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Evolution of host resistance: looking for coevolutionary hotspots at small spatial scales

Anna-Liisa Laine*

Metapopulation Research Group, Department of Biological and Environmental Sciences, University of Helsinki, PO Box 65 (Viikinkaari 1), 00014 Helsinki, Finland

Natural plant populations are often found to be extremely diverse in their resistance to pathogens. While the potential of pathogens in driving the evolution of resistance in hosts has been widely recognized, empirical evidence linking disease dynamics to host population genetic structure has remained scarce. Here I show that current coevolutionary selection for resistance can be divergent even on a very fine spatial scale. In a natural plant–pathogen metapopulation, disease occurrence patterns were highly aggregated over space and time within host populations. A laboratory inoculation experiment showed higher resistance within areas of the host populations where encounter rates with the pathogen have been high. Higher resistance to sympatric than to allopatric strains of the pathogen suggests that this change has taken place as a response to local selection. These results constitute evidence of adaptive microevolution of resistance resulting from disease epidemics in natural plant–pathogen associations, and highlight the importance of finding the relevant scale at which to address questions of current coevolutionary selection.

Keywords: coevolution; evolution of resistance; plant–pathogen interaction; *Plantago lanceolata*; spatial scale

1. INTRODUCTION

Host–parasite coevolution is potentially of great importance in producing and maintaining biological diversity. In natural plant–pathogen interactions, pathogens are widely recognized as potent forces influencing the diversity and distribution of resistance in plants (Dinoor & Eshed 1984; Burdon & Thompson 1995; Brown 2003). The benefits of genetic variation (polymorphism) of resistance in the host are obvious as it reduces the probability that a parasite can infect the next susceptible host encountered (Brown 2003), but actual genetic consequences of encounters with pathogens remain poorly documented (Thrall & Burdon 2003).

With the rising interest in coevolutionary dynamics using plant–pathogen interactions as model systems, it has become apparent that natural pathosystems contain overwhelming amounts of diversity in host resistance, both within and among host populations (e.g. Dinoor 1970; de Nooij & van Damme 1988; Parker 1988; Burdon & Jarosz 1991; Bevan *et al.* 1993; Antonovics *et al.* 1994; Thrall *et al.* 2001; Laine 2004). However, empirical data demonstrating adaptive changes in host resistance structure with respect to disease dynamics are scarce. Hence, it has been suggested that non-adaptive evolution, consisting of founder effects, random genetic drift and selection on correlated traits, is driving the resistance structure of plant populations (Parker 1991; Burdon & Thompson 1995; Laine 2004).

Studies of host–pathogen coevolution may be complicated by variation in selection intensity resulting from temporally and spatially variable disease encounter rates. The geographic mosaic theory of coevolution depicts geographical differences in selection intensity as an inherent part of the coevolutionary processes. Variation between habitats in the trajectories of natural selection, the occurrence of reciprocal selection in only some of the communities (coevolutionary hotspots), intermixed with sites with little or no coevolutionary activity (coldspots), produce a selection mosaic that continually changes through time (Thompson 1994, 1999). Geographical variation in selection trajectories has been verified for some interspecific interactions of hosts and parasites (Kraaijeveld & Godfray 1999; Lively 1999; Thrall & Burdon 2003), but the scale on which the selection mosaic is formed will vary from one system to another and most likely also within a single system. Hence, a key challenge of evolutionary studies is to direct research at the right spatial scale or we may miss important evolutionary signals.

Here I evaluate current coevolutionary selection on a spatial scale of a 1 m^2 quadrat within host populations of the herbaceous perennial plant, *Plantago lanceolata*, infected by the powdery mildew fungus *Podosphaera plantaginis*. The host populations are highly diverse in their resistance structure and resistance is expressed against specific strains of *P. plantaginis* (Laine 2004). Four years of survey data on within-host population disease dynamics showed that encounter rates between the host and the pathogen are highly aggregated over space and time over a very fine spatial resolution. I was able to identify areas within two host populations where selection intensity for resistance in the host has been high (coevolutionary hotspots) and areas where there has been little or no pressure for the host to evolve resistance (coevolutionary coldspots). Hosts were sampled from

*anna-liisa.laine@helsinki.fi

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these different areas and their resistance profile was scored against sympatric strains of *P. plantaginis* in a laboratory inoculation experiment. To test whether host resistance structure has evolved in response to local selection, hosts were also scored against allopatric strains of *P. plantaginis*. The specific aim of this study was to establish a link between within-population disease dynamics and the diversity and distribution of resistance phenotypes.

2. MATERIAL AND METHODS

(a) *The host–pathogen association*

Plantago lanceolata is a monoecious perennial herb that forms a rosette. The pollen of *P. lanceolata* is wind-dispersed mainly over relatively short distances (*ca* 1.5 m; Tonsor 1985; Bos *et al.* 1986) and it is an obligate outcrosser due to self-incompatibility induced by protogyny. Seeds are typically simply dropped to the ground, close to the mother plant (0.42 m; van Damme 1986) and they germinate the following summer or remain in the soil seedbank (Cavers *et al.* 1980). Clonal reproduction takes place via the production of side-rosettes (Cavers *et al.* 1980) and the incidence of side-rosettes is considered to be quite high (Mook *et al.* 1992). Field longevity estimates for *P. lanceolata* are highly variable, individuals may be more than 5 years old or there may be 99% mortality of a cohort in the first year (Roach 2003; Roach & Gampe 2004). The fungal pathogen, *P. plantaginis* (Erysiphales) is a powdery mildew that grows on the surface of the plant with only feeding roots, haustoria, inside the plant cells. It is an obligate pathogen requiring living host tissue throughout its life cycle. Powdery mildews exploit their host's nutrient supplies to the extent that infection may severely reduce the growth and progeny of infected individuals (Bushnell 2002). When infection coincides with other stressful environmental conditions, such as drought, it may even induce host mortality (Laine 2004).

The study system is located in the Åland Islands in southwest Finland, where the distribution of *P. lanceolata* populations is highly fragmented, with discrete local populations being separated by an unsuitable matrix consisting mainly of arable land, forests and roads (Nieminen *et al.* 2004). The system is surveyed annually for the occurrence of the powdery mildew in the context of metapopulation studies of the Glanville fritillary butterfly (e.g. Hanski 1999). During the growing season, clonally produced generations of the pathogen follow one another in quick succession of approximately 10 days, leading to local epidemics via aerially dispersed spores. In the Åland Islands, the phenology of the host results in distinct crashes in pathogen numbers as the majority of the plants die back to underground rootstocks at the end of the summer. The primary foci may be initiated from overwintering mycelial growth, resting spores or from a spore colonizing the population from outside. The incidence of *P. plantaginis* in this system is quite low at any given time, with less than 5% of the host populations being infected. The annual extinction rate of the pathogen is high, but this is roughly balanced out by frequent (re)colonizations (Laine 2004, 2005).

(b) *Field surveys of infection*

Originally, within-population surveys of disease dynamics were initiated to gather data to parameterize a model of local pathogen epidemics (Ovaskainen & Laine in press). Within-host population spread of the pathogen was studied in three host populations (875, 876 and 877) for 4 subsequent years (2000–2003) with a spatial resolution of a 1 m^2 quadrat that covered the entire host population. The number of infected individuals within each quadrat was first recorded in July when the epidemic starts to pick up, and again in September when the spread of the disease has ceased.

(c) *Sampling of host and pathogen lines*

I used the information on the dynamics of the pathogen within host populations to identify areas where potential selection pressure for resistance has been high among the areas where encounter rates have been high during the survey period. I similarly selected areas where the pathogen rarely or never occurred during the survey period. As the pathogen population 875 went extinct during the survey period, only populations 876 and 877 were included in this study. Hosts were sampled in August 2003 as seeds from the two populations according to the scheme represented in figure 1. The seeds were stored in dry conditions for three months to break dormancy and then grown to adults in greenhouse conditions with 16 h of light and a temperature of $+22\,^{\circ}C$. Of the maternal lines producing seedlings, one line was chosen to represent each 1 m^2 quadrat, except in population 877 where two hotspot quadrats are represented by two maternal lines, because there were flowering individuals in only 18 of the hotspot quadrats. Hence, altogether 40 maternal lines represent areas within the populations with a high disease prevalence in the past (hotspots) and 40 maternal line areas where the pathogen had rarely or never occurred during the period of the study (coldspots). Each genotype was cloned into a daughter rosette according to the method described by van der Toorn & ten Hove (1982). The leaves used in the experiment were of the same age, three month old ramets.

In July 2004, bulk samples of fungal material were collected as infected leaves from the sympatric pathogen populations 876 and 877, and from allopatric populations 1537, 7027 and 3269 located 10 km or further away. The infected leaves were placed on moist filter paper in Petri dishes. In the laboratory, mildew was propagated by brushing spores of the infected leaves onto detached healthy leaves. The plants used for the purification procedure were determined to be highly susceptible genotypes by earlier inoculation trials (Laine 2004). Inoculated leaves were kept in a growth chamber at $20\pm2\,^{\circ}C$ with a 16L/8D photoperiod. Single, discrete fungal colonies growing on the inoculated leaves were isolated and again spores were brushed onto healthy leaves. A widely accepted assumption in comparable studies is that isolates from single colonies are the product of one haploid, uninucleate spore (conidium), and hence are genetically homogenous (Nicot *et al.* 2002). To reduce the chance of obtaining mixed isolates, three successive inoculations from discrete colonies were carried out (cf. Persaud & Lipps 1995). Distinct and presumably genetically differentiated lines were chosen for the experiment based on their ability to infect the plants used for the purification procedure. In the experiment, four sympatric strains represented population 876 and four sympatric strains represented population 877, the three allopatric populations were each represented by two pathogen strains. Once the strains had been purified, repeated cycles of inoculations were performed to obtain adequate stocks of sporulating fungal material for the inoculation trials.

(d) *Identification of resistance types*

The hosts from populations 876 and 877 were scored with their respective four sympatric and six allopatric strains of *P. plantaginis*. A detached leaf from each host plant was exposed to a single pathogen strain. The leaf was placed in a 9 cm Petri dish on moist filter paper, and spores from an infected leaf were gently brushed with a fine paintbrush over the healthy leaves over the entire leaf surface (Nicot *et al.* 2002). Colonies of similar age and size were used for the inoculations in order to obtain as similar spore densities as possible. Inoculated dishes were placed in a growth chamber at 20 ± 2 °C with a 16L/8D photoperiod. Dishes were checked daily and the filter paper moistened with water when necessary. The infection status of each leaf was scored 10 days after the inoculation using a microscope. Individuals were scored as susceptible when there was mycelial growth and conidia on the detached leaf and no severe chlorosis. A chlorotic response, when there was no or very little mycelial growth, was scored as a resistant response.

(e) *Statistical analyses*

The difference in the number of resistance phenotypes in the hotspots and coldspots was analysed with a generalized linear model assuming a Poisson distribution of errors and a log link function (PROC GENMOD of SAS v. 8.02; Littell *et al.* 1996). The infection response of the host plant (resistant/susceptible) was analysed as a generalized linear mixed model with a binomial error distribution and a logit link function (GLIMMIX macro of SAS v. 8.02; Littell *et al.* 1996). The response of the host individual to the 10 pathogen strains (four sympatric and six allopatric strains) was treated as a repeated measures type of response in the model, and the individual was set as a random factor, nested within the population according to its source of collection. The 10 pathogen strains were defined as random effects in the model. Type of area within the host population (hotspot versus coldspot), the source of the pathogen strains (sympatric versus allopatric), and host population were all fixed effects in the model. Interactions 'pathogen strain'×'hotspot versus coldspot' and 'host population'×'allopatric versus sympatric' were included in the model.

As the disease occurrence patterns were highly aggregated within the host populations, hosts from the hotspot and coldspot areas had to be sampled often from proximate quadrats. In order to test whether the sample consisted of highly related individuals, a pairwise similarity of the hosts was calculated based on their resistance–susceptibility profile. For example, the similarity index of individuals '0000111011' and '0001101011' is 0.8, where '0' refers to a susceptible and '1' a resistant response to the four sympatric and six allopatric pathogen strains, respectively. The possible association between pairwise similarity indices of the hosts and their distances (m) was assessed using a Mantel test (Mantel 1967) as implemented in MANTEL for WINDOWS with 10 000 random permutations.

3. RESULTS

The disease occurrence patterns were highly aggregated over space and time within the two host populations (figure 1). The average infection prevalence for each 1 m^2 quadrat was first calculated as an average of the proportion of individuals infected in July and September in order to emphasize the duration of infection within season, and then averaged over the 4 years. In both populations, a high proportion of the host plants had never been infected (41 and 28% of the quadrats in 876 and 877, respectively), while average infection prevalence exceeded 20% in 6% of the quadrats (figure 1). A detailed analysis of the within-population disease dynamics is reported elsewhere (Ovaskainen & Laine in press).

Each host plant was assigned a resistance phenotype according to its response to the 10 pathogen strains. Resistance was expressed against specific strains of the pathogen, and individuals varied from being resistant to all strains tested to being resistant to only one of the strains. The frequency of resistance phenotypes did not differ between hotspot and coldspot areas of the host populations (19 versus 19), nor between the populations (39 versus 37; $\chi^2_1 = 0.05$, $p = 0.819$). No strong association emerged between the similarity of the hosts with respect to their resistance–susceptibility profile and distance (population 786: $r = -0.005$, $p = 0.375$, population 877: $r = -0.016$, $p = 0.348$; figure 2). In figure 2, the pairwise similarities among hosts from hotspots, among hosts from coldspots as well as among hosts from hotspots and coldspots are plotted as a function of distance. There is no evidence for hosts from hotspots or coldspots being more related than the hosts are in general within the populations. This result suggests that hosts representing the hotspot and coldspot areas of the two populations do form independent data units rather than a sample of closely related individuals.

The proportion of resistant responses was higher in hosts from the hotspot areas than in hosts from the coldspot areas in both populations (figure 3; table 1). In population 876, the average proportions of resistant responses were 54 versus 46% and in population 877, 54 versus 39% in hotspots and coldspots, respectively. This difference is statistically significant (table 1). In population 876, hosts from hotspots were more resistant than hosts from coldspots to three of the four sympatric pathogen strains and to all six allopatric pathogen strains. In population 877, hosts from hotspots were more resistant than hosts from coldspots to all sympatric pathogen strains and to five of the six allopatric pathogen strains. In the GLM model, the interactions term 'hotspot/coldspot× pathogen strain' was marginally significant ($p = 0.059$, table 1).

There was a tendency for resistance to be higher to the sympatric than to the allopatric strains of *P. plantaginis* (table 1: $p = 0.068$; figure 3). The difference was especially pronounced in population 876, where hosts were on average 22% more resistant to the sympatric pathogen strains (table 1: host population×allopatric/sympatric $F_{1,698} = 6.97$, $p = 0.009$; figure 3). In population 877, hosts from hotspots were 7% more resistant to the sympatric than to the allopatric strains, but hosts from the coldspots were 3% more resistant to the allopatric pathogen strains (figure 3).

4. DISCUSSION

The present results reveal a selection mosaic in a natural plant–pathogen interaction over a very fine spatial scale. Encounter rates between the host and the pathogen are highly divergent at a scale of some few metres due to a highly predictable seasonal spread of the local epidemic.

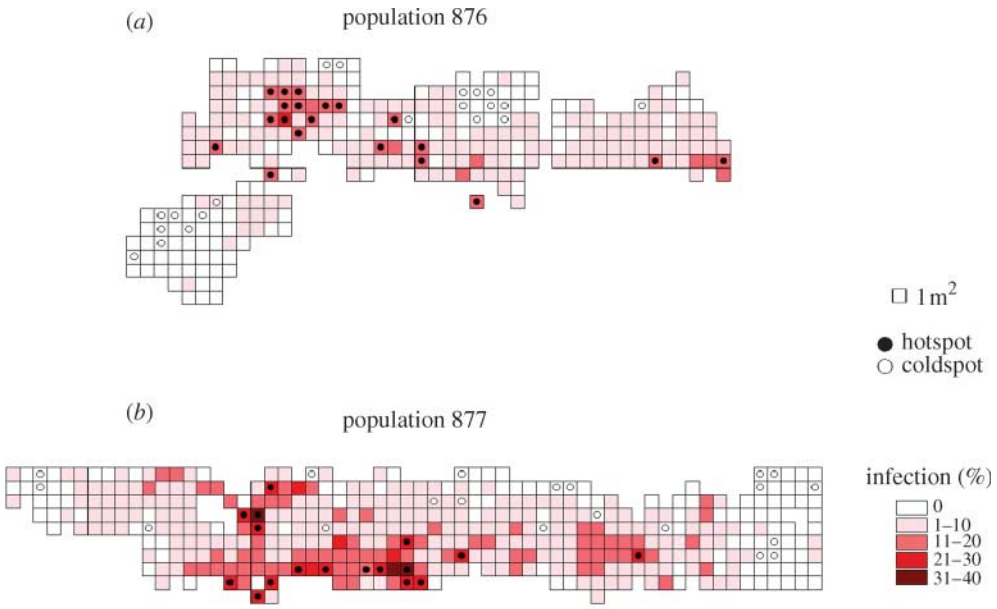

Figure 1. The average prevalence of the infection in populations (*a*) 876 and (*b*) 877 for years 2000–2003. The average infection was first calculated for one season as an average of the proportion of infected individuals in July and September, and then averaged over the 4 years. Hosts were sampled from 'hotspots' and 'coldspots' as seeds in August 2003.

Table 1. Differences in host resistance analysed with a generalized linear mixed model. (Wald's *Z*-statistic is given for random effects and the *F*-statistic for fixed effects.)

$\text{source}_{\text{ndf,ddf}}$	variance estimate for random effects	*Z* or *F*	*p*
host individual	0.183 ± 0.04	4.21	<0.0001
pathogen strain	0.032 ± 0.1	0.32	0.375
residual	0.849 ± 0.05	18.68	<0.0001
$\text{hotspot versus coldspot}_{1,77}$		4.49	0.037
$\text{allopatric versus sympatric}_{1,698}$		3.34	0.068
$\text{host population}_{1,77}$		1.93	0.169
$\text{host population} \times \text{allopatric/sympatric}_{1,698}$		6.97	0.009
$\text{hotspot/coldspot} \times \text{pathogen strain}_{18,698}$		1.58	0.059

The laboratory experiment showed that resistance of *P. lanceolata* to *P. plantaginis* was higher in areas within the host populations, where disease encounter rates have been systematically high than in areas where they have been low. This highlights the importance of disease dynamics in driving the diversity and distribution of host resistance genotypes. While the potential importance of this link has been widely recognized (Brown 2003; Thrall & Burdon 2003), this is the first evidence suggesting adaptive microevolution of host resistance following disease epidemics in natural plant–pathogen interactions.

Higher resistance against 90% of the tested pathogen strains in parts of the host population, where encounter rates with the pathogen have been high, is plausible evidence of microevolution. The idea of local selection driving the host population resistance structure was further supported by the fact that resistance was higher to sympatric than to allopatric strains of *P. plantaginis*. This difference was particularly pronounced in population 876. As the host plants were grown from seeds to adults under identical greenhouse conditions and daughter clones of these plants were used in the inoculation experiment, any environmental or maternal effects are highly unlikely. Therefore, a high level of genetic variability is the most likely explanation of the observed variation in resistance among the plants. There was a very weak negative association between the pairwise similarity of resistance profiles and distances separating the hosts. These results do not suggest that hosts sampled from hotspot and coldspot quadrats were more related than plants in general within these populations. Hence, the observed resistance patterns are not simply due to relatedness of individuals in closely adjacent quadrats. While possible chance associations or responses to other covarying environmental selection gradients between the spatially structured average host resistance and infections rates cannot be entirely discarded, the most likely explanation for the present results is disease-driven selection.

Using a spatially explicit model with Bayesian parameter estimation it was determined that the spatially aggregated disease dynamics result from two key processes: initial foci from which the epidemic begins occur in the same areas of the host populations over time and the spread during the growing season was mainly over short distances (Ovaskainen & Laine in press). The fine-scale selection mosaic may have formed through an interaction with the physical environment. The study period coincided with two years of severe drought when precipitation in August 2002 was 96% below average and in July 2003 was 71% below average. This may have

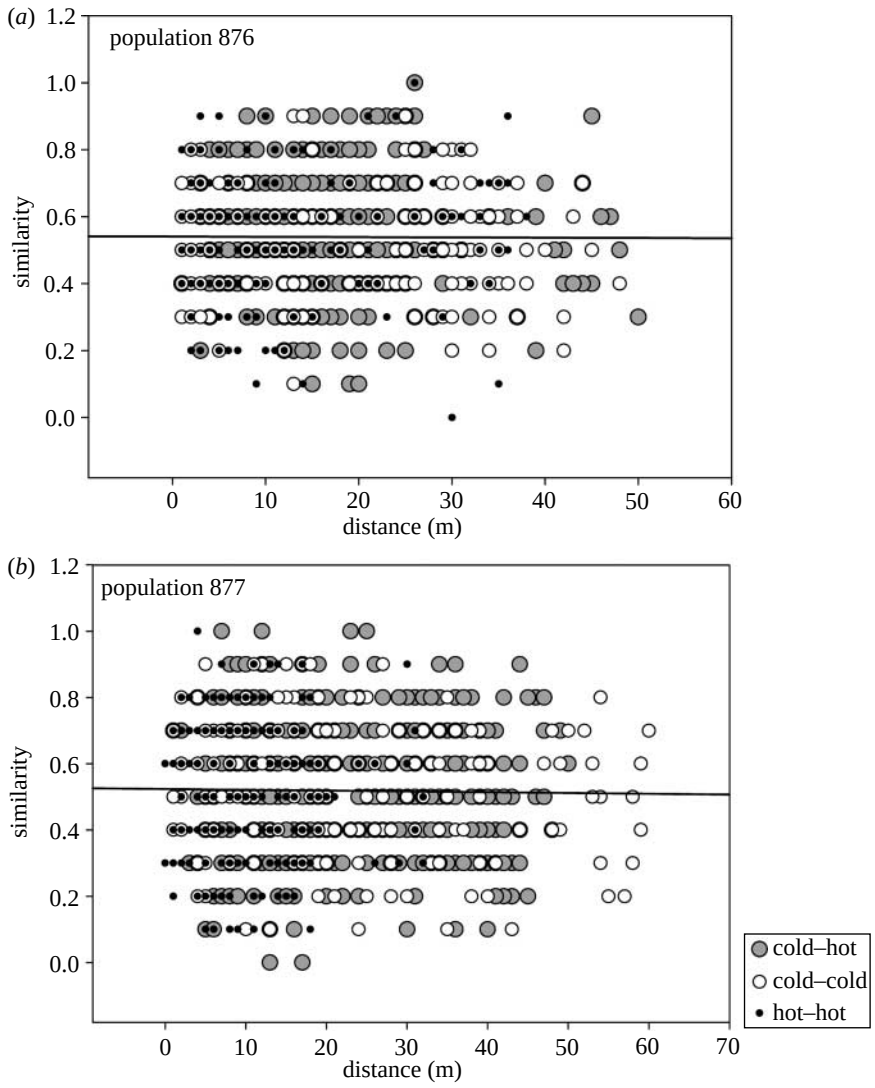

Figure 2. The pairwise similarities of resistance profiles of *P. lanceolata* individuals from populations (*a*) 876 and (*b*) 877 as a function of distance. A similarity index of '1' would indicate a comparison between plants that responded identically to all pathogen strains. Grey circles depict comparisons among hosts from hotspot and coldspot areas, white circles depict comparisons among hosts from coldspots and black circles depict comparisons among hosts from hotspots.

intensified natural selection, for while powdery mildews are widely recognized as stress factors reducing both growth and yields, infection alone does not necessarily induce host mortality. However, in these populations host density declined steeply during the study period and the decline was steepest in areas of the host population where infection was most prevalent (GLMM: $F_{1,348} = 11.85$, $p = 0.0006$; Laine 2004). More susceptible individuals are vulnerable to a larger number of local pathogen strains and they are presumably more heavily infected than more resistant individuals. It is likely that these more heavily infected individuals were more stressed at the end of the growing season, failing to store adequate nutrient supplies necessary for re-establishment in the following growing season. The higher mortality of susceptible individuals in areas where pathogen has been prevalent may have caused a local shift towards higher pathogen resistance. The decline in host density in the summers of low rainfall was accompanied with diminished recruitment possibilities, as seedlings are especially vulnerable to drought. Limited gene flow within as well as among populations may have further promoted local spatial structuring of the host populations. The recent shift

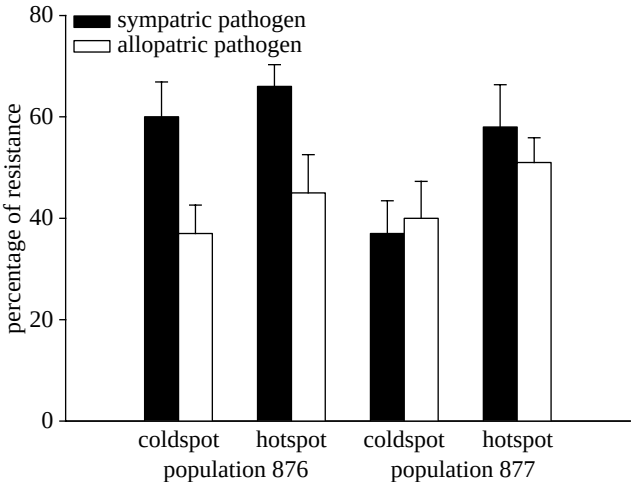

Figure 3. The average proportion of resistant responses of the host plant *P. lanceolata* against sympatric and allopatric strains of *P. plantaginis*. The host plants were sampled from two populations from areas where encounter rates with *P. plantaginis* had been low in the past (coldspots) and high in the past (hotspots). The error bars represent standard errors of means.

towards higher resistance is also supported by the lower average level of resistance detected in population 877 in a study conducted several years earlier (Laine 2004).

Although the exceptional weather conditions may have resulted in unusually strong selection for resistance, it is plausible that aggregated encounter rates between hosts and pathogens within host populations may be a common feature of natural plant–pathogen interactions. While detailed data on local disease occurrence patterns are scarce, the aggregated spatial pattern of infection could arise through an interaction between microclimate and pathogen development rates leading to marked differences in selection pressures over very short distances (Burdon & Thompson 1995). Many plant pathogenic fungi are highly sensitive to microclimatic conditions, possessing threshold values for temperature and humidity required for germination and spore production. Both temperature and moisture may vary over small spatial scales and translate into the disease process through the sensitivity of the organism involved (Jarosz & Davelos 1995; Truscott & Gilligan 2003), producing heterogeneous selection pressures over small spatial scales.

As self-fertilization and clonal reproduction are characteristic of many plant species, non-adaptive evolutionary change in resistance may be quite common, as previously suggested (Parker 1991; Burdon & Thompson 1995; Laine 2004). While these results do not allow the estimation of the relative strength of non-adaptive evolution and natural selection on host resistance, this study demonstrates that the strength of selection may be highly variable over very fine spatial scales as well as temporally variable through an interaction with the physical environment. The occurrence of local coevolutionary hotspots and coldspots in host–pathogen interactions is likely to promote the heterogeneity of host resistance that is evident in many natural plant populations (e.g. Dinoor 1970; de Nooij & van Damme 1988; Parker 1988; Bevan *et al.* 1993; Antonovics *et al.* 1994; Thrall *et al.* 2001; Laine 2004). In turn, this heterogeneity will have an impact on fundamental aspects of host–pathogen interactions such as the transmission of local epidemics and the evolution of virulence (Browning & Frey 1969; Wolfe 1985; DiLeone & Mundt 1994; Zhu *et al.* 2000; Thrall *et al.* 2001; Thrall & Burdon 2003). The results presented here highlight the importance of spatial and temporal data on disease occurrence when addressing questions of current coevolutionary selection.

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As this paper exceeds the maximum length normally permitted, the author has agreed to contribute to production costs.