19. LONG-TERM STUDY OF A PLANT–PATHOGEN METAPOPULATION

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19.1 INTRODUCTION

Although there has been a long-standing recognition that the numerical and gene frequency dynamics of natural populations may be affected by the interconnectedness of populations on a regional scale (Wright, 1931; Levins, 1969; MacArthur and Wilson, 1967), it is only since the early 1980s that attention has been given to the explicit study of interconnected sets of populations and to the theoretical exploration of the consequences of spatially explicit models for ecological and genetic processes (Silvertown and Antonovics, 2001). In the context of field studies, there is also increasing recognition that migration among interconnected populations and local extinction and recolonization are the rule rather than the exception in natural populations (Gilpin and Hanski, 1991; Hanski and Gilpin, 1996). Early metapopulation models assumed simplified within population dynamics driven largely by the effects of colonization, migration, and extinction (Levins, 1969; Caswell, 1978). More recently, with the advent of increased computational power, it has been possible to explore the consequences of within population dynamics on spatially extended systems and in multiple interconnected populations (Comins et al., 1992; Kareiva, 1994).

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The major feature to emerge from theoretical studies of spatially explicit systems is that conclusions regarding equilibrium states and dynamics derived from single populations are changed drastically when interactions among these populations are included. With regard to ecological dynamics, the best-known conclusion is that systems that show locally unstable population dynamics (such as would lead to extinction) can be stabilized readily when extended spatially (Comins et al., 1992; Antonovics et al., 1994; Molofsky et al., 2001). Conversely, it has been shown that changes in connectedness of populations can, in and of itself, drastically influence the overall prevalence of a species, without changes in the local dynamics (Carter and Prince, 1988; May and Anderson, 1990; Hanski, 1991). With regard to evolutionary dynamics, genetic change within populations can also be stabilized and allelic diversity can be maintained for extended periods in spatially explicit models (Frank, 1991). Many of the statistics of among population differentiation are also altered by explicit consideration of extinction and colonization processes (McCauley, 1993). Metapopulation structure can enhance the importance of kin selection or group selection by altering the local frequency of phenotypes (Gilpin, 1975; McCauley and Taylor, 1997; O'Keefe and Antonovics, 2002).

Short-term studies of a metapopulation can lead to estimates of population turnover and can be used to parameterize models that can be used as "surrogates" for experimental studies (Antonovics et al., 1998). Even single season studies of metapopulations can provide useful data for assessing distance dependence and size dependence of habitat occupancy and as a guide to conservation decisions (Hanski, 1991). However, whether a metapopulation is itself stable can only be determined if there are historical data on the state of the system at some point in the past or by long-term studies.

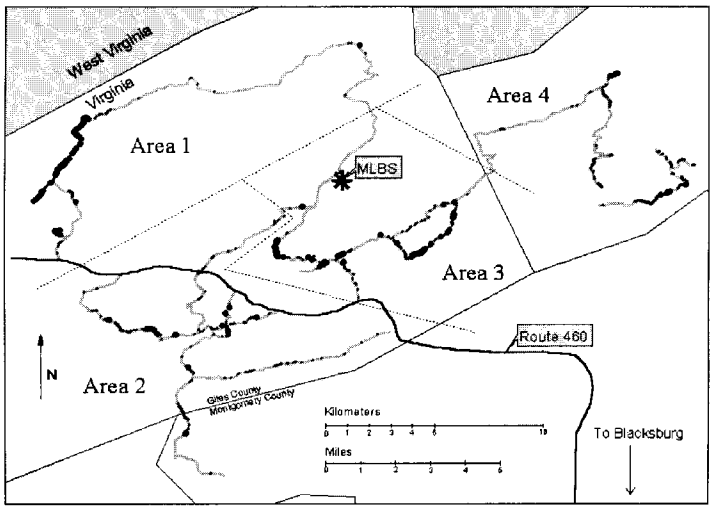

Fig. 19.1 Diagrammatic map of the census area showing the roads along which populations were censused (gray) and position of roadside segments that had healthy (small dots) or diseased (large dots) populations at some time during the census. Note that the scale results in an apparent overlap of populations that are often separated. Dotted lines separate the four "replicate" areas identified in the analyses. Populations were not censused along Route 460, which is a major highway; however, only rarely was the occasional plant seen along this highway.

We have been studying anther-smut disease caused by the fungal pathogen *Microbotryum violaceum* (= *Ustilago violacea*) in several hundred populations of the plant *Silene latifolia* (= *S. alba*) for 14 yr in the region of Mountain Lake Biological Station in western Virginia (Fig. 19.1). This chapter reports the results of these studies and discusses the factors that contribute to the overall stability of this metapopulation system.

19.2 THE STUDY SYSTEM

Life Cycles of Host and Pathogen

Silene latifolia, or white campion, is a short-lived perennial herb native to Europe commonly found in ruderal habitats throughout the northern regions of the United States and in upland areas farther south. Infection by *M. violaceum* results in the plant producing anthers that release fungal spores rather than pollen. In *S. latifolia*, which is dioecious, in addition to infecting the anthers in males, the pathogen induces the female flowers to produce stamens that bear diseased anthers. The ovary is aborted and sterile, although it is still visible as a rudimentary structure. The disease therefore has a large fitness effect by sterilizing the host, and diseased plants are identified easily in the field by their dark-smutted anthers. Anther smut is a relatively "slow" disease with a long latent period. The pathogen does not convert existing flowers into a smutted state, but grows into very young developing flower buds, which are then converted into smutted flowers. This process generally takes no less than 3 weeks, and sometimes more than 6 weeks from initial infection. Because *S. latifolia* in Virginia flowers from mid-May until early October, there are probably between one and three fungal generations per flowering season, depending when infection first takes place. The average life span of a plant that flowers is ca. 2 yr (see later). Initially, infected plants may be partially diseased, but the disease soon becomes systemic. The disease persists between seasons inside the overwintering rosette of the host plant.

The disease is transmitted when pollinators move from flower to flower. Because pollinators adjust flight distances to compensate for plant density, transmission at moderate plant densities depends on the frequency and not the density of infectious individuals, whereas at very high densities, when pollinators become limiting per capita, transmission rates decline (Alexander and Antonovics, 1992; Antonovics et al., 1995). Although the pathogen is actually vector transmitted, the frequency-dependent nature of the transmission and the expression of the disease in the sexual organs of the adult plants result in strong parallels between the biology of this host–pathogen system and other sexually transmitted diseases (Kaltz and Schmid, 1995; Lockhart et al., 1996).

There is substantial genetic variation in *S. latifolia* for disease resistance, and most populations are a mixture of genotypes that range from being almost completely resistant to completely susceptible (Alexander, 1989; Alexander et al., 1993; Biere and Antonovics, 1995). Although resistance has a high heritability (Alexander et al., 1993), the precise genetics underlying the resistance is not known. Additionally, large fitness costs are associated with resistance in the absence of the disease. More resistant plants flower later in the season and

produce fewer flowers (Alexander, 1989; Biere and Antonovics, 1995). Unexpectedly, the fungus appears to be relatively uniform with regard to its pathogenicity, and therefore this host–pathogen system does not follow the classical gene-for-gene scenario (Jarosz and Burdon, 1991). Whether this is because the disease has been recently introduced into the United States from Europe and has gone through a bottleneck is not known (see Section 19.4).

All the evidence indicates that *M. violaceum* on *S. latifolia* is host specific in the United States, although a recent host shift to *S. vulgaris* has been observed (Antonovics et al., 2002). We have found anther smuts on two other native species in the southeast United States (*S. virginica* and *S. caroliniana*). However, these anther smuts are phylogenetically and chromosomally quite distinct from the one on *S. latifolia* (Perlin, 1996; Perlin et al., 1997). There is no evidence for any cross-species transmission with the native species. Moreover, Antonovics et al. (1995b) showed that the anther smut on *S. virginica* in this area is isolated reproductively from the anther smut on *S. latifolia*.

Study Area and Census Methods

In the study area, *S. latifolia* is a ruderal species that is largely confined to roadsides. Its roadside distribution allows us to gain rapid access to many populations over a large area (25 km from north to south and 30 km from east to west), while at the same time being confident that we are missing very few populations.

Because the plant is distributed in patches of differing sizes and spacing, which may coalesce or separate due to colonization and extinction events, we do not define a population in terms of the patches themselves but count numbers of diseased and healthy individuals within contiguous 40-m segments of roadsides (Antonovics et al., 1994). Therefore, in formal terms, we collect data on a one-dimensional grid system at a local scale, but at a larger scale the topology of this grid follows the pattern of the roads in the area. Distances on curves are estimated on the right-hand side of the road in the direction that the census is being made. Local landmarks (unusual trees, driveways, telephone poles, etc.) are used to demarcate each segment.

We have counted the number of diseased and healthy individuals within each roadside segment since 1988. The main census is done prior to seed dispersal in June, and a recensus in August is restricted to checking a much smaller subset of the populations that have been recorded as extinct or that have been recorded as having lost the disease. Although we make no attempt to map individuals within a segment to a precise location, we note the location (approximate distance from start of grid unit and distance from edge of road) of either healthy or diseased individuals when there are very few in a grid unit; this helps us relocate those individuals in subsequent censuses and/or confirm their absence. Our census is therefore simple and rapid; field work can be completed by three crews of two to three people in less than 1 week.

The one-dimensional grid units of 40 m include perhaps one or two, but not many, "genetic neighborhoods" (i.e., areas within which genetic exchange is essentially random) as estimated from spore, pollen, and seed dispersal distances (Alexander, 1990). They may include several distinct patches of *S. latifolia* and sometimes these patches are contiguous between grid units.

However, rarely is there a continuous "swath" of *S. latifolia* that spans more than two grid units. By analyzing field data based on pooling two, four, or eight adjacent segments, we have shown that the patterns of disease incidence are remarkably robust over a grid scale of 40–160 m (Antonovics et al., 1998).

Throughout the study we take pains not to disturb the system by our own activities during the census. Because the flowers usually close before midday, we census between 5:30 and 11:00 A.M., during which time we can determine the disease status visually without touching the plants or trampling on the sites. In this way we avoid becoming disease dispersal agents ourselves.

Not all populations of *S. latifolia* occur at roadsides. Some populations also occur on waste ground or along field edges away from the actual road. There are relatively few so-called "off-road sites" (on average 5.9 % of occupied grid units in any 1 yr) but the number of diseased and healthy plants in these sites was also recorded. Sketch maps were used to identify these sites. However, because the area of these sites is very variable, they were not included in the following analyses.

General Characteristics of the Silene–Microbotryum Metapopulation

It is clear that our system does not fit the simple conceptualization of metapopulations presented by Levins (1969) (uniform populations, no distance dependence, instantaneous within population dynamics). The populations are very different in size, dispersal is limited, and within population dynamics is important relative to the annual time scale of the study. Moreover, as in many plant metapopulations, it is not possible to define "suitable habitats" of *S. latifolia* by a clear environmental discontinuity (see Chapter 18 for a discussion of the metapopulation concept as applied to plants). Previous studies have shown that colonization and extinction of the host and pathogen populations are frequent (Antonovics et al., 1994, 1998; Thrall and Antonovics, 1995; see also results). These colonization events enhance the degree of genetic differentiation among populations (McCauley et al., 1995). The growth rate of healthy populations is density dependent, with the disease having a negative effect on population growth (Antonovics et al., 1998). In particular, high levels of disease shift population growth rates from positive to negative values (Antonovics et al., 1998). The impact of the disease on population extinction is gradual; the disease results in a declining population growth rate, and a small population size in turn presages an increased probability of extinction. Using simulations models, Antonovics (1999) showed that the presence of the pathogen can more than halve the number of occupied segments in the metapopulation as a whole.

19.3 LONG-TERM TRENDS

Analysis

In the first year, 1988, data were gathered on a 0.1-mile (ca. four grid units) scale, and no recensus was carried out. Therefore, although data from this first year were valuable in indicating the high rate of turnover in the populations

and were a stimulus for embarking on the study, we did not use these data directly in the statistical analysis of population trends presented here.

To assess whether any long-term changes were general to the census as a whole, we divided the census region into four areas representing different valley systems and separated by high elevation areas (Fig. 19.1). Area 1 was the valley region of Big Stony Creek; Area 2 was the lowland area and foothills in the New River Valley; Area 3 was Clover Hollow and Route 700 up to the Biological Station; and Area 4 was Maggie Valley and the area east of Simmondsville, Route 42.

The scope of the census changed somewhat with circumstances. Thus access to one section of the census was denied by the land managers in 1994 (excluded nine grid units either side of the road). In Area 4, diseased sites present in the most northerly region were the result of artificial introduction of the disease from a spore dispersal experiment; this area was therefore not included in analyses of disease parameters. In 1998, we included a new section south of Area 4 when this became the focus of related demographic studies. Disease was present in this region. However, in order to avoid possible confounding effects, analyses of the long-term trends were based only on data from the roadside segments that were censused throughout the whole study period.

Host and Pathogen Occurrence

At a regional level, the abundance of a species can be assessed in terms of the number of populations as well as the average number of individuals within populations. Because the census was based on a grid system of roadside segments, we measured the number of host populations in terms of number of segments occupied and population size in terms of the number of individuals within each segment. The number of grid segments used in the analyses did not change, and therefore the former is a measure of regional abundance and the latter is a measure of local abundance (at the segment scale). We measured regional disease abundance as the fraction of segments occupied by *S. latifolia* that were diseased (we refer to this as "disease *incidence*") and the local abundance as the fraction of individuals that were diseased within each occupied segment (we refer to this as "disease *prevalence*").

The fraction of segments occupied by *S. latifolia* (Fig. 19.2A) did not change significantly overall ($P < 0.27$), but there was a significant area* year interaction ($P < 0.0001$). In Area 1 the occupancy declined significantly ($P < 0.0019$, $b = -0.0038$), while it increased in Areas 2 and 3 ($P < 0.015$, $b = 0.0029$ and $P < 0.0089$, $b = 0.0020$). There was no significant change in Area 4.

The average number of *S. latifolia* within each segment (Fig. 19.2B) declined markedly overall ($P < 0.0001$, $b = -0.0112$, $\log_{10}$ scale). The decline occurred in all four areas, significantly so in Areas 1–3 (P all < 0.0088), but not in Area 4 ($P < 0.13$).

The fraction of *S. latifolia* segments that were diseased, or "disease incidence," (Fig 19.3A) declined significantly overall ($P < 0.0001$; regression coefficient $b = -0.0153$, arcsin square root transformed data). Although disease incidence declined in Areas 1–3, the rate of decline differed among the areas (year*site interaction, $P < 0.0001$). Area 3 was particularly interesting in that it showed an initial increase in disease incidence, peaking in 1995

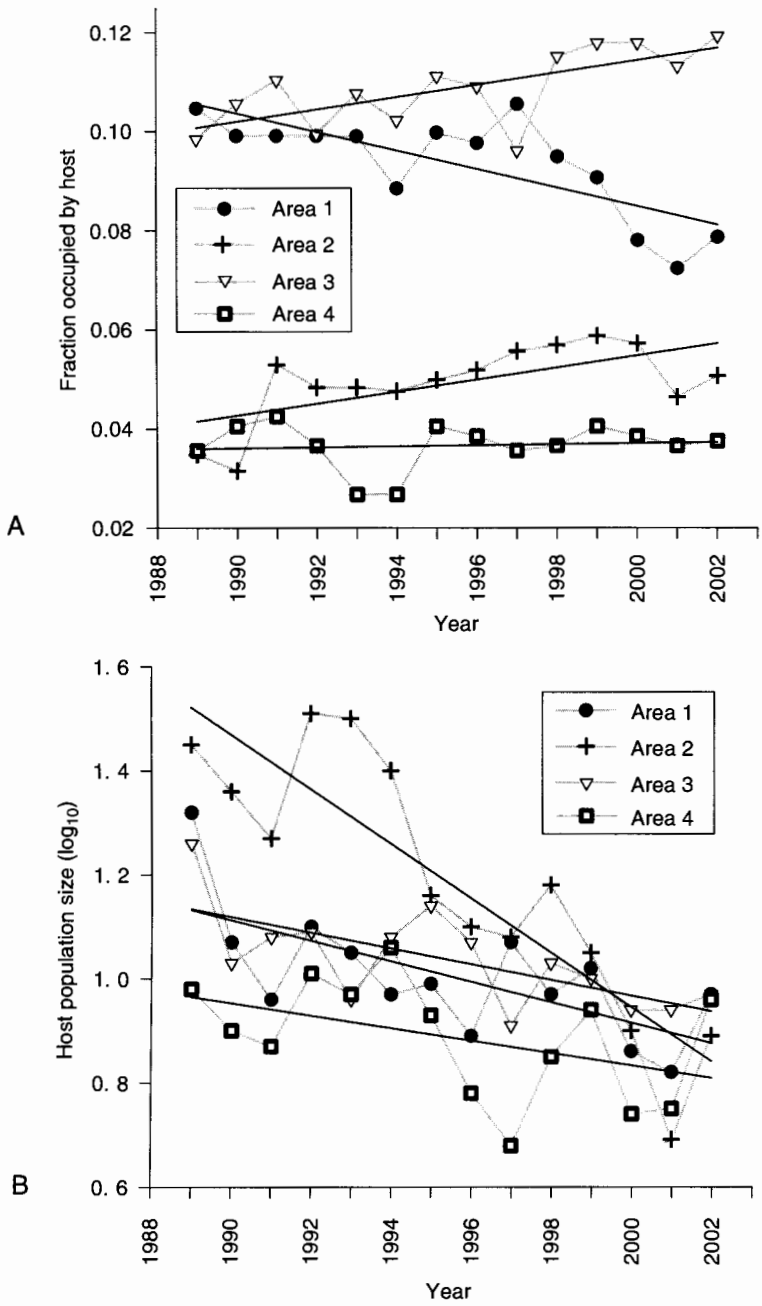

Fig. 19.2 (A) Fraction of roadside segments occupied by *S. latifolia* and (B) average number of *S. latifolia* individuals within each occupied segment in each year for the four areas of the metapopulation.

when nearly 35% of the populations were diseased, followed by a rapid decline. Three subareas were identified within this area on the basis of separation by long runs of unoccupied segments. All three subareas showed a similar pattern with disease incidence peaking in the mid-1990s and then declining (Fig. 19.4).

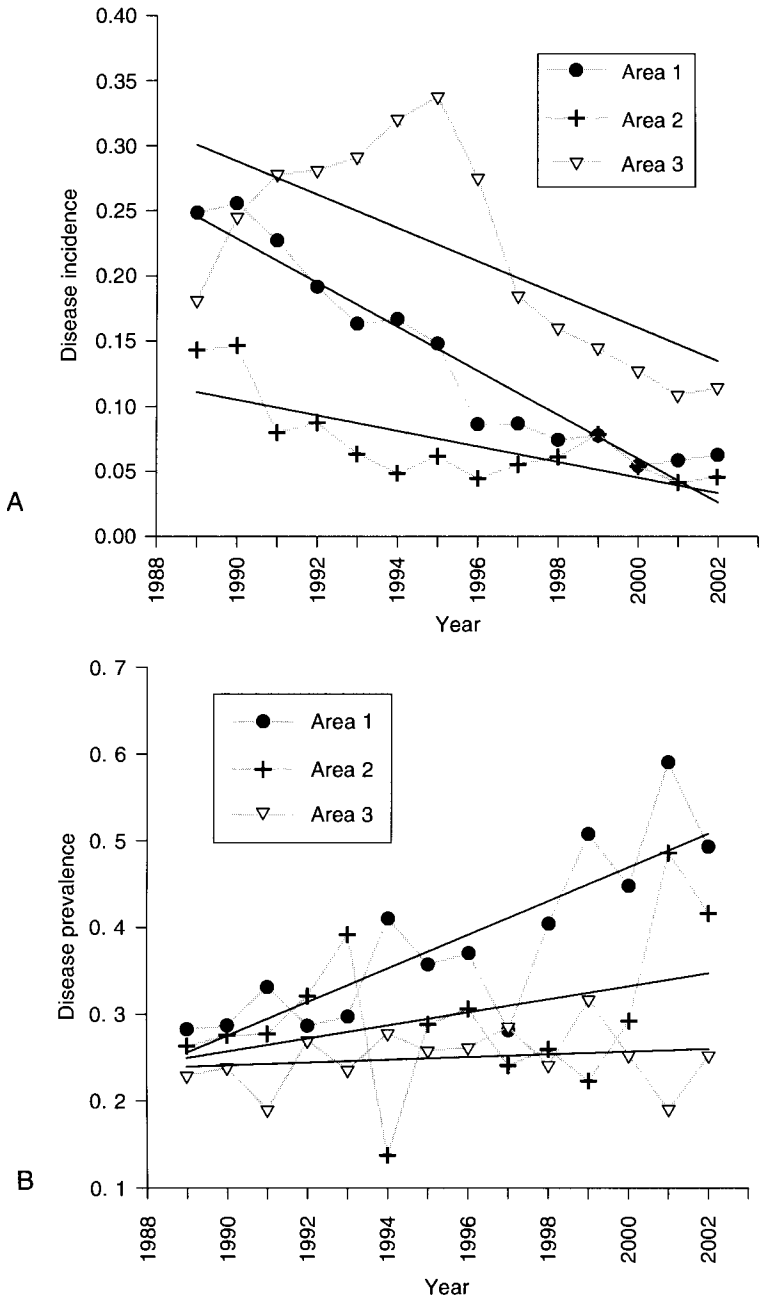

Fig. 19.3 (A) Fraction of *S. latifolia* populations that were diseased (disease incidence) and (B) fraction of individuals that were diseased (disease prevalence) within each diseased population for three areas of the metapopulation.

The fraction of individuals that were diseased, or "disease prevalence," within each diseased population (Fig. 19.3B) increased significantly overall ($P < 0.0042$, $b = 0.0051$, arcsin square root transformed data). All areas showed an increase in disease prevalence and the area*year interaction

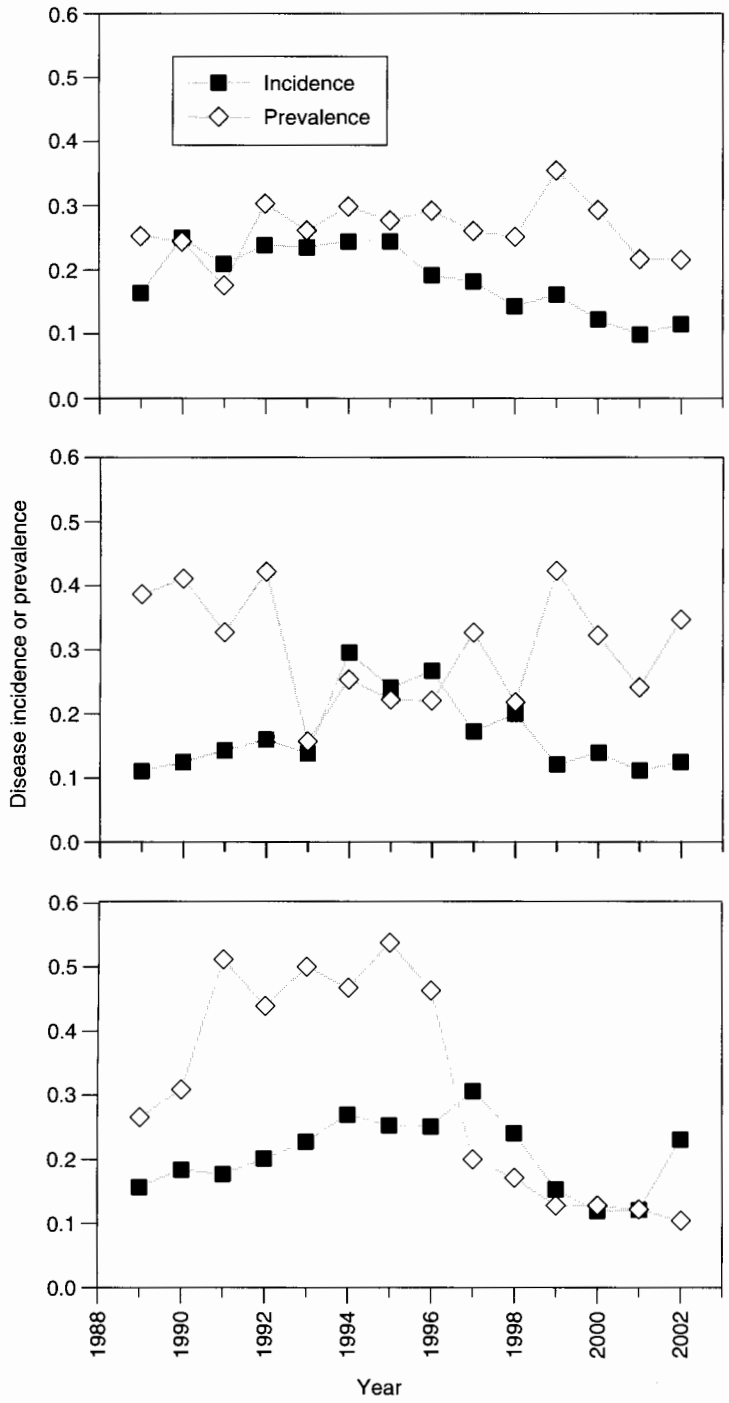

Fig. 19.4 Disease incidence and disease prevalence in three different sections of Area 3. Incidence is the fraction of roadside segments occupied by *S. latifolia* that contained at least one diseased plant, and prevalence describes the fraction of plants within each diseased segment that were diseased.

approached significance ($P < 0.061$). The increase was individually significant only in Area 1 ($P < 0.0005$, $b = 0.011$). The absolute number of diseased plants per segment decreased significantly ($P < 0.0037$, $b = -0.123$) and the area*year interaction was not significant ($P < 0.21$).

Within the subareas of Area 3 disease prevalence was positively but nonsignificantly correlated with incidence in two subareas ($r = 0.19, 0.38$; $P < 0.51, 0.17$), while in the other area they were negatively and nearly significantly correlated ($r = -0.50$, $P < 0.069$).

Host Colonization and Extinction

A host colonization was identified as the presence of a population in a roadside segment after a year when no plants were seen in that segment; the host colonization rate is therefore a compound measure that includes recruitments from the seed pool, recruitment of plants that had remained vegetative for a whole year, and immigration from other sites.

We calculated the colonization rates of the host *S. latifolia* as the number of new populations at time t per existing population at time $t - 1$. This "per capita" colonization rate does not take into account the number of empty segments available for colonization, as these were extremely numerous (1989: 6451, 1990–2002: 6616–6694) and did not vary appreciably with changes in host occupancy. Calculations on a "per unoccupied segment" basis (i.e., equivalent to Levins' "c" in the canonical metapopulation model, Levins, 1969) did not change the results appreciably. We included both healthy and diseased populations as sources because the latter also produced seed (except in the very rare case where there was 100% disease of females and/or males). Results (Fig. 19.5A) showed that the colonization rates of healthy populations declined over the time period of the study ($b = -0.0041$, $P < 0.040$) and that the rate of decline was not significantly different in the different areas (area*year interaction $P < 0.44$).

Host extinction was identified as the absence of a population in a roadside segment after a year when plants had been seen in that segment the previous year. Strictly speaking, it is an "apparent" host extinction rate because it refers to the absence of flowering individuals and does not preclude the persistence of the population as vegetative individuals or in the seed bank. Generally, most plants flower every year, except for very small individuals. When vegetative plants were occasionally seen, the population was not recorded as extinct; however, plants may have been missed because vegetative individuals are not very conspicuous. Results (Fig. 19.5B) showed that the extinction rates of the host tended to decline over the time period of the study, but this decline was not significant ($b = -0.017$, $P < 0.076$). The rate of decline was not significantly different in the different areas (area*year interaction $P < 0.21$).

Disease Colonization and Extinction

A disease colonization event was identified as the presence of the disease in a population of *S. latifolia* after a year when no disease had been seen in that population the previous year. Disease colonization is most probably by immigration,

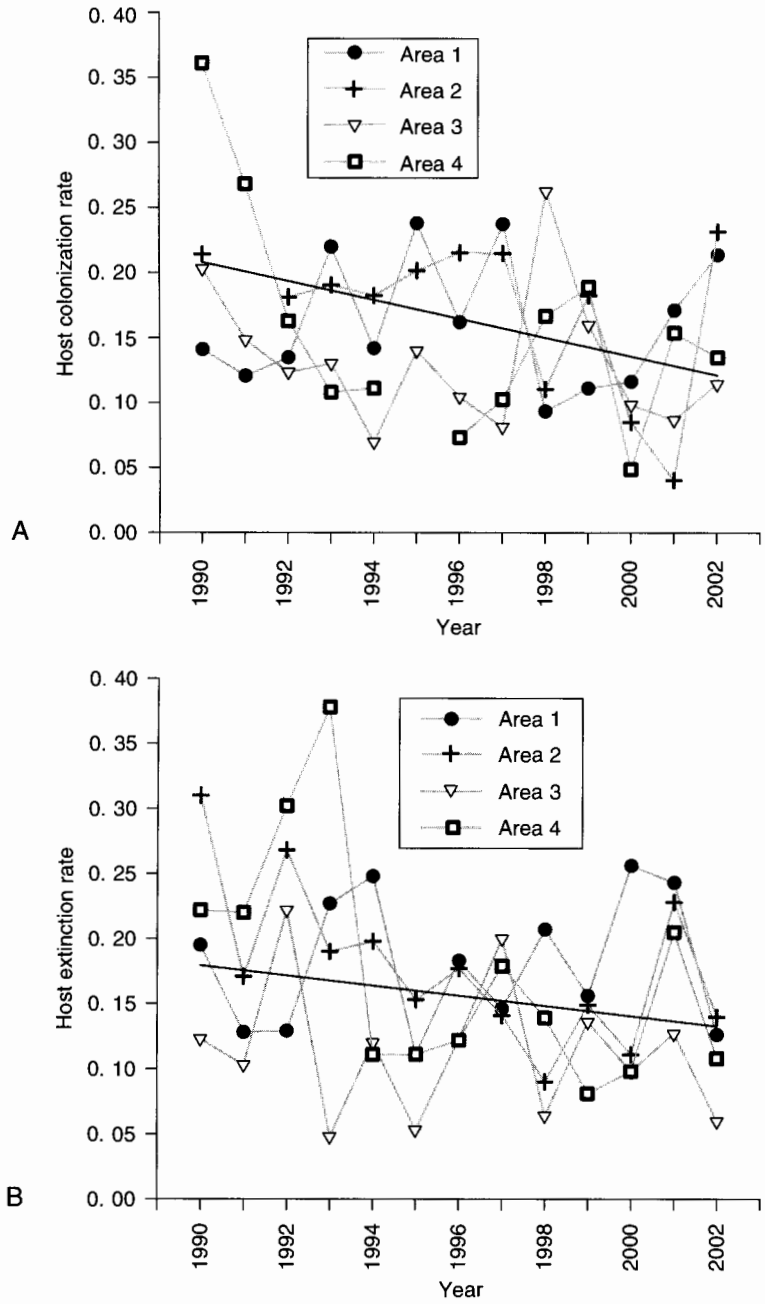

Fig. 19.5 (A) Colonization rate and (B) extinction rate of *S. latifolia* in each year for the four areas of the metapopulation. Colonization rate is measured as the number of new populations in a given year per existing population in the previous year. Extinction rate is measured as the number of populations that went extinct in a given year as a fraction of the number of populations in the previous year.

but the persistence of the disease in a vegetative plant (or a plant that was not flowering at the time of census) cannot be precluded. Across season soil-borne transmission and vertical transmission of the disease have never been observed. We calculated the colonization rate of the disease as the number of newly diseased populations at time t per existing population at time $t - 1$ divided by the number of healthy populations available for colonization in an area (i.e., Levins' "c"). Results (Fig. 19.6A) showed that the colonization rates of disease declined over the time period of the study ($b = -0.025$, $P < 0.029$) and that the rate of decline was not significantly different in the different areas (area*year interaction $P < 0.44$).

Disease extinction was identified as the absence of disease in a population that had been diseased in the previous year. Again this is an "apparent" extinction rate because the disease may have persisted in nonflowering individuals. Results (Fig. 19.6B) showed that the extinction rate of the disease did not change over the time period of the study ($b = 0.0094$, $P < 0.46$) and that the extinction rate was not significantly different in the different areas (area*year interaction $P < 0.20$). The correlation between disease extinction and colonization rate was not significant.

Disease Transmission Rates

Disease transmission rates were calculated using populations where disease had been present in two successive time intervals so as not to confound the estimates with disease colonization or extinction rates. Maximum likelihood methods were used to estimate the survival rate (S) and disease transmission rate (β) for each year by fitting the following model to the data (and minimizing the sum of squares of the log of predicted minus the log of observed):

$$Y_{t+1} = S(Y_t + X_t(1 - \exp(-\beta Y_t/N_t)) \qquad (19.1)$$

where X_t is the number of healthy plants in year t, Y_t, Y_{t+1} is the number of diseased plants in year t and $t + 1$, and $N_t = X_t + Y_t$. Note that the parameter β represents a within season transmission coefficient (assuming no summer mortality) and S represents overwinter survival. Equivalent analyses were also carried out using PROC NLIN in SAS (SAS Institute, 1999) and gave identical results.

The frequency-dependent transmission model always resulted in a better fit than the density-dependent model [where force of infection $= 1 - \exp(-\beta Y_t)$]; the latter also frequently produced unrealistic estimates of S (equal to or close to 1). A good fit was also obtained with a model where the force of infection was $= 1 - \exp(-\beta Y_t/N_t{}^*N_t)$, a model form appropriate for vector-based transmission, but because the relative values of S and β did not differ much between models, we present the results of the more familiar frequency-dependent model.

There was a strong colinearity in the estimates of S and β, such that high estimates of S were correlated with low estimates of β and vice versa. We therefore standardized the survival rate by taking the average over all years and including this average in the model to estimate β. Therefore, this estimate in effect represents an overall "cross-season" transmission coefficient that is a compound of the survival rate of diseased plants and the within season transmission.

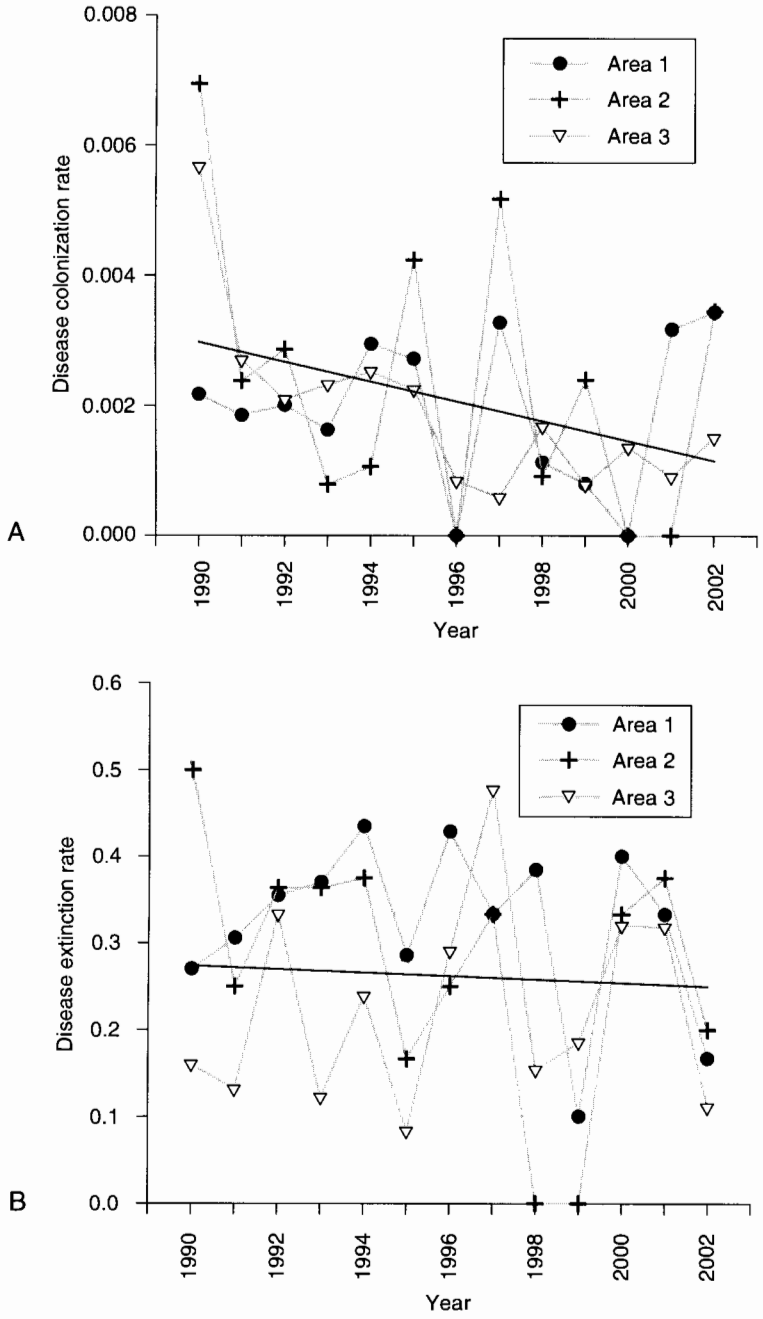

Fig. 19.6 (A) Colonization rate and (B) extinction rate of *M. violaceum* in each year for three areas of the metapopulation. Disease colonization rate is disease measured as the number of newly diseased populations in a given year per existing population in the previous year divided by the number of healthy populations the previous year. Disease extinction rate is measured as the number of populations that became healthy in a given year as a fraction of the number of diseased populations in the previous year.

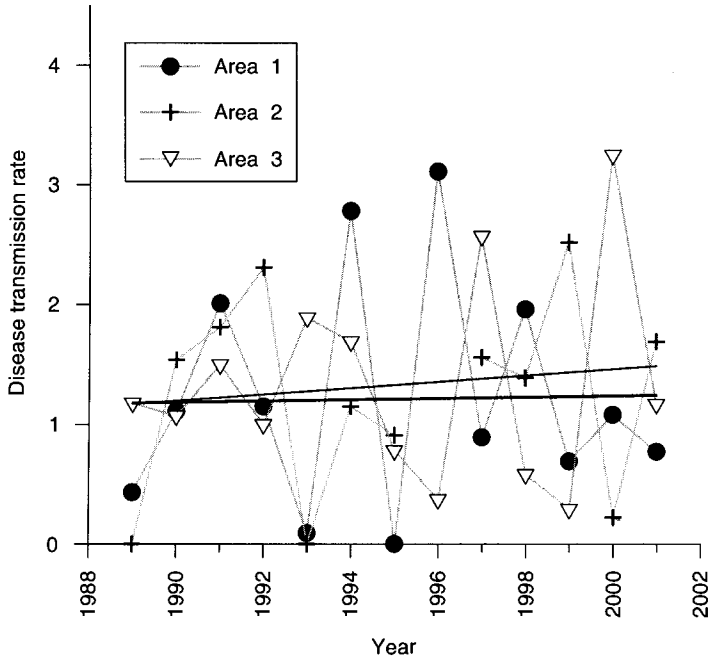

Fig. 19.7 Within population disease transmission rates per year for three areas of the metapopulation with diseased populations (see text for details of estimation).

Results showed that the transmission rates of the disease within populations (Fig. 19.7) did not change significantly over the time period of the study ($b = 0.015$; $P < 0.44$). Nor was there any evidence for a year*area interaction ($P < 0.93$); regressions for each area were slightly positive but did not individually approach significance, even when an outlier was removed ($P < 0.56$–0.82).

There was no significant relationship between disease transmission rates within populations and disease colonization rate (correlation coefficient, $r = -0.07$, $P < 0.83$). When an outlier was removed (1989 estimates), the relationship was positive ($r = 0.29$) but still not significant ($P < 0.36$). The relationship between disease transmission rates within populations and disease extinction rate was negative (-0.26) but not significant ($P < 0.40$) and was essentially unchanged when the 1989 estimate was removed. The same trends (greater colonization and lower extinction when the disease transmission rate was higher) were obtained when the analysis was carried out for each area individually, but these trends were not significant.

Weather Data

Prior to 1997, weather data at Mountain Lake Biological Station were gathered manually and were obtained from the National Climate Data Center. In 1994, a new weather station with automatic data acquisition was installed and run by the station. There was a close correspondence between weather data (monthly mean temperature, highest temperature, lowest temperature, and

precipitation) during the 2 to 3 yr period when both types of data were being gathered. Therefore the two types of data were averaged during this overlap period and were used to span the period 1988 to the present.

We investigated a specific set of weather variables that we thought might be related to host and pathogen colonization and extinction, as well as to within population disease transmission. Based on our natural history observations of field experiments, we hypothesized that hot dry summers would decrease disease transmission and hence disease colonization. We also hypothesized that cold winters and/or unusually cold weather in early spring would increase host extinction rates. For each year of the census, for the summer (June, July, and August), we calculated precipitation and mean daily maximum and minimum temperatures; for the winter (December, January, and February) we calculated mean daily maximum and minimum temperatures. We also calculated the minimum temperature in March, as this represents the incidence of unusually cold weather in the early spring.

Over the period of the census, there was a significant decrease in summer daily maximum temperatures ($r = -0.57$, $P < 0.033$) and an increase in summer and winter minimum temperatures ($r = 0.76$, $P < 0.0015$; $r = 0.56$, $P < 0.045$). Analysis of weather data at Mountain Lake Biological Station from 1972 to 2001 showed a gradual but nonsignificant increase in mean, maximum, and minimum summer temperatures (0.021, 0.023, and 0.018°C per year, respectively); the decrease in summer maximum temperatures since 1988 was therefore contrary to the longer term trend. Summer precipitation did not change systematically with year, but was correlated negatively with maximum summer temperatures ($r = -0.60$, $P < 0.023$). No other weather relationships showed a significant change with year.

With a few exceptions, the population parameters were not correlated with weather data. Host extinction was negatively correlated with winter minimum temperatures ($r = -0.76$, $P < 0.0042$), and disease colonization rate (but not transmission rate) was significantly negatively correlated with summer mean minimum temperature ($r = -0.60$, $P < 0.031$). A Bonferroni correction of the $P < 0.05$ criterion for significance (given that 30 correlations were estimated) results in a value of $P < 0.0017$. Under this criterion none of the aforementioned relationships would be deemed significant.

Examination of the change in incidence and prevalence in Area 3 where incidence was initially low and then peaked in the mid-1990s (see Fig. 19.4) showed no obvious or even suggestive relationship with the weather variables.

19.4 DISCUSSION

This study provides clear evidence that the *Silene–Microbotryum* metapopulation that we have been studying since 1988 is not in a state of "global stability." This result came very much a surprise with regard to our ongoing impressions of the populations. Indeed, analysis of the 15 yr of data was stimulated by an assessment of whether it was "worthwhile" continuing with the census, given the resources and effort needed to carry it out every year (our attempts several years ago to get funding for the study were unsuccessful!). Year-by-year observations did not give us the sense that diseased populations

were declining in frequency, as every year there were always reports of both disease extinctions and colonizations.

Several issues are raised by these data. First, what is the proximal cause of the decline? In particular, is it driven by changes in the external environment or is it intrinsic to the disease dynamics? Second, if it is the latter, is the instability related to the fact that both the host and the disease are relatively recent introductions into the United States? Finally, is the system moving toward some eventual equilibrium with host–pathogen coexistence or will the outcome be disease extinction?

It is well known from the crop literature that variation in weather can greatly influence the prevalence of disease. However, in the weather data we analyzed, only 2 out of a possible 30 correlations were significant. While the decrease in host–extinction rate with increasing winter minimum temperatures is hard to interpret causally, the increase in disease colonization rate with decreasing summer minimum temperatures is consistent with our own observations that disease transmission is highest at low temperatures and high humidity (Alexander et al., 1993). These low temperatures are most likely to occur during the night, which is also the period of greatest moth visitation (Altizer et al., 1998) and therefore the period most likely for the long distance transport of spores.

Other environmental changes unrelated to the weather may also have had an effect, although their relative importance is hard to judge. In Area 1, elimination of several heavily diseased off-road sites in 1995 by extensive relandscaping by a local lime-manufacturing company may have reduced the available disease sources. In one part of Area 2, road widening in 1990 eliminated five of six diseased sites and in 1995 it eliminated another two diseased sites nearby. However, it is doubtful that this had a cascading effect elsewhere in the area. The whole region of the census was also subject to early spring spraying to control gypsy moth (Sharov and Liebhold, 1998). However, because much of the spraying in this area has been with male mating pheromone whose effect is likely to be specific to gypsy moths (and which have not reached epidemic levels in the census area), the overall impact on moth pollinators (which are also disease vectors) has probably been small.

An alternative explanation for the disease decline is that it is intrinsic to the dynamics of the system as a whole. In a simulation of this host pathogen system (Antonovics et al., 1998), the disease could only be sustained in little over 50% of the runs. We have not reparameterized or reevaluated this model based on more recent data, but it is nonetheless interesting that our "best estimates" based on values from the earlier part of this census and from experimental studies often predicted that the disease would be lost from the metapopulation. Moreover, as the disease was lost, the prevalence of the disease within the remaining populations increased, as we have observed in this study. This is largely because newly founded populations with low levels of disease were no longer being produced. The decreasing disease incidence, the increasing prevalence within populations, and the declining of disease colonization rate observed here are all consistent with gradual disease extinction in the metapopulation.

In this region of Virginia there is extensive genetic variation in the host, yet no detectable variation in the infectiousness of the pathogen. Thus Antonovics et al. (1998) showed that if the simulation is carried out with a genetically

uniform host population with a resistance that is intermediate between that of the most susceptible and most resistant genotypes, with an exponential $\beta = 2.00$, and a survival of 0.50, then the metapopulation would persist about 90% of the time. (Analysis of census data gave an average value of β over all years of 2.89 and an average survival of 0.55, remarkably close to values used in the earlier simulations.) However, when the simulation was carried out with a genetically variable host population, persistence was much less frequent (ca. 40%). Introduction of the disease into a population led to a rapid local spread of the resistance gene and the generation of resistant populations that were not colonized readily by the disease. Populations only become readily available for colonization by the disease when the gene for susceptibility increased because of the cost of resistance (estimated to be about 25%; Biere and Antonovics, 1995).

In experimental populations of *S. latifolia* where individuals are not replaced over successive years, disease transmission showed an extremely rapid decline to almost zero within 2 yr, due to the fact that the only individuals remaining healthy were from genetically resistant families (Alexander, 1989; Alexander et al., 1995). Disease prevalence also dropped rapidly in experimental populations started with progeny of resistant genotypes but not in populations started with progeny of susceptible genotypes (Thrall and Jarosz, 1994a,b). Moreover, detailed demographic studies of extant diseased populations have shown low transmission rates (Alexander, 1990). It is therefore possible that the decline in disease colonization rates may be due to an increased level of disease resistance in the metapopulation as a whole.

It is relevant to place our metapopulation in a broader geographical and historical context, as this may help with the interpretation of the local changes. In a survey of over a thousand herbarium specimens of *S. latifolia* in the eastern United States, there was no evidence that the plant had been collected south of the Pennsylvania line before 1914 (Antonovics et al., 2003), apart from a collection made in 1896 on the Biltmore estate in North Carolina. Biltmore House was opened in 1895, and it is likely that the estate imported seeds from New England for hay or for the meadows. The first record in Virginia was in 1924, and it was not until the 1930s that collections in Virginia became frequent. The first record we could find for Giles County, where the majority of the metapopulation is located, was 1938. Therefore, the weight of the evidence is that the host plant has only been in the Mountain Lake area for perhaps less than 80 years.

The history of the disease is completely unknown. Previously, *M. violaceum* had been noted on *S. caroliniana* in Virginia and New York State and on several species of *Silene* in the western United States (Farr et al., 1989), but there is no record of it on *S. latifolia*, even though other fungal diseases are recorded for this species in the United States (Farr et al., 1989). None of the herbarium specimens we examined were diseased so they did not help resolve the question of the disease origins. The current distribution of *S. latifolia* and *M. violaceum* in the eastern United States was studied by A. M. Jarosz and E. Lyons (personal communication). They found that the disease was largely confined to the ridge and valley system of western Virginia (where 16% of 102 populations were diseased). Further in the northeast, they only found 1 diseased population (in Pennsylvania) out of 169, except for 3 diseased populations on Nantucket Island, Massachusetts. Diseased plants have been

known from Nantucket Island since the early 1980s (T. Meagher, personal communication). In the north central United States, a single diseased plant was found out of 387 populations sampled. The reason for the absence of the disease from more northern latitudes is unknown. In field experiments along a latitudinal gradient, A. M. Jarosz and E. Lyons (personal communication) showed that northern populations were susceptible to disease in their local areas, but that they were also somewhat more resistant than plants derived from seeds of a relatively susceptible parent from Mountain Lake that was used as a control. Artificial hand "pollination" with spores produced a higher incidence of disease than open visitation, suggesting a shortage of pollinators may limit disease transmission.

Given that the host has moved into this part of Virginia only recently and that the disease is near the southern edge of the current range of *S. latifolia*, yet is found sporadically in its former range, it is plausible that we may be seeing the movement of a disease "front" that is following the host as it colonizes new areas. The movement of this disease front may be driven by the evolution of more resistant populations in the wake of the disease. The spread of this disease in the United States may therefore be analogous to the spread of many other epidemics. In animal populations, "waves" of disease spread are often driven by the development of immunity in the wake of the epidemic, but a genetic component to this immunity has also been posited frequently. In the present metapopulation, this genetic component may be the major driving force. However, the issue of whether the changes we are observing are due to climatic and management changes or to intrinsic genetic and demographic factors cannot be determined by descriptive or simulation studies alone, but will require further experiments and more directed field studies of individual populations.