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Host susceptibility and spread of disease in a metapopulation of *Silene dioica*

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ABSTRACT

Hypothesis: In host–pathogen metapopulations, founder events and restricted gene flow cause differentiation in susceptibility among patches and populations of the host. Patterns of disease spread correspond to levels of susceptibility in host populations.

Organism: The host-plant *Silene dioica* and the sterilizing anther-smut fungus *Microbotryum violaceum*.

Study site: An archipelago in northern Sweden where populations of *S. dioica* constitute a hierarchically age-structured metapopulation and where *M. violaceum* is common.

Methods: Plants from patches within recently diseased island populations were transplanted into an experimental population where they were naturally exposed to the anther-smut. We also included two populations with a long history of disease.

Results: We found that susceptibility varied among recently diseased populations, while no significant differentiation in susceptibility was detected among patches within populations. Populations showing an increase in disease prevalence in the *in-situ* populations were more susceptible in the experiment than populations where the disease has remained at low levels. The more susceptible populations showed similar levels of susceptibility to the populations with a long history of disease.

Conclusion: We propose that the combination of restricted host and pathogen dispersal and high turnover rates of host patches within populations maintain variation for resistance and mediate host–pathogen co-existence at the metapopulation level.

Keywords: disease, evolution, metapopulation, *Microbotryum violaceum, Silene dioica*, susceptibility, turnover.

INTRODUCTION

The spatial structure of host-populations can have major impacts on ecological and evolutionary dynamics of plant–pathogen interactions. When host populations are continuously distributed, frequent dispersal and low extinction probabilities are likely to result in a high degree of synchrony in disease dynamics among local populations. Selection pressures and levels of host resistance and pathogen virulence may thus be

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relatively similar across populations (Burdon and Thrall, 1999; Carlsson-Granér and Thrall, 2002). In contrast, in metapopulations where populations are subjected to colonization/extinction dynamics and dispersal is restricted (Hanski and Gilpin, 1991), host and pathogen populations may show considerable differentiation in resistance and virulence (Thrall and Antonovics, 1995; Thrall *et al.*, 2001; Carlsson-Granér and Thrall, 2002). This is in part a result of founder effects associated with colonization and extinction processes, but asynchrony in disease dynamics and thus in selection pressures among populations may also contribute to differentiation (Frank, 1997; Thrall and Antonovics, 1995; Antonovics *et al.*, 1997; Burdon and Thrall, 1999). Moreover, opposing selection during colonization and growth of populations may cause differentiation in life-history characters, such as host-resistance, between populations of different ages but this metapopulation effect has rarely been considered in host–pathogen interactions (Olivieri and Gouyon, 1997). Clearly, if we are to understand how spatial structuring affects the evolutionary dynamics of plant–pathogen interactions, a thorough understanding of patterns of disease, and of resistance and virulence structures across multiple populations, is required. Nevertheless, few natural plant–pathogen interactions have been studied in such a spatial context (cf. Burdon and Thrall, 2001).

In this study, we survey patterns of host-susceptibility to the anther-smut fungus *Microbotryum violaceum* in an archipelago in northern Sweden where populations of the host-plant *Silene dioica* constitute a metapopulation (Carlsson-Granér, 1997; Giles and Goudet, 1997b). The anther-smut fungus, which sporulates in flowers and causes permanent sterility of host plants, has a potentially large effect on the genetic and numerical dynamics of host populations (Carlsson and Elmqvist, 1992; Alexander *et al.*, 1996; Giles *et al.*, 2000). The turnover dynamics of *S. dioica* in the studied archipelago are driven by successional changes occurring on islands that continually emerge and rise above the sea as a result of isostatic land uplift (Carlsson *et al.*, 1990). While the specific features of this system – islands emerging and rising at a constant rate – are unique to the land uplift archipelagos of formerly glaciated areas, the general feature that suitable habitats emerge and have limited longevity is typical for all successional systems. The archipelago thus provides an opportunity to study the dynamics of successional-bound host–pathogen systems with a major technical advantage: for those plants which appear predictably on islands of a given height, we can age populations on the basis of the current height of the islands and the land uplift rate (Carlsson *et al.*, 1990).

Where drift-migration dynamics are predominant forces acting on populations, the distribution of genetic variation is expected to be similar for all genes (Frank, 1997). Since host resistance has a genetic basis in the *Silene–Microbotryum* system (Alexander *et al.*, 1993; Carlsson-Granér, 1997), founder effects and restricted gene flow may give rise to differentiation in susceptibility among island populations and also among patches of *S. dioica* within islands as observed for neutral genes (Giles and Goudet, 1997a; Giles *et al.*, 1998). Differentiation among patches within islands is established during population expansion, which occurs through kin-structured colonization of new patches, initially by seeds from the original founders of the islands and subsequently by seeds from established plants. Short-distance seed and pollen dispersal restricts gene exchange among patches and populations and maintains the differentiation established at founding (Giles and Goudet, 1997a; Ingvarsson and Giles, 1999).

To evaluate how founder events and restricted gene flow affect patterns of susceptibility and disease dynamics in the archipelago, we selected five island populations that have become diseased in recent years. If these populations vary in susceptibility, it is more likely a result of chance founder effects than varying pathogen-mediated selection pressures. To explore the possibility that the patch colonization dynamics of *S. dioica* also results in

differentiation in susceptibility within islands, healthy plants for the experiment were sampled from separate patches within the islands. We also included two populations with a long history of disease, which enabled us to compare levels of susceptibility in the recently diseased populations with those in populations where the prevalence of the pathogen has remained stable for a long time. The plants were transplanted to a highly diseased experimental population where they were naturally exposed to the anther-smut. We used proportions of successful infections of transplants to address the following questions: (1) Are there differences in susceptibility to *M. violaceum* within and among recently diseased island populations of *S. dioica*? (2) Do differing levels of susceptibility seen in the experiment correspond to patterns of disease spread observed in the *in-situ* populations? (3) Is susceptibility in recently diseased populations showing an increase in disease prevalence comparable with populations with a long history of disease? The answers to these specific questions address a broader issue: Are there general mechanisms that maintain susceptibility in metapopulations bound to habitat patches with limited longevity?

MATERIALS AND METHODS

Plant–pathogen system

Silene dioica L. (Caryophyllaceae) is a dioecious, perennial and insect-pollinated herb that is relatively widespread in northern Fennoscandia. It occurs in fertile, disturbed habitats and is abundant in the deciduous phase of primary succession along the shores of the Gulf of Bothnia (Kurtto, 2001). *Silene dioica* reproduces when 2–3 years old, with males flowering at an earlier age than females, and has a life expectancy of 5–15 years in the archipelago (Carlsson and Elmqvist, 1992). It is primarily pollinated by bumblebees, which are the most efficient pollinators (cf. Elmqvist *et al.*, 1993), but other insects also visit its flowers (Westerbergh and Saura, 1994).

The anther-smut fungus *Microbotryum violaceum* (Ustilaginales; synonym *Ustilago violacea*) (Brandenburger and Schwinn) G. Deml. & Oberw. causes both male and female hosts to produce flowers with anther-like structures filled with teliospores (the ovaries become rudimentary in females). The teliospores are transmitted to the flowers of new hosts by flower visitors (e.g. Baker, 1947) but can also spread passively to seedlings and vegetative plants growing beneath diseased plants (Roche *et al.*, 1995; T.M. Pettersson and U. Carlsson-Granér, unpublished). Following transmission to new hosts, the teliospores germinate and undergo meiosis to produce haploid sporidia. Conjugation of sporidia of opposite mating types then produces a heterokaryotic infection hypha, which may infect the new host plant (Baird and Garber, 1979; Hood and Antonovics, 2000). Infection has no detectable effect on plant survival and infected plants thus remain in the population (Carlsson and Elmqvist, 1992).

Resistance in this host–pathogen system can be mediated both by active biochemical and passive morphological mechanisms – that is, host floral traits such as flowering time, size and number of flowers and stigma lengths that decrease the probability of visits by pollinators carrying the spores of *M. violaceum* or the number of spores deposited on the flowers (Elmqvist *et al.*, 1993; Alexander *et al.*, 1996; Carlsson-Granér, 1997; Giles *et al.*, 2000). Several caryophyllaceous host-species have been shown to vary in resistance both within and among populations (Alexander *et al.*, 1996; Carlsson-Granér, 1997; Ouborg *et al.*, 2000). In contrast, low genetic variability in microsatellite (Delmotte *et al.*, 1999) and electrophoretic markers

(Antonovics *et al.*, 1996), as well as in fitness-related traits (i.e. in infectivity, latency and spore-production), has been detected within and among populations of *M. violaceum* (Alexander *et al.*, 1996; Carlsson-Granér, 1997; Kaltz *et al.*, 1999; Ouburg *et al.*, 2000; Kaltz and Shykoff, 2002).

The studied metapopulation

We are studying the *Silene–Microbotryum* system in the Skeppsvik archipelago (63°44–48′N, 20°31–33′E) close to Umeå in northern Sweden. This area has undergone rapid land uplift since the end of the last glaciation; the current land uplift rate is about 0.9 cm per year (Ericson and Wallentinus, 1979). Today, the archipelago consists of approximately 100 islands of different ages within its 20 km^2 area. We have surveyed numerical changes in host and pathogen populations in the archipelago (i.e. host populations sizes and disease prevalences) since 1985 (Carlsson and Elmqvist, 1992; U. Carlsson-Granér and T.M. Pettersson, unpublished data), which together with the possibility of ageing the populations (Carlsson *et al.*, 1990) provides unique opportunities for studying metapopulation processes. This also makes it possible to evaluate whether the susceptibility of populations determined in this study correspond to observed patterns of disease spread.

Silene dioica establishes populations on young islands from a small number of seed founders (Giles and Goudet, 1997a). *Silene dioica* seeds have a limited floating ability [less than 1 h (Andersson *et al.*, 2000)] and seeds probably spread among islands attached to drift material or across the ice attached to dead shoots. Consistent with both alternatives, juvenile plants have been observed growing in drift deposited on the near-shore areas of the islands. Populations expand rapidly as *S. dioica* spread across islands and attain large sizes and high densities on islands of intermediate age. When later successional species establish and spread over islands, *S. dioica* populations decrease in size and after 200–500 years (~20–40 generations) eventually go extinct (Carlsson *et al.*, 1990). Similar turnover dynamics occur within islands but at a much higher rate as *S. dioica* colonize newly emerged substrate close to the seashore and decline and go extinct in older patches higher up on the islands (Carlsson-Granér, 1997). As a result of these successional changes, populations of *S. dioica* constitute a hierarchically age-structured metapopulation (Giles and Goudet, 1997b).

Microbotryum violaceum occupies a temporal window within that of its host. In general, *M. violaceum* is absent or rare in young populations and patches, but successfully colonizes dense populations and patches of young to intermediate age. Disease prevalence (percentage diseased individuals) is highly heterogeneous in populations (0–60%) and patches (0–80%) in this intermediate phase of population development. In old populations and patches, the disease is rare or absent (Carlsson *et al.*, 1990; Carlsson-Granér, 1997). Similar to patterns of dispersal in the host, *M. violaceum* spores are primarily dispersed over short distances (Pettersson *et al.*, 2000). However, since spores are found on 3–5% of the flowers in disease-free populations (B.E. Giles and P.K. Ingvarsson, unpublished), and the disease is widespread in the archipelago, spore dispersal among populations also occurs.

Transplantation experiment

Choice of populations

To evaluate whether founder events and restricted gene flow affect patterns of susceptibility, we selected five island populations in which disease had appeared in recent years. Because

there is a tendency for spatial structuring of host and pathogen populations in the archipelago (Carlsson-Granér, 1997; Giles and Goudet, 1997a), we sampled populations within a restricted area of the archipelago (Fig. 1). In two of the sampled populations (8 and 12), the disease prevalence increased from zero to 7% between 1985 and 1998, while the disease established but remained at very low levels in populations 5, 6 and 17 (Table 1).

As we wanted to compare levels of susceptibility in the recently diseased populations with those in populations where the disease has remained at high prevalence, two older populations close to the first five populations were also included in the experiment (Fig. 1). In these two populations (23 and 27), the pathogen was already present in 1985 and disease prevalence has been relatively high over the years (Table 1). Note that populations 6–8 and 17–23, respectively, are very close to each other (Fig. 1) but show very different patterns of disease spread and levels of disease (Table 1). Formerly, populations 17 and 23 were on two separate islands that have now fused as a result of land uplift (Fig. 1).

Sampling

To examine both within- and among-population variation in susceptibility, we sampled at least 20 healthy flowering plants from each of five patches per population, giving a minimum of 100 plants per population. Plants were sampled in patches with a diameter of

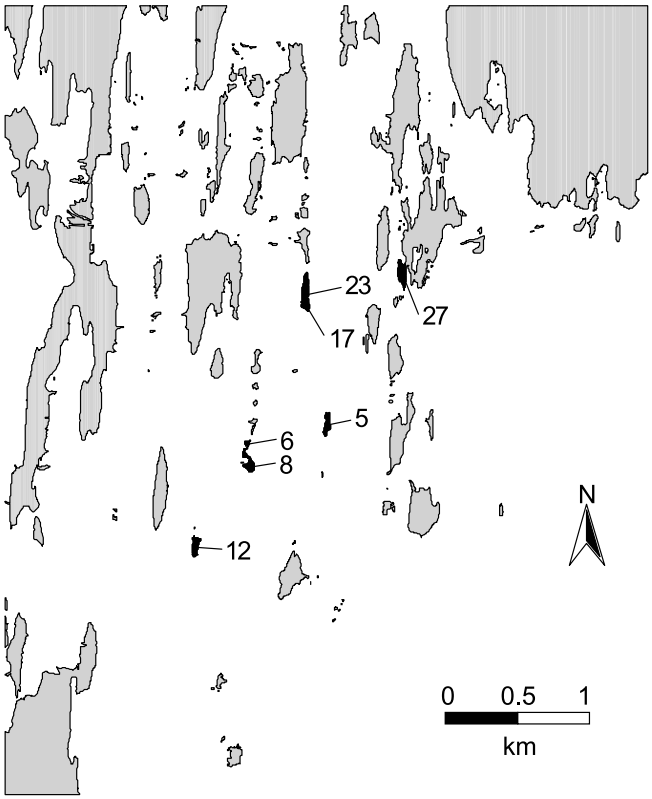

Fig. 1. The Skeppsvik archipelago in northern Sweden (63°44–48′N, 20°31–33′E). Numbers refer to populations of *Silene dioica* included in the transplantation experiment. Characteristics of populations are given in Table 1.

Table 1. Characteristics of the *Silene dioica* populations included in the study

Population	Age (years)	Population size		Disease prevalence (%)		
		1974	1990	1985	1990	1998
5	23 ± 3	0	1 500	0	0	0.5
6	41 ± 15	1	4 000	0	0.5	0.5
8	49 ± 14	20	4 000	0	8	7
12	63 ± 15	150	3 900	0	5	7
17	85 ± 15	2000	3 200	<0.5*	3	2
23	106 ± 15	4000	16 000	36	41	34
27	166 ± 15	300	4 600	14	18	13

Note: Estimations of ages, sizes of populations and disease prevalences in 1985 and 1990 are based on Carlsson *et al.* (1990) and Carlsson and Elmqvist (1992). Disease prevalences in 1998 were estimated by checking a minimum of 300 flowering plants per population for presence of disease symptoms.
* A few diseased plants were observed in population 17 in 1985.

~2 m in the intermediate aged part of the populations where *S. dioica* is more or less continuously distributed. This patch size was within the range of the estimated size of the breeding units (based on allozymes) of *S. dioica* on the islands (Giles *et al.*, 1998). The patches were chosen haphazardly with a minimum distance of 5 m between them. In mid-June 1998, approximately equal numbers of healthy female and male plants with flowering buds, but no open flowers, were carefully excavated from each patch. To ensure that plants chosen for sampling were healthy, one flower bud from each plant was checked for disease symptoms by a person who otherwise did not touch the plants. The excavated plants, which contained relatively small amounts of native soil, were placed singly in plastic bags and transferred to the experimental site where they were planted in 500 cm^3 pots filled with fertilized soil. The plants were then covered to exclude insects and kept close to the experimental area until the start of the experiment.

Experimental design

The plants were transplanted into a diseased high-density natural *S. dioica* population on the mainland. Eight years before this experiment, three isolates of the anther-smut were introduced to this formerly healthy host population. The fungal isolates were originally collected from three islands in the same area of the archipelago as our experimental populations. Disease spread has been rapid at this site and at the time of the experiment about 60% of the plants were diseased. Previous studies have not revealed any variation in infectivity among the original fungal isolates (Carlsson-Granér, 1997) and, in general, there seems to be little genetic variation in the anther-smut in the study area (B.E. Giles, K. Olsson, C. Halldén, T. Elmqvist and T. Sall, unpublished). This allowed us to design an experiment that tested only for variation in host susceptibility.

On 18 June 1998, pots with sampled plants were put in holes in the ground in the experimental population. One plant per population was planted at each of 100 stations in a grid system of 10×10 one square metre plots. Plants from patches within populations were randomly assigned to stations using a Latin square plan (Potvine, 1993). Within stations, plants from different populations were randomly assigned to one of nine positions in a 3×3 grid with 5 cm between pots. Damaged plants were replaced with plants from the respective

population/patch within the first 3 weeks of the experiment (118 replacements in total). Due to a lack of replacement plants, eight positions were left empty, giving a total number of plants in the experiment of 692. Plants were watered when needed. On 20 October, the plants were excavated and placed in a cold room at 2°C and complete darkness to induce flowering (cf. van de Pol, 1985).

Census protocols in the experimental population

There was some heterogeneity in flower density and disease frequency in the experimental population, which may influence the probability of infection of transplanted individuals (Alexander *et al.*, 1996; Biere and Honders, 1998). To control for this, we estimated the density of flowers and the frequency of diseased flowers around each transplant. This was done at two spatial scales, 1 and 9 m^2. From mid-June until late August, we did weekly censuses of the number of open flowers on all plants (natural as well as transplants) in the experimental area. Individual flowers last up to 5 days at peak flowering (U. Carlsson-Granér, personal observation) and new flowers are thus likely to have been counted at each census. After a few weeks, spittlebug nymphs (*Philaenus spumarius*) (Cercopidae), which feed by sucking plant sap in leaf nodes, became rather common on transplanted individuals. Due to the possibility that wounds made by the spittlebugs may serve as infection sites for *Microbotryum* spores, we also noted the presence/absence of these on the transplants. Peak flowering was between 29 June and 20 July for both transplanted and naturally occurring plants in the meadow population. At the end of the census period, ~9% of the transplanted individuals had not been recorded with open flowers. These plants had no obvious damage to their flowering stalks and are likely to have flowered between the weekly censuses.

Scoring of resistance

In this study, we did not distinguish between physiological and morphological resistance mechanisms, which are both under the genetic control of the plant (Alexander *et al.*, 1993; Carlsson-Granér, 1997). Instead, we determined how patch and population origin may influence the overall susceptibility of plants. The environmental conditions at the experimental site – moist and partly shaded – were similar to conditions in the intermediate aged part of the islands where the experimental plants were sampled. As a consequence, susceptibility determined in the experimental population was therefore expected to correspond to patterns of susceptibility in the *in-situ* populations (cf. Alexander, 1992). Susceptibility, scored as presence/absence of flowers producing spores, was determined when plants re-flowered after exposure to disease in the experimental population.

In early February 1999, the pots with transplants were transferred from the dark cold room into the greenhouse. The first flowers appeared in mid-March and the plants were checked for disease symptoms every second day during the flowering period. Once diseased flowers were observed on a plant, the plant was moved to a separate room in the greenhouse and covered to prevent contamination of other plants. In mid-June, the plants were transferred outdoors. Forty plants that remained vegetative during 1999 were transferred into the cold room once again in October 1999 and 10 of them flowered in 2000.

Statistical analyses

There were no significant differences in infection probabilities between transplants sampled on different occasions ($\chi^2 = 0.93$, d.f. = 2, $P = 0.63$) or between transplants with and without

observed flowers in 1998 ($\chi^2 = 1.74$, d.f. = 1, $P = 0.19$). We therefore included all plants for which we had scored disease status in the analyses. To reduce the number of factors in the main analysis, we first analysed the importance of neighbourhood characteristics (total floral density, density; frequency of diseased flowers) at the scales of 1 and 9 m^2, respectively, for probability of disease in transplants using generalized linear models with a binomial error distribution and a logit link [GLIM (Crawley, 1993)]. For each scale, the total floral density was calculated as the mean number of flowers (healthy and diseased) over all observations and the disease frequency as the proportion of those that were diseased. Since the area around each transplant is fixed, density of diseased flowers was represented by the interaction between total floral density and frequency of diseased flowers. Disease frequency at the 9 m^2 scale was the only factor that had a significant effect on the probability of disease in transplanted individuals; this factor was therefore included in the main analysis.

The effect of population and patch, nested within population, on the probability of transplants becoming diseased was analysed with a generalized linear model as above. In this model, we first included disease frequency of flowers in the 9 m^2 area around each transplant, presence/absence of spittlebug nymphs (*P. spumarius*) and sex of transplants, and then factors and interactions of main interest in this study, population and patch. The effects of the covariates were thus removed before testing the effect of population and patch. For significance testing, we referred to the change in deviance of each sequentially included term to the χ^2-distribution.

RESULTS

In total, 96% (662/692) of the transplanted individuals flowered after exposure to the disease in the experimental population and 17% (112) of these were diseased. In the five recently diseased populations, there were significant among-population differences in the proportion of transplants becoming diseased, while no detectable differences in susceptibility among patches within populations could be detected in the generalized linear model (Table 2).

The susceptibility of plants from the recently diseased populations in the transplantation experiment reflected the patterns of disease spread observed over the last 15 years in the *in-situ* island populations. Populations showing an increase in disease spread over the years (populations 8, 12) were on average more susceptible (i.e. a higher proportion of transplants became diseased) than populations where the disease became established but remained at a low prevalence (populations 5, 6 and 17) ($G = 8.34$, d.f. = 1, $P = 0.004$) (Table 1, Fig. 2). When comparing levels of susceptibility between the two more susceptible populations (8 and 12) and the two populations with a long history of disease (23 and 27), no difference could be detected ($G = 0.02$, $P = 0.89$) (Fig. 2). Note that the highest proportion of plants that became diseased per patch was always observed among plants from the more susceptible populations (8, 12, 23 and 27), while patches with the lowest proportion of diseased plants originated from the most resistant populations (6 and 17) (Fig. 2).

In addition to population origin, sex of transplants and environmental factors that were part of the experimental population (i.e. disease frequency and presence of spittlebug nymphs, *P. spumarius*) had effects on the probability of transplants becoming diseased (Table 2). The probability of infection increased with the frequency of disease in the neighbourhood and was also higher for males than for females, especially in high disease frequency areas (Table 2, Fig. 3). The presence of spittlebugs increased the infection rates in

Table 2. Generalized linear model of the effects of population, patch (nested within population), frequency of diseased flowers, sex and spittlebug nymphs (*Philaenus spumarius*) on the probability of *Silene dioica* plants from five recently diseased populations becoming diseased when transplanted to a diseased experimental population

Term	d.f.	Deviance change	*P*
Disease frequency	1	13.38	0.0003
Spittlebugs	1	0.14	0.7083
Disease frequency × Spittlebugs	1	4.06	0.0439
Sex	1	4.52	0.0335
Disease frequency × Sex	1	5.93	0.0149
Spittlebugs × Sex	1	5.23	0.0222
Populations	4	13.25	0.0101
Patches [populations]	20	18.54	0.5519
Populations × Disease frequency	4	2.91	0.5730
Populations × Spittlebugs	4	5.71	0.2218
Populations × Sex	4	5.95	0.2029

Note: For a description of the analysis, see Material and Methods.

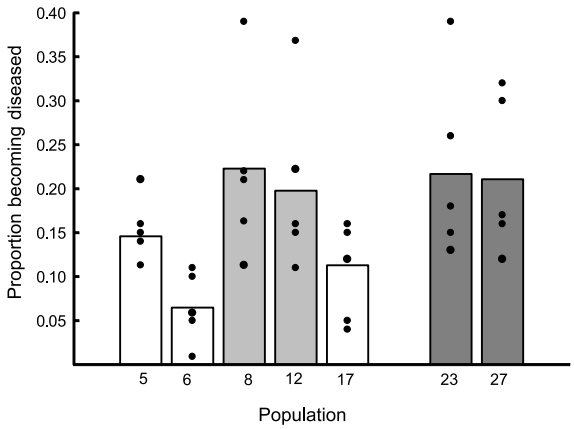

Fig. 2. Proportion of *Silene dioica* plants from seven island populations becoming diseased when transplanted to a diseased experimental population. Among the recently diseased populations (5, 6, 8, 12 and 17), white bars represent populations where the disease has remained at low prevalence and light grey bars populations where the disease prevalence has increased (see Table 2). Populations with a long history of disease (populations 23 and 27) are shown by dark grey bars. Dots represent the proportion of plants becoming diseased per sampled patch (five patches per island population).

males (Fig. 4) and in plants in neighbourhoods with a high frequency of diseased flowers, while no such effect was seen in females (even though the nymphs were more often observed on females) (Fig. 4) or in low disease frequency areas respectively (Table 2). Note that all interaction terms between these environmental factors and population origin were non-significant, indicating that the effects were equal over populations (Table 2).

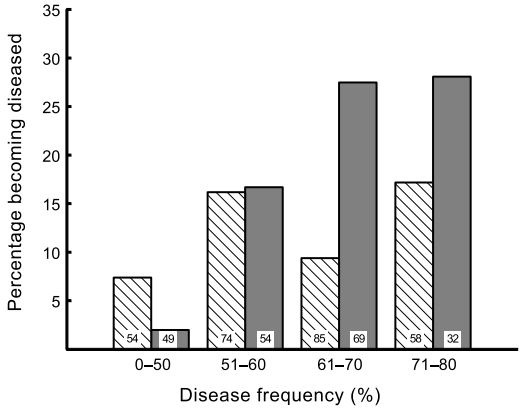

Fig. 3. Percentage of female (hatched bars) and male (grey bars) *Silene dioica* transplants becoming diseased as a function of the frequency of diseased flowers in a 9 m^2 area around each transplant. *N* in each frequency category given within the bars.

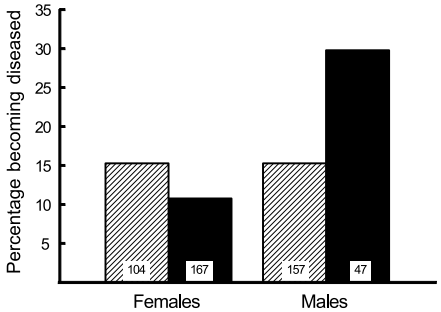

Fig. 4. Percentage of female and male *Silene dioica* transplants becoming diseased with *Philaenus spumarius* nymphs present (closed bars) and absent (hatched bars). *N* in each category given within the bars.

DISCUSSION

Founder effects and patterns of susceptibility

In accordance with the predictions of host–pathogen metapopulation models (cf. Burdon and Thrall, 1999), we found that levels of susceptibility to *M. violaceum* varied considerably among recently diseased *S. dioica* populations from the studied metapopulation. Because none of these host populations had experienced disease epidemics, differences in susceptibility could not be attributed to varying pathogen-mediated selection. Instead, it is likely that differences in susceptibility have been established during founding of the populations in the metapopulation, where new islands continually emerge and become colonized by plants. Large differences in susceptibility were, for example, observed between populations 6 and 8, which are of the same age and situated very close to each other (Fig. 1). Given that the susceptibility of plants in the transplantation experiment mirror genetically determined host-resistance as in previous studies of the *Silene–Microbotryum* system (Alexander *et al.*, 1996), our results strengthen the idea that founder effects have a large impact on the genetic

structure of *S. dioica* in the archipelago (cf. Giles and Goudet, 1997a; Ingvarsson and Giles, 1999). Variation in pattern of resistance among local populations in a metapopulation network has also been observed in the interaction between the plant *Linum marginale* and its rust pathogen *Melampsora lini* in Australia (Jarosz and Burdon, 1991; Burdon *et al.*, 1999; Thrall *et al.*, 2001). However, in that system it has not been possible to evaluate the importance of founder effects for the observed patterns of resistance.

In the archipelago, there are opportunities for gene exchange among populations through seed migration and pollen movement, which may erode the initial genetic structures created by founding (Whitlock and McCauley, 1990). However, allozyme studies have shown that although genetic differences of *S. dioica* are reduced with age of the islands, gene flow does not homogenize initial genetic differences between closely situated islands (Giles and Goudet, 1997a). Consistent with this, populations 17 and 23 in our study are only 50 m apart and are both older than 85 years, but still they differ in neutral characters (Giles *et al.*, 1998), levels of susceptibility and epidemiology.

Although the percentage of plants becoming diseased per patch varied (for example, between 11 and 40% in population 8), we did not detect any significant differentiation in susceptibility among *S. dioica* patches within populations. It must be noted, however, that with the low number of plants sampled per patch (i.e. there were rarely more than 20 flowering plants per patch) and the low number of patches that could be included, the power for detecting differences at the level of patches within islands was low. More recent and accurate estimates of breeding unit size indicate that breeding units are less than 1 m^2 (Ingvarsson and Giles, 1999; B.E. Giles and B.K. Epperson, unpublished data) and our within-patch samples may thus have contained plants from multiple breeding units. To better evaluate whether patterns of susceptibility/resistance vary among patches within islands, an experimental design with larger sample sizes and a more precise sampling of genetically distinct patches should be adopted in future studies.

Patterns of disease and evolutionary dynamics in the metapopulation

In host–pathogen interactions, numerical dynamics are often tightly linked to the genetic characteristics of populations – that is, host resistance and pathogen virulence (Antonovics, 1994; Thrall and Antonovics, 1995; Thrall and Burdon, 1997, 2000). We found that patterns of disease spread over the last 15 years in the island populations correlated with their observed susceptibility. Among recently diseased populations, populations that were most susceptible in the transplantation experiment were also those that have shown an increase in disease prevalence, while less susceptible populations have maintained a low disease prevalence. The levels of susceptibility in the recently diseased populations with more rapid spreading of the pathogen were also comparable to those seen in the two populations with a long history of high disease. This suggests that there is a potential for a further increase in disease prevalence over time in the more susceptible populations 8 and 12.

The high susceptibility of plants from populations with a long history suggests that populations may remain relatively susceptible, despite *M. violaceum* having a clear potential to increase levels of resistance in host populations (Alexander *et al.*, 1996; Giles *et al.*, 2000). Similar results were obtained in a previous study in the archipelago, where plants from populations with a long history of high disease levels were transplanted within their population of origin (Carlsson-Granér, 1997), and also in studies of anther-smut diseased populations of *Silene latifolia*, a close relative to *S. dioica* (Thrall and Antonovics, 1995). Using both

empirical and modelling approaches, Thrall and Antonovics (1995) showed that over time there may be a negative relationship between levels of disease and resistance frequency in the metapopulation, such that resistant populations will have zero or little disease whereas populations with susceptible plants will have the disease. They hypothesized that such a pattern is likely to build up in long-established systems where population turnover is high. In the archipelago, the rapid succession on the islands driven by the land uplift process and the relatively short generation time of *S. dioica* generate high turnover of patches within islands (U. Carlsson-Granér, unpublished). With high patch turnover, island populations may never be in demographic or genetic equilibrium, making selection less efficient within populations (Barton and Whitlock, 1997; Frank, 1997). Moreover, if newly founded populations are composed mostly of resistant or susceptible genotypes, selection may operate primarily at the level of populations rather than among individuals, which may slow the selection process (Thrall and Antonovics, 1995).

Another important consequence of the physical isolation of young patches from older ones, which may have a large impact on the evolutionary dynamics of the system, is that young, newly established populations and patches are normally healthy (Carlsson and Elmqvist, 1992; Carlsson-Granér, 1997) regardless of the resistance of the founders. Substantial host population growth, therefore, occurs before the arrival of the disease. Moreover, as most *S. dioica* seeds primarily disperse short distances within populations (Ingvarsson and Giles, 1999), seeds produced by females exposed to high levels of disease in patches of intermediate age will probably not contribute to population expansion in the younger, lower parts of the islands. Because long-distance seed migration can only occur from younger more open islands and lower more open parts of older islands where the water may reach and from where winter winds can carry standing dead shoots, susceptible plants may also be over-represented in the migrant seed pool. Therefore, although selection for resistance may occur locally in some diseased populations [given that variation in resistance has been introduced by colonization or gene exchange (Elmqvist *et al.*, 1993; Giles *et al.*, 2000)], as long as populations expand the movement of *S. dioica* may occur faster than pathogen-mediated selection can change the genetic composition of populations (Frank, 1997). In contrast, in the host *Lychnis flos-cuculi* in the southern part of Sweden where the land-uplift process and thus the turnover of host patches is much slower, highly diseased populations have shown greater resistance than nearby healthy populations, indicating selection for resistance (U. Carlsson-Granér and L. Ericsson, unpublished). This is consistent with recent simulation results showing that resistance against the anther-smut is more likely to evolve in systems with lower turnover rates than in more dynamic systems (U. Carlsson-Granér and P.H. Thrall, unpublished).

As proposed by Thrall and Antonovics (1995), given a low fitness cost of resistance, populations established by a few resistant plants may also remain relatively resistant. In *S. dioica*, floral characters mediating resistance to *M. violaceum* may be associated with a fitness cost (cf. Biere and Antonovics, 1996) – that is, resistant plants with small flowers and short stigmas may attract fewer pollinators (Galen, 1999), capture pollen less efficiently (Elmqvist *et al.*, 1993) and produce fewer seeds (Olsson, 1995) than susceptible plants with large flowers and long extruding stigmas. However, the fitness cost of resistance should be low in most populations of *S. dioica*, where the density of flowers and the frequency of pollinator visits are high, and hence reproductive output is primarily limited by resources (Carlsson-Granér *et al.*, 1998). Moreover, once a patch has reached its carrying capacity, the opportunity for seed recruitment is limited (U. Carlsson-Granér, unpublished). This may explain why population 17,

which has shown low disease prevalence over the years, still shows relatively limited susceptibility.

The fitness cost of resistance is, however, likely to be much higher in the initial stages of colonization of a new suitable patch. When *S. dioica* colonize young emerging islands, the chances of a colonizing female producing offspring is critically dependent on fertilization by occasional pollinators carrying pollen from conspecific plants on other islands. However, once a female in a new patch is pollinated and seeds are produced, the potential for seed recruitment in the low-density patch is high. Due to higher pollination success and seed production, susceptible plants may colonize an initially empty patch more rapidly than resistant plants. In species such as *S. dioica*, adapted to the early stages of primary or secondary succession, long-term persistence is crucially dependent on the ability to found and colonize new populations. Life-history characters that increase migration rates can thus be selected for even though within-population selection may operate in the opposite direction – that is, the so-called metapopulation effect (Olivieri and Gouyon, 1997). In this manner, metapopulation dynamics may favour susceptibility in the metapopulation as a whole even when the disease is widespread in the system.

Even though our results clearly show that level of host susceptibility may influence the dynamics of disease in populations, knowledge of susceptibility cannot fully explain the observed patterns of disease in the metapopulation. For example, plants from populations 23 and 27 had similar infection rates in the transplantation experiment, but the prevalence of disease has been about three times higher in population 23 than in population 27 for more than 15 years. This is not unexpected in a metapopulation where populations vary in size, density and distance to the nearest diseased population, which may affect both probability of disease establishment (Carlsson and Elmqvist, 1992) and rate of subsequent disease spread in populations (Burdon *et al.*, 1995; Ericson *et al.*, 1999; Smith *et al.*, 2003). The infection rates in populations could also be affected by spatial and temporal variation in factors such as weather, presence of spittlebugs and the sex ratio and frequency of disease in populations, as shown in our study (Figs. 3 and 4). Complex interactions between sex and other variables, as in our study, could explain the conflicting results regarding the relative susceptibilities of the sexes in previous studies (see Ågren *et al.*, 1999, and references therein). Note, however, that population 23 showed similar levels of susceptibility in a previous experiment where plants from this population were transplanted within the population of origin (Carlsson-Granér, 1997) and also in a recent inoculation experiment (Å. Granberg, U. Carlsson-Granér and B.E. Giles, unpublished). This suggests that the levels of susceptibility determined in our study correspond to the susceptibility of plants in the *in-situ* populations and further that the susceptibility of plants is similar over different pathogen strains from the archipelago.

CONCLUSIONS

We conclude that the variability in host susceptibility observed is consistent with founder effects. Moreover, the relatively high susceptibility observed in populations with a long history of disease suggests that initially susceptible populations may remain relatively susceptible. This pattern is not expected in a single large diseased host population, as selection pressure by *M. violaceum* will increase levels of resistance over time (Alexander *et al.*, 1996; Giles *et al.*, 2000), which may ultimately lead to loss of susceptible genotypes and purging of the pathogen from the system (Alexander *et al.*, 1996). In the studied metapopulation of *S. dioica*, founder effects, restricted dispersal of host and pathogen, and high turnover rates of host

patches are factors that maintain variation for resistance and mediate host–pathogen co-existence at the metapopulation level. Susceptible plants with a potential higher per-capita reproduction may further have a selective advantage over resistant plants in the initial stages of colonization. In systems subjected to colonization/extinction dynamics, this advantage may suffice to outweigh the fitness costs of susceptibility at later stages of succession when the disease spreads in populations, especially if colonization is non-random so that seeds produced in young patches are more likely to be carried to new patches of suitable habitat than seeds produced by older patches. Existing models of metapopulation dynamics do not deal with such a combination of patchiness within populations, limited longevity of these habitat patches, and non-randomness of the succession process. Exploring the dynamics of such systems is thus a challenging task to be tackled by future metapopulation models.

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