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ANTHER SMUT TRANSMISSION IN *SILENE LATIFOLIA* AND *SILENE DIOICA*: IMPACT OF HOST TRAITS, DISEASE FREQUENCY, AND HOST DENSITY

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Predicting the dynamics of hosts and pathogens in natural systems requires a thorough understanding of the disease transmission process. It has been argued that, unlike for airborne diseases, the transmission of vector-borne diseases such as the anther smut fungus *Microbotryum violaceum* is a function of the frequency rather than the density of disease in a population. However, recent models indicate that frequency-dependent transmission of vectored diseases is only expected if the time required for interplant movement (host search) by the vector is short relative to the time spent per visit (handling time). Hence, frequency dependence is only expected to dominate at lower ranges of interplant distances. We test this prediction by analyzing disease transmission of *M. violaceum* in relation to interplant distance in a natural population of the host species *Silene latifolia* and *Silene dioica*. We conclude that (1) The infection probability of uninfected hosts strongly increased with the frequency of diseased hosts within a patch, whereas the number of infected plants within a patch had no effect in either of the host species. (2) In agreement with predictions based on vector behavior, the probability of infection in patches of *S. dioica* increased more strongly with increasing disease frequency among patches with high average host density (i.e., small interplant distance) than among patches with low host density. This finding is consistent with the idea that transmission should shift from a more frequency- to a more density-dependent process when host search time increases relative to handling time. (3) In addition to local disease frequency and host density, individual host traits (host sex, size, and phenology) explained a significant part of the variation in infection probability within host species. The precise effects of individual host traits depended on local neighborhood characteristics. For instance, in *S. latifolia*, male hosts were on average more susceptible than female hosts, but the difference diminished with increasing disease frequency.

Introduction

Predicting the dynamics of hosts and pathogens in natural systems requires a thorough understanding of the disease transmission process in these systems (Anderson 1981; Getz and Pickering 1983; Thrall et al. 1993*a*). For passively dispersed diseases such as plant pathogens with airborne spores, transmission is often described as a function of the absolute number of diseased individuals ("disease density") in a population (Kermack and McKendrick 1927; Bailey 1975; Anderson and May 1979). A larger number of infected individuals in the population is expected to result in a higher probability that a healthy individual will come into contact with the disease. However, for many vector-transmitted diseases such as the anther smut fungus *Microbotryum violaceum,* which is transmitted by insect pollinators, it has been argued that disease transmission will be a function of the frequency rather than the density of diseased hosts in a population (Anderson 1981; Getz and Pickering 1983; Antonovics 1993; Thrall et al. 1993*a*). In such systems, the probability that a healthy host receives spores depends on the number of contacts with the vector and the per-contact probability that the vector carries spores. The rationale for frequency dependence is (1) that the per-contact probability of the vector carrying spores will be largely determined by the chance that the insect has previously visited a diseased host, i.e., on the frequency of diseased hosts in the population. (2) Many insect vectors tend to increase their average flight distance with decreasing plant density, so that the contact rate with infected individuals in the population is not necessarily lower if their density is reduced. Theoretical models have shown that, in contrast to density-dependent transmission systems, coexistence between hosts and pathogens that are transmitted in a frequency-dependent fashion requires disease-independent regulation of population size (Getz and Pickering 1983). Hence, conditions for stable coexistence in frequency-dependent transmission systems are generally more restricted than in density-dependent transmission systems, although later models have shown general cases for which these conditions may be relaxed (Thrall et al. 1993*a;* Antonovics et al. 1995; Thrall et al. 1995). Antonovics and Alexander (1992) provided the first experimental evidence that spore transmission of the anther smut fungus *M. violaceum* among its host *Silene latifolia* was indeed a positive function of frequency but not of the absolute number ("density") of diseased plants in the population.

However, as pointed out by Antonovics et al. (1995), strong frequency dependence is only expected if the time interval between the periods of vector-host contact (i.e., the time required for interplant movement or host search) is short compared with the time spent by the insects during each host contact (handling time). Frequency dependence is therefore expected to dominate more strongly in populations with high host density where interplant distances are short, than in sparse populations with low host densities. It is confusing that density, in its most widely used meaning

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in plant pathology, refers to the population size of healthy or infected hosts rather than to their true density (number per unit area), and these two parameters may or may not be interrelated. In an elegant experimental setup, Bucheli (1997) separated the effects of disease density (number of infected plants), disease frequency, and interplant distance on spore deposition by *M. violaceum* in the host *S. latifolia.* In agreement with expectations, spore deposition was determined by disease frequency in populations with small interplant distances but not in populations with large interplant spacing during at least part of the season. In this article, we take the approach one step farther and investigate the effects of number of infected plants, frequency of infected plants, and host density on the actual probability of infection, rather than on spore deposition patterns, in patches of natural populations of the host species *S. latifolia* and *Silene dioica.* Specifically, we ask, (1) Can we detect frequency-dependent disease transmission and at what spatial scale does it operate? and (2) Does the strength of frequency-dependent transmission decrease with average interplant distance within host patches? We investigate the relative importance of individual host traits (host size, host sex, and phenology) in addition to these population attributes for the probability of infection. Apart from the genetic makeup of a host plant with respect to specific resistance alleles, the probability that a host plant will become infected is often determined by additional host traits such as plant size, age, phenology, or nutritional status. Previous studies in the *Silene-Microbotryum* system have shown effects of, e.g., host biochemical factors (Ruddat and Calvin 1990; Ruddat et al. 1991), morphological traits such as flower or stigma size (Elmqvist et al. 1993; Shykoff et al. 1997), and life history traits such as timing of anthesis (Alexander 1990; Thrall and Jarosz 1994; Biere and Antonovics 1996; Biere and Honders 1996*b*) and host longevity (Thrall et al. 1993*b*). Interactions between effects of host traits and disease frequency or density would indicate that such host traits do not have fixed effects on infection probability but that their effects depend on characteristics of the local neighborhood.

Methods

Study Organisms

Silene latifolia Poiret (= *Silene alba* [Miller] Krause), white campion, Caryophyllaceae, is a dioecious, short-lived perennial from open, disturbed habitats and arable land with a typical moth-pollination syndrome, including heavily scented white flowers that open at dusk. *Silene dioica* (L.) Clairv., red campion, is a closely related dioecious perennial that mainly occurs in woodlands and mainly is pollinated by bumblebees (Jürgens et al. 1996). The anther smut fungus *Microbotryum violaceum* (Pers.:Pers.) Deml & Oberw. (= *Ustilago violacea* [Pers.] Fuckel) (Ustilaginaceae) (Deml and Oberwinkler 1982) causes a systemic infection that sterilizes its Caryophyllaceous host plants (Thrall et al. 1993*b*). Both genetics and population biology of the fungus have been studied extensively (for references, see Garber and Ruddat [1994] and Alexander et al. [1996], respectively). Diploid spores are produced in the anthers of infected hosts and are transmitted by insects that serve both as pollinators of the host and as vectors of the disease (Jennersten 1983). Although direct transmission through splash or wind dispersal is known to occur, it is generally less effective and spatially highly restricted (Roche et al. 1995). Ovaries of infected flowers are aborted. In dioecious host species, the flowers on female plants undergo a morphological sex reversal and produce stamens with spore-filled anther sacs. Diseased plants are easily recognized by the purple to brown mass of spores produced in their flowers. New infections occur when spores are transmitted to flowers of uninfected plants, germinate, and produce haploid cells of opposite mating type that conjugate to form an infectious dikaryotic hypha. These hyphae enter the host tissue and grow into the young anthers of newly developing flower buds, in which the life cycle is completed, often several weeks after infection. A large part of the inflorescences or branches within an inflorescence may remain undiseased during the year of infection. If the fungus manages to grow into the rootstock of the host before inflorescences die back at the end of the season and is able to survive the winter, hosts may become systemically infected and produce only diseased flowers in subsequent years.

Studies of fungal strains infecting *S. latifolia* and *S. dioica* have shown that there is strong genetic divergence between isolates sampled from these two host species, despite the fact that these strains are—in contrast to earlier reports (Baker 1947)—often able to cross-infect each other's host species (Biere and Honders 1996*a*). Bucheli (1997) showed strong host-specific differentiation between strains from the two host species on the basis of five microsatellite loci. In accordance, surveys of sporidial colony color, a polymorphic trait (Garber et al. 1978) whose genetics have been extensively studied by E. D. Garber and coworkers, have shown that strains from allopatric populations of *S. latifolia* in the Netherlands are almost invariably pink (+), whereas strains from *S. dioica* are predominantly yellow (y+) (Biere and Honders 1996*a*). Screening of sporidial colony color and RAPD markers of isolates from the sympatric host population used in our study also indicate that, despite potential cross-infection and the presence of interspecific host hybrids, different pathogen demes are maintained on the two host species (A. Biere and S. C. Honders, unpublished data), either through vector specificity or behavior, temporal or spatial host structure, or differential infection success.

Study Site

The field study was conducted in a population containing patches of both *S. latifolia* and *S. dioica,* located along an infrequently used 1200-m-long sandy road in the northern part of the Netherlands (6°30′N, 53°06′E) in 1993. The road shoulders, 7–11 m wide, are dominated by oak and elder and are bordered by agricultural fields and grassland. Flowering *Silene* in the population had been permanently marked since 1991. In 1993, the population consisted of 1755 flowering *S. dioica,* of which 7.4% were systemically infected by *M. violaceum,* and 1041 flowering *S. latifolia,* of which 17.0% were diseased at anthesis. In addition, 5.9% of the *Silene* plants in the population were scored as putative hybrids (Baker 1951) with 18.2% systemic disease. The host population was both spatially and temporally substructured with respect to the two host species. The mean distance of *S. dioica* hosts to their nearest conspecific neighbors was

Table 1 Logistic Regression of the Effects of Disease Frequency and Total Host Density at Increasing Areas around Focal Plants on Their Probability of Infection

		Univariate regression (direct + indirect effects)						Multivariate regression (direct effects)			
		Frequency of infected plants (F)		Density of infected plants (D)		Density total (N)		Frequency of infected plants (F)		Density total (N)	F × N
Radius (m)	Area (m²)	R^2 (%)	b	R^2 (%)	b	R^2 (%)	b	R^2 (%)	b	b	b
Silene dioica:											
0.5	0.8	1.7	+++	1.6	+++	3.9	−−−	5.8	·	−−−	++
1.0	3.2	2.9	+++	2.9	+++	3.6	−−−	6.9	·	−−−	+++
2.0	12.5	4.9	+++	3.1	+++	3.4	−−−	8.2	+++	−−−	+++
4.0	49.8	5.3	+++	3.5	+++	2.3	−−−	7.6	+++	−−−	+
8.0	197	3.1	+++	3.9	+++	1.1	−−−	4.2	+++	−−−	·
16.0	665	3.7	+++	5.1	+++	0.4	−	4.1	++	−	·
32.0	1523	3.6	+++	4.1	+++	0.0	·	4.7	·	·	−−−
64.0	3052	2.9	+++	2.4	+++	0.0	·	3.4	·	·	−
Silene latifolia:											
0.5	0.8	2.5	+++	1.8	+++	1.5	−−−	5.2	·	−−−	·
1.0	3.2	5.6	+++	3.7	+++	0.9	−−	8.4	++	−−−	·
2.0	12.5	6.8	+++	5.2	+++	0.3	·	9.0	+++	−−−	·
4.0	49.8	8.5	+++	5.6	+++	0.0	·	10.1	+++	−−−	·
8.0	194	6.9	+++	3.3	+++	0.0	·	78	+++	−	·
16.0	652	7.4	+++	4.7	+++	0.2	·	7.4	++	·	·
32.0	1536	7.5	+++	5.2	+++	0.4	·	7.9	·	+	·
64.0	3111	6.5	+++	4.5	+++	0.0	·	7.6	−	++	−−

Note. In univariate analyses effects of disease density are also estimated. R^2 (%): model $R^2 \times 100\%$; b: direction of effects (+/−) and their significance (·: ns; +/−: $P < 0.05$; ++/−−: $P < 0.01$; +++/−−−: $P < 0.001$). Data for *S. dioica* ($n = 1625$) and *S. latifolia* ($n = 864$).

0.32 m., whereas the mean distance to the nearest hybrid and heterospecific neighbor was an order of magnitude larger, 10.79 and 9.03 m, respectively. Similarly, for *S. latifolia* these distances were 0.66, 3.87, and 13.91 m, respectively. Differences in onset of flowering between the host species in this population were highly significant (Biere and Honders 1996*b*), *S. dioica* flowering on average 8 wk earlier than *S. latifolia*. Given the earlier-mentioned genetic differentiation between pathogen demes on the two host species even in the sympatric population, transmission dynamics of the fungal populations on the two host species are expected to proceed largely independent of each other, although considerable levels of gene flow between fungal populations of either host species and the fungal population on the relatively rare putative hybrid hosts cannot be excluded.

Censuses

Weekly censuses were performed from the last week of April until the last week of October 1993. Each flowering *Silene* was mapped to its nearest *X*- and *Y*-coordinate in a 0.1-m grid across the population. For each of these plants we recorded week of anthesis ("phenology"), disease status at anthesis, sex, and number of flower stalks ("plant size"). Changes in disease status were checked every third week. This allowed us to discriminate among initially healthy host plants that stayed healthy (no infection) and initially healthy host plants that became diseased (infection).

Data Analysis

Two approaches were used to study the impact of neighborhood characteristics and individual host traits on the probability of infection. The first approach was individually based. For each host, we calculated the total number of conspecific hosts and the frequency of infected conspecifics in circular areas of increasing diameter (0.5, 1, 2, 4, 8, 16, 32, and 64 m) around each host plant. Since the population has a linear structure, the area of the population within each radius increases with less than the square of each radius. For each radius, the average area around target plants is listed in table 1. The impact of the frequency of infected hosts and the total number of hosts in each of these areas on the probability of infection of a target plant was analyzed by logistic regression (SAS procedure LOGISTIC). Disease frequency and host density in the area that showed the highest generalized R^2 were used to estimate the impact of individual host traits (host size, sex, and phenology) and neighborhood characteristics (disease frequency and host density) jointly on an individual's probability of infection by stepwise logistic regression. A backward elimination procedure was used, starting from a full factorial model (SAS procedure LOGISTIC) with the restriction that lower-order interactions could not be removed if they were involved in higher-order interactions (Crawley 1993). Since the area around the host is fixed in this approach, the number of infected host plants in the area is a linear combination of disease frequency and host density and cannot be estimated independently. A second, patch-based, approach was taken to partition effects of the number of diseased hosts in a patch, disease frequency, and host density (host number per unit area) on infection rate. The population was subdivided into host patches using a nearest-neighbor joining method. Each host was connected to each of its nearest conspecific neighbors occurring at a distance of less than k. The networks created in this way joined groups of individuals whose nearest neighbor within the group occurred at a distance less than k, whereas the distance to the nearest neighbor of any other patch was larger than k. A value of $k = 3$ m was chosen, below which the fraction of single individuals in isolated patches sharply increased.

Table 2 Logistic Regression of the Effects of Individual Host Traits, Frequency of Diseased Hosts, and Total Host Density in 2- and 4-m Radii around Hosts of *Silene dioica* (n = 1625) and *Silene latifolia* (n = 864), Respectively, on Their Probability of Infection

	S. dioica (R^2 = 0.117)			*S. latifolia* (R^2 = 0.253)		
Source	b	W	P	b	W	P
FREQ (disease frequency)	−	0.4	0.5058	+	22.2	0.0001***
DENS (host density)	−	12.5	0.0004***	−	10.0	0.0015**
SEX (host sex)	−	3.4	0.0639	+	12.3	0.0005***
PHEN (host phenology)	−	2.8	0.0947	−	11.2	0.0008***
SIZE (host size)	−	2.1	0.1497	+	9.2	0.0024**
FREQ × SEX	−	0.1	0.7203	−	6.9	0.0088**
FREQ × PHEN	+	2.8	0.0921		...	...
FREQ × SIZE	+	5.5	0.0187*		...	...
FREQ × DENS	−	2.1	0.1493		...	...
DENS × SEX	−	1.7	0.1911		...	...
DENS × PHEN	+	14.0	0.0002***	+	8.0	0.0048**
DENS × SIZE	+	0.0	0.7804		...	...
SEX × PHEN	+	5.0	0.0256*		...	...
SEX × SIZE	+	2.2	0.1364		...	...
PHEN × SIZE	+	3.1	0.0802		...	...
FREQ × PHEN × SIZE	−	6.2	0.0127*		...	...
FREQ × DENS × SEX	+	4.4	0.0355*		...	...

Note. Model R^2, direction of effects (b), Wald χ^2 statistics (W), and significance of estimates (P) are indicated (* = 0.05, ** = 0.01, *** = 0.001). Dots indicate terms not included in the reduced model after stepwise removal of nonsignificant interaction terms. Positive effects of phenology indicate higher infection rates of late flowering hosts. Positive effects of host sex indicate higher infection rates of males.

In this way, the population was subdivided into 44 *S. dioica* and 74 *S. latifolia* patches containing more than one host individual, comprising 98.6% and 96.3% of the individuals of these host species in the population, respectively. The area of each patch was calculated from the polygon area formed by the outermost individuals with a border equal to one-half of the average nearest neighbor distance within the patch. For each patch, we calculated (1) the number and (2) the frequency of systemically diseased hosts in the patch, (3) the density of hosts per square meter in the patch, and (4) the infection rate (the proportion of hosts in the patch that were healthy at anthesis but that became infected over the course of the season). Only patches with at least one systemically diseased host and at least one initially healthy host were included in the analyses (*S. latifolia:* n = 25, *S. dioica:* n = 18). Impact of the number and frequency of diseased hosts and of host density on infection rate of patches was analyzed by multiple regression (SAS procedure GLM) with type III sums of squares. Number of host plants per patch, host density, and plant size were ln-transformed prior to analysis. All statistical analyses were performed using SAS 6.12 (SAS Institute, Inc.).

Results

Individual Approach: Interaction of Neighborhood Characteristics and Host Traits

In total, 17.7% of the 1625 *Silene dioica* and 24.4% of the 1041 *Silene latifolia* that were healthy at anthesis became infected during the season. Univariate regressions showed that infection probability increased with both the frequency and the density of systemically diseased individuals that served as inoculum source in areas of up to more than 50 m around individual host plants of the two host species (table 1). Disease frequency generally showed a higher coefficient of determination (R^2) than disease density in *S. latifolia,* but this was not generally the case in *S. dioica.* Direct effects of disease frequency and total host density showed different patterns for the two host species (multiple regressions; table 1). In *S. latifolia,* disease frequency in an area of 1–16 m around host plants showed a significant direct effect on infection probability. At this scale, disease frequency showed no interaction with total host density, which had a negative direct effect in areas up to 8 m and a positive direct effect at larger scale. In *S. doica,* however, disease frequency in areas up to 4 m around host individuals showed a positive interaction with total host density. This indicates that the infection probability of *S. dioica* hosts increased more strongly with disease frequency in areas with a high host density than in areas with low host density. The R^2 for effects of disease frequency and host density on infection probability showed a maximum in a radius of 2–4 m around host plants for both host species.

In *S. latifolia,* addition of information on specific host traits (table 2; host sex, size, and phenology) resulted in a large increase in the proportion of the variation in infection probability that could be explained among host plants (25.3%, compared with 10.1% when only information on disease frequency and host density was used; table 1). Early onset of flowering and large plant size resulted in a higher probability of infection, and male hosts had a higher probability of infection than females (table 2). The precise effects of individual host traits on an individual's probability of

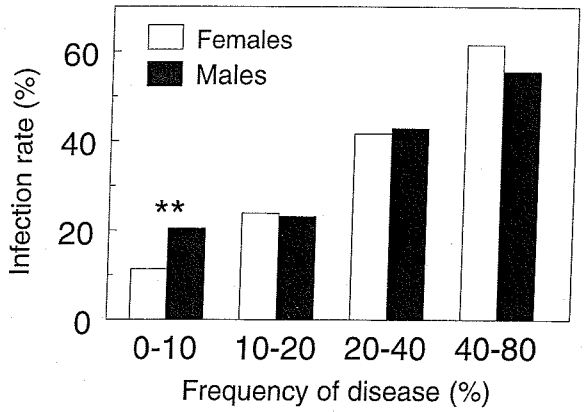

Fig. 1 Infection rate (proportion of initially healthy hosts becoming infected during the season) of female (grey bars) and male (black bars) hosts of *Silene latifolia* (n = 864) as a function of the frequency of diseased hosts in a 4-m radius around the host (class limits indicated). Significance of differences between sexes within a frequency class are indicated by asterisks (** $P < 0.01$).

infection appeared to depend on both host density and disease frequency. First, the negative interaction between disease frequency and host sex in *S. latifolia* (table 2) indicates that although females had an overall lower probability of infection than males, their infection probability increased more steeply with increasing disease frequency than for male hosts (fig. 1). In fact, differences in infection rates between males and females were only significant at low disease frequency (fig. 1). Second, the advantage of late onset of flowering in this species was more pronounced at low host density than at high host density (interaction density × phenology; table 2). In *S. dioica,* information on individual host traits made a relatively small additional contribution to the overall R^2 of infection probability (11.7% compared with 8.2% for neighborhood characteristics only) compared with *S. latifolia.* Effects of host traits on infection probability also tended to be more complex than in *S. latifolia* (table 2).

Patch Approach: Frequency- vs. Density-Dependent Transmission

The proportion of healthy hosts in a patch that became infected significantly increased with increasing disease frequency, both in *S. latifolia* (fig. 2*a;* F(1,23) = 44.27; $P < 0.0001$) and in *S. dioica* (fig. 2*d;* F(1,16) = 13.16; $P = 0.0023$). Infection rates were independent of the number of systemically diseased hosts in the patch for both host species (fig. 2*b, e*) but increased with the "true" density of infected plants (number of diseased plants per unit area) in the patch (fig. 2*c, f*). After removing effects of disease frequency and total host density, effects of true density of infected plants disappeared in both *S. latifolia* (F(1,21) = 1.63; $P = 0.22$) and *S. dioica* (F(1,14) = 2.27; $P = 0.15$). In *S. latifolia,* there was no indication that the strength of frequency dependence was affected by the average host density (interplant spacing) in a patch (interaction disease frequency × host density, F(1,21) = 0.0; $P = 0.99$). In *S. dioica* however, a significant interaction was found between disease frequency and host density (F(1,14) = 16.0; $P = 0.0013$). In accordance with the hypotheses based on vector behavior, infection rate strongly increased with disease frequency at high host density (short average interplant dis-

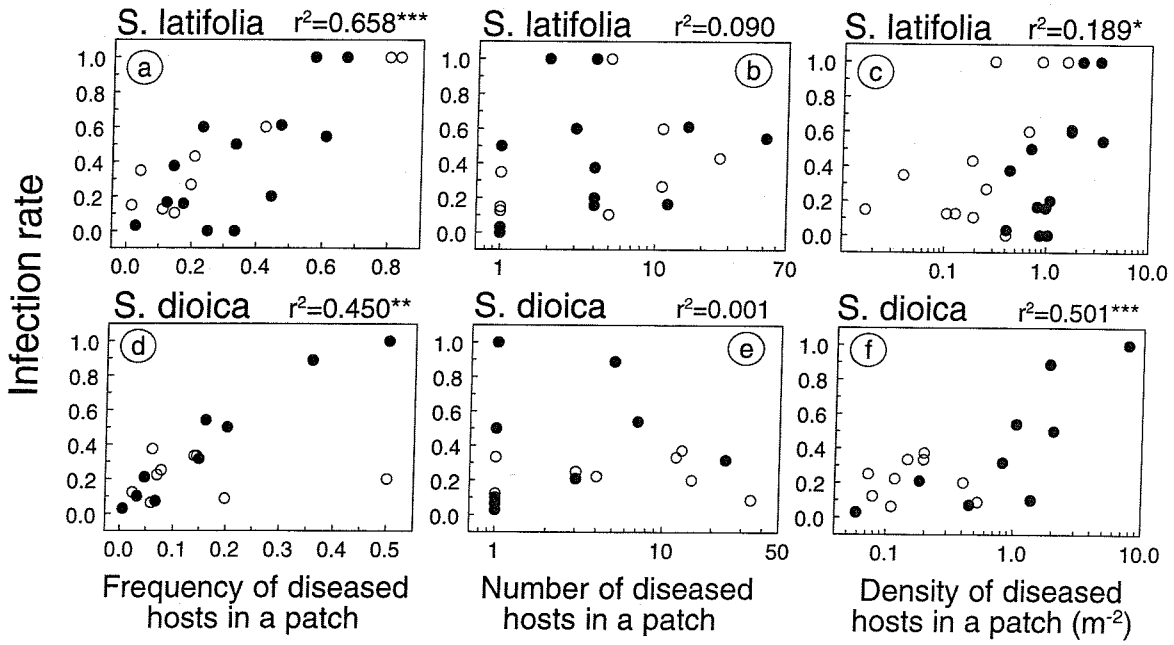

Fig. 2 Infection rate (proportion of initially healthy hosts becoming infected during the season) as a function of the frequency (*a, d*), number (*b, e*), and density (*c, f*) of diseased hosts per patch for *Silene latifolia* (upper panels) and *Silene dioica* (lower panels). For illustrative purposes, closed symbols are used for patches with higher than median host density (number of plants per unit area) and open circles are used for patches with lower than median host density.

Table 3 Multiple Regression of the Effects of Frequency of Diseased Hosts, Plant Density per Unit Area, and Number of Diseased Hosts per Patch on Infection Rate (Proportion of Initially Healthy Hosts in a Patch That Become Infected) in *Silene dioica* (R^2 = 0.894) and *Silene latifolia* (R^2 = 0.658)

	S. dioica				*S. latifolia*			
Source	df	MS	F	*P*	df	MS	F	*P*
FREQ: disease frequency	1	0.1309	9.78	0.0107*	1	2.0052	44.27	0.0001***
DENS: host density (area)$^{-1}$	1	0.0175	1.31	0.2785	1	...	...	
DIS: No. of diseased hosts	1	0.0462	3.46	0.0927	1	...	...	
FREQ × DENS	1	0.0384	2.87	0.1211	1	...	...	
FREQ × DIS	1	0.1163	8.69	0.0146*	1	...	...	
DENS × DIS	1	0.0047	0.35	0.5646	1	...	...	
FREQ × DENS × DIS	1	0.0767	5.74	0.0376*	1	...	...	
Error	10	0.0134			24	0.0453		

Note. Dots indicate terms not included in the reduced model after sequential removal of nonsignificant interaction terms. * = 0.05, *** = 0.001.

tance) but not at low host density (fig. 2*d*). In the joint analyses of number and frequency of infected plants and host density on infection rate (table 3), this interaction only showed up as a higher-order interaction among disease frequency, host density, and number of diseased plants in the population.

Discussion

Antonovics et al. (1995) have provided a general framework for interpretation of the effects of disease transmission processes on the infection probability of hosts, emphasizing that frequency- and density-dependent disease transmission should be viewed as two extremes of a general functional response curve. In vector-based transmission systems they showed that the probability of infection of a host may—depending on the details of vector behavior—range from being predominantly a function of the number of infected plants in the population (density dependence) to being proportional to the number of infected plants divided by the square of the total number of hosts (a form of frequency dependence). Our study shows that differences in infection rates among patches of *Silene latifolia* or *Silene dioica* were not related to the absolute number of infected host individuals in these patches or to their number per unit area when effects from the association with disease frequency were removed. This may indicate that encounter rates are not affected by population size of infected plants per se. This contrasts with results of Antonovics and Alexander (1992), who found that at a given frequency of infected plants the number of spores deposited on flowers of uninfected hosts actually decreased with the number of diseased and (since comparisons were made at fixed frequency) with the number of healthy hosts. This was interpreted as pollinator limitation; i.e., at an increase of both healthy and diseased hosts, the per capita spore deposition on uninfected hosts was reduced. Subsequent reanalysis of the data by Antonovics et al. (1995) gave a reasonable fit with predictions from their generalized model based on vector behavior. Our study was conducted in a natural population rather than in experimental arrays that were exposed to pollinators for a short period of time. Possibly, negative effects of the density of infected plants on infection rate were not observed under these conditions because there was more opportunity for vectors to partly adjust their population size to differences in host density at the level of host patches. In this respect it is interesting to note that in the analyses of the direct effects of disease frequency and host density on infection probability in *S. dioica,* high host density at a small spatial scale (less than 8 m around a host plant) had a negative effect on infection probability, but high host density at a larger scale had a positive effect on infection probability, possibly because at this scale larger densities attracted more vectors.

In both *S. dioica* and *S. latifolia* infection rate strongly increased with increasing disease frequency in patches with short average interplant distances. In *S. latifolia,* the pattern was similar for patches with larger interplant distances, but in *S. dioica* the increase in infection rate with disease frequency was much smaller for patches with larger average interplant distances. The latter is in agreement with earlier findings (Bucheli 1997) showing that disease frequency had a lower impact on spore deposition at large interplant spacing than at smaller interplant spacing in experimental populations of *S. latifolia* during part of the census period. Both observations are consistent with the idea that the expected increase in the time interval between successive visits, and hence in the ratio of search time to handling time, at larger interplant spacing will shift the disease transmission mode in a direction from more frequency dependence to more density dependence (Antonovics et al. 1995; Bucheli 1997). However, in our study this interpretation should be viewed with caution. In natural populations, the lower impact of disease frequency on infection rate at low host density may be related to factors other than vector behavior that are associated with host density. For instance, if host patches become less dense with age because of natural succession, and the frequency of resistant individuals increases with patch age as well, lower infection rates at larger interplant spacing may result even if the ratio of search time to handling

time of vectors were independent of host plant spacing. If the interactions between disease frequency and host density result from vector behavior, it also remains obscure why we observed such interactions in *S. dioica* but not in *S. latifolia.* The difference could not be attributed to larger overall interplant distances in *S. dioica.* In fact, overall interplant distances within patches of this species tended to be smaller than for *S. latifolia.* On the other hand, since the composition of prevalent pollinator species differs strongly between the two host species (Jürgens et al. 1996), differences in response to host density among vector species could contribute to the different types of interaction between disease frequency and host density observed for the two host species.

The individual host traits that we investigated explained a large additional part of the variation in infection probability among individual *S. latifolia.* In agreement with earlier studies in this species (Alexander 1989, 1990; Thrall and Jarosz 1994; Alexander and Antonovics 1995; Biere and Antonovics 1996; Biere and Honders 1996*b*), hosts with early onset of flowering and male hosts had significantly higher infection rates than hosts with late phenology and female hosts. In *S. dioica,* the same individual host traits explained a much smaller part of the variation in infection probability. It is interesting that effects of host traits on infection probability showed significant interactions with host density and disease frequency in the local neighborhood of hosts. Male hosts of *S. latifolia* had significantly higher infection rates than female hosts, but the difference decreased with increasing disease frequency. Hence, in areas of high disease frequency no differences in susceptibility between sexes could be detected. Such interactions illustrate another complexity in the prediction of infection dynamics of male versus female hosts. Disease incidence in female *S. latifolia* hosts in natural populations is often not different from or even higher than in male hosts (Zillig 1921; Alexander and Antonovics 1988; also Shykoff et al. 1996) despite the generally much higher infection rates of males observed in experimental field studies (Alexander 1989; Thrall and Jarosz 1994; Biere and Antonovics 1996). It has been argued that large and consistent differences in the survival ratio of diseased to healthy hosts among sexes could explain such apparent discrepancies. Our study shows that—depending on neighborhood characteristics—the magnitude of differences in infection rate itself may be small between host sexes, so that even small variation in survival rates of infected compared with uninfected hosts between sexes may result in variation in disease incidence among host sexes.

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