Molecular Ecology (2008) **17**, 3401–3415 doi: 10.1111/j.1365-294X.2008.03843.x

Population structure and diversity in sexual and asexual populations of the pathogenic fungus *Melampsora lini*

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Abstract

Many pathogens undergo both sexual and asexual reproduction to varying degrees, yet the ecological, genetic and evolutionary consequences of different reproductive strategies remain poorly understood. Here we investigate the population genetic structure of wild populations of the plant pathogen *Melampsora lini* on its host *Linum marginale*, using amplified fragment length polymorphism (AFLP) markers, two genes underlying pathogen virulence, and phenotypic variation in virulence. In Australia, *M. lini* occurs as two genetically and geographically divergent lineages (AA and AB), one of which is completely asexual (AB), and the other able to reproduce both clonally and sexually (AA). To quantify the genetic and evolutionary consequences of these different life histories, we sampled five populations in each of two biogeographical regions. Analysis of AFLP data obtained for 275 isolates revealed largely disjunct geographical distributions for the two different lineages, low genetic diversity within lineages, and strong genetic structure among populations within each region. We also detected significant divergence among populations for both *Avr* genes and virulence phenotypes, although generally these values were lower than those obtained with AFLP markers. Furthermore, isolates belonging to lineage AA collectively harboured significantly higher genotypic and phenotypic diversity than lineage AB isolates. Together these results illustrate the important roles of reproductive modes and geographical structure in the generation and maintenance of virulence diversity in populations of *M. lini*.

Keywords: avirulence, co-evolution, host–pathogen, metapopulation, Red Queen, virulence

Received 22 December 2007; revision received 1 May 2008; accepted 13 May 2008

Introduction

Host–parasite interactions are thought to be a major force driving co-evolutionary change and generating biological diversity (Thompson 2005). In host–pathogen systems, co-evolutionary dynamics are largely contingent upon interactions between host resistance and pathogen infection strategies. Genetic variation in these characters in both host and pathogen populations is common, and thought to be a crucial factor influencing disease dynamics in human (Cooke & Hill 2001), plant (Alexander *et al*. 1993) and animal (Bishop & Morris 2007) systems. However, the underlying micro-evolutionary processes that generate and maintain such diversity in natural host–pathogen interactions, and the epidemiological consequences of such variation, remain poorly understood (Thrall & Burdon 2003).

Pathogenic species exhibit extensive diversity in their modes of reproduction (i.e. relative level of sexual vs. asexual reproduction) (Kohn 1995; Milgroom 1996), with potentially profound consequences for levels of genetic variation within populations (Milgroom 1996), population growth rate (Heitman 2006), persistence within populations (Barrett *et al*. 2007), and evolutionary change (McDonald & Linde 2002). The role and significance of sexual reproduction in interactions between pathogens and their hosts in particular has been heavily debated (e.g. Keeling & Rand 1995; Fox *et al*. 1996; Lively *et al*. 2004; Salathé *et al*. 2006). For example, the Red Queen Hypothesis predicts that sexual reproduction

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in host species is an adaptation against rapidly evolving parasites because it enables hosts to respond quickly to the appearance of novel pathogen genotypes through recombination of resistance genes (Hamilton 1980; Clay & Kover 1996). By extension, the Red Queen Hypothesis also predicts that parasites infecting sexual hosts should be genetically more variable than those infecting asexual hosts because recombination of resistance genes will drive negative frequency-dependent selection on the corresponding virulence genes of parasites (Clay & Kover 1996; Ooi & Yahara 1999).

Spatial and environmental heterogeneity in the interactions between parasites and their hosts can have a major influence on co-evolutionary dynamics across landscapes (Thompson 2005). Many host species, particularly those in natural ecosystems, exist as interacting groups of small, geographically and genetically differentiated populations (Burdon 1992), subdividing pathogen populations into relatively small, discrete units. Such spatial and environmental heterogeneity among pathogen populations is likely to strongly influence spatial patterns of disease incidence and persistence (Thrall & Burdon 1997), with populations of many species shown to undergo frequent local extinctions and recolonizations (Antonovics *et al*. 1994; Ericson *et al*. 1999; Thrall *et al*. 2001a; Smith *et al*. 2003; Laine & Hanski 2006). Thus, advances in the understanding of broader evolutionary processes in pathogenic species are most likely to come from a metapopulation approach that accounts for populations structured into interconnected demes with ongoing local processes of extinction and recolonization.

Within such a metapopulation framework, variation in different micro-evolutionary forces among demes may act to generate and maintain resistance and virulence polymorphisms. Local host populations, particularly in natural ecosystems, often vary spatially in the identity and diversity of resistance genotypes present (Jarosz & Burdon 1991; Bevan *et al*. 1993; Laine & Hanski 2006), and local adaptation of pathogens to their hosts has been demonstrated as a strong driver of pathogen population genetic structure in a number of host–pathogen interactions (Greischar & Koskella 2007). Within populations, negative frequency-dependent selection, where pathogen (or host) genotypes have higher fitness when rare, is a common assumption in many models of host–parasite interactions, and may contribute to the maintenance of resistance and virulence polymorphisms within populations (Gillespie 1975; Clarke 1976; Anderson & May 1982). In addition, it is also likely that there is strong potential for nonselective factors, such as random genetic drift, founder events and selection on linked traits, to influence host and pathogen evolution (Parker 1991; Burdon & Thompson 1995; Salathé *et al*. 2005).

Comparative analyses of geographical variation in genes controlling pathogenic traits and neutral molecular markers can provide insight into the relative roles of natural selection and genetic drift in driving divergence among pathogen populations (Zhan *et al*. 2005). For example, when selection is important and favours different pathotypes or particular virulence genes in different populations, we would expect among-population variation in pathogenicity traits to exceed that of neutral markers. Conversely, when selection favours similar pathogenic traits or functional genes among populations, estimates of population differentiation calculated for the pathogenic traits are expected to be lower than estimates derived from neutral markers (Lewontin & Krakauer 1973; Latta 2004).

In this study, we focus on the fungal plant pathogen *Melampsora lini*. In Australia, *M. lini* infects *Linum marginale*, an endemic wild, herbaceous plant species. The interaction between *M. lini* and *L. marginale* follows a gene-for-gene model (Burdon 1994), and spatially structured variation in pathogen virulence has been demonstrated at scales ranging from individual populations to the entire range of the interaction in Australia (Burdon & Jarosz 1992; Burdon *et al*. 2002; Thrall *et al*. 2002). In Australia, isolates of *M. lini* have been shown to fall into two distinct lineages (termed AA and AB; Barrett *et al*. 2007) that differ in key genetic, reproductive and life history traits. Continental scale sampling and microsatellite genotyping shows that while lineage AA isolates have low overall genetic diversity and heterozygosity, lineage AB isolates maintain fixed heterozygosity at corresponding loci. Furthermore, while lineage AB isolates consistently have one allele in common with lineage AA isolates, the second allele is consistently singular to lineage AB. Similarly, sequence data show fixed heterozygosity in lineage AB isolates for ITS and β-tubulin loci, with one allele identical to the allele recovered from lineage AA isolates, and low nucleotide divergence between the A and B clades (< 2%; L.G. Barrett, P.H. Thrall, P.N. Dodds, M. van der Merwe, C.C. Linde and J.J. Burdon, under review). No intermediate genotypes have been detected to date. These results (Barrett *et al*. 2007) suggest that lineage AB is a fixed F_1 cross between two genetically divergent lineages of *M. lini* (i.e. a hybrid between lineage AA and a yet to be identified lineage BB), and that sexual recombination (i.e. meiosis) is unlikely to occur in lineage AB. In addition, the apparent inability of lineage AB isolates to effectively endure as resting spores (due to the lack of a sexual cycle) appears to drive disjunct geographical distributions among the lineages (Barrett *et al*. 2007), although the strength of this separation at local scales remains unclear.

We used a mixture of molecular and phenotypic approaches to explore the processes structuring genetic variation in *M. lini* across two distinct biogeographical regions (mountains and plains) in the state of New South Wales. These regions differ markedly in a range of factors likely to influence the geographical distributions of lineage AA and AB. The plains populations are comparatively hot and dry compared to the subalpine mountains populations; and the main growing seasons of the host are disjunct between

regions (Burdon *et al*. 1999; Barrett *et al*. 2007). Furthermore, in the plains populations, where host plants survive over summer largely as underground rootstock, pathogens survive between epidemics exclusively in the dormant teliospore stage (during which meiosis takes place) on senescent host stems. In contrast, in the mountains populations, pathogens rarely form telia, and instead overwinter largely in the clonal urediospore stage on dormant green host shoots (Barrett *et al*. 2007). Given that lineage AB isolates seem unable to effectively form resting teliospores (Barrett *et al*. 2007), these observations lead to the expectation that lineage AA and AB will be largely geographically disjunct among the mountains and plains. This scenario thus offers an excellent opportunity to explore how genetic diversity and structure in a pathogenic species might be influenced by mode of reproduction. We used amplified fragment length polymorphism (AFLP) markers to assess the geographical distribution of lineages AA and AB, and combined AFLP markers with phenotypic virulence data and allelic data from two avirulence (*Avr*) loci to examine patterns of genetic and genotypic diversity within and between lineages AA and AB. We further use these markers to evaluate the relative importance of local selection, neutral drift and gene flow in generating divergence within lineages and among local pathogen populations through comparison of estimates of population genetic structure.

Materials and methods

Study sites

The pathogen populations used in this study occur in two geographically and environmentally distinct areas (Fig. 1). One of these areas lies within the western plains region of New South Wales. Of the five plains populations examined here, three occur in a cluster to the north and east (Garra, Molong, Larras Lee), with the other two occurring further to the west and south (Gundagai, Marrar) (Fig. 1). Together these populations are part of a formerly patchy, but continuous, distribution of *Linum marginale* across the western plains region of New South Wales (an area that has been extensively cleared for agriculture). The second region encompasses the Kiandra and Wild Horse Plains, in the northern part of the Kosciuzko National Park (the mountains populations). While the five mountains populations are all within 20 km of each other, the populations are spatially discrete, and earlier studies show populations in this region are quite distinct with regard to pathogen virulence and host resistance structure (e.g. Thrall *et al*. 2001b) (Fig. 1).

Phenological and epidemiological patterns in the *Linum–Melampsora* interaction differ markedly between mountains and plains populations. In the mountains region, plants overwinter as underground rootstocks with a few short shoots protected from frost and snow by the surrounding

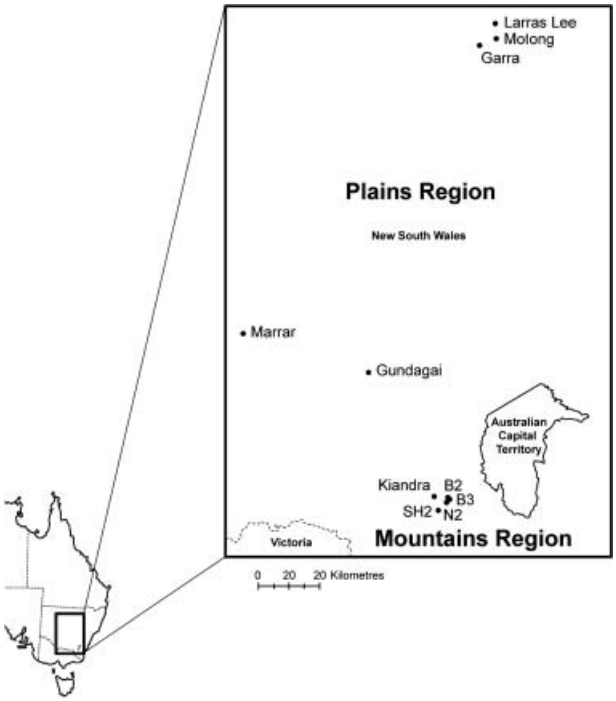

Fig. 1 Map with insert region showing locations of study regions and populations in New South Wales, Australia.

vegetation. With the coming of spring, fresh shoots develop and plants flower in mid- to late-summer before dying back with the onset of autumn frosts. Disease epidemics reach their peak in late summer, and the pathogen overwinters as dormant (clonally reproducing) uredial infections on occasional green shoots (Jarosz & Burdon 1991; Barrett *et al*. 2007). In the plains populations, very hot dry summers and mild winters result in a virtual reversal of the patterns observed in the mountains. Epidemics start in the autumn and reach their peak in early spring. In the plains populations, the pathogen survives the summer drought as dormant teliospores (specialized resting spores which are a necessary precursor to the sexual cycle) which are produced in large quantities as shoots senesce (Barrett *et al*. 2007).

Sample collection

Rust samples were collected from mountains populations in the summer of 2004/2005, and from plains populations in the spring of 2005 (mid-epidemic in both regions). At each site, 21–33 single pustule-derived individuals were recovered (Table 1) from 40 samples haphazardly collected from different infected plants by rubbing cotton buds across sporulating uredia. In the laboratory, urediospores were inoculated onto the universally susceptible *Linum usitatissimum* cultivar Hoshangabad. Inoculated plants were left in a humid atmosphere overnight before being transferred to a glasshouse. Approximately 1 week later, single pustules were isolated and put through up to three cycles of increase

Table 1 Lineage composition, AFLP and *Avr* multilocus genotypic diversity and virulence phenotypic diversity for plains and mountains populations of *Melampsora lini*

Region	Population	*n*	*n* (AA)	*n* (AB)	AFLP MLG	Vir Phen	Avr MLG	AFLP div	Vir div	Avr div	n_{cc}
Plains	Garra	31	30	1	16	7	5	0.89	0.69	0.38	23
	Gundagai	26	24	2	19	16	7	0.96	0.94	0.80	25
	Larras Lee	31	27	4	15	10	6	0.88	0.58	0.49	16
	Marrar	28	21	7	25	20	7	0.99	0.97	0.79	28
	Molong	25	21	4	23	10	7	0.99	0.86	0.56	25
	All	141	123	18	92	46	19	0.98*	0.92*	0.83*	119
Mountains	B2	25	0	25	6	5	5	0.48	0.52	0.49	13
	B3	26	0	26	7	9	3	0.47	0.76	0.22	14
	Kiandra	33	0	33	2	3	1	0.12	0.19	0	6
	N2	28	1	27	9	13	5	0.76	0.87	0.43	22
	SH2	22	0	22	3	4	2	0.48	0.45	0.31	11
	All	134	1	133	25	19	6	0.65*	0.74*	0.59*	84

n, sample size in each population; AFLP MLG, number of AFLP multilocus genotypes; Vir Phen, number of virulence phenotypes; Avr MLG, number of genotypes considering both *AvrP123* and *AvrP4*; AFLP Division, Simpson's diversity index for AFLP markers; Vir Division, Simpson's diversity index for virulence phenotypes; Avr Division, Simpson's diversity index for Avr genotypes; n_{cc}, number of clone corrected genotypes using all genotype and phenotype information. *Mountains and plains regions differ significantly at $P \leq 0.01$.

on Hoshangabad, to ensure that each isolate consisted of a single genotype, and that sufficient urediospores were available for DNA extraction and pathotype analysis.

Pathotype identification

We assessed the virulence of each of 275 isolates using the 'standard differential set' of 11 lines of *L. marginale* that has been used extensively in previous work assessing variation in virulence within *M. lini* populations (Burdon & Jarosz 1991; Jarosz & Burdon 1991; Burdon & Roberts 1995; Thrall *et al*. 2001b). Pathogenicity reactions were scored after 14 days, with the interaction between each pathogen isolate and individual differential host lines being classified as either virulent (1) or avirulent (0).

Individual pathotypes were thus defined by their unique combination of infectivity responses across the *L. marginale* differentials. The 10 pathogen populations were then analysed both in terms of the frequency and distribution of individual pathotypes.

DNA extraction

Prior to DNA extraction, approximately 100 mg of urediospores contained within an Eppendorf tube were allowed to germinate overnight in 100 μL of sterile water. Total genomic DNA was extracted from these urediniospores using a DNeasy plant mini kit (QIAGEN), following standard protocols.

AFLP analyses

Although microsatellite markers have been developed for *M. lini*, they proved too invariant for population genetic studies (Barrett & Brubaker 2006; Barrett *et al*. 2007). Here, we use an AFLP method adopted from Becerra Lopez-Lavalle & Brubaker (2007) with modifications as follows. We used eight *Eco*RI/*Mse*I primer combinations in the selective amplification (GC/GT; ACC/GT; ACT/GG; AGA/GG; GC/GA; ACC/GC; ACT/GC; AGA/GT) to generate markers ranging from 50 to 500 bp in size. In each case, the *Eco*RI selective primer was labelled with one of the fluorescent dyes FAM, VIC, PET or NED. Selective polymerase chain reaction (PCR) products were separated on an ABI 3130 automated sequencer (PE Applied Biosystems) with a GeneScan LIZ 500 internal size standard. Electropherograms were subsequently analysed using GENEMAPPER version 4.0 (PE Applied Biosystems). The intensity of each individual peak was normalized on the basis of the total signal intensity and the peak was considered only if its intensity exceeded a fixed threshold. To test the repeatability of AFLP results, 10 individuals from each sample were completely replicated; no differences between the two sets of samples were observed. Only samples that could be scored unambiguously by eye were retained for further analysis. The presence and absence of AFLP fragments in each sample was analysed using the software GENEMAPPER. Fragments obtained using each primer combination were scored as either present or absent for each size-specific locus. AFLP fragments were treated as dominant marker loci with two states, presence (1) or absence (0).

AvrP123 and AvrP4 amplification and sequencing

The avirulence genes *AvrP123* and *AvrP4* were initially cloned and characterized in the interaction between *M. lini*

and the cultivated flax species *L. usitatissimum* (Catanzariti *et al*. 2006), where multiple virulence polymorphisms are maintained via amino acid differences in the expressed *Avr* proteins (Dodds *et al*. 2006). *AvrP4* encodes a 95 amino acid protein with a predicted 28 amino acid cleavable secretion signal peptide and *AvrP123* encodes a 117 amino acid protein with a predicted 23 amino acid cleavable secretion signal peptide. For the interaction between *M. lini* and *L. marginale*, Barrett *et al*. (under review) demonstrated that both *AvrP123* and *AvrP4* are also recognized by *L. marginale* host plants, and that strong diversifying selection is acting at both these loci in Australian populations. This parallel study further demonstrated that lineage AB isolates are consistently heterozygous at these loci, and that haplotype diversity in lineage AB isolates at these loci is confined to alleles from the 'A' genome. One of these alleles (the 'A' allele), is very closely related to alleles recovered from lineage AA isolates. The second allele (the 'B' allele), which is highly divergent, displayed no variation.

Due to the lack of variation in 'B type' alleles, primers and PCR protocols were designed to amplify only the 'A type' alleles in lineage AB isolates (Barrett *et al*. under review). For *AvrP4*, we amplified a region including 180 bp of 5′ flanking sequence, 285 bp of open reading frame, and 103 bp of 3′ flanking sequence (F-CATCAAAATCTAACCCGTAC and R-TTGTTCAGGATAGATAGTGC). PCR amplifications were performed on a Hybaid Express thermocycler under the following conditions: 95 °C for 3 min, 34 cycles at 94 °C for 30 s, 56 °C for 45 s, 72 °C for 90 s, followed by a 4 °C holding step. For *AvrP123* we amplified a region including 119 bp of 5′ flanking sequence, 351 bp of open reading frame, and 128 bp of 3′ flanking sequence (F-ATTGTGAAC-CTTTTGAAGGAC and R-CGCCATGGTATTGTTCAGAC). PCR amplifications were performed as for *AvrP4* except for a 58 °C annealing temperature. PCR products were cleaned using Multiscreen PCR_{96} filter plates (Millipore) and sequenced directly using the forward primers in conjunction with BigDye™ Terminator Cycle Sequencing Reaction Kit (PE Applied Biosystems). Sequencing products were resolved on an ABI 3130 automated sequencer. To assist with alignment of different sequences, alignments were initially performed on the amino acid sequence, using conserved cystine motifs as reference points and then back-translated to the original nucleotides. We analysed *Avr* sequence variation by classifying amino acid variants as allelic variants. As noted above, all analyses based on data collected for lineage AB isolates are based on the 'A' haplotypes only.

Data analyses

We conducted analyses at both the between- and within-lineage levels, using different approaches. Analyses of genotypic diversity across different levels, and overall genetic differentiation [i.e. analyses of molecular variance (AMOVA) and Nei's genetic distance] were performed using all isolates. For comparative analyses of spatial genetic structure [i.e. θ^B and analysis of similarity (ANOSIM) comparisons] we treated the two lineages separately. Due to low sample size, for these analyses lineage AB isolates collected from the plains populations and the single lineage AA isolate collected from the mountains populations were excluded.

Genotypic diversity among lineages and populations was calculated based on the number of shared multilocus AFLP or *Avr* genotypes. Phenotypic diversity was calculated based on the shared number of virulence phenotypes. The genotypic or phenotypic identity of the different isolates was evaluated using the software GENOTYPE (Meirmans & Van Tienderen 2004). Genotypic diversity for lineages and populations was estimated via the number of unique multilocus genotypes, Nei's diversity index (Nei 1987) (calculated using GENODIVE; Meirmans & van Tienderen 2004). A bootstrap resampling method as implemented in GENODIVE was used to test whether these indices were significantly different among lineages.

For all other lineage and population-level genetic analyses, we used a clone-corrected data set. This approach was taken so as to minimize the effects of clonal reproduction during epidemics on estimates of genetic diversity and population differentiation. We identified clones based on variation between isolates in AFLPs, virulence phenotypes, and avirulence gene genotypes. AFLP genetic diversity was evaluated as the percentage of polymorphic loci.

To test for random associations among AFLP markers, multilocus linkage disequilibrium was measured by calculating the Index of Association (I_A; Maynard Smith *et al*. 1993), using the software MULTILOCUS version 2.2 (Agapow & Burt 2001). The observed I_A for each population and each lineage was compared with an expected I_A under random mating simulated through the reshuffling of data over 1000 permutations. I_A has an expected value of zero if there is no association of alleles at unlinked loci as expected in a randomly mating population.

The degree of genetic relatedness (AFLP data) among all pairs of populations was evaluated using unweighted pair-group mean analysis (UPGMA) based on estimates of Nei's genetic distance (1972) calculated using GENALEX version 6.0 (Peakall & Smouse 2005). The UPGMA tree was constructed using NT-SYSpc version 2.11 (Rohlf 1993). The genetic similarity among isolates (AFLP) within lineages was visualized using principal coordinate analysis (PCoA) based on Euclidean distances between AFLP multilocus genotypes using GENALEX 6.0.

To examine hierarchical partitioning of molecular variation among lineages and populations, we subjected AFLP to AMOVA using GENALEX 6.0. To test for genetic structure among populations within lineages, we used a Bayesian

approach to estimate an F_{ST} analogue among populations within each lineage (denoted θ^B) for the AFLP data, and the two *Avr* loci, as implemented in the software Hickory (Holsinger *et al*. 2002). The data were run with the default parameters (burn-in = 50 000, number of samples = 250 000, thinning factor = 50). The data were analysed using the *f*-free model, which does not assume any prior knowledge regarding the degree of inbreeding within populations. Comparisons of θ^B between data sets were performed by calculating the difference between paired random samples of the posterior distribution of θ^B for each data set, and declaring the difference in θ^B significant if the 95% credible interval of this difference excluded zero. This approach allowed us to estimate θ^B for both AFLP and *Avr* gene loci, and to formally test hypotheses regarding variation in genetic structure among neutral and selected markers. For comparative purposes, we also estimated genetic structure within lineages with AMOVA, using the software GENALEX 6.0.

To estimate levels of geographical structure in the virulence phenotypic data, we used ANOSIM (Clarke 1993). We used this approach because the nature of the phenotypic virulence data precluded more conventional approaches to measuring population divergence (e.g. F_{ST} or Q_{ST}, or their analogues). Each virulence phenotype is under direct genetic control, and is controlled by a limited but undetermined number of genes, each with major effects. Each individual response on each differential line is thus informative, but cannot be considered as an independent marker, because the phenotype is determined by multiple genes. At the same time, the data cannot be considered as a continuously distributed quantitative trait, because each phenotype is controlled by genes with major effects, and is quantified in a binary manner. Similar to AMOVA, ANOSIM can be used to assess similarity among individuals when grouped at different hierarchical levels. The method does not make assumptions about the nature of the data, and uses rank order of dissimilarity values among individual samples as its base. Under the null hypothesis of no differentiation, the test statistic, R, changes little when population labels are rearranged randomly. R values range between 1 (maximum separation among populations) and 0 (completely random grouping) (Clarke 1993). The associated test for significance was calculated over 1000 permutations. For comparative purposes, we also performed these analyses using the AFLP data set. ANOSIM was performed using the software PRIMER version 6 (PRIMER-E Ltd, Plymouth Marine Laboratory, Plymouth, UK).

To explore how much AFLP genetic variation between populations (within a lineage) was explained by geographical distance, matrices of pairwise genetic distance (Nei 1972) and geographical distance were subject to a Mantel test (1967) using GENALEX 6.0 (Peakall & Smouse 2005) with 10 000 permutations.

Results

Patterns of diversity among isolates

We recovered AFLP multilocus genotypes, virulence phenotypes and genotypes at the two *Avr* loci for 275 isolates of *Melampsora lini*, sampled from 10 natural populations across two geographical regions. Among the mountains populations, we recovered exclusively lineage AB isolates, with the exception of one lineage AA isolate in population N2. Among the plains populations, we recovered isolates from both lineages in all populations, although isolates belonging to lineage AA were predominant (population average: 87%; Table 1).

For the AFLP data set, a total of 208 AFLP markers were scored and retained for further analysis. Of these, 88 markers were polymorphic across the data set. Of the 208 fragments, 60 were unique to lineage AB, and four were unique to lineage AA. In total, 124 isolates were assigned to lineage AA, and 151 isolates to lineage AB. Genetic diversity as determined by AFLP was generally low, with only 12% and 14% of all scored AFLP markers being polymorphic within lineages AA and AB, respectively.

Pathogenicity analyses revealed extensive variation across all isolates, with 62 different virulence patterns detected across the 11 host differential lines. The number of host lines overcome by individual isolates ranged between 3 and 10. Across all isolates, we recovered 4 and 5 clade 'A' amino acid variants from *AvrP123* and *AvrP4*, respectively. The sequence data for each allele is deposited in GenBank (accession numbers for *AvrP123*: EU642492, EU642493, EU642494, EU642495; for *AvrP4*: EU642476, EU642478, EU642479, EU642482, EU642483). Isolates with identical AFLP multilocus genotypes frequently represented multiple virulence phenotypes, and isolates with the same virulence phenotype had different AFLP genotypes. Similarly, identical *Avr* genotypes harboured multiple virulence phenotypes, and vice versa (Table S1, Supplementary material).

Variation between lineages

The two lineages differed significantly in levels of genotypic diversity as determined by AFLP markers, *Avr* genes, and virulence phenotypes. In all three cases, lineage AA harboured significantly ($P < 0.01$) more genotypic variation than lineage AB (Table 2). Combining all of these marker systems, the total number of genotypes in lineage AA was 100 (of 124 isolates), compared to 61 (of 151) in lineage AB. For lineage AB, 18 of these isolates were recovered from plains populations, 16 of which were genotypically unique.

Overall, AMOVA revealed significant and high levels of structure in the data set ($\Phi_{PT} = 0.94$, $P = 0.001$), with 90% of the AFLP diversity attributed to differences among lineages, with the remainder among isolates within populations

Table 2 Summary statistics describing genotypic and phenotypic variation in 124 and 151 isolates of *Melampsora lini* belonging to lineage AA and lineage AB, respectively

Marker	Measure of diversity	Lineage AA	Lineage AB
AFLP			
	No. of isolates	124	151
	No. of CC* isolates	100	61
	No. of genotypes†	83	25
	Simpson's diversity†	0.98	0.68
	Shannon's index†	1.77	0.77
	I_A	0.71‡	1.47 ‡
	PLP	12	14.4
Avirulence loci			
	No. of genotypes†	14	9
	Simpson's diversity†	0.78	0.62
	Shannon's index†	0.8	0.53
Virulence phenotypes			
	No. of phenotypes	34	31
	Simpson's diversity†	0.90	0.28
	Shannon's Index†	1.22	0.95
	Mean virulence*	0.51	0.58

*CC, clone corrected using all genetic and phenotypic data. †Lineage AA and AB differ significantly at $P < 0.01$. ‡Observed I_A values differ significantly from that expected under random mating at $P < 0.01$. PLP, percent loci polymorphic; I_A, Index of association.

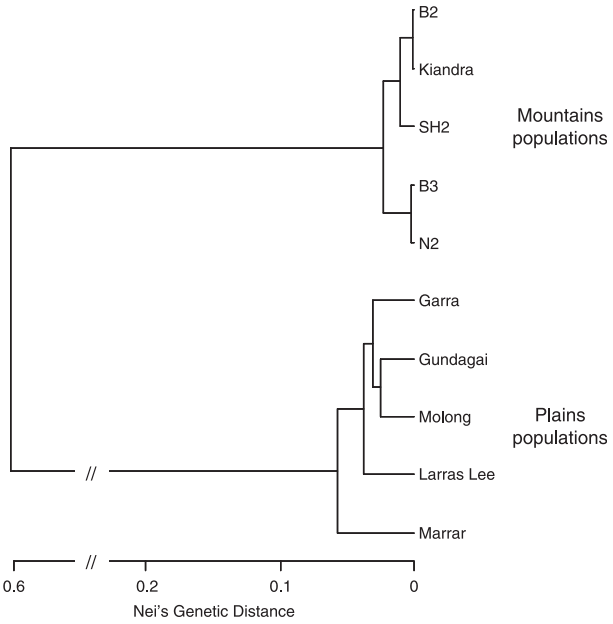

Fig. 2 UPGMA phylogram based on pairwise estimates of Nei's genetic distance (AFLP) among populations of *Melampsora lini*.

(6%), or between populations of the same lineage (4%). Consistent with these observations, the UPGMA phylogram based on the genetic distance among populations showed very strong separation between plains and mountains populations (Fig. 2).

Principal coordinate analysis of the Euclidean distance among lineage AA and AB isolates highlights clearly contrasting AFLP genetic structure within the two lineages (Fig. 3). Lineage AA isolates, while showing structure at the population level, form a relatively continuous cloud of points across axes 1 and 2 (explaining 36.5% and 20.1% of the variation, respectively). In contrast, lineage AB isolates form a series of four relatively discrete clusters along PCoA axis 1 (axes 1 and 2 explain 56.1% and 17.1% of all variation, respectively).

The I_A statistic revealed significant levels of nonrandom association among AFLP markers for both lineages AA and AB. However, while significant for both lineages, I_A was more than twice as high in lineage AB as in lineage AA. In conjunction with previous results showing fixed heterozygosity at microsatellite and *Avr* loci (Barrett *et al*. 2007), these elevated levels of linkage disequilibrium in lineage AB further support the suggestion that this lineage only reproduces clonally. Significant levels of I_A and the Bayesian estimate of the fixation index ($f = 0.7$) for lineage AA suggest frequent inbreeding within this lineage. The frequency distribution of the different AFLP multilocus genotypes within lineage AB further suggest that clonal reproduction is dominant in this lineage, with two distinct AFLP genotypes representing more than 70% of all sampled isolates (Fig. 4b). In contrast, while identical AFLP genotypes were present within the lineage AA samples, no single genotype reached frequencies of higher than 10% across populations (Fig. 4a).

Similarly, virulence phenotypes show a distinctly uneven frequency distribution in lineage AB compared to lineage AA, and the two lineages also displayed distinctly different virulence structures in terms of the identity and frequency of individual pathotypes (Fig. 5a). The specific differences among the two lineages in their ability to overcome different resistance genes are indicated by striking differences in the frequency with which pathogen isolates from the two lineages were able to overcome individual host differential lines (Fig. 5b).

Variation among populations

Within each lineage, among-population pathogen diversity as determined by all three marker systems varied widely (Table 1). Of the mountain populations (lineage AB), Kiandra was notable for having especially low levels of AFLP and virulence diversity, being dominated by a single clone corrected genotype. In contrast, diversities as indicated by virulence phenotypic diversity and *Avr* markers within

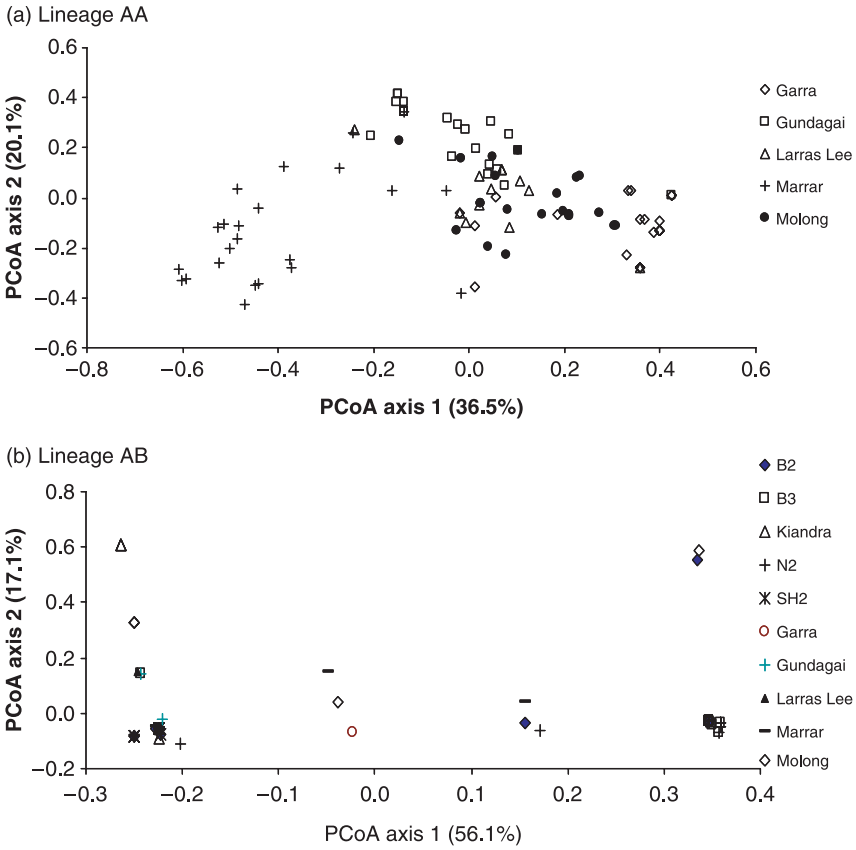

Fig. 3 Principal coordinates plots based on Euclidean distances among multilocus AFLP genotypes for all clone corrected isolates from plains and mountains populations. (a) Lineage AA isolates ($n = 100$), axes explain 56.6% (36.5 + 20.1) of the variation. (b) Lineage AB isolates ($n = 83$), axes explain 73.2% (56.1 + 17.1) of the variation.

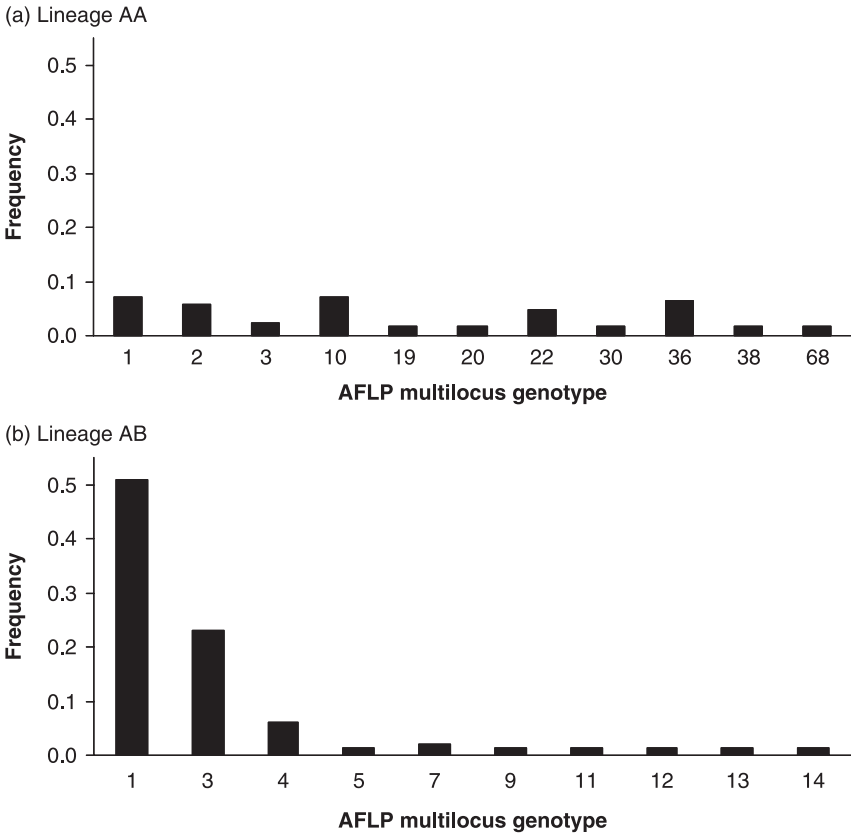

Fig. 4 Histograms of frequencies of different AFLP multilocus genotypes including more than one individual out of a total of 275 isolates of *Melampsora lini*. Lineage AA and AB isolates had no multilocus genotypes in common. (a) Lineage AA isolates. A total of 83 multilocus genotypes were recovered from 124 isolates, with 11 occurring more than once. (b) Lineage AB isolates. A total of 25 multilocus genotypes were recovered from 151 isolates, with 10 occurring more than once.

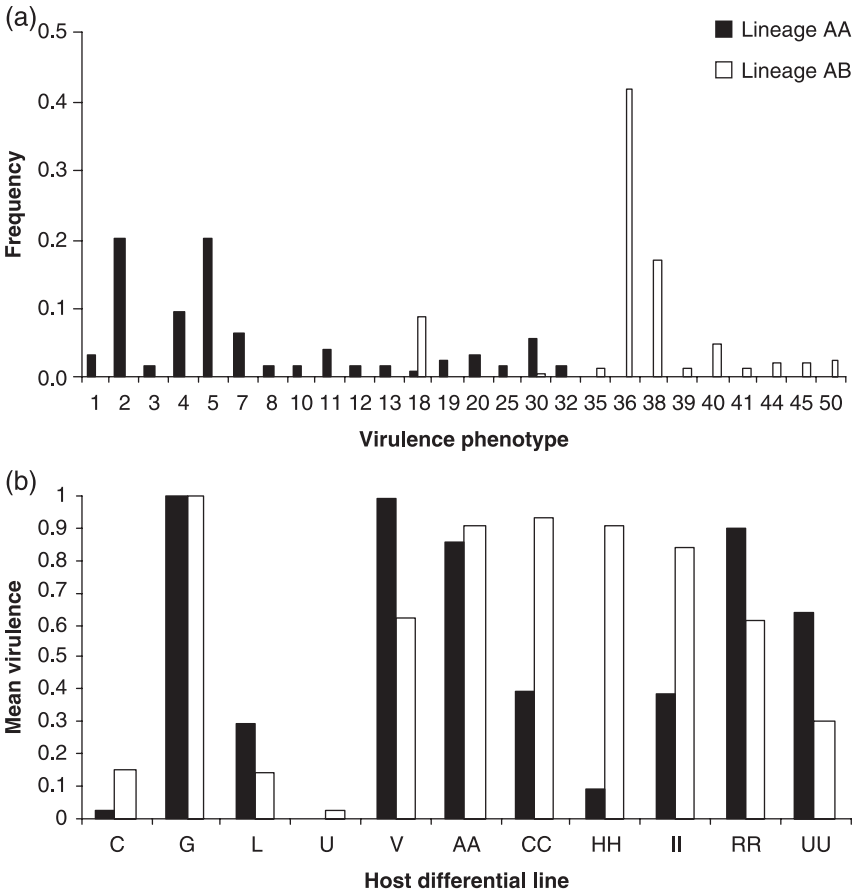

Fig. 5 Virulence profile of lineage AA and AB isolates. (a) Histograms of frequencies of different virulence phenotypes (including more than one individual) among 124 lineage AA and 151 lineage AB isolates of *Melampsora lini* as determined on 11 differential host lines collected from plains and mountains populations. Individual virulence phenotypes represent a unique combination of virulence specificities on the differential set, and have been assigned an arbitrary number as shown on the *x*-axis. (b) Mean virulence responses of isolates of *M. lini* belonging to lineage AA and AB on differential lines of the host plant *Linum marginale*.

population N2 were equal, or higher, than in several plains (lineage AA) populations. Within lineage AA, low levels of diversity were observed in the Larras Lee and Garra populations compared to other plains populations (Table 1).

Within lineages, estimates of divergence among populations revealed consistent patterns of genetic and phenotypic differentiation. Bayesian estimates of θ^B based on AFLP and avirulence gene data resulted in much lower deviance information criteria values for models allowing population structure than for models allowing no population structure (Table 3), providing evidence for population differentiation across all markers. For the AFLP data, estimates of population structure were high for both lineages ($\theta^B = 0.336$ for AA, $\theta^B = 0.367$ for AB). Strong population structure within lineages was also revealed for *AvrP4*, although the genetic structure among lineage AB populations was weaker ($\theta^B = 0.325$ for AA, $\theta^B = 0.186$ for AB). In contrast, estimates of population structure for *AvrP123* were much lower, although still significant, for both lineages ($\theta^B = 0.112$ for AA, $\theta^B = 0.046$ for AB). Posterior comparisons of θ^B revealed significantly lower levels of population differentiation for *Avr* loci compared to AFLP data, with the exception of the estimate of θ^B for *AvrP4* among lineage AA populations, which was not significantly different from estimates of population differentiation for AFLP markers ($\theta^B = 0.325$ and 0.336, respectively) (Table 3). Analysis of structure in the phenotypic virulence data using analysis of similarity also revealed significant spatial structure within both lineages (lineage AA: $R = 0.27$; lineage AB: $R = 0.12$), indicating moderate and low levels of separation among populations, respectively. Within lineages, Mantel tests revealed no significant association between the degree of population differentiation as estimated by AFLP markers (Φ_{PT}) and geographical distance (km) (lineage AA, $P = 0.08$; lineage AB, $P = 0.08$).

Relationships between patterns of phenotypic and genetic divergence among populations were not concordant among lineages. For lineage AA, pairwise comparisons of among-population differentiation as determined by AFLP multilocus genotypes (Φ_{PT}), *Avr* gene genotypes (Φ_{PT}), and virulence phenotypes (R) were not significantly correlated (Mantel tests of matrix comparisons, $P > 0.05$). In contrast, for clonal lineage AB, comparisons of pairwise differentiation among the different markers using Mantel tests revealed a number of statistically significant relationships (for AFLP vs. virulence phenotypes, $P = 0.01$, $R = 0.79$; for AFLP vs. *Avr* multilocus genotypes, $P = 0.01$, $R = 0.93$; for virulence phenotypes vs. *Avr* multilocus genotypes $P = 0.02$, $R = 0.8$).

Table 3 Estimates of population structure (θ^B), standard deviations (SD) and credible intervals (CRI) from Bayesian modelling for AFLP fingerprints and two avirulence loci for two genetic lineages of *Melampsora lini*. Deviance information criteria calculations (DIC) are provided for two alternative models (θ^B = variable and $\theta^B = 0$)

Marker	Lineage	θ^B	SD	95% CRI	DIC θ^B = variable	DIC $\theta^B = 0$
AFLP	AA	0.336	0.042	(0.220, 0.382)	362.13	785.27
	AB	0.367	0.068	(0.243, 0.502)	205.88	372.14
AvrP123	AA	0.112	0.041	(0.003, 0.154)	70.1	79.2
	AB	0.046	0.071	(0.016, 0.287)	114.41	122.66
AvrP4	AA	0.325	0.076	(0.198, 0.491)	104.73	264.73
	AB	0.186	0.068	(0.077, 0.339)	119.84	163.34

AvrP123 was significantly correlated to both AFLP genotypes ($P = 0.04$, $R = 0.88$) and virulence phenotypes ($P = 0.03$, $R = 0.75$). There were no significant relationships between *AvrP4* and either AFLP genotypes or virulence phenotypes ($P > 0.3$).

Discussion

The geographical mosaic theory of co-evolution predicts that traits influencing the outcome of co-evolutionary interactions will differ among populations, thereby generating a selection mosaic that varies across landscapes (Thompson 1994, 2005). In this context, our results have important implications for understanding how spatial, environmental and life-history variation influence the population and regional genetic structure of *Melampsora lini*. Through the integration of AFLP, *Avr* gene and phenotypic virulence data, we demonstrate strong genetic and pathogenic divisions among populations of *M. lini* within two biogeographical regions, driven by key differences between pathogen lineages in genetic composition and mode of reproduction. Within this broad genetic and geographical framework, selection and genetic drift interact to generate genetic and pathogenic divergence among local populations.

The geographical distribution of lineage AA and AB

Analysis of molecular and phenotypic virulence data at the regional scale confirm the strong genetic separation between the *M. lini* lineages AA and AB previously suggested by geographically broad sampling (Barrett *et al*. 2007). This pattern and the dominance of lineage AA isolates in the plains environment is likely a consequence of life-history differences associated with the hybrid origin of lineage AB. The loss or lack of the sexual mode of reproduction effectively restricts lineage AB isolates to the clonal (urediospore) stage of the lifecycle (Barrett *et al*. 2007). In the hotter, drier, plains populations, the regular occurrence of summer drought results in the widespread death or senescence of the host, *Linum marginale.* Without green host tissue, *M. lini* is unable to survive in the uredial stage, meaning that telial formation is critical for long-term persistence. A severe summer drought in the year prior to sampling during which isolates in three monitored plains populations survived exclusively as telia, suggests that lineage AB isolates recovered in plains populations are likely to have dispersed from more mesic sites during that growing season. In some years, summer rains may result in year-round persistence of green host tissue, reducing the likelihood of local extinction of lineage AB isolates in plains populations.

In contrast to the plains environment, host phenology is essentially reversed in the mountains region, and the striking dominance of lineage AB isolates in the mountains populations is more difficult to explain. The presence of only a single lineage AA isolate in the five mountains populations implies strong barriers to the establishment of this lineage in this environment. Given that mountains hosts are not universally or even unusually resistant to lineage AA isolates (L. G. Barrett, CSIRO Plant Industry, unpublished manuscript), this distribution is unlikely to be driven by among-region differences in host resistance. Instead, it is possible that an increased tendency to form telia during warm weather, while crucial to survival in the plains, may affect other fitness parameters relating to disease spread such as spore production and transmission. Consistent with this idea, glasshouse experiments demonstrate that lineage AB isolates are significantly less likely to form telia under warm conditions than lineage AA isolates (Barrett *et al*. 2007). Lineage AB isolates may therefore be better at exploiting host plants during the warm summer months, which coincides with the main growing season for *L. marginale* in the mountains.

Genetic and pathogenic structure within lineages

It is generally expected that populations able to reproduce sexually will generate and maintain higher genotypic diversity than asexual populations. Indeed, a number of studies show greater phenotypic and genetic diversity in sexual compared to asexual populations of plant pathogens (Milgroom 1996). Here, we demonstrate that levels of

genotypic diversity within lineages of *M. lini* are consistent with such expectations, with lineage AA harbouring significantly higher AFLP, *Avr*, and virulence diversity than lineage AB despite census populations being generally smaller and more isolated in the plains region where AA predominates (Burdon *et al*. 1999). The two lineages also differ strikingly in how variation is distributed among isolates. Under strict asexual reproduction, founding genotypes should diversify over time into discrete clonal lineages characterized by a complex of closely related genotypes. In contrast, the exchange of genetic information among individuals in sexually reproducing populations should result in a more homogenous population structure. Consistent with this expectation, PCoA of the AFLP data showed that genomic variation is distributed relatively evenly across isolates for lineage AA, while lineage AB isolates cluster into a few discrete genotypic groups.

For lineage AA isolates in the plains populations, the high diversity of genotypes in each population suggests that sexual reproduction is common, despite the strong potential for clonal multiplication during epidemics. Only at Larras Lee, and to a lesser extent, Garra, did we find evidence for clonal dynamics strongly influencing population structure. Generally, high genotypic diversity was maintained in plains populations despite evidence for significant levels of inbreeding. The low number of identical clones in these populations may reflect a high number of sexual recombinants as primary inoculum, or selection for different virulence phenotypes by individual host plants. While all populations were sampled during established phases of infection, these results represent only a snapshot of pathogen population structure during a single epidemic. Clearly, the clonal structure of populations may change throughout the growing season, with individual pathotypes having the potential to rapidly increase in frequency as epidemics progress (Jarosz & Burdon 1991; Burdon & Jarosz 1992).

Although the population structure detected for the mountains (lineage AB) populations was consistent with that expected under clonal reproduction, within-population diversity varied markedly, with some populations maintaining unexpectedly high levels of variation. For example, in population N2, 21 of the 28 isolates sampled were genetically unique, with AFLP and virulence diversity being higher than some of the sexually reproducing lineage AA populations. In contrast, we recovered only six genetically unique individuals from 33 sampled isolates in Kiandra, reflecting dominance by a single pathotype. Interestingly, this pathotype is also the most common pathotype across all mountains populations and has dominated Kiandra for many years (Burdon & Jarosz 1991; Thrall *et al*. 2001b; J. J. Burdon, CSIRO Plant Industry, unpublished data). Thus, while patterns of diversity in some populations suggest strong selection for variation in virulence, low levels of diversity in others agree with the general-purpose-genotype hypothesis (Lynch 1984) that predicts selection of a few asexual genotypes characterized by broad tolerances for fluctuating environmental or biotic conditions.

Although difficult to evaluate, given that the putative BB parent has not been detected, the presence of multiple shared *Avr* gene alleles between AA and AB isolates suggests that either multiple hybridization events, multiple origin of the same alleles, or somatic exchange of genetic information, must have occurred at some stage. Genetically discrete isolates within clusters of closely related AFLP genotypes, and isolates with identical AFLP multilocus genotypes, but different virulence phenotypes, are most likely to have been generated via stepwise patterns of mutation, following establishment of a founding genotype. Stepwise mutation has been demonstrated as an important mechanism for generating diversity in populations of asexual pathogen species (Jimenez-Gasco *et al*. 2004; Fisher *et al*. 2005; Hovmøller & Justetson 2007); however, horizontal gene exchange may also be important in driving divergence between otherwise very closely related isolates. Thus, although lineage AB *Avr* genotypes and AFLP multilocus genotypes on average are strongly linked, there were several examples where lineage AB isolates with identical AFLP genotypes carried different *Avr* gene alleles. While the generation of these genotypes via multiple hybridization events between identical AFLP genotypes cannot be discounted, horizontal gene exchange via hyphal fusion among genetically different individuals seems equally parsimonious. The contribution of such non-sexual genetic exchange to generating pathogen diversity has been clearly demonstrated in several species (Spiers & Hopcroft 1994; Burdon & Silk 1997; Friesen *et al*. 2006).

Higher overall diversity in plains compared to mountains populations could significantly impact on host–pathogen co-evolutionary interactions at the regional scale. As suggested by the Red Queen Hypothesis, where hosts and pathogens are naturally coevolving, high levels of diversity in pathogen populations may promote higher diversity through sexual reproduction in corresponding host populations (Haldane 1949). Thus, the results presented here are particularly intriguing when interpreted in light of host resistance variation and mating system in plains and mountains environments (Burdon *et al*. 1999; Thrall *et al*. 2001b). In the mountains populations (lineage AB), hosts are almost completely selfing. In contrast, in the plains environment, where the majority of isolates reproduce sexually, hosts show significant levels of outcrossing. These differences in host mating system are reflected in significant variation among regions in the diversity of resistance phenotypes, with host populations in the plains region showing consistently higher values. Furthermore, strong differentiation between pathogen lineages in virulence responses to individual host differential lines, and in the identity of associated virulence phenotypes between lineages, corresponds

to the observed divergence between mountains and plains host populations in the identity of resistance phenotypes (Burdon *et al*. 1999).

Genetic structuring among populations

Analysis of the AFLP data demonstrates relatively strong genetic divergence between populations of both lineages. This structure is maintained despite the fact that *M. lini* produces large numbers of urediospores that have the potential to disperse aerially over long distances. Indeed, the presence of lineage AB isolates in all plains populations at low frequencies, and the lack of evidence for any isolation by distance effects, suggests that long-distance spore dispersal is both stochastic and relatively frequent. Despite this, levels of among-population genetic differentiation are considerably higher than those reported for many wind-dispersed, foliar plant pathogens of cultivated crops, such as *Mycosphaeralla graminicola* (G_{ST} = 0.05; Linde *et al*. 2002), *Phaeosphaeria nodorum* (θ = 0.05; Pimentel *et al*. 2000), and *Tapesia yallundae* (θ = –0.008, Douhan *et al*. 2002). It is important to note that these agricultural examples largely represent pathogens of high-density crops, which can survive saprophytically to varying degrees.

Strong bottlenecks during epidemic troughs and the stochastic nature of recolonization processes are likely to be key factors driving divergence among populations (Brown & Hovmoller 2002). Given that *M. lini* is a biotrophic foliar pathogen, and occurs in small, fragmented and genetically diverse host populations, these kinds of stochastic demographic dynamics are common, with annual boom-and-bust epidemic dynamics likely promoting frequent local extinction, stochastic recolonization and subsequent genetic drift (Wade & McCauley 1988; Burdon & Jarosz 1992; Ingvarson *et al*. 1997; Pannell & Charlesworth 1999; Haag *et al*. 2006). Heterogeneity in terms of host availability determined by resistance structure and environmental conditions further limit opportunities for the establishment and persistence of pathogen migrants which are likely to be accentuated by clonal reproduction (lineage AB), and inbreeding during the sexual phase of the life-cycle (lineage AA).

Previous studies (largely within the mountains) suggest that selection for virulence in the *L. marginale–M. lini* interaction can strongly influence local patterns of genetic variation in *M. lini*. Considerable variation for host resistance exists within and among host populations (Burdon & Jarosz 1991; Jarosz & Burdon 1991) and comprehensive cross inoculation trials have demonstrated strong local adaptation of the pathogen to host populations (Thrall *et al*. 2002). Furthermore, pathogen virulence and host resistance are highly correlated, such that broadly virulent pathogens occurred more frequently in highly resistant host populations, whereas avirulent pathogens dominate susceptible host populations (Thrall & Burdon 2003). Consistent with expectations arising from these observations, we found significant structuring between populations, in both lineage AA and AB, for virulence phenotypes, and both *Avr* gene loci.

Direct comparisons of population structure revealed through phenotypic characters or genes under selection with that shown by neutral genetic markers can provide insight into the relative roles of drift and selection in driving genetic divergence among populations (Merilä & Crnokrak 2001). However, comparisons between different marker systems used to make inferences about local adaptation require that markers are comparable and unbiased. One concern is that the virulence phenotypes, although under direct genetic control, may underestimate the true level of divergence among populations relative to AFLP markers, particularly if either homoplasy or epistasis are common. Furthermore, the use of a standard set of host differential lines may also not provide a complete estimate of the diversity of pathogen populations when infecting their local hosts. Despite these caveats, our AFLP estimates of genetic structure provide a useful baseline against which to compare the structure of variation in phenotypic virulence characters, and *Avr* gene allele frequencies.

Most studies comparing phenotypic traits (Merilä & Crnokrak 2001; McKay & Latta 2002) or genes under selection (Conway *et al*. 2001; Abdel-Muhsin *et al*. 2003; Anderson *et al*. 2005) with neutral markers demonstrate higher levels of differentiation among populations for phenotypic traits than for neutral marker genes. Such findings are generally interpreted as evidence for local selection driving the divergence of specific traits or genes. In contrast, in the present study, data from two *Avr* loci and for virulence phenotypes showed levels of spatial genetic structure either significantly less, or similar to, structure determined by the AFLP markers. Both *AvrP123* (θ^B_{AA} = 0.05, θ^B_{AB} = 0.11) and *AvrP4* (θ^B_{AA} = 0.32, θ^B_{AB} = 0.19) are significantly differentiated among populations; however, with the exception of estimates of θ^B for *AvrP4* for lineage AA, these values are significantly lower than estimates for the AFLP markers. Similarly, global *R* statistics describing divergence among populations for AFLP markers and virulence phenotypes indicate that separation among populations was more than twice as strong for AFLP than for virulence markers.

The strong population genetic structure in the AFLP data compared to the *Avr* genes and virulence phenotypes could be a consequence of selection for particular virulence genes or phenotypes acting to constrain among-population divergence in virulence structure (i.e. selection for the same virulences in different populations). Thus, it is possible that selection imposed by host resistance structure is preventing population divergence beyond that seen for AFLP markers. Given the potential for very large annual fluctuations in demographic population size in *M. lini*, such a scenario is not implausible. However, even where selection is acting to limit divergence among populations, drift may also have

the potential to influence the pathogenic identity of local populations, particularly where individual virulence phenotypes of *Avr* gene alleles might be under only weak selection, or selectively neutral in local host populations (Salathé *et al*. 2005).

To evaluate the relative influence of drift vs. local selection in generating divergence among populations, we examined pairwise patterns of divergence among populations for the different genetic and phenotypic characters. In the sexual lineage AA, we found no significant relationships among populations for the degree of divergence as determined by any of the various genetic and phenotypic characters. These results demonstrate the potential for different evolutionary forces to act on these markers independently, and suggest that local patterns of selection contribute to phenotypic and *Avr* gene divergence among lineage AA populations. In contrast, for lineage AB, we found highly significant correlations between all phenotypes and genotypes at the population level. However, comparisons among populations for lineage AB are almost certainly complicated by a lack of sexual reproduction. Given that very strong linkages among all genetic elements in lineage AB are likely, unravelling the relative roles of drift and selection is highly problematic.

Conclusions

The pattern of variation in genetic and pathogenic structure of *M. lini* provides strong evidence that geographical structure across both regional and local scales is an important component in the generation and maintenance of virulence diversity in this system. The spatial distribution of the variation is consistent with evolutionary dynamics acting to structure diversity at two distinct spatial scales. At the regional scale, patterns of environmental heterogeneity interact with host and pathogen life-history traits to create two broad areas (mountains vs. plains) with distinct genetic and demographic dynamics. Importantly, correlated patterns of pathogen virulence diversity and host resistance diversity (Burdon *et al*. 1999) indicate contrasting rates of co-evolutionary response among the two regions, suggesting the possibility of a scenario where distinct co-evolutionary hotspots and coldspots (Thompson 2005) are generated through corresponding modes of host and pathogen reproduction. Within these regions local metapopulation processes act to structure the distribution of variation, driven by local patterns of selection and neutral genetic drift. A major goal for future studies will be to quantify the influence of these patterns on disease epidemiology, host mating system and host resistance.

Acknowledgements

We thank Caritta Eliasson for technical support, and Peter Dodds for providing assistance and expertise with the *Avr* gene component of this study. Three anonymous reviewers provided numerous constructive comments that significantly improved the quality of this manuscript.

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Luke Barrett's research has focused on the ecology and evolution of parasites and their hosts. Pete Thrall and Jeremy Burdon share common interests in the ecology and coevolutionary biology of natural plant-microbe interactions, with a long-standing interest in the spatial dynamics of host-pathogen systems. Adrienne Nicotra is interested in how architecture, morphology, and physiology interact to determine the ecological attributes of plant species; and in how these characteristics vary both within and between species. Celeste Linde's research focuses on the population genetics, evolution, phylogeography and molecular phylogenetics of plant pathogens.

Supplementary material

The following supplementary material is available for this article:

Table S1. AFLP multilocus genotypes, avirulence gene genotypes* and virulence phenotypes for isolates of *Melampsora lini* belonging to lineage AA or AB, collected from 10 populations across two regions. New AFLP mulitilocus genotypes and virulence phenotypes were assigned each time a unique fingerprint was encountered in the order listed below. *For lineage AB isolates we report only the 'A' allele. All lineage AB isolates are assumed heterozygous for a second invariant 'B' allele

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