The Ecology and Genetics of a Host Shift: *Microbotryum* as a Model System

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ABSTRACT: The need to prevent and cure emerging diseases often precludes their continuing study in situ. We present studies on the process of disease emergence by host shifts using the model system of anther-smut disease (*Microbotryum violaceum*) on the plant genus *Silene* (Caryophyllaceae). This system has little direct social impact, and it is readily amenable to experimental manipulation. Our microevolutionary studies have focused on the host shift of *Microbotryum* from *Silene alba* (= *latifolia*; white campion) onto *Silene vulgaris* (bladder campion) in a population in Virginia. Karyotypic variation shows that the host shift is recent and originates from the disease on sympatric *S. alba*. Analysis of the spatial pattern of disease shows that the host shift has been contingent on the co-occurrence of the two species at a local scale. Cross-inoculation studies show that families of the new host differ greatly in their susceptibility to the pathogen, indicating the potential for rapid evolution of resistance. Disease expression on the new host is frequently abnormal, suggesting that the pathogen is imperfectly adapted to its new host. In experimental populations, disease transmission within populations of the old host is greater than within populations of the new host. However, there is also a high transmission rate of the disease from the new host back to the old host, suggesting a feedback effect that increases disease prevalence in the community as a whole. Continuing studies of these populations are designed to determine whether this new host-pathogen system is likely to be self-sustaining and to quantify evolutionary changes in both the host and the pathogen.

Keywords: host shift, *Microbotryum*, *Silene*, emerging diseases, coevolution, resistance.

There has been increasing concern regarding the emergence of new diseases that result from the shift of a pathogen from its native hosts onto humans or crops and livestock. Although the issue of what constitutes "emergence" or "reemergence" remains controversial, there is continuing interest in diseases that may have their origins in wild populations. For example, in Southeast Asia in the past decade, seven new viral diseases of humans have been recognized that have their putative origin in wild bat populations (Mackenzie et al. 2001). Host shifts have also been a natural component of host-parasite interactions in wild populations (Thompson 1994) and, correspondingly, have been associated with high levels of parasite and pathogen diversification and host specificity. In this article, we use "host shift" in a phenomenological sense, in that we include any case where a disease is found to have moved to a new host (i.e., there has been novel interspecific transmission), regardless of the long-term outcome. In the nascent stages of this process, it seems not useful to make a terminological distinction between host shifts that eventually result in host-range expansions and those that result in specialization and host-race differentiation. Regarding host shifts of disease to humans and domesticated plants and animals, five types of questions have been the focus of study:

What is the original or reservoir host for the newly emerged disease? Phylogenetic studies are being extensively used to determine the origin of new diseases (Muller et al. 1993; Nichol et al. 1993; Sharp et al. 1994). Such studies are crucial for finding the sources of infection and therefore controlling or limiting disease outbreaks. However, these studies are essentially historical and not predictive, in that they do not make a priori statements about the likelihood of future host shifts.

How is disease transmitted from the old host to the new hosts? This question is important because it identifies risks that may lead to disease transfer between species. Overlapping species distributions of a potential new host with the old host is the most obvious prerequisite for a host shift. For example, HIV-1 and HIV-2 appear to have arisen through multiple, separate zoonotic transmission events, where direct human-simian interactions are common (Hirsch et al. 1989; Gao et al. 1999). Particular transmission modes will also affect the likelihood of a pathogen emerging on a new host. Sexually transmitted diseases are less likely to be transmitted across species (Lockhart et al. 1966), and the transmission of vector-borne diseases may

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be limited by vector behavior rather than by host susceptibility (Monath 1993). However, although some studies set out to determine the most important transmission agents, they rarely make quantitative assessments useful for understanding the population dynamics of the pathogen on its original and new host.

Are there genetic differences between the pathogen on the new host and that found in the old host? Identification of genetic differences between the pathogen on the original and new host may be important for understanding the basis of pathogenesis in the new host. For example, it has been demonstrated that a loss of function mutation in the *yopA* gene is responsible for increased virulence of the causative agent of plague and may have been associated with its shift to humans (Rosqvist et al. 1988). While host shifts are often attributed to genetic change, the evidence that such changes are solely responsible is usually circumstantial. Moreover, the issue of the importance of genetic variation in the new host is often considered to be a secondary or tangential issue.

Is the emerging pathogen self-sustaining on the new host? This question has important implications for determining the best strategy for controlling the disease. For example, although yellow fever virus can cause disease in humans, it is still primarily a virus of monkeys. Dengue virus, despite having the same vector, *Aedes aegypti*, and originally a primate host, is an established pathogen of humans (Holmes et al. 1998). Very few population-level studies have explained why some pathogens are able to persist on new hosts while others are not.

Can the disease be transferred to other hosts, especially laboratory organisms? Laboratory organisms may provide useful animal models for the disease or may be used in vaccine development and testing. Inoculation studies and passaging experiments are therefore often used to develop animal models for emerging diseases. Nevertheless, such passaging rarely reflects selective pressures acting on the pathogen in nature. Moreover, it is not possible or subject to ethical restrictions to passage new diseases through humans or other animals to study their immediate evolutionary changes.

In human disease, a wide range of environmental and behavioral changes, as well as genetic factors, have been identified as clear correlates of recent host shifts (Lederberg 1992; Morse 1993; Krause 1998; Stephens et al. 1998). However, a classification of reasons for a host shift into simple ecological or genetic categories fails to consider the dynamics of the process and therefore short circuits many of the important chains of causation involved in these events. For example, what degree of proximity of the new and old host is needed to facilitate a host shift? Are all pathogen genotypes equally likely to colonize a new host, or is genetic variance in pathogenicity important? Is there genetic variance for resistance to the disease in the new host, even though it may never have been previously exposed to the disease? Do especially susceptible individuals of the new host form "bridging" genotypes that permit colonization and subsequent adaptation to the rest of that species? Does colonization of the new host have feedback effects on disease prevalence in the old host? Do these feedback processes promote host shifts ecologically but limit them evolutionarily? These fundamental questions obviously extend not just to emerging disease in human and agricultural contexts but may also contribute to our understanding of the role of disease in conservation biology and to our understanding of the processes that lead to high levels of host specificity and species richness in parasite taxa (Price 1980; Thompson 1994).

To address these questions, we are using the anther-smut disease (*Microbotryum violaceum*, formerly known as *Ustilago violacea*), which attacks many natural populations of plants in the pink family (Caryophyllaceae), as a model system. This disease has some unique advantages for this type of research. Infection is readily identifiable in the field without special techniques. The disease is sterilizing and therefore has a large fitness effect on the host, but hosts are generally not lost through disease-induced mortality. The system is experimentally tractable and has been studied extensively (Deml and Oberwinkler 1982; Biere and Honders 1996; Carlsson-Graner 1997; Soldaat et al. 1997; Shykoff and Kaltz 1998). Importantly, while some species in the Caryophyllaceae are of horticultural importance (such as the carnations), they are never widely grown as crops. The disease is also innocuous to humans, and therefore sampling, transport, and field experimentation are possible with little risk of any social impact. The pathogen is also obligately parasitic with no other reservoir; therefore, the environment can be kept relatively pathogen free in both greenhouse and field studies.

The research described here focuses on the host shift of *Microbotryum* from *Silene alba* (= *Silene latifolia*; white campion) onto *Silene vulgaris* (bladder campion). Both of these short-lived perennial plants are native to Europe but are also common in fields and along roadsides in eastern North America. Both *S. alba* and *S. vulgaris* are perennials, have white flowers, and have overlapping flowering times. *Silene alba* is dioecious, while *S. vulgaris* has female and hermaphrodite individuals. The insect pollinators of both species include bumblebees (*Bombus*) and various moths (often in the Noctuidae; Marsden-Jones and Turrill 1957; Pettersson 1991; Altizer et al. 1998). *Silene alba* is commonly a host of *Microbotryum* in both Europe and North America. However, *S. vulgaris* had only been recorded as a host for *Microbotryum* in Europe (Zillig 1921) until we discovered diseased plants of *S. vulgaris* in Virginia.

Microbotryum is a basidiomycete that is now classified

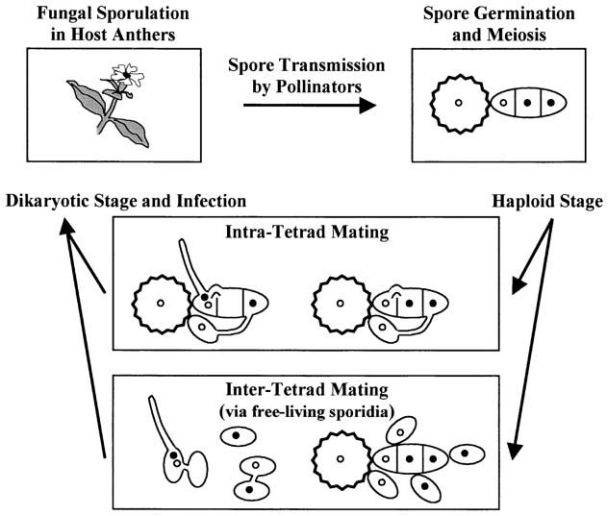

Figure 1: Life cycle of *Microbotryum violaceum*. Following vector transmission, the spore germinate on the surface of the healthy host and undergo meiosis and mating to produce an infection hypha. Note that two types of mating are possible (Hood and Antonovics 2000), namely, mating among the products of a single meiosis (intratetrad mating) or between haploid products of separate meioses (intertetrad mating). The latter could be either diploid selfing or outcrossing.

in its own order Microbotryales (Begerow et al. 1997) and is considered to be phylogenetically distinct from the grass smuts in the Ustilaginales. It is an obligate pathogen of over 100 species within the Caryophyllaceae (the pink or carnation family) and related plant families. In Europe, it has been recorded on 92 species and in North America on 21 species (Thrall et al. 1993). Although anther smuts from the Caryophyllaceae are often referred to as one species (*Microbotryum violaceum*), there has been substantial discussion about the validity of this species designation. It is clear from cross-inoculation studies that *Microbotryum* is actually a species complex where isolates from different species show considerable host specificity and thus consist of specialized host races or *formae speciales* (Zillig 1921; Goldschmidt 1928; Antonovics et al. 1996). Sometimes these host races have been given separate species names (Zillig 1921), and morphological differences among them have been recognized by some taxonomists (Vanky 1994, 1998). We will refer to this species complex simply as *Microbotryum*.

Anther smuts have an intriguing and convenient biology. Diseased plants are easily identified by the fact that the flowers produce dark-colored, spore-filled anthers instead of normal yellow, pollen-filled anthers. The disease is systemic in that the fungus spreads throughout the plant, and usually within one season all flowers produced by a plant become diseased. Diseased plants are sterile because no pollen is produced in the anthers, and the ovary becomes rudimentary. However, diseased plants of *S. alba* are only slightly affected in terms of their vegetative morphology or survival (Alexander and Antonovics 1995). In those species of *Silene* with female plants (e.g., *S. alba* and *S. vulgaris*), ovaries of diseased females are aborted, and flowers develop a male morphology with spore-producing anthers. Both males and females therefore transmit the disease.

The fungal spores are usually transmitted to healthy hosts by insect pollinators (Antonovics and Alexander 1992; Roche et al. 1995; Shykoff and Bucheli 1995; fig. 1). Once deposited onto flowers or leaf surfaces, the fungal spores (=teliospores) undergo meiosis and germinate to produce a germ tube (=promycelium). This germ tube consists of haploid cells of two mating types, A1 and A2. These may conjugate directly by intratetrad mating or produce a free-living haploid stage of yeastlike cells (=sporidia) that can conjugate. There is evidence that the mating system tends toward a predominance of intratetrad mating (Hood and Antonovics 2000) and that genetic exchange among fungal strains or genotypes is limited. Conjugation is required to form an infective dikaryotic mycelium that invades and spreads throughout the newly infected host plant. The mycelium enters the developing anthers, undergoes karyogamy, and differentiates to produce large numbers of diploid teliospores (Cummins and Day 1977; Batcho and Audran 1980).

The pathogen is easy to grow and culture. The yeastlike sporidial stage can be grown on standard microbiological media (we generally use potato-dextrose agar) and can be maintained indefinitely in silica gel in the freezer. Inoculations can be carried out with sporidial mixtures or with teliospores. Host plants are easily crossed and grown. This makes it possible to establish experimental populations for the study of disease transmission (Alexander 1989; Alexander and Antonovics 1995; Biere and Antonovics 1996) and population dynamics (Thrall and Jarosz 1994*a*, 1994*b*). *Microbotryum* has itself been the object of extensive genetic and molecular studies as a pathogenic fungus (Caten and Day 1977; Bej and Perlin 1989; Garber and Ruddat 1994).

Documenting the Host Shift

In the fall of 1998, as part of a general survey of *Microbotryum* on *Silene alba* in Virginia, we discovered diseased plants of *Silene vulgaris* in a large (~2 ha) field near the town of Broadway, Rockingham County. We considered this to be a putative host shift from *S. alba* to *S. vulgaris* because we had never encountered the disease on this species before. This is in spite of the fact that we have systematically observed *S. vulgaris* for over a decade as

part of our regular annual census of disease in populations of *S. alba*. Moreover, *S. vulgaris* has also been extensively studied in Virginia by Taylor et al. (2001) in the context of the population genetics of cytoplasmically inherited male sterility. There are also no previous records in the literature of the disease on *S. vulgaris* in the United States (Farr et al. 1989). An extensive search of regions within 20–30 km of Broadway Field revealed no other diseased populations of *S. vulgaris* but the frequent occurrence of *Microbotryum* on *S. alba*.

Genetic Epidemiology of the Host Shift

Spore collections were taken from diseased plants of each host species in Broadway Field and from diseased *S. alba* near Mountain Lake Biological Station (~130-mile-distance from Broadway) and from diseased *S. alba* in several European localities. Extensive karyotypic variation in *Microbotryum* was found by Perlin et al. (1992) and was used in this study to compare genetic similarity among strains of *Microbotryum* from the old and the new host. Chromosome size variation was quantified by pulsed–field gel electrophoresis. Comparison of *Microbotryum* from both hosts in Broadway Field and samples from *S. alba* in the other localities showed that the fungus from *S. vulgaris* is essentially identical to samples of *S. alba* from within the same field (Hood et al. 2002). Additionally, among the 26 samples within Broadway Field, there were 20 distinct karyotypes that often differed by the position of only one chromosome band. One karyotype was identical across two isolates from *S. alba* and three isolates from *S. vulgaris*, and another was identical between one isolate from each host.

The karyotype similarity of fungal strains infecting both *S. vulgaris* and *S. alba* in Broadway Field and the dissimilarity to the sample from other localities indicated that disease transmission has recently occurred (or is occurring) between these host species, and they are not two relatively divergent host races that are coincidentally sympatric (Hood et al. 2002). Given what we know about the distribution and natural history of disease occurrence on the two species, the host race on *S. alba* is almost certainly the progenitor of the disease on *S. vulgaris*.

Demography of the Host Shift

The Broadway locality is a hay field of ~2 ha, and it was first noted as having large numbers of diseased and healthy *S. alba* in 1997 (K. O'Keefe, personal communication). Its precise history before this date is not known, other than its having been used as a hay field for many years. In fall 1998, we observed disease infecting 10 *S. vulgaris* plants out of several thousand healthy *S. vulgaris* (interspersed with the large population of diseased and healthy *S. alba*). This count may be an underestimate since it was late in the season and many plants may have ceased flowering.

In spring 1999, we surveyed the field more systematically. We used circular plots of 2 m diameter and placed 15 of these at random within the field. Using data from these random plots, we estimated the field contained 33,000 healthy *S. alba*, 22,000 diseased *S. alba*, and 7,000 healthy *S. vulgaris* per hectare. We found no diseased *S. vulgaris* in these random plots. However, infected *S. vulgaris* was present in the field at low frequency, and we counted plant numbers in 15 circular plots of 2 m diameter centered on diseased and on healthy *S. vulgaris*. Within the plots centered on diseased *S. vulgaris*, there were an additional three diseased individuals. In the plots centered on healthy *S. vulgaris*, there were also two diseased individuals of that species. Therefore, minimally there were 20 diseased *S. vulgaris* in the population.

In spring 2001, we delimited a formal study area measuring 60 × 90 m (chosen so that the owner could continue to use of the rest of the field for hay). An exhaustive search (using five observers walking the study area systematically, row by row) found 121 diseased *S. vulgaris* (fig. 2). We counted every flowering plant of both species in 40 circular plots of 2 m diameter arranged on the intersection of the 10 × 10-m grid lines; this sampling area was 2.33% of the study plot as a whole. Extrapolating our census counts to the whole study plot, we estimated that in 2001 there were approximately 45,000 plants in the 1-ha area, of which 37% were healthy *S. alba*, 32% diseased *S. alba*, and 31% healthy *S. vulgaris*. Within the sample plots, we found two diseased *S. vulgaris* (a disease prevalence in *S. vulgaris* of 0.02%), which was close to the expectation of three plants based on the known count of 121 diseased *S. vulgaris* plants in the entire site.

Spatial Analysis of the Host Shift

We used contour maps to examine the spatial occurrence of diseased and healthy plants. The results (fig. 2) showed that there was no clear relationship between the number of diseased *S. vulgaris* plants and either the density of conspecifics or the density of diseased *S. alba*. However, when we used a simple disease transmission model to predict the number of diseased *S. vulgaris*, there was a remarkable correspondence between the predicted and observed distribution of plants. In the model, we assumed that if all the *S. vulgaris* had been healthy the previous year, then the number of diseased individuals in the following year would be proportional either to $\beta X_v Y_a$ (assuming density-dependent transmission) or to $\beta X_v Y_a$/total (assuming frequency-dependent transmission), where β = transmission coefficient, X_v = number of healthy *S.*

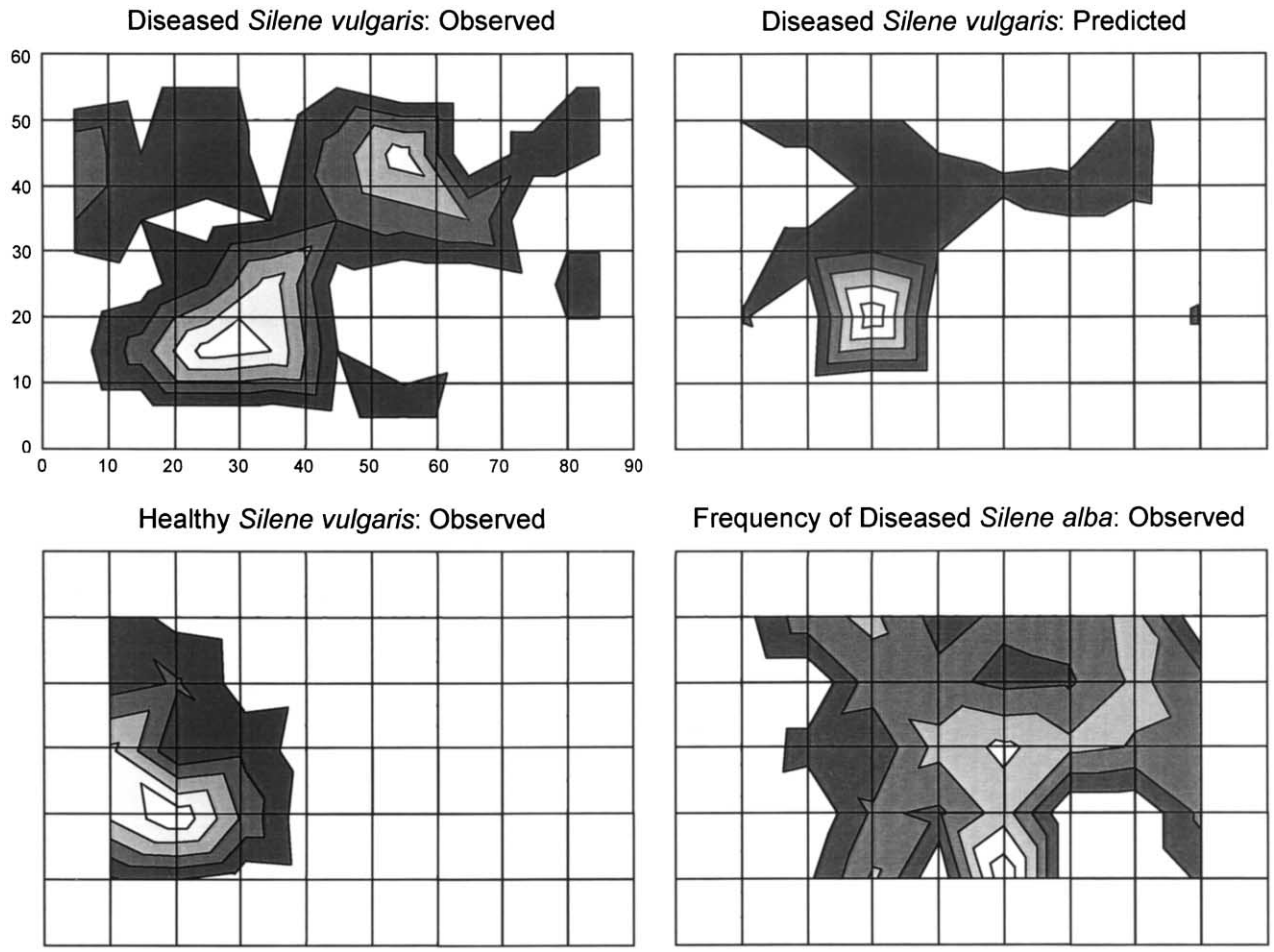

Figure 2: Spatial maps of the distributions of diseased and healthy hosts in Broadway Field based on grid sampling. Numbers on axes indicate distance in meters. *Top left*, observed distribution of diseased *Silene vulgaris* (shading indicates intervals in units of 2; data based on counts of number of diseased plants per 10 × 10-m quadrant). *Top right*, predicted distribution of diseased *S. vulgaris* using the same shading scale. *Bottom left*, observed distribution of healthy *S. vulgaris* (shading scale is in units of 10, based on counts of plants in circular 2-m-diameter plots). *Bottom right*, observed distribution of the frequencies of diseased *Silene alba* (shading scale in units of 0.2).

vulgaris, Y_a = number of diseased *S. alba*, and total = sum of all *S. alba* and *S. vulgaris*. Both these models produced a contour map that predicted the distribution of diseased *S. vulgaris*; we present the data for the frequency-dependent model (fig. 2). In this latter model, the regression of observed number per quadrat on predicted number was highly significant ($F = 15.86$, df $= 1, 26$, $P < .0001$); however, there was significant residual heterogeneity ($\chi^2 = 58.19$, df $= 25$, $P < .001$), indicating that the spatial patterns were not totally congruent.

Although the demographic data are as yet limited, we can draw three major conclusions from these studies. First, the host shift is being sustained either by repeated transmission from the old host or by transmission among plants of the new host, and it is not simply a transitory phenomenon; if anything, the number of diseased *S. vulgaris* is increasing based on the surveys of 1999 and 2001. Second, it is very likely that the co-occurrence of *S. vulgaris* with large numbers of diseased *S. alba* has favored the host shift at this site; we have found few other such large and heavily diseased populations of *S. alba* in this area. Third, within the study site itself, the probability of a host shift can be predicted using simple epidemiological principles; this argues strongly for using a dynamical context for interpreting spatial data on disease incidence.

Cross-Inoculation Studies

Genetic variation in both the pathogen and new host species will affect the probability of a cross-species transmis-

sion event. For example, genetic differences in host susceptibility may make it more likely that a temporary host shift will occur, yet may make it more difficult for the pathogen to persist because the host can evolve resistance. Differences in the pathogen may lead to only a small subset of pathogen genotypes colonizing a new host, and this may limit further evolutionary changes of the pathogen. To investigate genetic variation in the host and pathogen, we carried out inoculation experiments in the lab. Experimental field studies might be preferred, but they are especially difficult, requiring numerous relatively isolated subplots and/or extensive molecular epidemiology to trace specific pathogen strains.

To investigate genetic variation for disease susceptibility of the new host, seeds of *Silene vulgaris* were collected from 10 maternal families in each of four natural populations: the Broadway Field population (site of the host shift), two populations within 10 miles of Broadway, and a population 130 miles away near Mountain Lake Biological Station. The populations of *S. vulgaris* near Broadway and Mountain Lake were free of anther-smut disease. Seeds were surface sterilized and germinated on nutrient media under axenic conditions.

Two isolates of *Microbotryum*, from different diseased plants of *Silene alba* in Broadway Field, were used to inoculate these seedlings. Inoculum, consisting of 1,000 viable teliospores in 2 μL of water plus surfactant, was applied to the apical meristem between the two cotyledons 7 d after germination. Following inoculation, the seedlings were incubated at 18°C for 3 d and then transplanted into soil. Plants were completely randomized and maintained under greenhouse conditions until flowering. Disease status was determined on the date of the first flower, and the plant was then removed to avoid secondary infections of other plants. The experiment was repeated over two time intervals; the first run (or block) consisted of 1,200 seedlings divided among the family and inoculum treatments, from which 1,090 plants could be scored at flowering for their disease status. The second run consisted of 2,300 seedlings, from which 1,897 plants were scored. Because we wanted to look at variation in susceptibility in the new host, we did not carry out inoculations of *S. alba* with spores from *S. vulgaris* (but see "Experimental Populations").

Variation in the ability of *Microbotryum* to infect *S. vulgaris* was determined using the same methods. Fungal isolates were collected from separate diseased plants of *S. alba*: 10 were collected from *S. alba* in Broadway Field—one each from five populations within 40 miles of Broadway and one each from five populations near Mountain Lake Biological Station, 130 miles from Broadway. Seeds of *S. vulgaris* came from three maternal families from Broadway Field that ranged in susceptibility to anther smut from *S. alba* as determined in a previous experiment. The experiment consisted of a total of 3,117 seedlings of which 2,178 could be scored for disease when plants flowered.

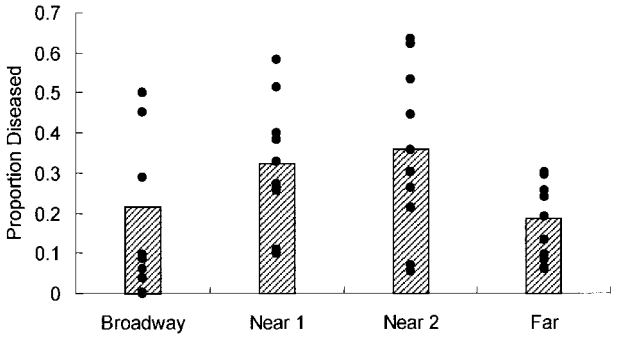

Figure 3: Variation of *Silene vulgaris* for susceptibility to *Microbotryum violaceum*. Bars represent weighted averages for each site, and the points represent different families within sites. *Near 1* and *Near 2* are sites within 10 miles of Broadway Field, and *Far* is 130 miles away. For statistics, see text.

Host and Pathogen Variation

There was significant variation among populations of *S. vulgaris* in their susceptibility to *Microbotryum* ($F = 3.14$, df $= 3, 36$, $P < .05$). There was also highly significant variation in resistance among families within populations ($F = 9.83$, df $= 36, 32$, $P < .001$). However, all the populations contained *S. vulgaris* families that were as susceptible to *Microbotryum* as those at Broadway Field itself (fig. 3). It was interesting that the Broadway Field site, but no other, had a substantial number of families that appeared to be completely resistant to *Microbotryum* (fig. 3). Whether this reflects that population's recent exposure to the disease from *S. alba* is not known.

For the pathogen, isolates sampled from *S. alba* at the site of the host shift did not have any greater ability to infect *S. vulgaris* than isolates from other populations in the vicinity or from much further away at Mountain Lake ($F = 0.55$, df $= 2, 17$, NS). Although there were significant differences in the infectiousness of strains from different populations, those strains collected from Broadway Field showed no heterogeneity in their infectiousness ($F = 1.62$, df $= 9, 29$, $P < .156$). As expected, the host families used in the experiment differed significantly in their resistance, but there was no significant host family by pathogen strain interaction ($F = 0.85$, df $= 18, 29$, NS).

Disease Expression on the New Host

In Broadway Field, we observed that the disease on the new host often resulted in an abnormal floral morphology. Frequently, there was a dramatic reduction of petals and the calyx (fig. 4). In the cross-inoculation experiment described above (where a diversity of fungal strains were used to inoculate *S. vulgaris*), all the plants that flowered were scored for floral morphology. The plants were scored on a 1–5 subjective scale for the normality of the first flower produced on the plant, including petal and calyx size. Diseased plants were also scored on whether the anther sacs were fully ruptured, allowing dehiscence of the fungal spores. Floral morphology was highly abnormal among the plants that became diseased compared with those that remained healthy (fig. 5). Furthermore, the anthers were nondehiscent in more than a third ($n = 179$) of the diseased plants.

These results indicate that *Microbotryum* may be "maladapted" to its new host, in that abnormal flowers may result in fewer pollinator visits and therefore reduced disease transmission. Our future studies will involve selection on the pathogen for normal disease expression by sequential passaging of the pathogen through *S. vulgaris*.

Population Dynamics

The dynamics of two-host–one-pathogen systems have been investigated by ecologists interested in pathogen-mediated interactions in plant and animal communities (Holt and Pickering 1985; Begon et al. 1992; Begon and

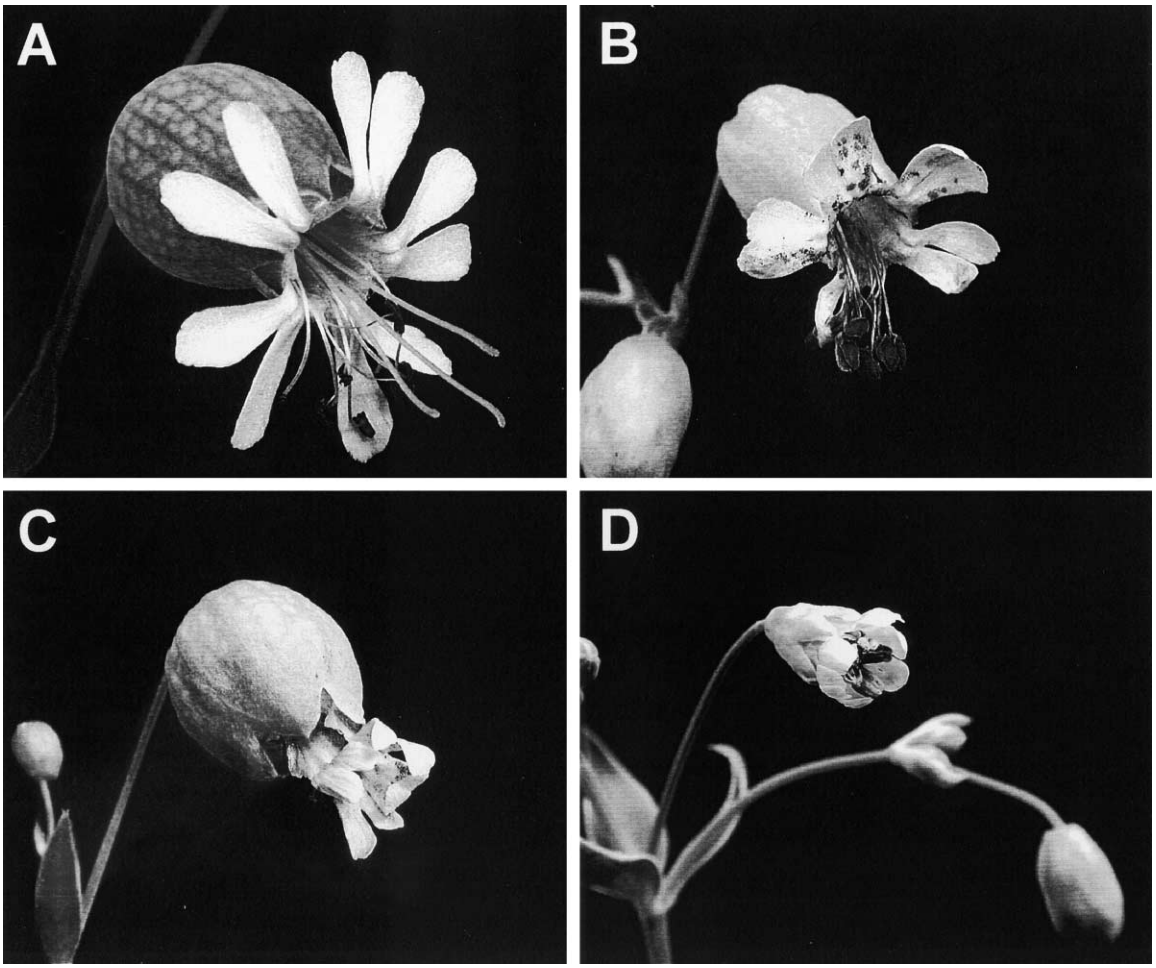

Figure 4: *Silene vulgaris* flower morphologies. All images are at equal magnification. *A*, Healthy hermaphrodite flower. *B*, Typical diseased flower showing slightly reduced-size and spore-filled anthers. Note the absence of female structures. *C*, Diseased flower showing highly deformed petals. *D*, Strongly reduced diseased flower with spore-filled but nondehiscent anthers. No further enlargement or growth of these flower structures occurred subsequent to the photographic documentation.

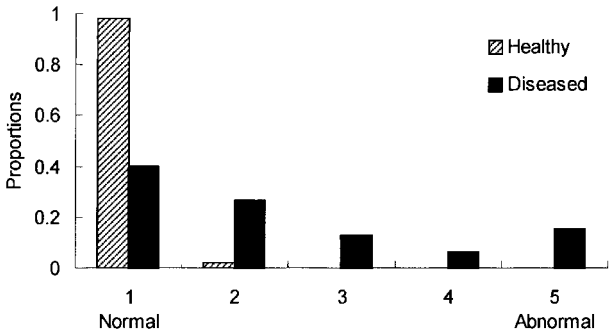

Figure 5: Frequency distributions of floral morphology types in healthy (n = 1,667) and diseased (n = 495) *Silene vulgaris*. Morphologies were scored on the following scale: *1*, normal; *2*, reduced petals; *3*, petals absent or highly deformed; *4*, flowers strongly reduced but anthers visible; *5*, flower development arrested at bud stage.

Bowers 1995). To describe the dynamics of a host shift using a microparasite model (Anderson and May 1981), at least four equations are required, namely, two for the numbers of healthy individuals in the old and in the new host (X_1, X_2) and corresponding equations for diseased hosts of the two species (Y_1, Y_2). We illustrate the simple form of these equations (i.e., with diseased-induced mortality, but no recovery, no immune class, and no disease-independent population regulation) in terms of total number of hosts (N_1, N_2) and number of diseased hosts (Y_1, Y_2) of each species:

$$\frac{dN_1}{dt} = r_1N_1 - \alpha_1Y_1,$$

$$\frac{dY_1}{dt} = \beta_{11}X_1Y_1 + \beta_{12}X_1Y_2 - (\mu_1 + \alpha_1)Y_1,$$

$$\frac{dN_2}{dt} = r_2N_2 - \alpha_2Y_2,$$

$$\frac{dY_2}{dt} = \beta_{22}X_2Y_2 + \beta_{21}X_2Y_1 - (\mu_2 + \alpha_2)Y_2,$$

where $N_i = Y_i + X_i$, r = rate of increase of the hosts, μ = mortality rate, and α = diseased-induced mortality. These equations contain four transmission coefficients, β_{11}, β_{12}, β_{21}, and β_{22}, representing transmission among individuals of the old host, transmission from the new host back to the old host, transmission from the old host to the new host, and transmission among individuals of the new host. Models of this form give a wide range of dynamics and equilibrium states (Begon et al. 1992; Begon and Bowers 1995), and one-parasite–two-species processes have been implicated in determining species abundance in nature (Tompkins et al. 2000)

With regard to emerging diseases, it can be seen that the conditions for the disease on the new host to increase when rare (i.e., Y_2 very small) simply requires that $\beta_{21}X_2Y_1$ be positive (i.e., some transmission, however small). If β_{22} is small, the persistence of the disease on the new host requires continual input of the disease from the old host. In this case, the old host is said to act as a reservoir for the disease. Because the term "reservoir" is sometimes used in a very nonspecific sense, for clarity, we term this a "source-dependent" host shift. If the disease on the new host is able to maintain itself without immigration from the old host, then we term this a "self-sustaining" host shift; operationally, the disease should persist on the new host if the old host is removed. This requires the rather obvious condition that not only is β_{22} positive but also that the new host population is sufficiently large (i.e., above the density threshold for disease increase); host shifts into low-density populations are unlikely to be self-sustaining.

Holt and Gomulkiewicz (1997) have considered the conditions for the spread of a single-locus mutation in a sink habitat (i.e., one where there is negative population growth). Translating their formulations into disease terminology, a new mutant will increase (i.e., disease in the new host population will become self-sustaining) only if the basic reproductive rate of the disease $R_o = \beta_{22}X_2/(\mu_2 - \alpha_2) > 1$. From this it follows that the "individual level" disease traits of infectiousness (β_{22}) and virulence (α_2) are per se insufficient to determine whether a host shift will be self-sustaining; local population dynamics are also critical. Moreover, the conditions for a source-dependent host shift are much less stringent than the conditions for a source-independent (i.e., self-sustaining) host shift. We therefore would expect source-dependent host shifts to be much more frequent than self-sustaining host shifts and that the transition between the two states is a critical step in host-pathogen coevolution.

Although we have ignored β_{12} and β_{11} in the above discussion, the values of these parameters will still be important in determining the frequency of the disease in the population. For example, it is possible to have the disease maintained on both hosts when they are together, even if it cannot be sustained on either when the hosts are in one-species populations. Moreover, back transmission from the new host may increase the prevalence of the disease in the old host, perhaps giving the false impression that high disease levels in the old host were necessary for the host shift to occur in the first place.

In the context of any specific disease, the form of the model describing a host shift will depend on biological details of the process, and this can only be inferred by empirical observations or experiments. For example, in many sexually transmitted diseases, the transmission pro-

cess is frequency rather than density dependent (as above), and disease symptoms often result in sterility rather than increased mortality. The above models also do not take into account mechanisms of population regulation other than those caused by the disease itself. And, of course, they do not take into account that host shifts may be between particular host and pathogen genotypes rather than equally between random members of the population. In the next section, we therefore describe the results of field experiments to quantify and understand the population dynamics of *Microbotryum* during its transition from the original to the new host.

Experimental Populations

Experimental populations have proved a useful tool for understanding population dynamics in the *Silene-Microbotryum* system (Thrall and Jarosz 1994*a*, 1994*b*; Alexander and Antonovics 1995) and in other plant-pathogen systems (Roy 1994). Here, we use the initial results of our experiments to estimate transmission rates within and between the two species (i.e., all four β's) as well as disease effects on demographic parameters. Our future goal is to parameterize theoretical models to see whether they predict the beginnings of a self-sustained host shift as well as to continue the experimental populations to assess genetic changes in the host and pathogen in response to their new association.

The populations were set up in the summer of 1999 on an abandoned golf course on the property of the Mountain Lake Hotel near Mountain Lake Biological Station, Virginia. Individual populations were separated by 50–100 m. Each population consisted of 64 individuals on a rectangular grid at 0.75 m spacing. For the disease treatments, 14 plants served as disease sources. These were grown from bulk seed collected from Broadway Field and were inoculated in the laboratory with six fungal isolates from *Silene alba* in that field. Given that diseased *S. alba* greatly outnumber diseased *Silene vulgaris* in Broadway Field, these isolates almost certainly represent a sample of the population that was originally the source of the host shift.

There were seven treatments in each of three blocks, defined by spatial position on the abandoned golf course as well as by successive planting times (over a period of 2 wk). The treatments were as follows: (1) healthy *S. vulgaris* with infected *S. vulgaris* as a disease source; (2) healthy *S. alba* with infected *S. alba* as a disease source; (3) healthy *S. vulgaris* with infected *S. alba* as a disease source; (4) healthy *S. alba* with infected *S. vulgaris* a disease source; (5) a mixture of healthy *S. vulgaris* and *S. alba* with infected *S. vulgaris* as a disease source; (6) a mixture of healthy *S. vulgaris* and *S. alba* with infected *S. alba* as a disease source; and (7) a mixture of healthy *S. vulgaris* and *S. alba* with no disease source, that is, a control to test for any among-plot disease transmission.

By far the majority of plants flowered (92.8%); the few that remained vegetative or died before flowering were omitted from the analysis.

Disease transmission occurred in all of the experimental plots, including transmission from *S. alba* to *S. vulgaris*, showing that we could successfully "emulate" the host shift we observed in Broadway Field. In the sentinel plots (i.e., without disease sources), four *S. alba* out of 96 became diseased, but no plants of *S. vulgaris* became diseased. The occasional disease in the sentinel *S. alba* indicates that there was a limited amount of long-distance spore transmission.

We calculated the within-year transmission rates for each experimental population based on the number of target-healthy individuals that became diseased or remained healthy by the end of the season. These measures were standardized by the number of diseased source individuals actually flowering in that season. Where these populations contained two target hosts, we estimated transmission for each host separately, and to calculate means, we weighted the data by the number of target plants that were scored.

To estimate the four transmission coefficients (i.e., β_{11}, β_{12}, β_{21}, and β_{22}), we used the following Nicholson-Bailey–type model (Hassell 1978):

$$Y_{t+1} = X_t\left[1 - \exp\left(-\frac{\beta_{ij}Y_t}{N_t}\right)\right],$$

where Y_{t+1} = number of diseased plants at the end of the summer; Y_t, X_t = number of diseased and healthy plants at the start of the season ($N_t = Y_t + X_t$); and β_{ij} = the transmission coefficient.

This "exponentially scaled" transmission coefficient takes into account the possibility of multiple infections on the same plant within the time interval t and $t + 1$ and limits the range of the coefficient between 0 and 1. We also used Y_t/N_t, instead of Y_t, to represent frequency-dependent transmission dynamics that have been shown in many studies of the *Microbotryum-Silene* system (Thrall and Jarosz 1994*b*; Antonovics et al. 1995; Biere and Honders 1998).

We carried out an ANOVA on the per capita probability of infection standardized to the frequency of diseased plants in the original experimental design ($= 1 - \exp[-\beta_{ij} \times (14/64)]$) because this had a normally distributed error variance. The probabilities of infection differed significantly among the experimental treatments ($F = 11.23$, df $= 1, 18$, $P < .0002$; fig. 6). The mean probability of a plant becoming diseased within the pure *S. alba* populations was 0.26, while the same probability within the

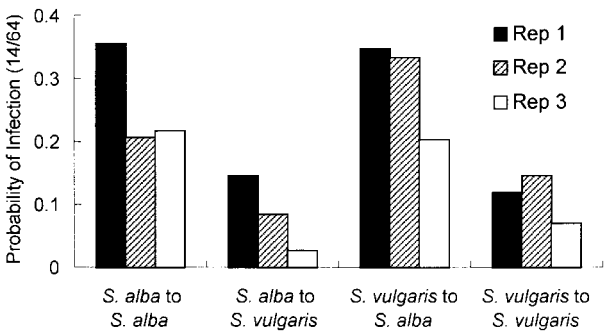

Figure 6: Probabilities of infection of healthy plants in experimental populations where each species served as a disease source for transmission to healthy individuals of the same or other species.

pure *S. vulgaris* populations was 0.11. Transmission probability from *S. alba* to *S. vulgaris* (from the old to the new host) was 0.08, while the reverse (transmission back to the old host) was 0.30. Transmission to *S. alba* from either host was much greater than transmission to *S. vulgaris* ($F = 32.61$, df $= 1, 18$, $P < .0001$). Although transmission from *S. vulgaris* back to *S. alba* appeared greater than transmission within *S. alba* itself (0.30 vs. 0.26), there was no significant source-species by target-species interaction ($F = 0.03$, df $= 1, 18$, $P < .87$). The probabilities of infection were the same in populations where only one species was a target versus populations that had target individuals of both species ($F = 0.27$, df $= 1, 17$, $P < .61$), showing that transmission rates to one of the species were not detectably influenced by the presence of the other species.

These results indicate that the new host, *S. vulgaris*, is considerably more resistant to anther-smut disease than the old host. However, transmission of the disease from the new host back to the old host is no less than within the old host itself. Therefore, the lower susceptibility of *S. vulgaris* to the disease is not accompanied by a corresponding reduction in transmission. Even though disease expression results in frequent floral abnormalities, *S. vulgaris* generally produces more flowers than *S. alba*, which may contribute to greater spore production on a per plant basis.

The overwintering mortality rate of *S. alba* that became diseased (32.2%) was much greater than that of *S. alba* that remained healthy (12.2%; $\chi^2 = 23.4$, $P < .001$). However, the mortality rate of *S. vulgaris* that became diseased (9.5%) was not significantly different from the mortality rate of healthy *S. vulgaris* (6.9%; $\chi^2 = 0.39$, $P < .53$). Analysis of the mortality of the source plants (which had been inoculated artificially) and the target plants (which became diseased) showed no significant differences (27.4% and 32.2% in *S. alba* and 8.9% and 9.5% in *S. vulgaris*; three-way $\chi^2 = 0.05$, $P < .82$). When the survival of all diseased plants (sources and targets) and the survival of healthy plants were compared, the three-way interaction was significant ($= 4.61$; $P < .032$). Contrary to many popular conceptions about the increased virulence of pathogens on novel hosts, this indicates that in terms of mortality, the disease has a lower impact on the survival of the new host *S. vulgaris* than it does on the survival of *S. alba*.

These studies therefore show that it is not only possible to investigate a host shift under seminatural experimental conditions but that we can also begin to parameterize models describing the dynamics of this process. Our results indicate that back transmission to the old host may be an important component of the dynamics and that the disease on the new host has a lower, not higher, virulence in terms of mortality effects. These populations are being maintained to study the long-term dynamics of pure and mixed populations of the two hosts and to examine whether the long-term fates are predictable by the two-host–one-pathogen models. Also, in combination with cross-inoculation studies, we can assess whether and at what rate evolutionary changes are occurring in the host and pathogen.

Since our goal is to understand the actual host shift at Broadway, a critical issue is translating our experimental results into field predictions. Already there seem to be some surprising discrepancies. The transmission rates observed in our experiments appear to be far higher than what can be occurring in the Broadway Field. If the transmission coefficients at the Broadway site were the same as in our experimental populations, we should be seeing numbers of diseased *S. vulgaris* at Broadway that are an order of magnitude greater than what we actually find. This is even though the densities are comparable, and the genotypes are from the same site. While the experimental and natural populations are exposed to their normal pollinators, the activity of these may be quite different at the two sites. We are currently carrying out studies of spore movement by pollinators between species, and these may help resolve this issue.

Ironically, had we had sufficient data from Broadway Field when we first contemplated these experiments, we may have been discouraged from carrying them out since the field data would have pointed to the need for an impracticably large number of target plants of *S. vulgaris* to detect any transmission at all. The ease with which we achieved the host shift in experimental populations and its rarity in natural populations is an enigma that we had not anticipated and which only future studies can resolve.

Conclusions

Using our model system, we have shown that there are multiple processes involved in a host shift and that studying the ecological and genetic dynamics of these processes is crucial to understanding both the proximate causes as well as the eventual fate of a host shift. Many questions as yet remain unanswered, especially those regarding evolutionary change of the pathogen on the new host. It is difficult to predict a priori the fate of this population. One can imagine several conflicting scenarios. For example, *Microbotryum* might differentiate into a new host race on *Silene vulgaris*, or the continual influx of the disease from *Silene alba* by cross-species infection may limit the degree of such differentiation.

To examine evolutionary changes, we are taking several approaches. First, we are continuing the population experiments described above; plants that die are being replaced by individuals grown from seed gathered from that same population in the previous year. Second, we are initiating passaging experiments in the lab and greenhouse to investigate the rate at which the pathogen adapts to the new host for infection ability and disease expression. In other experimental systems, evolutionary response to passaging has been rapid and often accompanied by decreased performance on the old host (Ebert 1998). If this is the case here, then the evolutionary component of this host shift may be very important. Third, diseased populations of *S. vulgaris* have been recorded in Europe where the disease appears to be self-sustaining, especially in high-elevation regions in the Alps. We will assess whether the disease in these regions is a singular, long-standing host shift or whether the process is a recurring one. Moreover, many species in the Caryophyllaceae are infected with *Microbotryum*, and it is possible that a host race other than that on *S. alba* is the progenitor of established populations of the disease on *S. vulgaris* in Europe (or even that the progenitor is no longer to be found in extant populations).

Many of the principles and processes we have outlined for *Microbotryum* are also applicable to host shifts onto economically important plants and animals as well as onto man. For example, if there had been the expectation that variation in "new host" resistance is commonplace (as we have found here), then studies of human variation in resistance to AIDS may have been initiated much sooner (Samson et al. 1996). Also, current controversies about the role of reservoir species in sustaining infections often raise the possibility that back transmission to the zoonotic host might also be occurring, but the role that this plays in the overall dynamics of a disease is rarely studied explicitly. Indeed, controversy can become focused on whether it is back transmission (to the wild species) or forward transmission (to the domesticated species) that is responsible for high disease incidence in an area. The controversy in England over whether badgers or cows themselves are the source of bovine tuberculosis is a case in point (Skuce et al. 1996; Woodroffe et al. 1999).

At a basic level, the processes involved in host shifts are similar to those involved in the colonization of a new habitat by any organism, with the important difference that in a pathogen system, the new "habitat" has both ecological and evolutionary dynamics that are driven by the host shift itself. All organisms to some degree "construct" their own environments (Lewontin 1983; Brandon and Antonovics 1995), and host-pathogen systems are a special case with the advantage that the theory of how pathogens affect their "environment" (i.e., their host populations) is well developed. Moreover, as we hope to have shown, there are situations where study of adaptation to a biotic environment may be experimentally more tractable and be based on firmer theoretical ground than the study of adaptation in seemingly simpler "abiotic" contexts.

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