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Natural history of *Arabidopsis thaliana* and oomycete symbioses

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Abstract Molecular ecology of plant–microbe interactions has immediate significance for filling a gap in knowledge between the laboratory discipline of molecular biology and the largely theoretical discipline of evolutionary ecology. Somewhere in between lies conservation biology, aimed at protection of habitats and the diversity of species housed within them. A seemingly insignificant wildflower called *Arabidopsis thaliana* has an important contribution to make in this endeavour. It has already transformed botanical research with deepening understanding of molecular processes within the species and across the Plant Kingdom; and has begun to revolutionize plant breeding by providing an invaluable catalogue of gene sequences that can be used to design the most precise molecular markers attainable for marker-assisted selection of valued traits. This review describes how *A. thaliana* and two of its natural biotrophic parasites could be seminal as a model for exploring the biogeography and molecular ecology of plant–microbe interactions, and specifically, for testing hypotheses proposed from the geographic mosaic theory of co-evolution.

Keywords *Hyaloperonospora parasitica* · *Albugo candida* · Downy mildew · White blister rust · Gene-for-gene · Innate immunity · Receptor-like proteins · Arms race · LRR · CATERPILLAR genes · *Boechera* · Geographic mosaic · Non-host resistance

Introduction

In the current age of molecular technology and big science questions, the practical aims for downy mildew research have remained essentially unchanged for more than a century. Investment in downy mildew research still seeks practical solutions for improved disease control, through better use of fungicides and to aid the breeding and deployment of downy mildew resistant crops.

Plant breeding, however, has been transformed over the past 20 years by molecular genetics, which has been providing knowledge of the genes that underlie natural variation in valued traits of species such as *Arabidopsis thaliana*, tomato and rice. Converting valued traits into knowledge of genes is an important step for genetic engineering, but more importantly, it provides the means to design the most precise molecular markers attainable for marker-assisted selection of valued traits in conventional breeding programmes (McCouch 2004). From the initial public investment in research, the knowledge gained has potential use across breeding efforts in different plant species. For instance, molecular genetics

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led to the discovery of numerous pathogen receptor-like genes that provide the molecular basis for downy mildew resistance in *A. thaliana* (Holub 2001, 2007). These major discoveries in turn have been instrumental in the early development of marker-assisted selection for disease resistance in crops (Aarts et al. 1998a; Botella et al. 1997; Michelmore and Wong 2008; Shen et al. 2002; Speulman et al. 1998).

In a similar process of scientific enquiry, conservation biology could also benefit significantly from the assimilation of molecular genetics research. Progress has been and will continue to be achieved in the protection of biological diversity without knowing much detail about the genetic variation within species. However, by analogy with the recent advances in plant breeding, knowledge about critical genes (e.g., that enable adaptability of a species) could provide useful indicators for improving the prospects for survival of animal and plant populations that are in most need of protection.

For example, the *geographic mosaic theory of co-evolution* has been proposed to explain how intimate interactions among multiple species will co-evolve across geographic landscapes and provides a major process in organising the earth's biodiversity (Thompson 1999a, b; Gomulkiewicz et al. 2007). The theory proposes a three-part hypothesis: (a) the species interactions will have different evolutionary trajectories in different populations thereby generating a *selection mosaic* of co-evolving traits among populations; (b) some of the interacting populations will be *hot spots* for reciprocal selection of the co-evolving traits, whereas *cold spots* will also be generated in which selection is unilateral or not occurring at all; and, (c) continual re-mixing of traits will occur across the geographic landscape (e.g., gene flow, genetic drift and local extinction of populations). Understanding the molecular basis of co-evolving traits will be essential for empirical testing of this tripartite hypothesis, for providing fundamental advances in evolutionary ecology, and ultimately for providing invaluable insight that will aid the more practical domain of conservation biology.

The purpose of this review is to describe how *A. thaliana* and two of its natural biotrophic parasites could be seminal as a model for exploring the biogeography and molecular ecology of plant–microbe interactions, and specifically, for testing hypotheses proposed from the geographic mosaic

theory of coevolution. This subject is relatively young, but stands on the shoulders of important biologists whose contributions provide a foundation for this review.

Divergent enquiry from early revolutions in plant science

Natural history and molecular biology have developed as polar disciplines (Wilson 1994; see 'Molecular wars' chapter, pp. 218–237), determined by the environments in which the respective practitioners have generally explored their chosen subjects (field versus laboratory), and contrasting methodologies. They also differ markedly in the relative accessibility to amateur biologists and a public audience.

Molecular biology is rooted in a revolution of experimental science that began in the mid-1800s and led by European scientists including the botanists Anton DeBary in Germany and Harry Marshall Ward in England (Ayers 2005). Their dream was 'the Cause', a new approach to botany that could equal chemistry and physics as a rigorous, experimental science. They incorporated both field observations and laboratory experiments in their studies of plant–microbe interactions catalyzed by the invention of light microscopy but pre-dating genetics. Biochemistry and biophysics would eventually provide a strong basis for experimental botany, as with the whole of biology. However, Gregor Mendel's discovery of genetics from his seminal experiments with plants soon provided a fundamental mathematical rigour that is unique to biology. It was therefore fitting that the 2nd International Downy Mildew conference was held in central Europe, and specifically in Olomouc, Czech Republic where Mendel had been a student and near the monastery in Brno where he conducted his now famous experiments.

Genetics is the cornerstone of molecular biology, used to illuminate the cause and effect mechanisms of proteins within organisms under strict laboratory environments. In recent years, whole genome sequences have become publicly available from pioneering use of species like *A. thaliana* and *Phytophthora sojae*. And, this in turn has galvanized a Linnaean-like approach by an international community of molecular biologists to name and classify all of the proteins encoded within an organism, one representa-

tive organism at a time (Clark et al. 2007; Holub 2006, 2007; Kamoun 2007; Tör 2008; Tyler et al. 2006; Win et al. 2007). This in effect is providing a powerful online catalogue of genes that enables further comparisons to be made among species (so-called comparative genomics). Researchers will continue to pursue their experiments largely in the laboratory. However, molecular biology is increasingly being enriched by biometricians and mathematicians who are enabling extrapolation of what has been learned from biochemical mechanisms in model organisms into other less tractable species, and predictive modelling in the emerging field of *molecular systems biology* which aims to advance understanding of the cause and effect associations amongst molecules of cellular processes (Holub 2007; Jönsson et al. 2006; Prusinkiewicz et al. 2007).

Natural history, on the other hand, is transforming into evolutionary ecology in its modern research form (Thompson et al. 2001). This young cross-discipline is rooted in comparative methods and mathematical modelling as its cornerstones, and builds on ecology as the ultimate arena for ‘systems biology’ research (Wilson 1994). Evolutionary ecology has progressed from the early revolutions led by Karl Linnaeus (the founder of taxonomy) and Charles Darwin (the founder of evolutionary theory) into a molecular age of being able to, for example, reassess classifications of species through the inference of familial history (or phylogenetics) of species with comparative analyses of informative DNA, RNA and protein sequences.

Phylogenetic trees provide the structure for bold new ventures led by evolutionary biologists, ecologists and amateur taxonomists to assemble information on the earth’s biodiversity in online encyclopaedias (www.eol.org; www.tolweb.org; www.nbn.org.uk). These publicly accessible libraries could eventually incorporate web links to vast amounts of genome-wide, DNA-based information from reference species and thereby stimulate growth of new research and educational tools that would join the polar disciplines together. This may require a new generation of cross-disciplinary biologists and creative educators.

At the moment, evolutionary ecology provides a powerful means for elucidating the specific and variable characteristics of habitats to which a species is unable to adapt and thereby render it vulnerable to local or global extinction. However, the molecular basis for the potential vulnerability of a species is

poorly understood. Phrased as a more positive question: what naturally variable genes provide a species with ‘genetic resilience’, by enabling it to adapt in a changing environment or to survive in a diversity of circumstances across its geographic range of habitats? In a nutshell, this question encapsulates a large gap that exists between the disciplines of molecular systems biology and evolutionary ecology, and defines molecular genetics of natural variation as a key means for bridging the gap.

Converging the enquiry from polar disciplines within plant science

Answers to this fundamental question, aimed at improving habitat conservation and species protection, will benefit from a molecular genetic approach to understanding how natural variation within wild populations enables a species to survive in increasingly variable habitats. This will require linking of our genome-wide (genomic) knowledge of organisms with an equally in-depth knowledge of suitable habitats. This approach should at the very least provide an illustrative guide to the minimal levels of genetic diversity required to sustain the survival of a species in a natural habitat, and thereby provide genetic indicators (e.g., disease resistance traits, highlighted below) for the direction and scale of public investment required for species conservation.

The Plant Kingdom provides many opportunities to probe this question. We can see and experience the magnificence of biodiversity in vascular plants that currently exists across our planet, in vast habitats such as precious tropical rainforests or in small patches of aged meadow (Silvertown 2005). As sedentary autotrophs, plants are supreme providers of sustenance, indispensable generators of diverse microhabitats for animals and microorganisms, and the backbone of terrestrial communities. They, along with their symbioses with microorganisms, make terrestrial life possible. Thus, understanding the genetic resilience of existing plant species is vital to human survival on Earth.

To address the question posed above, we will require genetically-based insights from three aspects of natural variation for a range of contrasting plant species representing different survival strategies. These insights include: 1. identifying the critical genes

that are naturally variable within and among established populations of a species; 2. mapping the allelic variation in these genes onto a geographic distribution of established populations; and 3. determining the selective factors that vary among microhabitats and drive fluctuations in natural variation in a given species.

Establishing a genetic model for co-evolved plant–microbe interactions

Since a major selective characteristic in a plant's microhabitat is often likely to be biological (e.g., symbiotic associations with parasitic or mutualistic microorganisms; Thompson 1999b; Gomulkiewicz et al. 2007), the remainder of this review considers where the natural history of *A. thaliana* and its common oomycete symbionts currently stands as an early model which a new generation of experimental botanists could soon be using to explore fundamental genetically-based questions at the forefront of conservation biology.

Host specialization is a characteristic that corresponds with a high degree of speciation in fungus-like oomycetes that cause downy mildew and white rust diseases in a wide range of plant genera (Constantinescu and Fatehi 2002; Göker et al. 2004; Riethmüller et al. 2002; Voglmayr 2008; Voglmayr and Riethmüller 2006). Speciation of these plant parasites caught the attention of botanists before biology had been transformed by genetics (Lindau 1901; Gäumann 1918). Their reports included downy mildew and white rust in *A. thaliana*, which was rediscovered much later in 1988 by Professor Paul H. Williams (University of Wisconsin-Madison) who was seeking a potential model for research on the molecular genetics of plant–microbe interactions (Table 1).

A visiting scientist working with Williams, Eckhard Koch, had attempted to infect *A. thaliana* with downy mildew and white rust using parasite isolates collected from brassica species but with no success because, as we can conclude with hindsight, the chosen isolates were constrained by a high degree of host preference (Fig. 1a). Williams, however, informed by his reading of the earlier German reports, was successful in acquiring support from the US National Science Foundation for a solo trip to collect *A. thaliana* seed and parasite material in Europe. He visited Germany,

France and England during May of 1988, and was successful in finding both downy mildew and white rust of *A. thaliana* in Kent (southeast England; Fig. 2) while visiting Professor Ian Crute, who had recently moved to a horticulture research institute at East Malling (now East Malling Research). Williams was unable to secure additional funding, but fortunately the seed of opportunity had been sown with both Koch (who found downy mildew on *A. thaliana* in Switzerland after completing his visit to UW-Madison) and Crute. They were each subsequently successful in establishing *A. thaliana* downy mildew and white rust as model pathosystems for molecular genetics research (Table 1).

With respect to the phylogenetics talks presented at the 2nd International Downy Mildew meeting by M. Thines and reviewed by Voglmayr (2008), I will refer to the two oomycete symbionts that Williams rediscovered in Europe on *A. thaliana* as *Hyaloperonospora arabidopsis* (Gäumann 1918; Göker et al. 2004; Rehmany et al. 2000; Constantinescu and Fatehi 2002; formerly *Peronospora parasitica* subsp. *arabidopsis*; abbreviated below as *Hpa*) causing downy mildew, and *Albugo candida* subsp. *arabidopsis* (abbreviated below as *AcA*) causing white rust.

Current molecular knowledge of *A. thaliana*–oomycete symbioses has largely been constructed from genetic analyses of interactions amongst a small sample of host accessions and a highly variable but also relatively small collection of *Hpa* isolates (Holub and Beynon 1997; Slusarenko and Schlaich 2003). The *Hpa* isolate names (e.g., Emoy2) reflect their origins, predominantly from the UK, with two prefix letters that indicate the geographic source (As = Aspatia, Cumbria; Bi = Biggar, Scotland; Ca = Canterbury, Kent; Ed = Edinburgh, Scotland; Em = East Malling, Kent; Go = Godmersham, Kent; Hi = Hilliers arboretum, Hampshire; Ma = Maidstone, Kent; Nu = Nunnery Walks, Cumbria; Wa = Wageningen, Netherlands; We = Weiningen, Switzerland), and two suffix letters indicating an accession available from *A. thaliana* stock centres suitable for cultivation of the particular isolate (Co = Columbia; Ks = Keswick-1; La = Landsberg *erecta*; Nd = Niederzenz; Oy = Oystese; Wa = Wassilewskija). Most of the *Hpa* isolates currently being used in research laboratories have been refined genetically by derivation from a single oospore and maintained subsequently by propagation of asexual inoculum (Holub and Beynon 1997).

Table 1 Two decades in the Linnaean genomics of *Arabidopsis thaliana*–oomycete natural history

Year	Milestone	References
1988	Paul Williams rediscovers downy mildew (DM) and white rust (WR) in European <i>At</i> , following an 80 year old trail.	Williams, PH (personal communication); Lindau 1901; Gäumann 1918.
1990	Natural variation of DM resistance in <i>At</i> is described as a model for molecular genetic investigation.	Koch and Slusarenko 1990.
1991	The DM isolate Emoy2 was borne from an oospore in a seedling of <i>At</i> 'Columbia'. This isolate would be used to establish genetics in the organism and provide the first reference genome of downy mildew parasites in 2007.	Holub 2006.
1995	Mutation of <i>NDR1</i> demonstrates that H.H. Flor's notion of disease resistance being conferred by single <i>R</i> genes can actually be a multi-genic process and involve common links in the signalling of defense against diverse pathogens. This established a precedent for using oomycete and bacterial pathogens of <i>At</i> in comparative laboratory experiments.	Century et al. 1995, 1997.
	Systemic acquired resistance to bacterial disease and DM in <i>At</i> is found to require salicylic acid.	Lawton et al. 1995.
	Natural variation in WR resistance provides a complementary model for molecular genetics of <i>At</i> –oomycete interactions.	Holub et al. 1995; Borhan et al. 2001.
1996	Mutation of <i>EDS1</i> demonstrates that species level barriers ('non-host' resistance) to biotrophic parasites can be amenable to mutation and genetic analysis. This gene and <i>PAD4</i> , which is also typically required for DM resistance in <i>At</i> , were found to encode lipase-like proteins.	Parker et al. 1996; Glazebrook et al. 1997; Falk et al. 1999; Jirage et al. 1999; Holub and Cooper 2004.
1997	The first DM resistance gene <i>RPP5</i> is cloned and found to encode a member of the previously described TIRNBS-LRR class of cytoplasmic receptor-like proteins.	Parker et al. 1997.
	Major <i>R</i> -gene clusters revealed on four chromosomes of <i>At</i> using a powerful combination of recombinant inbred <i>At</i> populations and DM isolates as physiological probes to map <i>RPP</i> loci.	Holub and Beynon 1997.
1998	The bacterial resistance genes <i>RPS4</i> and <i>RPS5</i> were cloned and demonstrated that non-host resistance in <i>At</i> can involve parasite recognition mediated by classic (receptor-like) <i>R</i> -genes. This established the use of a broadly virulent pathogen as a surrogate vector for transient expression to test the 'non-host' recognition of effector proteins. Interestingly, mutation of <i>RPS5</i> can interfere with recognition of some DM isolates.	Warren et al. 1998; Gassmann et al. 1999; Holub 2007; Rentel et al. 2008.
	<i>R</i> -like homologues provide a powerful class of molecular markers for map-based new oomycete resistance genes in <i>At</i> and in crops such as lettuce and potato.	Aarts et al. 1998a, b; Botella et al. 1997; Speulman et al. 1998.
	<i>RPP8</i> is cloned, and found to be a CC-NBS-LRR gene (similar to the first bacterial resistance genes described in <i>At</i>) and subsequently associated with viral resistance.	McDowell et al. 1998; Cooley et al. 2000; Takahashi et al. 2002.
	The multi-copy locus <i>RPP1</i> contains several DM resistance genes (TIR-NBS-LRR subclass) that differ in specificity.	Botella et al. 1998.
	DM resistance genes vary in how they confer defense via different regulatory proteins.	Aarts et al. 1998a, b; Eulgem et al. 2004.
2000	Whole genome sequence of <i>At</i> is announced as a public resource. The 'Linnaean genomics' age of plant biology is soon launched with ARABIDOPSIS 2010, an international community effort to ascribe a function for all of the genes in <i>At</i> by 2010.	AGI 2000; Holub 2007.
	A single DM resistance gene (<i>RPP7</i>) can confer accumulative (salicylic acid dependent and independent) defense responses.	McDowell et al. 2000; Tör et al. 2002; Eulgem et al. 2007.

Table 1 (continued)

Year	Milestone	References
	DM isolates collected from <i>At</i> appear to be phylogenetically distinct from brassica isolates: referred to hence as subsp. <i>arabidopsis</i> (<i>HpaA</i>) or <i>brassica</i> (<i>Hpb</i>).	Rehmany et al. 2000.
	<i>RPP13</i> is cloned, encoding a protein homologous to <i>RPP8</i> and providing the most extreme benchmark for allelic diversification of a receptor-like gene in <i>At</i> .	Bittner-Eddy et al. 2000; Rose et al. 2004.
2001	An <i>At-Phytophthora</i> model is described, providing <i>Agrobacterium</i> -mediated transformation of an oomycete for host–parasite research in <i>At</i> .	Roetschi et al. 2001.
2002	‘Gene-for-gene’ paradigm is established in the <i>At-HpaA</i> pathosystem. An outcross of <i>HpaA</i> enables genetic evidence for five independent <i>At</i> -recognizable effectors (<i>ATR1</i> , <i>ATR4</i> , <i>ATR5</i> , <i>ATR8</i> and <i>ATR13</i>) that correspond with different cloned DM resistance genes.	Gunn et al. 2002.
	<i>DM3</i> is cloned from lettuce, and found to encode a TIRNBS-LRR protein similar to several <i>RPP</i> genes in <i>At</i> .	Shen et al. 2002.
	The DM parasite of crucifer species (previously <i>Peronospora parasitica</i>) is renamed as <i>Hyaloperonospora parasitica</i> .	Constantinescu and Fatehi 2002.
	<i>SGT1b</i> and <i>RAR1/PBS2</i> provide evidence for highly conserved regulators (also found in monocots) and the likely involvement of proteolysis in defense signalling.	Austin et al. 2002; Muskett et al. 2002; Tör et al. 2002; Tornero et al. 2002; Warren et al. 1999.
	<i>A. thaliana</i> -oomycete molecular ecology is launched.	Damgaard and Jensen 2002.
2004	The first <i>At</i> -recognized effector (<i>ATR13</i>) is cloned from <i>HpaA</i> isolate Maks9 and found to encode a small secreted protein that exhibits a high degree of sequence variation amongst UK isolates of <i>HpaA</i> .	Allen et al. 2004.
	An <i>EDSI</i> -independent WR resistance gene (<i>RAC1</i>) is cloned and found to encode a TIR-NBS-LRR protein.	Borhan et al. 2004.
	The broad spectrum late blight resistance gene <i>Rb</i> is cloned and found to encode a CC-NBS-LRR protein.	Song et al. 2004.
2005	Enhanced downy mildew resistance (DMR) mutants are described, and launch the genetic analyses of induced accessibility for oomycete parasites in <i>At</i> .	Van Damme et al. 2005; Holub 2006.
2006	The first draft sequence of two oomycete genomes (<i>Phytophthora sojae</i> and <i>Ph. ramorum</i>) is announced.	Tyler et al. 2006.
	<i>ATR1</i> is cloned from <i>HpaA</i> Emoy2 and ignites the ‘Linnean genomics’ age of oomycete effector biology by revealing a conserved RxLR motif also found in <i>ATR13</i> , effector proteins from <i>Phytophthora</i> species, and secreted proteins from the human malarial pathogen <i>Plasmodium</i> .	Rehmany et al. 2005; Holub 2007; Win et al. 2007.
2007	Draft genome sequence of <i>HpaA</i> isolate Emoy2 is released by a <i>Phytophthora</i> consortium.	http://pmgn.vbi.vt.edu/

Noco2 is an exception, which was derived and has since been propagated from an original source of asexual inoculum.

Characterising the extent of phenotypic variation was an important step in establishing downy mildew and white rust of *A. thaliana* as model pathosystems for molecular genetics, following examples that Williams had established for assessing these diseases in vegetable brassica breeding (downy mildew exam-

ple in Fig. 3a; Holub et al. 1994, 1995). Incompatibility with *HpaA* isolates is common among *A. thaliana* accessions and much of the observed host resistance is *HpaA*-isolate specific and simply inherited (Fig. 3b; Holub 2007). The naturally variable *HpaA* isolates described above were therefore readily identified and differentiated according to their respective recognition by different combinations of downy mildew resistance (*RPP* = resistance to *Peronospora*

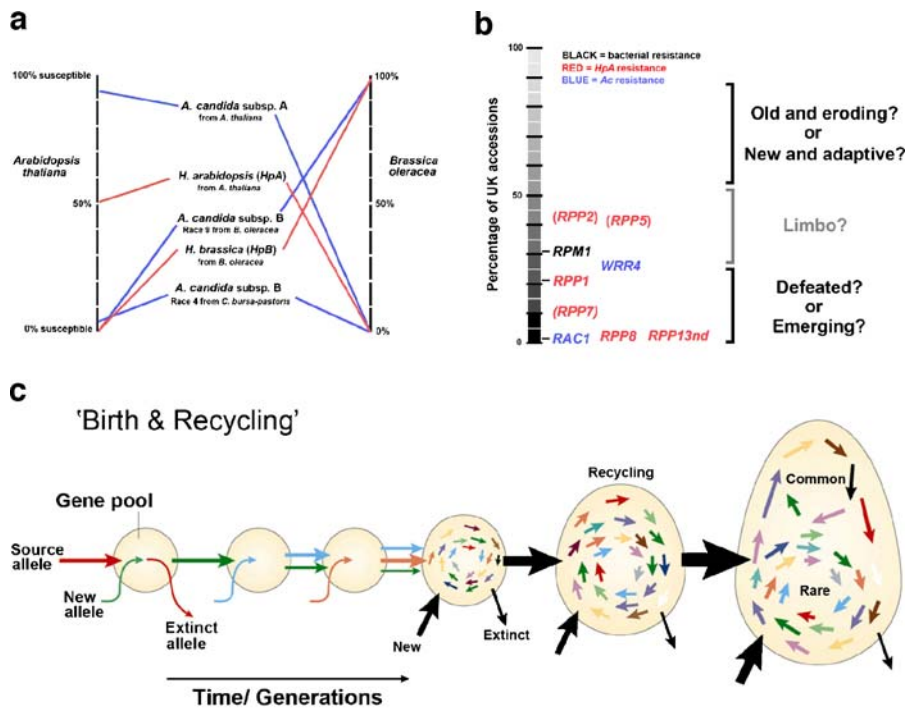


Fig. 1 *Arabidopsis thaliana* and its natural associations with parasitic oomycetes provide a rich and fascinating model for molecular and evolutionary ecology. *Arabidopsis thaliana* is a common host for two biotrophic oomycetes including *Hyaloperonospora arabidopsis* (*HpaA*, causing downy mildew) and *Albugo candida* subsp. ‘A’ (*AcA*, causing white blister rust); and will also be exposed under field conditions to recurrent but foiled attacks from closely related parasites (e.g., *H. brassica* and *A. candida* subsp ‘B’) that proliferate on brassica crops and other wild crucifers (e.g., *Capsella bursa-pastoris*). **a** The relative degree of compatibility to different parasite subspecies is compared in *A. thaliana* and *B. oleracea*; the estimates are based on the percentage of susceptibility within diversity collections of each host species (Holub et al. 1995; Holub, unpublished). **b** Many naturally variable pathogen recognition proteins (encoded by so-called *R*-genes) from just a few standard accessions of *A. thaliana* are now known which confer resistance to specific isolates of *HpaA* (red labels), *A. candida* (blue) or *Pseudomonas syringae* (black). All of the molecularly characterised examples are members of a large receptor-like ‘NB-LRR’ gene family (NB = nucleotide binding site; LRR = leucine rich repeat domain; Holub 2001). Preliminary analyses (Holub unpublished) indicate that the known functional proteins occur at a frequency of <50% in a UK diversity collection of 96 *A. thaliana* accessions, and many are potentially quite rare. Estimates for downy mildew and

white rust resistance genes here have been based on cumbersome F_2 allelism testing (populations derived from out-crossing UK accessions to the standard accessions containing the known *R*-genes), except where parentheses indicate cruder estimates based only on using a single standard isolate to survey resistance in the 96 accessions. These are overestimates; however, they are sufficient to pose questions (right margin) concerning the natural history of each variant R-protein, and more theoretical consideration of the origins and fate of allelic variation within a plant species. **c** For example (modified from Holub 2001), attempts to illustrate how the very earliest means of host defence (the first ‘source allele’), when an ancestral host–parasite relationship began to co-evolve, may have been replaced by a ‘new allele’ and presumably lost from the historical DNA-based record. Several rounds of this ‘arms race’ may have occurred before a final solution arose in vascular plants, with a highly adaptable capability of parasite recognition made possible by the accumulation in the gene pool of allelic variation in multiple copies of highly mutable NB-LRR proteins. These proteins enable individual plants, upon parasite detection, to rapidly elicit inducible and highly conserved (ancient) defence responses. It should be possible with population-level experiments to investigate whether recycling and ‘common versus rare’ variants of NB-LRR proteins actually occur in the molecular ecology of *A. thaliana*–parasite interactions

parasitica) genes in laboratory accessions of *A. thaliana* (Holub and Beynon 1997). This *HpaA* collection provided the ‘physiological probes’ for rapid, iterative mapping of numerous *RPP* loci relative to the molecular markers already available in recombinant

inbred populations of *A. thaliana* (Holub 1997). Targets for molecular characterisation were easily established (described below) on each of the five *A. thaliana* chromosomes that represented the breadth of phenotypic variation observed in inoculated cotyledons

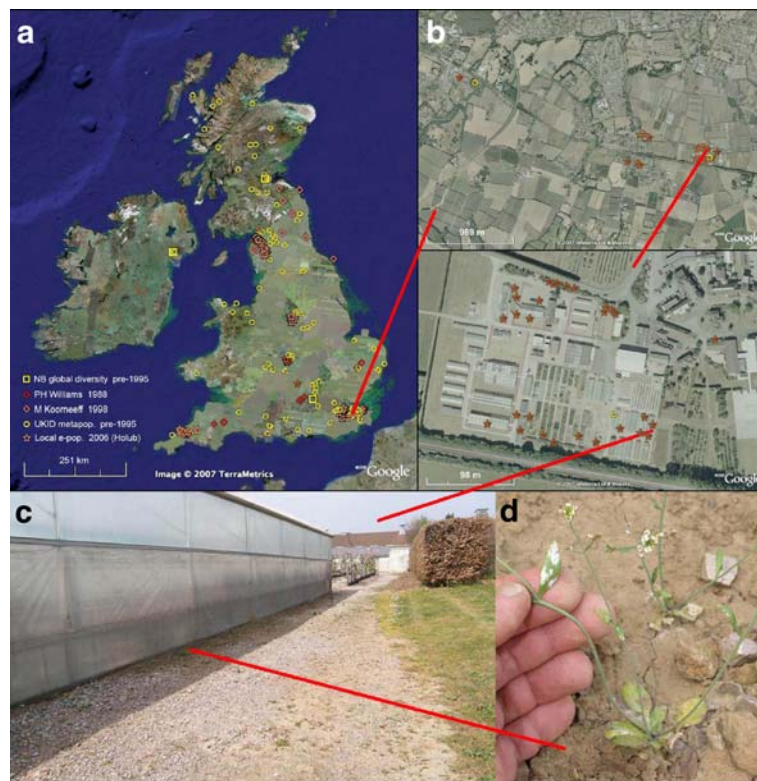


Fig. 2 The natural diversity and genomic history in *Arabidopsis thaliana* from the British Isles provides a unique resource for exploring molecular ecology of plants. **a** A large collection of *A. thaliana* has been assembled for a multi-national ARABIDOPSIS 2010 project which aims to generate a database of genome-wide, high density DNA sequencing from a global diversity collection of 1,152 accessions (Holub 2007). This collection includes all of the UK accessions currently available from public stock centres (Nordborg-Bergelson global diversity collection, and donations from P.H. Williams and M. Koorneeff) and a UK and Ireland diversity (UKID) collection assembled by E.B. Holub. This combined material provides a sample metapopulation of *A. thaliana* with a single accession representing the local population at each of ca. 150 locations. Extensive sampling of more than 100 established local

populations (>10 years old; ca. 1,200 accessions total) was conducted in 2006, and this material was also included in the first phase (low density genotyping) of the 2010 project. **b** Satellite photographs of one heavily sampled region in southeast England, where in 1988 P.H. Williams observed downy mildew and white blister rust in *A. thaliana* and where the molecular genetics of these pathosystems began 2 years later in I.R. Crute's laboratory at the horticulture research facility in East Malling, Kent (aerial view of buildings, lower panel). **c** Epidemics have occurred most years in established *A. thaliana* populations (>20 years old) that reside between polytunnels. **d** An example of white rust occurring on rosette (readily visible above as bright yellow tissue) and stem leaves of an individual plant

(Fig. 3b). It is important to note that natural variation exists in other characteristics of downy mildew or white rust resistance including: polygenic but isolate-specific in seedlings; broad spectrum in seedlings against the current *Hpa*-collection; and adult leaf resistance. Although these further examples are ecologically important, they have yet to be characterised at the molecular level.

Natural variation in *A. thaliana*–*AcA* interactions is comparatively sparse in providing clear examples of genotype-specific resistance (Fig. 1a; Holub et al. 1995), so this closely related pathosystem was less

attractive for the opening phase of molecular genetics research. As a consequence, this pathosystem remains undeveloped as a potentially excellent model for investigating such topics as species-level characteristics including host determinants of parasite speciation involving pathogen receptor-like genes (Fig. 1a; Rehmany et al. 2000; Borhan et al. 2008), and important aspects of compatible interactions including induced accessibility (pathogen-activated defence suppression) and source-sink dynamics affecting carbohydrate metabolism (Chou et al. 2000; Cooper et al. 2008; Holub and Cooper 2004; Tang et al. 1996).

juvenile rosettes can readily succumb to downy mildew or white rust, becoming chlorotic and stunted but plants are still capable of reproducing (albeit at severely reduced capacity). As plants get older, they are generally more able to ‘outgrow’ downy mildew or white rust infections with no obvious developmental effects, although the parasites themselves are able to survive endophytically (without symptoms) in older tissue of a compatible host. This suggests that under natural conditions, selection pressure by *HpaA* and *AcA* will be most acute during the establishment and competition amongst early juvenile rosettes in wild populations of *A. thaliana*, because debilitated plants may be less able to compete for resources with other non-diseased plants as well as being potentially more vulnerable to attack by secondary pathogens and saprophytes and more attractive to herbivores such as slugs and snails.

Using this rationale, the knowledge gained from molecular genetic analyses of downy mildew and white rust resistance in the cotyledon and seedling stage of *A. thaliana* should be relevant to theoretical considerations of host–parasite co-evolution (Fig. 1c). However, endophytic survival of *AcA* and *HpaA* throughout the life cycle of *A. thaliana* requires consideration in future studies in molecular ecology aimed at resolving the underlying selection drivers determining the observed distribution, epidemiology and maintenance of genetic variation in these symbiotic oomycetes. Similarly, host determinants of *HpaA* and *AcA* endophytism should also be targeted for molecular investigation; for instance, transgressive segregation for natural variation in *A. thaliana* can generate recombinant plants which exhibit hyper-susceptibility to downy mildew and white rust (high levels of sporulation and severe stunting) following inoculation of mature rosettes (Holub, unpublished). Such recombinants can presumably arise in natural populations but are presumably poor competitors when disease is prevalent and perhaps also under seemingly disease-free conditions.

Two decades in the Linnaean genomics of *A. thaliana*

Molecular milestones from *A. thaliana*-oomycete pathology have added appreciably to the Linnaean-like effort to name and classify gene families (Table 1; Holub 2001, 2006, 2007). For example, naturally variable genes that control *HpaA* isolate specific downy

mildew resistance were among the first pathogen recognition genes (so-called *R*-genes) to be molecularly characterised from plants. These abundant genes in monocots and dicots encode receptor-like proteins characterised by a leucine-rich repeat (LRR) domain that is typically highly mutable, and most known *R*-genes also contain a nucleotide binding (NB) site. A single accession of *A. thaliana* will contain more than 100 full length NB-LRR genes, many of which exhibit unusually high levels of allelic variation amongst accessions of the species collected from natural populations (Borevitz et al. 2007; Clark et al. 2007; Holub 2001). At least 10 such genes have been identified as genes conferring either downy mildew or white rust resistance and are distributed amongst each of the five *A. thaliana* chromosomes; at least half reside in major *R*-like gene clusters (Borhan et al. 2004, 2008; Holub 1997, 2001).

Laboratory researchers excelled at using artificial variation generated randomly by irradiation and chemical mutagenesis to reveal other key components of induced defence (Table 1). These components (e.g., *EDSI*, *NDRI*, *RARI/PBS2* and *SGT1b*) represent much smaller gene families more highly conserved than *R*-genes. Such genes have been shown to be essential for defence against a wide spectrum of pathogens, as first demonstrated by the discovery of *NDRI*, essential for resistance to plant pathogenic bacteria and downy mildew. Some of these genes have also been shown to represent essential and commonly-occurring components of induced defence across different families of vascular plants including monocots (e.g., *RARI/PBS2* and *SGT1b*).

It is important to remember that all of these innate defence genes, as with *R*-genes, have been identified and defined functionally in an experimental context. Each gene was discoverable because they were non-lethal and non-redundant (unique functional copy) in the specific combination of host and parasite genotypes selected by investigators for mutagenesis experiments. For instance, null mutation of *sgt1b* exhibits full susceptibility to some *HpaA* isolates (e.g., Cala2) in some genetic backgrounds of *A. thaliana* including the most popular laboratory accession Columbia (Tör et al. 2002). However, the same mutant exhibits residual resistance to other isolates of *HpaA* and strong resistance to all bacterial isolates tested thus far. Similarly, the effect of *sgt1b* mutation can also be masked in other genetic backgrounds such

as Landsberg *erecta* in an *RPP*-dependent manner (Tör et al. 2002). This effect may be due in some cases to redundant expression of a second copy of the gene (*SGT1A*) known to exist in Columbia *A. thaliana*, although this hypothesis is difficult to test decisively because double mutation of the gene pair (*sgt1a/sgt1b*) appears to be lethal (Azevedo et al. 2006; Holt et al. 2005).

In other words, the currently known *R*-genes and other defence components were essentially ‘low hanging fruit’ for discovery because, despite fortuitous examples like *SGT1b*, functional redundancy and mutational lethality present major obstacles that limit the scope of an approach to the dissection of defence mechanisms in plants based purely on molecular genetics. This is true even in the case of excellent model organisms like *A. thaliana*. The science has nevertheless progressed a long way towards addressing Albert Ellingboe’s challenge to identify the molecular ‘pieces of the (disease resistance) puzzle’ with genetics and mutational analyses (Ellingboe 1976, 2001; Holub 2006). Now however, complementary methods and techniques are required to extend knowledge incrementally from the low hanging fruit and fully reconstruct defence mechanisms in model systems (Glazebrook 2007; Kazan and Schenk 2007; Shen and Schulze-Lefert 2007; Staal and Dixelius 2007).

Nevertheless, for the purposes of this review, there is already sufficient molecular understanding of downy mildew resistance in *A. thaliana* to state what now seem to be three features underlying innate immunity in plants: (1) disease resistance typically involves the activation of an inducible defence process involving many other genes; (2) natural genetic variation in disease resistance is most prevalent at the level of receptor-like proteins (containing a highly mutable leucine-rich repeat domain), which acts as a trigger for the induced defence; and (3) exceptions to the above will arise from further investigation as a consequence of genetic background, plant tissue type and/or physiological age of tissue.

Linnaean genomics has also revolutionized our understanding of plant pathogenic oomycetes as a consequence of whole genome sequences that have become available for several *Phytophthora* species and *Hpa* (Table 1). There is now a fervent interest in revealing so-called effector proteins from parasitic microorganisms that somehow affect biochemical

responses in a potential plant host (Grant et al. 2006; Kamoun 2007; Tör 2008; Win et al. 2007). Microbial effectors that can be detected by matching R-protein activity were previously referred to in plant pathology literature as avirulence proteins or race-specific elicitors. Similarly, non-specific elicitors have come in recent years to be referred to as PAMPs (pathogen associated molecular patterns), an abbreviated term adopted from the research literature of innate immunity in animals. Many *ATR* (*A. thaliana* recognized) effectors were predicted in *Hpa* following the genetic identification of numerous *R*-genes conferring downy mildew resistance in *A. thaliana* and classical genetic analysis of avirulence in *Bremia lactuca* (Holub and Beynon 1997; Michelmore and Wong 2008). However, confirmation of a ‘gene-for-gene’ correspondence has only recently been confirmed for five cloned *RPP* genes, followed swiftly by the molecular identification of the first two *ATR* effectors (Table 1; Fig. 3b; reviewed by Holub 2007).

Downy mildew research in *A. thaliana* has benefited enormously over nearly two decades from healthy competition and collaboration amongst several research groups. However, our current knowledge (summarized in Table 1) would not have been achieved so quickly and coincident with the precedents from molecular investigations of bacterial resistance in *A. thaliana* without the original catalytic fieldwork provided by P. H. Williams in 1988. Two downy mildew resistance genes (*RPP1* and *RPP5*) would probably have been discovered; however, *Hpa* genetics and cloning of *Hpa* effectors would still only be a theoretical prospect if Williams had not conducted his European collection trip. Similarly, the involvement of major defence components such as *EDS1*, *NDR1* and *SGT1b* in bacterial resistance would have been revealed but the parallel with downy mildew resistance would likely have come much later. Hence, Williams provided a crucial link between the founders of experimental botany and the modern laboratories of molecular plant pathologists.

Establishing an experimental basis for molecular ecology of plant–parasite interactions

Arabidopsis thaliana–oomycete symbioses could provide the basis for making the next crucial link, between recent laboratory advances in molecular

biology of innate immunity in plants and genomics-based field experiments of molecular ecology (Holub 2007). Seven key resources will be: (1) access to microhabitats in the form of reasonably protected field locations that have been inhabited naturally by *A. thaliana*; (2) seed samples from these reference habitats collected from a high proportion of individuals at each location; (3) ‘DNA finger-printing’ of individuals derived from these initial seed lines involving genome-extensive sequence information to provide a base-line reference for future sampling; (4) access to comparable locations uninhabited by *A. thaliana* for hypothesis testing in future seed introduction experiments (including virgin sites which could provide suitable conditions for *A. thaliana*, and sites which are unsuitable for some unknown selective factor despite the initial appearance of the habitat); (5) satellite navigation (GPS) and photographic records enabling field biologists to accurately re-visit the marked populations; (6) public participation that must include land owners of reference sites, local and regional plant ecologists, and amateur naturalists; and (7) online access for researchers and educators to the public-sponsored data.

The best reference locations for field research will have been well-established (>10 years old, but ideally much older) natural populations of *A. thaliana*. In the British Isles for instance, *A. thaliana* is predominantly a winter annual (flowering May–June) throughout, but it can occur as a summer–autumn annual especially in wettest regions such as the Scottish highlands and the Hebrides (or even well-watered gardens with good slug control). The species relies largely on animals for seed dispersal to establish new populations, and human activity is particularly important so suitable locations for established populations will often be of man-made origin. Sites that support enduring natural populations of *A. thaliana* will include mural communities (described by Segal (1969) as vertical, such as the head, vertical face and foot of old stone or brick walls; and horizontal, such as cracks in urban pavements), ballast of disused and existing steam railway tracks, roadside verges, and cultivated areas such as historic gardens (Fig. 4).

All such habitats can be found throughout the British Isles, and even within regions such as the Lake District National Park that became a protected conservation area thanks to the investment and leadership of Beatrix Potter (Holub 2007; Thompson

2007). It will be fascinating to see whether the rich genomic history (DNA-based, whole genome) that is currently being accumulated from *A. thaliana* (Borevitz et al. 2007; Clark et al. 2007; Holub 2007) will enable us to resolve whether this species is a native in the British Isles or instead an archaeophyte (i.e., pre-Medieval introduction by one or more human migrations) like its close relative *Capsella bursa-pastoris* (Preston et al. 2004).

Mural populations of *A. thaliana* will be particularly interesting for molecular ecology, especially where *A. thaliana* can cycle continuously in consecutive years in essentially the same spot (Fig. 4a and b). Human disturbance represents a major threat to the survival of these self-maintaining mural populations. The linear nature of old wall and railway habitats provides pertinent opportunities to assess the accumulation and maintenance of genetic variation. It should be possible to collect evidence for multiple migration events or out-cross pollination events, as well as for genetic differences that correlate with contrasting features of microhabitat (e.g., heavy shade vs. full sunlight along a wall extending several kilometre; different exposure levels to atmospheric pollutants), and for possibly predicting historical movement of genetic variants along a wall. Although *A. thaliana* is highly self-fertile, pollen dispersal and outcrossing may be more important than expected for movement and maintenance of natural variation within established populations (Bakker et al. 2006), especially on walls.

In a given region, the total available length of wall habitats will far exceed the natural distribution of *A. thaliana*, so with appropriate permission from property owners, there is excellent potential for experimental introductions such as reciprocal transplants of seed from genetically distinct populations or ‘seeding’ of apparently non-diseased populations with oospores of genetically defined *HpaA* or *AcA* isolates. Defining the suitable environmental conditions for establishment and extended survival of *A. thaliana* in these natural habitats will, at the very least, be critical for judging the relative merits of experiments that are performed under controlled laboratory conditions, and whether sites chosen for ‘common garden studies’ (Mitchell-Olds and Schmitt 2006) are representative of habitat in a given geographic region.

In the British Isles, a good start has been made in assembling the key resources outlined above. A



◀ **Fig. 4** *Arabidopsis thaliana* habitats are typically of man-made origin in the British Isles including: **a** in the cracks of pavements; **b** on the head, vertical face and foot of old stone or brick walls; **c** in the ballast of existing steam railway tracks, **d** persisting in disused sections of railways; **e** in grassland meadows and roadside verges; and **f** in cultivated settings (e.g., a flower bed in the Cambridge University Botanical garden). The resident populations are typically well established in these locations; for example, ‘X’ literally marks the spot in **a** where three to five plants reside annually at this location on a street in Stratford-on-Avon, and similarly in **b** for the position on top of a drystone wall in the Lake District. White blister rust (*Albugo candida* subsp. A) is the disease most commonly visible during late spring in UK habitats (example shown in **b**, right panel); however, downy mildew (*Hyaloperonospora arabidopsis*) can also occur frequently during the juvenile rosette stage (October–March). Yellow arrowheads indicate the location of *A. thaliana* plants in each of these habitats

starter set of *A. thaliana* accessions has been available from public stock centres, and this has been extended with a metapopulation sample that was assembled over 5 years between 1988 and 1993 (Fig. 2a). The local population at each of the locations is represented by a single accession (seed from one individual). In 2006, GPS marking was initiated of field sites that were inhabited with established populations of *A. thaliana*, and seed was collected from at least five individuals per population (80% or more of individuals in each population; ca. 1,200 samples in total). Many of the sites had been sampled in previous forays, including wall populations in southeast England that P.H. Williams had visited in 1988. In most cases, populations were found in the same site as in previous forays indicating an age of at least 15–20 years. Such walls are often more than 100 years old. Many of the newly identified sites from 2006 were subsequently revisited in 2007 to confirm that the populations were also recurrent. Unfortunately, given the timing and logistics of handling seed samples, it was not possible to thoroughly assess disease and collect pathogen samples. The sole purpose was to accumulate potential sites of scientific interest and reference host material. Nonetheless, *AcA* was observed in ca. 70% of the established populations in both the spring of 2006 and 2007 (e.g., Figs. 2d and 4b), whereas *Hpa* was only found in a few locations (<2%). These observations at least identify sites for future host–parasite investigations.

For DNA fingerprinting, the complete collection of *A. thaliana* from the British Isles shown in Fig. 2a has been incorporated into a major international project led by US scientists, including sampling from

established populations in 2006 (Holub 2007). This project is aimed at providing laboratory researchers with a global diversity collection of 1,152 accessions, a large database of molecular markers (<10 kb spacing), and linkage analysis tools as a research capability for the mapping of genes that underlie natural phenotypic variation in *A. thaliana*. The first phase of the project, however, was to genotype more than 5,000 accessions (mostly European and USA samples) at 149 genome-wide loci containing SNP (single nucleotide polymorphism) or indel (small insertion or deletions) sequence variation. This relatively low-cost dataset provides a means for choosing accessions with unique genotypes that represent global diversity in an elite collection that will undergo the further high-density genotyping. All of the British accessions have been genotyped in the first phase, and ca. 20% are likely to be advanced for inclusion in the elite diversity collection. The low-density genotyping should provide sufficient information for assessing the frequency and distribution of genetic variation in UK *A. thaliana* at different geographic scales and in contrasting habitats, and for choosing prime diseased and non-diseased sites for future field research.

Access to *A. thaliana* seed stocks and DNA information will be possible via the North American and European stock centres (www.biosci.ohio-state.edu/pcmb/Facilities/abr/abrhome.htm; www.arabidopsis.info) and the central information website (www.arabidopsis.org). The UK National Biodiversity Network (www.nbn.org.uk) provides an exciting precedent for international efforts to release biodiversity information online, and will therefore provide a superb means for releasing information about reference field sites for *A. thaliana* in the British Isles.

Pump-priming molecular pathology in natural populations of *A. thaliana*

Although the seven key resources described above are at various stages of development, it is already possible to envisage how the molecular ecology of *A. thaliana*–parasite interactions will begin to unfold.

For instance, the conventional method of using standard parasite isolates (diagnostic for different *R*-genes) to assess wild accessions for compatibility phenotypes indicates that most of the currently known *R*-genes, conferring bacterial or oomycete resistance,

occur across the UK at a frequency well below 50%. This may suggest that the resistance alleles are transient, and are either newly-emergent alleles that have yet to provide a selective advantage under natural conditions, or are much older alleles that have been persistently defeated by a virulent pathogen across the species distribution (Fig. 1b). Alternatively, they may be relatively old alleles that have been recycled by undergoing fluctuations in frequency due to changes in virulence of the pathogen population (Fig. 1c; Holub 2001).

These hypotheses need testing by spatial and temporal experiments in populations of *A. thaliana* and should be extended to include other ecologically relevant examples of natural variation (e.g., polygenic and adult plant resistance). Seedling assays with *Hpa* and *AcA* isolates provide a simple and high-throughput means of generating phenotypic data (Fig. 5); however the results obtained will lack precision for genotyping purposes because uncharacterised R-protein/avirulent effector interactions may occur in the sampled material which can confound interpretations from the observed phenotypes. Additional data generated from bacterial assays may eventually be possible, using a broadly virulent isolate of *Pseudomonas syringae* as genetically engineered physiological probes to predictably deliver different avirulent effectors from downy mildew and white rust parasites (Holub 1997, 2007; Rentel et al. 2008).

The next step will be to generate phenotypic data using the existing UK seed stocks and remaining elite global diversity collection, to supplement the rich DNA-based dataset. Once the prime reference locations have been identified, the major investigation can commence with experiments designed to monitor changes in known *R*-genes and oomycete effectors. Low-cost genotyping and PCR-based assays that can be used to detect specific alleles will be instrumental as case studies in aiding progress, especially at fine levels of spatial distribution where populations may have, for example, extended along a kilometre or more of wall and are exposed to variable physical and biological conditions of microhabitat.

Concluding remarks

The natural history of *A. thaliana*–oomycete interactions was the topic of a reviews published a decade

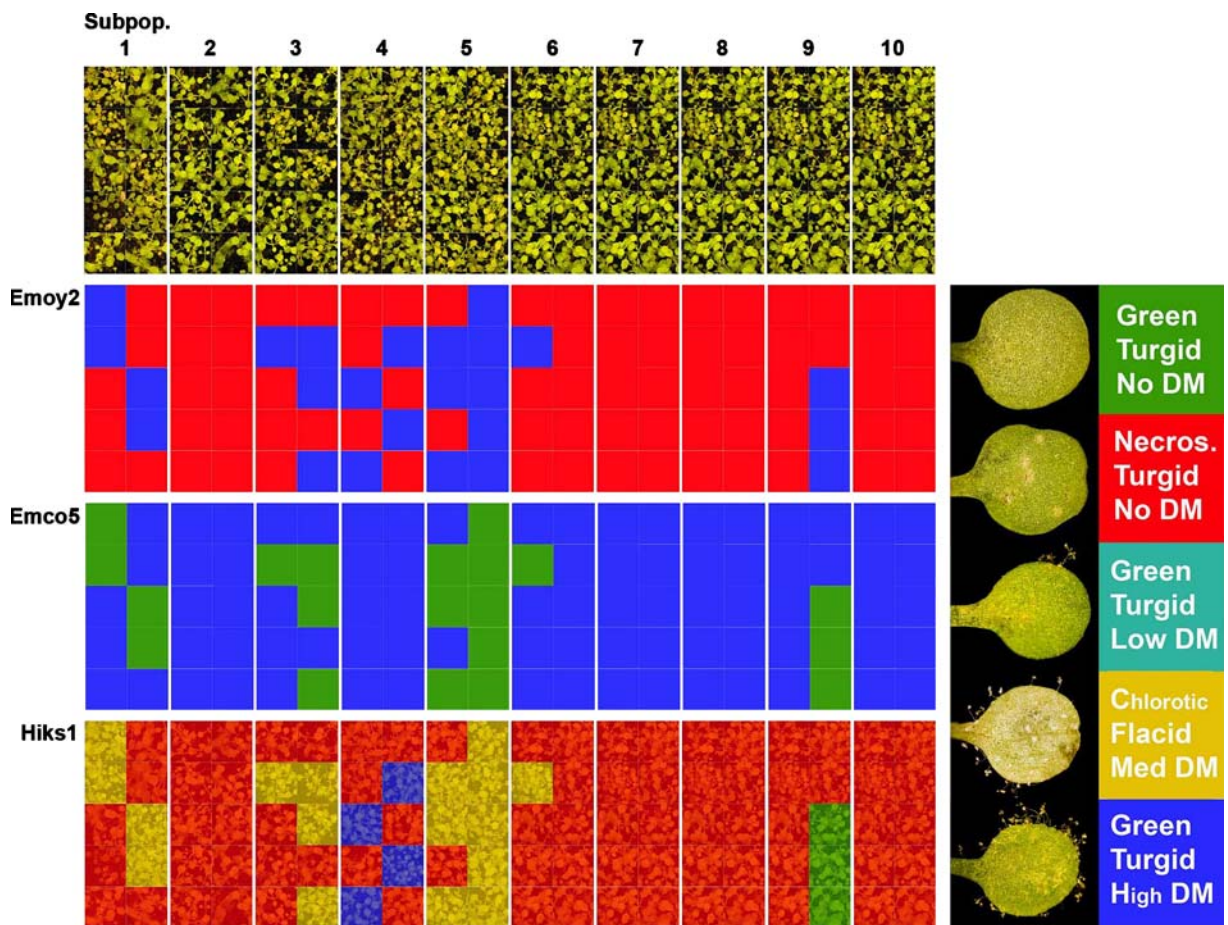


Fig. 5 *Arabidopsis thaliana* from the grounds of a horticulture research site in southeast England (located at East Malling Research) provides one important example of a well-established wild population (at least 25 years old) for investigating the molecular ecology of downy mildew. The reference isolate of *Hyaloperonospora arabidopsis* (*Hpa*-Emoy2) was derived in 1991 from oospore-infested leaf tissue, collected from *A. thaliana* plants that were growing naturally outside a polytunnel at this location (Holub 2006; see Fig. 2c). Seed samples were subsequently collected in 1993, from 200 plants that were distributed across the research site, and each seed line was increased by self-pollination to establish larger individual seed stocks of this sample population for use in laboratory experiments. In a pilot experiment, 100 seed lines (arranged as illustrated by top row of seedling photographs; divided in 10 subpopulations of 10 lines in each) were inoculated as 7 day-old seedlings with different *Hpa* isolates including Emoy2, Emco5 (also from the same East Malling site), and Hiks1 (from Hampshire, ca. 250 km west of East Malling). Interaction phenotypes (far right, vertical panel of cotyledon photographs, along with brief description and colour

coding) were recorded for each combination of seed line and *Hpa* isolate. The pattern of colour coding summarizes the observed phenotype from each combination (seed line × isolate) across the host population. The red phenotype is specifically indicative of downy mildew resistance mediated by *RPP1* on chromosome 3 (recognizing both Emoy2 and Hiks1), and appears to occur at a high frequency throughout this population. The only alternative phenotype observed with Emoy2 was full susceptibility, whereas two alternative resistance phenotypes were observed with Hiks1; the *RPP* gene(s) conferring these examples of resistance have yet to be determined. Interestingly, Emco5 inoculations revealed an opposite pattern of susceptibility and resistance compared with Emoy2. In this case, the GREEN or full immunity phenotype may be indicative of *RPP13* which is known to recognize Emco5 and is closely linked to *RPP1*; however, this too has yet to be determined). Nonetheless, these preliminary data indicate that two of the most important *RPP* genes from previous laboratory research most likely occur in this natural population of *A. thaliana* as well as the matching avirulence effectors within the resident *Hpa* population

ago (Holub 1997; Holub and Beynon 1997). However, the emphasis then was on laboratory research and the ‘hardcore’ molecular biology of innate immunity in plants. Molecular ecology of plant–parasite inter-

actions at that time was an aspiration for a small community of biologists. Times have clearly changed with the complete genome sequence of the first *A. thaliana* accession, rich DNA-sequence data from

another 20 *A. thaliana* accessions (Borevitz et al. 2007; Clark et al. 2007), and a dramatic fall in the cost of DNA sequencing. The aspiration has progressed from plausibility to a very real and exciting opportunity.

Much of the infrastructure is falling into place, behind major advances in the molecular genetics of downy mildew resistance in *A. thaliana* over the past two decades, and the discovery of large numbers of oomycete effectors providing raw material for another complementary round of investigation in the downy mildew parasites of *A. thaliana* and crops such as lettuce (Michelmore and Wong 2008; Tör 2008; Win et al. 2007). In parallel, the infrastructure for *A. thaliana* field biology is beginning to emerge, which has exciting potential for advancements in understanding the evolutionary context of a range of developmental and physiological processes in plants.

The well-developed and timely geographic mosaic theory of co-evolution provides the essential framework for constructive use of the resources described in this review. Mathematical modelling that combines ecological parameters (to explain spatial and temporal changes in populations) with evolutionary genetics (natural selection acting on multiple loci of interacting species) represents an enormous challenge for this exciting research field. However, a cross-disciplinary 'systems biology' approach is inevitable. Somehow, all of the public investment in laboratory research of *A. thaliana* will not make sense without further practical applications in plant breeding, and equally, without concerted contributions from a new generation of field botanists who are committed to aiding conservation biology. I would hope that the likes of A. DeBary and H.M. Ward would agree.

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