp. 241–253), are of vital importance in developing our understanding of nutrient constraints in peatlands and, potentially, even global carbon budgets. In making the connection, this article is not only a commentary on excellent research, but also a homage to the importance of mosses within the genus *Sphagnum*. These small, delicate, nonvascular plants dominate the cover and productivity of many peatlands globally, particularly those that are more ombrotrophic (i.e. 'rain-fed') or bog-like. Although peatlands occupy only approx. 2–3% of the terrestrial land surface (Bridgman *et al.*, 2001a), the imbalance between production and decomposition over thousands of years has led to an accumulated soil carbon pool that has been estimated to be one-third of the global total (Gorham, 1995). Within bogs, this peat is largely derived from partially decomposed *Sphagnum* tissue. *Sphagnum* tissue decomposes slowly because it has low concentrations of nitrogen and high concentrations of inhibitory compounds (Aerts *et al.*, 1999). The often-seen successional transition from minerotrophic (i.e. 'ground-water fed') fens to bogs is initiated by the increasing dominance of *Sphagnum* mosses that occur in raised hummocks above the water table (Vitt & Kuhry, 1992). Proton exchange from unesterified polyuronic acids in *Sphagnum* (Clymo, 1963), organic acid production during decomposition, and isolation of the peat surface from ground-water exchange lead to increasing acidification of peatlands. The increasing water stress on the nonvascular *Sphagnum* plants limits the growth of peat above the local water-table surface (Weltzin *et al.*, 2001). The extent to which succession in peatlands is driven by autogenic (internally driven) processes makes them unique among the world's ecosystems, and *Sphagnum* mosses are the primary plant species which drive these processes.

Adaptations of *Sphagnum* to low-nutrient environments

Bogs are extremely nitrogen-deficient ecosystems (Bridgman *et al.*, 2001b), and the ability of bog plants to efficiently utilize nitrogen is essential to their survival. *Sphagnum* mosses have very low tissue nitrogen concentrations and high nutrient use efficiency (Aerts *et al.*, 1999). The *Sphagnum* moss carpet scavenges from 50 to 90% of atmospherically deposited nitrogen (Li & Vitt, 1997), and Aldous demonstrated that vascular plants received < 1% of added atmospheric nitrogen at realistic dosage rates. Thus there may be effective nutrient partitioning between mosses and

vascular plants, allowing coexistence (Pastor *et al.*, 2002). Interestingly, most *Sphagnum* species also have very low nitrogen tolerances, sometimes with a positive growth effect from fertilization with low levels of nitrogen, but a toxic nitrogen effect after only a few years of even moderate nitrogen additions (Aerts *et al.*, 1992; Thormann & Bayley, 1997; Chapin, 1998; S. Bridgman, unpublished). Bog shrub species are more strongly nutrient limited, so upon fertilization one quickly sees a shift to shrub-dominated communities. In fact, high nitrogen deposition in more industrialized areas of England has been implicated in the loss of *Sphagnum* mosses from peatlands (Lee *et al.*, 1987). Thus, in many ways *Sphagnum* mosses represent an extreme in terms of plant adaptation to low-nutrient environments.

Nitrogen translocation

It is against this background that Aldous reports the first nitrogen translocation measurements in *Sphagnum*. This had previously been done using radioisotopes for carbon and phosphorus (Rydin & Clymo, 1989) but, for the reasons already described, nitrogen is probably the important element to examine. In a well designed experiment, Aldous followed the movement of a $^{15}\text{NH}_4^{15}\text{NO}_3$ tracer in *Sphagnum capillifolium* over the course of 2 yr in two bog sites in northern New York, USA with high atmospheric nitrogen deposition (1.0–1.3 g N m⁻² yr⁻¹ wet deposition) and two bogs in eastern Maine, USA with low deposition (0.2–0.4 g N m⁻² yr⁻¹ wet deposition). To calculate nitrogen translocation, she has developed a simple, but elegant model of the proportion of ^{15}N in old and new *Sphagnum* tissue over time. She hypothesized that nitrogen translocation would be greater in the Maine site with low nitrogen deposition, but found the opposite result, with the effect of atmospheric nitrogen deposition possibly confounded by differences in water-table dynamics among the sites.

Most importantly, Aldous unequivocally demonstrated the importance of nitrogen translocation in this important non-vascular plant. She found from 11 to 32% of ^{15}N was translocated into newly growing *Sphagnum* tissue over 1 yr in the low deposition Maine site, whereas from 64 to 83% of ^{15}N was translocated in the high deposition New York sites.

Aldous further estimated that between 0.5 and 11% of the annual nitrogen requirement of *S. capillifolium* was met by translocation. However, her calculations assume that all 'new' uptake for *Sphagnum* is derived from atmospheric deposition (her equations 9 and 10). While uptake of mineralized nitrogen in shallow peat has not been estimated yet for *Sphagnum*, given the translocation of ^{15}N in live stems shown in this study, it is reasonable to assume that *Sphagnum* mosses can be very effective competitors with microbes and vascular plant roots for available soil nitrogen in shallow peat. Aldous examined translocation in only the top 2 cm of stem, but Rydin & Clymo (1989) demonstrated

translocation of phosphorus and carbon over 7 cm of stem. Of course, the zone of active uptake of soil nitrogen would vary among species of *Sphagnum*, as different species visually have quite different lengths of apparently live stem. If one assumes no discrimination between ^{15}N and ^{14}N , multiple sources of available nitrogen, and that translocation is independent of the source of nitrogen, the larger values of translocation (i.e. 11–32% in Maine and 64–83% in New York) given by Aldous are more appropriate to build nitrogen budgets for this species. Given that approx. 9% of the nitrogen demand of *Sphagnum* is supplied each year by atmospheric nitrogen deposition (calculated from the data given in the paper), in the low deposition Maine site about one-third of the nitrogen requirements of *S. capillifolium* is supplied by either the atmosphere or translocation. Even more impressively, in the high deposition New York site, 73–92% of the nitrogen requirement is satisfied by these pathways. Thus, efficient scavenging of atmospheric nitrogen and translocation of internal nitrogen can supply almost all of the nutrient demand of this important species under at least some circumstances. An important consequence of this is that *Sphagnum* mosses will have spatially distinct nutrient pools from deeper rooted vascular plants, allowing coexistence (Pastor *et al.*, 2002).

Conclusions

Aldous presents a pivotal report on the nutrient dynamics of this important moss genus. Overall, one begins to get a coherent picture of *Sphagnum* nutrient dynamics. They have very low nutrient demands due to low tissue nutrient concentrations, high nutrient use efficiency, tight nutrient cycling (including translocation, despite the lack of vascular tissue), and spatially distinct nutrient pools from vascular plant, but they also have a very low toxic threshold for high atmospheric nitrogen deposition. *Sphagnum* mosses are a keystone species on an ecosystem scale. Understanding their ecological strategies to low nutrient availability is essential not only for predicting peatland dynamics but also for predicting their future role in global carbon budgets and global change.

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Key words: nitrogen translocation, *Sphagnum*, global carbon budgets, mosses, peatlands, soil carbon pool.

Meetings

Soil microbes and plants – raising interest, mutual gains

Impacts of Soil Microbes on Plant Population Dynamics and Productivity – the 8th New Phytologist Symposium, Helsinki, Finland, 10–14 June 2002

Ecologists are realising more and more that what goes on in soil, whether activities of plant roots themselves or associated soil organisms, has a very important bearing on the success of different plant species and on plant competition and community structure. The conference organisers set themselves the formidable task of bringing together scientists working at scales from the gene through to the ecosystem. Here, a few of the diverse contributions that may have challenged participants to think outside their usual conceptual boundaries are highlighted.

Mutualistic symbioses and extreme complexity

Mutualistic symbioses (mycorrhizas and N₂-fixers) provided a connecting theme through most sessions, reflecting the wide recognition of their likely importance in natural and managed ecosystems. The first major challenge was a timely one, directed at the 'received wisdom' about mutualistic symbioses, particularly arbuscular mycorrhizas (AM) (Fitter, University of York, UK). Current knowledge is grounded in reductive experiments, often on single plant species paired with single fungi, that commenced approx. 50 yr ago. Many of the questions that have been addressed, using increasingly sophisticated techniques, were outlined in the extremely influential book 'Endomycorrhizas' (Sanders *et al.*, 1975). The outcome is a corpus of general knowledge about the interactions between the symbionts – their growth, cellular and molecular interactions and physiology, and more recently functional diversity and nonnutritional benefits to their symbiotic partners (e.g. drought tolerance, interactions with pathogens and effects on soil structure). Now that we do have some 'general rules', they can and must be tested. This maxim should apply more widely than just to AM symbioses.

Fitter's challenges were that AM fungi represent a more diverse taxon than would be expected from the existence of *c.* 150 described species and that there is more evidence of

plant/fungus specificity than has been revealed so far, using those AM fungi that can be isolated as spores and nurtured easily in pot cultures. Thus, AM may not fit the accepted theory that mutualisms should have broad partner ranges and low α diversity (Law & Lewis, 1983; Douglas, 1998). Extreme genetic complexity is certainly implied in much recent research. To this must be added increasing evidence for diversity of function between different pairs of fungal and plant symbionts, revealed in pot experiments (Jakobsen *et al.*, 2002) and in analyses of gene expression in different ectomycorrhizal partnerships (Martin, INRA, France). The implications of such diversity in ecological interactions are enormous, and will impact on the feedback between plants and members of the soil community (including nutrient cycling organisms, pathogens and AM fungi) which can regulate the diversity of the plant communities (Bever, University of Indiana, USA; Bardgett, University of Lancaster, UK; Zobel, Tartu University, Estonia).

Taking research out into the field

Garbaye (INRA, France) urged the imperative to adopt new approaches to determining the importance of the multifunctional roles of ectomycorrhizal fungi in mature boreal and temperate forests, not just in seedlings. The challenges in these and all other ecosystems are considerable and, as Jones (Okanagan University College, Canada) showed, field bioassays in forests indicate that clearcutting may not reduce colonisation/diversity of ectomycorrhizal fungi as is widely believed based on glasshouse studies. The findings are highly relevant to the ways forests are managed. These pleas for investigations in the field, over wide areas and long time scales, would surely be supported by David Read and others advocating the need for field relevance.

Nutrient turnover and acquisition

The effects of herbivory on plant communities have traditionally been studied by looking at the performance of the plants and changes in community composition, but Bardgett (Lancaster University, UK) showed convincingly that feedbacks through the soil are crucial, involving plant nutrition, plant productivity, food supply for the above-ground grazers and return of faeces and urine to the soil. He combined data from field scale experiments, root biology and N cycling to test hypotheses on the effects of grazing by large above-ground herbivores on

plant productivity and species composition in both productive and unproductive sites. In Canada, lodgepole pine and western red cedar can grow in extremely nutrient poor environments in situations where the N budgets do not add up until, as Chanway (University of British Columbia, Canada) showed, the inputs from free-living diazotrophs are taken into account. Some of these N₂ fixers appear to be associated with the tuberculate, AM roots of red cedar.

In a logical extension of this focus on nutrient turnover and acquisition, Tibbett (CSIRO, Australia) highlighted the part played by ectomycorrhizal fungi (again) in plant exploitation of nutrient patches, such as those provided by seeds. The response of plants to patches in terms of plasticity in root growth has been relatively well explored (Robinson, 1994) and those plants that are apparently unresponsive to localised sources themselves may depend on the activities of their mycorrhizal symbionts (see also Farley & Fitter, 1999). The outcome may well depend on which of the functionally diverse assemblage of fungi actually colonise the roots.

Pathogens and transposons

Examples of ecology in action at smaller scales were provided by several studies of pathogens. The search for biocontrol agents, and identification of the attributes that make a good one, is essentially physiological/molecular ecology. The significant pathogen *Fusarium oxysporum* appeared as the target organism several times: in molecular and biochemical analyses of the antagonistic strategies adopted by *Pseudomonas* (Lugtenberg, Leiden University, The Netherlands); and by closely related non-pathogenic fusaria (Alabouvette, INRA, Dijon, France). Negative effects of AM fungi on *F. oxysporum* appear (at the larger scale) to be the predominant 'benefit' of the symbiosis in maintaining wild populations of the grass *Vulpia ciliata* (Watkinson, University of East Anglia, UK). Elucidating the mechanisms by which AM fungi antagonise *F. oxysporum* in or near *Vulpia* roots will need similar approaches to those used by the plant pathologists.

The 'life-cycle' of retrotransposons in plant genomes, the way in which they are transcribed in response to stress, the sites in which they are inserted, and the cost of synthesis of the resulting repetitive DNA (Schulman, University of Helsinki, Finland) add up to 'ecology of DNA' operating at a molecular level. Studies are yielding fundamental information about genome evolution, as well as fingerprints that can themselves be used as aids in larger scale plant ecology. Again at the level of genes, Martin (INRA, Champenoux, France) described his explorations of the transcriptome of *Pisolithus Eucalyptus* ectomycorrhizas with EST/cDNA arrays during formation of the mantle and Hartig net and establishment of new

symbiotic functions. Surprisingly, perhaps, no symbiosis-specific genes in either plant or fungus have been cloned and many genes are unaffected. Of the 15–20% that show a significant change in expression, most are involved in general activation of protein synthesis and energy metabolism; there are also increased levels of cell surface proteins probably involved in formation of the symbiotic interface. So far so good. But perhaps the most important message was that application of the same techniques to less well defined mycorrhizal interactions (*Populus/Laccaria* and Douglas Fir/*Laccaria*) gives different stories. This is yet another illustration of the need to test generalisations and be aware of the potential dangers of extrapolating directly from simple and easy-to-work-with 'model systems' to more complex ecological situations.

New techniques are being applied in molecular ecology too, leading to the identification of members of the Archea, not only in soil and water (Jurgens, University of Helsinki, Finland), but among the bacterial populations and biofilms in mycorrhizospheres (Bending, Horticulture Research International, UK; Bomberg, University of Helsinki, Finland; Sen, University of Helsinki, Finland). The next step is to find out what these organisms are doing in soil and in the hyphosphere.

Genetic modification

The usefulness and safety of genetic modification of both plants and microorganisms provided stimulating and controversial presentations. On the one hand, van Montagu (Ghent University, Belgium) expressed the view that genetic transformation of crops was the (only) way forward to cope with the future food needs of the world and that there were no ecological risks in following this path. This view was supported from the practical standpoint of breeding rice for different ecological situations (Datta, IRRI, Phillipines). However, the issue is not as clear cut as these proponents would wish, particularly for soil microorganisms, as shown by van Elsas (PRI, The Netherlands). Studies of the *Escherichia coli* genome show that it has evolved by acquiring remnants of genes and plasmids by horizontal gene transfer (HGT) over approx. 1 million years (a rather short time in evolutionary history) and most bacterial genomes are similar. A model system to investigate HGT revealed that the process appears to be 'a community event' involving groups of bacteria in regions of high activity, such as the rhizosphere. This finding suggests that there may be risks in releasing genetically modified microorganisms, although Hirsch (IACR-Rothamsted, UK) found no evidence for HGT between rhizobial strains persisting in soil for 5 yr. Is this time-scale long enough? What time-scale should be considered? The jury is still out on the benefits and risks of genetic modification to improve plant (crop) growth and performance

and it will be crucial to take evidence-based approaches to evaluating requests for release of transgenic organisms and to assess their ecological risks (Amijee, Pioneer Overseas Corporation, Belgium).

Final comments

Was anything missing from the symposium or, rather, what might be included in future meetings? Many presentations described heterogeneous distribution of organisms and the importance for them of heterogeneous supplies of nutrients in the rhizosphere and 'patches', but there was little or no discussion of the habitats in which soil organisms or roots live – varied sizes, continuity and tortuosity of the soil pore system or the physicochemical characteristics controlling water availability, atmosphere, pH, and so on. This information on the spatial and physicochemical environment is crucial to understanding the mechanisms underlying the feedbacks between plant productivity and soil biological activity in different ecosystems and soils. In future, biologists and soil physicists and chemists will need to get together at small meetings where they can discuss and exploit their somewhat different conceptual views of the soil environment to further increase our understanding.

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