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Research article

Tests for parasite-mediated frequency-dependent selection in natural populations of an asexual plant species

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Abstract. Genetic variation in plant populations for resistance to pathogens and herbivores might be maintained by parasite-mediated negative frequency-dependent selection (FDS). But it is difficult to observe the time-lagged oscillations between host and parasite genotypes that should result from FDS. To evaluate the potential for FDS, we tested for local adaptation of parasites to common clones, the role of host genetic diversity in resistance to parasites, and genetic correlations among fitness, parasitism, and the frequency of host clones. We studied three populations of *Arabis holboellii*, a short-lived apomictic (asexual by seed) plant attacked by rust fungi and insect herbivores. To estimate clone frequency, we used polymorphic allozyme markers on 200 individuals in each population in 1990 and in 2000. We also recorded levels of parasitism and host fitness (fruit production). Only the rust fungi showed evidence for local host adaptation; they usually increased in incidence as a function of clone frequency, and they tracked temporal change in clone frequency. In further support of FDS, parasitism was lower in populations with higher genetic diversity. However, total parasitism (herbivory and disease combined) decreased as host clone frequency and fitness increased. Thus, although the highly virulent rust pathogen showed potential for driving the cycles that result from FDS, this apparently does not occur in the populations studied because the host clones were also attacked by herbivores.

Key words: apomixis, *Arabis*, frequency-dependent selection, herbivores, pathogens, Red Queen, resistance

Introduction

How is genetic variation within populations maintained for traits that function in species interactions? In host–parasite interactions, genetic variation in host defense mechanisms is often greater than one might expect if directional selection by parasites was important in shaping these traits (Roy and Kirchner, 2000). One solution for why there is variation may be parasite-mediated frequency-dependent selection (FDS). Over time, FDS results in time-lagged oscillations between coevolving host and parasite genotypes (Nee, 1989; Dybdahl and Lively, 1995; Kaltz and Shykoff, 1998). This constant cycling

resulting from parasite-mediated selection is the essence of the ''Red Queen Hypothesis'' (RQH) (after Bell, 1982) for cross fertilization (Lively, 1996).

There are relatively few empirical tests of parasite driven frequency-dependent selection, despite the appeal of the RQH and the degree of theoretical attention it has received. The majority of the supportive empirical data has come from the snail-trematode systems studied by C. Lively and his associates (Lively, 1987, 1999; Jokela and Lively, 1995; Dybdahl and Lively, 1998). However, the evidence that FDS is a strong force structuring plant populations is equivocal (Parker, 1994; Clay and Kover, 1996; Roy, 1998). Two recent reviews (Kaltz and Shykoff, 1998; Van Zandt and Mopper, 1998) showed that only about half the plant studies yielded evidence that their parasites were adapted to their local hosts, a basic result of FDS. Here we outline a comprehensive evaluation of the potential for FDS in three populations of *Arabis holboellii*, a short-lived apomictic (asexual by seed) perennial plant that is attacked by rust fungi and multiple herbivores.

We used the entire community of parasites to assess parasite-mediated FDS in asexual *A. holboellii*. We use the term parasites when referring to concepts that cover both herbivores and pathogens (Price, 1980); however, because we recognize that there may be important biological differences among organisms, we also report our results separately for each parasite species.

Although it is difficult to observe the cycles predicted by FDS, there are several attributes of the cycles that should be observable at a single time point or across two time points within a cycle. Our tests of FDS were mainly focused on these attributes. Specifically, we tested the following predictions of FDS:

1. Parasites should be locally adapted to the common host clones (Haldane, 1949; Hamilton, 1980; Jaenike *et al.*, 1980; Antonovics and Ellstrand, 1984; Parker, 1989, 1992; Lively *et al.*, 1990; Alexander, 1991; Roy, 1993; Dybdahl and Lively, 1995). We tested for local adaptation in three ways: First, from the data we examined the relationship between incidence of parasites on clones and clone frequency. We interpreted a positive slope in the regression as an indication that parasites had adapted to common but not rare clones. A slope of zero was interpreted as random attack as expected when parasites have not adapted to different clones. A negative slope would also be expected in a homogenous parasite population but when common clones were resistant and rare clones were susceptible. Second, we determined whether common host clones were more often disproportionately over-parasitized and whether rare hosts were more often under-parasitized as exhibited in FDS cycles. Third, we determined whether there was a positive relationship between temporal changes in clone frequency and disease as would occur through time under FDS as parasites track hosts over short time periods. We measured host and rust

fungus frequency twice, once in 1990, and again in 2000, after about 2.5 host generations or half a cycle generated by FDS over time. To our knowledge, only one other study (Dybdahl and Lively, 1998) has repeated measurements on the same natural populations to examine predicted change.

2. Incidence of parasitism should be lower in host populations with high genetic diversity because the effective rarity of host genotypes increases in diverse populations (Adams *et al*., 1971; Harlan, 1976; Bremermann, 1980, 1983; Barrett, 1981; Alexander and Antonovics, 1988; Lively *et al*., 1990; Roy, 1993). However, time lags in cycles resulting from FDS might affect the relationship between parasitism and diversity of host genotypes. In populations where there is a common host genotype that happens to be disproportionately under-parasitized, host genotype diversity could be low, assuming that an under-attacked common genotype is a good competitor and consequentially would reduce the frequency of other genotypes (Dybdahl and Lively, 1995).
3. Host plant fitness should decrease as a function of host genotype frequency and parasitism. A negative genetic correlation between plant fitness and parasitism is expected for natural selection on host resistance (Rausher, 1996). If the fitness of a host genotype depends on its frequency, then selection is frequency-dependent (Ayala and Campbell, 1974). Thus, for parasite-mediated FDS to be occurring, host fitness should also decrease as a function of host genotype frequency.

Methods

Organisms

The host plants, *Arabis holboellii* var. *retrofracta* (Grah.) Rydberg (common name rockcress), are biennial to short-lived perennial plants in the mustard family that occur throughout western North America and into Greenland (Rollins, 1941). Reproduction in this species is entirely by seeds; the plants are tap-rooted and do not spread vegetatively. The populations under study are pseudogamously apomictic; pollen is necessary for successful seed set but does not fertilize the ovules (Roy, 1995). Because seed production is apomictic, all progeny produced by an individual are genetically identical and, thus, genetic clones of the parent plant (Roy and Rieseberg, 1989). We refer to independent plants as individuals to emphasize the lack of physiological connection.

The pathogens, *Puccinia monoica* (Pk.) Arthur and *P. thalaspeos* C. Schub., cause rust disease on *Arabis* species (Anonymous, 1960; Farr *et al*.,

1989). Infection by these pathogens severely decreases survival and reproduction in *A. holboellii* (Roy and Bierzychudek, 1993). *Arabis* plants become infected in the late summer to early fall by wind-borne spores. Over the winter, rust mycelium invades the hosts' meristematic tissue, causing a systemic infection that affects all future growth. Infection by these rusts is easily detected the following spring when masses of colorful spores cover the leaves.

There were four main types of herbivory on *A. holboellii* plants at our sites in 2000. We observed root galls, stem boring, and two types of damage on basal rosette leaves. Pea-sized galls just below the soil surface were formed by a weevil, *Ceuthorrhynchus* sp (Coleoptera: Curculionidae). A swollen stem below the basal rosette indicated stem-boring by larvae of another unidentified weevil. On leaves of the basal rosette we observed numerous windows, which resembled typical flea beetle damage except the holes did not extend through the leaves; these were probably caused by adults of a third weevil, *Rileyonymus relictus*. The edges of basal rosette leaves were also chewed by various species of grasshoppers (Orthoptera: *Melanoplus*) and unidentified leaf beetles (Chrysomelidae). Herbivory was recorded in early spring at the same time that we recorded disease incidence. Because this host species produces leaves just once per year at our sites, when we recorded damage in the spring, we had a record of the previous year.

We recorded whether each type of herbivory was present or absent on each plant, and except for the stem borer, we were also able to quantify the amount of herbivory. By measuring the extent of damage, we had more precision in assessing the effects of herbivory on plant fitness. For each plant we counted the number of galls, weevil-windows per basal rosette, and the number of chews (about 0.25 cm^2 each) per rosette.

Sampling

In 2000, we revisited the three populations studied in 1990 by Roy (1993) in Gunnison County, Colorado, and used the same sampling techniques to study the covariation of clone frequency, parasitism, and fitness. Sites were 1 ha meadows of sage brush (*Artemisia tridentata*), each occurring in a river valley separated from the other sites by a few kilometers (see Roy, 1992 for detailed site descriptions). Roy (1993) employed a systematic, stratified random sampling technique (Cochran, 1963) to determine incidence of rust infection and clone frequency, which entailed establishing 8 (–9) 25-m non-overlapping transects of 2 m width at each site. In 1990, the ends of these transects were permanently marked with metal spikes, so the sites could be revisited. In 2000, we used the same transects and techniques and recorded incidence of rust infection, herbivory, seed production, and clone frequency.

To obtain a random sample along each transect, at 1 m intervals data was recorded from the nearest *A. holboellii* plant, which was usually within 0.5 m of the transect.

Because diseased plants were rare (4.3–15.2% of the population), we also genotyped all of the infected plants within each 25 m × 2 m 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) was estimated by weighting the total infected sample of diseased individuals in each genotype by their proportion in the population at large (Roy, 1993).

Clone identification

Clones were identified by differences in allozyme banding patterns as in Roy (1993), except that cellulose gel electrophoresis was used in 2000 (procedures after Herbert and Beaton, 1993), and we added the enzyme phosphoglucomutase (PGM, 2loci). As in 1990, there were a large number of similar, but not identical, clones, thus we combined clones into classes that shared many alleles to increase sample sizes sufficiently to allow statistical analyses (Roy, 1993). The 45 unique clones were readily sorted into 9 distinct clonal groups, referred to as CGs (see Appendix of Roy (1993) for a list of CGs and their allozyme composition. The list for the 2000 census is similar and available from the authors upon request). Five of these CGs were based on being identical at a minimum of PGI and PGM (i.e., identical at ≥50% of the five variable loci scored). Clones grouped by PGI were found to differ in disease resistance (Roy and Bierzychudek, 1993). Because CGs differed in disease resistance, we hereafter simplify our terminology from clonal groups to clones except where indicated.

There are three main reasons why our pooling similar but not identical clones into clonal groups for analysis probably did not obscure patterns in the data. First, we observed some similar patterns among sites where one of the sites Cement Creek had only five clones by any measure. Second, the variation among clones that we clustered into clonal groups is quite small, and is likely to be the result of mutations accumulated during many generations of asexual reproduction (Roy and Siemens unpublished microsatellite data). Third, we know that the allozyme classes are correlated with pathogen resistance (Roy, 1993), and with glucosinolate concentrations (Siemens and Roy, unpublished data), one of the major classes of anti-herbivore compounds in crucifers (Wittstock and Halkier, 2002).

Testing the predictions

Are parasites locally adapted to common host clones?
We used regression analysis to examine the relationship between clone frequency and parasite incidence. We also performed this analysis for the incidence of each parasite, therefore we used sequential Bonferroni adjusted *p*-values to correct for type I errors associated with multiple tests (Rice, 1989). To satisfy assumptions of normality in parametric tests, parasite incidence and other response variables in this study were transformed when necessary.

To determine whether common host clones were more often disproportionately over-parasitized and whether rare hosts were more often under-paraisitzed, chi-square analyses were performed. We first determined whether clones were significantly common or rare compared to average clone frequency, then whether clones were disproportionately over- or under-diseased or fed upon within each population in each year.

To determine whether there was a positive relationship between temporal changes in clone frequency and disease, we regressed changes in clone frequency and disease across approximately three host generations or half a cycle generated by FDS over time. Generation time for *A. holboellii* at our sites is approximately 4 years (Roy, 1998), and data on disease was collected in 1990 and 2000. Only data on disease was collected in 1990 so we were not able to conduct these analyses for changes in herbivory.

Do genetically diverse populations have lower parasite incidence?
We first determined whether there were significant differences among the populations in clonal diversity. We used the Shannon–Weaver index for a measure of clonal diversity at each site, which were then compared by *t*-tests (Hutcheson, 1970 as cited in Zar, 1996, p. 157), adjusted by the sequential Bonferroni technique for multiple tests (Rice, 1989). To determine whether parasite incidence varied among sites we used a chi-square test on counts of attacked and non-attacked plants from the random samples.

To determine whether under-parasitized common clones were associated with low population genetic evenness we identified common clones using chi-square analyses as mentioned above under predictions for local adaptation, then we determined genetic evenness for each population from the Shannon–Weaver diversity index (Zar, 1996).

Do the parasites decrease host fitness and is fitness frequency-dependent?
In addition to clone identification and parasitism, we also recorded an estimate of plant fitness, seed production, by counting the number of mature fruits per plant (fruit number is positively correlated with seed number ($r = 0.71$) and weight per seed ($r = 0.75$)). We used multiple regression analysis to determine

the genetic correlation between mean clone fruit production and parasitism, and between clone fruit production and frequency, and to estimate selection gradients (Lande and Arnold, 1983; Rausher, 1992; Brodie III *et al*., 1995). These would be genetic and not phenotypic correlations because micro-environmental effects within a site were probably distributed randomly among and within genotypes that were spacially mixed. We also examined the phenotypic relationships between plant fitness and resistance.

Results

Are parasites locally adapted to common host clones?

Within each population, parasite incidence decreased as a function of clone frequency ($p < 0.001$, Figure 1a). Although this was the general pattern across all sites, separate analyses for each parasite revealed different patterns depending on parasite and site (Table 1). The relationship between disease incidence and clone frequency was actually positive at two of the sites (Taylor and Gold) across years ($p < 0.01$, Figure 1b). Since pathogens and herbivores attacked each clone these parasites are negatively genetically correlated, and the direction of selection would thus depend on the relative impact of the different parasites. We found a positive relationship between clone frequency and fitness (see below under the section on fitness), which suggests the herbivores have greater impact.

A deeper examination of the parasitism ratios also does not support a FDS pattern. If the rate of parasite evolution is about twice that of the host's then the ratio of over- to under-parasitism in common host genotypes should be 3:1 and for rare genotypes 1:3 (Kaltz and Shykoff, 1998). If there is no difference in generation times between parasite and host then we expect half of the host genotypes to be disproportionately under-parasitized and half to be over-parasitized (Dybdahl and Lively, 1995). This equal ratio would be true for common or rare host genotypes, or both types lumped together because the model is symmetric. We observed a 1:4 ratio of over- to under-parasitism for common clones, which was significantly different from the expected 3:1 ratio ($\chi^2 = 8.07$, df $= 1$, $p = 0.004$), and a 9:0 ratio for rare clones, which was significantly different from the expected 1:3 ratio ($\chi^2 = 27.0$, df $= 1$, $p < 0.001$). The same trends were found for pathogens and herbivores considered separately.

Temporal changes in host genotype frequency and parasitism should be positively correlated to reflect the dynamic oscillations resulting from FDS over time. The relationship between temporal changes in clone frequency and disease depended on population ($F_{2,10} = 2.976$, $p < 0.1$), mainly because of the dra-

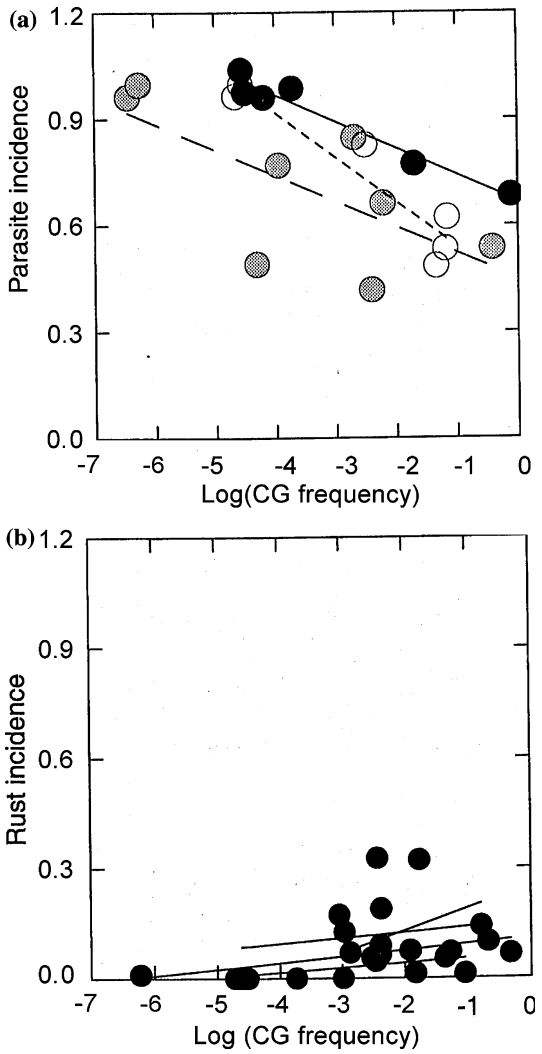

Figure 1. Relationship between host clonal group (CG) parasitism and frequency depended on parasite and site. (a) Parasite incidence (pathogens and herbivores) decreased at all sites in 2000 (different symbol fill and line patterns for each site: Gold Creek = shaded and dashes, Taylor = open and dots, Cement = black and solid). (b) Disease incidence increased as a function of CG frequency. Data were combined for Taylor and Gold sites and years 1990 and 2000. Each line is for a different combination of site and year to show that the same trend was observed for each. Statistical analyses in Table 1. CG frequency was log transformed because of the huge discrepancy among frequencies (see Figure 3).

matic difference between the Cement and Gold Creek sites (population-by-clone frequency interaction: $F_{1,7} = 7.259$, $p = 0.031$). The relationship at the Gold Creek site was positive, but the relationship was negative at the Cement Creek site, and there was no relationship at the Taylor River site (Figure 2).

Table 1. *F*-ratios from regression analyses of parasite incidence (disease and herbivory) on CG frequency, site, and CG-by-site interaction

Factor	Parasites								
	Rust	Rust (Taylor and Gold)	Rust (Cement)	Flea Beetle	Chewer	Galler	Borer	Flea-Beetle and Chewer	All parasites
CG freq	2.67	12.78**	0.47	0.36	0.33	0.26	0.09	3.38*	17.35**
Site	2.08			0.46	0.07			0.45	3.79*
CG freq-by-site	2.82*			0.84	0.93			0.98	276
Slope		+0.43	−0.21	−0.02	−0.02	−0.68	−0.17	−0.03	−0.01
R^2	19.6%	34.7%	4.5%	27.4%	6.3%	29.1%	0.0%	38.2%	63.0%

Also given are slope of the relation of parasite incidence on CG frequency (see text), and R^2, the variance explained by the model. The significant interaction for rust incidence led to analysis of different site combinations (in parentheses). Flea beetle and chew damage occur on basal rosette leaves and are usually correlated (e.g., $r = 0.33$, $p < 0.001$), hence these grouping and additional analysis. Year (for disease incidence) was never a significant factor and was therefore excluded from analyses. Blanks in the table occur because the galler and borer were sufficiently abundant for analysis only at one site, (Cement) or because sites were combined (Taylor and Gold for the analysis of Rust), or because an interaction term made it meaningless.
$*p < 0.1$; $**p < 0.01$; $***p < 0.001$ Bonferroni adjusted.

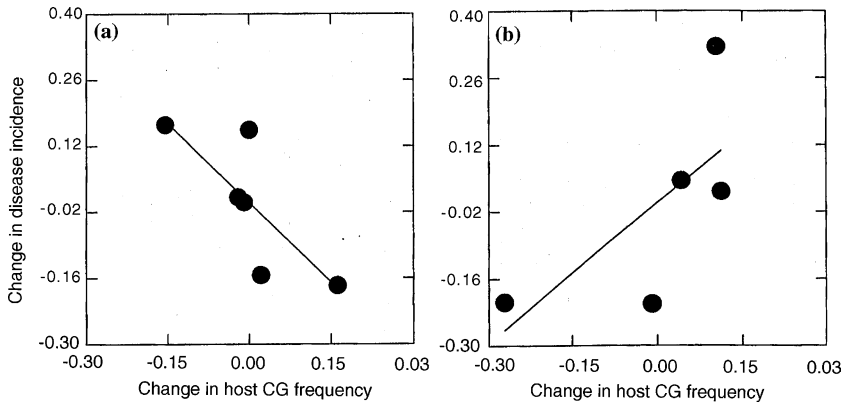

Figure 2. Relationship between temporal changes in disease incidence and CG frequency depended on site (population-by-CG frequency interaction: $F_{1,7} = 7.259$, $p = 0.031$). (a) Cement Creek site, (b) Gold Creek. There was no relationship at the Taylor River site (not shown).

Do genetically diverse populations have lower parasite incidence?

Parasite incidence in 2000 at Cement Creek (73.5%) was approximately 17% higher than the other sites ($\chi^2 = 13.58$, $p < 0.001$), which presumably reflects the lower clonal diversity at Cement Creek (Table 2). This general pattern held for 3 out of 4 types of herbivory, the exception being for the chewing damage, which was 75% higher at the site that had the highest clonal diversity, Taylor

Table 2. Population diversity and parasite incidence among sites and between years

	Site		
	Cement	Taylor	Gold
Number of individuals genotyped			
In 1990	455	219	230
In 2000	173	132	196
Clones[1]			
In 1990	6	27	22
In 2000	6	28	25
Unique clones			
In 1990	1	18	13
In 2000	2	17	15
Clones shared among:			
Cement			
In 1990	–		
In 2000	–		
Taylor			
In 1990	5	–	
In 2000	3	–	
Gold			
In 1990	2	9	–
In 2000	5	6	–
Shannon–Weaver H′			
In 1990[2]	**0.35 a**	1.21 b	1.04 b
In 2000	**0.36 a**	**1.09 b**	0.77 c
Evenness $J' = H'/H'_{max}$ 1990	0.22	0.75	0.65
J' in 2000	0.22	0.66	0.47
Parasite incidence[3]			
Rust in 1990	6.8 a	11.2 a	15.2 a
Rust in 2000	6.8 a	4.3 a	7.1 a
Gall-weevil in 2000	**17.2 a**	0 b	0.6 b
Stem-borer in 2000	**22.8 a**	1.7 b	1.2 b
Weevil windows in 2000	**45.9 a**	21.7 b	29.6 b
Chewers in 2000	24.0 a	**36.5 b**	17.8 a
Total parasite incidence 2000	**73.5 a**	57.6 b	56.2 b

Different lower case letters within rows indicate significant ($p < 0.05$) differences, which are in bold for notable comparisons between parasitism and diversity.

[1] Actual number of clones; for clonal groups (CGs) see Roy (1993).

[2] Multiple pair-wise *t*-tests to distinguish diversity indices (Zar, 1996) corrected for Type I errors with sequential Bonferroni adjustments (Rice, 1989).

[3] Chi-square and subsequent subdivided chi-square tests (Zar, 1996) determined significant ($p < 0.05$) differences among sites.

River. Disease incidence did not vary significantly among populations in either year; however, we expected for disease incidence to be much higher at the sites that happened to have high host genetic diversity because the density of the alternate host to the fungus happens to be 1 to 2 orders of magnitude higher at those sites (Roy, 1993).

We did not find any clear relationship between the level of parasite incidence on common clones and host population genetic diversity. There were two cases where the most common clone was under-parasitized (D in Cement 1990, and D at Taylor in 2000), and these two populations varied dramatically in clonal evenness, 0.22 and 0.657, respectively (Table 2, Figure 3). There was also dramatic variation in clonal evenness (range = 0.22–0.75) for the four remaining cases where the most common clone was not disproportionately under-parasitized.

Do the parasites decrease host fitness and is fitness frequency-dependent?

There was a strong negative genetic correlation between plant fitness (fruit production) and parasite incidence ($\beta = -0.80$, $r^2 = 63.7\%$, $F_{1,16} = 28.12$, $p < 0.001$), indicating the potential for natural selection on resistance (Figure 4a). This pattern held for disease considered separately ($\beta = -0.55$, $r^2 = 57.2\%$, $F_{1,9} = 12.02$, $p = .007$), however the pattern for herbivory varied depending on herbivore. We examined the quantitative measures of window and chew herbivory on plant fitness, and while the genetic correlation between weevil window damage and fruit production was negative ($\beta = -0.486$, $F_{1,\ 12} = 5.51$, $p = 0.037$), the relationship between chew damage and fruit production was positive ($\beta = 0.543$, $F_{1,\ 12} = 9.37$, $p = 0.01$). The positive relationship is expected under selection for compensatory responses to parasitism, or when there are preferences by parasites for more vigorous clones (Price, 1991). Sample sizes were too small to examine the genetic correlation between other types of herbivory and fitness. Phenotypic analyses showed a significant negative relationship between fruit production and parasitism for all parasites at each site, except for the chew damage at the Taylor River site (Table 3).

We did not find evidence for negative FDS. Instead, fruit production increased as clone frequency increased (Figure 4b, $\beta = 0.31$, $r^2 = 32.9\%$, $F_{1,16} = 7.85$, $p = 0.013$), suggesting an advantage associated with commonness, such as parasite resistance.

Discussion

To determine whether there was a potential for FDS and its cycles in populations of an asexual plant species, we investigated evidence for parasite adaptation to common host clones, lower parasitism in genetically diverse populations, and lower fitness for common clones. We found that the highly virulent fungal pathogens probably track common host clones in space and time, which should drive the cycles; however, our evidence also suggested that

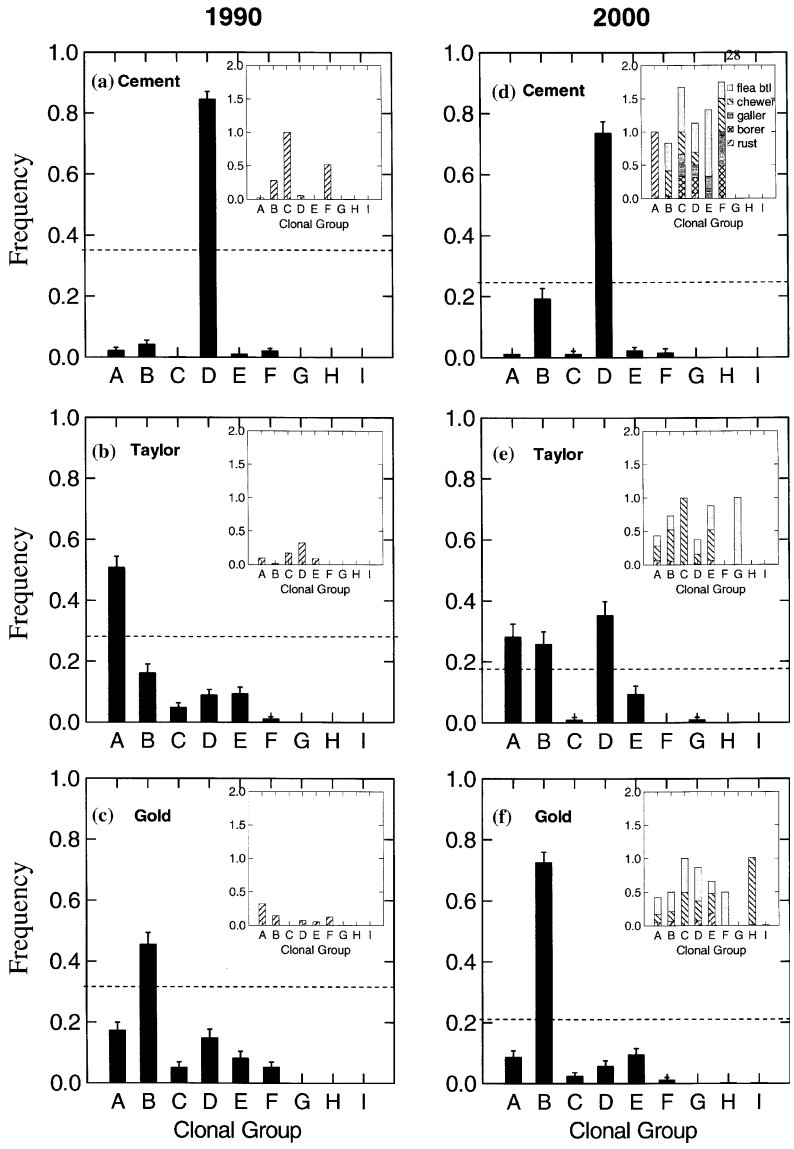

Figure 3. Frequencies of clonal groups (CGs) within site and year, and inset graphs are pest frequencies. Only parasitism by rust fungi was recorded in 1990. The horizontal dashed lines are population mean CG frequency, and at each site the frequency distribution of CGs was significantly different than this uniform distribution (smallest $\chi^2 = 142$, df $= 5$ to 7, $ps \ll 0.001$). Parasite frequencies exceed 1.0 because of multiple types of damage to CGs.

other factors prevent the cycles, such as the more abundant herbivores impacting rare clones more. Most field studies on the effects of parasites on host evolution have focused on a single parasite and a single host. Our results

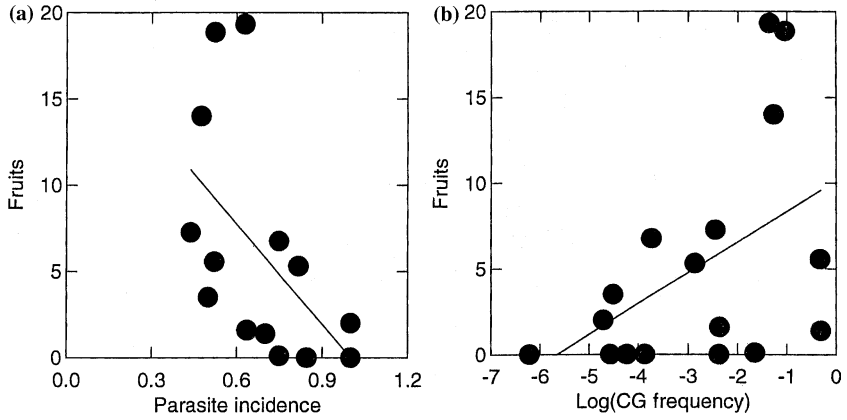

Figure 4. Plant fitness (fruit production) as a function of (a) pest incidence (data are CG means pooled for all sites, $r^2 = 63.7\%$, $p < 0.001$) and (b) CG frequency ($r^2 = 32.9\%$, $p = 0.013$).

Table 3. Phenotypic relationships between parasitism and host fitness (fruit production, which is correlated with number of seeds) in 2000

Site	Parasite	Parasitism	Test statistic	p[1]	Effect on fruit production
Cement	Rust fungi	Presence/Absence	$\chi_1^2 = 5{,}8$	0.016	Negative
	Weevil	#Windows/Rosette	$F_{1,\ 86} = 8.4$	0.005	Negative
	Chewer	#Chews/Rosette	$F_{1,\ 52} = 7.0$	0.011	Negative
	Stem borer	Presence/Absence	$\chi_1^2 = 5.8$	0.016	Negative
	Galler	#Galls/Plant	$F_{1,\ 35} = 5.1$	0.030	Negative
Taylor	Rust fungi	Presence/Absence	$\chi_1^2 = 18.9$	<0.001	Negative
	Weevil	#Windows/Rosette	$F_{1,\ 111} = 13.25$	<0.001	Negative
	Chewer	#Chews/Rosette	$F_{1,\ 112} = 0.75$	0.830	None
Gold	Rust fungi	Presence/Absence	$\chi_1^2 = 32.9$	<0.001	Negative
	Weevil	#Windows/Rosette	$F_{1,\ 98} = 5.2$	0.025	Negative
	Chewer	#Chews/Rosette	$F_{1,\ 94} = 5.2$	0.024	Negative

[1] Protected from Type I errors by sequential Bonferroni adjustments within each site (Rice, 1980).

suggest that it may be essential to study the whole system. The effects of some parasites may be negatively genetically correlated and disproportional. In the following sections we discuss the details that led us to this conclusion.

Local adaptation of parasites to common host clones

We checked for higher parasitism on common clones and temporal increases in parasitism as clones increased in frequency over time. From analyses of the incidence of herbivory and disease combined there was no indication of adaptation to common host clones in any of the host populations. Instead, we actually found evidence to the contrary that common clones had significantly less parasitism, which suggests that parasites have not been able to overcome

the resistance of these common clones, and that rare host clones are found by parasites and may be more susceptible. Thus the combined pathogen and herbivore results show no evidence for FDS.

However, when we examined herbivores and pathogens separately, we found some evidence for local adaptation by the fungal pathogens in two of three host populations. We found that disease incidence was positively correlated with clone frequency, and in the Gold Creek population, the pathogens were tracking host genotype frequencies through time. Yet in another test we did not find that common clones were more often disproportionately over-diseased, as should eventually happen with complete adaptation to hosts. The results actually suggested the opposite that common clones were more often disproportionately under-infected by the pathogens. Other unmeasured factors may prevent complete adaptation to common hosts and the cycles of FDS.

Dybdahl and Lively (1995) found that two of four locally common clones of the freshwater snail *Potamopyrgus antipodarum* were more often under-parasitized by the digenetic trematode (*Microphallus* sp.). They suggested that this result might actually be expected for FDS in snapshot studies conducted at one point in time because time lags should result in common clones that are sometimes disproportionately under-infected. However, with relatively large sample sizes, common host genotypes in snapshot studies should be more often over-parasitized according to FDS because the over-parasitized state occurs more often in the cycles.

Reciprocal transplant experiments of host clones among sites have also been conducted to determine whether parasites were locally adapted to common clones of *A. holboellii* (Roy, 1998). The main result was that host genotypes at their site of origin had less disease, less herbivory and higher fitness than foreign clones, which suggested that hosts were well adapted to their local environment. Thus no evidence of local adaptation by parasites was found. Other transplant studies with plants have come to similar conclusions (e.g., Parker, 1989; Kaltz *et al.*, 1999; Davidson 2000), which is also consistent with our current finding that locally common host clones had less total parasitism, and therefore appear to be more resistant. However, as mentioned above this was not the case for the fungal pathogens where a positive relationship between disease incidence and clone frequency was found at two sites, suggesting local adaptation to common host clones. Why is there a discrepancy between the field correlations and the reciprocal transplant experiments? To test for local adaptation, reciprocal transplant experiments introduce host clones from other populations with the assumption that local parasite populations have not encountered these foreign hosts. However, as Roy (1998) pointed out, reciprocal transplant experiments often confound origin and frequency of host clones as also occurred in the transplants of *A. holboellii*.

The relationship between temporal changes in host clone frequency and disease varied dramatically among sites. In an analysis, that only included the fungal pathogens because herbivory was not recorded in 1990, we observed the positive correlation expected from the cycles generated by FDS, but only at one site, Gold Creek. In our analysis, we used contemporaneous changes in disease incidence, but a stronger correlation is expected for delayed changes in disease because of the time lag in the FDS models (Dybdahl and Lively, 1998). Our use of contemporaneous measures may explain why no correlation was observed at the Taylor River site, however time lags are less likely to explain why a negative relationship was observed at another of our sites, Cement Creek. Several factors have been invoked to explain variation among populations or species in local adaptation (Kaltz and Shykoff, 1998; Lively, 1999), including variation among populations in virulence, elapsed time, migration, intensity of the interaction, and the genetic basis of infection and resistance.

Role of host genetic diversity in resistance to parasites

The ability of a parasite to overcome a particular host genotype should decrease in diverse populations because the absolute number of encounters of a single host genotype decreases, and because physiologically overcoming multiple defenses may be more difficult. In apparent agreement with this expectation we found the highest incidence of parasitism at the Cement Creek site, which had the fewest number of clones, the lowest clonal diversity (Table 3), and relatively low spatial heterogeneity in host genotype (Roy, 1993).

Why is FDS supported for effects of host genetic diversity, but not clonal frequency? The factors that reduced parasite incidence in diverse host populations should also have lowered parasitism on rare host clones. Statistically, our tests of the effects of host frequency were more rigorous because we had many more clones than populations. The effect of host population genetic diversity might be better evaluated with many more populations. No other plant studies exist that we are aware of that have adequate replication of host genotypes among populations and knowledge of the community of parasites.

Unlike the freshwater snail system studied by Lively and colleagues, we did not find an association between under-parasitized common clones and low population-genetic evenness. In a study of four lake populations of the freshwater snail *Potamopyrgus antipodarum*, each population had one dominant clone, and in the two populations where the common host clone was under-parasitized there was relatively low population genetic evenness in the host snail (Dybdahl and Lively, 1995). The under parasitized clones may have competitively reduced the frequencies of other clones in the snail system, but apparently not in this plant system.

Crucial for a rigorous evaluation of the presence of FDS is a check for a negative genetic correlation between host fitness and host frequency that is mediated by resistance to parasites. Infection of the freshwater snail by larvae of the trematodes, for example, results in sterilization of both males and females (Lively, 1999). Thus, higher infection rates also indicate lower fecundity of common host clones. Similarly, infection by the rust pathogens severely decreases survival and reproduction in *A. holboellii* (Roy and Bierzychudek, 1993). However, many parasites such as insect herbivores have less drastic negative impacts on hosts. Thus in addition to host genotype frequency and parasitism, it is also important to have independent measures of host fitness such as seed production. Since pathogens and herbivores attacked each clone, mean fitness of a clone allowed us to evaluate this important aspect of FDS. Once again, we found evidence for positive but not negative frequency-dependent selection. Host plant fitness (fruit production) increased as a function of clone frequency. This agrees with our finding that total parasitism decreased as a function of host clone frequency. Although disease incidence increased on common clones, apparently the relatively high incidence of herbivory overrides any negative frequency-dependent selection imposed by the pathogens. Further, the apparent strong negative impact by most herbivores on less frequent clones probably also overrides any selection by some herbivores for host compensatory responses.

Conclusion

Only the rust fungi showed evidence for local host adaptation, a basic result of FDS and the first prediction we listed (see Introduction); they usually increased in incidence as a function of clone frequency, and they tracked temporal change in clone frequency. However, total parasitism (herbivory and disease combined) decreased as host clone frequency increased, which is evidence against local adaptation for the majority of parasites in this system. In other evidence that supported the potential for FDS, parasitism was lower in populations with higher genetic diversity, the second prediction listed; however, studies are needed that include several populations to verify this result. In direct contrast to FDS and the third prediction, host clone fitness and frequency were positively correlated. Thus, although the highly virulent rust pathogen showed potential for driving the cycles that result from FDS, this apparently does not occur in the populations that we studied because the host clones were also attacked by several species of herbivores.

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