

## Resistance Variation in Natural Plant Populations

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### Abstract

The general outcomes of long-term trajectories of coevolutionary interactions between specific hosts and pathogens are set by the interaction of their life histories. However, within those outcomes the speed of co-evolutionary responses and the extent of their expression in the resistance/virulence structure of wild plant and pathogen populations respectively, are highly variable characters changing from place-to-place and time-to-time as a result of the interaction of host and pathogen with the physical environment. As a consequence, understanding of the role of diseases in the evolution of their hosts requires approaches that simultaneously deal with host and pathogen structures over multiple populations within a metapopulation framework.

**Keywords:** metapopulation; life-history characters; virulence; resistance; gene-for-gene

### INTRODUCTION

Pathogens are undoubtedly among the most potent forces shaping the structure and complexity of individual host populations and the ecosystems and communities in which they occur. As invasive organisms, the plant pathogens responsible for Dutch elm disease, *Phytophthora* die-back and American chestnut blight have had ecological effects of a similar magnitude to many infamous animal diseases. Even those pathogens that have evolved over long periods of time in the presence of their hosts may have substantial impacts on community structure through differential reduction in the vigour of their hosts (VAN DER PUTTEN *et al.* 1993; KNOPS *et al.* 1999; PACKER & CLAY 2000), while within individual species, interactions between host and pathogen may result in a complex mosaic of populations of quite different levels and diversity of resistance and virulence (BURDON & THRALL 2000).

To date, single population studies have dominated empirical and theoretical work on plant-pathogen systems. However, individual populations are ephemeral and provide a poor view of the true complexity of the coevolutionary trajectories of these associations. Indeed,

it is becoming increasingly clear that the maintenance of genetic polymorphisms in populations (MAY & NOWAK 1994; THRALL & ANTONOVICS 1995) and long-term coevolutionary trends, are strongly influenced by spatial structuring with coexistence of hosts and pathogens being possible in interconnected sets of populations (metapopulations) even when they fail to persist in single populations (THRALL & BURDON 2002). Understanding this complex matrix and its extensive subtle effects requires simultaneous consideration of both host and pathogen through time and space.

### Resistance in hosts; pathogenicity in pathogens

In the broadest sense, any mechanism that prevents infection – avoidance, qualitative resistance – or that reduces the impact of a pathogen once infection has occurred – tolerance, quantitative resistance – can be regarded as a form of resistance. The occurrence of disease resistance controlled by qualitative mechanisms in natural plant populations is well established. In contrast, the extent of diversity within populations with respect to other resistance mechanisms – quantitative, morphological and phenological – is poorly documented. Studies of three populations of *Plantago*

*lanceolata* infected by the necrotrophic fungus *Phomopsis subordinaria* show a near-continuous distribution of responses to individual isolates suggestive of the presence of many genes each responsible for minor phenotypic effects (DE NOOIJ & VAN DAMME 1988); while resistance conferred by differences in morphological features or phenological timing of host development has been demonstrated in natural systems (e.g. flower size and number in the *Silene* spp. – *Ustilago violacea*) (THRALL & JAROSZ 1994). However, these examples are exceptional as most work has concentrated on qualitative host-pathogen systems because of the relative ease with which both host and pathogen variation can be assessed and compared. It is these systems that are considered here.

#### **Interplay between environment, host-pathogen life history features and resistance-pathogenicity mechanisms**

Plant pathogens are an extremely diverse group of organisms that collectively cover a very broad taxonomic scope and range of life-history features. As a consequence, any one host species is likely to be involved in a range of quite distinctive interactions with different pathogens, each of which may have a different evolutionary trajectory. Such interplays between host and pathogen life history features have been investigated in depth in animal host-pathogen interactions using a comparative approach (LOCKHART *et al.* 1996). That study showed that diseases can be differentiated into distinct classes on the basis of transmission mode – a key point for selection with respect to pathogen persistence (e.g. sexually transmitted diseases differ from other infectious diseases for a wide range of characteristics, having smaller host ranges, longer infectious periods, and being less likely to increase host mortality). Clearly the particular combination of host and pathogen life-histories in a given interaction can provide important constraints on how the association co-evolves.

Perhaps more intriguing though, is the possibility that within individual species changes in life-history traits and environmental responsiveness may also induce significant changes in coevolutionary pathways (KOELLA & RESTIF 2001). No formal assessment of the empirical evidence for this has been conducted in plant-pathogen systems, but sufficient exists to suggest that over an extended environmental gradient such interplay would be accompanied by shifts in the intensity and nature of the reciprocal selective balance between host and pathogen.

This is perhaps best illustrated by a series of examples of host-pathogen associations occurring along an increasingly favourable environmental gradient (Figure 1). At one extreme, conditions favouring epidemic development occur infrequently and the pathogen exerts little selective pressure on its host. In these situations, the host largely avoids damage through escape and populations are dominated by susceptible phenotypes. An example of this is seen in the high susceptibility of wild relatives of small grain cereals growing in the Negev desert in Israel (DINOOR 1970). Shifts along the gradient towards conditions favouring pathogen growth and development place greater selective pressure on hosts as disease severity increases. The resultant changes in host resistance (number of resistance alleles; overall resistance levels) in turn favour changes in the virulence and aggressiveness of the pathogen population. A particular example of such environmentally driven change is seen in the increasing diversity and overall resistance of *Avena fatua* to *Puccinia coronata* along an 800 km transect from southern to northern New South Wales (BURDON *et al.* 1983). In this case, host and pathogen populations in the south were subject to a harsher environment of a shorter growing season with a more abrupt end. Changes in the resistance structure of host populations were paralleled by increasing diversity and virulence in the pathogen population (OATES *et al.* 1983).

As the environment becomes increasingly favourable this may affect other life history features of either host or pathogen – changes that in turn may have a ‘knock-on’ effect on the association. One such example, is seen in differences in the interaction between *Melampsora occidentalis* and *Populus trichocarpa* growing in the dry, cold interior of western Canada and on the moist, warm coastal Pacific coast (BURDON *et al.* 1996; HSIANG & CHASTAGNER 1993). In the interior, the rust occurs spasmodically and suffers major population crashes every autumn as the deciduous poplars shed their leaves. The interaction in this environment has favoured the emergence of resistance genes with major phenotypic effects (qualitative resistance). In contrast, in the coastal region where pathogen populations are constantly present, only quantitative resistance has been detected. These different scenarios have significantly different implications for the selective pressures host populations subsequently exert on those of the pathogen. In another example, changes in the mating system and phenology of *Linum marginale* between different metapopulations are correlated with major differences in the way in which resistance to *Melampsora lini* is partitioned within and among populations and in the

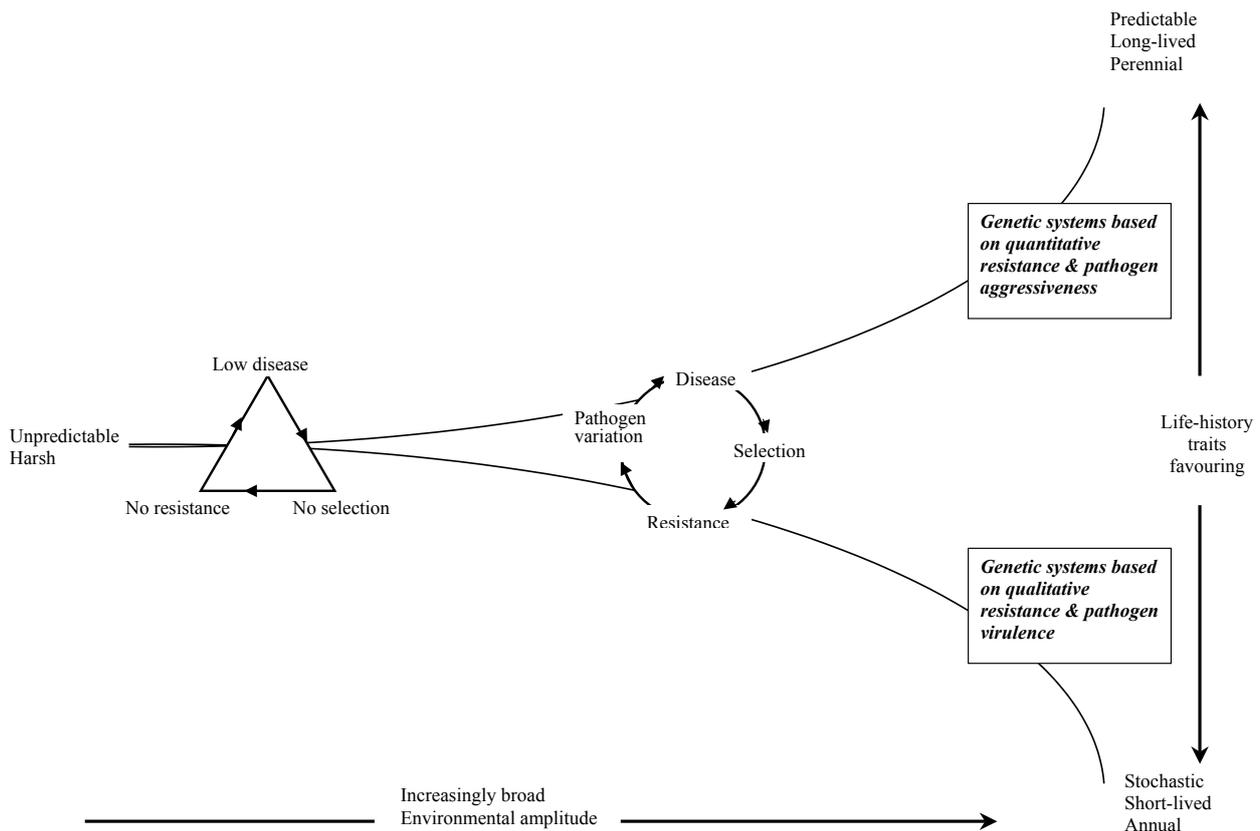


Figure 1. Schematic diagram relating the increasing diversity of possible host-pathogen evolutionary trajectories as environmental conditions become increasingly favourable and life-history features more exible

mean level of resistance. In populations with a mixed mating system (~28% outcrossing), more than 60% of individuals within families were heterozygous for one or more resistance alleles while in strongly selfing populations (< 3%) the comparable figure was less than 5%. Despite this, the inbreeding populations were more polymorphic although this variation was largely distributed among multilocus homozygous genotypes (BURDON *et al.* 1999).

Clearly, the way in which resistance is distributed in host populations and indeed, the type of resistances occurring in individual plant populations (qualitative, quantitative etc.) will be very strongly influenced by the interplay of life history features of both host and pathogen and its interaction with the environment. Undoubtedly, certain associations may follow unexpected evolutionary trajectories due to chance events or constraints imposed by other unrelated life history features. Notwithstanding this though, a detailed understanding of the causes of observed patterns of resistance in natural systems will only come with a greater appreciation of the importance of this interplay of life history features.

### Patterns of disease resistance in space

Landscapes are environmentally heterogeneous entities over which edaphic and meteorological factors vary from place to place. These factors act on hosts to affect the size of individual populations and their proximity to one other, and on pathogens to affect the incidence and severity of disease. Such populations do not exist in total isolation from one another, occurring instead in metapopulations containing numerous demes showing varying degrees of relatedness to each other (LEVINS 1969, 1970; HANSKI & GILPIN 1991, 1997). Evolutionary interactions occurring within metapopulations reflect a regional process involving different levels of gene flow, genetic drift and selection within and among demes (THOMPSON & BURDON 1992).

While a number of studies have examined host population resistance structures, the majority of these are restricted to simultaneous examination of only one or two populations. The only extensive detailed study available is that investigating interactions between *Linum marginale* and *Melampsora lini* in southern Australia. In one component of that study, invol-

ing 16 populations occurring in three distinct areas of a single metapopulation, considerable variation was detected among individual demes in their resistance response to 12 different pathotypes of *M. lini*. Although adjacent populations separated by only a few hundred metres often had markedly different resistance profiles, there was evidence of an isolation-by-distance effect such that host populations from the same area of the metapopulation had more resistance phenotypes in common than did populations from different areas (THRALL *et al.* 2001). The virulence structure of pathogen populations occurring at the same sites also showed considerable population-to-population variation but lack evidence of the spatial structuring detected in the host population. Given the air-borne nature of dispersal in *M. lini* this was not surprising.

Descriptions of differences in the resistance and virulence structure of host and pathogen populations respectively, and their placement in a spatial context, provides a solid grounding on which to further assess the extent of evolutionary interplay between the two partners. Thus patterns of local adaptation or maladaptation can provide insight into the spatial scale over which coevolution takes place (GANDON & VAN ZANDT 1998; KALTZ & SHYKOFF 1998). However, local adaptation is potentially mediated by a range of factors including spatial scale, the genetic basis of resistance and pathogenicity in the system concerned, and specific life history features such as relative dispersal distances (THRALL *et al.* 2002). As a consequence, in many host-pathogen associations a full assessment of this phenomenon requires comparison of the performance of pathogens both on the host population from which they were collected (sympatric comparisons) and all other host populations (allopatric comparisons). When this is combined with the need to provide adequate representation of each pathogen population, a full testing of the concept of local adaptation becomes a major exercise.

In the *L. marginale* – *M. lini* association such a comparison involving 6 host and pathogen population pairs has shown that while there is evidence of an overall trend for adaptation at a regional scale, there was no evidence to suggest that pathogens from more distant populations were necessarily less fit. Indeed, in line with the stochastic nature of interactions predicted for metapopulations, the actual outcomes encountered were greatly affected by the structure of individual host populations with resistant populations selecting for generally more virulent pathogens than more susceptible populations (THRALL *et al.* 2002).

### Changing patterns of resistance in time

Most host-pathogen associations are dynamic co-evolutionary processes in which changes to one partner in the association occur in response to, and in turn induce change in, the other partner. The generation and maintenance of variation in the pathogen partner of associations is relatively well understood with considerable complexity resulting from the interaction of migrational, recombination and chance mutation events that are subsequently subject to a diverse range of selective forces (Burdon 1994). In contrast, understanding of the origins of the diversity of resistance often encountered in plant populations is far less well documented with studies of temporal changes being particularly limited. Simple demographic/epidemiological experiments have documented significant mortality and a marked shift in the apparent relative resistance of survivors in a cohort of seedlings of *Filipendula ulmaria* subject to high disease loads over a five-year period (Ericson *et al.* 2002). However, detailed assessment of changes in the frequency of specific resistance phenotypes over the course of a major disease epidemic are extremely limited. One example involves a single population of *L. marginale* attacked by the rust pathogen *M. lini*. At the beginning of the epidemic this population was dominated by 5 resistance phenotypes (each > 10% of the population) with a further 10 present. Following the epidemic with its accompanying 78% mortality rate, the resistance structure of the host population became more diverse with a notable decline in the frequency of the previously common resistance phenotypes and an increase in the individual frequencies of the rarer phenotypes (Burdon & Thompson 1995). Such temporal fluctuations in the frequency of resistance phenotypes and hence shifts in the structure of individual host populations may occur as a direct result of the selective impact of the pathogen on individual host lines [e.g. in *Chondrilla juncea* populations attacked by the biological control agent *Puccinia chondrillina* (Burdon *et al.* 1981)] or perhaps more usually through a combination of these and other selective forces (Parker 1994).

In individual populations, epidemic peaks and troughs may explain year-to-year fluctuations in resistance structure. However, they do not explain the occurrence of different resistance phenotypes in different populations or the appearance of new phenotypes in any given population. In essence, where do these new resistances come from? Obviously, recombination of existing combinations of resistance genes and alleles giving rise to novel combinations, and gene-flow from

other populations broadens the range of resistance phenotypes in a population. However, analysis of the genetic basis of resistance in several wild populations has shown a marked tendency for gene combinations to be limited [in *L. marginale* – a mean of one gene per line (BURDON 1994); in *Glycine canescens*, two (BURDON 1987)]. More intriguingly though, in populations subject to extensive analysis, large numbers (> 100) of different resistance specificities have been detected (THRALL *et al.* 2001, and unpublished). These specificities are often only marginally different to each other and frequently are restricted to just one or a few populations. The high number of these phenotypes and their apparent relatedness raises significant questions regarding their origin and hence the speed with which the host may respond to continuing pathogen selective pressure.

A potential clue to the origins of these resistance specificities is the frequent distribution of resistances into allelic series (e.g. 23 and 30 alleles for resistance to *Puccinia sorghi* and *Melampsora lini* in 2 and 5 linkage groups in *Zea mays* and *Linum usitatissimum* respectively [HOOKER 1985; ISLAM & SHEPHERD 1991]). Detailed genetic studies of both of these interactions indicate that novel specificities may arise at relatively high frequencies [ $6.7 \times 10^{-3}$  to  $1 \times 10^{-2}$  at the Rp1 locus in maize (PRYOR pers. commun.)]. More recently a growing body of results from molecular studies of the architecture of individual resistance genes and relationships among alleles have confirmed that while the evolution of totally new resistance genes may be extremely rare events, the generation of new allelic specificities appears to occur at a much higher frequency (PRYOR 1987; MICHELMORE & MEYERS 1998; HULBERT *et al.* 2001). Some argument exists about the relative roles of equal and unequal exchange events at complex loci in this process, but there is very strong evidence showing that re-assortment of sequence polymorphisms by meiotic recombination is a principal force in resistance gene evolution.

The possibility that new resistance specificities may evolve at such a high rate (range of  $1 \times 10^{-2}$ – $10^{-3}$ ) has very significant implications for the co-evolutionary dynamics of host-pathogen associations. In disease-prone environments where selection pressures favouring resistance are high, the relatively high frequencies of these mechanisms generate scenarios in which large numbers of resistance alleles may be maintained in populations. In essence, they put hosts on a more equal footing with their pathogens.

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