Evolution, 56(7), 2002, pp. 1340–1351

LOCAL ADAPTATION IN THE *LINUM MARGINALE–MELAMPSORA LINI* HOST-PATHOGEN INTERACTION

PETER H. THRALL,[1,2] J. J. BURDON,[1] AND JAMES D. BEVER[3]
[1]*Centre for Plant Biodiversity Research, CSIRO–Plant Industry, GPO Box 1600, Canberra ACT 2601, Australia*
[2]*E-mail: P.Thrall@pi.csiro.au*
[3]*Department of Biology, Indiana University, Bloomington, Indiana 47405*

Abstract.—The potential for local adaptation between pathogens and their hosts has generated strong theoretical and empirical interest with evidence both for and against local adaptation reported for a range of systems. We use the *Linum marginale–Melampsora lini* plant-pathogen system and a hierarchical spatial structure to investigate patterns of local adaptation within a metapopulation characterised by epidemic dynamics and frequent extinction of pathogen populations. Based on large sample sizes and comprehensive cross-inoculation trials, our analyses demonstrate strong local adaptation by *Melampsora* to its host populations, with this effect being greatest at regional scales, as predicted from the broader spatial scales at which *M. lini* disperses relative to *L. marginale*. However, there was no consistent trend for more distant pathogen populations to perform more poorly. Our results further show how the coevolutionary interaction between hosts and pathogens can be influenced by local structure such that resistant hosts select for generally virulent pathogens, while susceptible hosts select for more avirulent pathogens. Empirically, local adaptation has generally been tested in two contrasting ways: (1) pathogen performance on sympatric versus allopatric hosts; and (2) sympatric versus allopatric pathogens on a given host population. In situations where no host population is more resistant or susceptible than others when averaged across pathogen populations (and likewise, no pathogen population is more virulent or avirulent than others), results from these tests should generally be congruent. We argue that this is unlikely to be the case in the metapopulation situations that predominate in natural host-pathogen interactions, thus requiring tests that control simultaneously for variation in plant and pathogen populations.

Key words.—Coevolution, disease dynamics, gene-for-gene pathosystem, metapopulation, Red Queen.

Received August 20, 2001. Accepted March 15, 2002.

Patterns of local adaptation in host-pathogen systems not only give insight into the spatial scales at which coevolutionary dynamics occur (Gandon et al. 1996; Gandon and Van Zandt 1998), but also provide crucial tests of many specific theoretical predictions. Indeed, models exploring the potential for pathogens to maintain host sexual reproduction (Hamilton 1980; Hamilton et al. 1990) assume that hosts and pathogens coevolve tightly through frequency-dependent cycling, leading to an expectation of strong patterns of local adaptation (Ladle 1992; Ebert and Hamilton 1996; Lively 1999). Although a majority of empirical studies appear to support local adaptation by pathogens to their hosts, patterns consistent with local maladaptation may sometimes be found due to populations cycling out of phase (Morand et al. 1996; Kaltz and Shykoff 1998). In other cases, no evidence has been found for adaptation at the scale of local populations (Davelos et al. 1996; Ebert et al. 1998; Imhoof and Schmid-Hempel 1998), potentially reflecting the consequences of coevolutionary processes occurring at either larger geographic (Thompson 1994, 1999) or smaller spatial scales (Karban 1989; Lively and Jokela 1996; Ebert et al. 1998).

The extent and intensity of host-pathogen interactions, and hence the extent of local adaptation, is mediated by a number of factors, including the spatial scale over which comparisons are made, the genetic basis of resistance and pathogenicity, the stochasticity of the physical environment, and specific features of both host and pathogen life histories. For example, results from computer simulations suggest that local adaptation depends on the relative migration rates of host and pathogen, with pathogens showing greater probability of being locally adapted when they migrate more than their hosts (Gandon et al. 1996). Similarly, local adaptation of pathogens might be expected to be strongest in associations where efficient pathogen survival mechanisms ensure intimate contact between host and pathogen over multiple pathogen generations and/or growing seasons and where disease tends to be endemic. The likelihood of observing local adaptation may be reduced when off-season survival is limited and individual pathogen populations are more ephemeral—in such situations, evidence of coevolution may be found at larger geographic scales (Thrall and Burdon 1997; Burdon and Thrall 2000).

In natural systems, host and pathogen populations may vary in the identity and diversity of genotypes present, conferring different levels of resistance or virulence, respectively (Bevan et al. 1993a,b; Thrall and Burdon 2001). Such patterns of among-population variation not only influence the ecological and coevolutionary dynamics of host-pathogen interactions, but may also complicate interpretation of results from studies of local adaptation. Local adaptation has generally been tested either by comparing the performance of pathogens on sympatric hosts to that on allopatric hosts or by comparing the performance of sympatric and allopatric pathogens on a given host population (e.g., Gandon and Van Zandt 1998; Kaltz and Shykoff 1998). Variation in host resistance can obscure tests using the former method, whereas variation in pathogen virulence can obscure the latter.

Few, if any studies have used both approaches simultaneously or examined local adaptation in plant-pathogen systems at the appropriate metapopulation scale at which most short-term coevolutionary change occurs. Here we use the interaction between *Linum marginale* and *Melampsora lini* to investigate patterns of local adaptation at several spatial scales within a well-defined metapopulation situation (Bur-

don et al. 1999; Burdon and Thrall 2000; Thrall et al. 2001). The *Linum-Melampsora* system is dominated by a gene-for-gene resistance/virulence structure, with pathogen populations undergoing frequent local extinction and recolonization. Prior studies of this system indicate that host and pathogen populations can vary substantially in average levels of resistance and virulence (Burdon and Jarosz 1992; Thrall et al. 2001). Throughout this paper, ''virulence'' is used in the standard plant pathology sense, meaning the ability of the pathogen to cause a susceptible response on a particular host line, and refers to the interaction between specific virulence and resistance genes. This is quite a different usage from the animal host-parasite literature, where virulence refers to the severity of effects on an infected host.

To evaluate coevolutionary patterns, we use cross-inoculations involving two host and pathogen populations from each of three metapopulation subregions. One expectation is that if pathogen populations are coevolving with their local hosts, there should be a significant relationship between average host resistance and pathogen virulence. We test for local adaptation of pathogens by comparing both the performance of individual pathogens on sympatric versus allopatric hosts and the performance of sympatric pathogens on their hosts to allopatric pathogens on the same hosts. We also develop a combined approach that simultaneously controls for variation in average host resistance and pathogen virulence. Previous studies have shown a nonrandom distribution of resistance among host populations, with nearby populations being more likely to share resistance phenotypes. In contrast, although there were major differences among pathogen populations with respect to which pathotypes were present, pathogen virulence was not related to distances between populations—a result most likely to reflect the greater dispersal of the pathogen (Thrall et al. 2001). This leads to the prediction that pathogens will generally be adapted to their hosts and that such adaptation will be increasingly apparent as the spatial scale of comparison increases. We explicitly evaluate this possibility using among-region, within-region, and between-population comparisons.

Materials and Methods

The Host

Linum marginale A. Cunn. ex. Planch is an herbaceous, perennial herb endemic to southern Australia. In subalpine areas, plants overwinter as underground rootstocks with or without a few short shoots protected from frost by the surrounding vegetation. With the arrival of spring, fresh shoots develop and plants flower in mid to late summer before dying back with the onset of autumn frosts. Seedling recruitment occurs mainly in spring and early summer. Although *L. marginale* shows significant variation in its mating system across its range, within the study area, populations are strongly inbreeding (Burdon et al. 1999).

The Pathogen

Melampsora lini (Ehrenb.) Lev. is an autoecious rust pathogen that, in Australia, is restricted to *Linum marginale*. This pathogen has substantial fitness effects on its host, causing 60–80% reductions in population size during severe epidemics (Jarosz and Burdon 1992). Thus, *M. lini* has the potential to provide strong selection pressure on host resistance. *Melampsora lini* is potentially capable of both sexual and asexual reproduction on *L. marginale*, but assessment of RFLP and pathogenicity variation within the study area found evidence of strong linkage disequilibrium, and hence an implied lack of sexual recombination (Burdon and Roberts 1995).

During the growing season, the pathogen occurs on living tissue as small localized, orange-colored uredial lesions. This stage of the life cycle is asexual and, under favorable environmental conditions, six to eight uredial generations follow one another in quick succession, leading to local epidemics. The asexually produced urediospores are wind dispersed and can infect either the same or different plants. The pathogen overwinters as very limited numbers of dormant uredial infections on occasional small shoots.

Study Sites

The host and pathogen populations used in the present study occur on the Kiandra Plain in the northern part of the Kosciuzko National Park or on the immediately adjacent Wild Horse Plain (Fig. 1). This open subalpine grassland is surrounded by extensive eucalypt forest in which *L. marginale* is rarely found. The populations studied were distributed in three groups located in the northern (G1, G3) and southern sections (SH1, SH2) of the Kiandra Plain and in the Wild Horse Plain (WHP1, WHP2; Fig. 1). The northern and southern population groups are approximately 10 km apart, and are 6.1 km and 4.6 km from the group located on Wild Horse Plain, respectively. Within each group, interpopulation distances are: G1-G3, 225 m; SH1-SH2, 775 m; WHP1-WHP2, 380 m.

Collection of Host and Pathogen Populations

In the summer of 1998–1999, seed was haphazardly collected from both healthy and infected individuals (minimum of 25 plants across the entire area) in each of the six host populations. Seeds from each plant were bagged separately and stored in the laboratory for six months to break dormancy.

At the same time as seed was collected, pathogen populations were sampled within each of the six host populations. At each site, approximately 25 rust samples were collected separately from different infected plants by lightly rubbing cotton buds across the surface of sporulating uredia. In the laboratory, samples were separately inoculated onto 10–15-cm tall seedlings of the universally susceptible *L. usitatissimum* 'Hoshangabad'. Inoculated plants were left in a saturated atmosphere overnight before being transferred to a naturally lit glasshouse. One week later, single infections were isolated and put through three cycles of increase to produce sufficient urediospores for pathotype analysis. This procedure ensured that each isolate consisted of a single pathogen genotype. A subset of 10 isolates was randomly chosen from each population for use in the local adaptation study.

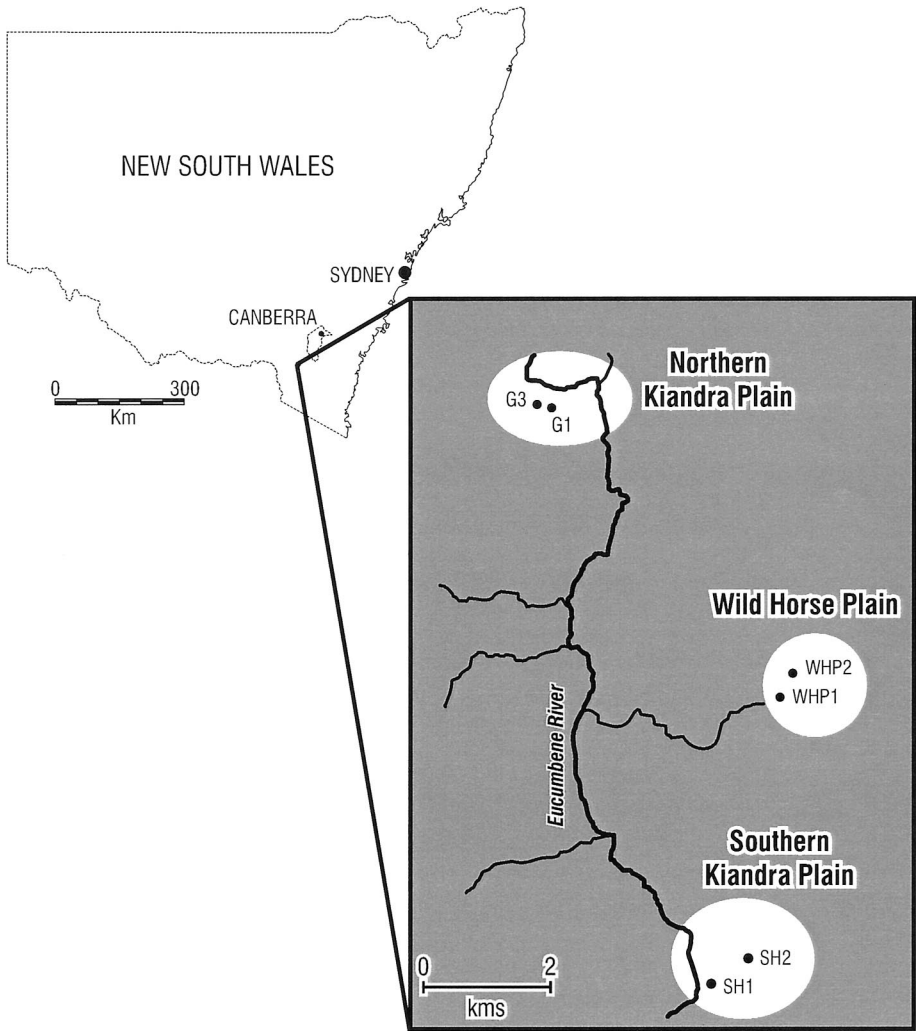

FIG. 1. Map showing the location of the study populations of *Linum marginale* and *Melampsora lini* on the northern and southern Kiandra Plain and the adjacent Wild Horse Plain.

Evaluation of Patterns of Local Adaptation

Our primary goal was to evaluate the extent to which pathogen populations were locally adapted to their host populations (assessed as the level of virulence expressed, where ''virulence'' refers to the ability of a particular pathogen isolate to attack a specific host resistance gene). We therefore determined the resistance structure of the six *L. marginale* demes by exposing 20 maternal plant lines per host population to 60 isolates of *M. lini* (10 isolates × 6 host populations in all possible pairwise combinations, giving a total of 7200 individual inoculation tests).

Multipathotype infection type reactions were determined using the same testing procedure as in earlier studies (Burdon and Jarosz 1991; Jarosz and Burdon 1991). This involved using shoots cut from young, vigorously growing host plants. Stems from each plant, plus one of *L. usitatissimum* 'Hoshangabad', were placed upright in holes pierced through the lid of 12-cm diameter disposable plastic tubs filled with tap water. Each tub (containing 14 host lines plus the control) was used in the determination of infection type responses to a single pathogen line.

Tubs were sealed in the bottom of 12-cm diameter, 55-cm tall plastic towers into which 10 mg of urediospores of *M. lini* dispersed in 100 mg of talc was injected with compressed air. The spore-talc mix was allowed to settle for 2–5 min before each set was lightly sprayed with water. The following day, tubs were transferred to a naturally lit greenhouse, where infections were scored 12–14 days later. Any tests giving ambiguous results were repeated. Five infection categories could be distinguished, ranging from those showing many large, freely sporulating uredia (+) through various forms of restricted growth (±, +/=, −) to those in which there was no macroscopic sign of infection (=; Lawrence and Burdon 1989). Partially resistant phenotypes (±, +/=) have been shown to be controlled by single dominant genes inherited in a simple Mendelian fashion (Burdon 1994).

General Assessment of Pathogen Virulence Pathotypes

Because we were interested in a broad view of local adaptation across the entire metapopulation, we also assessed the virulence of each isolate on a standard set of 11 *L. marginale* differential host lines (many of which are derived from

the Kiandra region). These lines have been used extensively in previous work assessing pathogen population structure (Burdon and Jarosz 1991, 1992; Jarosz and Burdon 1991; Burdon and Roberts 1995). For the purposes of pathotype identification, all resistant type reactions were pooled so that the interaction between a pathogen isolate and a particular host line was classified as either susceptible (+) or resistant (all other categories). The pattern of resistant and susceptible responses across the differential set provided a unique matrix that identified particular pathotypes. Using this approach, the pathotypic structure of the six pathogen populations was determined by screening an average of 23 isolates per population (range = 21–25), including the 10 used for the local adaptation study.

Using these raw data, pathogen populations were assessed both in terms of the frequency and distribution of individual pathotypes (essentially a multilocus assessment of homozygosity at the various virulence genes that correspond to the resistance genes/alleles in the lines of the differential set [virulence is a recessive character in *M. lini*; Flor 1955]) and in terms of their virulence (summed across all isolates) against specific differential lines.

Statistical Analyses

General patterns of local adaptation

The infection type data were investigated with a nested analysis, with populations nested within region and plant lines or pathogen isolates nested within host or pathogen populations, respectively. Note that for all analyses, individual host lines and pathogen isolates were treated as random effects, whereas host and pathogen regions and populations were assumed to be fixed effects.

Three approaches were used in the analysis: (1) the full dataset with all differences in infection reactions considered; (2) the dataset subdivided into three categories reflecting full propagule production (+), limited propagule production (±, +/=), and zero propagule production (−, =); and (3) the dataset divided into two categories according to the absence of an effective resistance gene in the host or avirulence gene in the pathogen (+) versus the presence of genes conveying at least some resistance. In all cases, fully susceptible reactions were scored as 1 and the most resistant category as 0, between which intermediate resistance categories were equally spaced. The first two approaches were analyzed using analysis of variance (SAS procedure GLM; SAS Institute 1989), whereas the third approach was analyzed using generalized linear models (SAS procedure CATMOD; SAS Institute 1989). Because the results of these analyses were qualitatively similar, we only present analyses using the intermediate approach with three infection categories. This most accurately reflects epidemiological outcomes, given that intermediate infection categories generate some spores. Because we focus on regional and population-level effects, assuming that plant line and fungal isolate effects are random (and therefore test over these higher-order interaction terms), and our dataset is highly replicated and completely balanced, the parametric assumptions are approximately met due to the central limit theorem (Sokal and Rohlf 1995).

Both plant and pathogen region and population main effects were significant and clearly nonlinearly related. The best fit for the relationship between mean plant population resistance and mean pathogen virulence was given by a second-order power function ($y = ax^{[b+c\ln(x)]}$, where x and y represent mean resistance and virulence, respectively).

Because the interaction between plant and fungal regions/populations was significant, we tested the specific hypothesis of local adaptation using three approaches. In the first, we assessed whether pathogens differed in their ability to infect plants from their own host population relative to plants from other populations. This was tested using separate one-way analyses of variance for each pathogen population followed by contrasts comparing infection of their sympatric hosts versus the average ability to infect allopatric host populations. The reciprocal question of whether pathogens were better able to attack their own host population than pathogens sampled from other host populations was also assessed with one-way analyses of variance for each plant population followed by sympatric versus allopatric contrasts. The same data is analyzed in these two different ways; therefore, significance of these tests was adjusted using the Dunn-Šidák method (Sokal and Rohlf 1995). Tests using the first approach factor out main-effect differences of fungal population but not main-effect differences of host, whereas tests using the second approach factor out main-effect differences of host population but not main effect differences of pathogen population. In tests using the third approach, we simultaneously factor out both these main effect differences by developing a series of contrasts within the nested analysis of the entire dataset (for similar analyses, see Bever 1994; Bever et al. 1997; Mills and Bever 1998). We first tested local adaptation among regions by comparing the average infection level of sympatric combinations with the average infection level of allopatric combinations. Because this effect was significant, we followed it with three specific contrasts that tested local adaptation of individual regions. The significance of these contrasts were controlled for multiple comparisons using the Dunn-Šidák method (Sokal and Rohlf 1995). We then tested for local adaptation within regions followed by tests within individual regions (again controlling for multiple comparisons).

Local adaptation and geographic distance

Two approaches were used to investigate the extent to which the ability of a pathogen population to attack a given host population was a function of physical distance between them: (1) an analysis of covariance in which pathogen population was assumed to be a class variable, linear distance to each host population was used as a covariate, and the mean virulence of each pathogen population × host population combination was the dependent variable (for a similar approach see Ebert 1994; Ebert et al. 1998); and (2) linear regression for each pathogen population separately. For these analyses, distance data were log-transformed and mean virulences were arcsine-square-root transformed.

Pathogen population diversity

Pathogen isolates were classified into individual pathotypes based on their interaction with the *L. marginale* differential set.

TABLE 1. Summary of descriptive data for the host and pathogen populations used in the local adaptation study. Population means for host resistance and pathogen virulence were calculated by pooling across all pathogen isolates or host lines, respectively. Superscripts indicate grouping according to a Tukey's comparison.

Population	Pathogen virulence	Host resistance	Pathotypes detected	Shannon-Weaver[1]	R Genes attacked[2]
G1	$0.812^{C,D}$	$0.269^{B,C}$	8	1.19	$5.1^{A,C}$
G3	0.861^{D}	0.472^{A}	16	2.29	6.0^{A}
SH1	0.530^{A}	0.085^{E}	8	1.40	4.2^{B}
SH2	$0.757^{B,C}$	$0.203^{C,D}$	7	1.32	5.0^{C}
WHP1	$0.831^{C,D}$	0.331^{B}	9	1.70	3.9^{B}
WHP2	0.707^{B}	$0.141^{D,E}$	7	1.14	$4.6^{B,C}$

[1] Diversity of pathogen populations based on pathotype frequencies.
[2] Number of lines attacked in the *L. marginale* differential set.

The differences in frequency of these pathotypes across pathogen populations was tested using Fisher's exact test (SAS procedure FREQ; SAS Institute 1989). In addition, a one-way analysis of variance was used to investigate whether differences existed among pathogen populations with respect to the mean number of host differentials against which pathogens were virulent. The frequency of individual pathotypes was also used to calculate a measure of pathogen population diversity (corrected Shannon-Weaver index; Hutcheson 1970).

RESULTS

Population Variation in Host Resistance and Pathogen Virulence

Overall, there was substantial variation among regions as well as among host and pathogen populations in mean levels of resistance and virulence respectively (Table 1). Host population G3 from the northern Kiandra Plain was significantly more resistant than all other populations (mean = 0.47, SE $\pm$ 0.032), whereas SH1 hosts (southern Kiandra Plain) were significantly less resistant than all others (mean = 0.09 $\pm$ 0.008). Moreover, pathogen population SH1 was significantly less virulent than all other pathogen populations (mean = 0.53 $\pm$ 0.038). Similarly, the most virulent pathogen population was G3 (mean = 0.86 $\pm$ 0.025), although its mean virulence did not differ significantly from either G1 or WHP1. This pattern resulted in a very strong positive asymptotic relationship between the overall resistance of a given host population and the virulence of its associated pathogen population (model parameter estimates: $a = 0.76$, $b = -0.28$, $c = -0.17$; adjusted $r^2 = 0.98$, $P = 0.001$).

There were strong interactions between the region and population of origin of hosts and pathogens (Table 2). Much of this effect fell within the interaction between the region of origin for hosts and pathogens (the host region $\times$ pathogen region effect in Table 2). However, plant and pathogen populations within regions also differed strongly in their interactions with pathogens and hosts from different regions and different populations within regions (the host region $\times$ pathogen population within region effect, pathogen region $\times$ host population within region effect, and the host population $\times$ pathogen population effect in Table 2).

Local Adaptation of Pathogen Populations

Using the approaches outlined in the Materials and Methods section, we addressed three specific questions relevant to the general prediction that *M. lini* should be locally adapted to populations of its host *L. marginale*.

Are individual pathogen populations better able to infect sympatric than allopatric hosts?

Because of the significant interaction between host and pathogen populations, we performed separate analyses for each fungal population. Each pathogen population exhibited significant variation in its ability to infect the six host populations (Table 3, Fig. 2). Contrast tests for differences in the ability of pathogens to attack their own versus other host populations were significant for four of the six pathogen populations (Table 3). For pathogen populations SH1, WHP1, and WHP2, mean virulence was greater on sympatric than allopatric host populations, whereas for G3 the reverse was true.

For pathogen populations WHP1 and WHP2, the significant contrast tests reflect the high resistance of host population G3 to these pathogens (e.g., pathogens from WHP1 showed high virulence on all host populations other than G3, and a Tukey's comparison showed no differences in the mean virulence of WHP1 across host populations with the exception of G3). Indeed, for both of the WHP populations, exclusion of G3 hosts from the analysis resulted in a nonsignificant test for local adaptation. In contrast, pathogens from population SH1 were better able to attack their own hosts than other host populations, regardless of whether host population G3 was excluded. In fact, resistance to pathogens from SH1 was generally quite high in all of the allopatric host demes.

Are individual pathogen populations better able to infect sympatric hosts than are other pathogens?

Except for SH1, there was significant variation in the response of host populations to the six pathogen populations (Table 4). Contrast tests for differences in the virulence of sympatric versus allopatric pathogen populations were significant for three of the pathogen populations (Table 4). For these populations (G1, G3, WHP1), pathogens consistently showed greater virulence on their sympatric hosts than the average performance of the five allopatric pathogen populations (Fig. 3). With respect to host populations G1 and WHP1, significant differences appeared to be primarily due to the very low virulence of pathogen population SH1 (although even when SH1 was removed, the contrast was close to significance for WHP1). For population WHP2, pathogens showed reduced virulence on their sympatric host relative to the other pathogens, although this difference was not significant (Table 4).

Is the average ability of pathogens to infect sympatric hosts better than the average ability of pathogens to infect allopatric hosts?

Overall, pathogens had greater ability to infect plants from sympatric than allopatric regions (Table 2). This pattern also

TABLE 2. Variation in resistance of the host plant *L. marginale* and virulence of the rust pathogen *M. lini* in relation to their region (NK, SK, WHP) and population of origin. All effects were tested assuming plant and isolate terms were random. When more than one mean square was combined to achieve the expected error mean square, the Satterthwaite approximation was employed to determine the appropriate degrees of freedom (SAS Institute 1989). Significance of contrasts were controlled for multiple comparisons using the Dunn-Šidák method.

Source	df	MS	*F*
Host region	2	31.82	19.65****
Host region error	130	1.62	
Pathogen region	2	12.49	12.40****
Pathogen region error	69	1.01	
Host region × pathogen region	4	1.60	5.89***
Local adaptation across regions: overall test	1	4.53	16.72****
Local adaptation across regions: NK vs. others	1	4.16	51.36****
Local adaptation across regions: SK vs. others	1	5.51	20.33****
Local adaptation across regions: WHP vs. others	1	1.76	6.47*
Host region × pathogen region error	226	0.27	
Host population within region	3	15.16	9.44****
Variation between NK populations	1	36.26	34.28****
Variation between SK populations	1	3.52	2.18
Variation between WHP populations	1	5.70	3.54
Host population within region error	207	1.61	
Pathogen population within region	3	7.85	7.91****
Variation between NK populations	1	0.94	0.95
Variation between SK populations	1	19.53	19.73****
Variation between WHP populations	1	3.08	3.11
Pathogen population within region error	67	0.99	
Host region × pathogen population within region	6	0.56	2.18*
Host region × pathogen pop within region error	228	0.25	
Pathogen region × host population within region	6	1.49	5.76****
Pathogen region × host pop within region error	269	0.26	
Host population × pathogen population	9	1.09	4.48****
Local adaptation within region: overall test	1	2.77	11.40***
Local adaptation within NK	1	0.69	2.84
Local adaptation within SK	1	2.31	9.51**
Local adaptation within WHP	1	0.28	1.16
Host population × pathogen population error	327	0.24	
Plant within host population within region[1]	114	1.51	9.45****
Isolate within pathogen population within region[2]	54	0.89	5.85****
Pathogen region × plant within host population[3]	228	0.16	1.11
Host region × isolate within pathogen population[4]	108	0.15	1.09
Path reg × path pop × host reg × plant pop × plant[5]	342	0.14	3.59***
Host reg × host pop × path reg × path pop × isolate[5]	162	0.13	3.45***
Error	6156	0.04	

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$.
[1] Tested over the pathogen region × plant within host population MS.
[2] Tested over the host region × isolate within pathogen population MS.
[3] Tested over the path reg × path pop × host reg × host pop × plant MS.
[4] Tested over the host reg × host pop × path reg × path pop × isolate MS.
[5] Tested over the error MS.

TABLE 3. Variation in virulence of individual pathogen populations across host populations. For each pathogen population, contrast tests were used to ask whether mean virulence was significantly different on sympatric versus allopatric host populations (superscripts indicate whether performance was greater on sympatric [S] or allopatric [A] hosts).

Pathogen population	Main effect of host population		Pathogen performance on sympatric vs. allopatric host populations	
	MS	$F_{5,54}$	MS	$F_{1,54}$
G1[S]	18.30	18.04****	0.48	2.35
G3[A]	6.69	4.12**	6.53	20.12****
SH1[S]	45.72	29.54****	24.13	77.97****
SH2[S]	21.56	12.96****	1.23	3.70
WHP1[S]	23.37	47.86****	0.89	9.09**
WHP2[S]	21.92	12.87****	1.84	5.39*

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$.

held true when each region was tested against the other regions individually. Similarly, pathogens had greater ability to infect plants from their own population than from the other population in the same region (Table 2), although local adaptation of pathogen populations at this level was only significant within the southern Kiandra Plain. In contrast to the previous results, when the main effects of both the overall resistance of plant populations and the overall virulence of pathogen populations were factored out, all tests were in the direction of local adaptation of the pathogens. This remained true when testing across the six populations without the hierarchical structure.

Relationship between Pathogen Virulence and Distance among Populations

Evidence for local adaptation is often expected to be coupled with significant negative relationships between pathogen

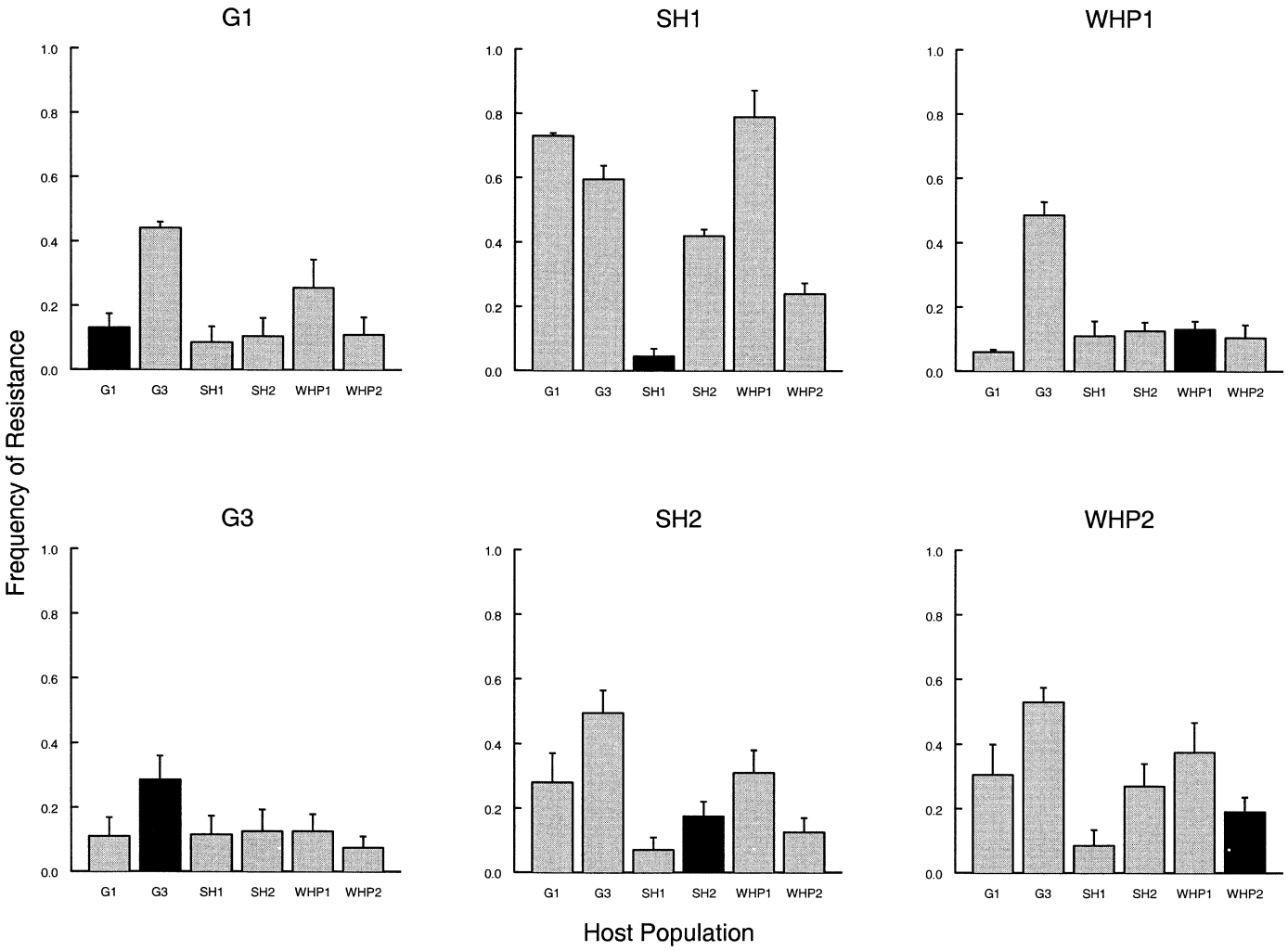

FIG. 2. Mean ability of pathogen populations to attack each of the six host populations. Each graph shows the resistance responses for host populations grouped by pathogen population. Black bars represent sympatric combinations, and gray bars represent allopatric combinations. Error bars represent standard error.

TABLE 4. Variation in the ability of individual pathogen populations to attack sympatric host populations relative to allopatric pathogen populations. For each host population, contrast tests were used to ask whether mean virulence of sympatric pathogens was significantly different from allopatric pathogen populations (superscripts indicate whether performance of sympatric [S] or allopatric [A] pathogens was greater).

Host population	Main effect of pathogen population		Performance of sympatric vs. allopatric pathogen populations	
	MS	$F_{5,54}$	MS	$F_{1,54}$
G1^{S}	31.57	21.46****	2.20	7.49*
G3^{S}	9.93	4.87***	7.07	17.36****
SH1^{S}	0.32	0.41	0.11	0.72
SH2^{S}	8.89	6.90****	0.27	1.04
WHP1^{S}	23.78	12.89****	3.36	9.11**
WHP2^{A}	2.48	4.02**	0.14	1.14

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$.

virulence and geographic distance, although this is not necessarily the case (Kaltz and Shykoff 1998). The analysis of covariance showed no main effect of distance, although there was a significant interaction between distance and pathogen population (Table 5), indicating that there was no consistent trend for more distant pathogen populations to perform worse than sympatric pathogens. This interpretation was confirmed by separate linear regressions of the mean performance of each pathogen population on the six host populations. Although only the regression for pathogen population SH1 was significant ($r^2 = 0.59$, $P < 0.05$), the relationship between distance and performance varied between negative (SH1, SH2) and positive (G1, G3; slopes varied from -0.22 ± 0.078 to 0.10 ± 0.063). This reflects the strong differences in overall susceptibility of these host populations and highlights the unpredictable patterns likely to be typical of metapopulations.

Occurrence and Distribution of Virulence Pathotypes

A total of 34 virulence pathotypes were detected using the *L. marginale* differential set (mean per population = 9.2).

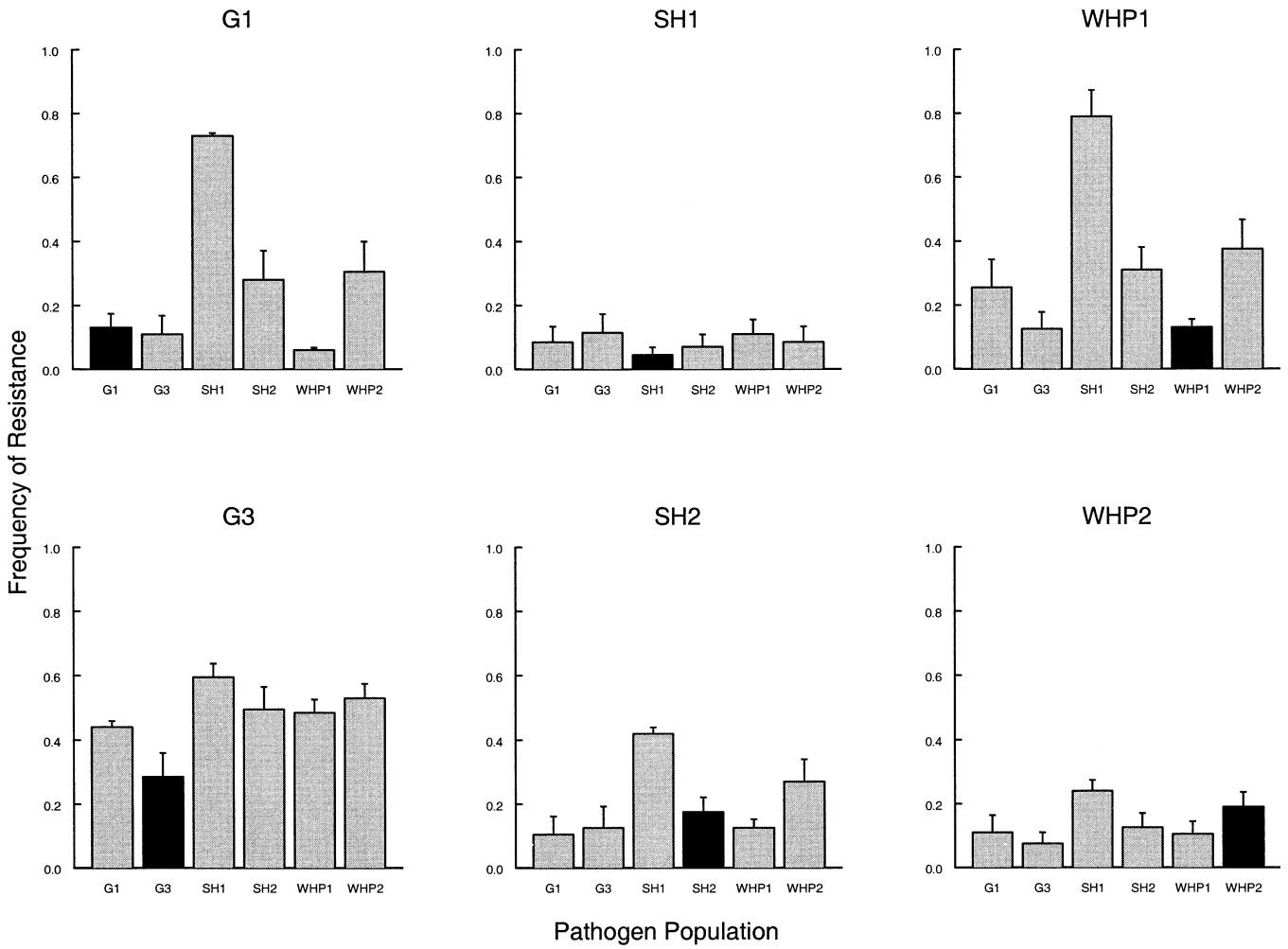

FIG. 3. Mean resistance of each host population to the six pathogen populations. Each graph shows resistance responses to the pathogen populations grouped by host population. Black bars represent sympatric combinations, and gray bars represent allopatric combinations. Error bars represent standard error.

Pathogen populations varied considerably in the number and frequency of these pathotypes (Fisher's exact test, $P < 0.0001$; Table 1), with some unique types existing in each population. Populations G1, SH2, and WHP2 were dominated (>50%) by a single pathotype (designated A in previous studies of the *Linum-Melampsora* system; Burdon and Jarosz 1991; Jarosz and Burdon 1991; Thrall et al. 2001). In contrast, although population G3 is separated from G1 by only 225 m, pathotype A was recorded at substantially lower frequencies in the former population. In fact, G3 had the highest overall diversity (16 pathotypes), with 68.8% of these being unique. Similarly, population SH1 was dominated by a completely different pathotype (pathotype A existed at < 5%) to SH2, although SH1 is only 775 m away. These data demonstrate that despite the high potential for among-population dispersal of *M. lini*, the six pathogen populations were genetically quite distinct.

A one-way analysis of variance revealed significant variation among pathogen populations in the mean number of resistance genes in the *L. marginale* differential set that could be attacked ($F_{5,132} = 11.99$, $P < 0.0001$). The distribution for pathogen population G3 showed the widest range of values, had the highest overall virulence of any of the pathogen populations, and was the only population with pathotypes able to attack more than seven of the host differentials (Fig. 4). These results correlated well with virulence estimates derived from inoculations using the six natural *L. marginale* populations ($r = 0.41$, $P = 0.001$), which clearly supports the value of the differential set in meaningfully identifying virulence pathotypes.

TABLE 5. Analysis of covariance with mean virulence of pathogen populations as the main effect, and linear distance between pathogen population and host population as the covariate. The interaction between pathogen population and distance was significant at $P < 0.05$.

Source	df	MS	*F*
Pathogen population	5	0.023	0.86
Distance	1	0.083	3.06
Pathogen population × distance	5	0.079	2.91*
Error	24	0.027	

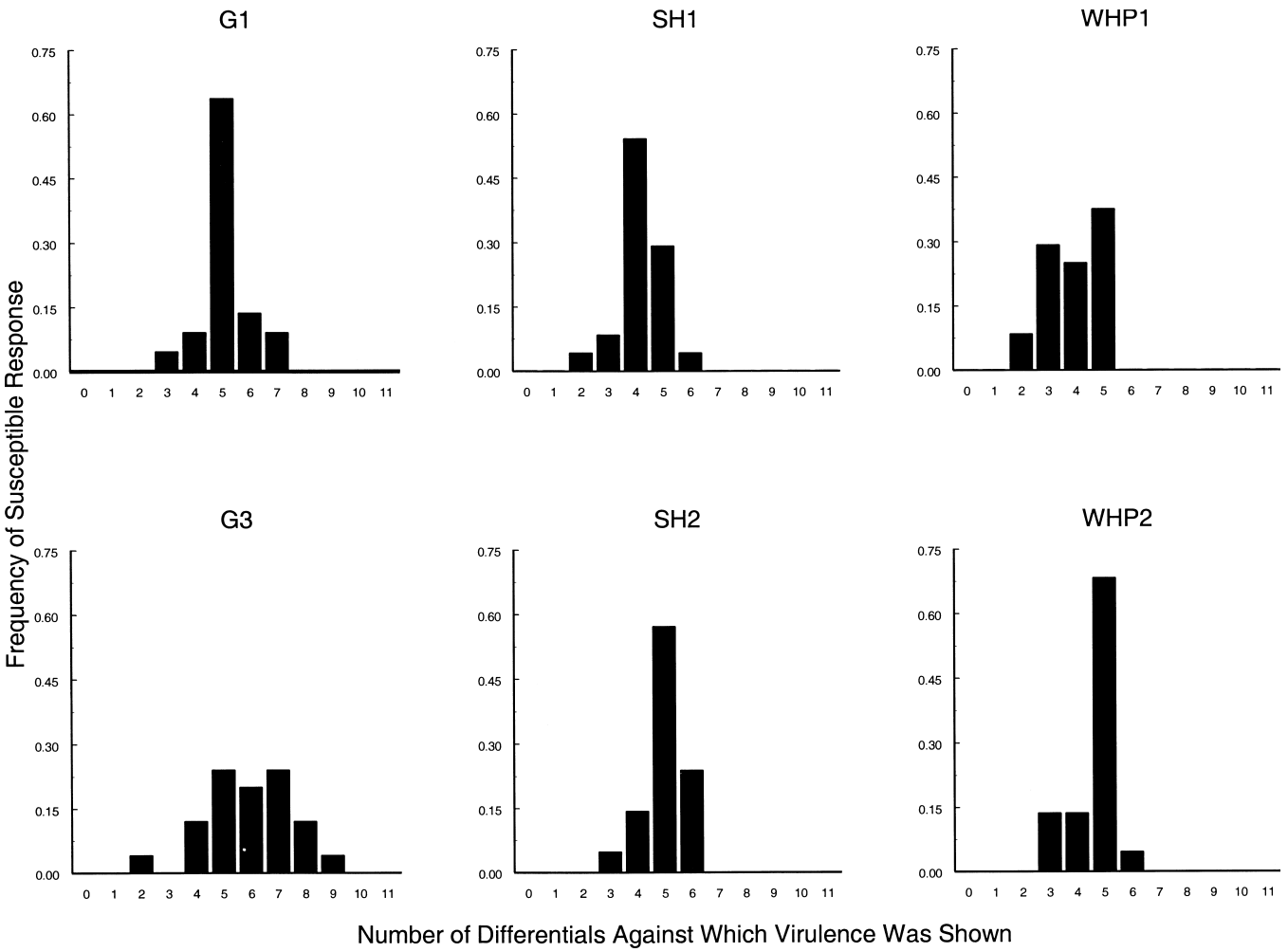

FIG. 4. Distribution of pathogen virulences (number of host lines that were susceptible) against the *Linum marginale* differential set for the six pathogen populations.

DISCUSSION

Patterns of Local Adaptation in the Linum-Melampsora System

Local adaptation is an average expectation that is not apparent in every population all the time (Gandon and Van Zandt 1998; Kaltz and Shykoff 1998; Lively 1999). Indeed, using a computer simulation, Lively (1999) showed that the magnitude of local adaptation fluctuates over time as a result of the frequency-dependent nature of host-pathogen dynamics. In metapopulations, where colonization and extinction processes and chance events (e.g., genetic drift) also play significant roles, detecting local adaptation becomes even more problematic. A reasonable test requires not only multiple populations located at a range of different distances from each other (Gandon and Van Zandt 1998), but also representative samples of each host and pathogen population. In the present study, we have evidence of local adaptation of pathogens to their hosts within a single metapopulation (Table 2), despite the often-large differences between individual populations in overall resistance or virulence. Indeed, this variation itself demonstrates the strength of the coevolutionary interaction as shown by the highly significant relationship between average resistance and average virulence of host and pathogen populations.

Local adaptation of pathogens is made possible by the independence of coevolutionary dynamics between populations. The hierarchical sampling of host and pathogen populations in this study allowed testing of these effects at multiple spatial scales. We observed strong differentiation of both plant and pathogen populations among the three regions (host region effect, pathogen region effect, Table 2) and clear evidence of local adaptation of the pathogen. Although we also obtained evidence of differentiation and local adaptation of plant and pathogen populations within regions, these differences were weaker than at the regional level. Furthermore, variation among pathogen populations within regions was weaker than that observed among host populations within regions. This pattern reflects the higher dispersal and greater stochasticity inherent in the life-history of *M. lini*. Indeed, an earlier study of host and pathogen populations within the Kiandra metapopulation found significant spatial structure with respect to the distribution of host resistance phenotypes. However, with respect to pathogen virulence, significant dif-

ferences were found between subregions of the metapopulation, but not within them (Thrall et al. 2001). This finding is consistent with the current study and is one of the conditions under which local adaptation of the pathogen might be expected (Gandon et al. 1996).

The spatial scale of dispersal, and hence differentiation, of pathogen populations is itself likely to determine the extent of local adaptation within a metapopulation. Thus, while differences in overall resistance were smallest for the two southern Kiandra Plain host populations, the pathogen populations in that region showed the greatest differentiation, consistent with the fact that the interpopulation distances were also greatest for this population pair. Moreover, it is between these two pathogen populations that we also found the strongest evidence for local adaptation (Table 2). These results illustrate both the potential influence of pathogen life history on the probability of observing local adaptation and the value of hierarchical sampling in a metapopulation context.

The *Linum-Melampsora* system is characterized by epidemic disease dynamics with boom-and-bust cycles and frequent local extinctions of the pathogen. *Linum marginale* populations are much less ephemeral (although there can be substantial numerical and genetic shifts following major epidemics; Burdon and Thompson 1995) and may be regarded as semistable assemblages upon which *M. lini* populations evolve. Given the stochastic nature of dynamics in the *Melampsora* component of this interaction, the strength of the positive relationship between host resistance and pathogen virulence underscores the potential for host variation to determine evolutionary trajectories of pathogen populations. Clearly, evolution in host populations harboring many resistance alleles (often correlated with average resistance; Thrall et al. 2001) may favor very different pathogen populations to those evolving in low-diversity host populations. Thus, in this *Linum-Melampsora* metapopulation, where hosts are long-lived and strongly inbreeding, founder events will play a large role in determining host population structure, leading to potentially quite different selection environments. In such situations, pathogen populations associated with more-resistant hosts may be both more diverse and more virulent. Conversely, plant populations with overall lower resistance appear to select for pathotypes with overall lower virulence, thereby generating the strong correlation between average virulence of pathogens and average resistance of hosts observed here. Selection in this direction must then result from a trade-off between virulence and some aspect of population growth or colonizing ability of pathogens. Such a trade-off would also play an important role in generating local adaptation in a gene-for-gene pathosystem because it would impede the evolution of super pathotypes capable of attacking all plant genotypes.

The expectation of a distance effect is based on the implicit assumption of a trade-off between adaptation to local hosts and performance on allopatric hosts (Kaltz and Shykoff 1998). Although a negative relationship between geographic distance and pathogen success is strong evidence of local adaptation (Ebert 1994; Ebert et al. 1998), its absence does not mean a lack of local adaptation. For example, Lively (1989, 1999) found strong patterns of local adaptation in populations of the snail *Potamopyrgus antipodarum* and its trematode parasite *Microphallus* sp. but no consistent distance effect. Similarly, Morand et al. (1996) examined data from a range of studies of schistosome parasites and snails and found that there was no consistent evidence of a negative relationship between compatibility and distance. Here we found a comparable result with a significant interaction between distance to the host population and pathogen population of origin, indicating that more distant pathogens could perform either better or worse than sympatric pathogens. This result is not unexpected in metapopulations where pathogens undergo frequent population crashes with attendant genetic drift and local extinction.

Some General Implications for Tests of Local Adaptation

The findings reported here raise a general issue regarding the interpretation of patterns of host-pathogen coevolution. Two alternative tests have been used previously with local adaptation being inferred when either parasites are better able to attack sympatric than allopatric hosts (Parker 1985; Lively 1989, 1996; Kaltz et al. 1999; Mutikainen et al. 2000) or parasites are better able to attack sympatric hosts than are allopatric pathogens (Ebert 1994; Ebert et al. 1998; Lively 1999). Some authors have noted that both tests are equally valid but provide different information on adaptive genetic structure (Gandon and Van Zandt 1998; Gandon et al. 1998; Kaltz and Shykoff 1998). Here we demonstrate that either or both of these tests of local adaptation may be inadequate when there are overall differences in resistance and/or virulence among the host and pathogen populations, respectively.

Specifically, assessing pathogen performance in sympatric versus allopatric host populations can provide a misleading test of local adaptation when host populations differ in overall resistance. For example, pathogen population G3 did significantly worse on its own highly resistant host population than on allopatric hosts (Table 3, Fig. 2). However, when differences in overall resistance were factored out, it showed strong local adaptation ($F_{1,342} = 18.38$, $P < 0.0001$) because it was better able to attack G3 hosts than could other pathogen populations (Table 4, Fig. 3). Alternatively, this same test can give a false-positive result. Pathogen population WHP2 was better able to attack its own host population than allopatric hosts (Table 3, Fig. 2); however, this effect was offset by the tendency of this pathogen population to be less virulent on its sympatric hosts than were other pathogen populations (Table 4, Fig. 3). These two factors counteract each other such that the combined test is no longer significant ($F_{1,342} = 3.29$). Similarly, testing whether pathogens are better able to attack sympatric hosts than are allopatric pathogens may also provide an inadequate test when the pathogen populations show overall differences in virulence. Thus, for pathogen population SH2 (Tables 3, 4), the combined test indicated significant local adaptation of the pathogen ($F_{1,342} = 6.03$, $P < 0.05$), although neither of the individual tests of local adaptation did so.

Gandon and Van Zandt (1998, p. 215) suggested that a potential solution to these problems is to pool information across the entire cross-inoculation experiment, resulting in the interpretation of local adaptation as ''a general pattern

that describes the adaptive genetic structure of both the host and the parasite.'' We agree but add that even with reciprocal cross-inoculation studies, tests of local adaptation in a single dimension (one pathogen population or the effects of pathogens on one host population at a time) must be interpreted with caution. In both cases, interpretation is based on the assumption that there are no intrinsic differences among populations in overall levels of host resistance or pathogen virulence. When these expectations are met, results will be generally congruent for both kinds of one-way tests for local adaptation. However, this expectation clearly does not match the patterns predicted for situations such as the *Linum-Melampsora* system, where metapopulation structure and stochastic local-history effects predominate. We therefore advocate a third approach that combines the previous two methods into a single test that controls for overall differences in virulence of pathogen populations and resistance of host populations. The hierarchical structure of our dataset allows us to implement these tests both between regions and between populations within regions.

Most theory has focused on within-population scales of local adaptation rather than how interactions are affected by the metapopulation matrix in which individual demes are generally embedded. In real-world systems where spatial structure is important, this is a critical component. Depending on the spatial scale of the interaction and the magnitude and frequency of colonization and extinction events, coevolutionary processes may occur at scales ranging from local patches to broader regional scales and beyond (Thompson 1994, 1999). Despite the inherently stochastic nature of processes occurring within local demes, by considering the interaction between multiple samples from multiple host and pathogen populations in an explicitly spatial context, it is possible to disentangle coevolutionary dynamics across the metapopulation. Using this approach in the *Linum-Melampsora* system, we demonstrate a complex coevolving interaction in which pathogens are both strongly locally adapted to their hosts and covary in overall virulence with the overall resistance of their hosts. The conjunction of these two patterns may well be a signature of coevolution within a metapopulation structure in pathosystems with gene-for-gene genetic architecture.

Acknowledgments

We are grateful to C. Eliasson for her able technical assistance, particularly with generating pathogen isolates and the glasshouse inoculation trials. PHT acknowledges the support of a Queen Elizabeth II Fellowship. We thank D. Ebert, P. Jarne, and two anonymous reviewers for helpful comments on earlier drafts of this paper. This paper is 15th in a series concerning the interaction between *Linum marginale* and *Melampsora lini*.

Literature Cited

Bevan, J. R., I. R. Crute, and D. D. Clarke. 1993a. Variation for virulence in *Erysiphe fischeri* from *Senecio vulgaris*. Plant Pathol. 42:622–635.

———. 1993b. Resistance to *Erysiphe fischeri* in two populations of *Senecio vulgaris*. Plant Pathol. 42:636–646.

Bever, J. D. 1994. Feedback between plants and their soil communities in an old field community. Ecology 75:1965–1977.

Bever, J. D., K. M. Westover, and J. Antonovics. 1997. Incorporating the soil community into plant population dynamics: the utility of the feedback approach. J. Ecol. 85:561–573.

Burdon, J. J. 1994. The distribution and origin of genes for race specific resistance to *Melampsora lini* in *Linum marginale*. Evolution 48:1564–1575.

Burdon, J. J., and A. M. Jarosz. 1991. Host-pathogen interactions in natural populations of *Linum marginale* and *Melampsora lini*. II. Patterns of resistance and racial variation in a large host population. Evolution 45:205–217.

———. 1992. Temporal variation in the racial structure of flax rust (*Melampsora lini*) populations growing on natural stands of wild flax (*Linum marginale*): local vs. metapopulation dynamics. Plant Pathol. 41:165–179.

Burdon, J. J., and J. K. Roberts. 1995. The population genetic structure of the rust fungus *Melampsora lini* as revealed by pathogenicity, isozyme and RFLP markers. Plant Pathol. 44:270–278.

Burdon, J. J., and J. N. Thompson. 1995. Changed patterns of resistance in a population of *Linum marginale* attacked by the rust pathogen *Melampsora lini*. J. Ecol. 83:199–206.

Burdon, J. J., and P. H. Thrall. 2000. Coevolution at multiple spatial scales: *Linum marginale–Melampsora lini*; from the individual to the species. Evol. Ecol. 14:261–281.

Burdon, J. J., P. H. Thrall, and A. H. D. Brown. 1999. Resistance and virulence structure in two *Linum marginale–Melampsora lini* host-pathogen metapopulations with different mating systems. Evolution 53:704–716.

Davelos, A. L., H. M. Alexander, and N. A. Slade. 1996. Ecological genetic interactions between a clonal host plant (*Spartina pectinata*) and associated rust fungi (*Puccinia seymouriana* and *Puccinia sparganioides*). Oecologia 105:205–213.

Ebert, D. 1994. Virulence and local adaptation of a horizontally transmitted parasite. Science 265:1084–1086.

Ebert, D., and W. D. Hamilton. 1996. Sex against virulence: the coevolution of parasitic diseases. Trends Ecol. Evol. 11:79–82.

Ebert, D., C. D. Zschokke, and H. J. Carius. 1998. Within- and between-population variation for resistance of *Daphnia magna* to the bacterial endoparasite *Pasteuria ramosa*. Proc. R. Soc. Lond. B 265:2127–2134.

Flor, H. H. 1955. Host-parasite interaction in flax rust: its genetics and other implications. Phytopathology 45:297–301.

Gandon, S., and P. A. Van Zandt. 1998. Local adaptation and host-parasite interactions. Trends Ecol. Evol. 13:214–216.

Gandon, S., Y. Capowiez, Y. Dubois, Y. Michalakis, and I. Olivieri. 1996. Local adaptation and gene-for-gene coevolution in a metapopulation model. Proc. R. Soc. Lond. B 263:1003–1009.

Gandon, S., D. Ebert, I. Olivieri, and Y. Michalakis. 1998. Differential adaptation in spatially heterogeneous environments and host-parasite coevolution. Pp. 325–342 *in* S. Mopper and S. Y. Strauss, eds. Genetic structure and local adaptation in natural insect populations. Chapman and Hall, New York.

Hamilton, W. D. 1980. Sex versus non-sex versus parasite. Oikos 35:282–290.

Hamilton, W. D., R. Axelrod, and R. Tanese. 1990. Sexual reproduction as an adaptation to resist parasites (A Review). Proc. Natl. Acad. Sci. USA 87:3566–3573.

Hutcheson, K. 1970. A test for comparing diversities based on the Shannon formula. J. Theor. Biol. 29:151–154.

Imhoof, B., and P. Schmid-Hempel. 1998. Patterns of local adaptation of a protozoan parasite to its bumblebee host. Oikos 82: 59–65.

Jarosz, A. M., and J. J. Burdon. 1991. Host-pathogen interactions in natural populations of *Linum marginale* and *Melampsora lini*. II. Local and regional variation in patterns of resistance and racial structure. Evolution 45:1618–1627.

———. 1992. Host-pathogen interactions in natural populations of *Linum marginale* and *Melampsora lini*. III. Influence of pathogen epidemics on host survivorship and flower production. Oecologia 89:53–61.

Kaltz, O., and J. A. Shykoff. 1998. Local adaptation in host-parasite systems. Heredity 81:361–370.

Kaltz, O., S. Gandon, Y. Michalakis, and J. A. Shykoff. 1999. Local maladaptation in the anther-smut fungus *Microbotryum violaceum* to its host plant *Silene latifolia*: evidence from a cross-inoculation experiment. Evolution 53:395–407.
Karban, R. 1989. Fine-scale adaptation of herbivorous thrips to individual host plants. Nature 340:60–61.
Ladle, R. J. 1992. Parasites and sex: catching the Red Queen. Trends Ecol. Evol. 7:405–408.
Lawrence, G. J., and J. J. Burdon. 1989. Flax rust from *Linum marginale*: variation in a natural host-pathogen interaction. Can. J. Bot. 67:3192–3198.
Lively, C. M. 1989. Adaptation by a parasitic trematode to local populations of its snail host. Evolution 43:1663–1671.
———. 1996. Host-parasite coevolution and sex. Bioscience 46: 107–114.
———. 1999. Migration, virulence and the geographic mosaic of adaptation by parasites. Am. Nat. 153:S34–S47.
Lively, C. M., and J. Jokela. 1996. Clinal variation for local adaptation in a host-parasite interaction. Proc. R. Soc. Lond. B 263:891–897.
Mills, K. E., and J. D. Bever. 1998. Maintenance of diversity within plant communities: soil pathogens as agents of negative feedback. Ecology 79:1595–1601.
Morand, S., S. D. Manning, and M. E. J. Woolhouse. 1996. Parasite-host coevolution and geographic patterns of parasite infectivity and host susceptibility. Proc. R. Soc. Lond. B 263:119–128.
Mutikainen, P., V. Salonen, S. Puustinen, and T. Koskela. 2000. Local adaptation, resistance, and virulence in a hemiparasitic plant-host plant interaction. Evolution 54:433–440.
Parker, M. 1985. Local population differentiation for compatibility in an annual legume and its host-specific fungal pathogen. Evolution 39:713–723.
SAS Institute. 1989. SAS/STAT user's guide. Ver. 6. 4th ed. SAS Institute, Cary, NC.
Sokal, R. R. and F. J. Rohlf. 1995. Biometry. 3rd ed. W. H. Freeman, New York.
Thompson, J. N. 1994. The coevolutionary process. Univ. of Chicago Press, Chicago, IL.
———. 1999. Specific hypotheses on the geographic mosaic of coevolution. Am. Nat. 153:S1–S14.
Thrall, P. H., and J. J. Burdon. 1997. Host-pathogen dynamics in a metapopulation context: the ecological and evolutionary consequences of being spatial. J. Ecol. 85:743–753.
———. 2000. Effect of resistance variation in a natural plant host-pathogen metapopulation on disease dynamics. Plant Pathol. 49: 1–8.
———. 2002. The evolution of gene-for-gene interactions: consequences of variation in host and pathogen dispersal. Plant Pathol. 51:169–184.
Thrall, P. H., J. J. Burdon, and A. G. Young. 2001. Variation in resistance and virulence among demes of a single host-pathogen metapopulation. J. Ecol. 89:736–748.

Corresponding Editor: P. Jarne