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Review

Co-evolutionary interactions between host resistance and pathogen effector genes in flax rust disease

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SUMMARY

Plant–pathogen co-evolutionary selection processes are continuous, complex and occur across many spatial and temporal scales. Comprehensive studies of the flax–flax rust pathosystem have led to the postulation of the gene-for-gene model, a genetic paradigm describing recognition events between host disease resistance proteins and pathogen effector proteins. The identification of directly interacting fungal effector proteins and plant disease resistance proteins in this pathosystem has facilitated the study of both the physical nature of these interactions and the evolutionary forces that have resulted in a molecular arms race between these organisms. The flax–flax rust pathosystem has also been detailed on the scale of interacting populations, and the integration of molecular- and population-scale datasets represents a unique opportunity to further our understanding of many poorly understood facets of host–pathogen dynamics. In this article, we discuss recent developments and insights in the flax–flax rust pathosystem and their implications for both long-term co-evolutionary dynamics in natural settings, as well as short-term co-evolutionary dynamics in agro-ecosystems.

INTRODUCTION

Interaction with parasites has been postulated to be a major driver of the evolution and maintenance of diversity in both plants and animals. Infection of hosts can lead to a reduction in fitness and selection for defence or avoidance mechanisms. Conversely, pathogens are selected to circumvent the continually evolving defences mounted by their target hosts. However, despite significant advances in our understanding of these interactions at both the molecular and population levels, there are still major questions to be resolved regarding the mechanisms of host resistance and pathogen virulence, their variation in space and time, and their long-term effect on host–pathogen co-evolution.

The interaction between flax and flax rust has been an important model system for understanding the genetic and molecular basis of host–pathogen interactions in plant diseases, as well as for understanding the co-evolutionary processes in natural disease systems. Flax rust, *Melampsora lini*, is a fungal pathogen that infects cultivated flax (*Linum usitatissimum*), as well as a number of related *Linum* species, including the native Australian flax *L. marginale* (Barrett *et al*., 2009; Lawrence *et al*., 2007). Rust pathogens are obligate biotrophs, which depend on living plant tissues for propagation. The invading fungal hyphae form specialized feeding structures, called haustoria, that extract nutrients from host mesophyll cells.

Working with the cultivated flax–rust system, Flor (1956) defined the gene-for-gene model which has proved to be widely applicable as the basic genetic paradigm of plant disease resistance. In this model, the outcome of infection is based on the interaction of dominant resistance (*R*) genes in the host and dominant avirulence (*Avr*) genes in the pathogen. This genetic interaction is now understood in terms of effector-triggered immunity (Chisholm *et al*., 2006; Dodds and Rathjen, 2010; Jones and Dangl, 2006), where R proteins constitute the recognition component of the plant immune system and detect the presence of specific pathogen effector (Avr) proteins, and trigger a defence response that prevents infection. These host and pathogen genes thus confer 'extended phenotypes' (Dawkins, 1999), that is their effects extend to the phenotype of another organism, implying close co-evolutionary interactions which have been the basis of much theoretical modelling (Sasaki, 2000; Thrall and Burdon, 2002). However, there are few experimental systems in which it is possible to evaluate theoretical predictions arising from different co-evolutionary scenarios (e.g. cyclical selection vs. an escalating arms race). Recent work in the flax–rust system has now delineated the molecular basis of gene-for-gene resistance and has revealed new insights into the evolutionary consequences of gene-for-gene interactions in wild

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systems. In this article, we summarize the current state of knowledge of molecular- and population-level interactions in the flax–rust disease system, and future directions for understanding how these organisms co-evolve.

MOLECULAR BASIS OF GENE-FOR-GENE INTERACTIONS

Resistance proteins

In cultivated flax, 30 genes that confer resistance to flax rust have been mapped to five loci (*K*, *L*, *M*, *N*, *P*), consisting of closely linked or allelic genes (Islam and Mayo, 1990). Nineteen *R* genes have been cloned from flax (11 from *L*, three from *M*, three from *N*, two from *P*), all of which encode intracellular Toll interleukin 1 receptor-nucleotide binding site-leucine-rich repeat (TIR-NBS-LRR) class proteins (Anderson *et al*., 1997; Dodds *et al*., 2001a, b; Ellis *et al*., 1999; Lawrence *et al*., 1995, 2010a). The *L* locus consists of a single gene with 13 allelic variants (*L*, *L1* to *L11*, and *LH*) distinguished by their reaction to rust strains carrying different *Avr* genes. The *N*, *M* and *P* loci are more complex, containing four, up to 15, and six to eight tandemly arranged paralogues, respectively.

Most variation between alleles and paralogues at these loci occurs in the LRR domain (Dodds *et al*., 2001a, b; Ellis *et al*., 1999, 2000), and domain swap experiments have confirmed that the LRR domain is important for determining R–Avr recognition specificity. For instance, chimeric L proteins consisting of the L2 LRR and either L6 or L10 N-termini express L2 recognition specificity (Ellis *et al*., 1999). In addition, the L6 and L11 proteins differ by 32-amino-acid polymorphisms, all in the LRR domain, and a recombinant protein with a chimeric L6/L11 LRR showed a novel recognition specificity (Dodds *et al*., 2006; Ellis *et al*., 2007). In general, LRR domains are horseshoe-shaped molecules (Fig. 1) composed of repeating leucine-rich units of approximately 24–30 amino acids (Kobe and Deisenhofer, 1995). Variable residues are exposed on a concave β-sheet surface and available for participation in R–Avr interactions. Indeed, the different *P* and *P2* specificities are a result of just six amino acid polymorphisms found in the LRR β-sheet region (Dodds *et al*., 2001a). Collectively, these results indicate that the LRR domain is the major determinant of Avr recognition specificities. Congruently, in the rice–rice blast pathosystem, the LRR domain of the rice *R* gene *Pi-ta* has been observed to interact directly with the corresponding Avr-Pita effector protein in a yeast two-hybrid assay (Jia *et al*., 2000). Likewise, domain swaps have shown that pathogen recognition specificity is controlled by the LRR region of the barley Mla resistance proteins and the Rx/Gpa2 proteins in potato (Rairdan and Moffett, 2006; Shen *et al*., 2003). Pull-down experiments have shown that the LRR domain of Arabidopsis RPP1 protein associates with the *Hyaloperonospora arabidopsidis* ATR1 effector (Krasileva *et al*., 2010).

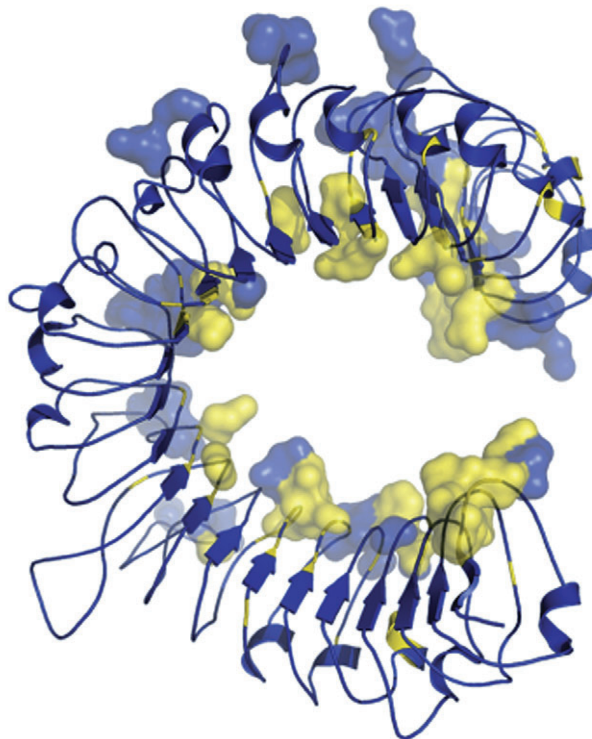

Fig. 1 A structural model of the leucine-rich repeat (LRR) domain of L5. Residues that may mediate interactions with AvrL567-A have been rendered as three-dimensional surfaces. Residues that are under significant ($P > 0.95$) positive selection are coloured yellow.

Although the LRR domain appears to be the primary mediator of recognition specificity, the TIR domain may also influence this function. TIR-NBS domain swaps between *L10* and *L2* or *L9* determined that L protein function requires co-adapted TIR-NBS and LRR regions, raising the possibility that these domains interact with each other (Luck *et al*., 2000). Indeed, positive selection has acted on the L TIR domain, suggesting that polymorphisms in this region are related to the function of the L proteins (Luck *et al*., 2000; M. Ravensdale, unpublished data). Hwang and Williamson (2003) have reported that intramolecular interactions between the coiled coil (CC) and LRR domains of the Mi resistance protein mediate downstream hypersensitive response (HR) signalling in tomato. Similarly, studies of the Rx resistance protein have demonstrated interactions between the CC-NBS and LRR domains, and between the CC domain and the NBS-LRR region (Moffett *et al*., 2002). Importantly, these interactions are disrupted in the presence of the cognate Rx effector ligand. In the case of the tobacco–*Tobacco mosaic virus* (TMV) pathosystem, the p50 fragment of the TMV replicase protein associates indirectly with the TIR domain of the N resistance protein through an intermediate protein (Burch-Smith *et al*., 2007), but

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Table 1 Characteristics of cloned effector genes and their protein products from flax rust, *Melampsora lini*.

Locus	Mature protein size (aa)	Number of cloned variants	Cognate *R* genes	Variation in rusts pathogenic on *Linum marginale*
AvrL567	127	12	*L5*, *L6*, *L7*	Not polymorphic
AvrM	184–349	6	*M*	Not polymorphic
AvrP123	88–94	6	*P*, *P1*, *P2*, *P3*	7 alleles
AvrP4	67	3	*P4*	13 alleles

aa, amino acid.

then appears to be recognized by binding directly to the LRR domain (Ueda *et al*., 2006). Collectively, these studies have led to hypothetical models of R protein function, in which the recognition of cognate effectors causes intramolecular conformational changes within the R protein, resulting in signal transduction (Rafiqi *et al*., 2009).

Avr effectors

Of the approximately 30 *Avr* specificities identified in flax rust via genetic studies, genes from four *Avr* families, representing nine recognition specificities, have been cloned to date: *AvrL567*, *AvrM*, *AvrP123* and *AvrP4* (Barrett *et al*., 2009; Catanzariti *et al*., 2006; Dodds *et al*., 2004). These were identified by screens for rust genes expressed during infection (*AvrL567*) or haustorially expressed sequence tags encoding secreted proteins (*AvrM*, *AvrP4* and *AvrP123*) that co-segregate with the *Avr* loci. Avirulence functions were confirmed by *Agrobacterium*-mediated transient expression in flax lines expressing the corresponding *R* genes, which induced HR, whereas expression in flax lines without these resistance genes resulted in no HR. Recently, direct confirmation that these genes are responsible for avirulence was achieved using *Agrobacterium*-mediated transformation of flax rust and RNA interference to silence *AvrL567* genes; transgenic rust isolates acquired virulence on flax plants containing *L5*, *L6* and *L7* (Lawrence *et al*., 2010b).

All the *Avr* gene variants encode small secreted proteins (Table 1) that are expressed in haustoria and appear to be translocated into plant cells (Catanzariti *et al*., 2006; Dodds *et al*., 2004). They are characterized by high levels of polymorphism associated with differences in recognition specificity. For instance, there are 12 variant forms of *AvrL567*, seven of which are recognized by L5, L6 or L7, whereas the other five are virulence alleles (Dodds *et al*., 2006). Likewise, several different alleles of *AvrP123* are differentially recognized by *P*, *P1*, *P2* and *P3*, and a recombinant allele showed a novel recognition phenotype (Barrett *et al*., 2009; Dodds and Thrall, 2009). None of the effector families isolated from flax rust share sequence similarity with each other or with other currently known proteins, although AvrP123 contains 10 cysteine residues that conform to the consensus spacing of the kazal family of protease inhibitors (Catanzariti *et al*., 2006).

Homologues of *AvrL567*, *AvrM* and *AvrP4* occur in the poplar rust (*M. larici-populini*) genome (http://genome.jgi-psf.org/Mellp1/Mellp1.home.html), and *AvrP4* homologues occur across 22 *Melampsora* species (Van der Merwe *et al*., 2009).

The flax R proteins are cytoplasmic, and transient expression of *Avr* proteins lacking N-terminal signal peptides results in an *R* gene-dependent HR, which indicates that Avr recognition occurs inside the plant (Catanzariti *et al*., 2006; Dodds *et al*., 2004). Thus, Avr proteins must be translocated into plant cells during infection and, indeed, immunolocalization has detected the AvrM protein inside host cells containing a haustorium (Rafiqi *et al*., 2010) Similarly, translocation of flax rust effectors appears to be independent of the pathogen, as transient expression of *AvrM* in resistant flax leaves results in an HR regardless of the presence of a signal peptide (Catanzariti *et al*., 2006). This suggests that plant-derived transport machinery may be exploited by these pathogens. Recently, Rafiqi *et al*. (2010) have demonstrated that the N-terminus regions of AvrM and AvrL567 are sufficient to direct the translocation of secreted green fluorescent protein (GFP) fusion proteins. Likewise, translocation of effectors produced by ooymycete pathogens is mediated by a conserved *N*-terminal RxLR motif (Whisson *et al*., 2007) and can occur independently of the pathogen (Dou *et al*., 2008).

Molecular basis of Avr–R interactions

The physical nature of Avr–R recognition has the potential to impact significantly on the evolution of these proteins. There are two hypothetical models that describe how pathogen effector proteins may interact with plant resistance proteins. The first is based on a direct physical interaction (receptor–ligand), and has been described in the rice—rice blast pathosystem, where Pi-ta interacts with Avr-Pita, in the *Arabidopsis thaliana*—*Ralstonia solanacearum* pathosystem, where RRS1 interacts with the avirulence protein PopP2, and in the tobacco–TMV pathosystem, where N interacts with the TMV replicase protein (Bernoux *et al*., 2008; Deslandes *et al*., 2003; Jia *et al*., 2000; Ueda *et al*., 2006). Alternatively, R proteins may recognize the presence of Avr proteins indirectly by detecting changes induced in other plant proteins by Avr proteins. In this scenario, R proteins guard the targets of Avr proteins. The guard hypothesis has been demon-

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strated in the *A. thaliana*—*Pseudomonas syringae* pathosystem, where RPM1, RPS2 and RPS5 recognize changes in RIN4 and PBS1 induced by the presence of bacterial effectors (Axtell *et al*., 2003; Mackey *et al*., 2002, 2003; Shao *et al*., 2003). More recently, the guard model has been modified to include the possibility that guarded host proteins may evolve as decoys, thus functioning only as dedicated effector detectors (van der Hoorn and Kamoun, 2008). R proteins that function as guards are likely to select against the pathogenicity function of Avr proteins, whereas R proteins that bind directly to Avr effectors will impose a strong selection pressure on these proteins to evade physical detection. When Avr recognition is not related to effector function, it is possible for mutations to occur that disrupt recognition without affecting effector function. Thus, 'guard'-type R proteins should provide stable, long-term resistance, whereas directly interacting R proteins should accelerate the evolution of new virulence phenotypes (Ellis and Dodds, 2003; van der Hoorn *et al*., 2002).

The diversification of both *R* and *Avr* allelic variants in the flax–rust system is suggestive of a direct protein–protein interaction and, indeed, yeast two-hybrid assays have confirmed this (Catanzariti *et al*., 2010; Dodds *et al*., 2006). For example, Dodds *et al*. (2006) co-expressed *L5*, *L6* and *L6L11RV* (a chimeric gene derived from *L6* and *L11*) with all 12 *AvrL567* variants in the yeast two-hybrid assay, and found a close correlation between Avr–R interactions in yeast and the induction of HR *in planta*. Likewise, M and AvrM interact directly in yeast, and this interaction correlates with the recognition specificities observed *in planta* (Catanzariti *et al*., 2010). Although M is approximately 80% identical to L5 and L6 at the amino acid level, AvrL567 and AvrM are unrelated. In addition, L5 and L6 are the most sequence diverged (87 polymorphisms; 59 in the LRR domain) of the L proteins and yet recognize an overlapping set of AvrL567 proteins, either as a result of convergent evolution, or because conserved sequences found in both L5 and L6 mediate these interactions. Collectively, these data suggest that the LRR domain is evolutionarily flexible and has evolved to directly recognize a diverse set of Avr effectors.

Protein sequence analysis of flax rust Avr effectors has revealed that these protein families are highly polymorphic, and appear to be under diversifying selection. For example, *AvrM* variants contain 14 polymorphic sites, as well as a number of deletions and truncations, and comparison with flanking sequence variation clearly indicated the effects of positive selection (Catanzariti *et al*., 2006). An initial study of *AvrP4* revealed seven polymorphisms concentrated in the C-terminal region of the protein that appeared to be the result of diversifying selection (Catanzariti *et al*., 2006), and comparison of AvrP4 homologues across 22 *Melampsora* species revealed significant positive selection in 15 codons located in the 3′ region (Van der Merwe *et al*., 2009). AvrL567 exhibits high amino acid sequence variability, with 27.5% of residues being polymorphic between variants, and DNA sequence analysis has revealed that this locus is also under significant positive diversifying selection (Dodds *et al*., 2006). Solution of the crystal structure of AvrL567 revealed that the side-chains of all the polymorphic amino acids are exposed on the surface of the molecule (Wang *et al*., 2007). Mutational analysis confirmed the role of several of these residues in controlling recognition specificity, and it appears that these specificities are mediated by multiple amino acid contacts in a quantitative manner (Wang *et al*., 2007). Consequently, evolution of virulent forms of AvrL567 could occur in a stepwise manner, where single amino acid changes in avirulent forms result in partially virulent forms (i.e. weakly recognized) that would be selectively advantageous to the rust, and subsequent amino acid changes could eventually result in complete virulence.

The *L* locus in flax is also highly polymorphic, with 131 sites (30 in the TIR-NBS region, 101 in the LRR domain) being under significant positive selection (M. Ravensdale, unpublished data). Wang *et al*. (2007) utilized the known structure of internalin A as a template for building a hypothetical structural model for the LRR domains of L5 and L6. These hypothetical L5 and L6 LRR models were then used to develop docking models for AvrL567-A–L5 and AvrL567-A–L6 interactions. This has resulted in a list of amino acid residues found in the LRRs of L5 and L6 that could make contact with AvrL567-A. Superimposition of the 101 positively selected LRR residues onto the model of potential interacting residues has highlighted specific regions that may be involved in the interaction (Fig. 1). These sites can be evaluated in domain swap and mutation experiments. As Avr567 interacts with L5 and L6 in yeast, it should be possible to use the yeast two-hybrid interaction to select for mutagenized L proteins with novel recognition specificities. A similar approach was successful in generating a variant of the potato Rx resistance protein with novel viral coat protein recognition specificities (Farnham and Baulcombe, 2006).

CO-EVOLUTION IN A NATURAL GENE-FOR-GENE SYSTEM

Gene-for-gene resistance in *L. marginale*

In addition to cultivated flax, *M. lini* also infects the related wild flax *L. marginale*, a short-lived perennial herb endemic to Australia. Both molecular and pathogenicity data indicate that the interaction between *M. lini* and wild flax represents a gene-for-gene association that is similar to, but evolutionarily differentiated from, the interaction between *M. lini* and cultivated flax. Of 15 pathogen isolates sampled from different *L. marginale* populations, only three were able to cause infection on a set of 25 *L. usitatissimum* rust resistance gene differential lines (Lawrence, 1989). In contrast, a set of 46 *L. marginale* lines, derived from

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across the geographical range of the native host, were all susceptible to at least several rust isolates, and six lines were susceptible to all isolates tested (Lawrence and Burdon, 1989). Furthermore, on *L. marginale*, isolates of *M. lini* display a variety of infection phenotypes, in terms of extent of sporulation and damage to the host plant, not seen on *L. usitatissimum*, causing, in some cases, the loss of older leaves at the bottom of the stem (Lawrence and Burdon, 1989). This differentiation between rust isolates from cultivated and wild flax is also seen in patterns of DNA variation in pathogen avirulence genes, with distinct *Avr* variants occurring in wild populations (Barrett *et al*., 2009; Dodds *et al*., 2006). The large variation in resistance and virulence phenotypes further suggests that *L. marginale* and *M. lini* have co-evolved for a long time, and therefore *M. lini* is unlikely to be a recent introduction to Australia (Barrett *et al*., 2008b; Lawrence and Burdon, 1989). As in the cultivated flax system, rust resistance in *L. marginale* results from single dominant genes, with a minimum of 17 different *R* genes or alleles (Burdon, 1994).

Impact of life history on host–pathogen interactions at the population level

Host–pathogen co-evolution is likely to be strongly influenced by life history factors, such as environmental conditions, effective population sizes (the number of individuals in a population that contribute offspring to the next generation) and pathogen dispersal mechanisms, which have been characterized extensively in the wild flax pathosystem. *Linum marginale* is found across Australia in various habitats, including eucalypt forests and savannah, open alpine areas covered with snow for several months a year, coastal sand dunes and along water courses in more arid inland areas (Lawrence and Burdon, 1989). *Melampsora lini* is also found across this extensive geographical and habitat range. Dikaryotic rust urediospores are wind dispersed and their rapid propagation can lead to local epidemics, with up to eight asexual reproduction cycles in a growing season. In colder and wetter environments (e.g. subalpine regions, referred to as the 'Mountains'), plants flower in mid- to late summer before the first autumn frosts induce a large fraction of them to die, causing significant and abrupt crashes in pathogen numbers. Here, plants overwinter as underground rootstocks with or without a few green shoots protected from frost by the surrounding vegetation. These shoots may carry occasional pustules over to the next growing season, but there is also a significant probability of local loss of the pathogen. In contrast, in environments with hot and dry summers and mild winters (e.g. drier inland regions, referred to as the 'Plains'), epidemics start earlier, last longer and are often more severe. The sexual cycle can be initiated late in the season as above-ground shoots die back during the summer drought. This results in the production of sclerotic diploid teliospores that are resistant to environmental extremes. The decline in pathogen numbers is not as drastic as in the 'Mountains' region.

Several lines of evidence indicate that the pathogen has the potential to impose severe selection on the population structure of native hosts. Disease incidence varies in space and time, ranging from virtually nonexistent to epidemic levels; such patterns are consistent with varying patterns of co-evolution across landscapes (Burdon and Thompson, 1995). Within local populations, the incidence, prevalence and disease severity start low and increase throughout the season, favoured by humid conditions that may result in epidemics with a prevalence of approximately 100% vs. approximately 20% for dry years (Jarosz and Burdon, 1992). The host survival rate is not affected by infection during the growing season, but over-winter plant survival may be as low as 20%–30% in years of severe epidemics, in contrast with 80%–90% for a growing season with low pathogen pressure (Jarosz and Burdon, 1992). In epidemic years, disease also affects host population demography and population structure (see below). Younger plants have fewer stems and, as a result, they are less prone to infections than older (and larger) individuals, increasing their chances of survival over the winter in years of severe epidemics (Jarosz and Burdon, 1992). Following severe epidemics, host population size is often greatly reduced. Smaller populations (<100 individuals) are less likely to be infected than medium or large populations (up to 5000 individuals) in years of mild epidemics (Burdon and Jarosz, 1992). This may result from bottlenecks occurring at the end of the growing season. If too few infected hosts support overwintering pathogens, the pathogen may go extinct. Given the reduced pathogen pressure, the host population size is likely to increase, rendering the host population more prone to infection.

Patterns of resistance and virulence at multiple geographical scales

Host populations represent discrete groups that are genetically and phenotypically differentiated. For example, extensive inoculation studies have shown that, in the 'Mountains' region, with regard to resistance structure, populations range from being nearly monomorphic to having at least 18 resistance phenotypes (Burdon and Thompson, 1995; Jarosz and Burdon, 1991). The number of resistance phenotypes in a population shows spatial and temporal variation, indicating that host populations follow different ecological and evolutionary trajectories despite close geographical distance. This suggests that selection or drift can be stronger than gene flow. Indeed, following severe epidemics, the resistance structure of host populations can be significantly more diverse (Burdon and Thompson, 1995). In turn, among-population variation in host resistance is a major determinant of the severity of pathogen epidemics (Thrall and Burdon, 2000).

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Taken together, it appears that *M. lini* exerts a mild to very strong selective pressure on populations of *L. marginale*, variable in space and time, shaping host population size, demography and genetic structure. Surprisingly, the predicted effects of selection are not consistently detected, e.g. after an epidemic in which a significant number of hosts die over the winter as a result of infection, the surviving hosts may not be more resistant to pathotypes from the previous year. Conversely, selection imposed by resistance on pathogen isolates at the local scale may be reduced by the large dispersal capacity of the pathogen. Therefore, selection may only be detected when looking at the metapopulation level, as a result of pathogen selective pressure competing with selection imposed by other environmental parameters at a local and short timescale.

Genetic analysis of *M. lini* isolates collected from *L. marginale* populations across Australia has revealed the existence of at least two distinct pathogen lineages (termed AA and AB). Lineage AB appears to have originated from hybridization between lineage AA and an extinct or as yet unidentified lineage BB (Barrett *et al*., 2007). The two lineages AA and AB are, for the most part, in geographical isolation from each other, with hybrids occurring mostly in areas of cool temperate climate with annual rainfall above 880 mm, whereas nonhybrids are found in hotter drier environments with under 640 mm of rainfall. No sexual reproduction was observed in the 'Mountains', where hybrids are prevalent, but extensive sexual reproduction was found in the 'Plains', where hybrids are present only at low frequencies (Barrett *et al*., 2007). Accordingly, AB isolates show a fixed pattern of heterozygosity (one A and one B allele at corresponding microsatellite loci). The extent to which differences in pathogen mating system are under genetic vs. environmental control is still unclear, although both field surveys and glasshouse inoculation studies indicate that telial formation (the precursor stage for sexual reproduction) is largely genetically determined (Barrett *et al*., 2008a). High temperatures appear to be the main environmental factor triggering telial formation in lineage AA (A. Nemri, unpublished data), and current studies have aimed to assess whether lineage AB isolates are able to proceed beyond telial formation to complete the sexual cycle. The implication is that differences in reproductive strategies and geographical distribution contribute to divergent evolutionary trajectories, and could result in the observed regional differences in virulence and diversity. Over time, this could lead to further specialization on hosts and subsequent host adaptation and, eventually, pathogen speciation.

Ecological differentiation within the host also occurs. Thus, within the 'Mountains' region, two morphologically discrete ecotypes (termed 'bog' and 'hill') occur at close geographical distances, but in different environments, e.g. in terms of soil moisture [A.L. Laine (University of Helsinki, Helsinki, Finland) and P.H. Thrall, unpublished data]. Populations of the 'hill' ecotype show distinctly more resistance than populations of the neighbouring 'bog' ecotype; their associated pathogen populations show correspondingly higher virulence (Thrall *et al*., 2001), but also have a environment-independent lower survival rate, as shown by transplant studies (Carlsson-Graner *et al*., 1999). Host differentiation in reproductive strategies is evident at larger geographical scales. Thus, the 'Plains' metapopulation exhibits a significant level of outcrossing, whereas plants in the 'Mountains' metapopulation are essentially inbred (Burdon *et al*., 1999). Such differences in mating system are associated with marked differences in the level and structure of resistance within and among host populations and metapopulations, which match observed differences in pathogen mating system, diversity and virulence.

Consistent with these local and regional patterns of differentiation in life history, phylogeny and variation in resistance and virulence in *L. marginale* and *M. lini*, adaptation between the pathogen and its host (a signature of co-evolution) has also been demonstrated at multiple spatial scales. This includes continental (Burdon *et al*., 2002; Lawrence and Burdon, 1989), among regions ('Plains' vs. 'Mountains'; (Barrett *et al*., 2007; Burdon *et al*., 1999) and within a single metapopulation ['bog' vs. 'hill' (Carlsson-Graner *et al*., 1999); or local adaptation (Thrall *et al*., 2002)]. For example, at the continental scale, rust isolates from Victoria and southern New South Wales are significantly more virulent on hosts from these regions than are isolates from other parts of Australia. Similarly, closely adjacent inbreeding host populations in the 'Mountains' metapopulation were found to be more similar with regard to the relative abundance of particular resistance phenotypes than they were with more distant populations. At very local scales, the mating system has also been demonstrated recently to have an impact on the within-population structure of resistance (A. Nemri, unpublished data). It is somewhat puzzling that, despite the broad dispersal ability of the pathogen relative to that of the host (as evidenced by the lack of distance-dependent distribution patterns for virulence phenotypes in associated pathogen populations; (Burdon and Jarosz, 1991; Thrall and Burdon, 2003), the most virulent pathotypes do not generally dominate host populations. This contrasts with agricultural crop systems in which supervirulent pathogens rise and disperse globally over relatively short timescales (often within a few years). This discrepancy may be explained by an evolutionary trade-off between infection strategies, which is suggested by glasshouse inoculation studies revealing a negative relationship between virulence and spore production (Thrall and Burdon, 2003). It is not known whether this negative correlation can be explained by avirulence effectors negatively contributing to pathogen fitness in their virulence form, although preliminary evidence suggests that there is no detectable fitness penalty to silencing *AvrL6* in a host background lacking *L6* (Lawrence *et al*., 2010b).

Partial infections, i.e. infections in which the pathogen cannot achieve maximum spore production, are commonly

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observed in glasshouse single-spore inoculation studies. Analysis of data from multiple populations in the *Linum–Melampsora* system has shown that the number of partial infection phenotypes relative to the total number of resistant responses is likely to be greater when hosts are challenged with sympatric rather than allopatric pathogen isolates (Antonovics *et al*., 2010). Some host populations have a high prevalence of partial resistance genes compared with the average across the metapopulation, whereas some pathogen populations contain primarily isolates that are unable to cause a severe infection regardless of host origin (Antonovics *et al*., 2010). The occurrence of partial infection phenotypes in different host–pathogen population pairs thus depends on the host, the pathogen and the interaction. These differences constitute an important, yet so far poorly understood, aspect of the co-evolutionary interaction between host and pathogen.

Avr gene diversity in natural populations

Melampsora lini isolates from the wild pathosystem contain homologues of the *AvrL567*, *AvrM*, *AvrP123* and *AvrP4* genes identified from the cultivated pathosystem. However, the patterns of selection differ between these genes. A geographically diverse set of isolates all contained the same *AvrM* variant, suggesting that *AvrM* does not contribute to the outcome of the gene-for-gene interaction, probably because there are no *R* genes in the *L. marginale* populations that recognize this *Avr* gene (Dodds and Thrall, 2009). Some variation has been observed amongst *AvrL567* homologues in rust isolates from *L. marginale*, but the complex nature of this multicopy locus has made genetic characterization at the population level more difficult. However, isolates of *M. lini* from *L. marginale* populations show extensive variation and a signature of strong positive selection at the *AvrP4* and *AvrP123* loci (Barrett *et al*., 2009). Transient expression of these *AvrP123* and *AvrP4* variants in *L. marginale* plants can trigger HRs in some host genotypes, suggesting that there is differential recognition of these genes by *R* genes in host populations (Barrett *et al*., 2009). It is not yet known whether these recognition specificities are mediated by homologues of the *P* genes from *L. usitatissimum*. However, there is among-population variation in the frequency of recognition of *AvrP123* and *AvrP4* variants, and also considerable year-to-year variation within populations of *L. marginale* (M. Ravensdale *et al*., unpublished data). Likewise, there is variation in the frequency of different *AvrP123* and *AvrP4* variants between rust populations (Barrett *et al*., 2009). This suggests the possibility of strong selection acting on the cognate *R* and *Avr* genes in this system. The spatial and temporal variation in the cognate *R* genes could explain the maintenance of the observed polymorphism at *AvrP4* and *AvrP123*.

CONCLUDING REMARKS

The combination of in-depth molecular- and population-level information makes the flax–rust disease system a powerful model for understanding host–pathogen co-evolution. Detailed knowledge of the molecular interaction between R and Avr proteins in cultivated flax provides a framework for understanding the selective forces underlying the population-level variation that is observed in the wild flax–rust interaction. There still remain many challenges to the integration of these data. For instance, although we know that *Avr* genes identified from cultivated flax rust also operate in the wild system, we do not yet know whether the corresponding *R* genes in *L. marginale* are also homologues of the cultivated flax *R* genes. It will be important in the future to correlate the phenotypic analysis of wild populations with DNA-based studies of the distribution of specific *R* and *Avr* genes. Likewise, much knowledge still needs to be gathered to enable the generalization of the findings from this system to other plant–pathogen interactions, including those occurring in agricultural crop systems. For the management of crop diseases, information on dispersal, disease dynamics and the virulence structure of rust populations is crucial. In the long term, the determination of how the selective pressure imposed by host genetic structure and other life history components shapes rust adaptation and evolution is also critical (Barrett *et al*., 2008b). Although considerable research effort has focused on major *R* genes, little is known about the epidemiological or evolutionary consequences of gene-for-gene interactions that result in partial infection, even though they have the potential to significantly impact disease dynamics and pathogen adaptation. The integration of the molecular understanding of gene-for-gene interactions with population genetics in the flax–rust system is now providing a powerful approach for measuring the effects of gene-for-gene co-evolution. However, such studies also need to account for other selective factors in the environment, i.e. life history, with which they probably interact. A specific experimental challenge will be to find the spatial and temporal scales at which one can verify predictions emerging from the arms race model in the context of co-evolving metapopulations of hosts and pathogens. This will be necessary not only to address issues such as the maintenance of host and pathogen diversity, but also to explain the modality of the arms race and the maintenance of sexual reproduction in the pathogen and outcrossing in the host with respect to the Red Queen hypothesis (Van Valen, 1973).

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GLOSSARY BOX

Definitions

Avr proteins: pathogen effectors that are recognized by host R proteins.

Co-evolutionary arms race in host–parasite interactions: an asymmetrical genetic interaction in which the fitness of the host is negatively correlated with the fitness of the parasite. To try to increase its fitness, the host evolves defence mechanisms, such as resistance, that have to be circumvented by the pathogen by evolving virulence mechanisms, such as effector-triggered immunity. This results in the constant accumulation of defence mechanisms on the host side and virulence mechanisms on the pathogen side. At the gene level, it is characterized by the rapid and continuous fixation of novel mutations with advantageous effect.

Diversifying selection: characterized by alleles at both extremes of a phenotype spectrum being selected at the same time, whereas individuals with alleles encoding intermediate phenotypes would be selected against.

Effectors: pathogen proteins that are produced to interfere with host processes and allow disease establishment.

Effector-triggered immunity: an immune response triggered by R–Avr recognition.

Metapopulation: a group of populations located at a geographical distance that permits the genetic exchange of propagules (pollen, seeds, spores) between them.

Positive selection: a type of directional selection in which one allele is favoured and rises from rare to predominant in a population. Genes under positive selection typically have a high ratio of nonsynonymous mutations (Ka/Ks).

Virulence (as used in plant pathology) describes the capability of causing infection, and aggressiveness refers to the reproductive output of the pathogen. Pathogen fitness is referred to as aggressivity.

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