The evolution of virulence and pathogenicity in plant pathogen populations

SOLEDAD SACRISTÁN AND FERNANDO GARCÍA-ARENAL

Depto. de Biotecnología, E.T.S.I. Agrónomos and Centro de Biotecnología y Genómica de Plantas, Universidad Politécnica de Madrid, 28040 Madrid, Spain

SUMMARY

The term virulence has a conflicting history among plant pathologists. Here we define virulence as the degree of damage caused to a host by parasite infection, assumed to be negatively correlated with host fitness, and pathogenicity the qualitative capacity of a parasite to infect and cause disease on a host. Selection may act on both virulence and pathogenicity, and their change in parasite populations can drive parasite evolution and host–parasite co-evolution. Extensive theoretical analyses of the factors that shape the evolution of pathogenicity and virulence have been reported in last three decades. Experimental work has not followed the path of theoretical analyses. Plant pathologists have shown greater interest in pathogenicity than in virulence, and our understanding of the molecular basis of pathogenicity has increased enormously. However, little is known regarding the molecular basis of virulence. It has been proposed that the mechanisms of recognition of parasites by hosts will have consequences for the evolution of pathogenicity, but much experimental work is still needed to test these hypotheses. Much theoretical work has been based on evidence from cellular plant pathogens. We review here the current experimental and observational evidence on which to test theoretical hypotheses or conjectures. We compare evidence from viruses and cellular pathogens, mostly fungi and oomycetes, which differ widely in genomic complexity and in parasitism. Data on the evolution of pathogenicity and virulence from viruses and fungi show important differences, and their comparison is necessary to establish the generality of hypotheses on pathogenicity and virulence evolution.

INTRODUCTION

It is widely accepted that virulent parasites are able to infect and damage a host. Therefore, virulence is the key property of pathogens, and understanding the evolution of virulence has been a major goal in pathology, including plant pathology, for a long time. The evolution of virulence may determine important phenomena such as the emergence and re-emergence of pathogens, host switch and host range expansion, and overcoming host resistance, which may compromise the success of control strategies for infectious diseases of people and domestic animals and plants. Moreover, virulence evolution may also modulate the important role of pathogens in shaping ecosystem composition and dynamics (Bull, 1994; Ebert and Hamilton, 1996; Frank, 1996; Read, 1994). In spite of the importance of virulence for human, animal and plant health and welfare, there has been little agreement among scientists of various disciplines on the definition of virulence beyond an intuitive meaning. Both in animal pathology and in evolutionary biology, virulence is usually understood as related to the harm that parasite infection causes to the host and, more precisely, has been defined as the detrimental effect of parasite infection on host fitness (e.g. Read, 1994). In plant pathology the definitions proposed by Vanderplank (1968) had a long-standing influence: the quantitative negative effect of a pathogen on its host was named aggressiveness, while the term virulence was used to describe the capacity of a pathogen to infect a particular host genotype, what in evolutionary biology is usually termed infectivity (Gandon *et al.*, 2002; Tellier and Brown, 2007). However, the American Phytopathological Society has adopted the convention of defining pathogenicity as the ability of a pathogen to cause disease on a particular host (i.e. a qualitative property), and virulence as the degree of damage caused to the host (i.e. a quantitative property), assumed to be negatively correlated with host fitness (D'Arcy *et al.*, 2001). These were indeed the traditional definitions of pathogenicity and virulence in plant pathology, which may be traced back at least to H. H. Whetzel (see Hunt, 1994). Thus, the phytopathological community seems to be returning to a terminology more in line with other scientists

interested in the biology of hosts and parasites. We follow this terminology here, except that for gene-for-gene and matching-allele interactions, virulence is used for the pathogen genotype that is able to overcome a resistance factor.

Plant pathologists, particularly those involved with diseases of crops, have devoted much effort to understanding the evolution of pathogenicity, largely because it determines the success and durability of resistant cultivars for the control of plant diseases. The evolution of virulence has been comparatively neglected, and its study has focused mostly on wild host–pathogen systems. We will deal here with the evolution of both pathogenicity and virulence, which may be not independent phenomena. We focus this review on mycelial pathogens (i.e. fungi and oomycetes) and on viruses, because a large body of literature has dealt with the evolution of virulence in the populations of these pathogens rather than with the molecular genetics of virulence or pathogenicity, extensively explored with phythopathogenic bacteria. More importantly, viruses and fungi represent extremes of genomic complexity, which may condition their evolutionary potential.

During the last 30 years the population genetics and dynamics of pathogenicity and virulence in pathogen populations have been extensively analysed. Experimental analyses have been less abundant. In the different sections of this review we will first present the conceptual background on which theoretical analyses are based and the predictions of theoretical models, and we will continue with the available experimental evidence, which we will discuss in relation to theoretical predictions.

THE EVOLUTION OF VIRULENCE

Although plant pathologists have devoted considerable attention to the evolution of pathogenicity, the evolution of virulence has generally been the subject of animal pathologists and evolutionary biologists. Explaining virulence is fundamental to understanding the life history of parasites. Virulence does not represent any clear advantage for parasites, which depend on their hosts for survival and fitness, so that it is not obvious why parasites harm their hosts. A commonly accepted hypothesis is that virulence is an unavoidable consequence of parasite reproduction within the infected host (Lenski and May, 1994). If so, virulence would be linked to within-host multiplication, which is a major component of parasite fitness, and would be a selectable trait. However, high virulence resulting in high host mortality and morbidity will negatively affect between-host transmission, which is another major component of parasite fitness. Consequently, virulence will result in trade-offs between within-host multiplication and between-host transmission, the basis of the so-called trade-off hypothesis. The general validity of the trade-off hypothesis and its central assumption of a positive correlation between parasite multiplication and virulence has been questioned for more than a decade. Other alternative hypotheses have been proposed to explain virulence, taking into account the diversity of the life cycles of parasites and the specificities of the host–pathogen interactions (Bull, 1994; Ebert and Bull, 2003). However, since the seminal work of Anderson and May (1982), formal analyses of the evolution of virulence have been based on the trade-off hypothesis, which has resulted in a large body of theoretical work aimed at understanding different aspects of pathogen evolution and host–pathogen co-evolution. Notably, the role of epidemiological and ecological factors in virulence evolution has been extensively modelled. In contrast, experimental analyses of assumptions of the trade-off hypothesis, or the role of epidemiological and ecological factors in virulence evolution, are comparatively scarce and have yielded conflicting results. For plant–parasite systems, reports of experiments specifically designed to address the role of different factors on virulence evolution are not frequent, and most evidence derives from experiments with fungi and oomycetes. Here we will focus on two major points of virulence evolution: the relationship between parasite fitness and virulence, and the relationship between the adaptation of parasites to hosts and virulence.

The relationship between parasite fitness and virulence

The central assumption of the trade-off hypothesis is that within-host multiplication, within-host transmission and virulence of parasites are positively correlated traits (Frank, 1996). A potential problem in experimental analyses of this hypothesis is how to estimate pathogen virulence and fitness (Kawecki and Ebert, 2004). Virulence is the detrimental effect of parasite infection on host fitness. In animal–parasite systems, virulence measurement is usually simplified as increased host mortality due to parasite infection (Frank, 1996). The underlying concept is that a reduction in the life span of the host results in a decreased fecundity and, hence, a decrease in its fitness. Most plant pathogens do not cause an immediate increase in mortality, and virulence is most often estimated as the effect of pathogen infection on plant fecundity (i.e. seed production) or on one of its correlates, such as plant size or biomass. These different measurements of virulence should not affect the outcome of the theoretical models (Day, 2002). Pathogen fitness is usually measured as fecundity, which has two important components: within-host multiplication and between-host transmission. Parasite fecundity is most often estimated as within-host multiplication, for instance spore production per leaf area (e.g. Salvaudon *et al.*, 2005) or virus accumulation in infected tissues (e.g. Sacristán *et al.*, 2005). Within-host multiplication is assumed to be positively correlated with between-host transmission, which is immediate for spore-producing microbes. For vector-transmitted plant viruses it has also been shown that transmission efficiency is positively correlated with virus accumulation in source tissues (Escriu *et al.*, 2000; Pirone and Megahed, 1966).

Reports on the relationship between parasite virulence and fitness yield conflicting results. A positive correlation between virulence and within-host parasite multiplication has been reported occasionally. Examples include *Albugo candida* on *Brassica campestris* (Fox and Williams, 1984), *Cryphonectria parasitica* on chestnut (Peever *et al.*, 2000), *Phytopthora infestans* on potato (Montarry *et al.*, 2006), *Rice yellow mottle virus* (RYMV) on rice (Fargette *et al.*, 2002), *Beet necrotic yellow vein virus* on sugar beet (Heijbroek *et al.*, 1999) and *Maize streak virus* (MSV) on maize (Martin *et al.*, 2005). However, there are also numerous reports showing that parasite multiplication and virulence are uncorrelated, or even negatively correlated, in fungi as well as in bacteria and viruses (e.g. Carr *et al.*, 2006; Escriu *et al.*, 2003; Gal-On, 2007; Handford and Carr, 2007; Imhoff *et al.*, 1982; Kover and Schaal, 2002; Robert *et al.*, 2002; Rodriguez-Cerezo *et al.*, 1991; Sacristán *et al.*, 2005; Shi *et al.*, 2002; Zhan *et al.*, 2002). Hence, there is no evidence to assume that a relationship between parasite reproductive capacity and virulence is a universal trend.

One difficulty in the interpretation of these reports in relation to the trade-off hypothesis is that virulence is estimated as a reduction in plant biomass due to infection, or as symptom severity, instead of as a reduction in plant fecundity, which is a better estimate of fitness. However, the relationship between biomass and seed production may be non-linear and depend on both genetic and environmental factors (e.g. Pagán *et al.*, 2007; Schürch and Roy, 2004). Hence, the relationship between seed production and biomass should be analysed prior to the use of biomass reduction as an estimate of virulence. This is even more important if virulence is estimated as symptom severity determined on visual scales, the most commonly used correlate of virulence (Jarozs and Davelos, 1995). Although for lesion-forming foliar pathogens host fitness is assumed to be negatively correlated with percentage leaf area covered by lesions (Zhan *et al.*, 2002), in a few examples the relationship between symptom severity and biomass production, or between symptom severity and seed production, has been determined. Examples are analyses of the effects of *Puccinia triticina* and *Mycosphaerella graminicola* on wheat (Robert *et al.*, 2004, 2005) and of *Pseudomonas syringae* on Arabidopsis (Kover and Schaal, 2002).

Most analyses of the relationship between parasite fitness and virulence consider the interaction of one parasite genotype with several host genotypes, or vice versa, and do not test for possible interactions between host and parasite genotypes in the expression of phenotypes. However, all traits of host–parasite interactions, including virulence, may depend on the genotypes of both host and parasite (Lambrechts *et al.*, 2006; Restif and Koella, 2003). When different genotypes of the plant host and the parasite have been analysed, a correlation between virulence and parasite fitness has been found to occur in some systems only for specific host–parasite genotype × genotype interactions, e.g. for *My. graminicola* on wheat (Zhan *et al.*, 2002), *Melampsora larici-epitea* on salix (Pei *et al.*, 2002) or *Cucumber mosaic virus* (CMV) on Arabidopsis (Pagán *et al.*, 2007). In other host–parasite systems, a correlation between parasite fitness and virulence is a more general trait. Thus, Salvaudon *et al.* (2005) showed, for the interaction between two strains of *Hyaloperonospora parasitica* and seven accessions of Arabidopsis, that the more virulent parasite strain was that with the highest fecundity, in concordance with the trade-off hypothesis, although which strain was the most virulent depended on the host genotype. A positive relationship between parasite fitness and virulence may also be inferred from the analysis of genotype × genotype interaction reported for *Silene inflata* and *Microbotryum violaceum* (Kaltz and Shykoff, 2002), or from the analysis of the interaction between Arabidopsis and *Pseudomonas viridiflava* (Goss and Bergelson, 2006).

All the above analyses have focused on the parasitic phase of the parasite life cycle. However, selection during inter-epidemic, non-infectious or saprophytic stages of the life history of the parasite may have a role in its fitness and virulence. For example, evidence for differential selection of *Rhyncosporium secalis* genotypes during the parasitic and saprophytic phases of the fungus life cycle have been reported, determining the genetic structure of the fungus population (Abang *et al.*, 2006). Conversely, a trade-off between virulence and survival in infected tubers during inter-epidemic periods was not found for the biotroph *Ph. infestans* (Montarry *et al.*, 2007). These examples illustrate the complexity of the factors that should be considered in analyses of virulence evolution.

Although reports are rare and heterogeneous regarding host–parasite systems and experimental approaches, current data indicate that a positive correlation between parasite fitness and virulence has been reported less often for plant–parasite than for animal–parasite systems (Ebert and Mangin, 1997; Fenner and Ratcliffe, 1965; Jensen *et al.*, 2006; Lipsitch and Moxon, 1997; Mackinnon and Read, 2004). It has been proposed that the trade-off hypothesis would not be of general validity to plant–parasite interactions because plant material is not a limiting resource to most plant parasites (Jarozs and Davelos, 1995). If so, plants could re-allocate their resources to diminish the harm of parasitism. Accordingly, in the few analysed instances, the lack of correlation between parasite fitness and virulence was explained by genotype-specific tolerance in the host plants (Kover and Schaal, 2002; Pagán *et al.*, 2007), tolerance being defined as the host's ability to reduce the effect of infection on its fitness (Jeger *et al.*, 2006). This is in agreement with the prediction that non-linear tolerance to parasites and herbivores would result in no clear relationship between parasite multiplication and host damage (Miller *et al.*, 2006). Tolerance has been involved more often in interactions of plants with natural enemies than for animals (Miller *et al.*, 2006). If this reflects a real difference between plant–parasite and animal–

parasite interactions or a difference in the interests of plant and animal scientists is unclear at present.

The relationship between parasite virulence and adaptation of parasites to hosts

Analyses discussed in the previous section considered the interaction of one host plant species with one parasite species. In fact, most theoretical and experimental efforts to understand the population biology of parasites have focused on specialist parasites, i.e. those able to infect and multiply in hosts of one or a few related taxa. However, a large fraction of plant pathogens are multi-host parasites (García-Arenal and McDonald, 2003; McDonald and Linde, 2002), i.e. generalists, able to infect and multiply in host species belonging to different taxa, even to different kingdoms (e.g. plant viruses that multiply in their insect vectors). The ability to infect different hosts conditions the epidemiology and pathogenicity of generalist parasites, and host range is predicted to be a major factor in the evolution of virulence (Frank, 1996). A generalist strategy provides the parasite with more opportunities for transmission and survival, but it is predicted that evolution would favour specialism: different hosts represent different selective environments and parasite–host co-evolution could result in functional trade-offs that would limit the generalist fitness in any one host (Kirchner and Roy, 2002; Woolhouse *et al.*, 2001). Trade-offs in the adaptation to different hosts could result in differentiation of the pathogen population according to host. Parasite adaptation to hosts can occur at two levels: specialization (i.e. adaptation to different host species), or local adaptation (i.e. adaptation to different populations of the same host species), and is manifested by improved fitness of the parasite population in its original host species or population (Kawecki and Ebert, 2004). Conditions for local adaptation and its effect on host–parasite co-evolution have been the object of many theoretical analyses (e.g. Gandon *et al.*, 1996; Gandon and Michalakis, 2002).

Evidence for local adaptation according to the above criterium has often been reported for plant parasitic fungi and oomycetes in wild host populations and in crops, analyses of viral systems being rarer. Thus, local adaptation has been shown for *Synchitrium decipiens* on *Amphicarpea bracteata* (Parker, 1985), *Melampsora lini* on *Linum marginale* (Thrall *et al.*, 2002), *Mi. violaceum* on *Silene dioica* (Carlsson-Granér, 1997), *Colletotrichum lindemuthianum* on *Phaseolus vulgaris* (Sicard *et al.*, 2007), *Podosphaera plantaginis* on *Plantago lanceolata* (Laine, 2005), *My. graminicola* on wheat (Ahmed *et al.*, 1995; Zhan *et al.*, 2002) or *Ph. infestans* from potato populations in France and in Morocco (Andrivon *et al.*, 2007). Other studies did not provide evidence of local adaptation, e.g. for *Puccinia podophylli* on *Podophyllum peltatum* (Parker, 1989), *Rh. secalis* on wheat (Abang *et al.*, 2006) or *Ph. infestans* on potato from different locations in France (Montarry *et al.*, 2006). Maladapation, i.e. poorer parasite performance in sympatric than in allopatric hosts, has been reported for *Microbotryum violaceaum* on *Silene inflata* (Kaltz *et al.*, 1999). Interestingly, local adaptation can occur in one host plant species and not in other, as reported for *Co. lindemuthianum* on *Ph. vulgaris* and *Phaseolus coccineus*, a system in which specialization also occurred (Sicard *et al.*, 2007). Restricted gene flow in both the parasite and the host, resulting in genetic differentiation of their populations, is a prerequisite for local adaptation, although gene flow between parasite subpopulations increases the potential for local adaptation, as long as migration does not homogenize populations (Gandon *et al.*, 1996; Gandon and Michalakis, 2002; Kawecki and Ebert, 2004; Morgan *et al.*, 2005). The instances in which no local adaptation or a maladaptation occurred have been explained on the basis of the reproduction system of the host, the dispersal system of the parasite or the cyclical nature of co-evolution. In these analyses of local adaptation no separate analyses of parasite fitness and virulence were made, and the underlying assumption is that these traits are positively correlated. The exception is the work on *Sy. decipiens* in *Am. bracteata* (Parker, 1985), in which it is reported than the proportion of fungal lesions that do not yield sori varies according to the host–parasite deme × deme interaction, but no specific data for virulence are reported. In some analyses two different components of parasite fitness, for example the efficiency of plant infection and within-host multiplication or growth, were analysed separately. In these instances, efficiency of infection and multiplication were found to be uncorrelated (Laine, 2005; Sicard *et al.*, 2007). Interestingly, efficiency of infection and within-host multiplication have also been reported to be uncorrelated in two analysed viral systems (Fargette *et al.*, 2002; Sacristán *et al.*, 2005). It would be interesting to analyse how common for plant parasites is a lack of correlation between efficiency of infection and within-host multiplication, as it would affect the relationship between fitness and virulence and, hence, virulence evolution. Serial passage experiments also provide evidence of adaptation of fungi, oomycetes and viruses to hosts (Ebert, 1998; Yarwood, 1979), although there are few experiments aimed to show adaptation to hosts in viruses. In one of the few experimental analysis of specialization in a plant virus, Sacristán *et al.* (2005) found that fitness of CMV field isolates was near to its optimum and could not be improved by serial passages. Data regarding efficiency of infection supported the hypothesis of specialization, while data of within-host accumulation or virulence did not, nor provided evidence of trade-offs.

More frequent is the evidence of genetic differentiation of virus populations according to host species or host population (García-Arenal *et al.*, 2001; Jeger *et al.*, 2006; Moury *et al.*, 2006), although in most reports data do not allow conclusions regarding whether genetic differentiation was due to adaptation to hosts or to any other factor. Exceptions are the analysis of adaptation of *Turnip mosaic virus* (TuMV) to infect *Raphanus* spp. (Oshima *et al.*, 2002), or the report of MSV isolates from maize

that differ genetically and were more virulent to maize than those of wild grasses (Martin *et al.*, 2001), both reports suggesting specialization.

Differential prevalence of a parasite over its host range in a particular ecosystem, as shown for *Barley yellow dwarf virus* on wild grasses (Power and Mitchell, 2004), can be evidence of specialization. An analysis of the prevalence of five generalist viruses on 21 species of wild plants showed a selective interaction between viruses and hosts and, more importantly, that host selectivity is a successful strategy for generalist viruses (Malpica *et al.*, 2006). Interestingly, this result is in agreement with the hypothesis that specialism is advantageous for parasites.

Differential pathogenicity on host genotypes (see below) can be considered as an extreme case of host adaptation. One of the few analyses of the relationship between pathogenicity and virulence was reported for RYMV. Isolates differing in pathogenicity on *Rymv-2* did not differ in virulence or fitness (Sorho *et al.*, 2005), suggesting that pathogenicity and virulence are under different genetic regulation.

THE EVOLUTION OF PATHOGENICITY

Because parasites must infect hosts for their survival and parasite infection limits host fitness, pathogenicity in parasites and resistance in hosts are targets for selection. Plants resist disease through a variety of preformed and induced barriers to infection (De Meaux and Mitchell-Olds, 2003; Nurnberger *et al.*, 2004), and pathogens use virulence factors to overcome plant defences and make infection possible. Plant immunity acts at different layers. One layer involves the recognition of conserved pathogen-associated molecular patterns, which triggers basal defences (PAMP-triggered immunity, PTI). Another layer of defence involves the recognition of pathogenicity effectors of the parasite that might have evolved to suppress PTI (Chisholm *et al.*, 2006; Jones and Dangl, 2006). Selection on both the resistance proteins that recognize the pathogenicity effectors and the pathogenicity factors may lead to an antagonistic host–pathogen co-evolution (Dawkins and Krebs, 1979; Stahl and Bishop, 2000). Two major models of host–parasite interaction determining the success of infection have been proposed: the gene-for-gene (GFG) and the matching-allele (MA) models, with different assumptions and predictions. GFG and MA models have generally been applied to plant and animal systems, respectively, but they may both be relevant to understanding the evolution of plant–pathogen interactions.

Evolution of pathogenicity under the gene-for-gene model

In plant–parasite systems, pathogenicity has been most often related and analysed in GFG interactions, first described in the flax – flax rust (*Me. lini*) system (Flor, 1955). In GFG interactions, plant resistance proteins (R proteins) recognize corresponding proteins of the pathogen, named avirulence (Avr) factors, either through direct R–Avr protein–protein interaction or indirectly through detection of changes in the host targets of Avr proteins, the so-called guardee proteins in the guard model (Jones and Dangl, 2006; McDowell and Simon, 2006). The recognition of the Avr factor by the host triggers defence responses leading to limitation of the spread of the pathogen from the infection site, often associated with localized host cell death or hypersensitive response (HR). In the absence of the Avr allele in the parasite or if the host has not have the resistance *R* allele, the parasite is not recognized by the host, resistance is not triggered and the host is infected. Accordingly, a key feature of the GFG model is that universal pathogenicity occurs, i.e. there are parasite genotypes able to infect all host genotypes (Agrawal and Lively, 2002).

Although different classes of plant R proteins have been described (Dangl and Jones, 2001), most of them contain nucleotide binding site (NBS) and leucine-rich repeat (LRR) domains, and there is evidence that the latter is involved in pathogen recognition (Jones and Dangl, 2006; McDowell and Simon, 2006; but see also Burch-Smith *et al.*, 2007). No function other than resistance is known for this protein class (Dangl and Jones, 2001). In contrast to R proteins, Avr factors are diverse. For many of them a function has yet to be found (Catanzariti *et al.*, 2007), but those for which a function is known are determinants for the parasite's fitness, even as pathogenicity effectors that play important roles in host infection (Skamnioti and Ridout, 2005; van't Slot and Knogge, 2002). Hence, plant immune systems have evolved to recognize pathogen proteins with an important role in the pathogen life cycle.

Variability of avirulence genes

Avirulence factors were first identified in viruses, following the development of reverse genetic approaches for RNA viruses in the early 1980s. The first Avr factor identified in a plant pathogen was the capsid protein (CP) of *Tobacco mosaic virus* (TMV): reverse genetics experiments showed that the CP determined the elicitation of the HR defence response triggered by the *N′* resistance gene in *Nicotiana* spp. (Knorr and Dawson, 1988; Saito *et al.*, 1987). Since then, virus-encoded proteins with each possible role in the virus life cycle have been shown to act as Avr factors (Maule *et al.*, 2007). For instance, within the genus *Tobamovirus*, the helicase domain of the RNA-dependent polymerase (RdRp) of TMV is the Avr factor for the *N* gene in *Nicotiana*, and the movement protein of *Tomato mosaic virus* is the elicitor of the *Tm2*- and *Tm2*^{2}-encoded HR reaction of tomato, in addition to the above-mentioned TMV CP and *N′* gene (Meshi *et al.*, 1989; Padgett *et al.*, 1997; Weber and Pfitzner, 1998). Within the genus *Potyvirus*, the NIa protease of PVY is the elicitor of *Ry* in potato (Mestre *et al.*, 2003), the P3 protein of *Soybean mosaic virus* elicits *Rsv1*

in soybean (Hajimorad *et al.*, 2005), or the cylindrical inclusion helicase of TuMV elicits *TuRB01* of *Brassica* (Jenner *et al.*, 2000). All these proteins interact with host factors and are required for completion of the virus life cycle within the infected host and, thus, can be considered as pathogenicity effectors.

Much progress has been made also in studies of the molecular genetics of avirulence in bacterial pathosystems, particularly in interactions with Arabidopsis (McDowell and Simon, 2006). Several bacterial Avr proteins, delivered into the plant cell using type III secretion systems, have been shown to be pathogenicity effectors, for instance by having enzymatic activity and modifying host proteins, and mutations in *Avr* genes impaired infectivity or multiplication in susceptible hosts (Grant *et al.*, 2006). By contrast, few fungal Avr genes have been cloned thus far, most of them encoding novel proteins (Fudal *et al.*, 2007) with no obvious function. In a few cases the hypothesis of a function as effectors is strongly supported, for example: the barley powdery mildew AVR_{k1} and AVR_{a10} genes, which directly contribute to infection success (Ridout *et al.*, 2006); *Avr2* and *Avr4* from *Fulvia fulva* (anamorph: *Cladosporium fulvum*), which protect the fungus from the action of the defence mechanisms of the plant (Rooney *et al.*, 2005; van den Burg *et al.*, 2006); and *SIX1* of *Fusarium oxysporum*, deletion of which leads to a reduced virulence on susceptible lines (Rep *et al.*, 2005). In addition, a pathogenicity effector role may be assumed based on evidence for evolutionary conservation of *Avr* genes in fungi and oomycete species (Skamnioti and Ridout, 2005). The genomics of filamentous fungi has made great advances in recent years (Weld *et al.*, 2006), and the genomes of several phytopathogenic fungi have been sequenced or will be so imminently (Xu *et al.*, 2006). This will facilitate functional studies of Avr proteins and the identification of additional *Avr* genes based on similarity with known avirulence effectors (Tyler *et al.*, 2006).

In GFG interactions, host–pathogen co-evolution will lead to pathogens altering their Avr factors to avoid R-dependant recognition as well as the host evolving new specificities in their R proteins to identify the corresponding Avr factors. There is ample evidence for allelic polymorphisms at *R* and *Avr* loci in plants and pathogens, respectively (e.g. Parker and Gilbert, 2004; Thrall *et al.*, 2001). It has been argued that the mechanism of recognition of Avr by R will determine Avr evolution. Thus, direct recognition of Avr by R can lead to relatively rapid evolution of new virulence phenotypes by alteration of the Avr structure without affecting its virulence role (Van der Hoorn *et al.*, 2002). A direct physical interaction between Avr and R proteins has been shown for the AvrPita–Pi-ta pair in the rice blast fungus, *Magnaporthe grisea*, and rice (Jia *et al.*, 2000). According to predictions, the AvrPita proteins from natural isolates of *Ma. grisea* virulent on *Pi-ta* plants differ from one another by several mutations (Orbach *et al.*, 2000). In the flax—flax rust system, in which a direct Avr–R interaction has also been shown, diversifying selection has led to extreme levels of polymorphism at the *AvrL567* locus in different rust strains, leading to qualitative differences in recognition specificity by the corresponding R genes (Dodds *et al.*, 2006). Diversifying selection and high levels of polymorphism were also reported for the *Atr13* and *Atr1* loci of *Hy. parasitica* and the corresponding *RPP13* and *RPP1* resistance loci of Arabidopsis, respectively (Allen *et al.*, 2004; Rose *et al.*, 2004). Differential recognition of *Atr1* alleles by *RPP1* alleles has been shown (Rehmany *et al.*, 2005), and it has been suggested that the encoded proteins might interact directly (Jones and Dangl, 2006). Many analysed Avr–R systems seem to conform to the guard model of indirect recognition, where the R protein recognizes changes in the virulence target after interaction with Avr. While direct recognition would lead to relatively rapid evolution of new virulence phenotypes, it has been argued that indirect recognition can lead to balancing selection in *Avr* and *R*. If guardee proteins are virulence targets for the pathogens, and the guard protein (i.e. the R protein) recognizes changes in the guardee due to interaction with Avr, resistance could not be circumvented by mutations in Avr without affecting its virulence functions (Van der Hoorn *et al.*, 2002). Hence, purifying selection is predicted to act on Avr. Alternatively, the pathogen could overcome host detection by discarding the Avr gene, if its function can be provided by other genes of the pathogen. This situation will result in the presence of ancient polymorphisms in R genes, and is well exemplified by the Arabidopsis–*Ps. syringae* system (Mauricio *et al.*, 2003; Stahl *et al.*, 1999). According to the predictions above, evidence of purifying selection has been reported for several families of type III effectors of this bacterium, although there is also evidence for diversifying selection in domains of some gene families (Rohmer *et al.*, 2004). Examples of fungal systems that conform to a model of indirect interaction are the R/Avr gene pairs *Cf-2/Avr2* and *Cf-9/Avr9* of tomato and *Fu. fulva* (Rivas and Thomas, 2005). Avr-2 is a cystein protease inhibitor, inhibiting the tomato cystein protease Rcr3, which is guarded by Cf-2 (Rooney *et al.*, 2005). According to predictions, *Fu. fulva* races virulent on *Cf-9* have large deletions in *Avr9* or express truncated proteins, and races virulent on *Cf-2* arise due to single insertion–deletions that generate truncated proteins (Rivas and Thomas, 2005). Thus, available data on the evolution of Avr genes in cellular pathogens agree with predictions according to the recognition mechanism of Avr by R.

In plant–virus interactions, data do not support differences in Avr evolution linked to the mode of Avr–R recognition. In the tobacco–TMV system, the p50 helicase domain of the RdRp is necessary for N oligomerization and activity (Mestre and Baulcombe, 2006) and p50 directly interacts with the TIR domain of N (Burch-Smith *et al.*, 2007). However, no diversifying selection in p50 has been described. Rather, evidence supports strong negative selection on p50, as avr on *N* is extremely rare, occurring only in *Obuda mosaic virus*, a tobamovirus species with a restricted geographical

distribution (García-Arenal and McDonald, 2003). *Rx* resistance of potato to *Potato virus X* (PVX) is elicited by the virus CP, and requires the interaction of *Rx* with a Ran GTPase activating protein, although this interaction does not fit the guard hypothesis (Tameling and Baulcombe, 2007). Virulence on *Rx* is conferred by mutations at two positions in PVX CP, but mutants leading to resistance breaking were shown to be selected against (Goulden *et al.*, 1993). In nature only one strain of PVX, PVX-HB, has been described to be pathogenic on *Rx* with, again, a limited geographical distribution (see García-Arenal and McDonald, 2003). In addition, engineered mutations on *Rx* change its recognition pattern expanding it to new PVX strains and new viruses (Farnham and Baulcombe, 2006). Therefore, data suggest that diversification on PVX CP and Rx can be constrained by fitness penalties. Elicitation of *HRT*-resistance in Arabidopsis by the CP of *Turnip crinkle virus* (TCV) requires interaction with a NAC protein, according to the guard model (Ren *et al.*, 2000). Virulence on *HRT* has not been found in TCV, although this has not been explored extensively. Regardless, at odds with the *N* and *Rx* resistances, which have been extensively used in tobacco and potato cultivars for decades, resistance to TCV is not frequent in Arabidopsis accessions (Dempsey *et al.*, 1997), and it is not known if TCV is an important pathogen exerting a selection pressure on Arabidopsis wild populations.

Thus, the hypothesis that the mechanisms of Avr recognition by the host plant determine *Avr* evolution (Van der Hoorn *et al.*, 2002) is supported by evidence from some host–cellular parasite systems, but not from virus–host systems. More evidence both from cellular and viral parasites is needed to test its general validity.

The processes resulting in evolution of Avr genes also differ between viruses and cellular parasites. Changes in recognition of viral Avr by R proteins depend of one or a few amino acid substitutions (Harrison, 2002; Maule *et al.*, 2007). Available data concern RNA viruses, which have spontaneous mutation rates several orders of magnitude higher than DNA-based microbes (Drake and Holland, 1999; Malpica *et al.*, 2002), so that a rapid generation of mutants should be expected. Hence, selection of avr in plant RNA viruses seems to be countered by intrinsic or extrinsic factors. In contrast, for fungi and oomycetes there is evidence that Avr genes are selected for high mutability and vary according to multiple mechanisms. Reported mechanisms resulting in conversion of Avr to avr include point substitutions, insertions and deletions, as in *Avr-Pita* of *Ma. grisea* (Orbach *et al.*, 2000); mutations leading to truncation of the encoded protein, as with the frame shift mutations and point mutations resulting in premature stop codons described in *Avr2*, AVR_{k1} and AVR_{a10} of *Fu. fulva* and *Bl. graminis* f. sp. *hordei* (Luderer *et al.*, 2002; Ridout *et al.*, 2006); or deletions of large fractions of the *Avr* gene, as reported for *NIP1* of *Rh. secalis* (Schúrch *et al.*, 2004). Alleles of this gene and also of *Atr13*, *Avr3a* and *AvrL56 7* from *Hy. parasitica*, *Ph. infestans* and *Me. Lini*, respectively, are subject to diversifying selection (Allen *et al.*, 2004; Armstrong *et al.*, 2005; Dodds *et al.*, 2006). The genomic context can influence the high potential of virulence/avirulence genes to mutate. Thus, in *Ma. grisea*, *Avr-Pita* is located close to a telomere (Orbach *et al.*, 2000). Transposable elements can also have a role in Avr gene expansion and diversification, by disrupting the expression of Arv genes or by hitchhiking the sequences nearby when they multiply and proliferate in the genomes. For example, the barley powdery mildew AVR_{k1} and AVR_{a10} genes pertain to a gene family with more than 30 homologues in the fungal genome that is closely associated with sequences homologous to the retrotransposon CgT1 (Ridout *et al.*, 2006), and isolates with point mutations that cause a frame shift and fusion with a CgT1 sequence in both Avr genes are virulent. In addition, in *Ma. grisea*, insertion of the Pot3 transposon into the promoter of *Avr-Pita*, or of the retrotransposon MINE in the avirulence gene *ACE1* resulted in gain of virulence (Fudal *et al.*, 2005; Kang *et al.*, 2001). The pathogenicity islands described in prokaryotes are groups of clustered genes that can undergo rapid, radical changes, frequently flanked by transposable elements, which may contribute to their proliferation in the genome (Kim and Alfano, 2002). Similarly, two miniature impala transposable elements flank the *SIX1* gene from *Fu. oxysporum*, and can be considered as characteristic of such pathogenicity islands (Rep *et al.*, 2005). Genetic or molecular evidence of clusters of *Avr* genes that may be interspersed with various transposable elements have been described in *Phytophthora* (Jiang *et al.*, 2005, 2006), *Bl. graminis* f. sp. *hordei* (Brown and Jessop, 1995; Jensen *et al.*, 1995; Ridout *et al.*, 2006) and *Leptosphaeria maculans* (Balesdent *et al.*, 2002; Fudal *et al.*, 2007).

Therefore, the evolvability of *Avr* genes seems to be different for viruses and mycelial parasites. This difference could be related to different costs of pathogenicity in the two parasite groups. The cost of pathogenicity, which is treated in the next section, is a major and most debated question in host–parasite co-evolution.

Costs of pathogenicity

Much theory on host–parasite co-evolution is based on GFG systems in plants. The classical model of GFG co-evolution was proposed by Leonard in 1977, and has set the ground for later ones (Agrawal and Lively, 2002; Frank, 1994, 2000; Leonard and Czochor, 1980; Parker, 1994, 1996; Pietravalle *et al.*, 2006; Thrall and Burdon, 2002). Leonard's model is based on indirect frequency-dependent selection, in which host allele frequencies determine those of the pathogens and vice versa. Under this model, non-trivial equilibria in the frequency of *R* and *avr* (i.e. equilibria in which gene frequencies are different from 0 or 1) require that both resistance and pathogenicity have a fitness cost for the host and the pathogen, respectively. Tellier and Brown (2007) have shown that the equilibrium predicted under this model is unstable, and requires that the cost of pathogenicity (commonly referred to

as the cost of virulence) for the parasite is much smaller than the cost of not infecting a resistant plant, assumed to be about 1 in a GFG system. A condition for stable equilibriium is that, in addition to indirect frequency-dependent selection, negative direct frequency-dependent selection on resistance and avirulence occurs, i.e. that the effect of an allele for resistance or virulence on fitness decreases as its frequency in the population increases (Tellier and Brown, 2007). Different sets of factors can result in direct frequency-dependent selection. The solution of the various models proposed by Tellier and Brown (2007) under different scenarios requires fitness costs of both resistance and pathogenicity and, importantly, that these costs are small (less than 10%). Because of their importance on host–pathogen co-evolution, and on pathogenicity management, much effort has been devoted to the analysis of the costs of resistance in hosts and of pathogenicity in parasites, with conflicting results. We will not deal here with the costs of resistance, and direct the reader to reviews of this subject (Bergelson and Purrington, 1996; Bergelson *et al.*, 2001; Brown, 2003; Mauricio, 1998), but will limit our discussion to the costs of pathogenicity.

An approach to evaluate fitness costs of pathogenicity has been to analyse the dynamics of *avr* genes in field populations of parasites. Assuming the cost of pathogenicity, there will be a decline in *avr* frequency in the absence of the corresponding *R* gene, and thus unnecessary pathogenicity will be selected against. Data from studies with fungi provide conflicting results (Leach *et al.*, 2001; McDonald *et al.*, 1989). For instance, selection against *avrXa7* in *Ma. grisea* in the absence of the corresponding *R* gene suggests a cost for this pathogenicity factor (Vera Cruz *et al.*, 2000). In addition, selection against unnecessary avr on *Sr6* resistance in *Puccinia graminis* f. sp. *tritici* in Australia, or against unnecessary avr on *Mla6* resistance of *Bl. graminis* f. sp. *hordei* in the UK, allowed Grant and Archer (1983) to estimate selection coefficients of 4–6% for both systems. By contrast, unnecessary avr genes are present in populations of *Bl. graminis* fsp. *hordei* and *tritici*, and avr did not segregate with fitness traits (Bronson and Ellingboe, 1986; Brown and Wolfe, 1990). Also, unnecessary avr on *R5*, *R9* and *R10* resistance of lettuce increased in Swedish populations of *Bremia lactucae* although these genes were not used in lettuce cultivars in Sweden, suggesting no cost of pathogenicity (McDonald *et al.*, 1989). We are not aware of analyses of the frequency of unnecessary avr in virus populations. However, there is ample evidence from several different systems that genotypes pathogenic on a particular *R* gene do not become prevalent in the virus population even with extensive use of cultivars with that *R* gene, so that resistance has been effectively durable (García-Arenal and McDonald, 2003). In fact, resistance to viruses is durable more often than not, in spite of pathogenic genotypes being reported in the field or in the laboratory (García-Arenal and McDonald, 2003). This is in clear contrast to the short life of most resistance factors deployed against fungi or bacteria (McDonald and Linde, 2002), suggesting higher costs of pathogenicity for viruses than for cellular plant pathogens. Data from field populations, however, should be viewed with caution, as selection against unnecessary *avr* genes could be countered by a number of factors. Most studies from natural populations do not check for conditions necessary for selection being the major determinant of *avr* frequency. For instance, selection on *avr* could be countered by random genetic drift due to small effective population sizes associated with population bottlenecks during the parasite life cycle, spatial or host-associated structure of its population, etc. In addition, linkage disequilibrium may be important, and unnecessary *avr* genes could be maintained in the population by hitchiking with alleles at other loci that determine higher parasite fitness. Linkage disequilibrium could be particularly important on plant parasitic fungi or viruses, in which sexual processes may be limited.

Another approach to analyse pathogenicity costs has been experimental. Evidence for a possible cost of pathogenicity came from mutagenic experiments with rusts of flax (*Me. lini*) and wheat (*Pu. graminis* f. sp. *tritici*) in which a correlation between the disruption of pathogenicity by mutations and a decrease in fitness was found (Flor, 1958; Luig, 1979). However, the effects on fitness due to second-site mutations could not be discarded. Experimental evidence for the costs of avr in viruses is more abundant. Different mutations have been described in the CP of TMV that disrupt the elicitation of *N'* (Culver *et al.*, 1994). Nevertheless, these mutations cause incorrect folding of the CP and, hence, are expected to have a fitness penalty. Also, all experimental mutants in the protease domain of the NIa protein of *Potato virus Y* (PVY), the Avr factor for *Ry*-resistance, resulted in virulence. However, no field isolate of PVY has been described to overcome *Ry* as elicitation of *Ry* seems to require a functional protease domain, which is also necessary for viability of the virus (Mestre *et al.*, 2003). Specific experiments to estimate avr costs have been reported for genotypes of *Raspberry ringspot virus* overcoming *Irr* resistance in raspberry, which have a decreased transmission both by nematodes and through the seed in alternative hosts (Hanada and Harrison, 1977; Murant *el al.*, 1968). For *Pepper mild mottle virus* genotypes overcoming *L3* resistance in pepper, competition experiments with Avr genotypes on susceptible pepper allowed an estimate of the fitness of avr genotypes relative to Avr of about 0.6 (A. Fraile *et al.*, unpublished data). Similarly, M. Molina *et al.* (personal communication) found a high penalty for avr on *L3*, but the analyses of chimeras between Avr and avr genotypes showed that the penalty was only in part determined by mutations in the CP (the Avr factor for *L3*; Berzal-Herranz *et al.*, 1995) and that other genomic regions also determined the fitness of avr genotypes. For TuMV, genotypes overcoming *TuRB01* resistance in rape were out-competed by Avr ones in susceptible hosts. Importantly, assays included engineered *avr* mutants with no second-site mutations, and thus provide unequivocal evidence

for a cost of pathogenicity due to a pleiotropic effect of the *avr* mutation (Jenner *et al.*, 2002b). Similarly, Jenner *et al.* (2002a) reported fitness costs and a high rate of reversion for mutations in TuMV resulting in avr on a second resistance gene, *TuRB04*. From the data in Jenner *et al.* (2002a,b) the fitness of avr mutants relative to Avr ones, on both genes, can be estimated to be about 0.50. It is worth noticing that these pathogenicity costs are much higher than assumed in theoretical models of GFG co-evolution (Tellier and Brown, 2007), and, hence, may violate model assumptions.

Therefore, the scenario is that pathogenicity costs may be small, if any, for mycelial pathogens, where no unequivocal evidence of costs has been reported, while costs may be quite high for viruses. This important difference between both groups of parasites could be explained by a variety of factors. It has been hypothesized that effective population sizes are smaller for plant viruses than for plant parasitic fungi (Harrison, 1981). Indeed, small effective sizes, several orders of magnitude below the census, have been estimated for population bottlenecks during viral colonization of the host plant or during horizontal transmission (Ali *et al.*, 2006; French and Stenger, 2003; Sacristán *et al.*, 2003). Estimates of effective population sizes for fungi are rare, but indicate no such gross difference with census sizes (Leslie and Klein, 1996; Zhan *et al.*, 2001).

A major determinant for the difference in pathogenicity costs between fungi and viruses could be the nature of their genome. In the genomes of RNA viruses there are few neutral sites, and most mutations, including nucleotide substitutions, are deleterious (Sanjuán *et al.*, 2004a). The small genomes of RNA viruses are tightly packed with information: there is overlapping of coding and regulatory sequences and of different coding sequences, and the few encoded proteins perform different functions in the virus life cycle, imposing different selection pressures on the corresponding genes (García-Arenal *et al.*, 2001). In addition, epistatic interactions occur among and within genes (Escriu *et al.*, 2007; García-Arenal *et al.*, 2001; Martin *et al.*, 2005; Sanjuán *et al.*, 2004b), which limit the variability of viral proteins. Thus, the plasticity of plant virus genomes could be low, in spite of high mutation and recombination rates (Drake and Holland, 1999; Froissart *et al.*, 2005; Malpica *et al.*, 2002), and mutation to pathogenicity would have high costs. This would not be case for fungi, in which genome complexity allows for high levels of redundancy, alternative metabolic pathways and multiple regulatory elements that could reduce the effects of mutational perturbations. Accordingly, the fitness effects of mutations are much smaller in eukaryotes, including fungi, than in RNA viruses (Sanjuán and Elena, 2006). As mentioned above, multigene families for Avr factors have been described, which would result in few penalties for mutation in an individual gene or even for its whole loss. In agreement, frameshift mutations and deletions are common mechanisms for variation of fungal Avr genes, as pointed out above. Hence, differences in genome size, structure and plasticity may determine differences in the evolution of pathogenicity of viruses and cellular pathogens.

Evolution of pathogenicity under the matching-allele model

The MA model was originally based on self–non-self recognition in invertebrates. Its key feature is that infection requires a specific match between host and parasite genes. Therefore, in a pure MA system, pathogenicity on all host genotypes (i.e. 'universal pathogenicity') cannot exist, an important difference with the GFG model. Also, the MA model predicts that polymorphisms for pathogenicity and resistance will be easily maintained by negative frequency-dependent selection, and that there is no such thing as costs of pathogenicity (Agrawal and Lively, 2002). It has been argued that pure GFG and MA models are extremes of a continuum, within which the MA model should be modified to admit partial infection (i.e. the parasite infects, but reproduces less effectively, and the host suffers less intensely from parasitism than in a 'full infection'). Within this continuum, costs of pathogenicity exist as a function of the degree of success of partial infection (Agrawal and Lively, 2002; Parker, 1994). Although data from plant–parasite systems have generally been analysed under the GFG model, it has been pointed out that a modified MA model could fit plant–pathogen interactions, even for systems usually analysed under the GFG model. For instance, isolates of *Rh. secalis* from Australia showed different degrees of specialization, some isolates being able to infect few barley genotypes, with high severity, and some being able to infect many genotypes, but with low severity (Jarosz and Burdon, 1996).

At least two specific types of plant–parasite interaction may well fit a pure or modified MA model. One type are the plant–fungal interactions in which host-specific toxins (HSTs) act as virulence factors (Wolpert *et al.*, 2002). HSTs need a target in the host, according to the MA model. A well-known example is the strict association of virulence to oats and production of the HST victorin by *Cochliobolus victoriae*: all isolates that produce victorin are virulent, whereas mutants or segregating progeny not producing the toxin are not. Susceptibility in oats to *Co. victoriae* and to victorin is linked and determined by a single dominant allele at the *Vb* locus (Wolpert *et al.*, 2002). The molecular evolution of the toxins of *Pyrenophora tritici-repentis* has been analysed in most detail. The HST ToxA is produced by the wheat pathogens *Py. tritici-repentis* and *Phaeosphaeria nodorum*, and interacts with the product of the dominant plant gene *Tsn1* to induce necrosis. It has been hypothesized that the single copy gene encoding the secreted ToxA protein in cultivar-specific virulent strains of *Py. tritici-repentis*, an emergent wheat pathogen, could have been horizontally transferred from the ancient wheat pathogen *Ph. nodorum* (Friesen *et al.*, 2006). Evidence for diversifying

selection supports the hypothesis that evolution of the ToxA locus is driven by selection imposed by the host. The distribution of ToxA alleles and deletions may reflect the distribution of different Tsn1 alleles in the corresponding host populations (Stukenbrock and McDonald, 2007). A second HST, ToxB, is encoded by six genes in pathogenic races of *Py. tritici-repentis*, and flaking sequences suggest a role of transposable elements in this high copy number. Interestingly, non-pathogenic races have a single copy gene homologue, *toxb*, under a different transcriptional regulation (Martinez *et al.*, 2004). Fungal genotypes that do not produce HSTs can infect the host plant, albeit with reduced efficiency, and hence their evolution should be considered under a modified MA model.

The other situation is the plant–virus interactions determined by recessive resistance (i.e. lack of susceptibility) genes, which confer immunity to virus infection. All characterized genes for recessive resistance encode translation initiation factors (eIF). Mutations leading to resistance in eIF can be countered by mutations in the virus, thus acquiring pathogenicity on the mutated host gene. In most systems, the virus-encoded protein covalently linked to the 5′ terminus of the genomic RNA (VPg) must interact correctly with the corresponding eIF for infectivity, but other 'pathogenicity' determinants have been described, e.g. 3′ untranslated region of *Melon necrotic spot virus* (Robaglia and Caranta, 2006). The best analysed instance of plant–virus interaction under this system is that of PVY with alleles at the *pvr2* locus of pepper. *pvr2* encodes eIF4E, and alleles differ by a small number of nucleotide substitutions (Robaglia and Caranta, 2006). Up to 11 amino acid changes in the central region of PVY VPg have been described to result in overcoming the three characterized alleles of *pvr2*, and there is evidence that positive selection on these sites leads to diversification of the VPg. Overcoming of one *pvr2* allele does, or does not, confer pathogenicity on the other according to the mutation (Ayme *et al.*, 2007; Moury *et al.*, 2004), and hence the system corresponds to an MA interaction. Fitness of the various mutants overcoming allele $pvr2^3$ was analysed in different hosts: pepper genotypes homozygous for $pvr2^3$, or for the susceptibility allele $pvr2^+$, and in the susceptible host *Nicotiana clevelandii*. The various pathogenic VPg mutants differed in fitness in all three hosts, but some of them were as fit in susceptible pepper and *Ni. clevelandii* plants as the non-pathogenic wild-type (Ayme *et al.*, 2006). As these experiments were done with mutants derived from an infectious cDNA clone of wild-type PVY, effects of second site mutations can be discarded. A second well-characterized system is recessive resistance in rice, conferred by *Rymv1*, encoding eIF(iso)4G (Albar *et al.*, 2006), to RYMV. Different mutations in RYMV VPg determine pathogenicity on the different resistance alleles at *Rymv1*. One of the few analyses of unnecessary pathogenicity in a plant virus showed that a high percentage (~17%) of isolates of RYMV from Africa were pathogenic either on allele *Rymv1-2* or *Rymv1-3*, and that fewer (~5%) were pathogenic on both (Traoré *et al.*, 2006). No fitness penalty for pathogenicity on *Rymv1-2* was found in passage competition experiments with non-pathogenic genotypes (Sorho *et al.*, 2005). Hence, although the interaction of viruses with recessive resistance in host plants is most often described under the assumptions of the GFG model, these two well-characterized systems show that it better conforms to the MA model for the evolution of pathogenicity, as no universal pathogenicity occurs and pathogenicity may occur without a fitness penalty.

CONCLUSION

Extensive theoretical analyses of the factors that shape the evolution of both pathogenicity and virulence have been reported in the last three decades, with predictions on the outcome of pathogenicity and virulence evolution under different scenarios. Experimental work has not followed the path of theoretical analyses. Plant pathologists have shown greater interest in pathogenicity than in virulence, possibly because the extensive use of qualitative resistance to control infectious diseases of crops, and in the last 10 years understanding the molecular basis of pathogenicity, has made enormous progress. However, little is known on the molecular basis of virulence, probably because virulence determinants in hosts and parasites might be quantitative and less amenable to genetic and molecular dissection than determinants of pathogenicity. It has been proposed that the mechanism of Avr recognition by hosts will affect the evolution of pathogenicity factors but much experimental work is still needed to test these hypotheses. In fact, the huge success of molecular plant pathology in recent years has been accompanied by an increasing gap between plant pathologists with a molecular and a population orientation, and advances in understanding the mechanisms of pathogenicity have not resulted in a proportional understanding of its evolution in pathogen populations. We hope this review will show that theoretical models set the directions for experimentation, and that advances in molecular genetics provide the means for it. Understanding plant–parasite evolution would greatly benefit from more communication between molecular- and population-orientated plant pathologists.

A second major goal of this review was to bring together and to compare data derived from cellular plant pathogens and from viruses. Another important gap between plant pathologists occurs between those dealing with cellular pathogens and those dealing with viruses. This gap seems to be growing and, despite the fact that progress in understanding plant–virus interactions has not lagged behind and often has preceded understanding of plant–bacteria or plant–fungi interactions, reviews of plant–pathogen interactions tend to ignore results from the virus field, to the degree that the excellent review of Jones and Dangl (2006) on plant immunity does not include viruses in its initial list of

plant pathogens. However, the comparison of viruses and cellular pathogens, which are so different in genomic complexity and regulation and in their parasitism, is necessary to establish the generality of hypotheses or their field of application, as well as to reinforce or disprove conjectures on plant–parasite interaction and evolution.

Last, in this review we wanted to stress the many gaps and limitations in our current knowledge on pathogenicity and virulence evolution. A major question that is in urgent need of experimental analysis is the relationship between the different components of parasite fitness and virulence. Data show that a positive correlation between within-host multiplication and virulence is not universal, but do not at present allow us to define if a correlation between fitness and virulence depends on the parasite and/or host life history, on environmental factors or on both. Thus, virulence should not be used as a measure of parasite fitness, as it often is in reported analyses. Moreover, assumptions of a positive correlation between the two major components of parasite fitness, within-host multiplication and between-host transmission, often do not consider that the relevant parameter for parasite fitness is the number of new infections per infectious host, which could result in trade-offs between both fitness components. Pathogenicity and efficiency of infectivity of inoculum are two other traits that may affect the within-host and between-host reproduction of the parasite. The few available data suggest that these traits are under different genetic control than virulence, but more knowledge is needed on the relationship between pathogenicity, inoculum infectivity, within-host multiplication and virulence. Molecular genetics could provide the tools to analyse the genetic determinants and the possible trade-offs among the various components of parasite reproductive potential, pathogenicity and virulence.

Other areas of research that would benefit from future interactions between molecular and populational plant pathologists would be the relationship between host–pathogen recognition and the evolution of pathogenicity, including analyses of the costs of resistance and pathogenicity and whether host–parasite interactions correspond to a GFG or an MA model. Current evidence suggests some trends, but derives from few pathosystems, which limits the validation of hypotheses. Applying current knowledge on the mechanisms of plant–parasite interactions to test hypotheses and predictions on the evolution of virulence and pathogenicity should be a rewarding area of research in the near future.

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