Published in final edited form as:

Curr Opin Plant Biol. 2014 April ; 18: 24–30. doi:10.1016/j.pbi.2013.12.003.

Genomic variability as a driver of plant–pathogen coevolution?

Talia L Karasov^{1,4}, Matthew W Horton², and Joy Bergelson^{1,3}

¹University of Chicago, Chicago, IL 60637, USA

²Gregor Mendel Institute, Dr. Bohr-Gasse 3, 1030 Vienna, Austria

³Department of Ecology & Evolution

⁴Committee on Genetics, Genomics and Systems Biology

Abstract

Pathogens apply one of the strongest selective pressures in plant populations. Understanding plant–pathogen coevolution has therefore been a major research focus for at least sixty years [1]. Recent comparative genomic studies have revealed that the genes involved in plant defense and pathogen virulence are among the most polymorphic in the respective genomes. Which fraction of this diversity influences the host–pathogen interaction? Do coevolutionary dynamics maintain variation? Here we review recent literature on the evolutionary and molecular processes that shape this variation, focusing primarily on gene-for-gene interactions. In summarizing theoretical and empirical studies of the processes that shape this variation in natural plant and pathogen populations, we find a disconnect between the complexity of ecological interactions involving hosts and their myriad microbes, and the models that describe them.

Introduction

Pathogens proliferate within plant tissues by secreting effectors — virulence-associated proteins, RNAs and metabolites — that down-regulate basal defenses. Plants, in turn, employ myriad defense mechanisms to prevent pathogenic proliferation. Studies of plant–pathogen co-evolution have repeatedly implicated one class of interaction, the gene-for-gene interaction, as a primary interface at which pathogens evolve to evade detection, and plants evolve to improve detection. In this interaction, plants encode resistance (*R*) products that either directly or indirectly recognize the action of specific effectors. This recognition, which requires the presence of a specific *R* gene and a specific effector, induces localized cell death (hypersensitive response; HR) and a systemic defense response [2]. In this review we will focus primarily on the well-studied gene-for-gene interaction, relating to other means of plant–pathogen interaction only tangentially.

Plants encode hundreds of *R*-genes with differing specificities, and pathogens encode dozens to hundreds of effectors [3,4,5]. These genes vary widely in copy number, single nucleotide polymorphisms (SNP) [4,6,7], and expression levels [8] among individuals. This variation has the potential to impact disease epidemiology; host resistance

polymorphisms can dampen pathogen spread [9] and virulence polymorphisms can determine host range [10]. In this review, we describe genomic variability in plants and microbes, and the mechanisms that generate and maintain this variability. We highlight recent breakthroughs that reveal complexities critical to the evolution of naturally occurring plant–microbe interactions.

Multiple processes maintain genomic variation

Both plant *R*-genes and pathogen effectors are located in genomic regions that undergo exceptional rates of mutagenesis. As a result, *de novo* diversity is generated quickly, providing material to allow rapid adaptation on the part of each species, but simultaneously creating variability that is maladaptive or neutral. Determining the impact of this diversity remains a central challenge.

Mutagenesis and selection in pathogens

Infection loci in pathogens are hypermutagenic—Effectors frequently contain high SNP diversity, many small insertions and deletions (indels), and presence-absence polymorphisms [4•,11] among isolates. As an example, whole genome sequencing of 19 phylogenetically diverse isolates of *Pseudomonas syringae* identified 58 type III secreted effectors (T3SE), only five of which were common to all isolates [4•]. Effectors in the Xanthomonads also vary widely among species and pathovars [12]. How is this variability generated?

In general, effectors are expected to mutate frequently, enabling pathogens to avoid detection within extant hosts or adapt to new hosts [13–15]. These virulence loci are often located in genomic islands or on separate chromosomes [13] that can be reshuffled, transferred between pathogens or lost from the genome [16]. In bacteria, effectors and pathogenicity islands tend to insert into tRNA genes that are targets for integration and excision. For oomycetes and fungi, effectors commonly lie in gene-sparse, repeat-driven regions, which presumably allows duplications, deletions, or gene fusions to occur without major fitness penalties [15].

Indeed, the loss and gain of effector-containing plasmids and islands can be observed after a few *in planta* passages [17]. This rampant exchange of genetic material can result in chimeric genomes, as exemplified by *Xanthomonas* species, which contain genes from groups as disparate as the Archaea and Eukarya [18]. It can also result in the immediate transfer of virulence to a previously non-pathogenic strain [13]

Selection on variability in pathogens is different between agricultural and natural ecosystems—Our view of how selection shapes variation at virulence genes is biased by agricultural systems, where most studies have focused. Several agriculturally relevant pathogens have undoubtedly experienced selection. For example, the recent deletion of the avirulence locus *AvrLm1* has nearly swept to fixation (~90%) in the fungus that causes stem canker in rapeseed [19]. Similarly, *P. syringae* pv. *tomato* is starting to overcome field resistance conferred by the tomato genes *Pto* and *Prf* [20]. Indeed, even though naturally occurring effector loci harbor extensive variability [21], most agricultural

research suggests that effectors quickly sweep to fixation in a manner consistent with an arms-race (but see [22]). This is inconsistent with the variation that is generated and segregates in nature. What explains the discrepancy?

First, plant–pathogen interactions are expected to differ between agricultural and natural ecosystems. Both theoretical and empirical studies have demonstrated that genetically heterogeneous plant populations, the norm in natural communities, are less susceptible to disease than plants grown in monoculture [9], as is common in agriculture. This lack of host diversity provides a uniform host environment for the pathogen, allowing for the selective sweep of effector loci beneficial in that one host background.

More importantly, as the major determinants of host specificity, effectors contribute to niche expansion [10]. Consistent with this, the effector repertoire of a single pathovar, found on a single host, is less extensive than the effector repertoire of the pathogen species when all hosts are considered [4••]. While selective sweeps may be apparent as a pathovar adapts to a particular host-genotype (i.e. in an agricultural setting), they should be less obvious when considering adaptation in the face of genetically variable hosts and pathogens. What's more, the presence of effectors can shape interactions within the microbial community [23]. The complexity of species interactions in natural communities complicates analyses of plant–pathogen interactions, a point described in more detail below.

Mutagenesis and selection in plants

Resistance loci in plants are hypermutagenic—*R*-genes are the most polymorphic loci in plant genomes [6,24], largely because they occur in clusters of paralogous copies [5,25]. Their high sequence similarity and repetitive sequences (e.g. the leucine rich repeat (LRR) domain common to most *R*-genes) predisposes them to slippage and non-allelic homologous recombination, and leads to their frequent turnover and diversification (fusion, duplication, or deletion) [26,27]. Furthermore, the highest frequency of deleterious mutations, including premature stop codons or indels resulting in frameshifts, fall within members of the NBS-LRR, F-box and Receptor-like kinase (RLK) gene families [8], all of which have been implicated in disease resistance.

R-genes exhibit extreme variability not only at the sequence level, but also at the expression level. In *A. thaliana* the NBS-LRR gene family shows the highest levels of differentially methylated regions [28••] and gene expression differences [8] among individuals.

Plant resistance loci frequently show a signature of balancing selection—

Allelic variation in many *R*-genes is likely to be ecologically and functionally relevant. Population genetic analyses in several plant species provide insight into the adaptive significance of this variation, identifying dozens of *R*-genes likely to have undergone diversifying selection [29–32]. For example, elevated *Ka/Ks* ratios are common in the LRRs of paralogous *R*-genes and suggest the rapid diversification of LRRs within a species [31]. Indeed, it has recently been shown that a diverse repertoire of functional *R* alleles, capable of recognizing a quickly evolving pathogen, occurs not only within, but also between, host species [33••]. Finally, elevated *Ka/Ks* ratios among orthologs suggest that related species may have evolved specificities to different virulence factors.

Interestingly, *R*-genes and other immune genes frequently exhibit a signature resembling balancing selection (Figure 1, Table 1) [34,35••,36,37,38••,39–46]. A clear example of this signature surrounds the gene *RCR3* in the wild tomato species *Solanum peruvianum*. *RCR3* is a cysteine protease cleaved by effectors from several pathogen species. Several complex haplotypes at *RCR3* coexist within populations, and the proteins encoded by these different haplotypes are cleaved at similar rates by the pathogen effectors. These protein variants differ, however, in the level of defense response induced by this cleavage. These haplotypes exhibit high values of Tajima's *D*, suggesting a role of balancing selection in maintaining quantitative variation in the stimulation of a defense response [35••]. This example is notable in revealing the evolution not of sites involved in pathogen recognition, but instead in the level of the response triggered by this recognition [41]. Huard-Chauveau *et al.* [38••] similarly show a signature suggestive of balancing selection acting on the expression level of a gene involved in resistance to *Xanthomonas campestris*.

Several of the balanced resistance polymorphisms studied to date appear to be millions of years old, even persisting as trans-species polymorphisms [36,43,47–49]. The long-term maintenance of these polymorphisms indicates the ubiquity and stability of the dynamics that generated them.

Why does host–pathogen coevolution result in signatures of balancing selection?

Many processes generate signatures of balancing selection

What does the ubiquitous signature of balancing selection indicate about host–pathogen dynamics? The signature of balancing selection itself does not reveal the dynamics that produced it; very different adaptive processes can generate similar genomic signatures (Figure 2). Balancing selection indicates the maintenance of co-occurring alleles, which may result from heterozygote advantage, frequency-dependent selection, or environmental fluctuations that alternately favor different resistance and virulence alleles [50,51]. Local adaptation [52–54] and metapopulation dynamics [55] can also yield a signature that resembles balancing selection when a global population is considered, further complicating analyses.

How do we determine the dynamics underlying a signature of balancing selection?

Two alternative approaches have been adopted to disentangle the processes behind a signature of balancing selection. First, finer analysis of molecular variation can point to particular dynamics [56]. Stahl *et al.* [47] suggested that the ancient balanced polymorphism at *RPM1* in *A. thaliana* was maintained by frequency-dependent selection due to a skew in the molecular variation segregating within *R* and *S* classes. By embedding an ecological model within a coalescent framework, such a skew was shown to be consistent with the fluctuations that result from frequency dependence. In contrast, high F_{st} values associated with variation at the *RCR3* locus in tomato suggest that its variability has been maintained by local adaptation [35••].

Second, one can ask whether the assumptions of models generating balancing selection are consistent with particular host–pathogen systems. Most models require tradeoffs in resistance and virulence [57,58,59], and evidence for these tradeoffs is mounting. For example, *R*-genes increase the fitness of infected plants [60] but incur an energetic cost that reduces fitness in the absence of infection [49]. Similar tradeoffs have been observed in pathogens [61].

In one of the few studies that have investigated these dynamics in field conditions, Thrall *et al.* [62••] tracked the allele frequencies of resistance genes and effectors over a six-year period in the Australian native flax (*Linum marginale*) and its fungal rust pathogen (*Melampsora lini*). They found rapid adaptation of the host and cycling polymorphisms in both the host and the pathogen. After challenging host populations from multiple years with pathogen strains from multiple years, they failed to find any systematic increase in pathogen virulence over time, suggesting the lack of an arms race. In another field study, Tack *et al.* [63] found that the interaction between *Plantago lanceolata* and its obligate pathogen *Podosphaera plantaginis* is shaped by frequency dependent selection on resistance traits. Although their study populations spanned hundreds of kilometers and exhibited genetic isolation by distance, most phenotypic variation in defense was maintained within host populations. Both of these studies point to the longterm maintenance of polymorphisms locally.

Plants interact with many pathogen species simultaneously

Pathogens rarely specialize on one host species

Whereas most studies of coevolution focus on the dynamics of tightly interacting pairs, host–pathogens in nature lie on a spectrum of specificities, with tight coupling accounting for fewer than half [64]. Without a tight, obligate association, it is not given that a pathogen will evolve in response to changes in a particular host species. Kniskern *et al.* [65] illustrate this point in showing that the ubiquitous pathogen *Pseudomonas syringae* is maladapted to its *A. thaliana* host in the Midwestern USA. That is, Midwestern *A. thaliana* are more resistant to the local *P. syringae* strains than to non-native *P. syringae* strains. It appears that *P. syringae* has tailored its infectivity to an alternative host or hosts rather than to the weedy, ephemeral, *A. thaliana*, which is unlikely to be a major host reservoir for this pathogen.

Hundreds/thousands of microbial species simultaneously interact with a plant

Another level of complexity lies in the enormous microbial diversity that shares a single host plant. *A. thaliana*, for example, hosts a microbial community consisting of thousands of species of microbiota [66]. The composition of this community is influenced by the surrounding environment, by the host genotype and by other microbes in the community [23,66,67].

A pathogen strain within that community will encounter not only host defenses but also those thousands of other microbial species [66,67], themselves competing and cooperating for the extraction of resources [23,68••]. All of these interactions complicate coevolutionary dynamics and call into question the relevance of models that consider the coevolution of a host with a single pathogen species.

Conclusion

Resistance and virulence genes exhibit exceptional levels of variation, in part due to the genomic processes that generate it and in part due to selection that promotes its maintenance. The persistence of this variation is unexpected on the basis of the dynamics that we see in agriculture, yet in nature there are clear indications of diversifying and balancing selection in action. Given the genetic and ecological complexities that we have touched upon, it is surprising that polymorphisms are maintained for long periods of time. While precious few examples exist to tease apart natural dynamics, we suggest that community level dynamics play a key role in explaining the ubiquity of virulence and resistance polymorphisms. Consideration of systems containing multiple hosts and pathogen species, interacting as assemblages, is an area ripe for future studies.

References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

- of special interest
- of outstanding interest

1. Flor HH. Current status of gene-for-gene concept. *Annu Rev Phytopathol.* 1971; 9:275–280.
2. Jones JD, Dangl JL. The plant immune system. *Nature.* 2006; 444:323–329. [PubMed: 17108957]
3. Kamoun S. A catalogue of the effector secretome of plant pathogenic oomycetes. *Annu Rev Phytopathol.* 2006; 44:41–60. [PubMed: 16448329]
- 4••. Baltrus DA, Nishimura MT, Romanchuk A, Chang JH, Mukhtar MS, Cherkis K, Roach J, Grant SR, Jones CD, Dangl JL. Dynamic evolution of pathogenicity revealed by sequencing and comparative genomics of 19 *Pseudomonas syringae* isolates. *PLoS Pathog.* 2011; 7:e1002132. Through sequencing the genomes of 19 pathovars of *Pseudomonas syringae* and subsequent functional testing, the authors identify the full complement of effectors in these diverse strains. This study reveals the rapid turnover of effector genes in *P. syringae* genomes even between closely related isolates, suggesting that these genes are frequently lost and gained, and that *P. syringae* can adapt rapidly to new hosts. [PubMed: 21799664]
5. Michelmore RW, Meyers BC. Clusters of resistance genes in plants evolve by divergent selection and a birth-and-death process. *Genome Res.* 1998; 8:1113–1130. [PubMed: 9847076]
6. Borevitz JO, Hazen SP, Michael TP, Morris GP, Baxter IR, Hu TT, Chen H, Werner JD, Nordborg M, Salt DE, et al. Genome-wide patterns of single-feature polymorphism in *Arabidopsis thaliana*. *Proc Natl Acad Sci U S A.* 2007; 104:12057–12062. [PubMed: 17626786]
7. Guo YL, Fitz J, Schneeberger K, Ossowski S, Cao J, Weigel D. Genome-wide comparison of nucleotide-binding site-leucine-rich repeat-encoding genes in *Arabidopsis*. *Plant Physiol.* 2011; 157:757–769. [PubMed: 21810963]
8. Gan X, Stegle O, Behr J, Steffen JG, Drewe P, Hildebrand KL, Lyngsoe R, Schultheiss SJ, Osborne EJ, Sreedharan VT, et al. Multiple reference genomes and transcriptomes for *Arabidopsis thaliana*. *Nature.* 2011; 477:419–423. [PubMed: 21874022]
9. Zhu Y, Chen H, Fan J, Wang Y, Li Y, Chen J, Yang S, Hu L, Leung H, Mew TW, et al. Genetic diversity and disease control in rice. *Nature.* 2000; 406:718–722. [PubMed: 10963595]
10. Baltrus DA, Nishimura MT, Dougherty KM, Biswas S, Mukhtar MS, Vicente J, Holub EB, Dangl JL. The molecular basis of host specialization in bean pathovars of *Pseudomonas syringae*. *Mol Plant Microbe Interact.* 2012; 25:877–888. [PubMed: 22414441]
11. Cai R, Lewis J, Yan S, Liu H, Clarke CR, Campanile F, Almeida NF, Studholme DJ, Lindeberg M, Schneider D, et al. The plant pathogen *Pseudomonas syringae* pv. tomato is genetically

- monomorphic and under strong selection to evade tomato immunity. *PLoS Pathog.* 2011; 7:e1002130. [PubMed: 21901088]
12. Bogdanove AJ, Koebnik R, Lu H, Furutani A, Angiuoli SV, Patil PB, Van Sluys MA, Ryan RP, Meyer DF, Han SW, et al. Two new complete genome sequences offer insight into host and tissue specificity of plant pathogenic *Xanthomonas* spp. *J Bacteriol.* 2011; 193:5450–5464. [PubMed: 21784931]
 13. Ma W, Dong FF, Stavrinides J, Guttman DS. Type III effector diversification via both pathoadaptation and horizontal transfer in response to a coevolutionary arms race. *PLoS Genet.* 2006; 2:e209. [PubMed: 17194219]
 14. Lovell HC, Jackson RW, Mansfield JW, Godfrey SA, Hancock JT, Desikan R, Arnold DL. In planta conditions induce genomic changes in *Pseudomonas syringae* pv. phaseolicola. *Mol Plant Pathol.* 2011; 12:167–176. [PubMed: 21199566]
 15. Raffaele S, Kamoun S. Genome evolution in filamentous plant pathogens: why bigger can be better. *Nat Rev Microbiol.* 2012; 10:417–430. [PubMed: 22565130]
 16. McCann HC, Guttman DS. Evolution of the type III secretion system and its effectors in plant–microbe interactions. *New Phytol.* 2008; 177:33–47. [PubMed: 18078471]
 17. Neale HC, Slater RT, Mayne LM, Manoharan B, Arnold DL. In planta induced changes in the native plasmid profile of *Pseudomonas syringae* pathovar phaseolicola strain 1302A. *Plasmid.* 2013; 70:420–424. [PubMed: 23895800]
 18. Lima WC, Paquola AC, Varani AM, Van Sluys MA, Menck CF. Laterally transferred genomic islands in Xanthomonadales related to pathogenicity and primary metabolism. *FEMS Microbiol Lett.* 2008; 281:87–97. [PubMed: 18318843]
 19. Gout L, Kuhn ML, Vincenot L, Bernard-Samain S, Cattolico L, Barbetti M, Moreno-Rico O, Balesdent MH, Rouxel T. Genome structure impacts molecular evolution at the AvrLm1 avirulence locus of the plant pathogen *Leptosphaeria maculans*. *Environ Microbiol.* 2007; 9:2978–2992. [PubMed: 17991027]
 20. Kunkeaw S, Tan S, Coaker G. Molecular and evolutionary analyses of *Pseudomonas syringae* pv. tomato race 1. *Mol Plant Microbe Interact.* 2010; 23:415–424. [PubMed: 20192829]
 21. Stukenbrock EH, McDonald BA. Population genetics of fungal and oomycete effectors involved in gene-for-gene interactions. *Mol Plant Microbe Interact.* 2009; 22:371–380. [PubMed: 19271952]
 22. Araki H, Tian D, Goss EM, Jakob K, Halldorsdottir SS, Kreitman M, Bergelson J. Presence/absence polymorphism for alternative pathogenicity islands in *Pseudomonas viridiflava*, a pathogen of *Arabidopsis*. *Proc Natl Acad Sci U S A.* 2006; 103:5887–5892. [PubMed: 16581904]
 23. Barrett LG, Bell T, Dwyer G, Bergelson J. Cheating, trade-offs and the evolution of aggressiveness in a natural pathogen population. *Ecol Lett.* 2011; 14:1149–1157. [PubMed: 21951910]
 24. Clark RM, Schweikert G, Toomajian C, Ossowski S, Zeller G, Shinn P, Warthmann N, Hu TT, Fu G, Hinds DA, et al. Common sequence polymorphisms shaping genetic diversity in *Arabidopsis thaliana*. *Science.* 2007; 317:338–342. [PubMed: 17641193]
 25. Meyers BC, Shen KA, Rohani P, Gaut BS, Michelmore RW. Receptor-like genes in the major resistance locus of lettuce are subject to divergent selection. *Plant Cell.* 1998; 10:1833–1846. [PubMed: 9811792]
 26. Nagy ED, Bennetzen JL. Pathogen corruption and site-directed recombination at a plant disease resistance gene cluster. *Genome Res.* 2008; 18:1918–1923. [PubMed: 18719093]
 27. Wicker T, Yahiaoui N, Keller B. Illegitimate recombination is a major evolutionary mechanism for initiating size variation in plant resistance genes. *Plant J.* 2007; 51:631–641. [PubMed: 17573804]
 - 28•. Schmitz RJ, Schultz MD, Urich MA, Nery JR, Pelizzola M, Libiger O, Alix A, McCosh RB, Chen H, Schork NJ, et al. Patterns of population epigenomic diversity. *Nature.* 2013; 495:193–198. A genome-wide profile of the methylomes and transcriptomes of *A. thaliana* accessions. NBS-LRR genes exhibit extreme variability in methylation and transcription levels, with differential methylation at these genes higher than any other class of genes in *A. thaliana* genomes. [PubMed: 23467092]
 29. McDowell JM, Dhandaydham M, Long TA, Aarts MG, Goff S, Holub EB, Dangl JL. Intragenic recombination and diversifying selection contribute to the evolution of downy mildew resistance at the RPP8 locus of *Arabidopsis*. *Plant Cell.* 1998; 10:1861–1874. [PubMed: 9811794]

30. Botella MA, Parker JE, Frost LN, Bittner-Eddy PD, Beynon JL, Daniels MJ, Holub EB, Jones JD. Three genes of the *Arabidopsis* RPP1 complex resistance locus recognize distinct *Peronospora parasitica* avirulence determinants. *Plant Cell*. 1998; 10:1847–1860. [PubMed: 9811793]
31. Dodds PN, Lawrence GJ, Ellis JG. Contrasting modes of evolution acting on the complex N locus for rust resistance in flax. *Plant J*. 2001; 27:439–453. [PubMed: 11576428]
32. Parniske M, Hammond-Kosack KE, Golstein C, Thomas CM, Jones DA, Harrison K, Wulff BB, Jones JD. Novel disease resistance specificities result from sequence exchange between tandemly repeated genes at the Cf-4/9 locus of tomato. *Cell*. 1997; 91:821–832. [PubMed: 9413991]
- 33•. Yang S, Li J, Zhang X, Zhang Q, Huang J, Chen JQ, Hartl DL, Tian D. Rapidly evolving R genes in diverse grass species confer resistance to rice blast disease. *Proc Natl Acad Sci U S A*. 2013 By cloning rapidly evolving R-genes from several grass species into rice the authors find that ~25% of R-genes from other species confer resistance in rice to strains of a rice pathogen. These results indicate that R-genes in a plant species can confer resistance to effectors found in pathogens of other plant species.
34. Rose LE, Langley CH, Bernal AJ, Michelmore RW. Natural variation in the Pto pathogen resistance gene within species of wild tomato (*Lycopersicon*) I. Functional analysis of Pto alleles. *Genetics*. 2005; 171:345–357. [PubMed: 15944360]
- 35•. Horger AC, Ilyas M, Stephan W, Tellier A, van der Hoorn RA, Rose LE. Balancing selection at the tomato RCR3 Guardee gene family maintains variation in strength of pathogen defense. *PLoS Genet*. 2012; 8:e1002813. Analyzing patterns of sequence and functional variation at the *RCR3* resistance locus in tomato species, the authors find evidence of selection on the interaction between an immune gene and its guard. This paper importantly illustrates that balancing or diversifying selection acts at several locations in immune response pathways, not only at the level of pathogen detection. [PubMed: 22829777]
36. Rose LE, Michelmore RW, Langley CH. Natural variation in the Pto disease resistance gene within species of wild tomato (*Lycopersicon*) II. Population genetics of Pto. *Genetics*. 2007; 175:1307–1319. [PubMed: 17179076]
37. Mauricio R, Stahl EA, Korves T, Tian D, Kreitman M, Bergelson J. Natural selection for polymorphism in the disease resistance gene *Rps2* of *Arabidopsis thaliana*. *Genetics*. 2003; 163:735–746. [PubMed: 12618410]
- 38•. Huard-Chauveau C, Perchevied L, Debieu M, Rivas S, Kroj T, Kars I, Bergelson J, Roux F, Roby D. An atypical kinase under balancing selection confers broad-spectrum disease resistance in *Arabidopsis*. *PLoS Genet*. 2013; 9:e1003766. Among the first studies to identify a resistance locus involved in quantitative resistance. This study reveals that signatures of balancing selection persist not only at qualitative trait loci (HR-associated). [PubMed: 24068949]
39. Allen RL, Bittner-Eddy PD, Grenville-Briggs LJ, Meitz JC, Rehmany AP, Rose LE, Beynon JL. Host–parasite coevolutionary conflict between *Arabidopsis* and downy mildew. *Science*. 2004; 306:1957–1960. [PubMed: 15591208]
40. Bakker EG, Toomajian C, Kreitman M, Bergelson J. A genome-wide survey of R gene polymorphisms in *Arabidopsis*. *Plant Cell*. 2006; 18:1803–1818. [PubMed: 16798885]
41. Van der Hoorn RA, De Wit PJ, Joosten MH. Balancing selection favors guarding resistance proteins. *Trends Plant Sci*. 2002; 7:67–71. [PubMed: 11832277]
42. Bittner-Eddy PD, Beynon JL. The *Arabidopsis* downy mildew resistance gene RPP13-Nd, functions independently of NDR1 and EDS1 and does not require the accumulation of salicylic acid. *Mol Plant Microbe Interact*. 2001; 14:416–421. [PubMed: 11277440]
43. Gos G, Slotte T, Wright SI. Signatures of balancing selection are maintained at disease resistance loci following mating system evolution and a population bottleneck in the genus *Capsella*. *BMC Evol Biol*. 2012; 12:152. [PubMed: 22909344]
44. Tian D, Araki H, Stahl E, Bergelson J, Kreitman M. Signature of balancing selection in *Arabidopsis*. *Proc Natl Acad Sci U S A*. 2002; 99:11525–11530. [PubMed: 12172007]
45. Rose LE, Bittner-Eddy PD, Langley CH, Holub EB, Michelmore RW, Beynon JL. The maintenance of extreme amino acid diversity at the disease resistance gene RPP13, in *Arabidopsis thaliana*. *Genetics*. 2004; 166:1517–1527. [PubMed: 15082565]

46. Bergelson J, Kreitman M, Stahl EA, Tian D. Evolutionary dynamics of plant R-genes. *Science*. 2001; 292:2281–2285. [PubMed: 11423651]
47. Stahl EA, Dwyer G, Mauricio R, Kreitman M, Bergelson J. Dynamics of disease resistance polymorphism at the Rpm1 locus of *Arabidopsis*. *Nature*. 1999; 400:667–671. [PubMed: 10458161]
48. Kuang H, Woo SS, Meyers BC, Nevo E, Michelmore RW. Multiple genetic processes result in heterogeneous rates of evolution within the major cluster disease resistance genes in lettuce. *Plant Cell*. 2004; 16:2870–2894. [PubMed: 15494555]
49. Tian D, Traw MB, Chen JQ, Kreitman M, Bergelson J. Fitness costs of R-gene-mediated resistance in *Arabidopsis thaliana*. *Nature*. 2003; 423:74–77. [PubMed: 12721627]
50. Tellier A, Brown JK. Stability of genetic polymorphism in host–parasite interactions. *Proc Biol Sci*. 2007; 274:809–817. [PubMed: 17251091]
51. Charlesworth D. Balancing selection and its effects on sequences in nearby genome regions. *PLoS Genet*. 2006; 2:e64. [PubMed: 16683038]
52. Holub EB. The arms race is ancient history in *Arabidopsis*, the wildflower. *Nat Rev Genet*. 2001; 2:516–527. [PubMed: 11433358]
53. North A, Pennanen J, Ovaskainen O, Laine AL. Local adaptation in a changing world: the roles of gene-flow, mutation, and sexual reproduction. *Evolution*. 2011; 65:79–89. [PubMed: 20731716]
54. Nemri A, Barrett LG, Laine AL, Burdon JJ, Thrall PH. Population processes at multiple spatial scales maintain diversity and adaptation in the *Linum marginale*–*Melampsora lini* association. *PLoS ONE*. 2012; 7:e41366. [PubMed: 22859978]
55. Damgaard C. Coevolution of a plant host–pathogen gene-for-gene system in a metapopulation model without cost of resistance or cost of virulence. *J Theor Biol*. 1999; 201:1–12. [PubMed: 10534431]
56. Grenfell BT, Pybus OG, Gog JR, Wood JL, Daly JM, Mumford JA, Holmes EC. Unifying the epidemiological and evolutionary dynamics of pathogens. *Science*. 2004; 303:327–332. [PubMed: 14726583]
57. Brown JK, Tellier A. Plant–parasite coevolution: bridging the gap between genetics and ecology. *Annu Rev Phytopathol*. 2011; 49:345–367. [PubMed: 21513455]
58. Tellier A, Brown JK. Spatial heterogeneity, frequency-dependent selection and polymorphism in host–parasite interactions. *BMC Evol Biol*. 2011; 11:319. [PubMed: 22044632]
59. Frank SA. Models of plant–pathogen coevolution. *Trends Genet*. 1992; 8:213–219. [PubMed: 1496557]
60. Gao L, Roux F, Bergelson J. Quantitative fitness effects of infection in a gene-for-gene system. *New Phytol*. 2009; 184:485–494. [PubMed: 19659661]
61. Wichmann G, Bergelson J. Effector genes of *Xanthomonas axonopodis* pv. *vesicatoria* promote transmission and enhance other fitness traits in the field. *Genetics*. 2004; 166:693–706. [PubMed: 15020460]
62. Thrall PH, Laine AL, Ravensdale M, Nemri A, Dodds PN, Barrett LG, Burdon JJ. Rapid genetic change underpins antagonistic coevolution in a natural host–pathogen metapopulation. *Ecol Lett*. 2012; 15:425–435. This is one of the only studies to date to track the coevolution of plant and pathogen populations over time. The authors find a clear lack of arms-race dynamics (in contrast to agricultural systems) and instead find the cycling of resistance and virulence polymorphisms. [PubMed: 22372578]
63. Tack AJ, Thrall PH, Barrett LG, Burdon JJ, Laine AL. Variation in infectivity and aggressiveness in space and time in wild host–pathogen systems: causes and consequences. *J Evol Biol*. 2012; 25:1918–1936. [PubMed: 22905782]
64. Barrett LG, Kniskern JM, Bodenhausen N, Zhang W, Bergelson J. Continuum of specificity and virulence in plant host–pathogen interactions: causes and consequences. *New Phytol*. 2009; 183:513–529. [PubMed: 19563451]
65. Kniskern JM, Barrett LG, Bergelson J. Maladaptation in wild populations of the generalist plant pathogen *Pseudomonas syringae*. *Evolution*. 2010; 65:818–830. [PubMed: 21044058]
66. Bodenhausen N, Horton MW, Bergelson J. Bacterial communities associated with the leaves and the roots of *Arabidopsis thaliana*. *PLoS ONE*. 2013; 8:e56329. [PubMed: 23457551]

67. Lundberg DS, Lebeis SL, Paredes SH, Yourstone S, Gehring J, Malfatti S, Tremblay J, Engelbrektson A, Kunin V, del Rio TG, et al. Defining the core *Arabidopsis thaliana* root microbiome. *Nature*. 2012; 488:86–90. [PubMed: 22859206]
- 68••. Mendes R, Kruijt M, de Bruijn I, Dekkers E, van der Voort M, Schneider JH, Piceno YM, DeSantis TZ, Andersen GL, Bakker PA, et al. Deciphering the rhizosphere microbiome for disease-suppressive bacteria. *Science*. 2011; 332:1097–1100. This study identifies the cause of *Rhizoctonia solani* disease suppression on beet plants in a field in the Netherlands. The authors determine that a toxin produced by a co-occurring *Pseudomonad* suppresses the expansion of the pathogenic fungus. The results of this study illustrate the profound effect of the microbial community on the interaction between a host and its pathogen. [PubMed: 21551032]

Figure 1.

Evaluating the allele frequency spectrum to assess evidence of selection on a locus. Balanced polymorphisms can be distinguished by the (excess) intermediate frequency SNPs that arise on the deepest branches (a) of a genealogy. In this example, because of the long branches, the genealogy on the right is expected to have a higher number of intermediate frequency SNPs than the tree on the left. These common SNPs will tilt Tajima's D (b), a commonly used test of selection, towards positive values, which are consistent with balancing selection (or complex population structure) and can be distinguished from neutrality using permutations or an outlier approach.

Figure 2.

Processes that maintain variation in plant genotypes. This figure presents the hypothetical expected allele frequency, and genomic signatures (outlier statistics) resulting from three distinct selective scenarios capable of maintaining diversity at a resistance locus. Only scenarios (a) and (c) are classically considered scenarios of balancing of selection. Most studies to date have been unable to distinguish between scenarios (a) and (b) in molecular evolution analyses.

Table 1

Examples of selection on *R* gene variation. This table summarizes the pattern of variation observed in intraspecies comparisons of *R*-gene orthologues in several plant species. $Ka > Ks$ is interpreted as evidence of diversifying selection, while an increased time to coalescence and elevated Tajima's *D* are signatures consistent with balancing selection. This table does not distinguish between the different processes that generate a signature resembling balancing selection, (see Fig. 1)

Species	Gene	Polymorphism type	Spp. recognized	Recognized effector	Putative selection	Evidence for selection	Reference
<i>Arabidopsis thaliana</i>	27 <i>R</i> -genes	Various	NA	NA	Balancing selection	~25% exhibit elevated Ka/Ks values, elevated Tajima's <i>D</i> , low F_{st}	[40]
<i>A. thaliana</i>	<i>RKS1</i>	SNPs	<i>Xanthomonas campestris</i>	NA	Balancing selection	Elevated Tajima's <i>D</i> 5' of gene	[38••]
<i>A. thaliana</i>	<i>RPM1</i>	Deletion of locus	<i>Pseudomonas syringae</i>	<i>avrRpm1</i>	Balancing selection	Increased time to coalescence of presence absence polymorphism	[47]
<i>A. thaliana</i>	<i>RPP13</i>	SNPs	<i>Hyaloperonospora arabidopsidis</i>	<i>ATR13</i> , others NA	Balancing selection, diversifying selection	$Ka > Ks$, increased time to coalescence	[42,45]
<i>A. thaliana</i>	<i>RPS2</i>	SNPs	<i>Pseudomonas syringae</i>	<i>avrRpt2</i>	Balancing selection	Increased time to coalescence at the 5' end of the LRR	[37]
<i>A. thaliana</i>	<i>RPS4</i>	SNPs	<i>Pseudomonas syringae</i> , <i>Colletotrichum higginsianum</i> , <i>Ralstonia solanacearum</i>	<i>avrRPS4</i> , others NA	Positive selection (selective sweep)	Reduced variation between alleles	[46]
<i>A. thaliana</i>	<i>RPS5</i>	Deletion of locus	<i>Pseudomonas syringae</i>	<i>avrPphB</i>	Balancing selection	Increased time to coalescence of presence/ absence polymorphism	[44]
<i>Capsella rubella</i> , <i>Capsella grandiflora</i>	Nine <i>R</i> -genes	Various	NA	NA	Balancing selection, diversifying selection	~20% exhibit increased time to coalescence, low F_{st}	[43]
<i>Lactuca sativa</i> (lettuce)	Several genes within RGC2 locus	SNPs, indels	NA	NA	Balancing selection	Trans-species polymorphism	[48]
<i>Lycopersicon</i>	<i>Pto</i>	NA	<i>Pseudomonas syringae</i>	<i>avrPto</i> , <i>avrPtoB</i>	Balancing selection	Elevated Pn/Ps levels, trans-species polymorphism	[34,36]
<i>Solanum peruvianum</i>	<i>RCR3</i>	SNPs	<i>Cladosporium fulvum</i> , <i>Phytophthora infestans</i>	<i>avr2</i> , <i>EPIC1.2B</i>	Balancing selection (possibly local adaptation)	Elevated Tajima's <i>D</i> 3' of gene, high F_{st}	[35••]