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The potential for rust infection to cause natural selection in apomictic *Arabis holboellii* (Brassicaceae)

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Abstract. Few studies have examined the potential for pathogens with complex life cycles to cause selection on their required alternate (=intermediate) hosts. Here we examine the effects of two fungal pathogens on an herbaceous mustard, *Arabis holboellii*. One pathogen species uses *A. holboellii* as a primary host, the other uses it as an alternate host. This plant-pathogen system is especially interesting because the host, *A. holboellii*, is apomictic; thus individuals reproduce exact copies of themselves. Despite this mode of reproduction, *A. holboellii* populations are surprisingly genetically diverse. Could frequency dependent selection by pathogens be maintaining clonal diversity? This study assesses the potential for selection by pathogens. In a controlled greenhouse experiment we show that there is heritable variation in *A. holboellii*'s resistance to the rust, *Puccinia monoica*, and that host fitness is severely reduced by *P. monoica* infection in both the greenhouse and under natural conditions. Field observations indicate that host clones are also differentially susceptible to the short-cycled rust, *P. thlaspeos*, and that host fitness is reduced by infection to this pathogen as well. Although the preconditions for pathogen-mediated selection are present, frequency-dependent selection by pathogens is unlikely to be important in structuring populations of *Arabis holboellii* because multiple host genotypes are susceptible to the same inoculum and the pathogen has a long generation time.

Key words: *Arabis* – Alternate hosts – Frequency-dependent selection – Pathogens – *Puccinia*

Many authors have recognized the potential of pathogens to shape the genetic structure of natural plant populations, and, ultimately, the composition of the communities to which these populations belong, through selection on resistance alleles (Harper 1969; Harlan 1976; Burdon 1987; Burdon and Müller 1987; Augspurger 1988; Alexander 1992; Parker 1992). Most of this recent attention has been focused on fairly simple pathogen-host relationships. However, many plant diseases have complex life cycles that involve at least two hosts, a primary one on which they undergo meiosis, as well as an alternate host that is also necessary for completion of the pathogen's life cycle. Few studies have examined the evolution of resistance in these alternate hosts.

Here we examine the effects of two fungal pathogens on an herbaceous mustard, *Arabis holboellii*. One pathogen species uses *A. holboellii* as a primary host; the other uses it as an alternate host. This plant-pathogen system is especially interesting because *A. holboellii* is apomictic; individuals produce seed asexually, bypassing meiosis, and thus reproduce exact copies of themselves. Despite this mode of reproduction, *A. holboellii* populations are surprisingly genetically diverse. For example, in 3 populations in Colorado surveyed electrophoretically at 7 polymorphic loci, the number of *A. holboellii* clones per population ranged from 6 to 27 (Roy 1993b). In the same populations, the incidence of rust infection varies among clones from 0–100% (Roy 1993b).

These observations suggest the hypothesis that frequency-dependent selection by its pathogens might contribute to *A. holboellii's* observed clonal diversity. In order for pathogen-mediated selection to maintain balanced polymorphisms in resistance alleles within populations, several conditions must be met. First, resistance to the pathogen must be a heritable trait. Second, there must be genetic diversity within the pathogen population. Third, host genotypes must be differentially resistant to pathogen genotypes. Finally, the hosts that lack resistance must experience decreased fitness as a consequence of infection. If there are no genetic differences among host genotypes in resistance, if the pathogen is broadly virulent, or if there are no fitness consequences of infection, then frequency-dependent selection is not likely to maintain resistance alleles in populations (Barrett 1988; Parker 1992).

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This study was designed to assess the potential for pathogen-mediated selection to occur in these populations by determining whether some of the preconditions listed above were met. We addressed the following specific questions:

1) Is resistance to *Puccinia monoica,* a rust with a complex life cycle, heritable?

2) Does rust infection affect host fitness (survival and reproduction) in the greenhouse?

3) Under natural conditions are clones of *A. holboellii* differentially resistant to *Puccinia monoica* and *P. thlaspeos,* and what are the consequences of infection for host fitness?

Materials and methods

The hosts

Arabis holboellii Hornem. is a relatively common, biennial-perennial monocarpic mustard found throughout the western U.S. and ranging into Canada and Greenland (Rollins 1941). Allozyme analysis of the genetic structure of progeny arrays (Roy and Rieseberg 1989) and emasculation and crossing experiments (Böcher 1954; Roy 1992) indicate that *A. holboellii* is a pseudogamous apomict. The consequence of apomixis is that all progeny from a single mother are genetically identical. We refer to groups of individuals with identical allozyme phenotypes as clones. Families are progeny arrays grown from seed from the same physiological mother. Because different physiological mothers may be genetically identical, it is possible to have multiple families representing the same clone.

The pathogens

Puccinia monoica (Pk.) Arth. causes systemic rust disease on *Arabis* species (Arthur 1912; Anonymous 1960; Arthur 1962; Farr et al. 1989). *P. monoica* has a "typical" rust life cycle: it must alternate between hosts in different genera and it has five different spore states (i.e., it is heteroecious and macrocyclic). After spending part of its life cycle on *Arabis* or on a few other genera in the Brassicaceae, it switches to a grass host in the genus *Trisetum, Koleria* or *Stipa* (Arthur 1962; Farr et al. 1989). The grass host is referred to as the primary host because this is the host upon which meiosis occurs (Petersen 1974; Agrios 1988). The pathogen need not spend most of its life cycle on the "primary" host and, in fact, tenure on this host is short in the *Arabis-Puccinia* system (Roy 1993b).

The closely related *Puccinia thlaspeos* C. Schub. (= *P. holboellii*) also causes systemic infection in *Arabis* (Anonymous 1960; Farr et al. 1989). *P. thlaspeos* differs from *P. monoica* by having a simpler, life cycle. It has only three kinds of "spore stages" (spermatia, teliospores, basidiospores) instead of five, and completes its entire life cycle on *Arabis* (i.e., it is microcyclic and does not alternate hosts).

Under natural conditions both pathogens cause similar abnormal growth in the host. Infected plants are taller, more branched and have more leaves than uninfected non-flowering host plants (Roy 1993a).

Measuring broad-sense heritability of resistance: greenhouse experiment

Determining whether host genotypes differ in resistance to pathogen infection is difficult under natural conditions; environmental heterogeneity can influence both an individual's probability of infection and its response to infection (Burdon 1985; Augspurger 1988; Antonovics and Alexander 1989). We measured heritability of resistance and the fitness effects of infection in the greenhouse so that we could use known inoculants for heritability estimates, and reduce variability from sources other than infection.

Because *A. holboellii* is apomictic, it was possible to measure genetic variance for resistance directly (Falconer 1981). In experiments where progeny from different clones are randomly arranged with respect to each other and the environment, the expected effect of the environment on the phenotype is zero, and all variation among clones can then be attributed to genetic differences. This kind of heritability estimate is referred to as "broad-sense heritability" (Falconer 1981).

Experimental design

Two mother plants were chosen at random from each of two sites. Seeds from these plants were grown to make the families used for the inoculation study. Later, it was determined that these four families represented 3 clones, 2 of them common, and one rare. Each family consisted of about 160 genetically identical individuals, with approximately 40 individuals from each family receiving one of 4 inoculation treatments (3 different rust inoculants and a control). Table 1 lists the sources of host plants and the rusts, and gives the number of progeny from each parent in the treatment groups.

The seeds from each wild collected mother were sown into seedling plug trays (400 plugs per tray, plugs were 2.7 × 6.4 cm) in planting medium composed of sand, vermiculite and peat moss in a ratio of 2:1:1 by volume. All planting took place within a 48 h period (October 17–18, 1989). Seedlings were grown for 41 days, then were transplanted over a 48 h period (November 28–29, 1989) into individual pots (5.5 cm by 8 cm). The final potting mixture was composed of sand, pea-gravel, vermiculite and peat in a 1:1:1:1 ratio by volume, with an approximate pH of 7.0. After transplanting, the pots were placed in an unheated but cooled greenhouse (winter temperatures 0–24° C, summer 10–37° C). Treatments, individuals, tray number, and tray position were all randomized. After five weeks (January 1–2, 1990) the plants were censused and dead individuals replaced if available. The pots were then rearranged into treatment groups and inoculated (this was necessary to avoid cross-infection, see below under inoculation). Two weeks after inoculation the plants were returned to the randomized design and not moved again until the experiment was terminated one year later (January 2, 1991).

The plants were censused approximately every 4 weeks for infection (present or absent), survival (alive or dead), and reproduction (yes or no, and number of seeds produced). The plants were

Table 1. Number of individuals inoculated in each treatment and source information for host seeds and rust spores

		Treatment			
	Source	Inoc. #1 Cement[a]	Inoc. #2 Taylor[b]	Inoc. #3 Gold[c]	Control
Seed family					
Holb 1 (A3)	Taylor	40	40	40	25
Holb 2 (D1)	Cement	35	36	35	29
Holb 3 (D1)	Cement	37	36	33	36
Holb 4 (C5)	Taylor	40	39	40	25

[a] The Cement Creek site is south of Crested Butte, Colorado and 4 km up the Cement Creek road (forest route 740) from its intersection with highway 135

[b] The Taylor River site is 7.2 km east of Almont, Colorado on the Taylor River road

[c] The Gold Creek site is 1 km north of Ohio City, Colorado on the Gold Creek road

not fertilized, but an insecticide (75% Orthene, 15 ml soluble powder per 4.2 liters of water) was applied to all plants whenever insects such as aphids were observed on any plants (approximately bi-monthly).

Inoculation

We caused infection on *Arabis* by using diploid teliospores of *Puccinia monoica* collected from the grass *Koleria nitida* Nutt in September 1989. Teliospores germinate to form haploid basidiospores, the stage which causes infection. Infection causes abnormal growth (extremely elongated stems and smaller leaves), and the eventual formation of bright yellow rust spermatia (haploid sex cells) and aeciospores on the leaf surface (Roy 1993a).

Each inoculant was prepared from a single blade from a randomly chosen, infected *Koleria* individual from each of 3 sites (including the sites where the seeds were collected). The sites where spores were collected were separated by mountains and by distance (by at least 20 km as the crow flies). Although inoculum was prepared from a single blade of grass, it is likely that each spore suspension contained a mixture of pathogen genotypes. Nonetheless, each spore suspension had a distinct allozyme pattern. (Enzymes were assayed electrophoretically on 12% starch gels. Teliospores were ground with a glass rod either in distilled water (best) or a 1.0 molar phosphate buffer, the resultant solution was loaded onto a chromatography paper wick which was inserted into a starch gel. The following enzymes were resolved on gel and electrode buffer system one of Soltis et al. (1983): malate dehydrogenase [MDH], isocitrate dehydrogenase [IDH], 6-phosphogluconate dehydrogenase [6–PGD], acid phosphatase [AcPH], phosphoglucomutase [PGM]. The following enzymes were resolved on system eight minus of Rieseberg and Soltis (1987): triosephosphate isomerase [TPI], aldolase [ALD], phosphoglucoisomerase [PGI]. All enzyme stains followed Soltis et al. (1983)).

Inoculants were prepared by scraping teliospores off the grass blades with a razor blade and placing them in a microcentrifuge tube along with 1.0 ml of distilled water and 0.05 µl Triton × 100. The tube was shaken in a vortexer for 1 min, and any remaining clumps of spores were broken up with a glass rod. This suspension was then emptied into a small flask and the volume in the flask brought to 25 ml. An estimate of spore density in each suspension was obtained with a Fuchs-Rosenthall Ultra Plane Haemocytometer. Afterwards, all solutions were diluted to the same number of spores per ml as in the least dense suspension (ca. 1.1×10^6 spores per 25 ml of suspension) and incubated for 2 h at 18° C prior to inoculation to allow the spores to imbibe water and to begin germinating. For inoculation, 0.5 µl of spore suspension were placed directly on each plant. The inoculated plants were placed under plastic under the greenhouse bench for 72 h, then misted, and left under cover for another 24 h. Basidiospore formation from teliospores took between 24 and 48 h, and another 48 h were allowed for basidiospore germination and infection. Controls were treated exactly as above except the water dropped on the plants contained no spores.

After inoculation, plants were kept separated in treatment groups for two weeks to eliminate potential cross-infection during basidiospore formation. The fact that none of the controls became infected post-separation indicates that the initial segregation procedure was sufficient to prevent cross-infection. We did not worry about cross-infection after the basidiospore stage, because the next infectious spore state (aeciospores) can only infect the primary host, which was not present.

Infection and fruit production were monitored every time the plants were watered (every 2–3 days). The plants were individually scanned for infection and seed production; if they were infected a colored tag was inserted into their pot, and seed pods were collected as they matured. Every 4–6 weeks a complete census was done of all of the individuals in the greenhouse. At this time the tagged plants were recorded as being infected and all plants were checked for survival (alive or dead).

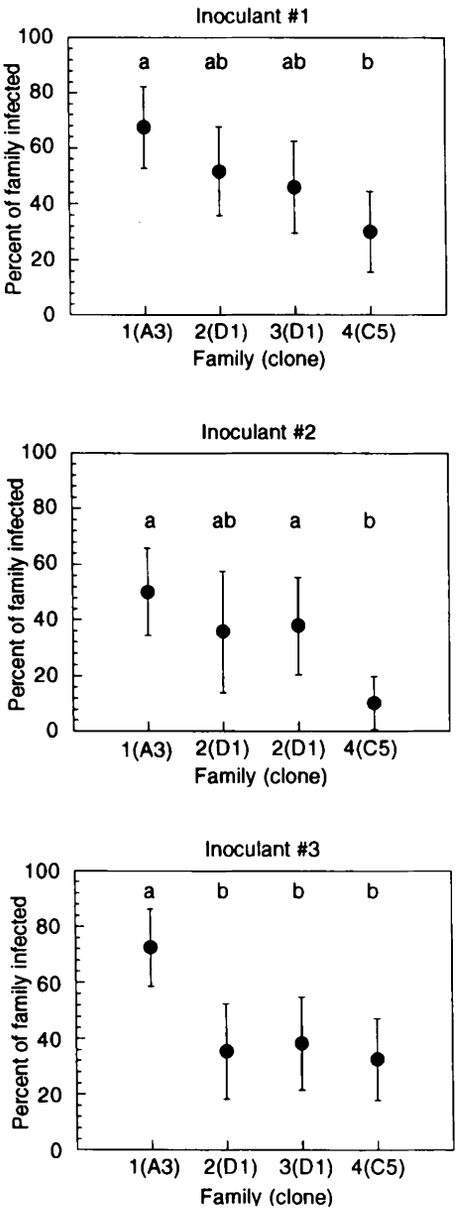

Fig. 1. Percent infection ±95% confidence intervals caused by 3 different inoculants of *Puccinia monoica* in 4 families of *Arabis holboellii*. Family 1 belongs to clone A3, families 2&3 belong to clone D1, and family 4 belongs to clone C5 (for a complete description of clones see Roy 1993). Letters at the top of the graph indicate significance of ≤ 0.0025 when they are different from each other

Resistance for each family was determined by calculating the percent of individuals infected under each of the 4 inoculation treatments. The significance of differences in percent infection among families (and clones) was assessed by comparing 95% confidence intervals; nonoverlapping confidence intervals are significant at $P \leq 0.0025$. Log-likelihood analysis was used to assess the importance of family, clone, and treatment on infection.

Effects of infection on survival and reproduction in the greenhouse

Survival was analyzed hierarchically using BMDP 1L and 2L (Dixon et al. 1988). For the first level of analysis we used stepwise logistic

regression to divide the variables that were likely to affect survival (family, treatment, infection, reproduction) and their interactions into groups of those that did affect survival and those that did not, then eliminated insignificant effects from subsequent analyses. In the second level of analysis we compared survival curves using a product-limit estimate of the survivor function (Dixon et al. 1988); comparisons were tested for significance with the Mantel-Cox statistic. The effects of treatment level (3 different rust inoculants and a control) on survival were tested in stratified analyses in which the data were grouped by a classification variable (infection class, family, reproduction class) into strata. Stratified analyses are similar to those using covariates except that in stratified analyses the test statistic is an overall statistic and the strata are categorical, whereas in covariate analysis of survival curves, statistics are produced separately for each covariate group and the covariate is continuous (Benedetti et al. 1988).

Reproduction (production of viable seeds) is likely to be influenced by interactions with variables other than infection, such as family, so we tested for the effects of these variables in all analyses. We first used logistic regression to determine which of the categorical variables (family, treatment, infection) and their interactions had the greatest effects on whether or not a plant reproduced. After determining whether there were differences between the reproductive and nonreproductive groups, we then asked whether there were differences in seed production due to treatment and infection among the plants that did reproduce. For this second set of analyses we were able to use analysis of variance (ANOVA) because once the nonreproductive plants were removed from the data set, the distribution of seed number and their residuals were approximately normal.

Differences among host clones in resistance: field experiment

Because resistance and the consequences of infection may be quite different under natural versus greenhouse conditions (Burdon and Müller 1987; de Nooij and van Damme 1988; Antonovics and Alexander 1989; Alexander 1992) we also observed infected and uninfected plants of *A. holboellii* in the wild. However, in the wild it was not possible to determine which pathogen genotype infected a particular host clone. Instead, we were limited to asking whether different host clones were differentially susceptible to the two rust species, which we could distinguish. In the field it was possible to classify infected plants as infected by *Puccinia monoica,* which has characteristic yellow aeciospores in the spring, or by *P. thlaspeos,* which has black teliospores that appear at the same time of year. If host clones do not differ in their resistance to the two different rust species, it is probably unlikely that they will respond differently to different genotypes of the same pathogen species. Of course, finding differential resistance to the two species is no guarantee of differential resistance to different pathogen genotypes of the same species.

Host specificity of *Puccinia monoica* and *P. thlaspeos,* and their effects on *Arabis holboellii* fitness, were monitored at 3 sites. Two of the three clones of *Arabis holboellii* chosen for the greenhouse experiment (A3 and D1) were common clones at these 3 field sites (for example, clone D1 made up approximately 85% of the population at Cement Creek, 6% at the Taylor River, and 11% at Gold Creek (Roy 1993b)); the third clone, C5, is only known from the Taylor River area. At each of these study sites an average of 300 randomly selected individuals of *A. holboellii* were genotyped (by electrophoresis at 7 polymorphic loci) and were monitored through the summer for infection (infected or "uninfected", and species of rust if infected), survival, and reproduction (number of fruits). The association of rust species with particular host clones was only calculated for Cement Creek because more plants were genotyped there, enabling statistical analysis of clonal infection by both rust species. Fitness consequences of infection by the different rust species were pooled over all clones and all sites. The population sampling procedures and electrophoresis methods are detailed in Roy 1993b which describes patterns of genetic diversity and disease incidence in 3 populations of *A. holboellii,* but does not address the fitness consequences of infection.

We used log-likelihood ratio tests to determine whether rust species were preferentially associated with particular host clones at Cement Creek. After performing an overall test of independence on the frequency counts, unplanned tests of homogeneity were used to compare pairs of clones for heterogeneity of infection (Sokal and Rohlf 1981). The effect of the different rust species on the survival and reproduction of the hosts was analyzed in multiway contingency tables using log-linear models (Sokal and Rohlf 1981).

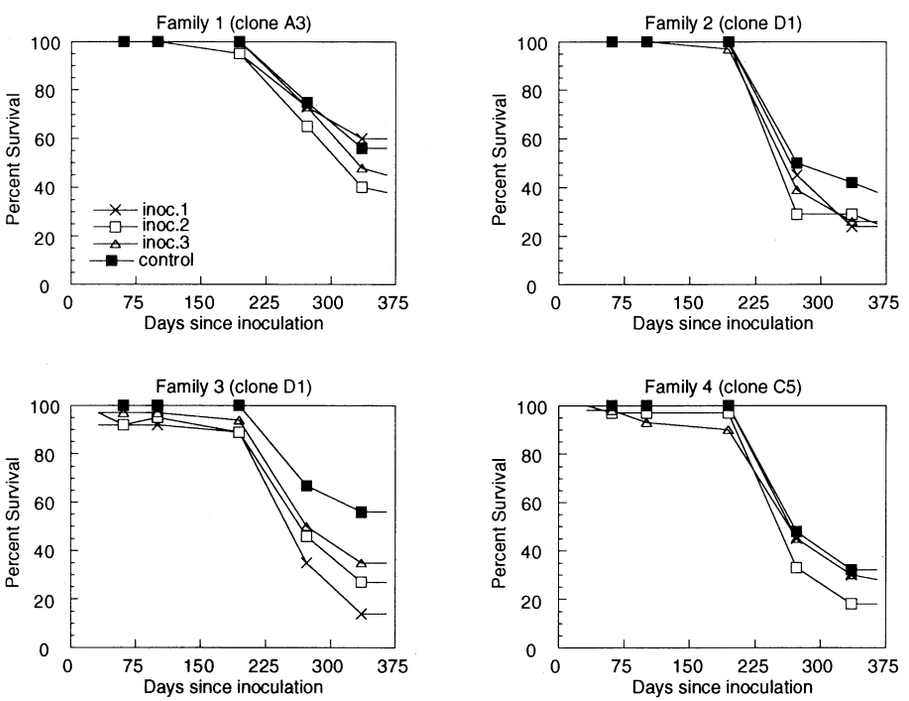

Fig. 2. *Arabis holboellii.* Family survival curves. Within inoculation treatments, families differ significantly in survival, Mantel-Cox, 4 d.f., = 14.24, $P = 0.0007$

Results

Broad-sense heritability of resistance: greenhouse experiment

If resistance is heritable (in the broad sense), and if there is variation in resistance, then there should be significant differences in infection among families, and this is in fact what we found. A log-likelihood analysis of the effects of family, inoculation treatment (excluding the controls), and their interactions on infection in *A. holboellii* showed that infection varied significantly among families and treatments (3 d.f., chisq. = 35.96, $P < 0.001$; 2 d.f., chisq = 8.77, $P = 0.01$, respectively). There was no significant interaction between family and inoculation treatment (4 d.f., chisq. = 5.54, $P = 0.47$). Across all treatments, family 1 (clone A3) was the most susceptible, family 4 (clone C5) was the most resistant, and families 2 and 3 (both clone D1) were intermediate in resistance between the other two families (Fig. 1).

The results are the same if the two families that represent the same clone are combined, and the analysis is re-done for clones instead of family. Clone and inoculation treatment were significant effects (2 d.f., chisq. = 35.97, $P < 0.001$; 2 d.f., chisq = 10.28, $P = 0.006$, respectively), but there was no significant interaction between clone and inoculation treatment (4 d.f., chisq. = 5.24, $P = 0.26$, see Fig. 1).

To test whether differences in resistance might be due to maternal effects, we compared infection in the two families (i.e. from two distinct physiological mothers) representing the same genetic clone (Fig. 1, families 2&3, clone D1); these two families did not differ from one another in resistance, as they might have if differences were maternal.

Effects of infection on survival and reproduction in the greenhouse

For analysis, inoculated plants are divided into two classes, those that became obviously infected and those that did not, the "uninfected" plants. Plants in the "uninfected" class did not have obvious signs of infection, but could have had "cryptic" infection; thus "uninfected" plants may differ from the controls.

Infection affected plant survival. Survival curves differed significantly among inoculation treatments (Mantel-Cox statistic, 4 d.f. = 2.91, $P = 0.012$), among families

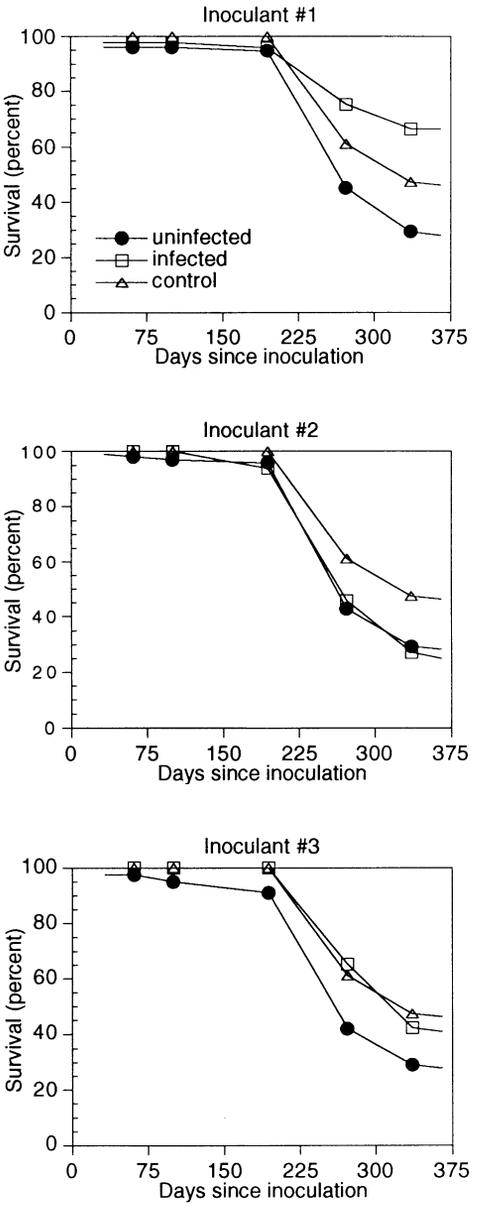

Fig. 3. *Arabis holboellii.* Survival curves of infected and "uninfected" plants (all families combined) for all rust inoculation treatments; each graph includes the combined curve for the controls. Overall test of infected versus "uninfected" survival curves is significant (Mantel-$Cox_4 = 15.65$, $P = 0.004$)

Table 2. Mean survival time (days ± SE) of "uninfected" versus infected plants of *A. holboellii* within treatments. Individual comparisons of mean survival times used Wilcoxon rank sum test because survival time was not normally distributed

Treatment	*N*	"uninfected"	Infected	Z	$P > \|Z\|$
Inoc.# 1	146	299.0 ± 8.1	316.0 ± 5.6	1.24	0.215
Inoc.# 2	144	302.0 ± 6.3	305.0 ± 7.3	0.02	0.985
Inoc.# 3	145	295.0 ± 8.1	326.0 ± 5.1	2.79	0.052
Control	110	327.0 ± 4.1	---	---	---

Table 3. Summary of reproduction and survival in controls versus inoculated plants (both infected and "uninfected"). Different letters following numbers within a column indicate significant (≤ 0.05) differences between variables; all tests of differences between means calculated with Wilcoxon rank sum tests

	% that set seed	X days to first seed	X days survived
Controls	78.2 ± 0.04^a	176.2 ± 2.99^a	327.0 ± 4.10^a
Inoculated, "uninfected"	71.4 ± 0.03^b	164.0 ± 1.77^b	297.5 ± 9.28^b
Inoculated, infected	38.2 ± 0.06^c	162.6 ± 2.38^b	315.3 ± 9.55^b

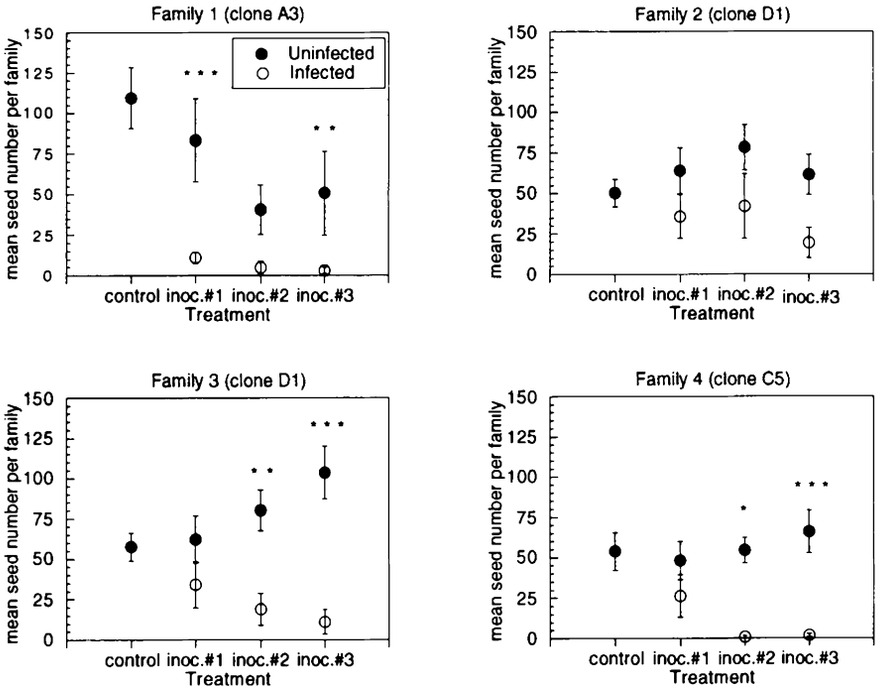

Fig. 4. *Arabis holboellii*. Mean seed number per family ± SE, by inoculation treatment and infection class ("uninfected" or infected). Significance levels for differences between mean seed number of infected and "uninfected" classes calculated by Wilcoxon rank sum test: $*P \leq 0.05$, $**P \leq 0.01$, $***P \leq 0.001$

within inoculation treatments (Mantel-Cox statistic, 4 d.f. = 14.24, $P = 0.007$, Fig. 2), and between infection classes within treatments (Mantel-Cox statistic, 4 d.f. = 15.65, $P = 0.004$, Fig. 3). However, infected plants tended to live longer than "uninfected" plants (Tables 2 and 3, Fig. 3), a result we did not expect.

Not only did the survival curves and mean survival times differ, but so did the timing of death in control plants versus inoculated plants (both infected and "uninfected"), with most mortality in infected plants occurring before the plants had reproduced, and most mortality in the controls and "uninfected" treatment plants occurring after reproduction. *Arabis holboellii* plants tend to die after flowering both in the greenhouse, and under natural conditions (Roy 1992). For this reason we expected the category of plants with the highest reproduction to have the shortest survival time. This was not the case here (Table 3). The controls reproduced the most, but also survived the longest. However, although control plants reproduced more than treated ones (Table 3), they also flowered about 9% later than any of the treatment plants (infected or "uninfected"); thus, it is possible that the mortality usually associated with flowering was delayed in the controls. Survival dropped in all treatments between days 200 and 265 (Fig. 2, Table 3). This drop was caused by post-flowering mortality and perhaps also by the high summer temperatures in the greenhouse.

Infection was the single most important factor in determining whether or not a plant set seed. In general, infected plants within an inoculation treatment produced far fewer seeds than their "uninfected" counterparts, and "uninfected" plants within an inoculation treatment usually produced as many seeds as the controls. Family was the second most important factor, as shown by stepwise logistic regression using presence or absence of seed production as the dependent variable, and family, treatment and infection as independent variables. The chi-square for infection was 66.41 ($P < 0.001$), and the chi-square for family was 20.29 ($P < 0.001$). Inoculant 3 caused the greatest reduction in seed number and inoculant 1 the least (Fig. 4). The reproductive outputs of the two families from the same clone (2&3, clone D1) were more similar to each other than either was to the other families (Fig. 4).

Differences among host clones in resistance: field experiment

There is a strong association between clones of *A. holboellii* and the species of rust infecting them at Cement Creek. The 3 clones differed significantly (log-likelihood ratio test, $G_4 = 221.86$, $P < 0.001$) in their susceptibility to the two rust species (Fig. 5). Clones B4 and F2 were more often infected by *P. thlaspeos* (and were not statistically different from each other by an unplanned homogeneity test), whereas clone D1 more often had *P. monoica*. In fact, clone D1 appeared to be resistant to the *P. thlaspeos* genotypes (races) present at this site, since only 2 out of 153 infected individuals (well within error of clone identification) had *P. thlaspeos* present and, unlike the other clones, there were no plants of clone D1 with both rust species present.

The presence of both species of rusts on some plants (Fig. 5) is evidence that multiple spore infections occur with some frequency in nature. Interestingly, there may be a tendency for infection by both species of rust to occur more often on clones B4 and F2 than infection by *P. monoica* alone (Fig. 5).

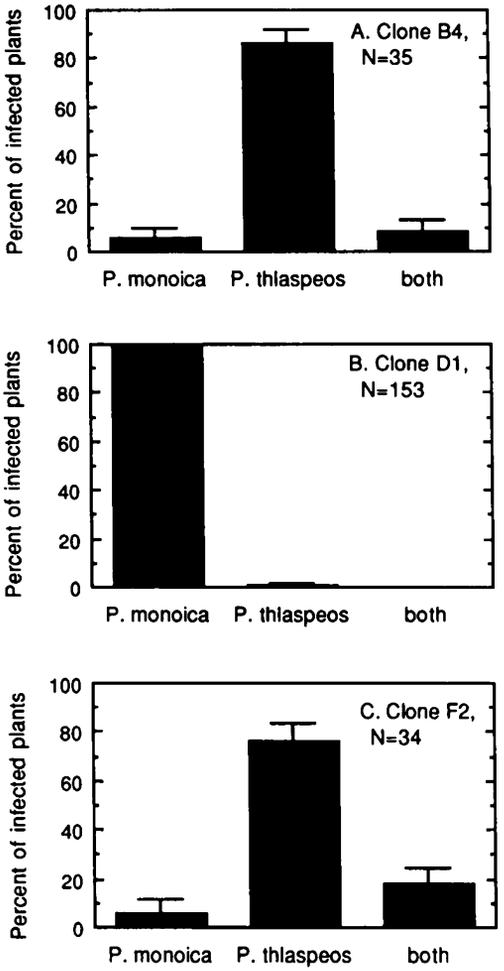

Fig. 5A–C. Association of rust species with clones at Cement Creek (percent of infected sample of each clone that is infected by one or both rusts ± standard error of the proportion). **A** Clone B4. **B** Clone D1. **C** Clone F2. The clones differ significantly in their susceptibility to the two rusts (log-likelihood ratio test, $G_4 = 221.86$, $P \ll 0.001$)

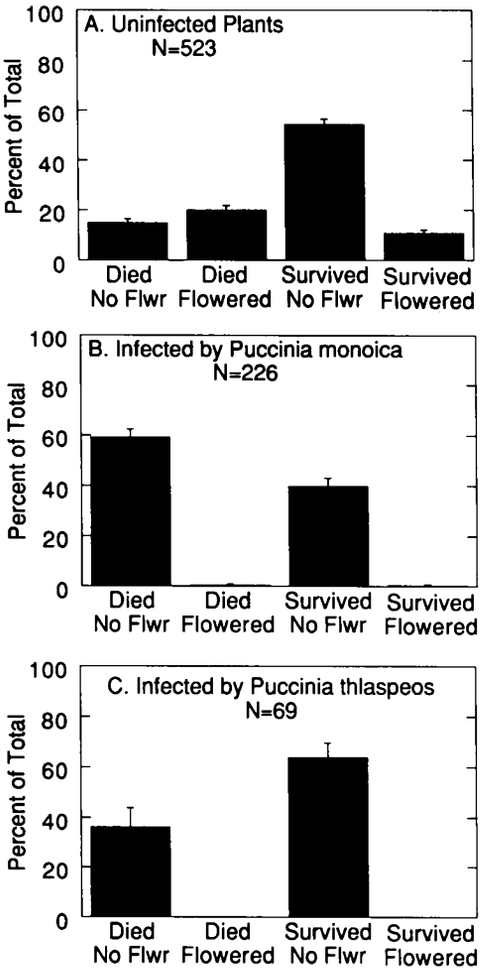

Fig. 6A–C. Relationship between infection and fitness in *Arabis holboellii*, over all host clones and all sites. **A** Uninfected plants, $N = 523$. **B** Plants infected by *Puccinia monoica*, $N = 226$. **C** Plants infected by *P. thlaspeos*, $N = 69$. Survival and reproduction depend on the species of rust present (log-likelihood ratio test, $G_1 = 11.86$, $P = 0.001$)

Effects of infection on host survival and reproduction in the wild

The pathogens strongly decrease host fitness under natural conditions. The relationship between infection (by each rust species) and reproduction/survival, summed over all clones and sites, is shown in Fig. 6. Uninfected plants flowered 31% of the time versus 0.4% of those infected by *P. monoica*, and 0% of those infected with *P. thlaspeos*. Survival was relatively high for uninfected plants (65%) and for those infected by *P. thlaspeos* (64%), but was only about 40% for those infected with *P. monoica*. The survival and reproduction of individual infected plants depended on the species of rust present (log-likelihood ratio test, $G_6 = 236.4$, $P < 0.001$), with most of the difference being in survival; those plants infected by *Puccinia thlaspeos* lived longer than those infected by *P. monoica* (log-likelihood ratio test, $G_1 = 11.86$, $P = 0.001$).

For uninfected plants, survival and reproduction were not independent of clone genotype (log-likelihood ratio test, $G_{12} = 27.25$, $P = 0.007$). On the other hand, the fate of infected plants was independent of clone genotype (log-likelihood ratio test, $G_{12} = 6.85$, $P = 0.867$); all clones experienced similar low fitness when infected by the same rust species.

Discussion

In this examination of the potential for pathogen-mediated selection to cause host evolution, we have discovered that several of the necessary preconditions exist. We have established that *Arabis holboellii* clones vary in their resistance to *Puccinia monoica*, both in the field and in the greenhouse, and therefore that resistance is a heritable trait. We have also found that infection decreases plant fitness by depressing reproduction.

Infection's effects on survival differed between the greenhouse and the field. Under natural conditions, 40% of the infected plants died by the end of the summer (after 1 year about 70% of these plants were dead), but in the greenhouse, infected plants lived as long or longer than

uninfected plants did. We also observed other differences between the 2 conditions: under natural conditions, no more that 1% of infected plants set seed, whereas 38% did so in the greenhouse. In nature *P. monoica* produces sexual structures (spermatia) or fruiting structures (aecia) on the host's leaves, whereas in the greenhouse these structures were rare on leaves, and occurred primarily on flowers, which infected plants in nature rarely produced.

In general, the effects of infection were less severe in the greenhouse than in the field. This was not unexpected, since plants in the field are often under severe water and perhaps nutrient stress. An additional explanation for the different behaviors of field and greenhouse plants may lie with another aspect of the greenhouse environment. In the wild, inoculation occurs in early fall when hosts are no longer actively growing, and infection progresses over the winter. Plants emerge infected from melting snow in the spring. Our inoculations were performed during the coolest part of the year, January, in an unheated greenhouse, but plants were able to grow continuously afterwards, perhaps depriving the fungus of the early advantage it experiences in the fall in the field.

These conditions – heritability of resistance and negative effects of infection on fitness – are sufficient to drive the evolution of resistance in the host population. But can they explain the high levels of host genetic diversity observed in these populations (Roy 1993b)? One mechanism often suggested for the maintenance of genetic diversity is frequency-dependent selection. In theoretical treatments, the ability of frequency-dependent selection to maintain genetic variation in populations depends on a very tight genetic correspondence between the host and the pathogen, such that each host resistance allele is matched by a virulence allele in the pathogen (Clarke 1976; Jaenike 1978; Hamilton 1980; Barrett 1988), a condition often referred to as race-specific resistance. If resistance to infection is largely or entirely race-nonspecific, then the probability that frequency-dependent selection by pathogens can maintain host genetic diversity through selection on resistance alleles is decreased. This is because frequency-dependent selection depends on rare host genotypes being able to escape infection; there can be no escape in rarity if the pathogen population can be supported by more than one host genotype, because the quantity of inoculum will never decrease (May and Anderson 1983; Parker 1992). None of the *A. holboellii* clones were infection free, and all clones were at least partially susceptible to all inoculants. Thus it is unlikely that frequency-dependent selection will occur, or if it does, it is likely to be weak.

However, the conclusion that resistance to *P. monoica* is race-nonspecific must be a tentative one. In this greenhouse experiment, there was a high probability that each plant was inoculated with a mixture of pathogen genotypes rather than with a single one. This is because the only infectious stage of this fungus on *Arabis* is the haploid basidiospores that germinate from sexually-derived teliospores; the binucleate and diploid stages of the fungus do not infect this host. Because each plant was inoculated with large number of haploid spores (ca. 88), it is likely that each experimental plant was exposed to a similar array of pathogen genotypes. Nonetheless, these results are interesting because simultaneous infection by multiple genotypes, as was performed in this study, is exactly what takes place naturally in the field (Van der Plank 1975).

Frequency-dependent selection models generally assume that each host can be infected by only a single pathogen genotype at a time (Parker 1992). For *A. holboellii* and its relationship with *P. monoica,* this assumption is clearly incorrect. Indeed, we have shown that not only can host individuals support more more than one pathogen genotype, they not infrequently support more than one pathogen species in the wild. It is not clear how the interaction of different pathogen species or genotypes in this system may alter host response and selection, and we are unaware of any empirical studies on the consequences of multiple infections.

Because of *Arabis'* probable non-specific resistance, and the occurrence of infection by multiple genotypes, the potential for frequency-dependent selection in this system appears to be low. An additional consideration is the length of the pathogen's generation time. For both of these rust species, there is only one generation per year on *Arabis,* rather than the much higher numbers typical of some other plant and animal parasites. This long pathogen generation time means that the pathogen population will be relatively slow to respond to changes in the host's population genetic structure, and further decreases the probability of frequency-dependent selection (May and Anderson 1983).

If resistance in *Arabis holboellii* is indeed race-nonspecific, then it conforms to a pattern observed in some other studies of alternate hosts. For both barley rust (Segal et al. 1980; Anikster and Wahl 1979) and wheat rust (Green 1971), resistance is less race-specific in the alternate host than in the primary host. One mechanism that has been suggested to explain the differences in resistance between alternate and primary hosts is that the spore stage that causes infection of the alternate hosts is typically monokaryotic (uninucleate) and may be less specialized than its binucleate counterparts (Green 1971; Anikster and Wahl 1979). This does not appear to be an adequate explanation, however, because both of the rusts that infect *A. holboellii* cause infection with uninucleate spores (basidiospores), yet resistance to the two rusts appear to differ.

If conditions are inappropriate for the action of frequency-dependent selection by pathogens, what then might be the factors maintaining genetic diversity in *Arabis* populations? Work on natural populations of *A. holboellii* suggests several alternatives: 1) diversity might be maintained by continual production of new clones (Roy and Rieseberg 1989; Roy 1993b), 2) there may be a cost of resistance, or 3) some other frequency-dependent factor, such as herbivory, may be a more significant selective force (Roy 1992; Roy unpublished data).

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