

LOCAL MALADAPTATION IN THE ANTHR-SMUT FUNGUS *MICROBOTRYUM VIOLACEUM* TO ITS HOST PLANT *SILENE LATIFOLIA*: EVIDENCE FROM A CROSS-INOCULATION EXPERIMENT

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Abstract.—Conventional wisdom holds that parasites evolve more rapidly than their hosts and are therefore locally adapted, that is, better at exploiting sympatric than allopatric hosts. We studied local adaptation in the insect-transmitted fungal pathogen *Microbotryum violaceum* and its host plant *Silene latifolia*. Infection success was tested in sympatric (local) and allopatric (foreign) combinations of pathogen and host from 14 natural populations from a metapopulation. Seedlings from up to 10 seed families from each population were exposed to sporidial suspensions from each of four fungal strains derived from the same population, from a near-by population (< 10 km distance), and from two populations at an intermediate (< 30 km) and remote (< 170 km) distance, respectively. We obtained significant pathogen × plant interactions in infection success (proportion of diseased plants) at both fungal population and strain level. There was an overall pattern of local maladaptation of this pathogen: average fungal infection success was significantly lower on sympatric hosts (mean proportion of diseased plants = 0.32 ± 0.03 SE) than on allopatric hosts (0.40 ± 0.02). Five of the 14 fungal populations showed no strong reduction in infection success on sympatric hosts, and three even tended to perform better on sympatric hosts. This pattern is consistent with models of time-lagged cycles predicting patterns of local adaptation in host-parasite systems to emerge only on average. Several factors may restrict the evolutionary potential of this pathogen relative to that of its host. First, a predominantly selfing breeding system may limit its ability to generate new virulence types by sexual recombination, whereas the obligately outcrossing host *S. latifolia* may profit from rearrangement of resistance alleles by random mating. Second, populations often harbor only a few infected individuals, so virulence variation may be further reduced by drift. Third, migration rates among host plant populations are much higher than among pathogen populations, possibly because pollinators prefer healthy over diseased plants. Migration among partly isolated populations may therefore introduce novel host plant resistance variants more often than novel parasite virulence variants. That migration contributes to the coevolutionary dynamics in this system is supported by the geographic pattern of infectivity. Infection success increased over the first 10-km range of host-pathogen population distances, which is likely the natural range of gene exchange.

Key words.—Caryophyllaceae, coevolution, gene flow, host-parasite coevolution, local adaptation, *Ustilago*.

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In coevolutionary interactions between host and parasite, the biotic aspect of the environment (host for parasite and vice versa) continually degrades. Because of this reciprocal, antagonistic relationship, host-parasite coevolution is considered as an arms race of adaptation and counteradaptation (Dawkins and Krebs 1979). Who will have the upper hand in this arms race is determined by the evolutionary rates of the two players. It is conventional wisdom that parasites have greater evolutionary potential than their hosts (Hamilton et al. 1990). Parasites usually have shorter generation times than their hosts, and this, together with high reproductive rates, will increase rates of evolution (Hafner et al. 1994) and thereby the turnover of novel adapted parasite variants. This process will be further accelerated if parasites have intrinsically high mutation rates, as shown for pathogenic bacteria (LeClerc et al. 1996).

Coevolutionary host-parasite interactions may be responsible for spatial genetic differentiation of host and parasite populations. Local host-parasite coevolutionary dynamics are commonly considered to follow negative frequency-dependent, time-lagged cycles of host and parasite genotypes (Hutton and Law 1981; Bell and Maynard Smith 1987; Nee 1989; Hamilton et al. 1990; Frank 1996). Parasites will evolve the ability to attack the locally most common host genotype(s),

thereby generating an advantage for rare resistant host types that will in turn increase in frequency. Given their higher evolutionary rates, parasites are expected to respond faster to changes in the genetic composition of their local host population than *vice versa*, and it has been suggested that parasite local adaptation may constitute a general rule (Ebert and Hamilton 1996).

Specializing on the most common host types in its own population is likely to reduce the parasite's mean performance on hosts from foreign populations, either simply because other host types are common in other populations (Morand et al. 1996; Kaltz and Shykoff 1998) or because there are additional genetic trade-offs/costs coupled with performance of particular virulence types on different host types (Clarke 1979; Parker 1994). This geographic pattern of increased mean performance on sympatric over allopatric host populations is a widely used definition of local adaptation of parasites (Ebert and Hamilton 1996; Lively 1996; Gandon et al. 1998). Similarly, parasites can be considered as locally adapted if their fitness on the local host population is superior to that of potentially competing foreign parasites (Gandon and Van Zandt 1998).

Experimental tests of local adaptation only rarely follow the process of parasite adaptation to local hosts within a

population (Dybdahl and Lively 1998). Usually a snapshot of a population is taken by sampling parasite and host at a given point in time and comparing performance of the parasite on sympatric versus allopatric hosts. This provides an indirect test of whether the parasite has undergone the process of adaptation to its local host population. From the frequency-dependent dynamics of coevolutionary cycles, it is predicted that the more rapidly the parasite tracks common local hosts the more likely it will be found to perform better on local than on foreign hosts in such experimental snapshots (Kaltz and Shykoff 1998). Artificial cross-infection in a common garden situation (e.g., Karban 1989; Lively 1989) or transfer into the natural sites (e.g., Davelos et al. 1996; Roy 1998) provide experimental approaches to test for local adaptation.

Although documented in a variety of host-parasite systems including plant-herbivore, plant-pathogen, and animal systems (Kaltz and Shykoff 1998; Van Zandt and Mopper 1998), the pattern of local adaptation of parasites is not universal. A number of studies find no difference between parasite performance on sympatric and allopatric host (e.g., Parker 1989; Ennos and McConnel 1995; Davelos et al. 1996; Dufva 1996), possibly for the following reasons. Local adaptation may be present at a higher or lower scale than the investigation considers, but undetected at the scale investigated. The host populations chosen for the experimental sympatric-allopatric comparison of parasite performance may not be differentiated for the relevant genetic characters. In addition, temporal variation in host-parasite dynamics within populations together with spatial variation in resistance among host populations (Morand et al. 1996) may translate into large variation in parasite performance on both sympatric and allopatric hosts (Kaltz and Shykoff 1998). Therefore large numbers of host and parasite populations may be required to detect a statistically significant pattern of local adaptation.

Alternatively, parasites may truly not be adapted to their local hosts. For example, local coevolutionary processes may not be sufficiently powerful to produce the pattern of local adaptation in host-parasite systems that are dominated by migration-drift dynamics. In metapopulation systems where parasite and host populations experience regular extinction and recolonization (Antonovics et al. 1994; Frank 1997; Thrall and Burdon 1997) or exchange enough genes to overcome local adaptive processes (Dias 1996), genetic characteristics of both hosts and parasites will not reflect local selective processes. In addition, fluctuations in host or parasite populations may be affected by local selective forces not involved in the host-parasite interaction. Particularly in highly selfing or asexual organisms, where the whole genome is in linkage disequilibrium, this may lead to (apparently) non-adaptive changes in the frequency of resistance or virulence types in a population (Parker 1991; Burdon and Thompson 1995).

It is commonly assumed that the host arsenal against more rapidly evolving parasites with shorter generation times is limited. One option is the rearrangement of already existing alleles by sexual recombination. This can potentially generate unique combinations for resistance against rapidly coevolving parasites each generation (Levin 1975; Jaenike 1978; Hamilton 1980). Clearly, the effect of sexual recombination will entirely depend on the diversity of the available material,

and ultimately only mutation can generate variation at resistance loci de novo. However, as a more proximate force, migration has been suggested as a source of raw materials for host adaptation, which may even reverse the evolutionary advantage usually enjoyed by parasites (Thompson 1994).

Although migration homogenizes populations, thereby counteracting adaptation to local conditions (Slatkin 1985), even populations with regular introduction of new gene combinations can maintain different resistance and virulence types if selection is strong (Frank 1991). Migration can thus play an important role in introducing new resistance types into host populations (Ladle et al. 1993; Judson 1995). A recent model suggests that migration influences the relative evolutionary rates of hosts and parasites in a metapopulation (Gandon et al. 1996). When parasites migrate more than hosts, parasites will usually be locally adapted to their sympatric host populations. Under some conditions where hosts migrate more than their parasites, however, hosts may be more resistant against sympatric than against allopatric parasites, resulting in a pattern of local parasite maladaptation (Gandon et al. 1996; 1998). Maladapted parasites are less able to infect host individuals from their local population than allopatric hosts. The one previous system where both host and parasite population structure and pattern of local adaptation have been investigated supports these model conclusions, finding parasites that migrate more than their hosts (Dybdahl and Lively 1996) and that are locally adapted (Lively 1989).

Here we investigated local adaptation in the pollinator-transmitted anther-smut disease, *Microbotryum violaceum*, on its perennial host plant *Silene latifolia*. This obligate fungal pathogen of the family Caryophyllaceae sporulates in flowers of infected plants, thereby sterilizing them. There are several reasons to expect that evolutionary rates of this parasite may not be faster than that of its host. The fungal life cycle from deposition to sporulation can be completed within 30 days, but latency can last for several months (Alexander et al. 1993; O. Kaltz, unpubl. data). Furthermore, diseased plants may survive for several years, serving only to disperse fungal spores. Thus, host and parasite generation times may be similar in this system. Passive short-distance transmission of spores occasionally leads to infection of seedlings, but transmission by flower-visiting insects appears to be the rule (Alexander 1990; Roche et al. 1995). Because pollinators avoid diseased plants (Real et al. 1992; Shykoff and Bucheli 1995) more pollinators should carry pollen than fungal spores, which renders interpopulation movement of spores less likely than that of pollen. Furthermore, because seeds are never infected with this fungus (Baker 1947), seed dispersal results in the movement of host but not parasite genes. In addition, the reproductive systems of parasite and host differ. The dioecious host plants are obligate outcrossers, whereas the fungus though capable of outcrossing is apparently mostly selfing (Baird and Garber 1979; Delmotte et al. 1999; Kaltz and Shykoff 1999). Pollinator behavior that transports host genes more effectively than parasite genes, together with the strongly selfing breeding system of the fungus, combines to generate interpopulation gene flow of the host plants two orders of magnitude higher than that of the parasites (Delmotte et al. 1999).

We used host plants and fungal strains from 14 populations to compare fungal infection success on sympatric and allopatric hosts in a cross-inoculation experiment. This experiment was also designed to test the effect of geographic distance between allopatric host populations and the host population of origin on infection success. Because the conditions are met for more rapid evolutionary rates of hosts than of their parasites, we predicted local maladaptation by parasites in this system, such that parasites should have, on average, lower infection success on sympatric than on allopatric hosts.

MATERIALS AND METHODS

Study Organisms

Silene latifolia (= *S. alba* [Miller] Krause; = *Melandrium album* [Miller] Garcke) of the family Caryophyllaceae is a dioecious weedy perennial, found on roadsides and borders of fields, that flowers from early May to late October. Infection with the anther-smut fungus *Microbotryum violaceum* (= *Ustilago violacea* Pers.; Ustilaginales: Basidiomycetes) is common in this and other species of the Caryophyllaceae (Thrall et al. 1993). This obligate pathogen grows endophytically and sporulates in the anthers of flowers of infected plants, thereby replacing all pollen with diploid fungal teliospores (Audran and Batcho 1981). In female plants, infection with the fungus induces abortion of female organs and concomitant production of spore-bearing anthers (Baker 1947). The main disease transmission route is via insects (mostly noctuid moths) that visit flowers for nectar and usually serve as pollinators (Baker 1947; Alexander and Antonovics 1988; Jennersten 1988; Roche et al. 1995). After fungal spores are deposited on a new host, a series of developmental steps are necessary for successful infection. During germination teliospores undergo meiosis and produce a two- to four-celled haploid promycelium (Hood and Antonovics 1998) from which single-celled sporidia bud off in a yeastlike manner. To establish an infection, sporidia of opposite mating type (a1 and a2; Day and Garber 1988) must conjugate to form a dikaryon from which an infection hypha grows into the host. A detailed description of the fungal life cycle can be found in Day and Garber (1988). Systemically infected plants are sterile and recovery from disease is rare (Alexander and Antonovics 1988).

The ability of fungal hyphae to penetrate host plant surface is not restricted to floral tissue, and infection via leaves or flower buds is also possible. Under natural conditions, passive transmission of spores by wind or rain and infection of plants at the vegetative stage is a possible, but relatively rare option occurring only over short distances (Roche et al. 1995). Inoculation of plants at the seedling or rosette stage has been widely used to investigate variation in resistance or infectivity (e.g., Baird and Garber 1979; Alexander 1989; Biere and Honders 1996; Shykoff and Kaltz 1997), and plant crosses more resistant to artificial inoculation are also more resistant when exposed to spores by insect transmission in the field (Alexander et al. 1993).

Disease can be present in a plant population for several years. Prevalence is generally low (less than 10%), but can reach up to 50% (O. Kaltz, unpubl. data; see also Table 1). Longevity of host populations are generally determined by

TABLE 1. Plant population size and disease prevalence for the 14 *Silene latifolia* populations from which seed families and strains of *Microbotryum violaceum* were collected for a cross-inoculation experiment. For location of the populations, see Figure 1.

| Population | Symbol (plant, fungus) | Population size | Disease prevalence |
|---------------|---------------------------|-----------------|-----------------------|
| Weiningen | A, a | 224 | 0.32 |
| Oetwil | B, b | 323 | 0.05 |
| Riehen | C, c | <300 | <0.05 |
| Friedlingen | D, d | <500 | <0.05 |
| Märkt | E, e | 101 | 0.51 |
| Blotzheim | F, f | 552 | 0.05 |
| Magstatt | G, g | <400 | <0.05 |
| Waltenheim | H, h | <500 | <0.05 |
| Kembs | I, i | 318 | 0.05 |
| Hombourg | K, k | 86 | 0.08 |
| Ottmarsheim 1 | L, l | <500 | <0.05 |
| Ottmarsheim 2 | M, m | 232 | 0.1 |
| Balgau | N, n | <1000 | <0.05 |
| Gerstheim | O, o | 180 | 0.11 |

factors other than the pathogen, such as succession or destruction of the habitat, for example, by human impact (Antonovics et al. 1994; O. Kaltz and J. Shykoff, pers. obs.). Infected plants invest less in roots than do healthy plants (Shykoff and Kaltz 1998) and this may explain higher mortality of infected plants in some years (Thrall and Jarosz 1994a; O. Kaltz, unpubl. data).

Collection and Processing of Plant and Fungal Material

From late June to early October 1996, we collected seeds and fungal spores from 14 naturally infected *S. latifolia* populations extending over a 170-km distance along the Rhine Valley in northern Switzerland, France and Germany (Fig. 1, Table 1). From each population, we collected all available capsules containing ripe seeds from each of 10 female plants. Because a female may receive pollen from different male plants the progeny of these females constitute a mixture of half and full siblings. Seeds were stored in paper bags at room temperature. On January 22, 1997, we germinated the seed families in petri dishes containing 1.2% water agar. Until the start of the inoculation experiment seedlings were kept in a climate room under a 16 h light regime at 21°C.

Seeds and fungal spores were collected from each population on the same day. From each of four (only two were available in the Magstatt population) diseased plants from each population we collected flower buds that contained mature spores and were just about to open. We used flower buds (instead of expanded flowers) to avoid contamination with spores from other sources. Buds were dried in microcentrifuge tubes with lids open for one week. Then we dissected buds, harvested the teliospores, and stored them in plastic tubes at room temperature. We used teliospores from only one flower bud per plant individual. Teliospores from the same flower bud are presumably identical (Day 1980) and will be referred to as "strain" hereafter. During December 1996, we isolated one mating-type a1 sporidial colony and one mating-type a2 colony from each of the 54 teliospore strains using a standard isolation technique (Day and Jones 1968; see also Shykoff and Kaltz 1997); teliospores from a given strain were suspended in sterile water and appropriate

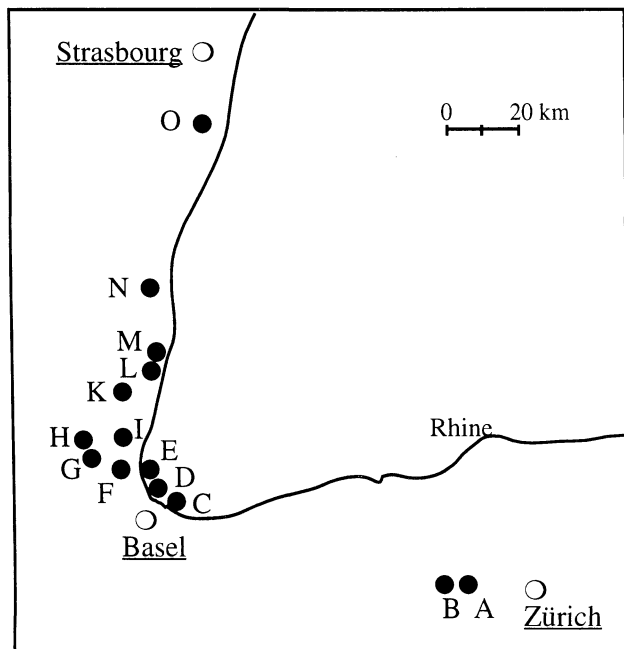


FIG. 1. Location of 14 natural populations (A to O) of *Silene latifolia* harboring the pathogen *Microbotryum violaceum* along the Rhine Valley in the bordering region of France, Switzerland, and Germany. Seed families and fungal strains from these populations were used for a cross-inoculation experiment.

dilutions (500–100 teliospores) spread on complete solid medium (5 g yeast extract, 20 g glucose, 20 g agar per liter distilled water). Teliospores germinate and undergo meiosis, and the resulting haploid sporidia replicate in a yeastlike manner. After one week of incubation at room temperature, the sporidial colony originating from a single teliospore was removed with a toothpick, suspended in sterile water, vortexed, and again plated on complete medium. The resulting sporidial colonies originate from single sporidia. For mating type determination, two suspensions of sporidia from a given colony were mixed with sporidia from either an a1 or an a2 reference colony in a microcentrifuge tube. After 24 h at 10°C, the mating type was inferred from the presence of conjugations between sporidia of opposite mating type in one suspension and absence in the other. We isolated one mating-type a1 sporidial colony and one mating-type a2 colony from each of the 54 teliospore strains. Each of these pairs of sporidial colonies originated from the same teliospore colony, that is, from the same meiosis. After isolation and mating type determination, the 108 sporidial colonies were stored on complete solid medium at 5°C. On January 30, 1997, the colonies were replated on complete medium and grown at room temperature to have the necessary amounts of sporidia for the inoculation procedure. On February 8, 1997, we prepared stock suspensions of the colonies to be used for inoculation. Densities in these stocks were $1.5\text{--}2 \times 10^6$ sporidia/ μl , as measured with a Neubauer[®] haemocytometer. To prevent further replication of sporidia, we kept these suspensions at 2°C during the three days of inoculation (February 9–11, 1997).

Experimental Design and Inoculation Procedure

The goal of this experiment was to compare infection success in sympatric combinations (fungus and plant from the same populations) with infection success in allopatric combinations (fungus and plant from different populations). Limitations in space and time did not allow us to perform all of the 196 possible combinations between plant and pathogen populations. We restricted the matrix of inoculation combinations as follows: Each of the four fungal strains from a given population was confronted with host plant seedlings from its own population (sympatric combinations) and from three allopatric populations (Fig. 2). This design was balanced such that each plant and pathogen population encountered its sympatric and three allopatric opponents. In addition, for each fungal population, allopatric plant populations were chosen at three distances: near (< 10 km away from the fungal population), intermediate (10–30 km away), and far (> 30 km away). Distances were calculated as the shortest possible stepping-stone distance. We attempted to have each plant population appearing once as near, intermediate, and far allopatric host for the different fungal populations. However, precise assignment to the three distance categories was not possible for all fungal and host populations at the same time (e.g., the nearest neighboring population to the Gersheim population is 53 km away). We gave priority to the fungal populations, confronting them with plant populations in the three distance categories where this was possible.

We carried out the inoculations between February 9 and 11, 1997, when seedlings began developing the first leaves after the cotyledons. Because not all inoculations could be carried out at once, we performed 14 inoculation series. For a given series the seedlings from a single plant population were exposed to the different fungal treatments as follows: Thirty-four sterile petri dishes (5-cm diameter) were filled with 5 ml tap water. We carefully removed the seedlings from the water agar and added one seedling per seed family to each petri dish. Petri dishes were arranged in 17 pairs, and each of 16 pairs received one of the 16 fungal inocula, that is, a mixture of sporidia of opposite mating type from a particular strain taken from the stock suspensions (four fungal populations \times four strains, according to Table 2). The 10 plants in an individual petri dish received approximately $1.5\text{--}2 \times 10^7$ sporidia from each of the a1 and a2 sporidia of a given strain. We split fungal treatments into two petri dishes to minimize the risk of losing an entire treatment combination (e.g., by breaking the petri dish), but also in an attempt to minimize potentially confounding petri dish effects (e.g., if a particular petri dish environment influences infection). The remaining two petri dishes received no sporidia, and thus served as a control to test whether contamination occurred during inoculation or subsequent planting of seedlings.

Because there was considerable variation in seed germination among seed families, we did not have enough seedlings from all 10 families in some populations. Families with few seedlings were not used, and instead we added more seedlings from other families of this population to each petri dish. This resulted in a general overrepresentation of particular seed families, but ensured equal representation of families across petri dishes (i.e., fungal treatments). In addition,

| Fungal population | Plant population (number of seed families) | | | | | | | | | | | | | |
|-------------------|---|--------|--------|--------|-------|--------|--------|-------|-------|--------|--------|-------|--------|-------|
| | A (6) | B (10) | C (10) | D (10) | E (9) | F (10) | G (10) | H (9) | I (9) | K (10) | L (10) | M (7) | N (10) | O (9) |
| a | 0 | 4 | | 72 | | | | | | | | | | 166 |
| b | 4 | 0 | | | 72 | | | | | | | | | 110 |
| c | | | 0 | 4 | | | | | 14 | | | 30 | | |
| d | 72 | | | 0 | 4 | | 14 | | | | | | | |
| e | | | | | 0 | 4 | | 15 | | | | | 40 | |
| f | | 72 | 8 | | | 0 | | | | | 21 | | | |
| g | | | | 14 | | | 0 | 2 | | | | | | 90 |
| h | | | | | 15 | | | 0 | 7 | | | | | 35 |
| i | | 78 | | | | | 6 | | 0 | 11 | | | | |
| k | | | | | | 17 | | | | 0 | 4 | | | 74 |
| l | | | 29 | | | | | | 15 | | 0 | 2 | | |
| m | 99 | | | | | 23 | | | | 6 | | 0 | | |
| n | | | | | | | 37 | | | 21 | | 15 | 0 | |
| o | | | 99 | | | | | 88 | | | 70 | | | 0 |

FIG. 2. Matrix of combinations of populations of the fungus *Microbotryum violaceum* and its host plant *Silene latifolia* in a cross-infection experiment. For each combination stepping-stone geographic distances (km) between fungus and plant populations are given. Each fungal and plant population was used in four combinations. Each of four (two for population g, three for population k) strains per fungal population were confronted with a total of 16 seedlings taken from up to 10 seed families per plant population (for details, see text). Sympatric combinations are marked in dark, allopatric combinations in light shading. For location and name of populations, see Figure 1, Table 1.

only three fungal strains of the Hombourg population could be used, because for one strain we had accidentally prepared two sporidial colonies of the same mating type.

After adding the sporidia, the petri dishes were placed on a shaker platform and agitated for 60 min at moderate speed to facilitate contact between sporidia of opposite mating type on the seedling surface. After incubation for 24 h at 12°C in a climate room, seedlings were transferred to the greenhouse and planted into plastic multipot containers (0.1-liter volume per plant). We planted only the eight most vigorous (those with expanded leaves and well-developed roots) of the 10 seedlings per petri dish. During planting sessions care was

taken to avoid secondary contamination of seedlings with sporidia from other treatments. We exchanged toothpicks used for placing seedlings into the pots and disinfected hands with 95% alcohol before planting seedlings from another plant × fungus combination. Ten days after inoculation the young plants were exposed to additional artificial light from 07:00 h to 12:00 h to enhance growth and induce flowering as soon as possible. On March 10, 1997, when rosettes had begun to form, plants were transplanted to larger pots (0.9-liter volume) and randomized over a 100-m² greenhouse space where they were kept until flowering. We scored flowering plants as either diseased (with the fungus sporulating

TABLE 2. Proportion of diseased plants for sympatric and allopatric combinations of populations of *Silene latifolia* and *Microbotryum violaceum* in a cross-inoculation experiment. For name and location of the populations, see Figure 1, Table 1.

| Fungal population | Plant population | | | |
|-------------------|------------------|------------|----------|----------|
| | Sympatric | Allopatric | | |
| a | 0.35 (A) | 0.59 (B) | 0.26 (D) | 0.22 (O) |
| b | 0.47 (B) | 0.42 (A) | 0.63 (E) | 0.19 (N) |
| c | 0.34 (C) | 0.33 (D) | 0.45 (I) | 0.10 (M) |
| d | 0.47 (D) | 0.41 (E) | 0.64 (G) | 0.37 (A) |
| e | 0.22 (E) | 0.63 (F) | 0.31 (H) | 0.46 (N) |
| f | 0.21 (F) | 0.49 (C) | 0.42 (L) | 0.40 (B) |
| g | 0.16 (G) | 0.44 (H) | 0.25 (D) | 0.29 (O) |
| h | 0.31 (H) | 0.39 (I) | 0.50 (E) | 0.35 (N) |
| i | 0.34 (I) | 0.56 (G) | 0.19 (K) | 0.44 (B) |
| k | 0.29 (K) | 0.32 (L) | 0.33 (F) | 0.47 (O) |
| l | 0.21 (L) | 0.36 (M) | 0.29 (I) | 0.46 (C) |
| m | 0.17 (M) | 0.32 (K) | 0.51 (F) | 0.50 (A) |
| n | 0.51 (N) | 0.26 (M) | 0.53 (K) | 0.77 (G) |
| o | 0.37 (O) | 0.36 (L) | 0.30 (H) | 0.48 (C) |

in the flowers) or healthy (symptomless, healthy flowers were produced). Diseased plants were immediately segregated to avoid secondary infection of neighbor plants.

Data Analysis

Variation in Infection Success among Fungal and Plant Populations

Analysis of deviance was used to investigate variation in the proportion of diseased plants among plant and fungal populations, strains within fungal populations, and whether there were fungus \times plant interactions. In this analysis we used infection status (diseased/not diseased) as response variable to carry out a logistic regression with the logit link function of the GENSTAT statistical package (Payne et al. 1987). Terms were entered into the model in analogy to SAS Type II (SAS Institute 1988) model fitting: to calculate the deviance caused by the main effect of plant population, this effect was fitted after entering the effects of fungal population and strains within population. To calculate the effects of fungal terms, these were fitted after entering plant population; nested terms and interactions were fitted after the main effects. All factors were considered random. For hypothesis testing we used mean changes in deviance from the overall mean (two times log-likelihood ratio divided by the degrees of freedom; analogous to mean squares obtained from least-square methods) to carry out pseudo- F tests (e.g., Schmid and Dolt 1994). Only the deviance caused by highest-order term in the model (fungal strain \times plant population interaction) was directly tested against the χ^2 distribution.

Infection Success in Sympatric versus Allopatric Combinations of Fungus and Plant

Two approaches were used to investigate infection success in allopatric versus sympatric combinations of fungus and plant. First, three t -tests were performed as follows. For the first t -test (linewise comparison of sympatric versus allopatric combinations, Fig. 2), we calculated the mean difference in the proportion of diseased plants between sympatric and al-

lopatric hosts separately for each of the 14 fungal populations. These differences were obtained by averaging over sympatric versus allopatric differences of individual strains. Differences in infection success of individual strains were calculated by combining the number of diseased and healthy plants over the three allopatric populations and subtracting the overall allopatric proportion of diseased plants from the sympatric proportion of disease. Proportions were arcsine-transformed before calculating differences. A one-sample t -test (Zar 1984) then tested whether the 14 mean differences were significantly different from zero, that is, whether, on average, infection success on allopatric hosts differed from that on sympatric hosts.

The second t -test (columnwise comparison) was to test whether, on average, resident fungi were superior on their own host plant populations to foreign fungi on those same populations. To this end, we calculated the relative infection success of fungal strains on a given plant population by dividing their value of proportion of diseased plants by that of the strain with the highest infection success on this plant population. Then, for each of the 14 plant populations, we calculated the mean relative infection success of sympatric and allopatric fungal populations by averaging relative infection success across strains and populations. These 14 pairs of (arcsine-transformed) means were compared with a paired t -test. This columnwise comparison asks whether sympatric fungal populations are invisable by the allopatric fungi tested. To further determine whether plants were more resistant to sympatric versus allopatric fungi we carried out a third t -test comparing the proportion of healthy plants when exposed to sympatric and allopatric fungi. We calculated the mean sympatric-versus-allopatric differences in proportion of healthy plants for the 14 plant populations as described for the first t -test. Finally, we investigated the correspondence between different measures of local adaptation: We tested whether the difference in infection success of fungal populations in sympatry versus allopatry was correlated, first, with the resistance of host plant populations against their local and foreign fungi and, second, with the relative performance of sympatric fungi versus allopatric fungi on the same plant population.

In a second approach we compared infection success in the diagonal (sympatric combinations) of the combination matrix (Fig. 2) with the off-diagonal (allopatric combinations). To this end, we repartitioned the variation in the proportion of diseased plants explained by the fungus \times plant population interaction (29 df, see Table 2) from the analysis of deviance described above into an sympatric-versus-allopatric effect (1 df) and a residual term (28 df). This residual term represents the remaining among-combination variation nested within diagonal and off-diagonal and was therefore used as error term to test for a significant difference in infection success between sympatric and allopatric combinations.

Relationship between Infection Success and Geographic Distance between Host and Pathogen Populations

Whether the performance of fungal populations was a function of the geographic distance between their population of origin and the population against which they were tested was

TABLE 3. Analysis of deviance testing the effects of fungal population, strain nested within population, and plant population on the proportion of diseased plants of *Silene latifolia* after inoculation with *Microbotryum violaceum*. To compare infection success of sympatric versus allopatric combinations of fungus and plant the fungus \times plant population was partitioned into an effect "sympatric versus allopatric" and a residual. This analysis used a logistic regression approach. Mean deviance (MD) is equal to twice the log-likelihood ratio divided by the degrees of freedom. The effects used as denominator for hypothesis tests are presented in the denominator column.

| Source | df | MD | Denom. | F | P |
|---|------|-------|--------|------|-------|
| Fungal population (1) | 13 | 2.76 | (2) | 1.86 | 0.067 |
| Strain(fungal population) (2) | 39 | 1.481 | (6) | 1.06 | 0.39 |
| Plant population (3) | 13 | 6.106 | (4) | 2.1 | 0.047 |
| Fungus \times plant population (4) | 29 | 2.904 | (6) | 2.07 | 0.003 |
| Allopatric vs. sympatric | 1 | 10.46 | (5) | 3.97 | 0.056 |
| Residual (5) | 28 | 2.634 | | | |
| Strain \times plant pop.(fungal pop.) (6) | 117 | 1.402 | (*) | | 0.003 |
| Error | 2329 | 1.282 | | | |

* Deviance tested against χ^2 distribution.

investigated as follows. First, using the arcsine-transformed overall proportions of diseased plants (combined over strains within fungal populations) for each of the 56 combinations of host and pathogen population we carried out a one-way analysis of variance with plant population as explanatory variable. The residuals from this analysis were used as response variable in an analysis of covariance with fungal population as explanatory variable and the log-transformed geographic distance between host and pathogen populations as the covariate. We also included the second-order term of the covariate and tested for variation in slope among fungal populations by adding fungus \times covariate interactions. This analysis was carried out with the SAS statistical package (SAS Institute 1988) and sequential SAS Type I sums of squares (order of terms fitted: fungal population, distance linear, distance second order). To analyze distance effects in more detail we also investigated the influence of distance on residual infection success separately for the three categories of interpopulation distance.

RESULTS

Of the 3616 planted seedlings (inoculated and controls), 737 died during the first 10 days after planting, and another 21 plants died later. Thirty-nine percent of the 2424 inoculated plants that had flowered by the end of September 1997, when we terminated the experiment, produced diseased flowers. One of the 198 flowering control plants was found diseased in late September, thus indicating that secondary infection was a rare event.

Variation in Infection Success among Fungal and Plant Populations

We found marginally significant variation in the proportion of diseased plants among plant populations as well as an almost significant effect of fungal population (Table 2). Among plant populations, proportions of diseased plants ranged from 0.22 (population M) to 0.56 (population G). Among fungal populations, infection success ranged from 0.28 (population g) to 0.51 (population n). It should be noted here that, because analysis of deviance is based on a maximum-likelihood technique, *P*-values are not exact and interpretation of such marginal significance should be exercised with care. However, there was a highly significant plant \times

fungal population interaction. Variation in infection success among the 56 combinations of fungal and plant populations (Table 3) ranged from 0.10 (combination c \times M) to 0.77 (n \times G). Furthermore, proportions of diseased plants produced by the different fungal strains on plants from the different plant populations ranged from zero to one, and strains from the same population performed differently on different hosts (significant strain \times plant population interaction, Table 2).

Infection Success in Sympatric versus Allopatric Combinations of Fungus and Plant

We found a 20% higher average infection success of parasite strains on allopatric hosts (proportion diseased plants averaged across fungal populations = 0.40 ± 0.02 SE, $N = 14$) than on sympatric hosts (proportion diseased plants = 0.32 ± 0.03 SE, $N = 14$).

The rowwise paired *t*-test (the difference in performance on sympatric vs. allopatric hosts of the different fungal populations, i.e., among rows in Fig. 2) revealed that, on average, this fungus was less infectious for sympatric than allopatric hosts ($t_{13} = 3.17$, $P = 0.007$). Eleven fungal populations had lower mean infection success on sympatric than on allopatric hosts, and only three populations tended to perform better on sympatric than allopatric host populations (Fig. 3). The fungal population that revealed the greatest maladaptation was less than half as successful at infecting sympatric (proportion disease = 0.17) than allopatric hosts (proportion disease = 0.46). In contrast, the fungal population that was the most locally adapted produced only 7% more infection on sympatric (proportion disease = 0.47) than on allopatric hosts (proportion disease = 0.40; Fig. 3).

The second, columnwise paired *t*-test, based on relative infection success of the different fungal strains on the same host plant population (i.e., among columns in Fig. 2), revealed a similar pattern: On average, sympatric (resident) fungal populations were less successful at infecting their local host plants than were allopatric (nonresident) fungi ($t_{13} = 2.66$, $P = 0.02$; Fig. 4). This mean resident disadvantage was obtained for 11 of the 14 fungal populations. The analogous paired *t*-test comparing mean proportion of healthy plants in sympatric versus allopatric combinations also found a higher level of resistance against sympatric than allopatric fungi in 11 of the 14 plant populations ($t_{13} = 1.87$, $P = 0.04$, one-

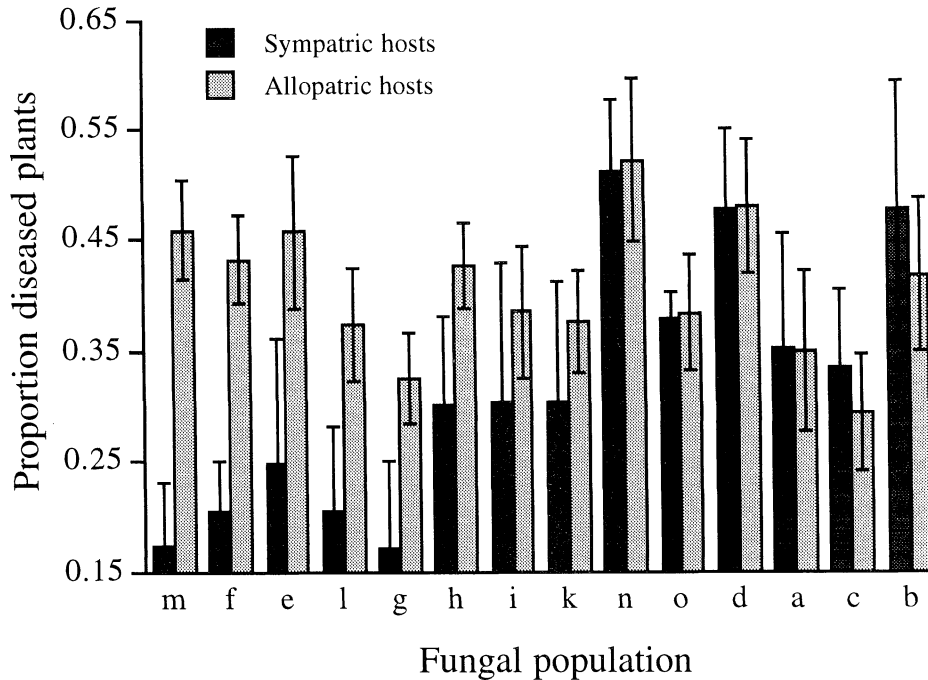


FIG. 3. Proportion of diseased plants of *Silene latifolia* inoculated with *Microbotryum violaceum* for sympatric and allopatric combinations of pathogen and host. Means and standard errors were calculated across fungal strains and host populations. For location and name of populations, see Figure 1, Table 1.

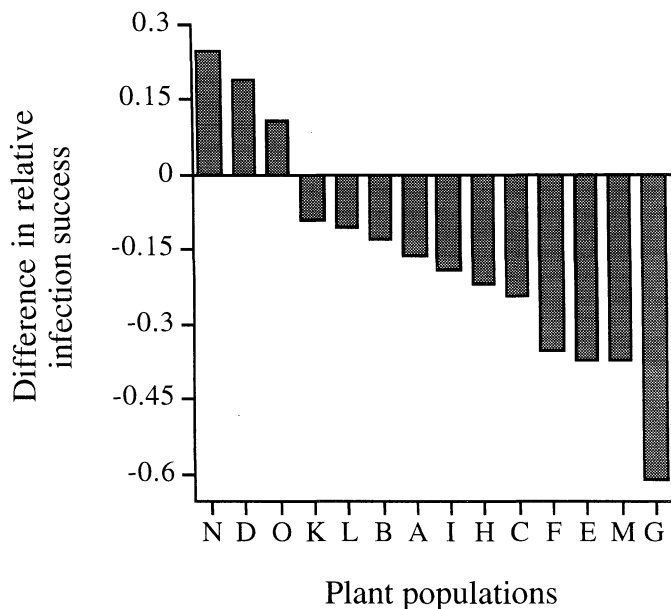


FIG. 4. Mean relative infection success (i.e., difference in relative proportion of diseased plants) of sympatric versus allopatric strains of *Microbotryum violaceum* on plants from 14 *Silene latifolia* populations. Differences were calculated from arcsine-transformed overall mean proportions of diseased plants produced by sympatric and allopatric strains. Positive values, relative infection success sympatric strains > allopatric strains; negative values, relative infection success allopatric strains > sympatric strains.

tailed). We found a correspondence between the different measures of local adaptation: There was a significant negative correlation between the difference in infection success of fungi on sympatric versus allopatric host plants and the difference in resistance of these host plants against their local versus foreign fungi (Pearson correlation coefficient: $r = -0.58$, $P < 0.031$, $N = 14$). That is, the level of local plant maladaptation corresponded with the level of local plant adaptation across the 14 populations. There also was a significant positive correlation between the difference in infection success on sympatric versus allopatric hosts and the difference in relative infection success of sympatric versus allopatric fungi (Pearson correlation coefficient: $r = 0.54$, $P < 0.048$, $N = 14$). That is, fungi performing less well on their local than on foreign hosts were also less successful on their local hosts than were foreign fungi. This implies that locally maladapted parasite populations would be invisable.

In a second approach we compared sympatric and allopatric combinations as diagonal and off-diagonal rather than performing row- or columnwise comparisons. This analysis, by repartitioning deviance explained by the plant \times fungal population interaction (Table 2), found a marginally significant difference in the proportion of diseased plants in sympatric versus allopatric combinations ($P = 0.056$, Table 2).

Relationship between Infection Success and Geographic Distance between Host and Pathogen Populations

Controlling for the main effect of plant population on the proportion of diseased plants ($F_{13,42} = 1.36$, $P = 0.22$) and using the residuals in an analysis of covariance, we found a significant second-order effect of geographic distance be-

TABLE 4. Sequential (SAS Type I) analysis of covariance testing the effects of fungal population and geographic distance (linear and second-order term) between populations of *Microbotryum violaceum* and *Silene latifolia* on the residual proportion of diseased plants in a cross-inoculation experiment. Residuals had been obtained from fitting the effect of plant population on the proportion of diseased plants in a one-way analysis of variance.

| Source | df | Mean square ($\times 10^{-2}$) | F | P |
|-----------------------|----|-------------------------------------|------|-------|
| Fungal population | 13 | 1.52 | 1.15 | 0.349 |
| Distance | 1 | 1.74 | 1.32 | 0.258 |
| Distance ² | 1 | 8.06 | 6.10 | 0.018 |
| Error | 40 | 1.32 | | |

$R^2 = 0.36$.

tween the parasite and host populations on residual infection success (Table 4). None of the two fungal population \times covariate interactions were significant (F -values < 1) and therefore were removed from the full model. For interpopulation distances of less than 10 km there was a positive effect of distance on residual infection success (linear regression for distances between 0–10 km: $F_{1,24} = 5.29$, $P = 0.031$). Of the 12 fungal populations that were tested against host populations located less than 10 km away from their population of origin (Fig. 2), nine had higher infection success on these nearby populations than on their sympatric hosts (Fig. 5). For more distant host-parasite combinations this distance effect leveled off or even decreased (Fig. 5). For distances between 10–30 km, there was a significant second-order effect of distance on residual infection success (sequential regression: linear term: $F_{1,11} = 0.31$, $P = 0.59$; second-order term: $F_{1,11} = 6.66$, $P = 0.026$), whereas for distances larger than 30 km we found a significant, negative linear effect of distance on residual infection (linear regression: $F_{1,14} = 5.37$, $P = 0.036$).

DISCUSSION

The fungal plant disease *Microbotryum violaceum* provides an example of an average pattern of a locally maladapted pathogen. Over a set of pathogen and *Silene latifolia* host populations this fungus was, on average, less able to infect sympatric than allopatric hosts, which is a finding in contrast to most other host-parasite systems (Kaltz and Shykoff 1998). Furthermore parasites performed, on average, worse on their own sympatric population than did foreign parasites on plants from that same population. Thus, parasites were locally maladapted both in terms of their performance and their invasibility (Gandon and Van Zandt 1998).

Variation in Infection Success

We found that populations of the host plant *S. latifolia* varied in their overall susceptibility to *M. violaceum*. Intrapopulation variation in host plant resistance has been previously shown for this host-parasite system (Alexander 1989; Alexander and Antonovics 1995), and among-population variation is known from a closely related host plant species (Carlsson-Granér 1997). Little evidence exists to date for variation in infectivity of this fungus on *S. latifolia* (Alexander et al. 1993; Shykoff and Kaltz 1997; but see Alexander

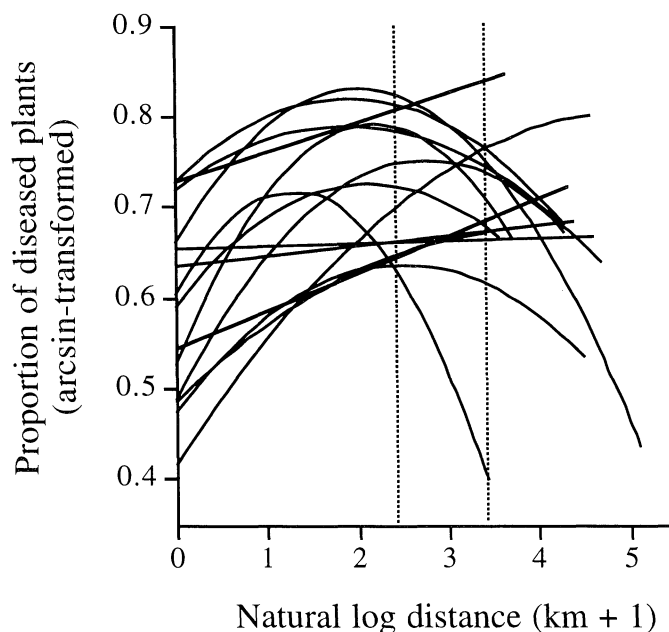


FIG. 5. Infection success of *Microbotryum violaceum* after inoculation of *Silene latifolia* plants as a function of geographic distance between host and pathogen populations. Curves represent linear or second-order polynomial fits separately for each fungal population across its sympatric and three allopatric host populations (i.e., four data points). Vertical dotted lines mark distances of 10 km and 30 km, respectively.

and Maltby 1990), possibly because of weak statistical power or because in North America, where most experiments were carried out, this fungus suffered a severe bottleneck either through introduction or host-switching (Antonovics et al. 1996). Here we found strong interaction effects between pathogen and host at both the fungal population and strain level (Table 2). Such evidence for virulence variation in the native European populations of this fungus reflects variation in phenotypic effects of different fungal strains on its host plants (Shykoff and Kaltz 1998). Clearly these pathogen \times host interactions in infectivity and resistance within and among populations provide an opportunity for coevolutionary processes that can generate patterns of local adaptation or maladaptation.

Infection Success in Allopatric versus Sympatric Combinations

Parasite-host coevolutionary interactions are expected to generate cycles of adaptation and counteradaptation, with new resistance and infectious types arising and spreading (Haldane 1949; Hamilton 1980). Time-lagged cycles of host and parasite types are then driven by negative frequency-dependent selection (Hutson and Law 1981; Bell and Maynard Smith 1987; Nee 1989; Hamilton et al. 1990; Frank 1996). Critical to the coevolutionary dynamics of these cycles is the length of the time-lag between genetic change in the host and parasite response. The higher the evolutionary rates of the parasites, the shorter is the time-lag. When parasites evolve faster than their hosts, an overall pattern of local adaptation will arise, with the majority of parasite populations

having the appropriate infectivity types for their local host populations, although all parasite populations will at some point be lagging behind their hosts (Nee 1989; Kaltz and Shykoff 1998).

In our host-parasite system, however, the average finding was of poorer pathogen performance on local host plant populations. Of the 14 fungal populations, five showed no strong reduction in infection success on sympatric hosts, and three even tended to perform better on sympatric hosts (Fig. 3). This pattern is consistent with coevolutionary cycling where parasites evolve relatively more slowly than their hosts. Local adaptation (or maladaptation) in host-parasite system should indeed only emerge as an average phenomenon (Ebert and Hamilton 1996; Morand et al. 1996; Gandon and Van Zandt 1998; Kaltz and Shykoff 1998) when it results from coevolutionary cycles as explained above. Additional factors may contribute to the average nature of the phenomenon. For example, time may simply not have been sufficient for adaptation in a given population. Furthermore, because coevolution occurs in sympatry and not allopatry, parasite performance may vary considerably on foreign hosts chosen for allopatric combinations (Morand et al. 1996).

High variance in infection success among the different combinations of fungus and host may also explain the slight inconsistency between the paired *t*-test approach, which gave strong significant differences between allopatric and sympatric combinations, and the analysis of deviance (Table 2). Partitioning of the "plant \times fungus population" variation into its sympatric versus allopatric components revealed only a marginally significant effect, presumably because the sympatric-versus-allopatric effect was tested against the remaining variation among the combinations within the two categories (Table 2). However, we feel that the question of parasite performance in sympatric versus allopatric hosts is best addressed by a paired *t*-test because the comparison of diagonal and off-diagonal in the analysis of deviance cannot account for the paired aspect of the question.

At this point, it should be noted that our finding of local parasite maladaptation is unlikely to result from biased sampling of seeds from only healthy plants in our host populations. Systemically infected plants are sterile and de facto removed from the population. Thus one might argue that reduced infection success on sympatric versus allopatric is simply an artifact of sampling more resistant genotypes. However, given the mostly low disease prevalence in our populations (Table 1) and low transmission rates in general (Alexander and Antonovics 1988), not all plants may have been sufficiently often exposed to the fungus to become infected. Furthermore, with strongly overlapping generations, offspring of infected plants should be present among healthy individuals, particularly because more susceptible plants flower earlier in the season and produce more flowers and fruits (Alexander and Antonovics 1995; Biere and Antonovics 1996). Hence, it appears reasonable to conclude that our samples were relatively unbiased. Finally, we tested for local adaptation by looking at many populations at a single point in time rather than following the process. This necessarily considers the relationship between the pathogen and those hosts currently available for infection, that is, healthy plants. Performance on healthy plants is a measure of both the path-

ogen's current state of adaptation and its ability to exploit its local host population. In this respect it is irrelevant whether or not seed samples were biased. As outlined above, if *M. violaceum* were rapidly evolving and ahead of its host in the coevolutionary arms race, it should be locally adapted most of the time during the coevolutionary cycle and thus in most populations. Local adaptation should be found even though this parasite is selecting for increased resistance in *S. latifolia* hosts and had increased host plant resistance levels before we collected seeds. Thus, our finding of poorer performance in sympatric combinations implies that the parasite *M. violaceum* has lower evolutionary rates in the coevolutionary arms race.

What Causes Local Maladaptation in this Pathogen?

First, a possible explanation for lower evolutionary rates in *M. violaceum* than in its host *S. latifolia* is the difference in their breeding systems. Sex may generally provide hosts with the necessary variation to cope with coevolving parasites. By rearranging existing alleles at resistance loci, potentially novel genotypes may be generated that are resistant against the common parasite type in the population. This argument, known as the Red Queen hypothesis (Bell 1982), may explain the maintenance of sex in hosts (Jaenike 1978; Hamilton 1980) despite its two-fold cost (Maynard Smith 1978). However, to what extent host and parasite sexual recombination contributes to their relative evolutionary rates has rarely been addressed. It would be interesting to determine whether outcrossing by hosts of highly inbred parasites could provide the hosts with a sufficiently strong advantage to be ahead in the coevolutionary arms race most of the time. Morand et al. (1996) predict complex coevolutionary dynamics for the case of sexual hosts and asexual parasites, but nonetheless local adaptation of the parasite is expected under some conditions (S. Morand, pers. comm.).

Silene latifolia is dioecious, and therefore obligately outcrossing, so it undergoes higher levels of sexual recombination than its highly selfing pathogen (Baird and Garber 1979; Bucheli et al. 1998; Delmotte et al. 1999). Allozyme data indicate that *S. latifolia* populations in our study area are effectively randomly mating (Delmotte et al. 1999). In contrast, despite its potentially mixed mating system, *M. violaceum* appears to be predominantly selfing, presumably because simultaneous arrival of teliospores from different sources is rare, initial developmental processes of the fungus preclude outcrossing (Hood and Antonovics 1998), or self mating partners are preferred over outcross ones (Kaltz and Shykoff 1999). Interestingly, fungal populations from the closely related species *Silene dioica* are more outbreeding, with less excess of homozygotes in natural populations than the fungus on *S. latifolia* (Bucheli 1997). Higher infection rates in local than foreign plants was found in two out of three populations of *S. dioica* in a transplant experiment (Carlsson-Granér 1997).

Particularly when hosts and parasites have similar generation times, breeding system variation will be most important in determining relative evolutionary rates (Bell and Maynard Smith 1987). In our system, the generation time advantage of *M. violaceum* may indeed be low. Sporulation after infec-

tion of a new host can occur within one month, although latency can be very long (Alexander et al. 1993). Furthermore, the fungus can complete only one generation per year in areas with short flowering seasons. Diseased plants can survive and produce spores for many years. Similarly plants are perennial, so cohorts of seedlings and new fungal infections may be produced by old individuals, thus increasing realized generation times.

Second, virulence variation in *M. violaceum* may be reduced by stochastic effects. Indeed, *S. latifolia* populations often harbor only a few infected host plants, and disease prevalence can remain stably low over several years (Antonovics et al. 1994; Kaltz and Shykoff, pers. obs.). Median disease prevalence in our study populations was 0.06 (Table 1), indicating that disease spread within populations is indeed limited. For this obligate parasite, a unique virulence type will be lost from the population if its host plant dies before successful transmission. Diseased plants sometimes experience higher mortality risk (Thrall and Jarosz 1994a; Alexander and Antonovics 1995; O. Kaltz, unpubl. data), possibly because infected plants invest less in root stocks (Shykoff and Kaltz 1998). Drift, together with selection for increased host plant resistance, may even entirely purge the fungus from a population (Antonovics et al. 1994; Thrall and Jarosz 1994b; Thrall and Antonovics 1995).

Differential migration rates of *M. violaceum* and its host *S. latifolia* may represent a third reason for low relative evolutionary potential of this pathogen. Coevolutionary dynamics and local differentiation or adaptation of host and parasite within individual populations may not be independent of processes at the metapopulation level (Thompson and Burdon 1992; Thompson 1994; Thrall and Burdon 1997). Migration introduces new genotypes into populations and can thus provide raw materials for coevolution. Indeed, migration may compensate the evolutionary advantage usually enjoyed by rapidly evolving parasites or even render evolutionary rates of hosts greater than that of their parasite (Thompson 1994). A theoretical formalization of this idea has shown that, all else being equal, patterns of local adaptation of either host or parasite may be generated in favor of the player migrating more (Gandon et al. 1996, 1998).

In our system, migration is likely to be responsible for introducing new resistance variation into plant populations faster than fungal populations can respond. First, plants migrate by pollen and seeds, although pollen flow occurs over larger distances than does seed flow (McCauley 1994, 1997). *Microbotryum violaceum* is transmitted mainly by pollinators (Roche et al. 1995). Despite long dispersal distances of pollinators, these discriminate against diseased plants (Real et al. 1992; Shykoff and Bucheli 1995) and so are more likely to carry host plant genes than parasite genes among populations (Delmotte et al. 1999). That differential gene flow of hosts and parasites is responsible for local maladaptation is revealed in the geographic pattern of infectivity (Fig. 5). *Silene latifolia* populations show a pattern of isolation by distance (Delmotte et al. 1999). Generally, if populations are connected by gene flow, parasite performance on different host populations should correlate with geographic distance between these populations and the population of origin of the parasite (Ebert 1994; Gandon et al. 1996).

We obtained a significant nonlinear effect of distance on infection success, with infection success increasing over the first 10-km range of population distances, but leveling off or even decreasing for more distant combinations of pathogens and hosts (Fig. 5). This decrease in infection success could explain why fungal populations from the edges of our supposed metapopulation (populations a, b, n, o; Fig. 1) showed little or no local maladaptation in this experiment (Fig. 3), simply because these fungi were tested in combinations with very distant host populations (Fig. 2). The populations from the center of our metapopulation (populations C–M, Fig. 1) were used for combinations of short or intermediate distances between host and pathogen (Fig. 2). In particular distances of 10 km or less may represent the typical range of genetic exchange among host plant populations. Therefore these populations are most likely to express coevolutionary patterns resulting from selection-migration equilibrium within the metapopulation. It was indeed these central populations showing the most local fungal maladaptation (Fig. 3), possibly because of higher migration rates in the host. However, although increasing infection success over short geographic distances is consistent with migration-selection processes within the metapopulation, decreasing infection success over larger distances is difficult to explain with metapopulation dynamics alone. Long-distance combinations in our experiment mainly involved fungi and/or hosts from the northern and eastern edges of the metapopulation (Figs. 1, 2). These edge populations may experience other environmental conditions than populations in the center or receive gene flow from outside the limit of the metapopulation considered. Consequently, local evolutionary and coevolutionary processes may be different in these populations. Therefore, over larger geographic scales host plants may have become sufficiently differentiated and present a relatively less appropriate host environment for foreign fungal strains.

Altogether, our study represents a well-supported example of a pattern of parasite maladaptation. Other studies have pointed in the same direction, but did not measure parasite adaptation directly. For example, Sork et al. (1993) found less herbivore damage of oak seedlings planted in their own versus foreign sites. However, their experiment did not consider a specific plant-herbivore interaction, so different herbivore species could have been involved at the different sites. Furthermore, seedlings may have been adapted to their own microhabitat, thus rendering them less susceptible to herbivores close to their natal site than far from it. Similarly, by reciprocally transplanting seven clones of the plant *Arabis holboellii* from three sites Roy (1998) found lower levels of rust or herbivore attack for local than for foreign host clones in two of the three sites. In another example, bumblebees from three regions suffered lower mortality after exposure to sympatric than allopatric trypanosome parasites. This vertically transmitted parasite was less virulent on sympatric than allopatric hosts, possibly because bumblebees migrate more than their parasites (Imhoof and Schmid-Hempel 1998). Lower migration rates of parasites than hosts may also explain local maladaptation of blood parasites of Canary Island lizards (*A. Oppliger*, pers. comm.).

In conclusion, maladaptation of *M. violaceum* to its local populations of *S. latifolia* indicates that parasite-mediated

selection for host resistance is more effective than is host-mediated selection for new virulence types in the pathogen. We suggest that the evolutionary potential of this pathogen is limited by the combined effects of its selfing breeding system, low population size, and, in particular, limited dispersal among populations. As a consequence, new infectious variants in the pathogen cannot arise as rapidly as resistance is generated in the host, so that the plant is ahead in the coevolutionary arms race. Within- and among-population coevolutionary processes, here mediated by insect behavior, are very complex. This study shows that geographic patterns of local adaptation in host-parasite systems may therefore nevertheless emerge as an average phenomenon across several populations.

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