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PATTERNS OF RUST INFECTION AS A FUNCTION OF HOST GENETIC DIVERSITY AND HOST DENSITY IN NATURAL POPULATIONS OF THE APOMICTIC CRUCIFER, *ARABIS HOLBOELLII*

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Abstract.—It is often assumed that genetic diversity contributes to reduced disease incidence in natural plant populations. However, little is known about the genetic structure of natural populations affected by disease. Here I present data from three apomictic (asexual) populations of *Arabis holboellii* infected by the rusts *Puccinia monoica* and *P. thlaspeos*. An average of 300 host individuals per population were genotyped (using seven variable allozyme loci) and scored for disease presence. *Arabis holboellii* populations are genetically diverse; the number of clones detected per population ranged from 6 to 27. There was substantial variation in frequency of host clones within and among sites, and significant variation among clones in susceptibility to the different rusts. Contrary to predictions based on frequency-dependent selection theory there was not a consistent positive relationship between clone frequency and disease incidence within any of the populations (Spearman's $r = -0.096$, $P > 0.5$). In addition, clonally diverse populations did not necessarily have decreased disease incidence. The population with the lowest overall (both pathogens combined) disease incidence ($7.5 \pm 1.9\%$) had the smallest number of clones (6), the lowest spatial variability, and the highest *Arabis* density. By comparison, another population had 22 clones, high spatial variability, low *Arabis* density and significantly more disease overall ($16.8 \pm 2.7\%$). Although this study does not eliminate the possibility of frequency-dependent pathogen attack in these populations, the results suggest that it is likely to be weak or intermittent.

Key words.—*Arabis,* density, epidemiology, frequency-dependence, plant pathogens, *Puccinia.*

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Genetically variable clonal populations are difficult to explain under traditional ecological theory, because the most productive and competitive clone should drive all of the others to extinction (Bell, 1982; Sebens and Thorne, 1985; Begon et al., 1986). However, there are several ways genetic variability in clonal populations could be maintained, including frequency-dependent selection by pathogens, heterogeneous environments favoring one clone then another, the continuous generation of new clones from sexual progenitors, rapid mutation, and coexistence of similar clones for short times due to neutral stability (Bell, 1982; Sebens and Thorne, 1985). One of the least studied of these mechanisms is the potential for pathogens or parasites to exert frequency-dependent selection.

Two predictions are typically made concerning populations thought to be undergoing pathogen-mediated frequency-dependent selection:

Prediction 1: Within populations, genotypes (clones) occurring at low frequency should have a fitness advantage ("rarity advantage") (Haldane, 1949; Jaenike et al., 1980; Hamilton, 1980; Antonovics and Ellstrand, 1984; Parker, 1989; Lively et al., 1990; Alexander, 1991; Parker, 1992). That is, genotypes with rare resistance alleles will escape infection until the resistant genotype is common enough in the population that selection on the pathogen favors strains that can overcome the resistant hosts.

Prediction 2: Populations with more variation in resistance should have less disease than more uniform ones (Adams et al., 1971; Harlan, 1976; Bremermann, 1980, 1983; Barrett, 1981; Alexander, 1988). Decreased disease incidence is generally expected in variable versus uniform populations because the absolute number of hosts susceptible to a given pathogen genotype will be lower, the distance between susceptible plants will be greater, making pathogen

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transmission difficult, and pathogen propagules landing on resistant hosts will be lost to the system (Burdon, 1987; Alexander, 1988).

The distance between susceptible plants can be large because either a) host resistance alleles are diverse and thus the frequency of individuals in a susceptible genotype is low, or b) because the actual physical proximity (density) of hosts is low. Thus, host density, like host frequency, is typically predicted to be positively associated with disease incidence because more and closer host 'targets' are assumed to improve the probability of pathogen transmission (Anderson and May, 1979; Burdon and Chilvers, 1982; Alexander, 1988; Burdon et al., 1989). Nevertheless, host density is not always positively associated with disease incidence (Burdon and Chilvers, 1982; Burdon, 1987).

The present study is part of a larger effort to determine whether frequency-dependent selection by pathogenic rust fungi is structuring natural populations of the apomictic (asexual) crucifer, *Arabis holboellii. Arabis holboellii* displays heritable variation in resistance to rust infection, and host fitness is reduced by infection (Roy, 1992). For example, in a single season, uninfected plants flowered about 31% of the time versus 0.4% for those infected by *Puccinia monoica* or never for those infected by *P. thlaspeos.* Survival of uninfected plants and of those infected by *P. thlaspeos* was relatively high, 65% and 64% respectively, but declined to 40% or less for those infected by the more common rust, *P. monoica.* The goals of the present study were to determine, in natural populations of *Arabis holboellii,* (1) the extent of clonal variability, (2) whether clones varied in frequency and in disease incidence within and among populations, and (3) the effects of host density on disease incidence.

The fact that *Arabis holboellii* is clonal has important implications for this study. First, it should be possible to assess disease incidence at the clonal level, in situ, because each genotype in a given population is represented by many replicate, genetically identical individuals. I am assuming that the environmental influences on infection probability are spread among these replicated genotypes and that differences in infection at the clonal level therefore reflect genetic differences in susceptibility (Falconer, 1981). Second, the clonal nature of the plants may make it easier to detect frequency-dependent effects. This is because a population composed of clones originating from different parental stocks may be more strongly differentiated than a sexual population in which there may be more genomic sharing (Tibayrenc and Ayala, 1988).

Materials and Methods

The Hosts

The hosts, *Arabis holboellii* (Brassicaceae), are biennial to short-lived perennial plants occurring on well-drained soils throughout North America and extending north into Greenland (Rollins, 1941). Reproduction in *A. holboellii* may be sexual or apomictic (Böcher, 1947, 1951, 1969; Nygren, 1954). I have verified, by emasculation and crossing experiments (Roy, 1992; unpubl. data) and by electrophoresis of progeny arrays (Roy and Rieseberg, 1989), that the plants in the study populations reproduce by pseudogamous apomixis. In pseudogamous apomicts, pollen is necessary for successful seed production (probably required for endosperm formation) but does not fertilize the ovules (Richards, 1986; Asker and Jerling, 1992). The genetic consequence of this form of apomixis is that all progeny from a single mother are identical to each other and to their mother (Richards, 1986). *Arabis holboellii* plants do not spread vegetatively, but they are nevertheless clonal (sensu Buss, 1985; Ellstrand and Roose, 1987), because all the members of a family are genetically identical. I have chosen to call these independent plants individuals instead of ramets to emphasize that there is no physiological connection between plants. I refer to groups of individuals with identical allozyme phenotypes as clones.

The Pathogens

The pathogens, *Puccinia monoica* (Pk.) Arth. and *P. thlaspeos* C. Schub. [listed as *P. holboellii* in Anonymous (1960) and Arthur (1962)], cause systemic rust disease on *Arabis* species (Anonymous, 1960; Farr et al., 1989). Although these rusts are closely related to each other (Savile, 1974; Cummins and Hiratsuka, 1983), and may rep-

resent a progenitor-derivative species pair (Cummins and Hiratsuka, 1983), they have dissimilar life cycles (Fig. 1) and the differences may have epidemiological consequences. *P. monoica* has a "typical" rust life cycle: it alternates hosts and it has five different spore states (i.e., it is heteroecious and macrocyclic). For about four weeks of the year it occurs on the primary host, a grass belonging to any of the genera *Trisetum, Koleria,* or *Stipa*; then it must switch to its alternate host, *Arabis* or a few other genera in the Brassicaceae to complete its life cycle (Arthur, 1962; Farr et al., 1989). *P. thlaspeos,* however, is microcyclic, having only three kinds of "spore stages" (spermatia, teliospores, basidiospores) instead of five; it completes its entire life cycle on *Arabis.*

In the field, these rusts are easily distinguished in mid-May by the color of the spores and spore-bearing structures on the undersides of leaves on infected plants. At this time *P. thlaspeos*' teliospores are black, whereas *P. monoica*'s aeciospores are bright yellow.

The Sites

Three sites at similar elevations (approximately 2,700 m), but located in different drainage systems (to minimize intersite gene flow) were selected for monitoring in Gunnison County, Colorado. All three sites were in sagebrush (*Artemisia tridentata*) meadows approximately 10,000 m^2 in size, and bounded by forest on three sides and by a road on the fourth side (see Roy, 1992, for detailed site descriptions).

Population Sampling

Large scale population sampling was necessary to determine how clonal variation was partitioned within and among sites and for calculations of population and clonal disease incidence. In April of 1990 I established at each site eight nonoverlapping, randomly chosen, linear transects (nine at Gold Creek) 25 m long by 2 m wide. A random sample of 200 individuals per site were permanently marked with aluminum tags along the eight transects (25/transect).

To minimize the potential for introducing spores between sites and thus changing the genetics of the pathogens present in the pop-

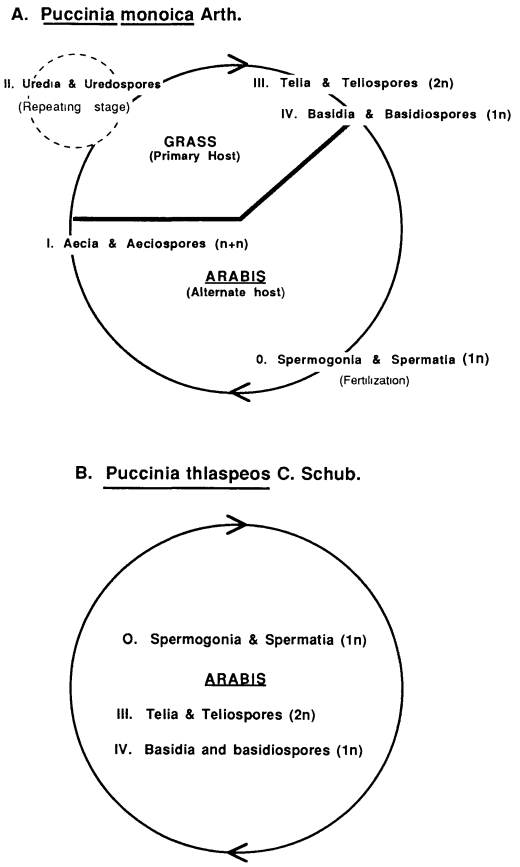

FIG. 1. *Arabis* rust life cycles. A. *Puccinia monoica.* This macrocyclic rust alternates between a grass (of the genera *Koleria, Trisetum,* or *Stipa*) and *Arabis* (or other mustard species depending on the rust race). During the spermatial stage (stage 0), cellular fusion (plasmogamy) occurs between opposite mating types, and dikaryotic (n + n) aeciospores (Stage I) are formed shortly after fusion. The aeciospores disperse to the primary host, a grass. Not long after infecting the grass, the pathogen produces urediospores (Stage II), which are capable of infecting other grass individuals. It is during stage II that the pathogen's populations expand greatly. Eventually, nuclear fusion occurs (karyogamy), and diploid teliospores are formed at Stage III. The teliospores germinate in the next rains to form basidia and basidiospores (Stage IV). Meiosis occurs in the basidia, so the basidiospores are haploid. The basidiospores disperse from the grasses and cause infection on *Arabis,* completing the cycle. The spermatial and aecial stages occur in the early spring, the uredial stage during mid-late summer, and the telial and basidial stages take place late summer–early fall. B. *Puccinia thlaspeos.* This microcyclic rust has only three known spore stages and is probably derived from the macrocyclic species, *P. monoica* (Cummins and Hiratsuka, 1983). *P. thlaspeos* does not alternate hosts, and is only found on mustard species such as *Arabis.* Life cycle terminology follows that of Petersen (1974).

ulations, I always changed clothing, shoes, and notebooks between sites and thoroughly cleansed my hands and measuring equipment with alcohol.

Clone Identification

Clones were identified by differences in allozyme banding patterns. I used allozymes because it is relatively easy to screen large numbers of individuals with them, and I expected that if clones differed in resistance, then they would probably also have different allozyme phenotypes because in apomictic taxa all loci act as a single linkage group. Inoculation tests on a small number of clones indicated that this was a reasonable assumption since there was variation in resistance among different allozyme phenotypes, and identical allozyme phenotypes had the same degree of resistance (Roy, 1992). For electrophoresis, small amounts of leaf tissue (approximately 1 cm^2) were removed from the marked individuals at each of the sites on May 8, 1990. The tissue samples were stored on ice for two days during transportation from the field sites to the laboratory, then kept in an ultralow freezer (−80°C) until use. Electrophoretic procedures followed Roy and Rieseberg (1989). Of the 9 putative loci scored, 7 were variable (an additional 15 loci were resolved in tests, but were not polymorphic or were too difficult to score). To facilitate accurate scoring of banding patterns and comparisons among gels, marker individuals with known banding patterns were included in each gel.

Clone identity was determined by sorting the allozyme banding patterns of phosphoglucoisomerase (PGI, 3 loci), triosephosphate isomerase (TPI, 2 loci), isocitrate dehydrogenase (IDH, 2 loci) and leucine aminopeptidase (LAP, 2 loci), and identifying unique patterns (see Appendix). Clonal diversity was estimated for the three sites using standard diversity indices [Shannon-Weaver and Simpson's Diversity corrected for finite sample size (Peet, 1974)].

Because there were a large number of similar, but not identical, clones, I also combined clones into classes that shared many alleles to increase sample sizes and statistical power for some analyses. The 41 unique clones were readily sorted into seven distinct clonal groups, hereafter referred to as CGs (see Appendix for a list of CGs and their clonal make-up). Five of these CGs were based on being identical at a minimum of PGI and LAP (i.e., identical at 57% or more of the seven variable loci scored). The other two clonal groups ("E" and "G") are more heterogeneous and represent individuals that may be sexual (based on higher levels of homozygosity and less linkage than in known apomictic *Arabis*).

Disease Incidence

Disease incidence (measured by percent of individuals infected) was determined for each population, and for each clone and clonal group that occurred in all three populations. I classified each genotyped individual for presence or absence of infection. Infected plants are easily identified because the systemic rust infections cause distorted growth in the host and the undersides of the leaves are covered with black (*P. thlaspeos*) or yellow (*P. monoica*) spores. Population disease incidence was measured by counting the number of infected individuals encountered in the random sample of 200 individuals from each of the sites. Standard errors of the proportions were calculated according to Cochran (1963, p. 64).

I was interested in learning whether host clones occurred in different frequencies within these populations and whether frequency of infection (percent infected) varied within and among clones. Because diseased plants were rare (7.5 to 16.8% of the population), the random sample did not provide an adequate sample for assessing disease incidence within clones. Therefore, I also genotyped all of the infected plants within each transect (hereafter referred to as the complete infected sample to differentiate it from the random sample). Because the frequency of infection within the population (percent infection in the random sample) and the distribution of genotypes in the infected class (complete infected sample) were necessarily estimated from different, but overlapping samples, actual percent infection per genotype (=clone or clonal group, depending on the analysis) was estimated by weighting the total infected sample of diseased individuals in each genotype by their proportion in the population at

TABLE 1. Clonal diversity of *Arabis holboellii* at three sites.

	Site		
	Cement Creek	Taylor River	Gold Creek
Number of individuals genotyped	455	219	230
Number of clones	6	27	22
Number of unique clones	1	18	13
Clones shared with:			
Cement Creek	—	5	5
Taylor River	5	—	9
Gold Creek	5	9	—
Number of clones/sample size	0.01	0.12	0.10
Simpson's Diversity Index, $\tilde{D}$	0.40	0.92	0.88
Shannon-Weaver, H′	0.80	2.8	2.4
Population disease incidence	7.5 ± 1.9	10.9 ± 2.2	16.8 ± 2.7

large. Standard errors were calculated using Gaussian error propagation analysis (Bartsch, 1974, pp. 475–476), because each proportion in the equation was subject to error.

To determine whether there was a relationship between clone frequency and disease incidence I performed two different statistical tests: Spearman's rank correlation on the ranks of clone frequency and disease incidence, and a two-way test of independence to determine whether disease incidence was independent of clone frequency. For the independence test I classified each clone as rare or common by whether it fell above or below the average frequency for all clones (across all sites), and by whether it was above or below average in terms of infection (averaged over all sites); a chi-square test was used to evaluate significance.

Host Density

Density of *Arabis* was measured in randomly placed 1 m^2 plots (15–48 per site in 1990). Density of the grass *Koleria nitida* Nutt. (the primary host for *P. monoica*) was estimated by counting the number of reproductive stems encountered in spring of 1991 in each of the eight random transects at each site (the reproductive stems were produced during the fall of 1990). Reproductive individuals of *Koleria* were counted instead of all plants because it is extremely difficult to identify nonflowering grasses; thus, this measure is probably an underestimate of actual density.

RESULTS

Clone Frequency and Variation in Disease Incidence

Arabis holboellii populations are genetically diverse; the number of clones per population ranged from 6 to 27 (Table 1 and Appendix). Across all three sites a total of 41 distinct clones were found (Table 1 and Appendix). Five clones were common to all populations, but each population also contained between 1 and 18 unique clones (Table 1). Clonal diversity, as measured by two standard indices (Table 1), was very similar at the Taylor River and Gold Creek sites, which were both much more diverse than Cement Creek.

Spatial variation in the genetic composition of the sites can be evaluated visually by mapping the spatial distribution of the clonal groups (CGs) along the transects, as in Figure 2. Cement Creek is dominated by a single CG (=clone D1 in this case). The Taylor River site is dominated by CG A on the north side of the site but is occupied by a combination of CG A, B, and D on the south side, perhaps indicating the existence of an environmental or historical gradient. Gold Creek is by far the most heterogeneous, with the genotypes spread fairly evenly throughout.

Disease incidence (as measured by percent of individuals infected) varied significantly among sites, clones, and clonal groups. At the population level, the disease incidences at Cement Creek and Taylor River were similar (7.5 ± 1.9 and 10.9 ± 2.2 respectively) and significantly lower than

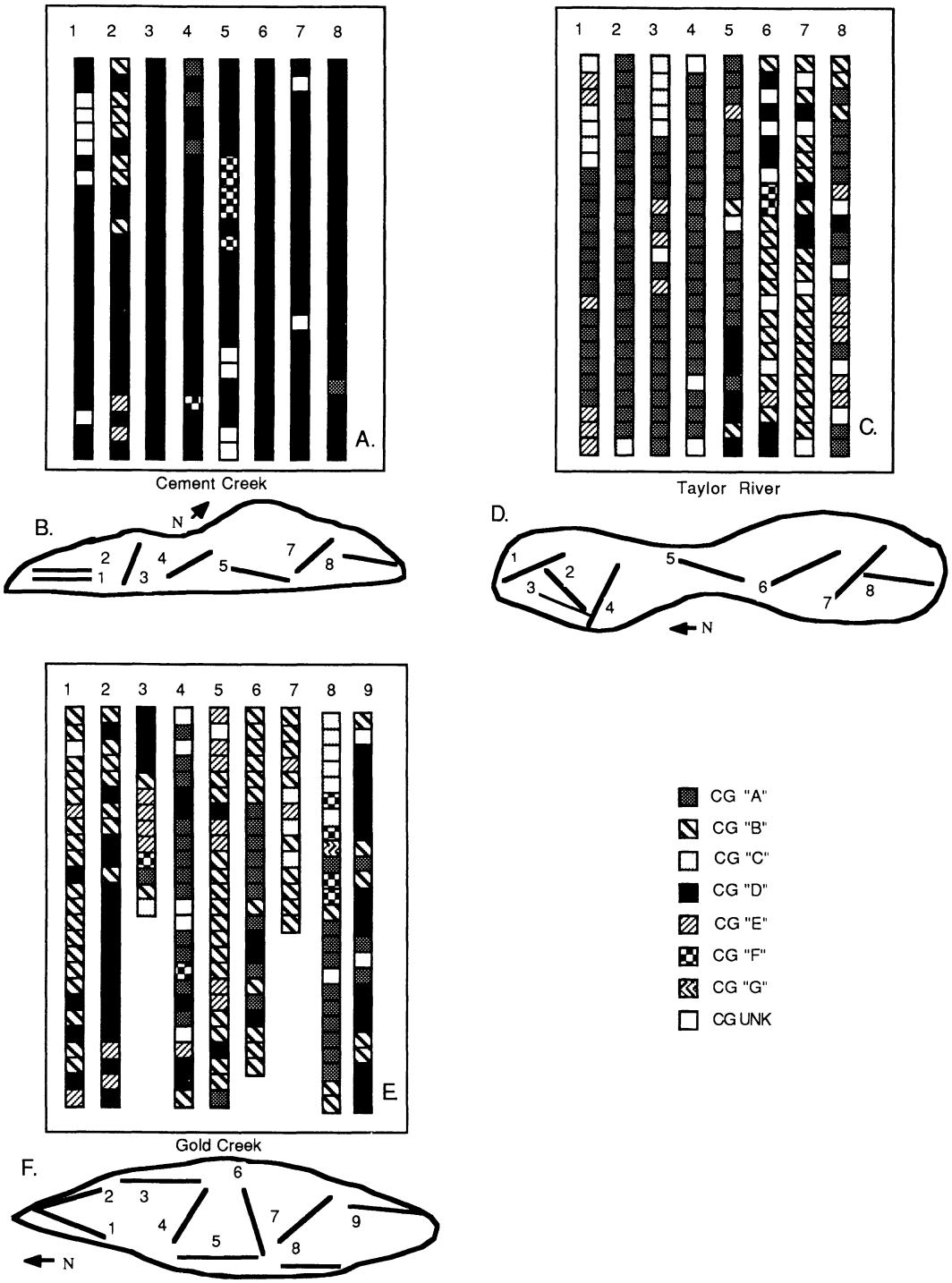

FIG. 2. Maps showing distribution of clonal groups (CGs) in the random sample of 200 *Arabis holboellii* from each of three sites. (A) Cement Creek CGs (B) map of transects at Cement Creek, (C) Taylor River CGs, (D) map of transects at Taylor River, (E) Gold Creek CGs, (F) map of transects at Gold Creek. The placement of transects and their relationship to each other within a site are only approximately to scale. The number identifying each transect is placed at the end of the transect corresponding to the top of the genotype "histogram" shown for each transect.

TABLE 2. Clone frequency (estimated percent of all individuals sharing identical electrophoretic phenotypes, see methods for calculation) and their associated disease incidence (percent infection) at three sites in 1990. Only the clones shared among all three sites are shown. Standard errors calculated with Gaussian error analysis.

Clone	Variable	Site		
		Cement Creek	Taylor River	Gold Creek
A3	Clone frequency	2.1 ± 1.1	6.1 ± 1.8	13.3 ± 2.4
	Disease incidence	2.6 ± 2.3	10.2 ± 6.2	34.9 ± 8.0
B4	Clone frequency	4.2 ± 1.3	4.0 ± 1.5	24.3 ± 3.5
	Disease incidence	27.9 ± 10.1	0.0	1.8 ± 1.3
C1	Clone frequency	0.1	4.8 ± 1.5	5.1 ± 1.9
	Disease incidence	100.0	17.2 ± 9.1	0.0
D1	Clone frequency	84.6 ± 2.5	5.9 ± 1.3	10.78 ± 2.5
	Disease incidence	5.5 ± 1.4	49.2 ± 12.9	10.3 ± 4.8
F2	Clone frequency	2.1 ± 0.8	1.0 ± 0.8	5.2 ± 1.8
	Disease incidence	52.0 ± 19.2	0.0	12.6 ± 7.5
Population disease incidence		7.5 ± 1.9	10.9 ± 2.2	16.8 ± 2.7

disease incidence at Gold Creek (16.8 ± 2.7). Contrary to prediction, disease incidence was lowest at the site with the least genetic diversity (Cement Creek, see Tables 1, 2, 3, and Fig. 2). Disease incidence was low at the Cement Creek site because the site was dominated by one common clone, D1 (85% of the sample), which had low disease incidence (5.5%) (Table 2, Fig. 2).

There was substantial variation for disease incidence among the five clones that occurred at all three sites, ranging from 0 to 52% (Table 2). At the level of clonal groups, the incidence of infection also varied strongly within and among sites (Table 3). The fact that disease incidence per clone (or CG) was not consistent across sites (Tables 2 and 3) suggests that there were differences in the composition of the pathogen populations present at the sites, or that the same electrophoretic phenotype may have different resistance phenotypes among different populations. Contrary to expectation, however, within populations there was no apparent relationship between a clone's frequency and its disease incidence (Spearman's rank correlation, $r = -0.096$, $P > 0.5$; chi-square test of independence, χ^2, 1 $df = 0.277$, $P = 0.599$).

Host Density

Host density varied strongly among sites (Table 4), with Cement Creek having 8 to 16 times higher *Arabis* density than the other two sites. Contrary to expectation, population disease incidence was lowest in this high density population. Infection by *Puccinia thlaspeos,* the autoecious rust (passes from *Arabis* to *Arabis*), was highest at the Cement Creek site where *Arabis* density was highest, and infection by *P. monoica,* the heteroecious rust (passes from *Koleria* to *Arabis*), was highest where *Koleria* was the most dense (Gold Creek).

DISCUSSION

Prediction 1: Clones Occurring at a Low Frequency Should Have a Fitness Advantage

A widespread assumption about genotypes within populations undergoing frequency-dependent selection is that common genotypes will suffer disproportionately more than rare ones (Haldane, 1949; Williams, 1975; Maynard Smith, 1978; Hamilton, 1980; Lively et al., 1990). In this study, however, disease incidence within a clone was not typically proportional to that clone's frequency within a population (Tables 2 and 3). For example, clone D1 was the most common clone at Cement Creek (84.6 ± 2.5% of the population), but experienced very low disease incidence (5.5 ± 1.4%), whereas clone F2 was rare, and its disease incidence was much higher (52.0 ± 19.2%). One hypothesis to explain this pattern is that common clones (e.g., D1 at Cement Creek) are common precisely because they have thus far escaped infection, either through resistance or through a fortuitous lack of a pathogen population capable of

TABLE 3. Frequency (estimated percent within a population, see methods for calculation) of clonal groups (CGs; CGs are formed by pooling clones with similar electrophoretic banding patterns, see Appendix and methods) and associated disease incidence (frequency of infection) at three sites in 1990. Standard errors calculated with Gaussian error analysis.

Clone	Variable	Site		
		Cement Creek	Taylor River	Gold Creek
A	CG frequency	2.1 ± 1.1	50.7 ± 3.7	17.2 ± 2.7
	Disease incidence	2.6 ± 2.3	9.8 ± 2.5	32.1 ± 6.9
B	CG frequency	4.2 ± 1.3	16.1 ± 2.9	45.5 ± 3.9
	Disease incidence	27.9 ± 10.1	1.3 ± 1.3	14.1 ± 3.1
C	CG frequency	0.1	4.8 ± 1.5	5.1 ± 1.9
	Disease incidence	100.0	17.2 ± 9.1	0.0
D	CG frequency	84.6 ± 2.5	8.9 ± 1.9	14.8 ± 2.9
	Disease incidence	5.5 ± 1.4	32.6 ± 9.4	7.4 ± 3.5
E	CG frequency	1.0 ± 0.8	9.3 ± 2.2	8.1 ± 2.3
	Disease incidence	0.0	8.9 ± 4.7	5.5 ± 4.0
F	CG frequency	2.1 ± 0.8	1.0 ± 0.8	5.2 ± 1.8
	Disease incidence	52.0 ± 19.2	0.0	12.6 ± 7.5
Population disease incidence		7.5 ± 1.9	10.9 ± 2.2	16.8 ± 2.7

infecting them. Theoretically, over time, a pathogen strain virulent on the common clone will be selected for and the common clone will become heavily infected and decline. Across a large number of clones, however, the expectation is that, on average, there should be a positive relationship between clone frequency and disease incidence. In these clonally diverse populations there was not a consistent positive relationship between clone frequency and disease incidence (Tables 2 and 3, Spearman's $r = -0.096$, $P > 0.5$).

Studies of selection by pathogens in natural populations are relatively few in number. Many of these studies have not detected frequency-dependence (e.g., Parker, 1989; Alexander, 1991, reviewed in Fritz and Simms, 1992), or have shown that other factors such as selection on characters linked to resistance can control variation in resistance (Parker, 1988*a*), or that morphological defenses can be adequate to protect genetically uniform clonal populations from disease (Parker, 1988*b*). There are, however, two study systems involving clonal organisms where there is evidence for frequency-dependent pathogen attack. Lively et al. (1990) surveyed parasite loads in clonal and sexual genotypes of fish in the genus *Poeciliopsis* in three populations. In this system, incidence of parasitism was strongly correlated with genotype commonness. In clonal plants, the best direct evidence for pathogen-mediated frequency-dependent selection comes from an example of bio-

TABLE 4. Density (individuals m^{-2} ± SE) of the hosts *Arabis holboellii* and *Koleria nitida* and percent of *A. holboellii* infected by the two rust species, *P. monoica* and *P. thlaspeos*, at three sites. *Puccinia monoica* requires *Koleria* to complete its life cycle, whereas *P. thlaspeos* occurs only on *Arabis*.

Species	Variable	Site		
		Cement Creek	Taylor River	Gold Creek
Arabis[a]	Density	16.1 ± 5.4	2.2 ± 0.4	2.1 ± 0.3
Arabis	% infected[b] by *P. thlaspeos*	29.07 ± 2.73	15.79 ± 5.95	2.78 ± 2.70
Koleria[c]	Density	0.01 ± 0.001	0.32 ± 0.01	1.21 ± 0.04
Arabis	% infected by *P. monoica*	70.93 ± 2.73	84.21 ± 5.95	97.22 ± 2.70
Population disease incidence		7.5 ± 1.9	10.9 ± 2.2	16.8 ± 2.7

[a] *Arabis* density is reported as the mean from 15, 45, and 48 random 1 m^2 quadrats per site in 1990, respectively.
[b] % infection by the different rusts is calculated from the complete sample of infected plants (see materials and methods). Individuals that were infected by both species of rust were divided evenly between the two rust species for the percentage calculations.
[c] *Koleria* density is reported as mean from eight random 25 by 1 m transects per site by counting every reproductive stem encountered in spring of 1991 (reproductive stems were produced during the fall of 1990).

control where a rust fungus (*Puccinia chondrillina*) is being used to control an introduced apomictic weed (*Chondrilla*). Studies in both North America and Australia have shown that disease incidence changes host frequency, and that host frequency depends on disease incidence (Burdon et al., 1981; Supkoff et al., 1988). In both the *Poeciliopsis* and *Chondrilla* studies there were only a small number of clones in the host populations. In the study on *Poeciliopsis* there were two asexual clones and an unknown number of sexual genotypes, and for *Chondrilla* there were three clones. Even if frequency-dependent selection is acting, it may be much more difficult to observe the effects in more complex systems, such as the populations described herein.

Frequency-dependent selection is cyclic and dynamic. A resistant host genotype should increase in frequency until a pathogen race virulent on the resistant clone is selected for (or migrates in), then the frequency of the clone will decline until it is rare enough to "escape" disease, whereupon the now rare host genotype will again be able to increase in frequency. Because frequency-dependent selection is cyclic, and because in natural populations there may be numerous host and pathogen genotypes, frequency-dependence is difficult to evaluate simply by determining current host frequencies and disease incidence as was done in the current study. In a given population, different host genotypes are likely to be in different parts of the frequency-dependent cycle. For example, one genotype may be rare but still be heavily infected because the pathogen population has not yet crashed, or a common genotype may be uninfected because there is not a pathogen genotype in the population capable of infecting it. For this reason, a descriptive study that examines a population at only one point in time can only establish whether or not disease varies with host frequency, and cannot determine whether frequency-dependent selection is occurring. The consistent lack of correlation between clone frequency and disease found in the current study does, however, suggest that frequency-dependent selection may be weak or infrequent. Now that it has been established that clones do vary in frequency within these populations, a reciprocal transplant experiment, using rare and common clones, is currently underway to more explicitly test for frequency-dependent selection.

There are, however, reasons to believe that frequency-dependent selection may indeed be weak in this system. One potential cause for weakened frequency-dependence are pathogen genotypes that are capable of infecting more than one host genotype (Barrett, 1988; Parker, 1992). In this situation, even when a host clone becomes rare, disease incidence on this rare clone may stay high if the pathogen population is maintained by living on other host clones. A concurrent experiment under controlled conditions revealed that, although there are significant differences in susceptibility to *Puccinia monoica* among clones, all four pathogen inoculants tested were capable of infecting all three clones tested (Roy, 1992). The fact that more than one genotype of pathogen can infect more than one host genotype of *Arabis holboellii* would clearly tend to weaken frequency-dependence.

It is also possible that the rust-host associations noted here are not the product of selection on resistance alleles. In apomictic hosts there is complete linkage disequilibrium; thus, it is possible for selection on other characters to change resistance associations nonadaptively (Burdon, 1985; Burdon and Müller, 1987; Parker, 1988*a*, 1991). For example, in wild oats with high levels of linkage disequilibrium due to selfing, Burdon and Müller (1987) found that differences in disease resistance were associated with differences in seed germination. Thus, in wild oats, selection on germination would also change resistance. It is possible that selection by pathogens on resistance in *Arabis* may be swamped by selection on other traits. Although rust infection decreases fitness of *Arabis* (Roy, 1992), it is likely that the overall effect of rust disease on the populations is small because only a few percent of the plants in each population are infected at a time. In addition, herbivory on *Arabis* has the potential to be a stronger selective force than pathogen attack (Roy, 1992, and unpubl. data) and therefore may cause correlated, non-adaptive change in rust resistance alleles. An experiment in progress should help estimate the importance of her-

bivory and its interaction with infection on selection in *Arabis*.

Prediction 2: Populations with More Variation in Resistance Should Have Less Disease Than More Uniform Ones

The prevalent view in both evolutionary biology and plant pathology is that host genetic diversity is one of the primary forces controlling disease incidence in populations (Adams et al., 1971; Browning, 1974; Harlan, 1976; Bremermann, 1980, 1983; Burdon and Shattock, 1980; Barrett, 1981, 1988; Alexander, 1988; for a review including negative evidence see Kranz, 1990). In the present study only three populations were characterized due to the amount of work involved in determining multilocus genotypes; thus, it is difficult to generalize about the effects of host genetic diversity on population level disease incidence. Although not conclusive, it is interesting that the predicted relationship between genetic diversity and disease incidence was not borne out in any of the populations, that is, high clonal diversity within populations was not associated with decreased disease incidence. For example, the Cement Creek population, which had the lowest overall (both pathogens combined) disease incidence (7.5 ± 1.9%) had the smallest number of clones (six), the least amount of clonal genetic diversity (Table 1), the lowest spatial heterogeneity (Fig. 2), and the highest density (Table 4). By comparison, Gold Creek had 22 intermingled clones, much lower *Arabis* density, but significantly higher disease incidence (16.8 ± 2.7%).

The Relationship of Host Density to Disease Incidence

Host density is clearly an important and complex factor in the *Arabis-Puccinia* pathosystem. It is unusual for there to be a negative relationship between host density and disease as was found at the population level in this study of *Arabis* (Burdon and Chilvers, 1982; Burdon et al., 1989). However, the amount of disease caused by the most common rust on *Arabis, Puccinia monoica,* is clearly related to density of the primary host, *Koleria,* from which the inoculum is received, and not simply the density of *Arabis*. For infection by *P. monoica* to become common in *Arabis* populations, there must be sufficient density of both the primary and alternate hosts to facilitate transmission. Frequency-dependent selection could occur at any density, but it is unlikely for population level disease incidence of *Puccinia monoica* to be high, regardless of the level of genetic diversity in the population, unless the density of both host species is high. The relationship between infection and host density is likely to be positive for the other rust, *P. thlaspeos,* because *Arabis* is its only host, and indeed, the densest population of *Arabis* (Cement Creek) supported the largest population of this less common rust species (Table 4).

Clonal Diversity

The number of genotypes per population uncovered in this study ranged from 6 to 27 (Table 1) and the average number, 18.3, was slightly higher than the average of 16.1 reported by Ellstrand and Roose (1987) for several species and populations of asexual plants. What is the origin of the clonal variation in these populations? In an earlier study (Roy and Rieseberg, 1989), electrophoresis of progeny arrays from *Arabis holboellii* collected at the Cement Creek site revealed no recombination, suggesting that the clonal variation uncovered there may have resulted from polyphyletic origin of the apomicts from sexual parents combining different alleles. The current survey of two other populations uncovered several individuals that may be sexual (Appendix, CG "E"). Their banding patterns are more homozygous than the typical fixed heterozygote pattern for apomictic plants, and one group, CG "G," appear to be heterozygotes combining different "E" patterns. It would not be too surprising for sexual individuals to occur at the Gold Creek and Taylor River sites, as diploids are rarely apomictic (Bierzychudek, 1985; Asker and Jerling, 1992) and I have made diploid chromosome counts from Taylor River plants (Roy, 1992 and unpubl. data). Other workers have reported occasional sexual plants of *A. holboellii* on the basis of chromosome number and embryology (Böcher, 1951, 1969), and on the basis of putative hybrids with other species (Rollins, 1983). Because pollen of

proven apomicts retains high viability at these sites (Roy, unpubl. data), it is possible that apomictic pollen can fertilize sexual plants and give rise to both sexual and apomictic progeny (Nogler, 1975; Asker, 1979). Progeny tests of the putative sexual plants are necessary to determine their breeding systems. If any of these more homozygous plants are sexual, then there are somewhat fewer apomictic clones than reported in Table 1 (CGs E and G are the most likely sexual genotypes and account for 9 of the 41 clones).

Conclusions

The study populations are clearly dissimilar; the primary host of one of the pathogens is nearly absent at one site (Cement Creek) yet quite abundant at another (Gold Creek), different host clones dominate the various sites (Fig. 2), and the pathogen populations are also apparently variable in composition and abundance (Tables 2 and 3). In some cases, host clones appear to be disease-free, suggesting a lack of pathogen genotypes capable of infecting them. Although this study does not eliminate the possibility of frequency-dependent pathogen attack, the lack of correlation between clone frequency and disease incidence within populations does suggest that it may be weak or intermittent. If the environment is sufficiently heterogeneous in space and time, due to a combination of, for example, intermittent pathogen attack, historical reasons, and physical attributes, then different clones could have differential success often enough to maintain polymorphic populations (Maynard Smith, 1978; Falconer, 1981). Alternatively, the likelihood of sexual reproduction in two of these populations suggests the possibility that clonal diversity may be maintained by continuous generation of new clones and not, or only secondarily so, by selection, pathogen-mediated or otherwise.

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Appendix

Description of clonal groups (CGs) and clones, including information on their numbers at the three sites. Each clone has a two part identifier, a letter for the clonal group to which it belongs (defined by banding pattern at PGI and LAP) and a unique number for individual clones within each clonal group. The letters under each allozyme locus represent the bands present for each clone (the bands include heteromers as well as alleles). Because these banding patterns often represent unbalanced heterozygotes (due to polyploidy), they are coded to reflect dosage. If the band was strong, then the letter is underlined. UNK means unknown.

Clone	Allozyme locus						Site		
	PGI 2 and 3	IDH 2	TPI 1	TPI 2	LAP 1	LAP 2	Cement	Taylor	Gold
Clonal group A									
A 9	abc	a	ab	ac	cde	ab	0	37	0
A 11	abc	a	ab	c	cde	ab	0	1	0
A 23	abc	a	abc	abc	cde	ab	0	0	3
A 10	abc	a	abc	bc	cde	ab	0	4	0
A 12	abc	a	abc	c	cde	ab	0	2	0
A 4	abc	abc	a	bc	cde	ab	0	2	0
A 22	abc	abc	abc	a	cde	UNK	0	3	0
A 25	abc	abc	abc	abc	cde	ab	0	1	0
A 2	abc	abc	abc	ac	cde	ab	0	6	0
A 3	abc	abc	abc	abc	cde	ab	6	16	41
A 26	abc	abc	abc	abc	cde	ab	0	0	1
A 1	abc	abc	abc	bc	cde	ab	0	21	5
A 24	abc	abc	ab	ac	cde	ab	0	1	0
A 14	abc	abc	abc	ac	cde	ab	0	2	0
A 15	abc	abc	abc	abc	cde	ab	0	9	0
A 17	abc	abc	abc	bcc	cde	ab	0	17	0
A 20	abc	c	a	ac	cde	ab	0	1	0
Total A							6	123	50
Clonal group B									
B 9	cde	a	abc	abc	cde	b	0	0	3
B 11	cde	a	abc	abc	cde	b	0	0	12
B 4	cde	a	c	bc	cde	b	51	8	53
B 8	cde	abc	abc	abc	cde	b	0	0	1
B 1	cde	abc	abc	abc	cde	b	0	5	30
B 10	cde	abc	abc	abc	cde	b	0	0	5
B 6	cde	abc	c	bc	UNK	UNK	0	0	1
B 7	cde	abc	abc	abc	cde	b	0	14	0
B 3	cde	abc	abc	bc	cde	b	0	0	1
Total B							51	27	106
Clonal group C									
C 1	ace	abc	abc	abc	cf	ab	3	14	11
Clonal group D									
D 5	bcde	a	abc	abc	bce	b	0	4	0
D 4	bcde	a	ac	abc	bce	b	0	2	8
D 1	bcde	abc	abc	abc	bce	b	346	23	25
D 3	bcde	abc	abc	abc	bce	b	0	1	0
Total D							346	30	33
Clonal group E									
E 7	a	a	a	abc	e	b	0	6	3
E 2	a	a	a	bc	e	b	0	16	0
E 8	a	a	a	bc	e	a	0	0	1
E 6	a	c	c	bc	e	b	2	0	0
E 9	abc	abc	abc	abc	e	a	0	0	1
E 4	abc	abc	abc	abc	ce	a	0	0	9
Total E							2	23	14

APPENDIX. Continued.

Clone	Allozyme locus						Site		
	PGI 2 and 3	IDH 2	TPI 1	TPI 2	LAP 1	LAP 2	Cement	Taylor	Gold
Clonal group F									
F 2	abcde	a	abc	bc	de	ab	47	2	14
Clonal group G									
G 1	ac	a	abc	abc	cde	b	0	0	1
G 2	ac	a	abc	abc	e	a	0	0	1
Total G							0	0	2
Grant total							455	219	230