Biodiversity

Robbie Waugh

Investigating natural and induced biological diversity and biological complexity underpins plant and environmental sciences and impacts upon a wide range of issues that we face today. For example, the spectrum of biological diversity in broad acre crops such as potatoes and barley is intentionally manipulated, and frequently minimised by plant breeders during the process of developing and releasing new and better crop varieties. This is necessary to meet the current demands of regulators, growers and end users throughout the production and processing chain. In contrast, assessing and managing diversity is frequently the objective when attempting to maintain the viability and vitality of natural and managed environments. There, a diversity and balance of life forms is considered key to effective long-term function, including the ability to recover from a variety of anticipated or unanticipated events.

In the following short articles it will become clear just how central the issue of biodiversity is to the research we conduct in each of the science programmes at SCRI. They cover topics that range from the impact of genetic manipulation on the 'metabolome' of specific potato varieties, to a broad appraisal of the diversity of the vegetation currently present in Scotland's arable– grass ecosystems. It will also become apparent that the type of data upon which the articles are based reflect a similar diversity of both approach and resolution, highlighting the impact that technological and analytical advances are having in furthering our understanding of biodiversity and consequently in generating knowledge. Importantly, each short story has practical outcomes that will benefit specific end users either in government, commerce or society.

Metabolome biodiversity in crop plant species – when, where, how much and so what?

Louise V.T. Shepherd, Gary Dobson & Howard V. Davies

Plant breeding drives the production of the new varieties required to compete successfully in the complex global agricultural marketplace, with increasing emphasis on the use of early landrace varieties and wild species to introduce the new genes and alleles required to improve yield, pest and disease resistance, quality and nutritional value. Targeted analysis of key compounds has provided the cornerstone for assessing the quality, nutritional value and safety of cultivated crop species and a benchmark against which new generations of crops and advances in production systems can be evaluated.

Profiling approaches which use 'omics' technologies (transcriptomics, proteomics and metabolomics) provide a coverage of gene and protein expression and metabolite composition that is unsurpassed compared to traditional targeted approaches. Metabolomics in particular is being used to identify significant sources of variation in the composition of crop and model plants caused by genetic background, breeding method, growing environment (site, season), genotype ×



Figure 1 Statistical separation of *S. tuberosum* (old and modern cultivars), *S. phureja* and Chilean landraces, using data from the analysis of tuber composition by GC-MS.

environment interactions and crop cultural practices, to name but a few. While breeders have long been aware of such variation from tried and tested targeted analytical approaches, the broad-scale, so-called 'unbiased' analyses provided by profiling technologies offer a major upside to our understanding of the true extent of variation in compounds relevant to breeding targets.

We have used Gas Chromatography–Mass Spectrometry (GC–MS) to assess compositional variation in a range of potato cultivars (both new and old), Chilean landraces and two diploid potato cultivars developed from *Solanum tuberosum* var. Phureja. As an example, Fig. 1 shows a principal components analysis (PCA) of GC–MS data from a collection of potato genotypes where the abundance of *c*. 100 metabolites is used to distinguish most of the landraces from both old and new cultivars, and indeed some of the modern





cultivars from older material and *S. phureja* lines. This type of information can help target the selection of wild species to deploy (or potentially avoid) in potato breeding programmes.

In a study funded by the EU, and in collaboration with Dr Eugenia Barros at CSIR (Council for Scientific and Industrial Research), Pretoria, South Africa, we have used metabolomic analysis (in this example Direct Injection Mass Spectrometry [DI-MS]) to assess potential safety issues related to potential compositional differences between a specific genetically modified (GM) potato developed to express a protein conferring resistance to specific insect pests and its non-GM counterpart (the cv. Spunta). Fig. 2 shows a PCA that clearly distinguishes the three potato varieties (cvs Spunta, Mnandi and BP1; circled) included in the field trials, and between crops of the same variety grown at three different locations (Ceres, Kokstad and Roodeplaat) in South Africa. However, it was not possible to distinguish between the GM and non-GM Spunta grown in any of the locations. Thus, the DI-MS profiling approach showed that both genetic background and growing location were major drivers of compositional variation, but that the GM event did not

result in any detectable compositional changes. This is valuable information, which can be used to support the risk assessment process.

Temporal and spatial variation in gene and protein expression and metabolite composition between cultivated varieties of the same crop species is an indisputable reality. Indeed such variation underpins variety development and product differentiation and the knowledge derived from 'omics' can be harnessed to support more effective breeding. Arguably, data on the sources, drivers and extents of natural variation in raw and processed materials can help to define the boundaries of foods with a 'history of safe use', a concept often used in risk assessment but poorly defined experimentally.

Scotland's wild arable plants

Geoffrey R. Squire, Graham S. Begg, Cathy Hawes, Pietro P.M. Iannetta, Euan James, Alison J. Karley & Mark W. Young

A long-term study of the vegetation in Scotland's arable–grass ecosystems will soon be complete. The aim of the study is to design systems that exploit the synergy between crops and wild plants, such as in-field



Wood vetch (Vicia sylvatica) on mountain ledges in Corrie Fee, within the Cairngorms National Park

weeds. Weeds can be both a burden through reducing yield and a benefit through supporting ecological processes not provided by the crop alone. They are also a largely untapped source of new economic products. Here we report on three facets of the study that were published in 2010.

The arable flora is buffered by the seedbank – a reserve of seeds buried in the soil. The rate and degree to which the composition of the seedbank may be shaped by proactive field management, as opposed to soil and other local conditions, was, however, previously uncertain. Therefore data were collected on seedbanks from a survey of 110 fields covering a range of management preferences (organic, integrated, and general commercial practice) and environments in the east of Scotland. The results showed that while the environment had an influence, the type of management applied to fields had the greatest effect on the composition of the seedbank. The seedbank was also shown to be a more discriminating indicator of the weed flora in a field or region than the emerged



Figure 3 Micrographs of the nitrogen fixing root nodules of two wild legume species (top) *Trifolium campestre* and (bottom) *Astragalus danicus*, showing the symbiotic bacteria stained blue. The nodules are 2 mm in length.

weed communities. Our overarching finding was that the seedbank can *in principle* be managed to achieve a composition in which the beneficial groups of plants are well represented. Effort is now directed towards several of the plant groups that will bring both ecological and economic benefit.

One such group is the wild legumes (Family Fabaceae). High inputs of mineral nitrogen (N) fertiliser have reduced the wild legumes in agricultural land, but they are still thought to fix N in semi-natural systems. Consequently, they could yet provide a major source of N to future crop systems since alternatives to mineral N are needed to offset its high cost and adverse environmental impact. However, not all members of the Fabaceae form the root nodules - the outcome of a 'symbiosis' between the plant and bacteria called 'rhizobia' that enables them to fix atmospheric nitrogen. To assess their potential, samples were obtained of many of the wild legumes from within and around agricultural land and tested for their ability to form symbioses. The initial results are promising in that many of the species do in fact nodulate (Fig. 3). The evolutionary relationships between the 'rhizobia' in different legume species are currently being examined to assess the diversity and ecological function within the group. The practical development of this work was boosted in 2010 by the EU 'Legume Futures' project, which will design legume-based rotations throughout Europe, and a SPARK award to examine the amino acid composition of current crop-legume varieties.

We have also made progress this year with one of the main analytical problems in this topic, which is to quantify the links between time-series in different parts of the biota. Time-series are important since something that happens early in an individual's life cycle or trajectory can affect its later performance. With machine learning experts at the Josef Stefan Institute in Slovenia, we have applied the methods of dynamic time warping (DTW) to compare a large number of paired trajectories of crop and weed cover. DTW is an approach used in voice recognition, where a reference sample of a person's speech is compared with a test sample. When our trajectories for crop and weed cover were quantified by DTW, and then further probed using machine learning methods, it was apparent that the main factor driving the combination was the crop, rather than features of weed management. It appears from this first analysis that DTW may have further uses in analysing the trajectories of the entities (e.g. plants, insects, fields) that occur in managed ecosystems. The practical management of seedbanks and weed flora at field and regional scales will be facilitated as part of an EU project (called PURE) awarded in 2010.

Genetic diversity in modern barley varieties

Luke Ramsay, William T.B. Thomas, Jordi Comadran, Dave F. Marshall, Paul Shaw & Robbie Waugh

The genetic diversity of the barley varieties that are grown widely in Scotland and elsewhere in the UK has been shaped by past selection for yield and quality of the crop in the UK environment and for the demands of associated end-user industries. However, although it forms only a subset of the variation present in the species as a whole, the diversity that exists in elite UK material has been sufficient to power significant breeding progress over the last 20 years. In order to study this genetic diversity and to investigate its relationship with variation in agronomic and economic traits, we led a large coordinated project on the



Figure 4 Phylogenetic tree using SNP marker data showing the divisions between crop types in modern UK barley varieties

'Association genetics of UK elite barley' (AGOUEB) funded by BBSRC and RERAD and supported through the Defra SA LINK programme. The project





Figure 5 Diversity scan of the long arm of chromosome 4H of barley using a sliding window of 10 adjacent loci with a step of one plotted against the cumulative marker number.

Key: Two-rowed spring = orange; two-rowed winter = blue; (adjusted full data set = black); Position of vernalisation gene = Vrn2

also involved two academic collaborators (NIAB and the University of Birmingham) as well as industrial collaborators and sponsors from all sections of the industry: growers, maltsters, brewers and distillers. Importantly, the project involved all commercial UK– based barley breeding programmes.

The project used genomics resources to monitor variants of genes involving individual base changes (or single nucleotide polymorphisms, SNPs) in barley genes as markers to determine the genetic diversity in elite barley varieties. Over 3000 such SNP markers were assessed on over 1000 barley lines to determine patterns of diversity. These data have provided an unparalleled insight into the genetic variation in modern barley varieties. Overall, the data showed clear groupings that related to known divisions between spring and winter-sown bio-types and between two-rowed and six-rowed varieties within the winter material (Fig. 4). Moreover, as the markers used are variants of genes that have been accurately positioned in linear order along the barley genome, the results allowed much more detailed interrogation of how diversity changes along the chromosomes both within and between these crop bio-types. For example, the comparisons highlighted reduced variation in winter barleys at the end of chromosome 4H that corresponds to the position of one of the vernalisation genes that delays flowering until spring in winter-sown barleys (Fig. 5). This correlation between allelic forms at

specific markers and differences in a particular trait can be extended to a genome wide analysis in order to find associations between other genomic regions and many different agronomic and economic traits. In AGOUEB such associations have been found using yield and guality from historical data derived from official trials results together with data from new trials undertaken specifically for the project. Some of the other traits studied include disease resistance and specific morphological characters used in the Distinct, Uniform and Stable tests (DUS) as part of the official variety registration. This has highlighted the importance of tightly defined genomic regions in the control of certain traits and in some cases has even allowed the identification of variants of the actual gene that causes the measured differences in the targeted trait. Such results highlight the possibilities that this type of study can offer and gives breeders the tools to screen and utilise variants of genes from more diverse barley germplasm (landraces, wild barley) in future breeding programmes.

The AGOUEB project has characterised the variation that barley breeders have been utilising over the last 20 years within the UK and North-Western Europe to produce improved varieties. This characterisation is now allowing other follow-on projects to study the genetic control of resistance to fungal diseases and of nitrogen use efficiency in barley in more detail, and has also developed the tools and understanding to aid breeding companies to produce future varieties using the genetic diversity within the current adapted elite gene pool.

The impact of invasive plant pathogenic *Phytophthora* species on biodiversity **David E.L. Cooke**

The influence of plant pathogens on biodiversity is not always immediately obvious and, as a consequence, has tended to be overlooked. The aptly named *Phytophthora* (from the Greek – plant destroyer) is, however, one pathogen that is changing this view. The best known of the 100 or so species, *Phytophthora infestans*, was inadvertently introduced to Europe from Central or South America in the 19th century and caused the devastating



Figure 6 Sporangia of *P. kernoviae* emerging from the stomata of an infected host plant, in this case bilberry.

potato late blight disease. Although P. infestans does not directly impact UK biodiversity it is, unfortunately, one of a succession of invasive species that are having more serious consequences. Phytophthora ramorum and Phytophthora kernoviae were first recorded in the UK in 2002 and 2003 respectively, and between them have caused hundreds of economically damaging outbreaks across the UK horticultural industry. Plant health legislation has been enforced by teams across the UK who are working hard to prevent the spread of both pathogens. Nonetheless, movement into managed gardens and subsequently, natural ecosystems has occurred, primarily through infection of an invasive plant species, Rhododendron ponticum. This highly susceptible host has been shown to disseminate spores that cause disease (Fig. 6) on nearby native hosts. Severe epidemics in Japanese Larch plantations have also been observed in 2009 and 2010 affecting 1,900 hectares of forestry across South West England, South Wales, Northern Ireland and a recent case in Western Scotland. This progression to a landscape-scale disease in some areas of the UK is a serious concern, particularly in light of infections of heather and bilberry (or blaeberry) at some sites. These plant species alone are of great value to Scotland's biodiversity (Fig. 7), supporting a wide range of insect and bird species; the capercaillie, for example. At SCRI we are leading a collaborative project (with SASA, Fera, CEH and the University of St Andrews) to examine the threat of P. ramorum and P. kernoviae to the biodiversity of Scottish heathlands. We are currently investigating the susceptibility of key heathland plant species to these pathogens, using high-



Figure 7 Bilberry is a key component of many Scottish ecosystems but is under threat from invasive *Phytophthora* species.

throughput sequencing to develop molecular markers to track *P. kernoviae* inoculum and using existing DNA markers to understand the diversity and spread of *P. ramorum*.

The above studies focus on invasive Phytophthora species. However, it is important to remember that all pathogens originate from natural ecosystems in which they have co-evolved with their native plant hosts. In recent years extensive surveying, enhanced by molecular detection tools, has revealed many new Phytophthora species. Some are destructive foliar pathogens but others, in particular root infecting species, may have a more subtle and perhaps even beneficial impact on ecosystem function. A recent study in the rainforests of Central America, for example, suggests a strong influence of native below ground microbial pathogens on tree species abundance. Tools are required to characterise rapidly the pathogens involved which has led us to develop a molecular method for studying Phytophthora diversity by amplifying diagnostic DNA sequence signatures (or DNA-barcodes) from water and soil. The system has been validated on Scottish natural and semi-natural habitats and has revealed considerable Phytophthora biodiversity and sequence signatures of several new species. This test has great potential in future studies using next generation sequencing to probe soil and water for this important group of damaging plant pathogens.



