Biodiversity

Robbie Waugh

Biodiversity describes the variation of life forms that exist within a population, a species, an ecosystem or a geographical region. Measures of biodiversity are frequently used to assess the health of a 'biological system' and although commonly associated with conservation biology, biodiversity research has broad application in plant and environmental sciences. It provides a framework for understanding and quantifying the molecular variation in individual genes and the richness of species in complex ecosystems, and may guide management interventions to maintain or enhance the viability of both species and communities. Biodiversity is the driving force of evolution, and its efficient exploitation is the basis of crop plant improvement. Not surprisingly then, across SCRI, considerable energy is being expended on the quantification and interpretation of biodiversity to address a wide range of biological questions.

Here we provide four examples of biodiversity research being conducted across the research programmes. In the first, Joanne Russell and colleagues show how a clear relationship exists between genetic distances and geographic distances among populations of barley landraces and discuss how these observations shape our understanding of the domestication and evolution of the species, with potential implications for plant breeding. By walking through an example project, in the second, Iain Milne and colleagues illustrate how the visualisation tools they have developed have become essential components in the analysis of highly complex molecular diversity datasets. Ali Karley and colleagues then describe how interactions between insects and microbes can shape interactions between herbivores and other components of an agroecosystem while, in the final contribution, the importance of understanding pest and pathogen diversity is described by Alison Lees and colleagues. These serve to illustrate the breadth of activity being conducted under the biodiversity banner.

Relationship between genetic distances and geographic distances among populations of barley landraces

Joanne R. Russell, Ian K. Dawson, Allan Booth, Andy J. Flavell[1], Brian J. Steffenson[2], Eva Weltzien[3], Salvatore Ceccarelli[4] & Robbie Waugh

Within barley an enormous amount of natural diversity exists, which has been shaped by the processes of domestication, cultivation and breeding. Different views have been proposed about the domestication of barley, with some authors stressing a single origin in the Fertile Crescent and others suggesting more complex domestication events. One of the most effective means of understanding the origins of domestication and cultivation is by comparative sampling of wild and cultivated accessions from regions where their ranges overlap. We have assembled and studied a collection of approximately 500 stratified, geographically referenced and matched samples of landrace (cultivated) and wild barley from across Jordan and Syria (Fig. 1). We used a high resolution genotyping platform that provides accurate measures of molecular biodiversity across the entire barley genome to explore the origins of barley cultivation. We observed clear

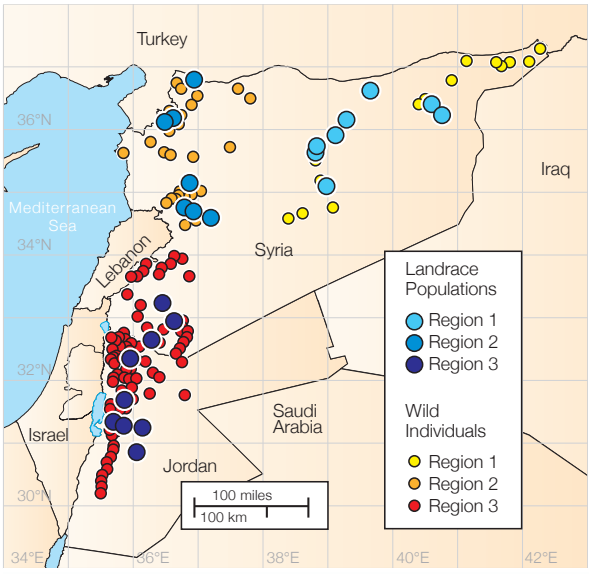

Figure 1 Geographic locations of sites in Jordan and Syria from which landrace and wild barley accessions were sampled for an analysis of SNP variation. Twenty-four landrace populations, comprising 317 accessions in total and 131 individual wild plants differentiated into three geographical regions: Region 1, North-eastern Syria; Region 2, North-western Syria; Region 3, Jordan and southern Syria.

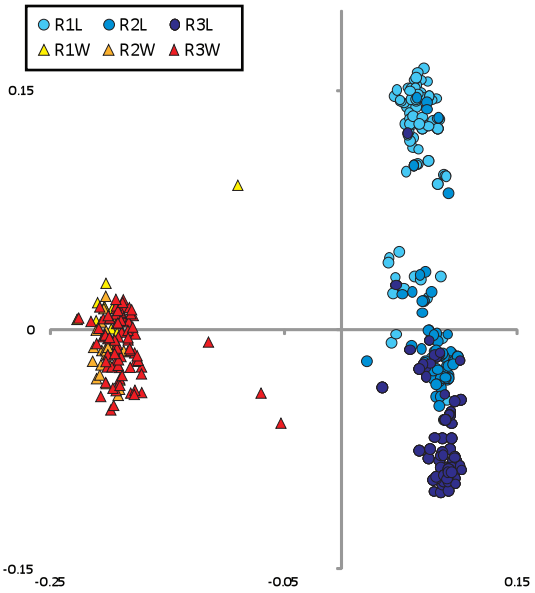

Figure 2 Principal coordinate analysis for 1135 SNP loci in landrace (*N* = 317) and wild (*N* = 131) barley accessions sampled from Jordan and Syria. Landrace and wild accessions were clearly differentiated, except for four 'wild' individuals that placed unusually in ordination. The first principal axis (horizontal) accounted for 35% of all variation, the second principal axis (vertical) for 13%. For information on sample designations see Fig. 1.

evidence for geographic structuring of genetic variation in both wild and landrace barley (Fig. 2). Differentiation was however less pronounced in wild barley. Three geographical regions were clearly identified and cross comparisons indicated that landrace populations from all three regions were most similar to wild material from Jordan and south Syria. An important observation is that landrace and wild accessions were 'not-matched' in north-western and north-eastern Syria, as would have been anticipated if cultivated material was of local origin. At the same time, a high degree of structuring in landrace accessions suggests that these cultivated stands have been present at their current locations for a considerable period of time. This distinctness has been maintained in spite of human exchange of germplasm that could potentially swamp any pre-existing landraces and suggests strong pressure towards local adaptation to varying environmental conditions in the two countries.

Gene flow between cultivated and wild populations after domestication has been suggested as an important mechanism for adaptation in barley and has been

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proposed to be relatively common. Our analysis highlighted four unusual 'wild' individuals consistent with minor directional interaction between wild and landrace barley (Fig. 2). However overall, landrace and wild material act essentially as independent entities despite geographic overlap. This may reflect variation in flowering time and maturity, with the brittle rachis of wild barley meaning that in mixed stands wild heads will shatter and seed be dispersed before the cultivated seed is collected. This research provides insight into the process of domestication of barley in the Fertile Crescent. Comparisons of the observed patterns of molecular diversity in this material with that in advanced cultivars will help identify chromosomal regions that have been subject to human and environmental selection during the process of domestication.

Visualising genetic diversity

Iain Milne, Paul Shaw, Linda Milne, Micha Bayer, Gordon Stephen & David F. Marshall

The increasingly complex data sets generated through new DNA sequencing and genotyping technologies, such as described above for barley, require a new generation of software tools to aid analysis and interrogation. The sheer volume of data imposes limitations on our ability to inspect the results of analyses and look for patterns that reflect either quality control issues or biologically meaningful structure in the data. Therefore, to support and enable analyses of genetic and genomic data we have developed a suite of computational visualisation and analysis tools. To illustrate their value, in this report we follow a typical project that starts with the discovery of single nucleotide polymorphism (SNP) markers, is followed by their use for genotyping to assess patterns of diversity in germplasm collections, and ends with comparative genomics – an approach used to identify putative regional gene content.

The process starts with using second generation sequencing technologies to sequence the transcriptome from two or more plant lines from any plant species. The output of this exercise, more than 1,000,000 individual sequences, are either aligned against an existing sequence (such as an Expressed Sequence

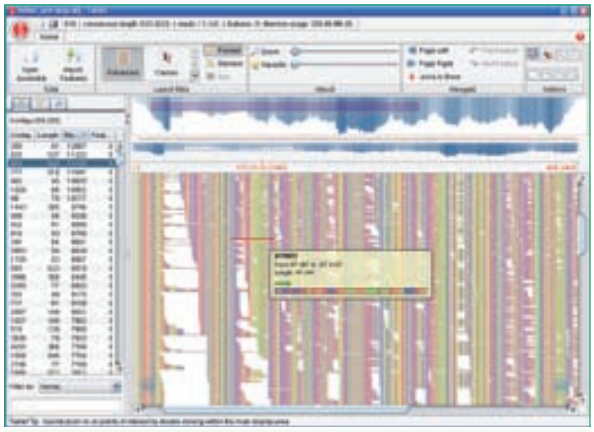

Figure 3 *Tablet* displaying an assembly of singletons that are left over from a *Mosaik* mapping of barley read using Solexa technology from seven different barley lines.

Tag assembly) or assembled (i.e. joined together into longer contiguous sequences) *de novo*. The output may result in more than 50,000 assembled transcripts, which can be analysed for polymorphic nucleotide sites that are then 'marked up' as potential SNPs. These assemblies can be visualised with *Tablet* (Fig. 3), software we specially designed to cope with this scale of data, allowing us to view and navigate the millions of aligned DNA fragments and identify which SNPs are most suitable for translation into high-throughput SNP genotyping assays.

The resulting avalanche of high-quality genotypic data presents us with new challenges in data maintenance and analysis. In collaboration with colleagues at the University of Dundee we are developing a new implementation of our existing marker database

Figure 4 160 SNPs from chromosome 6H of barley are displayed within *Flapjack*, where multiple graphical views allow us to visualise the associated genotype, phenotype and QTL data simultaneously.

Figure 5 *Strudel* rendering homologous mappings between high-density linkage maps across multiple genomes of *Brachypodium*, barley and rice.

infrastructure, *Germinate 2,* to cope with these demands. *Germinate 2* allows us to store, query and visualise a broad range of associated information, including passport data, trait information, pedigrees and the results of analyses. We developed *Flapjack* (Fig. 4) – a revolutionary new graphical-genotyping and manipulation tool – to visualise the genotype data from many thousands of lines at many thousands of SNPs, and we are working with collaborators around the world to ensure that *Flapjack* is compatible with major new genotypic data sets from maize, rice and *Arabidopsis* as well as genetic analysis and simulation packages.

High-throughput genotyping technology enables us to rapidly identify key genomic regions that control agriculturally important traits through either conventional Quantitative Trait Loci (QTL) analysis or association analysis. But we want to identify genes – not regions. Comparative mapping is facilitated by our visualisation tool *Strudel* (Fig. 5), which enables the rapid identification of the gene content of orthologous genomic regions (derived from a common ancestor) across multiple species. In addition, *Hordeum Relator* visualises the relationships between DNA sequences from various species related to barley, including rice, *Brachypodium*, oat and sorghum. Based on this set of integrated databases, web resources, and Java desktop applications, our software – although primarily developed to meet the needs of ourselves and our collaborators – is made freely available to all, and is used by researchers in over 40 countries around the world.

Insect herbivore–microbe interactions: impacts on insect fitness and behaviour

Ali J. Karley, Scott N. Johnson, Emily Clark & Lindsay S. McMenemy

Insect herbivores are key components of agroecosystems, both as consumers of crop and non-crop plants and as a food source and host for a wide range of natural enemies. Interactions between insects and microbes often shape how insect herbivores interact with other parts of the system. In particular, microbes are frequently harboured or transmitted by insect herbivores and fulfil a range of roles as symbionts, parasites or pathogens. We study the biology of plant sap-feeding aphids to unravel the complex interactions between insect herbivores, their bacterial symbionts and the plant viruses that they transmit, to determine the role of microbes in regulating insect populations in agroecosystems.

Many herbivorous insects possess microbes known as symbionts that live within the insect and perform vital functions that are essential for insect survival. In aphids, most species harbour a 'primary' bacterial symbiont, *Buchnera aphidicola*, which lives in specialised organs within aphid tissues and produces amino acids that the aphid cannot produce. Aphids can also harbour one or more types of 'secondary' bacterial symbiont whose functions are less certain. There is increasing evidence that these secondary bacteria play a significant role in aphid interactions with other organisms, particularly by altering aphid susceptibility to natural enemy attack. In the Cabbage aphid (*Brevicoryne brassicae*), we have identified different bacterial types, split into two dominant groups (Group 1 and Group 2), in aphid lines collected from Fife, Tayside and North Yorkshire. We have developed quantitative molecular methods to determine the relative abundance of secondary bacteria associated with the Cabbage aphid and shown that frequency of Group 1 and Group 2 secondary bacteria compared to *Buchnera* is variable between aphid lines. Experimental work indicates that Group 2 bacteria are associated with reduced aphid fecundity (Fig. 6a) and with increased egg production in emerging *Diaeretiella rapae*, the hymenopteran wasp species that parasitises cabbage aphids (Fig. 6b). Changes in aphid fitness associated with the presence of particular secondary bacteria types are likely to affect the ability of aphids to act as vectors of plant pathogens.

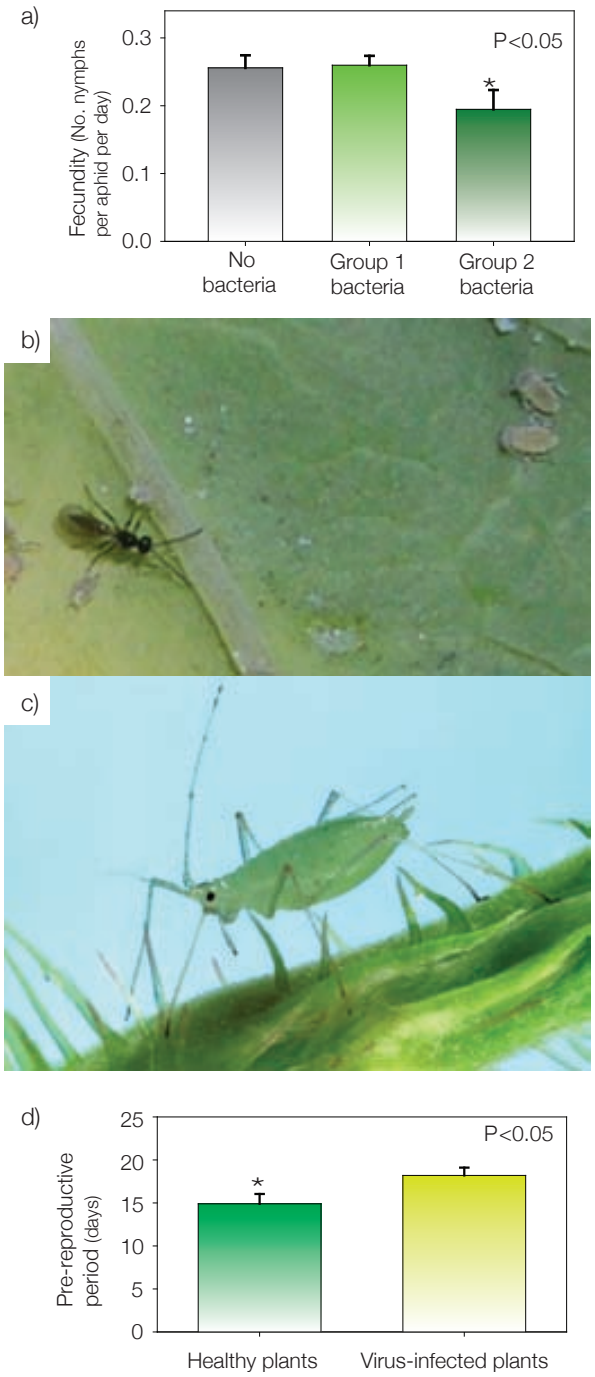

Figure 6 a) Impact of secondary bacteria on reproduction by the Cabbage aphid, *Brevicoryne brassicae.* b) The hymenopteran wasp *Diaeretiella rapae* together with Cabbage aphid nymphs. c) Large raspberry aphid, *Amphorophora idaei.* d) Impact of RLSV infection on large raspberry aphid pre-reproductive period.

Moreover, plant pathogens themselves can change plant chemistry in a way that makes crops more attractive to insect vectors. We have shown that the large raspberry aphid, *Amphorophora idaei* (Fig. 6c), preferentially colonises plants infected with one or more of the viruses it vectors, such as *Raspberry leaf spot virus* (RLSV). However, the plant virus impairs aphid performance by slowing its development so that the time taken by the aphid to start reproducing, the pre-reproductive period, is extended (Fig. 6d). This developmental delay may cause the aphid to disperse from the plant soon after initial colonisation, thereby facilitating the spread of the plant pathogen. Thus, characterising how microbes influence insect herbivores in arable and horticultural crops is providing us with an insight into how we might exploit ecosystem processes to ensure productive systems and maintenance of biodiversity.

Understanding pathogen biodiversity as a key to controlling crop diseases

Alison K. Lees, David E.L. Cooke, Brian Fenton & Adrian C. Newton

Crop pathogens and pests are an unwelcome but important component of the biodiversity found in managed ecosystems. They are diverse and adaptable, meaning that they are able to respond quickly to disease management practices and changing climate and therefore to reduce agricultural sustainability. Evolution of pests and pathogens, and the migration of new invasive species, leads to a constantly changing threat to crop production. In particular, the ability of pests and pathogens to overcome genetic resistance that has been bred, or engineered, into crops and the development of resistance to agrochemicals is problematic. Our work seeks to understand these threats, and the factors that drive population changes, with the goal of effective disease management. We illustrate this approach by discussing examples of our work on three very different host–parasite interactions. In each case we have developed and used DNA fingerprinting methods (microsatellite markers) to allow us to monitor and understand diversity.

Phytophthora infestans (Fig. 7) is the cause of potato late blight which results in serious reductions in yield and quality of ware and seed potato crops on a global scale. By monitoring several thousand isolates of *P. infestans,* sampled predominantly from commercial crops, we identified a dramatic shift in the UK population with an increase in the frequency of a particularly aggressive A2 mating type strain that is able to overcome some sources of host resistance. The implications of the changes have been reported to the potato industry and management practices altered accordingly.

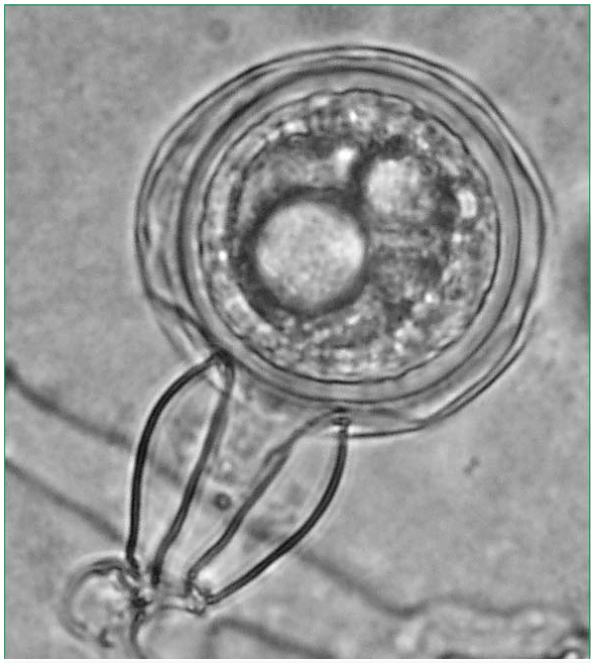

Figure 7 Image of an oospore of *Phytophthora infestans* – a long lived soil-borne survival structure which is a source of increased biodiversity in the population.

The fungal pathogen *Rhynchosporium secalis* causes 'rhynchosporium' or 'scald', the most problematic disease currently challenging sustainable barley production. Major genes for resistance to *R. secalis* in barley varieties are readily overcome, but partial resistance genes can provide effective resistance in the field. *R. secalis* is being monitored to understand how populations change in response to use of varieties with major resistance genes, partial resistance, and even non-symptomatic resistance and how barley variety mixtures affect population structure. Studies so far show that *R. secalis* is clearly a very variable pathogen with a very high capacity for adaptation.

In addition, natural variation in the *P. infestans* and *R. secalis* genes that govern the ability of the pathogens to infect and cause disease is being investigated so that the likely durability of corresponding resistance genes in the potato and barley gene-pools can be inferred, thus making efficient use of host biodiversity.

The peach–potato aphid is an important vector of potato viruses and can readily adapt to insecticides.

In a parallel situation to late blight we have followed the relative success of different genotypes each year and have shown that a single genotype, or clone, denoted type O, has come to dominate the UK population almost certainly due to the presence of a mutation causing resistance to dimethyl carbamate, a very commonly used insecticide. As the growth rate of clone O is no better than other genotypes (Fig. 8) it is also likely to have maintained good defences against attack by parasitoids in the field. Aphid genes are responsible for this phenotype as these *M. persicae* have no secondary symbionts. It appears that, like the potato plant itself its pests and pathogens effectively use asexual reproduction to multiply well adapted gene combinations.

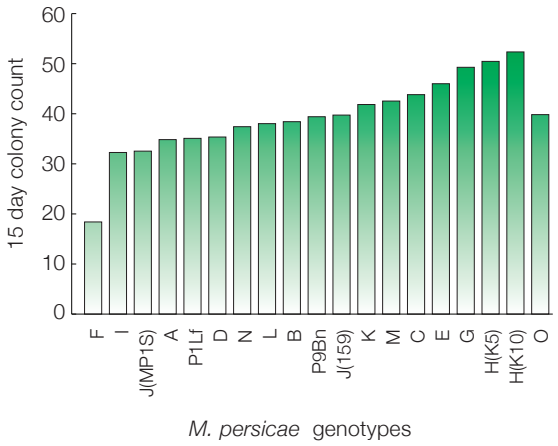

Figure 8 Variation in growth rate of different *M. persicae* genotypes (as characterised using SSR markers) measured as number of individual aphids per colony after culture on oilseed rape for 15 days.

Perspectives

As illustrated above, it is clear that agriculture faces many challenges to sustain production as climatic zones shift and changes to agricultural systems accelerate. Consequently, new biotic and environmental stresses continually threaten crop production. A solid understanding, continued monitoring and appropriate use and/or management of biodiversity, from genes to agroecosystems, will remain a key component of agriculture's ability to adapt.