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Spatial and temporal pattern of a pollinator-transmitted pathogen in a long-lived perennial, *Silene acaulis*

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ABSTRACT

Questions: Does the spatial structure or rate of spread of anther-smut disease differ among plots with low versus high density of the host *Silene acaulis* or low (<6%) versus high (25%) disease frequency? What is the demographic pattern of seedling recruitment and host mortality in *S. acaulis*? How do the temporal dynamics of anther-smut disease differ among hosts that vary in lifespan and population persistence?

Organisms: *Silene acaulis*, a long-lived alpine tundra perennial, and *Microbotryum violaceum*, a pollinator-transmitted fungus that causes anther-smut disease.

Field site and time: Plots ranged in elevation from 3658 m to 3964 m on Pennsylvania Mountain Field Station owned by Colorado State University, Park County, Colorado, USA. Six plots (6 × 10 m) were spatially mapped for 5–8 years and three of these plots were monitored for seedling recruitment and host mortality for 8 years.

Methods: The spatial structure of healthy, diseased and juvenile plants was monitored in natural plots that ranged in host density from 3 to 27 plants per square metre and from 5–25% in initial disease frequency. The spatial pattern of diseased plants was analysed using Moran's *I*, and a join-count method was used to analyse nearest-neighbour relationships of juveniles and healthy or diseased adults. Data on rate of disease spread and loss were monitored in all plots. Demographic data on seedling recruitment and host mortality, as well as spore dispersal patterns, were monitored in three of the six plots.

Results: Infections were stable regardless of host density or initial disease frequency; less than 1% of healthy individuals became infected and less than 1% diseased individuals lost infection over the 8-year study period. Diseased plants were significantly clustered at the scale of less than 1 m in five of six plots; Moran's *I*, a measure of spatial autocorrelation, ranged from 0.1 to 0.6. There was a trend for higher autocorrelation values in plots with lower host density. The number of new seedlings was consistently greater than either the number of deaths or new infections. The annual rate of disease spread in *S. acaulis* is lower than that reported for four other hosts of the same disease. Among-species comparisons suggest that the spatial clustering of diseased plants, rarity of vegetative infection of juveniles, short flowering time and high temporal stability of *S. acaulis* populations contribute to the relatively low rate of disease spread in *S. acaulis*.

Keywords: Caryophyllaceae, *Microbotryum violaceum*, plant–fungus interactions, *Silene acaulis*, spatial autocorrelation, *Ustilago violacea*.

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INTRODUCTION

Multi-year studies describing spatial and temporal change in natural plant–pathogen systems are a powerful method of studying the combined effects of demography and spatial structure on host–pathogen interactions and in developing models of host–pathogen evolution (reviewed in Burdon and Thrall, 1999; Antonovics *et al.*, 2002). Models of vector-transmitted diseases suggest that pathogen populations are most likely to go extinct when host death rate is greater than disease spread. Neither host nor pathogen will persist when disease spread is greater than host population growth rate, but co-existence is possible when the rate of disease spread is greater than host mortality, but less than population growth rate (Thrall and Jarosz, 1994; Thrall *et al.*, 1995). In addition, the spatial structure of disease has the potential to strongly affect the rate of disease spread and consequently the pressure a pathogen exerts on its host (Burdon *et al.*, 1989; Thrall and Burdon, 1999; Brown and Bolker, 2004). Additional factors such as life histories of host and pathogen are also likely to strongly affect disease dynamics (Thrall *et al.*, 1993). Hence, investigations of multiple hosts of the same disease are also warranted.

We investigated the host–pathogen dynamics of *Silene acaulis* and the fungus *Microbotryum violaceum*, which causes a systemic infection known as anther-smut disease. We studied the rate of disease spread and the spatial structure of *M. violaceum* within a population of *S. acaulis* in Colorado over 8 years, from 1992 to 2000. Specifically, we determined the spatial structure of healthy, diseased and juvenile plants, the rate of disease spread and loss, host mortality and population growth rate, and the spore-dispersal gradient. Furthermore, we gathered demographic and spatial information from six plots that varied in host density to determine how host density relates to disease frequency, spatial distribution of the disease and rate of disease spread.

The *Silene–Microbotryum* system is unusual in that disease dynamics have been studied in natural populations of several host species, which provide an important starting point for understanding how host life history may contribute to disease dynamics. The dynamics of anther-smut disease have been studied most thoroughly in four host species other than *S. acaulis*, *Silene virginica*, *Silene latifolia* (= *S. alba*), *Silene dioica* and *Viscaria vulgaris* (= *Silene viscaria*). In all hosts of *M. violaceum* studied so far, including *S. acaulis*, infected plants are reproductively sterilized (they are unable to produce either pollen or seeds), but the disease does not kill the plants. While this aspect of the interaction does not differ among species, other aspects do vary considerably. For example, pollinators differ among the host species and the pathogen is spread by pollinators, which pick up and transmit fungal teliospores that are produced in the anthers of the flowers (Jennersten, 1988; Roche, 1993; Antonovics *et al.*, 1996). Moreover, there are differences in lifespan and population persistence among the host species. The most extensive lifespan studies have been on *S. latifolia* and *S. dioica*. These two host species have a two-fold difference in lifespan, with *S. latifolia* individuals typically living less than 5 years (Carlsson *et al.*, 1990; Carlsson and Elmqvist, 1992; Alexander *et al.*, 1996). These species also differ markedly in their population persistence. In *S. latifolia*, 20% of the populations go extinct in any year (Antonovics *et al.*, 1994; Thrall and Antonovics, 1995), whereas *S. dioica* populations persist for several decades and some populations have persisted over 350 years (Carlsson *et al.*, 1990; Giles and Goudet, 1997).

This study contributes to a comparative approach by describing the host–pathogen dynamics in a species with a dramatically different life history. *Silene acaulis* is a cushion plant (moss-like growing habit) with a short flowering period; individuals produce flowers over a period of 2 weeks. In contrast, the other species listed above have elongated stems

and leaves and shorter-lived species can flower over a period of 2–5 months. In addition, the lifespan of *S. acaulis* individuals has been measured to be as long as 300 years in some populations (Morris and Doak, 1998), which is 6–20 times longer than that for other species studied. These differences in host life history allow us to begin addressing the question of whether differences in the lifespan and population persistence of host species of *M. violaceum* are related to disease spread and ultimately the co-existence of the host and pathogen. In this paper, we address two primary issues: (1) the spatial structure and temporal dynamics of *S. acaulis* and *M. violaceum*, and (2) what patterns are revealed by making comparisons across host species that might explain differences in dynamics of anther-smut disease.

MATERIALS AND METHODS

Natural history of the study species

Silene acaulis L. ssp. *subacaulescens* (F.N. Williams) C.L. Hitchc. & Maguire has a gynodioecious breeding system consisting of females and hermaphrodites. Both sexes can be systemically infected by *Microbotryum violaceum* (Pers.) Deml. & Oberw. (= *Ustilago violacea* Pers.). In infected plants, ovules are aborted and stamens develop to full size (in both sexes), but the anthers are filled with diploid spores rather than pollen (Fisher and Holton, 1957). The diploid spores are transmitted from plant to plant by pollinators (Kevan and Parmelee, 1972). Queen bumblebees (*Bombus sylvicola*) are the most common pollinator of *S. acaulis* in Colorado (Shykoff, 1992; L. Delph and D. Marr, personal observation). Spores that are transmitted to flowers germinate on floral styles, undergo meiosis, and then mitotically bud until they fuse with a compatible mating type (Fisher and Holton, 1957). Floral infection is the major mode of transmission for this disease (Alexander and Antonovics, 1988; Jennersten, 1988), although some infections may occur from spores germinating in wounded vegetative tissue. In addition, vegetative infection occurs via spore dispersal on juveniles in *S. latifolia*, *S. virginica* and *S. dioica* (Alexander, 1990; Carlsson and Elmqvist, 1992; Roche *et al.*, 1995), although this has not been shown in *S. acaulis*. It is difficult to detect *M. violaceum* hyphae within plant tissue; therefore, we have relied on fungal sporulation in the flowers for scoring presence and absence of infection.

Silene acaulis is patchily distributed, and is most common in dry, rocky areas (fellfields) that are free from taller plants. In the Rocky Mountains, these habitats primarily occur on upper slopes and ridges (Billings, 1987). Fellfields can remain stable for hundreds of years until enough soil has accumulated to support the invasion of taller plants (Zwinger and Willard, 1986). Individual *S. acaulis* plants have been estimated to live for over 100 years in Colorado (Benedict, 1989) and for over 300 years in Alaska (Morris and Doak, 1998). It is not known whether strains of *M. violaceum* can live as long as *S. acaulis*. In *S. dioica* and *S. latifolia*, infected individuals often remain diseased until the host dies (Carlsson, 1995; Alexander *et al.*, 1996). Populations of *M. violaceum* within *S. latifolia* are dynamic in that an individual can be infected with multiple fungal strains, and in experimental populations individuals commonly changed disease status (e.g. diseased to healthy) between years (Hood, 2003).

Range of host and pathogen

At the broadest geographic scale, *S. acaulis* is a common member of arctic and alpine tundra throughout the northern hemisphere. The distribution of *M. violaceum* has not been

explicitly studied, but appears to occur throughout the range of *S. acaulis*. Reports of its presence have been noted in Alaska (W. Morris, personal observation), western and eastern Canada (Saville and Parmelee, 1964; Kevan and Parmelee, 1972; Hermanutz and Innes, 1994), the Rocky Mountains in Colorado (Marr, 1997), the Swiss Alps (Bucheli *et al.*, 2000), Norway (L. Delph, personal observation), Finland (P. Mutikainen, personal observation) and Russia (Kevan and Parmelee, 1972). We have studied *S. acaulis* and *M. violaceum* at several sites located in central Colorado in the Mosquito Range of the Rocky Mountains. Both species also occur on mountains throughout the Front Range (Indian Peaks, Central Area and Southwest Section) and Park Range (five mountains in the Mosquito Range) (D. Marr and L. Delph, personal observation). Overall, *S. acaulis* and *M. violaceum* have a widespread distribution and, at least within the Mosquito Range of the Rocky Mountains, host and pathogen commonly co-occur.

Method of collecting temporal and spatial data

We chose four sites that represent the altitudinal gradient where *S. acaulis* and *M. violaceum* co-occur on Pennsylvania Mountain (Park County, Colorado 39°15′N, 106°15′W): Slope (3658 m in elevation), Flat (3701 m), False Summit (3923 m) and Summit (3964 m). The lowest site, Slope, is approximately 2.5 km from the highest site, Summit. Pollen flow occurs among these sites, although there is genetic structure on the scale of less than 3 m that is probably caused by localized seed dispersal (Gehring and Delph, 1999). Within sites, plots were chosen to represent a typical patch for that local area. In total, six plots were spatially mapped. One plot was established at each of the False Summit and Summit sites and two plots were established at each of the Slope and Flat sites. Slope plots were separated by 15 m and Flat plots were separated by 20 m. Each plot was 6×10 m, except for two plots that were enlarged to accommodate plant distribution (see Table 1). The number of *S. acaulis* plants per plot ranged from 157 to 1606, and disease frequency ranged from 5 to 25% (Table 1). Grids were constructed by placing string at 0.5 m intervals. All flowering and non-flowering individuals were drawn on grid paper relative to the plant's size and position within the plot (Fig. 1). Individual plants were tagged by nailing a small aluminium tag next to the plant, which allowed us to follow the plant year after year. The sex of each plant, disease status (infection gain and loss) and mortality were recorded each year from 1992 to 1997. New seedling recruits were recorded annually in three of the plots from 1992–1997, 1999 and 2000. In 1995, unusually high snowfall in the spring delayed flowering for a month at most sites and snow cover prevented plants at the Summit from leafing out and flowering at all in that year. In both 1996 and 1997, plants at the Summit were still recovering from the 1995 season; they had very small leaves and few plants produced flowers. Thus spatial and temporal analyses are limited to the years 1992–1994 for the Summit plot.

Spatial analysis

The spatial pattern of diseased plants was analysed in all of the plots using Moran's I, a commonly used method of measuring spatial autocorrelation (Sokal and Oden, 1978; Cliff and Ord, 1981; Real and McElhany, 1996). Moran's I is analogous to Pearson's correlation coefficient with I values ranging from -1 to $+1$. A value of zero indicates random spatial pattern (no predictive value of variables), $+1$ indicates clustering of like-variables, and -1 indicates hyperdispersion. The response variable for disease status was healthy or diseased.

Table 1. Spread of anther-smut disease in six plots of *S. acaulis* that were spatially mapped for 3–8 growing seasons on Pennsylvania Mountain, Colorado

Plot	Host density plants per m^2	Disease frequency	Altitude (m)	Plot size (m)	Number of years mapped (actual dates)	Average % plants gained infection per year (mean ± 1 SE)	% years with no new plants infected	% new infections per year	Average % plants lost to infection per year (mean ± 1 SE)	Total no. *S. acaulis* individuals in 1996	Total no. diseased individuals in 1996
Southeast Slope 1	6	0.05	3658	8 × 13	8 (1992–2000)	0 ± 0	100	0	0.8 ± 1.85	694	18
Southeast Slope 2	3	0.20	3658	6 × 10	8 (1992–2000)	0.8 ± 0.79	43	0–2.3	0.7 ± 1.75	157	23
Southeast Flat 1	4	0.06	3701	6 × 13	8 (1992–2000)	0.3 ± 0.46	71	0–1.3	0 ± 0	332	12
Southeast Flat 2	9	0.25	3701	6 × 10	5 (1992–1997)	0.6 ± 0.65	25	0–1.7	0.2 ± 0.38	516	115
False Summit	4	0.18	3923	6 × 10	5 (1992–1997)	0.6 ± 0.44	25	0–1.2	0.7 ± 1.2	213	35
Summit	27	0.20	3964	6 × 10	3* (1992–1997)	0.1 ± 0.13	33	0–1.2	0.3	1606*	297*

Note: Gain of infection refers to plants that produced healthy flowers in previous years, then started producing smutted flowers. Loss of infection refers to plants that produced smutted flowers in previous years, then started producing all healthy flowers (recovered). Only unidirectional switches have been observed so far (i.e. no plants have been observed to switch from diseased to healthy back to diseased again). Total number of *S. acaulis* includes both flowering and non-flowering individuals. Plant density is based on total number of plants in a plot. Disease frequency was calculated as the number of diseased plants per total number of flowering plants. *From 1995 to 1997, very few plants flowered in the Summit plot due to heavy snowfall; therefore, changes in disease status could only be determined between 1992 and 1994. SE = standard error.

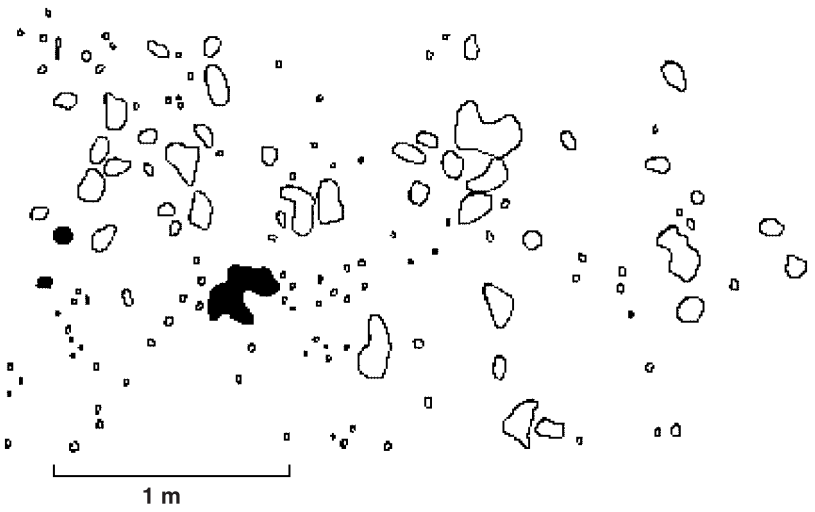

Fig. 1. A representative map showing the relative size and position of *S. acaulis* individuals in a section of the Slope low-disease plot on Pennsylvania Mountain in Colorado. Solid black individuals have anther smut disease and open individuals are healthy. The scale bar represents 1 m.

We calculated Moran's *I* for spatial pattern of disease using two connection schemes, neighbour and distance-based connections, which differ in their definition of spatial relationships among data points. Neighbour-based connections consider the status of neighbours (diseased or healthy) and ignore Euclidean distance between the neighbour and focal individual. The neighbour definition we used was Gabriel connectedness, which defines nearest-neighbours as two plants that do not have any other plants located within the circle whose diameter is the line between the plants (Gabriel and Sokal, 1969). Defining location based on status of neighbours allows points to be irregularly spaced (Odland, 1988; Real and McElhany, 1996), which is appropriate for pollinator-transmitted diseases because pollinators adjust their flight distances by plant location rather than absolute distance (Antonovics and Alexander, 1992; Real and McElhany, 1996). A limitation of relying solely on neighbour connections is that neighbours are defined at different spatial scales depending on host density (Cliff and Ord, 1981). In other words, all connections are considered equal whether relatively near or distant. In contrast, distance-based connections consider physical distance from the focal individual, which provides an absolute scale to compare sites that differ in density. Although each connection scheme differs in how it defines spatial relationships among data points, we found that the spatial structure of disease status was similar whether neighbours were analysed using Gabriel connectedness (Gabriel and Sokal, 1969) or the Euclidean distance-based measure (Odland, 1988). Information regarding size and shape of plants is ignored in both neighbour- and distance-based matrices, but the similarity in results between the two connection schemes suggests that omitting this information is not biasing the analysis.

For ease of comparing plots differing in density and enabling comparisons of spatial structure in this system with other plant–pathogen systems, we describe the analysis and results for only the Euclidean distance-based measure of Moran's *I*. Specifically, we used the Euclidean distance measure lag *h*, which considers a plant joined to every plant located within a radius of distance *h*. Distance classes were increased in non-overlapping intervals

of 0.2 m or 0.3 m depending on sample sizes per distance class. A preliminary analysis showed that this interval scale allowed detection of spatial pattern and had sufficient numbers of point-pairs per distance class to meet distribution and variance assumptions (Cliff and Ord, 1981; Sokal and Thomson, 1987; Odland, 1988). Significant differences from zero were determined by comparing the observed Moran's I value with the probability of obtaining that value from 10,000 randomizations of the point labels. A Bonferroni correction was used to account for the number of tests being done. There was no measurable change in disease spatial structure across years in any of the plots; therefore, spatial analysis is presented for only the 1994 maps so that analysis of the summit plot is comparable to that of the other plots.

Spatial relationships of juveniles and adults

Diseased plants cannot produce seed, and previous work has shown that adult plants have a localized genetic structure (Gehring and Delph, 1999). We were interested in whether the location of juveniles was affected by spatial pattern of adult diseased plants. Specifically, we tested whether juveniles were likely to have either healthy or diseased adults as nearest neighbours. A join-count method developed for completely mapped data, analogous to 2×2 contingency tables, was used to determine whether plants segregated by juvenile status (Dixon, 1994). Juveniles were defined as individuals that had true leaves and had emerged since 1992. The nearest neighbour for each juvenile was classified as juvenile, healthy adult or diseased adult, thereby generating a list of observed juvenile–juvenile, juvenile–disease, juvenile–healthy and disease–disease joins. The observed number of joins was compared with the null hypothesis that the possible join combinations were randomly distributed among the given set of points. With completely mapped data, a single plant may share one or more neighbours with another plant, which violates the assumption of independence among cells. Therefore, the expected counts are derived by random labelling of the data points based on the total numbers of juvenile and diseased plants (Dixon, 1994). A measure of the degree of segregation, s, analogous to log-odds ratios, was used to identify significant differences between observed and expected counts (Dixon, 1994). High positive s values indicate clustering of like-values (excess of disease–disease or juvenile–juvenile), and high negative s values indicate associations with non-like values (excess of disease–juvenile); s values are not bounded by 1 (Dixon, 1994). Because of low sample size at greater distance comparisons, only nearest-neighbour joins were considered.

Spore dispersal

Open-pollinated flowers were collected haphazardly within Slope and Flat sites on 29 June and 5 July 1994 during peak spore deposition (Marr, 1997). Flowers were collected after they had received most of the pollen and fungal spores they were likely to acquire before closing completely. Each flower was placed in a tube filled with 70% EtOH. Styles were mounted in fuchsin gelatin (Kearns and Inouye, 1993), and spore counts were made under a light microscope at 400× total magnification. Spore deposition and distance to the nearest diseased plant was determined for 122 flowers in the Slope and Flat plots. Data were pooled across plots because flowers had been collected within the same week; a previous study showed that for these plots timing of flowering had the largest effect on spore deposition pattern (Marr, 1997). Spore number was log-normal transformed. A linear regression between number of

spores per flower and distance to the nearest diseased plant was used to estimate the spore-dispersal gradient.

RESULTS

Rate of disease spread, new seedlings and host mortality

The average gain and loss of infection per year was less than 1% in each plot (Table 1). Rate of disease spread was similar among plots despite marked differences in host density and disease frequency (Table 1). The number of new seedling recruits was highly variable among years, and ranged from 3 to over 100 new juveniles per plot per year (Fig. 2). Death rate was low; the average rate of mortality per year was 1% (Fig. 2). Mortality was higher among juveniles than adults ($\chi^2 = 23.6$, d.f. = 1, $P < 0.0001$). Of the 82 plants that died in three plots between 1992 and 2000, 63 were juveniles and 19 were adults. Total juvenile mortality in the three plots was 12% (63 of 529 new recruits died), and total adult mortality was 2% (19 of 825 adults died), over the 8-year time period. Overall, *S. acaulis* populations increased in size, and *M. violaceum* populations (measured by number of plants that produced smutted flowers) showed either no change or a slight increase in population size.

Spatial pattern of disease

Diseased plants were significantly clustered at the scale of less than 1 m in five of six plots (Fig. 3). In all plots, positive spatial autocorrelation was strongest at the smallest interval (either 0.2 or 0.3 m), and dropped off to randomness by 1 m. In terms of neighbours, diseased plants were significantly clustered at the distance of one neighbour, and then quickly dropped off to randomness in spatial pattern beyond 2–3 neighbours in most plots. The pattern of disease did not show strong directional gradients or anisotropism; therefore, isotropic results are presented in Fig. 3 (i.e. the average Moran's *I* over all directions – north,

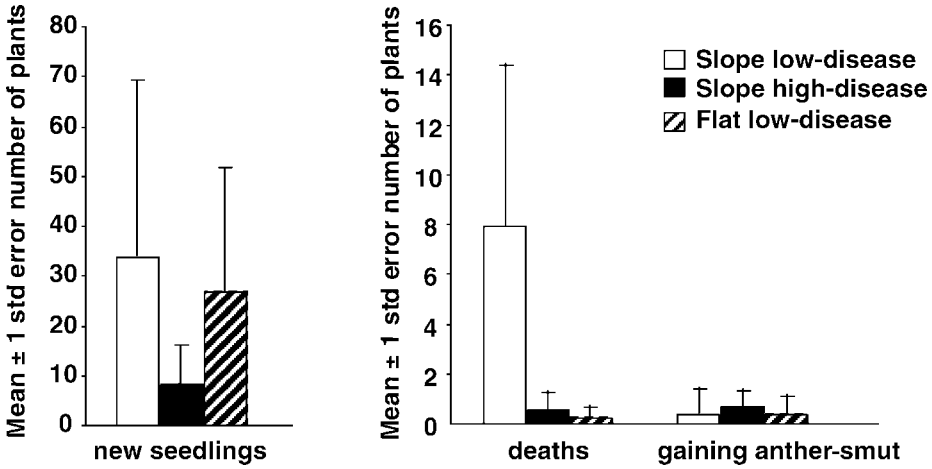

Fig. 2. Mean (± 1 standard error) number of seedling recruits, host mortality and plants becoming infected with anther-smut disease for 1993 to 2000 for three plots. The total number of *S. acaulis* individuals in each plot is listed in Table 1.

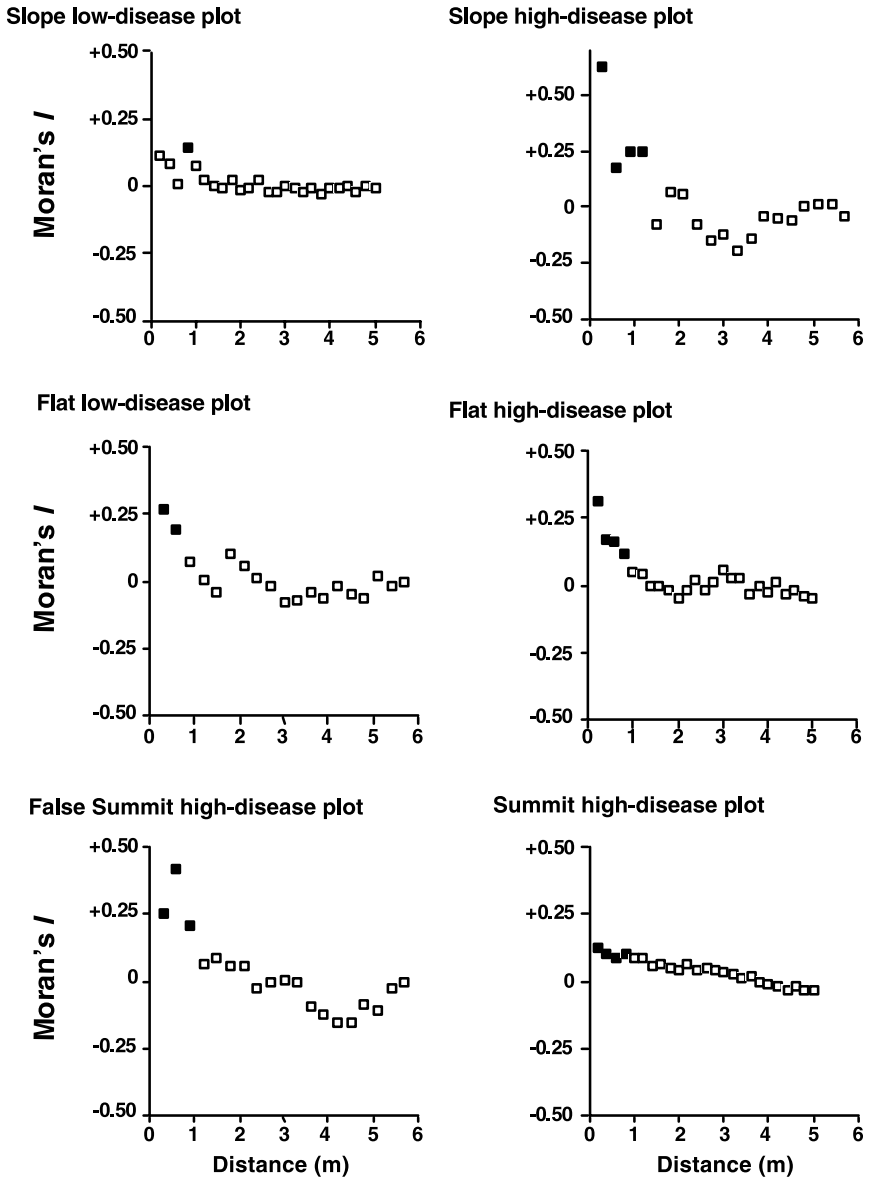

Fig. 3. All-directional spatial correlogram of healthy and diseased *S. acaulis* in six plots. Note that the scale of Moran's *I* in the Slope high-disease plot is slightly different from that in the other plots to accommodate the unusually high *I* value in the first distance class. Positive Moran's *I* values indicate that diseased plants are clumped. Values near zero indicate random spatial structure (i.e. no predictive value of knowing the disease status of plants at that distance interval). A lag interval of 0.2 m was used, except in the plots Slope high-disease and False Summit, which had fewer plants. In these lower density plots, longer lag distances (0.3 m) were needed to ensure adequate sample sizes per lag interval. Black squares indicate significant differences from zero ($P < 0.01$).

south, east and west). Weak anisotropy indicates that the disease is not spreading predictably in only one direction (Burdon *et al.*, 1989). Clustering of diseased plants tended to be higher in plots with the lowest host density (Slope high-disease and False Summit high-disease) and weaker in plots with high host density (Fig. 3), although this trend could not be tested statistically.

Spatial pattern of juveniles

Juveniles were significantly more likely to be located near other juveniles in 2 of 3 plots, and there was a trend in this direction in the third plot (Table 2). Juveniles were significantly less likely to be located near either diseased (2 of 2 plots) or healthy plants (3 of 3 plots). Plots differed in the strength of juvenile–disease clustering. In the Slope high-disease plot, juveniles were rarely found near diseased plants (5 observed *vs* 15 expected juvenile–disease joins), whereas the Slope low-disease plot showed weaker segregation of juveniles and diseased plants (12 observed *vs* 17 expected juvenile–disease joins).

Spore dispersal gradient

At times of peak spore deposition, flowers closest to diseased plants (less than 0.5 m) received more spores than flowers more distant from diseased plants ($F_{1,120} = 13.39$, $P < 0.0004$) (Fig. 4). Linear regression between spore number and distance explained 32% of the variance in spore deposition.

DISCUSSION

The dynamics of *M. violaceum* in the *S. acaulis* study population were remarkably subtle over the 8-year study period. Plants that were infected at the beginning of the study continued to produce smutted flowers each year, and plants that produced healthy flowers

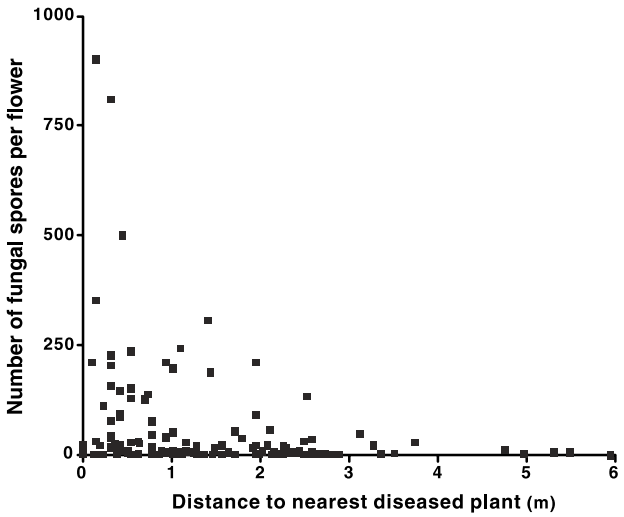

Fig. 4. The relationship between fungal spore deposition on open-pollinated flowers and distance to the nearest diseased plant.

Table 2. Join-count analysis for three plots of *S. acaulis* to determine whether new recruits tend to cluster near adult plants (either healthy or diseased)

Site and disease level within plot	Healthy adults *vs* New recruits	Observed joins	Expected joins	*Z* statistic (1 d.f.)	Degree of clustering (*s*)	Diseased adults *vs* New recruits	Observed joins	Expected joins	*Z* statistic (1 d.f.)	Degree of clustering (*s*)
Slope–low	HA–HA	235	219.63	1.56	0.17	DA–DA	4	1.13	2.23	1.45
	NR–HA	99	151.37	** −9.71	−0.83	NR–DA	12	16.87	** −5.10	−0.36
	NR–NR	156	103.63	**6.03	0.83	NR–NR	241	236.13	1.46	0.36
Slope–high	HA–HA	60	62.95	−0.63	−0.14	DA–DA	18	8.49	**3.58	1.7
	NR–HA	21	29.05	** −4.13	−0.81	NR–DA	5	15.51	** −5.60	−1.47
	NR–NR	21	12.95	2.33	0.81	NR–NR	37	26.49	**3.35	1.47
Flat–low	HA–HA	105	83.91	**3.15	0.54	Not enough				
	NR–HA	55	77.09	** −5.13	−0.61	diseased plants				
	NR–NR	92	69.91	**3.39	0.61	for comparison				

Note: Nearest-neighbour connectedness was used to define joins. The expected number of joins is based on the null hypothesis that juveniles and adults are randomly scattered among the plant locations. *Z* statistics compare observed and expected counts, and significance levels are based on two-tailed probabilities (*$P < 0.01$, **$P < 0.001$). Data for 1992–1999 were combined to increase sample sizes of new recruits. HA = healthy adults; NR = new recruits; DA = diseased adults.

remained healthy. The low 0–1% annual rate of disease spread was consistent across plots that varied ten-fold in host density and from 5 to 25% in disease frequency, suggesting that these factors play a limited role in disease spread in *S. acaulis*. Monocyclic pathogens, those that reproduce once per growing season, often have a lower rate of spread than polycyclic pathogens (Agrios, 1997), but the annual rate of disease spread observed in *S. acaulis* is lower than that for anther-smut disease infecting shorter-lived hosts of the fungus (Table 3). The demographic pattern was consistent across the three plots in that the number of new seedlings per year was greater than both average mortality and new infections (Fig. 2). The mean number of deaths and new infections per year were nearly identical in two of the plots, and the mean number of deaths was over 18 times higher than the number of new infections in the third plot. Based on models of vector-transmitted diseases explained earlier, this demographic pattern of relatively high population growth rate and low rates of mortality and disease spread best match predictions of host population persistence and the possible co-existence of host and pathogen (Thrall and Jarosz, 1994; Thrall *et al.*, 1995). Successful disease transmission may require conditions favourable to fungal growth that do not occur in most years (see below) or require long-distance migration of a pathogen strain to a susceptible host. In either case, given the high survivorship of adults and low rate of recovery, one successful infection potentially results in a stable environment for the fungal strain for many years, and this should contribute to the persistence of anther-smut disease in the population.

Spatial structure of anther-smut disease

Diseased individuals of *S. acaulis* were clustered at scales of less than 1 m. This spatial pattern is similar to the scale of clustering observed in natural populations of both *S. latifolia* and *S. virginica*. In *S. latifolia*, disease was significantly clumped at the scale of 1 m in one natural population (Moran's $I = 0.2$) (Real and McElhany, 1996). In *S. virginica*, spatial structure was analysed in quadrats and diseased plants were significantly clumped at the scale of 2.5 m (Morasita's I, range = 2.5–3.8) (Antonovics *et al.*, 1996). One strength of our study is that we have shown that this pattern holds for six different plots that varied widely in host density. Both theoretical models (Burdon *et al.*, 1989; Thrall and Burdon, 1999) and experimental studies with *S. latifolia* have shown that a clumped distribution of diseased plants reduces the rate of disease spread. Biere and Antonovics (1996) found a 37% higher spread of the disease in experimental *S. latifolia* populations with a random distribution (Moran's $I = 0$) than in those with a highly clumped distribution (Moran's $I = 1$). Due to the extremely low rate of disease spread observed over 8 years in *S. acaulis*, we were not able to determine whether rate of spread was lower in plots with strong clustering than in plots with weaker clustering.

Processes that lead to such spatial structuring are thought to include environmental heterogeneity (Burdon *et al.*, 1989), spore-dispersal gradients (Roche *et al.*, 1995) and the genetic structure of resistance (Burdon and Thompson, 1995). It is possible that environmental heterogeneity has an effect on the distribution of the disease in our study populations. We noted in one year (1995), when there was exceptionally high water run-off during flowering (caused by heavy late-spring snow-fall), that smutted individuals tended to occur in patches of high moisture, where water was standing or even running. Given the rarity of such events [once in 10 years (C. Galen, personal observation)], long-term monitoring combined with experimental manipulations would be needed to test whether soil moisture is important for disease spread.

Table 3. Summary of within-population dynamics for five hosts of anther-smut disease

Host	Population studied	Host lifespan (years)	Rate of disease spread		Rate of host recovery per year	Overall host mortality per year	Vegetative infection (% of juveniles infected per year)	Disease frequency in population
			Between years	Within years				
Silene virginica	N	<5[a,b]	6–14%[a]	—	2–15%[a]	24–70%[a,b]	23–63%[a]	0–59%[a]
Silene latifolia	I	<5[c]	4%[c], 8%[d]		2%[c]	30–83%[e,f]	2.5–41%[d,g]	0–35% common, up to 80% rare[c]
	N			24.4%[q]				17.0%[q]
Silene dioica	N	<10[h,i]	0–4%[h]	17.7%[q]	—	11–22%[h]	0–8%[h]	0–60%[h,l,q]
Viscaria vulgaris	N	<15[j]	2%[l]	—	—	5%[j]	—	0–70%[k,l]
Silene acaulis	N	<100[m,p]	0–1%[n]	0%	0–0.2%[n]	0–5%[n]	0[n]	0–28%[n,o]

Note: For disease spread, the annual rate of successful infections (percentage of successful infections from one year to the next) is reported. For each category, a range is provided if available. Population studied refers to whether the host and fungus were recently introduced (I) or native (N) to the region studied.
[a] Antonovics *et al.* (1996), [b] Dudash and Fenster (1997), [c] Alexander and Antonovics (1988), [d] Roche *et al.* (1995), [e] Thrall and Jarosz (1994), [f] Alexander and Antonovics (1995), [g] Alexander (1990), [h] Carlsson and Elmqvist (1992), [i] Carlsson (1995), [j] Skogsmyr (1993), [k] Jennersten *et al.* (1983), [l] Jennersten (1988), [m] Benedict (1989), [n] D. Marr and L. Delph (this study), [o] Hermanutz and Innes (1994), [p] Morris and Doak (1998), [q] Biere and Honders (1996).

In addition to environmental heterogeneity, the spore-dispersal gradient appears to have some effect on the spatial distribution of the disease in our study population. We found that the spore-dispersal gradient corresponded closely with the scale at which diseased plants are most strongly clustered. However, large numbers of spores were occasionally observed on flowers quite distant from any diseased plant, and most plants receive at least some fungal spores and yet do not become infected (Marr, 1997; Bucheli *et al.*, 2000). Hence, spore-dispersal gradients do not completely explain the clustering of the disease. In *S. latifolia*, spore deposition was higher for plants located near existing diseased plants, as was the probability of new infection (Alexander, 1990; Roche *et al.*, 1995). Given the small number of new infections we observed, we were not able to directly test the extent to which exposure to spores affects probability of new infection. We observed variation in the degree of clustering of the disease, with spatial autocorrelation values ranging from 0.1 to over 0.6. There was a trend for the greatest clustering of diseased plants to occur in plots with the lowest host density, and clustering was weakest in plots with higher densities. Spore-dispersal gradients depend on pollinator behaviour (Roche *et al.*, 1995; Altizer *et al.*, 1998), and pollinator behaviour is affected by plant density (Waddington, 1983; Jennersten and Nilsson, 1993). Hence, one hypothesis would be that pollinator behaviour varied among sites differing in host density and this impacted the clustering of the disease.

Gehring and Delph (1999) have shown that positive spatial autocorrelation of isozyme alleles exists at distances of less than 3 m in this *S. acaulis* population. They also found that density affected this autocorrelation: clustering of genetically similar individuals had higher correlation values in low-density sites than in high-density sites. This is the same pattern we observed for the clustering of disease in this population. We do not have information on the distribution of resistant and susceptible individuals. However, the similarity in spatial scale of both the genetic structure of the host and spatial structure of the disease suggests that the distribution of resistance, as mediated by gene flow, could also be a factor affecting the patchiness of the disease. Spatial clustering of disease resistance at a scale of metres has been documented in a population of *Phlox paniculata* infected with powdery mildew (McElhany, 1997), and genetic variation in resistance to anther-smut disease has been observed in *S. latifolia* (Kaltz and Shykoff, 2002) and *S. dioica* (Elmqvist *et al.*, 1993).

Comparison among hosts of anther-smut disease

Anther-smut disease is unusual in that it has been studied in five host species, and together these hosts vary from short-lived perennials (<5 years) that colonize disturbed habitats to long-lived perennials (<100 years) that occur in very stable environments, such as the alpine tundra (Table 3). Overall, the dynamics of the disease are more extreme in shorter-lived than in longer-lived hosts. For example, the annual rate of disease spread ranges from as high as 24% within one season in *S. latifolia* to an average of 0.4% per year in *S. acaulis* populations studied for 8 years. Similarly, vegetative infection of juvenile individuals ranges from a high of 63% to 0% (Table 3). Although the sample size of species is small at this point, both the annual rate of disease spread and amount of vegetative infection are correlated with host lifespan (Spearman's $r = -0.95$, d.f. = 4, $P = 0.01$ and $r = 0.95$, d.f. = 4, $P = 0.05$, respectively). These correlations suggest that either lifespan or traits associated with lifespan affect the interaction between *M. violaceum* and its hosts. This pattern has been predicted in theoretical models (Thrall *et al.*, 1993), but data on infection rates in the longest-lived species have been limited. Below, we make comparisons among species and

discuss how transmission to seedlings is likely to impact disease spread. In addition, we discuss how differences in the colonization and extinction of host populations may affect the host–pathogen interaction.

Although floral transmission is probably the most common mode of anther-smut transmission, transmission via spore deposition on seedlings occurs in *S. latifolia, S. virginica* and *S. dioica* (Alexander, 1990; Carlsson and Elmqvist, 1992; Roche *et al.*, 1995; Antonovics *et al.*, 1996). Infections of seedlings can be as high as 41% in *S. latifolia* and 63% in *S. virginica* (Table 3). In *S. latifolia* and *S. dioica*, juveniles located adjacent to diseased plants had the highest probability of becoming diseased (Alexander, 1990; Carlsson and Elmqvist, 1992; Roche *et al.*, 1995). In contrast, *S. acaulis* juveniles flowering for the first time [which takes a minimum of 5 years in this population (L. Delph and D. Marr, unpublished data)] have never been observed to be diseased. Furthermore, in *S. acaulis* plant size is correlated with age (Benedict, 1989) and diseased plants tend to be larger than healthy plants (Marr, 1997). This indicates that older plants are more likely to be infected than younger plants. Unlike other hosts, *S. acaulis* individuals infected with *M. violaceum* do not produce more flowers (Marr, 1997). The spatial arrangement of juveniles and adults shows that juveniles are less likely to establish near diseased adults than healthy adults. Given that higher spore deposition occurs on nearest neighbours of diseased plants, the establishment of juveniles farther from adults may decrease their exposure to fungal spores. Overall, there is no evidence to date that juveniles are infected by anther-smut and the short flowering time makes within-season disease transmission impossible (i.e. plants infected early in the season begin producing flowers with fungal spores later in the same growing season). Together, these two factors probably contribute to the lower rate of disease spread in *S. acaulis*.

Life-history characteristics contribute to metapopulation dynamics, and these dynamics are likely to affect the rate of spread of the disease. For example, in the short-lived perennial *S. latifolia*, an extensive decade-long census of 400 populations in Virginia, USA has shown that the anther-smut disease frequently goes extinct in local populations (mean rate of 30%) (Antonovics *et al.*, 1994; Thrall and Antonovics, 1995; Alexander *et al.*, 1996). In addition, host populations are ephemeral, with mean annual colonization and extinction rates of 20%; hence host patches must be close enough to allow frequent migration of spores (Antonovics *et al.*, 1994; Thrall and Antonovics, 1995). As a consequence, neither the host nor the pathogen is far removed in time from the processes of colonization and extinction, and such metapopulation dynamics may contribute to the high rate of disease spread in *S. latifolia*.

Colonization and extinction of host populations also appears to affect the colonization and persistence of *M. violaceum* with its host *S. dioica*. In a series of island populations in the Swedish archipelago, the populations of *S. dioica* can be aged accurately (Carlsson *et al.*, 1990). The disease is rarely found in young or old populations, being most common in middle-aged populations (Carlsson, 1995). Young populations are genetically more differentiated than middle-aged populations, apparently because of colonization dynamics (Giles and Goudet, 1997). Carlsson (1995) suggested that the colonization process influences disease dynamics by determining the genetic structure of the host and pathogen. Migration of pollen or fungal spores among islands is low (Giles and Goudet, 1997), although frequent enough that nearly all middle-aged populations are infected (Carlsson, 1995). As succession progresses on these islands, the extinction of the pathogen occurs at a higher rate, perhaps due to a combination of increasing fragmentation of host populations (Carlsson, 1995) and an increasing number of resistant hosts (Elmqvist *et al.*, 1993). The relative stability of the host populations and low rate of migration among populations suggest that, in addition to metapopulation

dynamics, local population dynamics contribute to the population genetic structure of *S. dioica* and *M. violaceum* and hence the rate of disease spread.

In contrast to *S. latifolia* and *S. dioica*, the colonization and extinction of *S. acaulis* populations occur gradually over the course of centuries, determined in part by the rate of soil formation and climate. In the Rocky Mountains, much of the alpine soil has developed over the last 12,000 years since the Wisconsin glacial period (Retzer, 1974; Billings, 1987). Based on evidence from pollen and plant macrofossils, the forest boundary defining the alpine tundra in southern Colorado (which is climatically sensitive) has been at its current elevation for the past 2000 years (Fall, 1997). The specific age of the *S. acaulis* population in this study is unknown, but it certainly has a longer history than the populations of *S. latifolia* or *S. dioica* that have been studied.

In summary, *S. acaulis* has the lowest mean rate of disease spread [although most flowers receive fungal spores (Marr, 1997)] and the lowest measured rate of host recovery of any of the hosts of *M. violaceum*. Factors that contribute to the low rate of disease spread in *S. acaulis* appear to include the spatial clustering of diseased plants, the rarity of vegetative infection of juveniles, short flowering time, and the high temporal stability of *S. acaulis* populations. Patterns of disease spread revealed by comparisons among the five hosts of *M. violaceum* suggest that host lifespan, the timing of infection, and local- and meta-population dynamics all contribute to the variation observed in the rate of spread of the disease.

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