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Coevolution at multiple spatial scales: *Linum marginale–Melampsora lini* – from the individual to the species

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Abstract. Coevolutionary processes are intrinsically spatial as well as temporal, and occur at many different scales. These range from single populations dominated by demographic and genetic stochasticity, to metapopulations in which colonisation/extinction dynamics have a large influence, and larger geographic regions where phylogenetic patterns and historical events become important. We present data for the genetically and demographically well-characterised plant host–pathogen interaction, the *Linum marginale–Melampsora lini* system, and use this to demonstrate the varying nature of resistance and virulence structure across these spatial scales. At the within population level, our results indicate considerable variability in resistance and virulence, but little evidence of coordinated changes in host and pathogen. Studies involving comparisons among multiple demes within a single metapopulation show that adjacent populations often have asynchronous disease dynamics and large differences in diversity and frequency of resistance and virulence phenotypes. Nevertheless, at this scale, there is also evidence of spatial structure in that more closely adjacent host populations are significantly more likely to have similar resistance phenotypes and mean levels of resistance. At larger scales, comparisons among adjacent metapopulations indicate that quantitative differences in host mating system and other life history features can have further major consequences for how host and pathogen variation is packaged. Finally, comparisons at continental and among host-species levels show variation consistent with specialisation and speciation in the pathogen.

Keywords: epidemiology, gene-for-gene resistance, metapopulation, pathogen, virulence

Introduction

Coevolution between plants and their fungal pathogens occurs in heterogeneous environments where host and pathogen populations tend to be discontinuously distributed and abiotic conditions vary from place to place, here favouring the growth and development of one partner, there favouring the other. It also occurs in the biotic context of interacting life history features of both partners that may shift subtly from region-to-region in the landscape. The

coevolutionary process is fuelled by the interplay between demographic and genetic features, epidemiological 'boom and bust' cycles in pathogen numbers leading to genetic drift, extinction and recolonization events, and the fitness consequences of pathogen attack and the genetic response of plant populations to such selection pressures.

This recognition of the important influence of heterogeneous environments on evolutionary dynamics is not new, with a history stretching from the pioneering population genetics work of Wright's stepping-stone, mainland–island and adaptive landscape models (Wright, 1932, 1943, 1982), to the initial metapopulation formulation of Levins (1969, 1970) and its subsequent development. In studies of interspecific interactions, the importance of a broad spatial view encompassing multiple populations has become increasingly recognized (Burdon *et al*., 1990; Thompson, 1990; Burdon, 1993; Antonovics *et al*., 1994, 1997; Thrall and Antonovics, 1995; Gandon *et al*., 1996; Lively, 1999), but it was left to Thompson (1994, 1997, 1998) to draw together the many disparate threads of numerous coevolutionary studies to propose an overarching hypothesis – 'the geographic mosaic theory of coevolution'. This hypothesis envisages coevolution as a process characterized by (i) variation among populations in the intensity and or direction of selective processes (a selection mosaic; Thompson, 1998); (ii) the occurrence of 'hotspots' of coevolutionary activity intermixed with sites of lesser activity; and (iii) the mixing of coevolved traits as a consequence of migration, genetic drift, extinction, recolonization and genetic recombination. As Thompson (1994) has pointed out ''the challenge for studies in coevolution is to understand how different ecological and genetic conditions and different configurations of population structure favour different modes and outcomes of the coevolutionary process''.

Here we use one of the genetically best defined natural coevolutionary interactions – that occurring in Australia between the rust fungus *Melampsora lini* and its native host plant *Linum marginale* – to probe the consequences of these interactions at a series of spatial scales. These range from intra-populational effects governed by limited seed dispersal and mating-system induced neighborhood effects, through inter-deme differences among the multiple populations of a single metapopulation, to differences between adjacent metapopulations occupying distinctly different environmental niches. Within one of these metapopulations, hybridization between two distinct ecotypes of *L. marginale* raises the distinct possibility of evolutionary 'hotspots' in which new resistance combinations or even new specificities may rise. Equally, the results of comparisons among host metapopulations with differences in the breeding system have significant implications for the resistance structure of individual populations and may well accord with differences in the rate of 'micro-evolutionary' responses of host and pathogen. Finally, at the regional and continental scale, differences in resistance and virulence occur over 100s

and 1000s of kilometres suggesting the first steps in a further process of separation and ultimate speciation.

The *Linum marginale–Melampsora lini* host–pathogen system

Here we consider the coevolutionary consequences of interactions between the endemic Australian plant *L. marginale* and its host-specific rust pathogen, *M. lini*, at a series of spatial scales. This native interaction occurs in a broad range of environments across a wide swathe of southern Australia, from sub-alpine herbfields and semi-arid rangelands of the southern interior of the continent, to coastal communities. Throughout this range *L. marginale* is a herbaceous perennial although its phenology is greatly influenced by the environment in which it grows. Thus in the sub-alpine regions of the Kosciuzko National Park, New South Wales, plants overwinter as underground rootstocks with or without a few short shoots protected from the frost by surrounding vegetation. In spring, fresh shoots develop and plants flower in mid to late summer before dying back in autumn. In the drier interior, on the other hand, this pattern is largely reversed with summer survival of plants largely being achieved via protected rootstock. Plants in this environment grow through winter and flower in early to mid spring. Population sizes typically range from less than a hundred through to several thousand.

Not surprisingly, the phenological development of the pathogen *M. lini* reflects that of its host. In the sub-alpine zone, pathogen populations build up to epidemic levels during the summer growing season before undergoing a precipitous crash as winter sets in. Although *M. lini* is an autoecious rust, survival in this environment appears to be entirely asexual (Burdon and Roberts, 1995). In contrast, in hotter drier regions, pathogen epidemics occur during the late winter–spring. In these regions the possibility of over-summer survival through a combination of asexual and sexual means appears most likely.

Linum marginale has no specialized means of seed dispersal with most, if not all, seeds being shaken loose from the capsule to fall close to the parent plant. *Melampsora lini*, on the other hand, is an aerially dispersed rust pathogen with a steep dispersal gradient that results in most spores landing within the same host population (J.J. Burdon and A.M. Jarosz, unpublished data). However, some spores are likely to travel considerable distances.

In studying this coevolutionary system we have investigated interactions within and among demes within a single metapopulation occurring on a 31 km^2 area of sub-alpine grassland at Kiandra in the Kosciuzko National Park (Fig. 1). In this area *L. marginale* is composed of two distinct ecotypes, one occurring on hillsides ('hill' type), the other in boggy areas along stream courses ('bog' type). Occasional hybridization events occur where two such

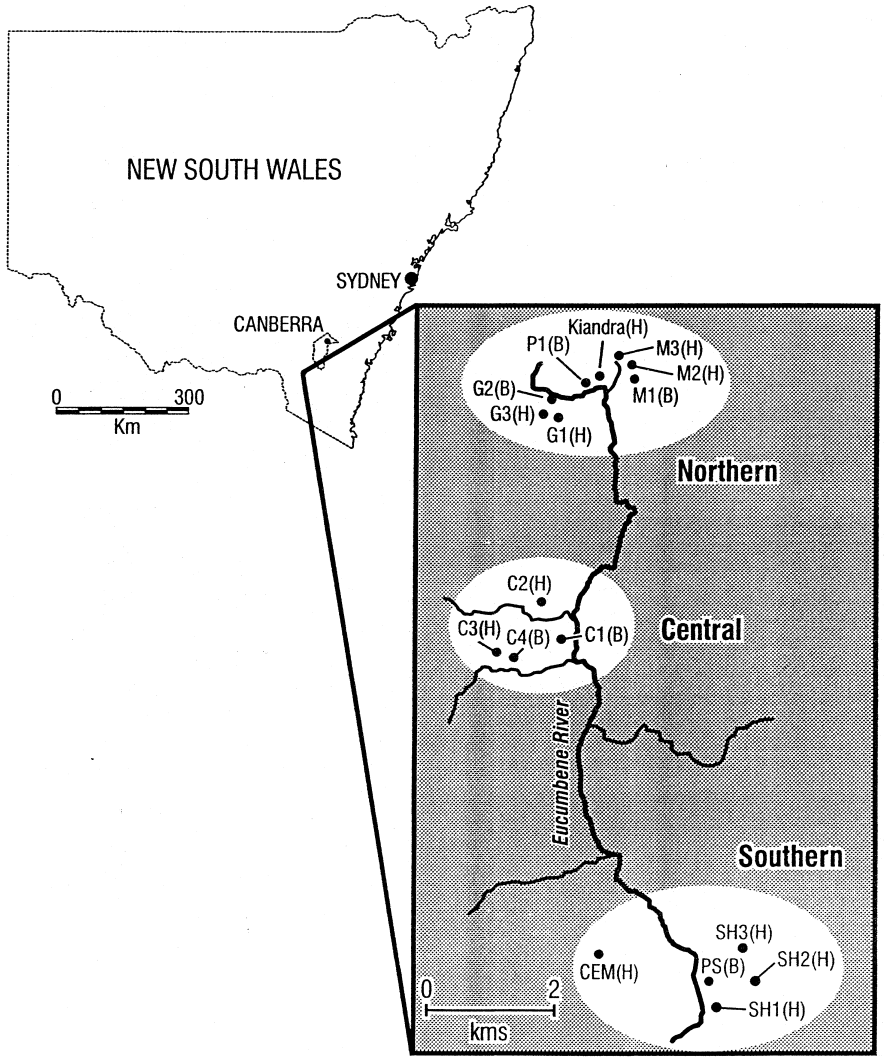

Figure 1. Schematic diagram of the Kiandra (=Mountains) metapopulation showing the distribution of individual host–pathogen demes in the southern, central and northern zones. A potential evolutionary hot-spot in which hybridization between the 'bog' and 'hill' ecotypes of *L. marginale* is occurring is found between c3 and c4. Bog [B] and hill [H] type populations are identified.

populations come into close contact, leading to the development of small hybrid swarms (Carlsson-Granér *et al.*, 1999). Patterns observed within the Kiandra Mountains metapopulation have been contrasted with the behaviour of the same host–pathogen interaction in an adjacent metapopulation occurring on the central plains of New South Wales (the Plains metapopulation – closest population ca. 100 km away). Finally a comparison of patterns of host

resistance and pathogen virulence across the continent (ca. 3000 km), and between Tasmania and south-eastern Australia (ca. 700 km), provides a yet larger scale of interaction.

The spatial scales of coevolution

A basic building requirement for any kind of coevolutionary accommodation between organisms is genetically based variation in both partners for characters of relevance. In host–pathogen systems this may encompass a range of features that influence virulence and/or aggressiveness in the pathogen or resistance in the host. The latter may include a range of morphological (e.g. floral structure, attractiveness to pollinators and potential pathogen vectors; Alexander, 1989; Thrall and Jarosz, 1994) or phenological features (e.g. timing of germination or flowering) although in *L. marginale* the primary mode of resistance to attack by *M. lini* appears to be biochemical. Indeed, detailed assessment of F_1 progeny and F_2 and F_3 segregating families of crosses among various *L. marginale* lines clearly indicate that resistance is controlled by single dominant genes inherited in a simple Mendelian fashion (Burdon, 1994). In contrast, significant phenotypic variation in pathogen virulence has been demonstrated using a standard set of host lines carrying different genes or alleles for resistance. The genetic basis for these differences have not been shown in Australian populations of *M. lini* but in his classic study of the 'gene-for-gene' interaction between host and pathogen, Flor clearly demonstrated the genetic basis for such differences in *M. lini* (Flor, 1942).

Within population effects

To take part in the coevolutionary interaction, each player has to be able to place their prospective partner under sufficient selective pressure as to impose fitness effects. In the *Linum–Melampsora* metapopulation occurring on the Kiandra Plain, long-term host demographic and pathogen epidemiological data has shown such pathogen-imposed effects, with host population crashes of 68–77% being correlated with major pathogen epidemics (Jarosz and Burdon, 1991, 1992). However, as has been observed in other host–pathogen systems (Burdon *et al.*, 1995; Ericson *et al.*, 1999) host and pathogen behaviour within local populations is unpredictable in time and intensity. Epidemics of *M. lini* sometimes follow each other year-on-year, while at other times epidemic peaks are followed by years of little, if any, pathogen activity (Jarosz and Burdon, 1992).

These marked and stochastic swings in the size of pathogen populations have a direct effect on pathogen population diversity and structure. In one deme (P1) of the Kiandra metapopulation (Fig. 1), several pathotypes fluctuated in incidence, being present some years and absent in others in an unpredictable fashion – a pattern consistent with random genetic drift occurring while the population size is small during and immediately following the winter crash. However, in another host population (Kiandra – separated from P1 by only 225 m), one particular pathotype dominated (comprising 45–80% of the population) for many years although the structure and phenotypic diversity of the remaining fraction of the pathogen population showed considerable unpredictability (Burdon and Jarosz, 1992; Burdon, 1996).

At the scale of the single population, the interaction of pathogen-induced selection and host resistance is also frequently difficult to interpret. In the Kiandra population of *L. marginale*, a period of several low disease years allowed the build-up of the host population such that in 1989, the year of a major pathogen epidemic, the local spatial distribution of host genotypes within the population was well-developed (Burdon and Thompson, 1995; Fig. 2). As a consequence of the epidemic, 79% of the host population died, and there was significant change in the frequency of the commonest resistance phenotypes (those occurring at $>5\%$ prior to the epidemic) leading to a more even frequency and spatial distribution of host phenotypes.

Simple theoretical models often envisage a process of local frequency-dependent selection leading to a continuing spiral of rises and falls in the fre-

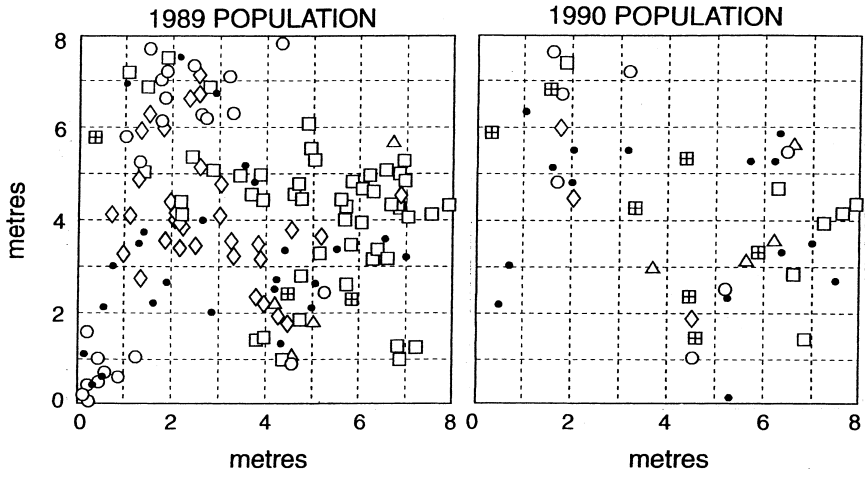

Figure 2. Spatial distribution of the five most common resistance phenotypes of *L. marginale* in the year of a major epidemic of its host specific pathogen *M. lini* (1989) and in the subsequent year (1990). The different resistance phenotypes were defined by their response to attack by 9 different pathotypes of *M. lini* (redrawn from Burdon and Thompson, 1995). The common host resistance phenotypes are identified by the different symbols; all other phenotypes are marked by a dot.

quency of particular resistance genes in response to similar cycles in the corresponding virulence genes in the pathogen population (Person, 1966; Jayakar, 1970). However, changes in the frequency of resistance phenotypes detected in the Kiandra population could not be attributed directly to differences in their susceptibility to the most common pathotypes occurring in the population during the epidemic. Indeed, those host phenotypes that rose in frequency in the population were the more susceptible ones (attacked by a greater percentage of the common pathotypes) while the two most resistant lines that together made up 78% of the pre-epidemic *Linum* population declined to a combined frequency of just 11.5% (Burdon and Thompson, 1995). Explanations for these changing patterns of resistance in the *L. marginale* population cannot be conclusive but possibly represent maladaptive changes resulting from linkage between resistance genes and other traits under even more intense selection. Such a pattern has been observed in other single population studies of plants and their pathogens (Parker, 1991).

At the scale of the individual population, spatial structure is detectable in the distribution of host resistance phenotypes (Burdon and Jarosz, 1991; Burdon and Thompson, 1995; see Fig. 2), but this is more likely to reflect neighbourhood effects resulting from limited seed dispersal rather than any tight causal interaction with the pathogen population. In essence, the host-pathogen association in an individual population shows several of the basic features required for reciprocal interaction and evolution between the antagonistic players. However, because such individual populations are all ephemeral, their individual fate is inevitably extinction rather than long-term coevolution. Moreover, the unpredictability imposed on the interaction at this highly restricted scale by year-to-year environmental heterogeneity, and by genetic drift and gene-flow in both host and pathogen populations, mean that individual populations alone provide poor guidance as to the overall coevolutionary direction of the evolving metapopulation.

Hybrid zones – hotspots of evolutionary activity?

Hybridization between locally adapted plant populations can have significant evolutionary consequences, including the creation of novel genotypes, changes in genetic diversity and transfer of adaptations between populations (cf. Riesenberg and Wendel, 1993). The sites of such hybridization activity may therefore represent important 'coevolutionary hot-spots' (Thompson, 1994, 1997) in which the host may develop new combinations of resistance or may even generate new alleles through enhanced intra-genic recombination due to chromosomal differences between parental types (Pryor and Ellis, 1993). Hybridization and subsequent introgression may also be a way whereby

resistance genes are transported among locally adapted populations. These scenarios may also affect pathogen populations by conferring a selective advantage on those individuals with an appropriate combination of virulence genes. As a consequence such hot spots may be important sites for the evolution of virulence in pathogens.

Linum populations can be classified into one of two morphologically distinct ecotypes ('bog' and 'hill') on the basis of overall size, numbers of basal shoots, and differences in floral morphology. These differences have been confirmed by allozyme studies showing strong genetic divergence at more than 14 loci. The possibility of evolutionary hot-spots occurs in the Kiandra (Mountains) metapopulation where a limited number of hybrid zones have been found at which bog and hill ecotypes of *L. marginale* come into close contact. In these zones hybrids are identifiable as morphological intermediates (confirmed using allozymes) between the two parent populations.

In one such situation that has been examined in detail, Carlsson-Granér *et al.* (1999) found that bog plants were generally susceptible to pathogen isolates taken from the bog, hill or hybrid populations, but that the hybrid population exhibited a mean frequency of resistance similar to the more resistant hill plants (Fig. 3). Similarly, even though the general pattern of the

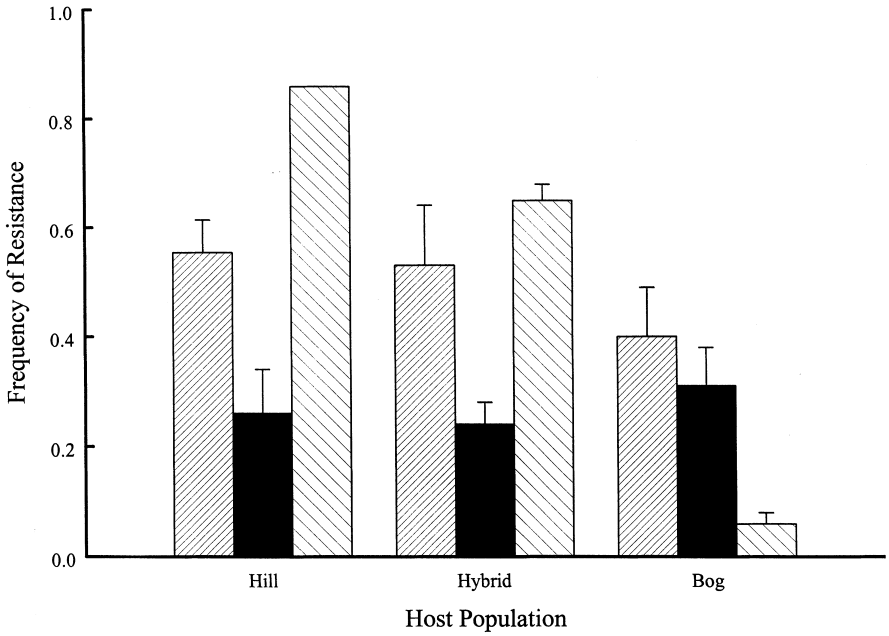

Figure 3. Pattern of resistance to *M. lini* in bog, hill and their associated hybrid populations of *L. marginale* to pathogen isolates taken from the three areas of the Mountains metapopulation respectively (densely fill bars = hill isolates; closed bars = hybrid isolates; lightly filled bars = bog isolates).

three pathogen populations were broadly similar (one pathotype dominating multiple minor pathotypes), the virulence structure differed considerably between the bog and other two populations. Thus the frequency of virulence in the bog population towards a standard set of 10 host lines, each carrying a different resistance gene (the *Linum* differential set – half of which includes resistance genes from the Kiandra region; see Burdon and Jarosz, 1991) was generally low for all but two genes. In contrast, the hill and hybrid pathogen populations possessed intermediate or high frequencies of virulence (20% or greater) to at least half of the differential lines.

In general, *Linum* populations within the Kiandra metapopulation have negligible rates of outcrossing (Burdon *et al*., 1999). However, if special features of the micro-environment associated with these bog-hill hybrid interfaces promotes outcrossing between bog and hill plant populations, new combinations of host resistance become possible. In that case, one might expect a corresponding change in the virulence structure of associated pathogen populations. Certainly, given that only a single hybrid situation has been assessed, caution must be applied in interpreting results. However, the idea that the hybrid zone could be an active site of coevolutionary change is supported by the observation that although a number of pathotypes occurred in all areas, 30% of the pathotypes present in the hybrid population were unique to that population (equivalent percentages for hill and bog populations were 21 and 20% respectively). While further hybrid populations have not been assessed as yet, a comprehensive study of bog and hill populations in the Kiandra metapopulation has confirmed the existence of significant differences in host resistance and pathogen virulence structures between these two ecotypes (see below; Thrall *et al*., 2001).

Among deme within metapopulation effects

Within the Mountains metapopulation, of which the Kiandra population discussed above is part, considerable variation was observed among the dynamics of 13 individual local host and pathogen populations. These populations were distributed into three distinct areas – the southern, central and northern zones – within which individual host populations were separated by distances ranging from 100 m to 1.5 km, while among the zones, separation distances varied from 3 to 10.5 km (Fig. 1). A further complication is provided by the occurrence of two distinct ecotypes of *L. marginale* in the area. These 'bog' and 'hill' ecotypes are specialized to their respective environments and occur scattered across the metapopulation area.

Over a 4-year period, epidemic development varied across years and populations. Some years were generally favourable for pathogen development while

others were less so. However, set against this general physical environmental backdrop, there was considerable asynchrony among populations in the severity of epidemics in particular years with adjacent populations showing similar disease incidence in some years and very dissimilar levels in others (Fig. 4).

Detailed study of the pathogenic structure of individual pathogen populations also detected considerable variation and general unpredictability among

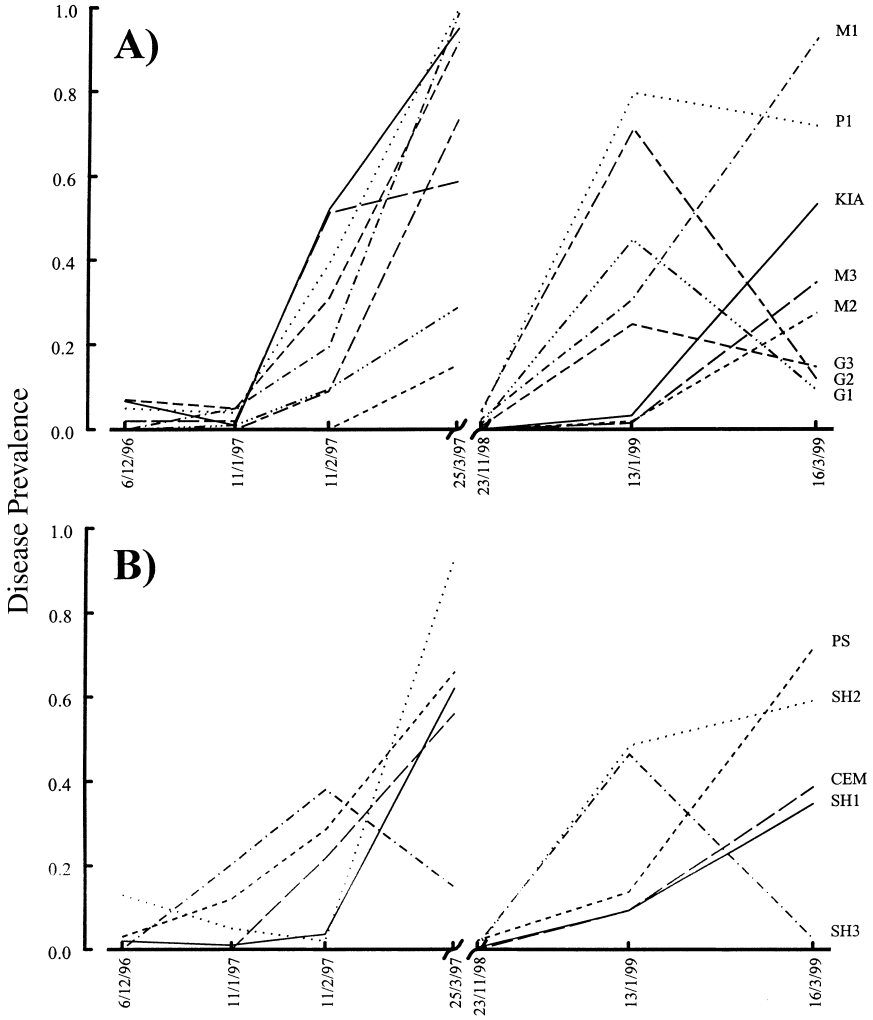

Figure 4. Asynchronous patterns of disease development in populations of *L. marginale* growing in the (a) northern and (b) southern regions of the Mountains metapopulation over the period December 1996–March 1999. Disease prevalence was assessed by scoring presence/absence of pustules on a minimum of 100 randomly chosen plants from each population.

different demes. While the pattern of virulence shown was similar in many pathogen populations, the actual pathotypes that were present varied considerably. Even the commonest pathotype (A) which had a frequency of $> 20\%$ in the metapopulation as a whole showed considerable variation in occurrence and prevalence among adjacent host populations (Fig. 5a), among the different regions of the metapopulation (Fig. 5b), as well as between the two *Linum* ecotypes (Fig. 5c). Indeed, pathotype N that dominated populations in the Central region of the metapopulation, and was present in the northern region, was totally absent from southern populations. Even within regions, considerable variation existed between pathogen populations growing on host populations of the same ecotype (e.g. compare Kiandra and G3 which are only 950 m apart; Fig. 5a) and certainly between 'hill' and 'bog' populations compared either as individual adjacent pairs or across the metapopulation as a whole (Fig. 5a). In fact, χ^2 goodness-of-fit tests showed that the most common pathotypes (those occurring at $> 5\%$ across the metapopulation) were all non-randomly distributed with respect to host ecotype, with pathotype A being the only one more common in hill populations than bogs (Table 1). In keeping with the absence of pathotype N from the Southern populations, it was the only pathotype non-randomly distributed among the three subregions. Although the overall distribution of minor pathotypes (making up 40% of the total number of isolates) did not vary among regions, a significantly greater percentage of hill pathogen populations were composed of these minor races than bog populations, reflecting the overall greater diversity of hill pathogen populations (Table 1).

Asynchrony in disease incidence and severity over space and time has also been observed in a number of other multi-population systems (Burdon *et al.*, 1995; Ericson *et al.*, 1999) where it has been used to argue that the variable selective forces thus generated are likely to result in variable population resistance structures. In the studies described here, we have tested that proposition explicitly by challenging host populations with a range of pathogen isolates from the metapopulation.

Within 16 demes of *L. marginale* considerable within and among population differentiation was detected in resistance. Again there was considerable difference between the two *Linum* ecotypes with populations of hill types being consistently more resistant than those in the bogs (Thrall *et al.*, 2001). However, even when these ecotypic differences are factored out, there is still clear evidence of a non-random spatial distribution of resistance. Individual Mantel tests of the independence of inter-population distance matrices and matrices containing among-population differences in resistance responses, showed that resistance to several of the pathotypes was significantly non-random in hill populations (isolate S2: $r^2 = 0.10$, $p = 0.03$; isolate S1: $r^2 = 0.30$, $p = 0.008$; isolate C7: $r^2 = 0.09$, $p = 0.04$; isolate G3: $r^2 = 0.26$,

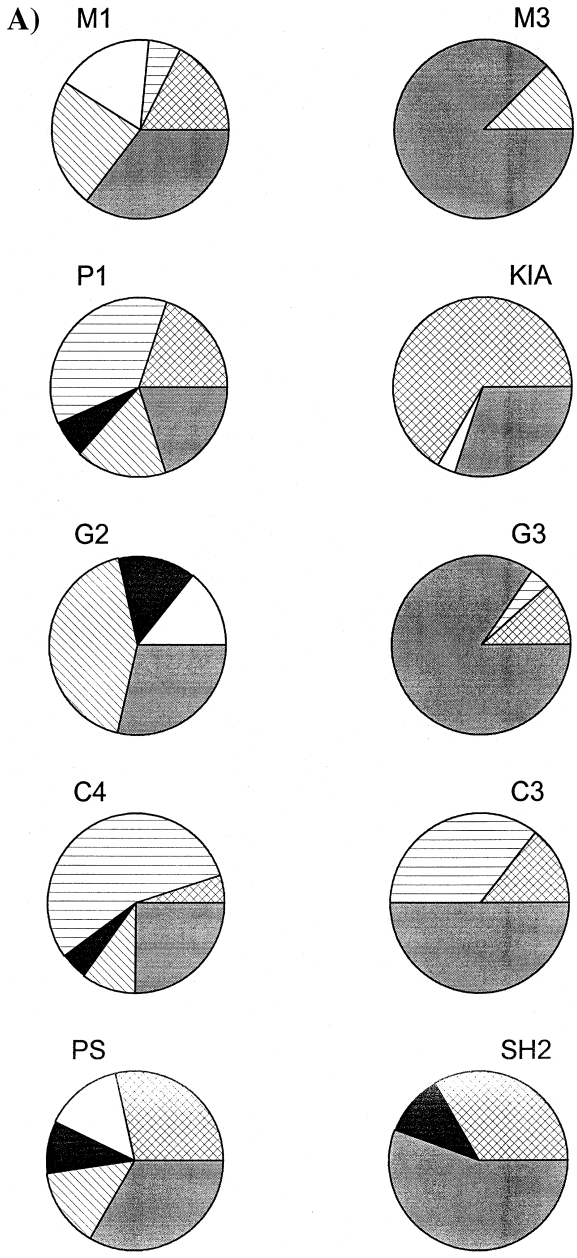

Figure 5. Frequency distribution of five most frequent pathotypes of *M. lini* in the Mountains metapopulation. (a) comparison of individual pairs of adjacent bog and hill demes; (b) frequency distribution of pathotypes according to metapopulation sub-region; (c) frequency distribution according to the ecotype of the host population (cross-hatched lines = pathotype A; diagonal lines = pathotype E; open = pathotype K; horizontal lines = pathotype N; solid black = pathotype U; grey = all other pathotypes combined).

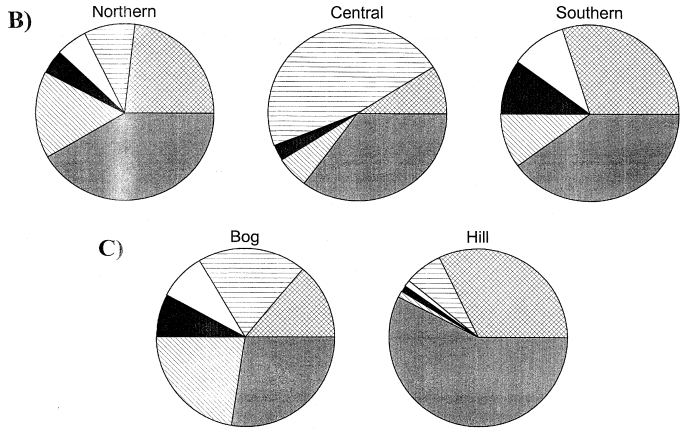

Figure 5. (continued)

$p = 0.008$), with populations closer together being more similar in resistance to those pathotypes. Thus, for example, hill populations in close proximity to one another have more resistance types in common, and more similar mean resistance responses to challenging pathogen isolates, than do more distant demes (Fig. 6). This indicates at least weak isolation with distance, with gene-flow being sufficient to redistribute genes among populations within parts of the metapopulation but insufficient to carry them rapidly across the entire area. By

Table 1. The χ^2 goodness-of-fit tests for the distribution of the five most common pathotypes of *M. lini* occurring in the Mountains metapopulation. The 'minor' category consists of the pooled data for all pathotypes with frequencies of <0.05 across the metapopulation as a whole. (Pathotype designations are based on the pathogenicity of isolates on the *Linum* differential set; Burdon and Jarosz, 1991)

(a) Distribution among the three subregions (Northern, Central, Southern) of the metapopulation (figures in brackets are expected values)

Pathotype	Northern	Central	Southern	χ^2
A (0.22)	32 (30.1)	3 (7.4)	9 (6.5)	NS
N (0.14)	13 (19.9)	16 (4.9)	0 (4.3)	32.22 ($p < 0.001$)
K (0.13)	22 (18.5)	2 (4.5)	3 (4.0)	NS
Minor (0.51)	71 (70.5)	13 (17.3)	18 (15.2)	NS

(b) Distribution among the two ecotypes (bog, hill) of the metapopulation

Pathotype	Bog	Hill	χ^2
A (0.22)	16 (25.1)	28 (18.9)	7.76 ($p < 0.01$)
N (0.14)	23 (16.6)	6 (12.4)	5.82 ($p < 0.025$)
K (0.13)	26 (15.4)	1 (11.6)	16.90 ($p < 0.001$)
Minor (0.51)	51 (25.9)	52 (44.1)	25.86 ($p < 0.001$)

way of contrast, the relative lack of spatial structure in the pathogen populations reflects their greater dispersal ability (Thrall *et al.*, 2001).

The overall picture that results from this assessment of the dynamics and structure of the *Linum–Melampsora* host–pathogen interaction metapopulation is one of considerable unpredictability in which even closely adjacent populations may show evidence of asynchrony and mismatching of evolutionary trajectories. In this system, the occurrence of strong local differences in disease severity and pathogen population structure provides a strong selection mosaic. In turn, this is responsible for the marked spatial differences in resistance structure observed. The 'glue' holding these divergent elements together in an interacting metapopulation is the exchange of genetic material between overlapping sets of adjacent populations across the entire metapopulation.

Among metapopulation effects

Comparison of the two distinct, yet adjacent metapopulations of the *L. marginale–M. lini* interaction gives some insight into the extent of the evolutionary

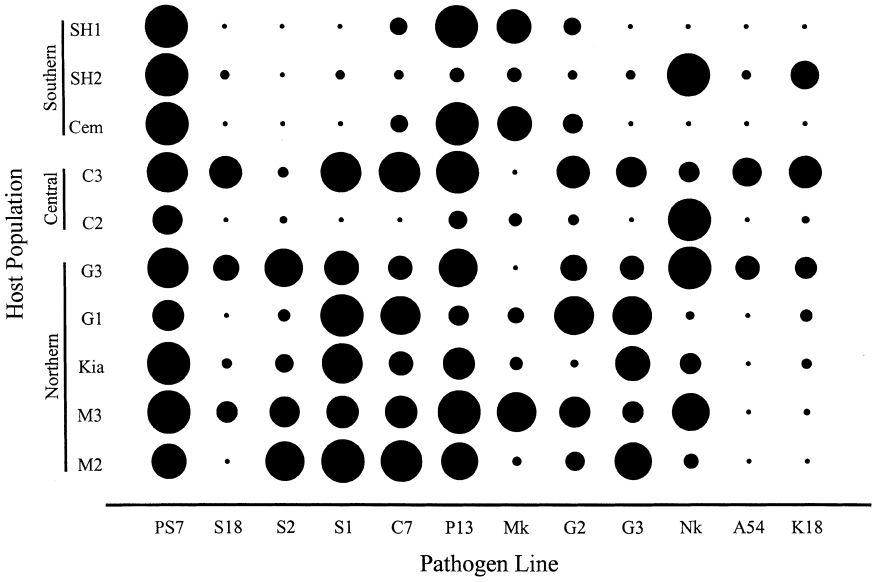

Figure 6. Mean resistance response of 10 populations of the hill ecotype of *L. marginale* growing in the Mountains metapopulation to 12 isolates of the pathogen *M. lini* collected from the same area. The ten host populations were distributed among the southern (3), central (2) and northern (5) regions of the metapopulation. Mean resistance response was measured as the percentage of plants showing resistant responses to each pathogen isolate (ca. 20 plants per population). Redrawn from Thrall *et al.*, 2001.

flexibility of host and pathogen species as they respond to each other under different sets of conditions. As noted earlier, the two metapopulations (Plains and Mountains) differ in the annual cycle of disease development with disease epidemics in demes of the Plains metapopulation having mostly run their course before those in the Mountains metapopulation have started to develop. Compounding this difference imposed by the physical environment, is a marked switch in the mating system of *L. marginale* with demes in the Mountains metapopulation being highly inbred while those on the Plains showing appreciable levels of outcrossing (Burdon *et al*., 1999). Mean levels of resistance, and the number and evenness of distribution of resistance phenotypes within populations, differed among populations within the two metapopulations. In general, values for these parameters were consistently higher in the Plains than the Mountains metapopulation but the differences were rarely significant. In contrast, significant differences in the frequency of individual virulences were detected between the two pathogen metapopulations with that in the Plains carrying virulences that were absent in the Mountains and vice versa.

The contrasting mating systems of the Plains and Mountains metapopulations have a significant impact on the way variation in general, and resistance in particular, is 'packaged' in populations in the two areas. Despite the higher levels of heterozygosity and outcrossing of the Plains populations, gene diversity (determined by isozyme analysis) was actually higher in the Mountains populations. The latter populations were more polymorphic although a greater proportion of this variation was 'frozen' in a number of discreet multilocus homozygous genotypes. Moreover, resistance tests of multiple progeny from single mothers showed that in nearly all cases they had the same resistance phenotypes (Table 2). In contrast, in the Plains metapopulation there was considerable evidence for continuing recombination through outcrossing as shown by the isozyme data which were supported by the higher mean levels of within-family variation in resistance (Table 2; Burdon *et al*., 1999).

No comparable information is available concerning the mating system of the two pathogen metapopulations. However, work on pathogen populations oc-

Table 2. Measures of differences in the breeding system of *L. marginale* populations occurring in the Mountains and Plains metapopulations. Equilibrium outcross rates were estimated using allelic variation in three enzyme systems while the mean level of within family variation for resistance was estimated from the response of circa 20 family lines per population to 6 different pathotypes of *M. lini* (Burdon *et al*., 1999). Data are based on 4 Mountain and 5 Plains populations

	Metapopulation	
	Mountains	Plains
Equilibrium outcross rate	0.03	0.28
Mean level of within family variation for resistance	0.06	0.64

curring in the Mountains metapopulation found clear evidence of a clonal structure with very strong linkage disequilibrium between different RFLP markers (Burdon and Roberts, 1995). Similar data are unavailable for the Plains metapopulation but circumstantial evidence suggests a mixed mating system may occur. Indeed, the occurrence of substantial telia formation (required for sexual reproduction), the greater evenness of distribution of the different pathotypes in the Plains population, and the greater proportion of polymorphic virulence loci in those populations are all consistent with the occurrence of some sexual recombination (Burdon *et al*., 1999).

In recent years there has been considerable interest in the possibility that pathogens may be a driving force in the evolution of sex (Levin, 1975; Hamilton, 1980; Bell, 1982; Hamilton *et al*., 1990). Here the differences in breeding system between Plains and Mountains metapopulations may reflect a continuing higher selection pressure being exerted on the host population in the Plains. Certainly, pathogen demes in the Plains may suffer population crashes of a similar magnitude as those occurring in the Mountains and hence ineffective resistance genes may be restored to effectiveness through local pathogen extinction (Burdon *et al*., 1996), but the more prolonged period of potential interaction each season in the Plains may have favoured a more open recombination system.

The extent of likely interactions between the adjacent Plains and Mountains metapopulations is difficult to predict although given the 'out of phase' phenology of development of both host and pathogen, migration events may be relatively infrequent. However, as has often been shown to be the case for agricultural systems, events occurring at the lower limit of detection can result in abrupt shifts in the direction of selection (c.f. changes in the virulence of wheat stem rust in Australia as a consequence of long-distance migration and somatic hybridization; Watson and Luig, 1959; Watson, 1981). Thus, during the period of these studies at least one pathotype appeared in the Kiandra metapopulation that was pathogenically and biochemically unlike any other pathotypes in that metapopulation. This pathotype, that carried a highly unusual virulence and a unique isozyme fingerprint, was first detected in the Kiandra population in 1988 where it rose in frequency over a 3-year period to approximately 38% of the population before suddenly disappearing. Its origin remains a mystery but migration from another metapopulation such as that on the Plains remains the most probable explanation (J.J. Burdon, unpublished data).

Evolutionary trends at the continental scale

At the continental scale, distinct differences in the pathogenicity of *M. lini* have been observed between isolates collected in western and eastern Australia, such

that those from the west formed a discrete group that were avirulent against most resistance phenotypes found in eastern Australian populations of *L. marginale*. Because of marked differences in accessibility between eastern and Western Australia (WA), comparisons could only be made using 4 pathotypes and 4 *Linum* accessions from Western Australia, 26 pathotypes and 27 host phenotypes from mainland eastern Australia, and 7 pathotypes and 11 host accessions from Tasmania. Despite this, differences between the pathogenicity of isolates from the two sides of the Australian continent were very clear (Table 3). On average, 75% of pair-wise combinations of WA pathogen isolates and WA host lines showed susceptible reactions (pathogen virulent) (Table 3a). In contrast, less than 18% of pair-wise combinations of WA pathogen isolates with hosts from eastern Australia were virulent (Table 3b). The eastern Australian isolates of *M. lini* showed the reverse pattern, being virulent in 53% of combinations involving hosts from the east and only 18% in those involving hosts from the west (J.J. Burdon, unpublished data).

There were also significant differences in the mean percentage of virulent pair-wise combinations between *M. lini* isolates from Tasmania and Western Australia. Rather surprisingly these ran counter to expectation with Tasmanian pathotypes showing significantly less virulence against host lines from Tasmania than to those from Western Australia ($\chi^2_1 = 17.65$, $p < 0.001$). Comparisons of Tasmanian and eastern mainland pathogen isolates, on the other hand found no significant differences ($\chi^2_1 = 3.53$, $0.1 < p < 0.05$). Such differences essentially define major epidemiological regions and supra-metapopulation blocks, in which evolutionary trajectories in the pathogen populations are very different. This is likely to reflect the combined effects of large-scale environmental differences, and historical chance events interacting with genetic drift and isolation.

Table 3. Pathogenicity of major regional groupings of *M. lini* on *L. marginale* lines (a) Pathogenicity of Western Australian isolates of *M. lini* towards *L. marginale* hosts from two areas (figures in brackets are expected values) (b) Pathogenicity of East Australian mainland isolates of *M. lini* towards *L. marginale* hosts from two areas (figures in brackets are expected values)

	Avirulent	Virulent
(a)		
Western Australian hosts	4 (12)	12 (4)
East Australian mainland hosts	89 (81)	19 (27)
	($\chi^2_1 = 24.49$; $p > 0.001$)	
(b)		
Western Australian hosts	89 (56)	19 (52)
East Australian mainland hosts	331 (364)	371 (338)
	($\chi^2_1 = 46.60$; $p > 0.001$)	

The species level and beyond

Beyond the differentiation that has developed in the spectrum of pathogenicity shown by the *M. lini* populations in Western and eastern Australia, comparison of the virulence of *L. marginale* derived isolates of *M. lini* with isolates derived from cultivated flax (*L. usitatissimum*) shows a further gulf of differentiation and reciprocal specialization. Thus *L. marginale* isolates were avirulent on all members of the *L. usitatissimum* differential set except those that carry no resistance genes (e.g. cv. Hoshangabad) or one particular resistance gene present in some *L. usitatissimum* lines (the 'N' gene; Islam and Mayo, 1990). Similarly, *L. usitatissimum*-derived pathogen isolates were avirulent on all *L. marginale* lines known to be carrying a resistance gene (Lawrence and Burdon, 1989; Burdon, unpublished data).

Melampsora lini has been reported from a range of *Linum* species, a temperate – subtropical genus of c180 species that are arranged into 5 taxonomic sections. Within these species, early work with *Melampsora* isolates from *L. catharticum, L. alpinum, L. striatum* and *L. tenuifolium* (all from a different taxonomic section than *L. usitatissimum*) suggested that they were restricted in their host range, being unable to attack *L. usitatissimum* (Buchheim, 1915, cited in Laundon and Waterston, 1965). Indeed, at various times the form that occurs on *L. catharticum* has been recognized as sufficiently different as to warrant subspecific (*M. lini* var. *liniperda*) or specific (*M. liniperda*) rank although this is not currently seen as a useful distinction (Laundon and Waterston, 1965). Indeed, if asexual urediospores are used as the inoculum, a similar separation in pathogenicity can be demonstrated between *M. lini* occurring on *L. lewisii* and *L. usitatissimum*. Intriguingly though, Arthur (1907, cited in Laundon and Waterston, 1965) found that this distinction appeared to disappear in the sexual stage of the pathogen's life cycle. Thus when basidia from germinating teliospores collected from *L. usitatissimum* were used as inoculum, *L. lewisii* plants became infected.

The complexity of these coevolutionary interactions between *M. lini* and its various potential hosts await detailed investigation, however, an exciting feature of the *Linum–Melampsora* interaction is the enormous amount of understanding that is now developing regarding the molecular structure of individual resistance genes (Ellis *et al*., 1995, 1999). As more of these genes in *L. usitatissimum* are cloned, resistance-gene specific probes will become available that will allow the distribution of particular resistance gene families to be traced within and among sections in *Linum*. This will facilitate the tracking of coevolutionary trajectories at the phylogenetic level and, in turn, should help our understanding of events occurring at the boundary between coevolution and speciation.

Conclusions

In line with expectations of the geographic mosaic theory of coevolution (Thompson, 1994, 1999), diversity in host resistance and pathogen virulence structures clearly occurs over a variety of spatial and temporal scales ranging from broad geographic patterns down to sharp differences among adjacent populations. In the latter situation, the available evidence strongly suggests that evolution results from a complex interaction between colonization-extinction processes and asynchronous dynamics in a metapopulation context. As a consequence, a detailed understanding of the long-term trajectory of coevolutionary dynamics between host and pathogens is only likely to result from studies that investigate multiple populations over multiple seasons in conjunction with the development of models that investigate the interplay of host and pathogen life history attributes against a backdrop of varying dispersal scales (Thrall and Burdon, 1997, 1999).

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