

# Resistance variation within and among host populations in a plant–pathogen metapopulation: implications for regional pathogen dynamics

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## Summary

**1** Studies conducted on natural host–pathogen metapopulations have revealed considerable diversity of host resistance phenotypes within populations. The resistance structure of non-infected populations has, however, been largely ignored and the role of among-population variation in resistance profiles in the dynamics of natural pathogen populations is poorly understood.

**2** The *Plantago lanceolata*–*Podosphaera plantaginis* pathosystem in the Åland Islands in south-west Finland is characterized by the highly fragmented distribution of the host. Only a small fraction of the host populations is infected at one point in time and pathogen turnover rate is high. I studied a sample of these populations to find out whether adjacent host populations are differentiated in their resistance structure and whether variable levels of disease resistance among host populations could be linked to disease incidence patterns.

**3** Results show striking differences in the resistance structure both within and among host populations. Sixteen resistance phenotypes were identified in a sample of 64 host individuals. Populations varied from one in which all sampled individuals represented a different resistance phenotype, to one in which half showed identical resistance responses.

**4** There was no association between local resistance composition and the geographical distance between populations, suggesting that within-population processes, such as founder effects and genetic drift, largely determine local resistance structure.

**5** Non-infected populations showed significantly higher mean levels of resistance than infected populations, suggesting that differences in the mean level of resistance among host populations may retard the spread of the fungus and thereby decrease the probability of regional epidemics.

*Key-words:* disease resistance, host–pathogen interactions, metapopulation, plant epidemiology, *Plantago lanceolata*, spatial pattern

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## Introduction

Increasing evidence suggests that natural host–pathogen systems support heterogeneity in the host's resistance structure (de Nooij & van Damme 1988; Burdon & Jarosz 1991; Bevan *et al.* 1993; Antonovics *et al.* 1994; Burdon *et al.* 1999; Ericson *et al.* 2002), but the number of studies conducted on natural pathosystems consisting of several host populations remains limited. To date, almost nothing is known about how patterns of

disease resistance link to variation in the incidence and severity of natural disease epidemics (Thrall & Burdon 2000). It is possible that a highly diverse resistance structure may at least partly explain why damaging regional epidemics are so rare in natural systems (Dinor & Eshed 1984; Bevan *et al.* 1993), while they are common in agricultural pathosystems, in which the hosts tend to be genetically more uniform (Browning & Frey 1969; Wolfe 1985; Zhu *et al.* 2000).

At some spatial scale, most plant species have a patchy distribution due to their sedentary lifestyle and environmental heterogeneity (Silvertown & Lovett Doust 1993). The evolutionary interplay between plants

and their pathogens takes place in this fragmented spatial context, with heterogeneity in both biotic and abiotic factors. While the importance of heterogeneous environments on evolutionary dynamics has been recognized for a long time (Wright 1943, 1982), it was Thompson's theory of the geographical mosaic of coevolution that more recently tied together processes operating over space and time, determining the outcome of coevolutionary interactions (Thompson 1994, 1999).

The notion of coevolution as a geographical mosaic involves (i) variation in the intensity and direction of selection processes among populations (selection mosaic); (ii) coevolutionary 'hotspots', which are the subset of communities in which reciprocal selection actually takes place, intermixed with sites with less activity, the so-called 'coldspots'; and (iii) continual geographical mixing of traits, resulting from the selection mosaic, coevolutionary hotspots/coldspots, gene flow, random genetic drift, and population turnover (Thompson 2001). Gene flow between coevolutionary hotspots and coldspots, and between communities in which different coevolved traits are favoured, may swamp local selection (Thompson 1999). As a result, the geographical mosaic of coevolution may routinely produce local and transient mixtures of apparently maladaptive traits. The interplay between these processes, operating at different scales, may produce a variety of outcomes affecting both the resistance and the virulence structure of hosts and their pathogens, respectively.

Distribution and dynamics of natural pathogen populations are often characterized by patchiness and asynchrony of neighbouring populations. Previous studies have established the importance of spatial configuration of the host populations for the occurrence of pathogens (e.g. Burdon *et al.* 1995; Thrall & Burdon 1997; Burdon & Thrall 1999; Ericson *et al.* 1999), yet the 'evolutionary quality' of host populations remains poorly understood in this context, although several studies stress the importance of linking evolutionary and spatial dynamics in host-pathogen associations (e.g. Antonovics *et al.* 1994; Thrall & Burdon 1997; Burdon & Thrall 1999; Carlsson-Granér & Thrall 2002). Natural host populations show differentiation in their resistance structure at several scales: within a single host population (de Nooij & van Damme 1988; Parker 1988; Bevan *et al.* 1993), among host populations (Dinoor 1970; Thrall *et al.* 2001), and among metapopulations (Burdon *et al.* 1999). How the complex resistance-susceptibility structure of populations affects the dynamics of pathogens remains largely unknown. In particular, only a few studies have addressed the resistance structure of non-infected host populations within host-pathogen metapopulations (Thrall & Antonovics 1995; Carlsson-Granér 1997).

In agricultural systems it has become evident that increasing resistance diversity within populations leads to reduced pathogen prevalence (Browning & Frey 1969; Wolfe 1985; DiLeone & Mundt 1994; Zhu *et al.* 2000). Most of what is known about natural systems

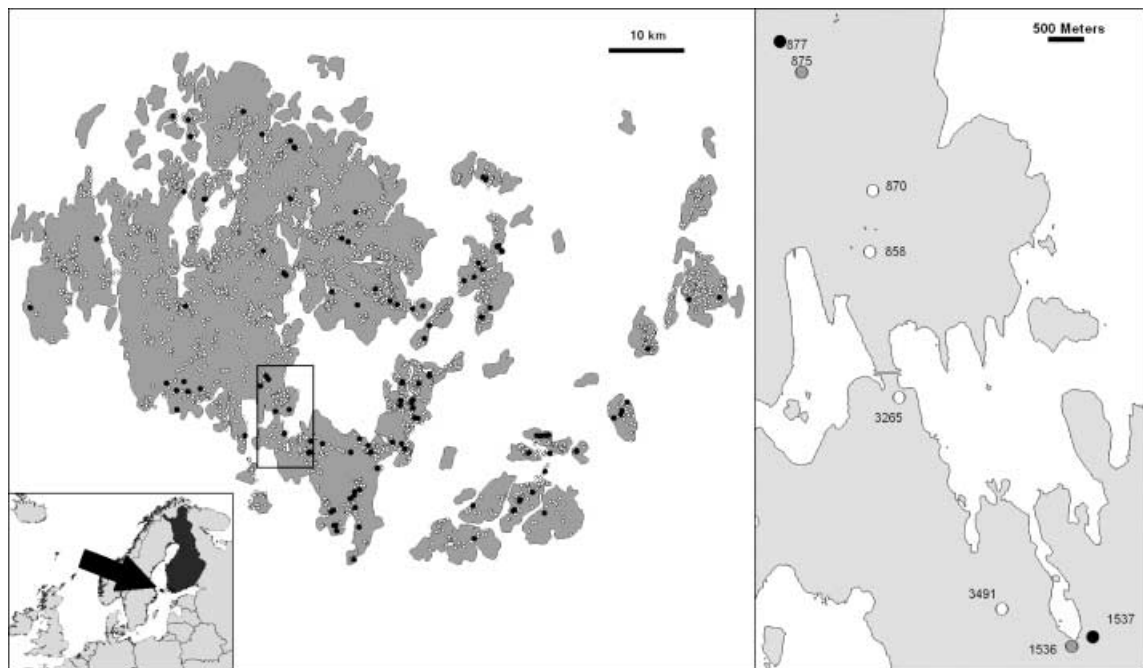
comes from studies that have taken an experimental approach. Using a series of experimental host populations with dissimilar genetic compositions, several studies have shown that disease levels are higher in less diverse host stands (Thrall & Jarosz 1994; Schmid 1994) and that disease declines in resistant populations while the host and the pathogen seem to coexist in susceptible populations (Alexander *et al.* 1996). Thrall *et al.* (2001) provided the first evidence of the importance of host resistance on pathogen dynamics in natural systems in their study of *Linum marginale* infected by *Melampsora lini*. They demonstrated a negative relationship between resistance diversity in host populations and disease prevalence in the following year. Carlsson-Granér & Thrall (2002) showed that host resistance varied as a function of population connectedness, which could explain why disease prevalence decreased in the more continuous host populations. Antonovics (2004) proposed that an increased level of disease resistance in the metapopulation as a whole could partly explain why disease colonization rate has declined in the *Silene-Microbotryum* pathosystem in SW Virginia, USA.

In this study, I address the question of whether variable levels of disease resistance among host populations can be linked to disease incidence patterns in a natural pathosystem. The *Plantago lanceolata*-*Podospaera plantaginis* system in the Åland Islands in SW Finland is particularly well suited to examine this question for several reasons. The large host population network has served as a model system for metapopulation dynamic studies of the Glanville fritillary butterfly for over a decade (Hanski 1999; Nieminen *et al.* 2004) and has been surveyed in greater detail than perhaps any other natural system at a comparable spatial scale (30 by 50 km). Annual surveys of this system have yielded unique data on the occurrence of the pathogen as well as on the distribution of non-infected but presumably suitable host populations. As no previous studies of the resistance structure of the host in this system have been conducted, my first goal was to identify the number and frequency of resistance phenotypes, defined based on their infection responses to a set of pathogen strains, both within and among populations. Secondly, I investigated whether neighbouring *P. lanceolata* populations were more likely to share a similar resistance phenotype composition than far-away populations. Finally, I analysed whether the mean level of resistance was related to the disease status of the host population.

## Materials and methods

### THE HOST

*Plantago lanceolata* L. (Plantaginaceae) is a herbaceous perennial whose inflorescences develop from the axils of the basal rosette leaves (Sagar & Harper 1964). Pollen is dispersed by wind, and the plant is an obligate outcrosser due to self-incompatibility induced by



**Fig. 1** Occurrence of *P. plantaginis* (black dots) in *P. lanceolata* populations in the Åland Islands in 2002. White dots depict non-infected host populations. The panel on the right shows the eight populations (four infected, four non-infected) used in the inoculation experiment, with degree of shading indicating the level of infection. The pathogen strains used in the inoculation experiment were collected from the heavily infected populations 877 and 1537.

protogyny. As the seeds ripen, they are dropped to the ground close to the mother plant (Bos 1992; van Damme 1992). *Plantago lanceolata* is capable of reproducing clonally via the production of side rosettes from the axillary meristems (Sagar & Harper 1964).

The study system in the Åland Islands comprises about 3000 local *P. lanceolata* populations. The host populations are typically small, discrete entities that occur in dry meadows ranging from dry rocky outcrops with naturally sparse vegetation to more diverse grazed meadows on deeper soils. The host populations are typically delimited by unsuitable habitat, such as roads, arable land and forest edge, which enforces a fragmented structure on the pathosystem (see Fig. 1; Hanski 1999). Even within host populations the distribution of plants is highly patchy. Host densities within populations may vary from one host individual to up to 170 hosts  $m^{-2}$  (A.-L. Laine, unpublished results). This variation is likely to reflect environmental heterogeneity as well as clonal reproduction in *P. lanceolata*.

#### THE HOST–PATHOGEN ASSOCIATION

*Podosphaera plantaginis* is an obligate powdery mildew fungus in the order Erysiphales within the Ascomycota (Yarwood 1978), which in Finland appears to be restricted to *P. lanceolata* (A.-L. Laine, unpublished data). During the summer growing season (June to August), the pathogen is first visible on living host tissue as small, localized (non-systemic) white powdery lesions. By late summer the lesions have spread, covering almost the entire leaf surface. This stage in the

pathogen's life cycle is asexual, and clonally produced conidia are dispersed by wind to the same or different plants. Some four to seven conidial generations follow one another, leading to a local epidemic. In August the sexually produced resting spores, cleistothecia, begin to appear. At the end of the growing season, the pathogen goes through a major decline as most host individuals die back to rootstock.

Powdery mildews obtain nutrients from the plant by sending haustoria (feeding organs) into the epidermal cells of the plant organs. The fungus has a prodigious ability to sporulate, providing potentially overwhelming amounts of inoculum. To achieve this, powdery mildews exploit the host's nutrient supplies, reduce photosynthesis, impair growth and reduce yields to the extent that when infection coincides with other stressful environmental conditions, it may lead to mortality of the infected host individual (Agrios 1997; Bushnell 2002). In agriculture, powdery mildews are known to cause severe damage to their hosts leading to economic losses in a wide range of hosts (Bushnell 2002).

Data are lacking on fitness effects on natural host populations due to infection by powdery mildews. To understand how infection affects host density in the *Plantago-Podosphaera* pathosystem data were collected at the population level from two infected host populations in three successive years, 2001–03. The host populations were surveyed twice during each growing season, first when the epidemic started picking up in early July and again at the end of the growing season when the spread had ceased in September. The entire populations were surveyed with the spatial resolution

of 1-m<sup>2</sup> quadrats, within which all host individuals were counted and the number of hosts with powdery mildew recorded.

#### CENSUS OF DISEASE IN NATURAL POPULATIONS

Disease prevalence in natural populations of *P. lanceolata* in the Åland archipelago has been recorded systematically in early September of each year since 2000. At this time the fungus is most visible, with entire plants being covered by white mycelia supporting the conspicuous black cleistothecia. The network of meadows containing the 3000 populations is surveyed by 40 students who record the prevalence of *P. plantaginis* on a qualitative scale within host populations (1 = a small proportion of the host population infected, 2 = disease has spread to cover local areas of the host population, 3 = the majority of host individuals are infected; Nieminen *et al.* 2004). The students collect a leaf sample from each infected site for subsequent microscopic identification.

#### THE STUDY POPULATIONS

Eight *P. lanceolata* populations were chosen for this study. These populations are located on the southern part of the main Åland island, where *P. lanceolata* meadows are abundant. The populations are similar in size (average size = 0.21 ha, range 0.15–0.28 ha), and occur along 10 km of roadside, thus constituting a roughly linear array (Fig. 1). During the period 2000–02 the populations at either end have been heavily infected by *P. plantaginis*, with up to 80% of the host individuals supporting infection. The populations situated closest to these have also been infected, albeit at a lower level, with only 30–40% of the hosts having been infected by late summer in 2000–02. The four populations that are situated in the middle of the array have all remained non-infected throughout the study period (Fig. 1). Four pathogen isolates used in this study were obtained from the two heavily infected marginal populations, two isolates from each. This choice was motivated by the fact that these populations represent likely sources of colonists into the six other populations due to their proximity and abundant spore production.

#### COLLECTION OF HOST POPULATIONS AND CLONING OF MATERNAL LINES

Seeds were collected from 12 individuals in each of the eight host populations in August 2001 and placed into paper envelopes. The seeds were stored in dry conditions over winter to break dormancy. Before the seeds were germinated, they were surface sterilized in 30% NaClO for 20 minutes, then washed twice with sterile water. Of the maternal lines producing seedlings, eight from each population were chosen randomly for the experiment so that each maternal line was represented

by only one individual. Each genotype was cloned into four rosettes according to the method described by van der Toorn & ten Hove (1982). Twelve-week-old mother plants growing in pots with a perforated bottom were placed in pots with vermiculite. After 9 weeks the mother plant was cut from the roots it had developed in the pot of vermiculite. Roots in the vermiculite yielded new rosettes, which were planted into pots with sand-rich humus. The plants were grown under glasshouse conditions, with 16 hours of light and a temperature of +22 °C.

#### COLLECTION, PURIFICATION AND PROPAGATION OF PATHOGEN ISOLATES

Bulk samples of fungal material were collected in August 2002 as infected leaves from populations 877 and 1537 (Fig. 1). The leaves were placed on moist filter paper in a Petri dish. The mildew was propagated in the laboratory in a settling tower (height = 50 cm, diameter = 16 cm) by blowing the infected leaves over detached leaves obtained from individuals in the same populations, derived from seeds grown under glasshouse conditions. Inoculated leaves were kept in a growth chamber at 20 ± 2 °C with a 16L/8D photoperiod. Single, discrete fungal colonies growing on the inoculated leaves were isolated and again blown over detached 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 that they are genetically homogeneous (Nicot *et al.* 2002). To reduce the chance of obtaining mixed isolates, four successive isolations from single colonies were carried out (Persaud & Lipps 1995). Once the strains were purified, repeated cycles of inoculations were performed to obtain adequate stocks of sporulating fungal material for the inoculation trials.

#### IDENTIFICATION OF RESISTANCE PHENOTYPES

Resistance responses were scored in a cross-inoculation experiment using the four pathogen strains isolated as described above. A 5-cm leaf segment from each of the eight host plant lines from each study population was exposed to a single pathogen line. I similarly exposed one leaf from a susceptible line (as determined by previous inoculation trials) to demonstrate pathogen viability. The leaves were placed in a 14-cm Petri dish on moist filter paper, and the dish was inoculated in a settling tower, where conidia from an infected leaf were blown over the top and then left to settle for 2.5 minutes. 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. Colonies of similar age and size (diameter *c.* 1 cm) were used for the inoculations in order to obtain similar spore densities in all inoculation trials. To control for slight variation in spore density among inoculations, all inoculations were repeated

four times. Leaf material for each replicate was obtained from a naïve host clone. Infection status was scored after 10 and 14 days using a microscope. Individuals were scored as susceptible when there was mycelial growth and conidia on the leaf segment and no severe chlorosis. When no mycelial growth could be observed under a microscope or if it had died off at an early stage due to surrounding leaf chlorosis, the individual was classified as resistant.

#### STATISTICAL ANALYSES

The change in host density for the years 2001–02 and 2002–03 was measured as the net reproductive rate:

$$R_0 = N_{t+1}/N_t$$

where  $N_t$  is the number of host plants at time  $t$  and  $N_{t+1}$  is the number of host plants the next year. This value was log-transformed for the analysis. The impact of infection prevalence within quadrats on the density of *Plantago* was analysed as a mixed model ANOVA in the PROC MIXED routine in SAS assuming a normal distribution of errors and an identity link function (SAS Institute 1999). Fixed factors in the model were infection prevalence in  $N_t$ , *Plantago* density in  $N_t$ , which was log-transformed for the analysis, the year and the host population. In the model quadrats were treated as random factors and the change in host density is treated as a repeated measures type of response to account for temporal autocorrelation within quadrats.

Each of the 64 plant individuals was assigned a phenotype according to its infection response to the four pathogen strains tested, which could be susceptible '0' or resistant '1'. An individual was classified as susceptible if pathogen germination and sporulation was successful in any of the inoculation trials. For example, '0000' refers to a plant that is susceptible to all four pathogen strains. The similarity of the phenotypic composition among populations was estimated as:

$$P = \sum_i \text{minimum}(p_{1i}, p_{2i})$$

where  $P$  is the percentage similarity between populations 1 and 2,  $p_{1i}$  is the percentage of phenotype  $i$  in population 1 and  $p_{2i}$  is the percentage of phenotype  $i$  in population 2 (Krebs 1999). Possible association between pairwise similarity indices of populations and their geographical distances was assessed using a Mantel test (Mantel 1967) as implemented in GENEPOP 3.1 using 10 000 random permutations (Raymond & Rousset 1995).

The number of resistance phenotypes in the two categories of populations, infected and non-infected, was analysed as a generalized linear model assuming a Poisson distribution and a log link function using PROC GENMOD of SAS version 8.02 (SAS Institute 1999). The infection response of the host plant was analysed as a generalized linear mixed model with a binomial error distribution and a logit link function

using the GLIMMIX macro of SAS version 8.02 (SAS Institute 1999). The response of a host individual to the four pathogen strains was treated as a repeated measures type of response in the model, in which the individual was considered to be a random factor, nested within the population according to its source of collection. The infection status (infected or not) of the host population and the host population (nested within the infection status) were both fixed effects. The four pathogen strains were treated as random effects in the analysis. They were nested within the Petri dish effect, equivalent to a block effect in experimental design, as each Petri dish was exposed to a single pathogen strain. Replication number, of which there were four, was included in the model to determine whether it would explain any variation in data. When significant, interactions were included in the model.

## Results

### EFFECTS OF INFECTION ON HOST MORTALITY

In both populations *Plantago* density declined during the study period, but the decline was steepest in the infected quadrats. In 2001–02 the average  $\ln R_0$  in infected quadrats was  $-0.72$  and in the non-infected quadrats  $-0.29$ , compared with  $-1.11$  and  $-0.96$  in 2002–03. This difference is statistically significant (Table 1) and indicates that mortality is highly dependent on environmental conditions. The other factors included in the model, initial *Plantago* density and population, were not significant (Table 1).

### CENSUS OF DISEASE IN NATURAL POPULATIONS

Two features became strikingly clear from the disease surveys. First, the pathogen is quite rare in the pathosystem, with 5% of the host populations being infected in years 2001 and 2002, and only 2% in year 2000. Secondly, the dynamics of *P. plantaginis* is characterized

**Table 1** Results of a mixed model ANOVA assessing the impact of infection prevalence on the survival of *P. lanceolata* in two populations. The plant density was surveyed in 1-m<sup>2</sup> quadrats covering the entire population (see Material and methods). n.d.f. = numerator degrees of freedom; d.d.f. = denominator degrees of freedom

Source				
Fixed factors	n.d.f.	d.d.f.	<i>F</i>	<i>P</i>
Infection prevalence	1	348	11.85	0.0006
<i>Plantago</i> density	1	348	0.35	0.555
Year	1	348	21.34	< 0.0001
Population	1	648	1.64	0.2
Random factors				
	Estimate	SE	<i>Z</i>	<i>P</i>
Quadrat	3.46	0.68	5.09	< 0.0001
Residual	118.57	9.77	12.13	< 0.0001

**Table 2** The number of old, new and extinct populations of *P. plantagin* in the pathosystem in the Åland Islands in years 2001 and 2002. 'Totals' is the number of infected populations in each year

Year	Old	New	Extinct	Total	Infected (%)
2000				56	1.8
2001	29	119	27	148	4.6
2002	38	116	110	154	4.8

by high population turnover from one year to another. During the 3-year survey period, over half of the infected populations went extinct between years. On the other hand, the extinctions were balanced by colonizations of previously non-infected host populations, so that the number of infected populations actually increased during 2000–02 (from 56 to 154; Table 2). The occurrence of the disease is highly aggregated (Fig. 1; A.-L. Laine, unpublished data). A more thorough analysis of the epidemiology and incidence of disease will be conducted once more comprehensive data are available.

#### RESISTANCE PHENOTYPE COMPOSITION

The inoculation method was considered to be appropriate, as 93% of the inoculation replicates gave identical scores. Sixteen resistance phenotypes were identified in the sample of 64 plants based on their pattern of resistance/susceptibility to four pathogen isolates. All of the eight host populations were diverse in their resistance phenotype composition; indeed, in population 3265 each individual tested responded in a different manner to the four pathogen strains (Table 3) and no host population showed fewer than five resistance phenotypes. There was a tendency for non-infected populations to support a greater number of resistance phenotypes (6, 6, 7 and 8) than infected populations (5, 5, 5 and 6), although this trend was not statistically significant (d.f. = 1,  $\chi^2 = 0.75$ ,  $P = 0.39$ ). Individuals that were resistant to all four pathogen strains were found only in the non-infected populations, while individuals susceptible to all of the studied strains were most abundant in the infected populations (Table 3). The most common phenotype was the one that was susceptible to all studied strains, which comprised 25% of the 64 host individuals, and 30% were susceptible to three of the four *P. plantagin* strains. By contrast, only 6% of the hosts were resistant to all four pathogen strains and 15% to three of the four. More than half of the phenotypes (nine) were expressed by no more than three or fewer individuals. In the Mantel test comparing the phenotypic similarity and geographical distance matrices of the host populations, the probability of finding a value greater than the one observed was  $P = 0.18$  and a value smaller than the observed one  $P = 0.82$ . Hence, no positive or negative association emerged between the geographical distance and the resistance phenotypic composition (Fig. 2).

**Table 3** The distribution of plant resistance phenotypes to *P. plantagin* in eight *P. lanceolata* populations. '0' refers to a susceptible and '1' to a resistant response to the four pathogen strains tested (order of pathogen strains: 877H, 877 L, 1537B and 1537C)

Phenotype	Population							
	877	875	870	858	3265	3491	1536	1537
P1 (0000)	4	2	2	1	1		3	3
P2 (0001)	1	1		2	1	2		1
P3 (0010)		2					1	
P4 (0100)		2			1			
P5 (1000)			1	1	1		2	
P6 (0011)	1		1		1	1		
P7 (0110)		1						
P8 (0101)	1							1
P9 (1010)	1							1
P10 (1001)			1			1	1	1
P11 (1100)				1				1
P12 (1011)				1		1		
P13 (0111)				1				
P14 (1101)				1	1	1		
P15 (1110)			2		1		1	
P16 (1111)			1		1	2		

#### COMPARISON BETWEEN INFECTED AND NON-INFECTED POPULATIONS

A mean level of resistance was calculated for each host population based on the resistance responses of each individual within each host population against all tested pathogen strains (Fig. 3). The level of resistance supported by the infected host populations was significantly higher than that of the non-infected populations (Table 4). The level of resistance varied very little among populations in the same infection category (Fig. 3a). Individuals within host populations differed significantly in their resistance (Table 4). The four pathogen strains differed in their ability to infect host populations, which is indicated by the significant

**Table 4** Results of the generalized linear mixed model assessing the impact of various factors on the level of resistance of *P. lanceolata* to *P. plantagin*. n.d.f. = numerator degrees of freedom; d.d.f. = denominator degrees of freedom

Source				
Fixed factors	n.d.f.	d.d.f.	F	P
Infection status	1	784	7.65	0.006
Population	6	784	0.31	0.93
Session	3	784	0.16	0.93
Random factors				
Random factors	Estimate	SE	Z	P
Host individual	2.06	0.51	4.02	0.0001
Pathogen strain	0.16	0.01	18.97	< 0.0001
Pathogen × Population	0.61	0.26	2.37	0.009
Petri dish	0.61	0.19	0.19	0.0007
Residual	0.74	0.04	18.97	< 0.0001

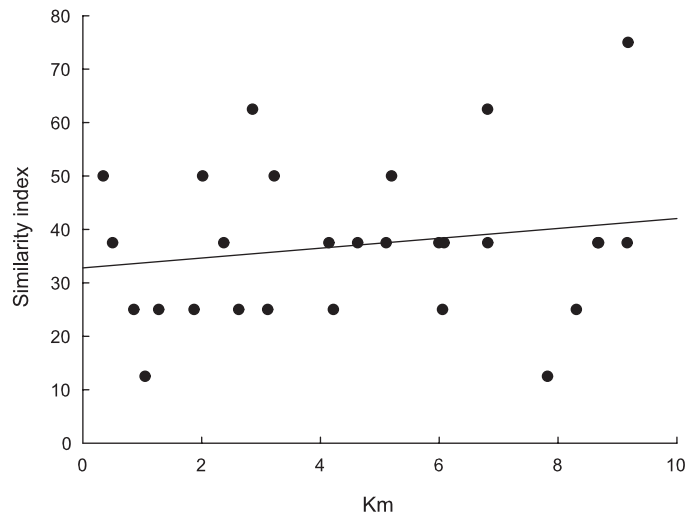


Fig. 2 The pairwise similarity in the phenotypic composition of *P. lanceolata* populations, measured using the minimum percentage similarity, shows no association with the geographical distance separating the populations.

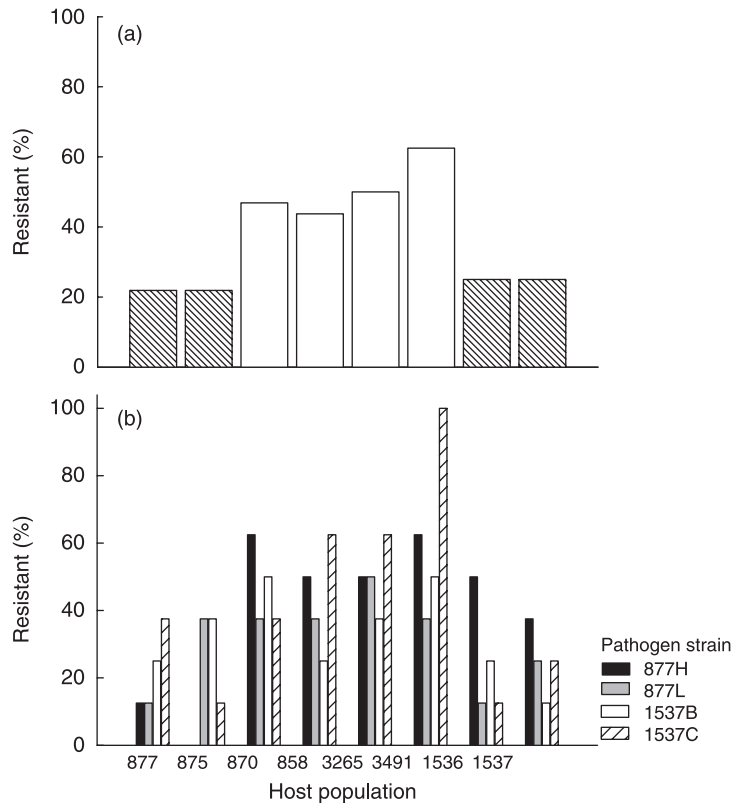


Fig. 3 (a) Mean level of resistance of the eight *P. lanceolata* populations as scored in the inoculation experiment. Naturally infected populations (Fig. 1) are shown by hatched bars. (b) Resistance of the host plants within each of the eight host populations to the four *P. plantagin* strains.

‘Population’–‘Pathogen’ interaction (Table 4, Fig. 3b). On the other hand, while the performance of the four pathogen strains varied among individuals and populations (Table 4), overall there was little variation in their virulence. Strains 877H and 877L were virulent on 59% and 69% of the hosts, respectively, whereas strains 1537B and 1537C were virulent on 67% and 56% of the hosts.

### Discussion

The present results demonstrate that non-infected host populations were significantly more resistant to the mildew than infected host populations. Regional surveys on the incidence of the fungus showed that only 5% of the host populations was infected at a given time and that the disease dynamics are characterized by

high extinction and colonization rates. These findings strongly suggest that there is considerable variation among host populations within the plant-pathogen metapopulation that will influence interactions between the host and the fungus, including the spatial dynamics of the pathogen. Below, I will discuss the possible causes of the observed variation in resistance and implications for pathogen dynamics.

#### DIVERSITY AND DISTRIBUTION OF RESISTANCE PHENOTYPES

*Plantago lanceolata* populations turned out to be highly diverse in their resistance responses to the four pathogen strains obtained from the two heavily infected natural populations. A diverse composition of resistance phenotypes was observed both within and among the eight host populations. Of the 16 phenotypes identified in this study, more than half were represented by only three or fewer individuals. Within-population diversity ranged from all eight individuals representing a unique resistance phenotype to half of the individuals sharing the same phenotype. Overall, the level of susceptibility was high; the most common phenotype was susceptible to all four pathogen strains and 55% of the host individuals were susceptible to at least three of the four *P. plantagin* strains. High diversity of resistance phenotypes agrees with the results of previous studies on natural host-pathogen systems, in which diversity has been measured at different spatial scales (Dinoor 1970; de Nooij & van Damme 1988; Parker 1988; Bevan *et al.* 1993; Burdon *et al.* 1999; Thrall *et al.* 2001). In the present study, the frequency of resistance phenotypes was uneven in the host populations but, in contrast to the results of Thrall *et al.* (2001), no evidence was found for a greater similarity in the resistance phenotypic composition of neighbouring than far-away populations. A similar seemingly random distribution was observed by Davelos *et al.* (1996) in rust infection of the clonal plant *Spartina pectinata*.

#### CAUSES OF RESISTANCE DIVERSITY

A study on local epidemics of *P. plantagin* revealed that in years of severe drought (2002 and 2003) when population densities declined everywhere, the decline was steepest in those parts of host populations where infection was prevalent. This clearly suggests that there is a fitness cost of being infected, although the effects of infection on the host will be complicated by interactions with other species (Laine 2004) and varying environmental conditions. No crosses have yet been conducted to confirm that the phenotypic differences among plants, as measured in the inoculation experiment, represent genetic differences. However, the seeds from which the plants used in the experiment were derived were surface sterilized and the plants were grown under identical glasshouse conditions; hence,

any environmental effects are unlikely. Therefore, a high level of genetic variability is the most likely explanation for the observed variation in resistance among the plants. The resistance of *P. lanceolata* against *P. plantagin* can be classified as race specific, as resistance was expressed against specific pathogen isolates instead of against all strains tested (Burdon *et al.* 1996).

Evolutionary theory would predict infected host populations to be more resistant than non-infected populations (Van Valen 1973; Clay & Kover 1996). However, in a metapopulation with high pathogen population turnover, disease may not persist locally long enough for resistance to evolve. Also, in a spatially structured pathosystem processes other than natural selection may have contributed to the resistance structure of host populations, as hypothesized by Thompson's theory of geographical mosaic coevolution (Thompson 1999). In the *Plantago-Podosphaera* pathosystem on the Åland Islands, the four infected populations were, on average, more susceptible to disease than the four non-infected populations. Studies on the anther-smut fungus *Microbotryum violaceum* by Carlsson-Granér (1997) and Thrall & Antonovics (1995) have found a similar trend. The local population differentiation and seemingly random distribution of resistance phenotypes suggest that gene flow among local host populations may be restricted and that within-population processes (such as drift and founder effects) may be important in defining the resistance structure of local populations. Many features in the reproductive biology of *P. lanceolata* support this notion. Previous studies have shown that although the pollen of *P. lanceolata* is wind-dispersed, pollen flow occurs mainly over very short distances (up to 1.5 m; Tonsor 1985a,b; Bos *et al.* 1986) and the majority of mature seeds land very close to the mother plant (0.42 m; van Damme 1986). Furthermore, a substantial fraction of new recruits in *P. lanceolata* populations are clonal ramets (Sagar & Harper 1964; Van Groenendael & Slim 1988).

Ennos (2001) concluded that genetic structure resulting from founder effects during population establishment and regeneration may be an important cause of differentiation among local plant populations. Local variation in resistance against one particular strain of *P. plantagin* may be generated by stochastic proliferation of host genotypes during the establishment of new local populations (Frank 1997; Thrall & Burdon 1997). Non-adaptive evolution due to random genetic drift may also occur in small populations of *P. lanceolata*, in which effective population size is likely to be very small (cf. Parker 1985). Non-random associations between disease resistance and other ecologically important traits have been observed in a number of plant species where recombination is restricted by clonal reproduction (Burdon *et al.* 1980). If natural selection on these correlated traits is stronger than that exerted by pathogens, disease resistance may evolve in a non-adaptive or even maladaptive manner (Parker 1991).

Hence, the observed differentiation in the resistance structure of the host populations in this study may at least partly be non-adaptive.

#### HOST POPULATION DIFFERENTIATION: IMPLICATIONS FOR PATHOGEN DYNAMICS

Restricted gene flow and local population differentiation are characteristics of many plant species, and these features are likely to profoundly affect the occurrence, size and genetic composition of fungal pathogen populations (Parker 1985; Gandon & Michalakis 2002). While many factors will influence the occurrence of *P. plantaginis* in any given *P. lanceolata* population, the diversity of resistance phenotypes and differences in the mean level of resistance among host populations are likely to have an impact both on the within-season as well as between-seasons dynamics of the pathogen populations (Thrall & Burdon 2000).

In this study non-infected host populations were more diverse in their resistance phenotypic composition and had a significantly higher mean level of resistance than the infected *Plantago* populations. Experimental studies of natural pathosystems and results from studies of agricultural systems show that the probability of pathogen colonization and persistence is reduced with increasing resistance diversity (e.g. Schmid 1994; Thrall & Jarosz 1994; Alexander *et al.* 1996; Zhu *et al.* 2000). At the level of individual populations, resistance diversity will reduce the probability of establishment by immigrant pathogen spores simply because of the possibility that the virulence of a spore landing on a host does not match the specific resistance alleles (Burdon *et al.* 1996). In populations where the mean level of resistance is high there is an even lower probability of encountering matching resistance alleles. Also, even if a spore has successfully established within a host population, its further spread will be delayed by the diversity of resistance alleles it encounters. While almost all *Plantago* populations contained at least one individual that was susceptible to each of the pathogen strains, this mechanism may have prevented, at least partly, the establishment of an epidemic in the populations that remained non-infected during the survey period. Finally, strain-specific resistance genes will reduce the rate of development of an epidemic in any given population, and this, in turn, will reduce the number of spores dispersing to new host populations (Burdon *et al.* 1996). All these factors are likely to contribute to the rarity of regional epidemics in natural situations.

To summarize, a wide range of factors, including host population size, density, suitable microclimate and distance to the nearest spore source, will influence exactly which host populations will become infected. As previous studies have shown, chance may also play an important role in determining the distribution of pathogens (Carlsson-Granér 1997). Variation in the virulence of pathogen lines may also affect the success of colonization events. While there was little variation

in the overall virulence of the four pathogen isolates used in this study, they each were clearly distinct in their inoculation success among the tested host plants. It remains a challenge to determine how variable the local pathogen populations are in this system. The eight host populations studied here varied significantly in their mean level of resistance, which adds to the heterogeneity of the fragmented pathosystem. In the present sample, those host populations that were naturally infected showed significantly lower overall resistance than uninfected populations. It may be concluded that diversity of resistance phenotypes and differences in the mean level of resistance have the potential to decrease the likelihood of pathogen colonization and thus the development of regional epidemics in natural pathosystems.

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