Coevolution of host and pathogen populations in the *Hordeum vulgare*–*Rhynchosporium secalis* pathosystem

(virulence/resistance/selection)

B. A. McDONALD*[†], J. M. McDERMOTT*, R. W. ALLARD*, AND R. K. WEBSTER[‡]

Departments of *Genetics and [‡]Plant Pathology, University of California, Davis, CA 95616

Contributed by R. W. Allard, February 24, 1989

ABSTRACT Isolates of *Rhynchosporium secalis* collected from two experimental barley populations were scored for putative isozyme, colony color, and virulence loci. Allelic frequencies, multilocus haplotype frequencies, and multilocus genetic structure differed in the two populations of *R. secalis*; haplotypes also differed widely from each other in virulence. The average virulence of isolates collected from the more resistant host population was greater than the average virulence of the isolates collected from the less resistant host population; also the least virulent haplotype, which made up 19% of the pathogen population collected from the less resistant host population, accounted for only 0.3% of the isolates collected from the more resistant host population. It was concluded that the genetic systems of the barley host and fungal pathogen interacted in a complementary fashion and that the genetic structures of both the host and pathogen populations were shaped by coevolutionary processes featuring interactions among loci affecting many different traits, including interactions among host resistance genes and pathogen virulence genes.

It is now widely accepted that the coevolution of plants and their pathogens can be understood only in the context of integrated host–pathogen systems (e.g., see refs. 1 and 2). However, empirical studies of host–pathogen systems have usually focused on the host; this is partly because the host is the partner of economic importance in the pathosystem and partly because plant pathogens have often been perceived as less tractable to genetic analysis than their hosts. *Rhynchosporium secalis* (Oud.) Davis, the causal organism of the scald disease of barley (*Hordeum vulgare* L.), is a haploid imperfect fungus, which is transmitted from generation to generation of the barley host through seed-borne mycelial inoculum; however, within single growing seasons it spreads from plant to plant by short-distance rain-splashed dispersal of conidia (3). It is likely that occasional migrations of *R. secalis* take place between barley populations (i) as a result of contamination of seeds by mycelium during harvesting, cleaning, or planting operations; (ii) as a result of longer-distance dispersal of conidia by windswirling during rainstorms; or (iii) from barley crop residues or infected volunteer plants in planting areas. Barley composite cross II (CCII) and composite cross V (CCV) are closed experimental populations, which have been grown at Davis, California, since 1929 and 1941, respectively, in isolated plots that had not been seeded to barley for a number of years. Thus, it is likely that the populations of *R. secalis* that occur on CCII and CCV are, like their host populations, also tightly closed and that they have coevolved in intimate association with CCII and CCV. *R. secalis* populations in California are highly variable for virulence as defined on a set of 14 barley differentials (4).

The synthesis and evolutionary histories of CCII and CCV have been described in detail (5, 6) and both populations are known to be polymorphic at many loci, including loci that govern resistance vs. susceptibility to *R. secalis*. There have also been a number of studies of the evolutionary responses of CCII and CCV to *R. secalis* (7–11) but much less information is available concerning the genetic structure of the populations of *R. secalis* that have evolved on CCII and CCV (12). In this paper, we report the results of a study in which isozyme, colony color, and virulence variants were used to investigate the genetic composition and population structure of samples of *R. secalis* collected from generation 56 of CCII and generation 44 of CCV during the 1985–1986 growing season.

MATERIALS AND METHODS

Field Plantings. CCII and CCV were seeded in a pair of plots (10 × 10 m), separated by a 4-m alley, in a field that had not been seeded to barley for several years. For purposes of collecting fungal isolates, the two plots were each divided into 16 subplots measuring 1.25 m (width) × 5.0 m (length). Rainfall was above average (692 mm; 42% higher than the normal of 492 mm) in 1985–1986, providing excellent conditions for development of scald from seed-borne inoculum; conditions were particularly favorable for spread of the scald disease within the main plots during nine rainy periods, each ranging from 1 to 3 or 4 days and distributed throughout the growing season.

Collection of Leaf Tissue and Generation of Isolates. The populations of *R. secalis* isolated from CCII and CCV were designated RSII and RSV, respectively. In an attempt to obtain representative samples of *R. secalis* from both CCII and CCV, 20 or more scald-infected leaves were collected from plants distributed throughout each of the 32 subplots. This collection strategy allowed us to test for differences in the distribution of genetic variation within subplots, among subplots, and between RSII and RSV. Leaves from the different subplots were placed in paper envelopes and allowed to dry at room temperature (21°C) for 8 weeks. The dried leaves were then wet for 10 sec in 70% ethanol, surface sterilized for 90 sec in a 0.5% sodium hypochlorite solution, pressed dry between paper towels, and placed on a plastic screen that rested on rubber bands above damp filter paper in a Petri dish. The Petri dishes were placed in an incubator (15°C) for 72 hr to induce sporulation, at which time isolates were collected from distinct lesions that had produced macroscopically visible mycelium on their borders. Small pieces of mycelium were transferred to potato dextrose agar (PDA)

Abbreviations: CCII, barley composite cross II; CCV, barley composite cross V; PDA, potato dextrose agar; PGI, phosphoglucosomerase; PGM, phosphoglucomutase; LAP, leucine aminopeptidase; BGLU, β -glucosidase; COL, color.

[†]Present address: Department of Plant Pathology and Microbiology, Texas A&M University, College Station, TX 77843.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Petri dishes with a sterile needle. An average of three clonal isolates per leaf were transferred to PDA culture tubes, which were incubated at 15°C for 6 weeks and then transferred to a 4°C cold room for storage until they were analyzed. A total of 1331 isolates were collected, 677 from RSII and 654 from RSV.

Data Collection. Isolates were scored for three different types of characters: colony color, isozymes, and virulence vs. avirulence (12). Colonies of the isolates were either black or cream-colored after 6 weeks of growth in PDA culture tubes. Isolates were assayed on starch gels for four isozyme systems following methods described by McDermott *et al.* (12): phosphoglucose isomerase (PGI; glucose-6-phosphate isomerase; D-glucose-6-phosphate ketol-isomerase, EC 5.3.1.9), phosphoglucomutase (PGM; α -D-glucose 1,6-phosphomutase, EC 5.4.2.2), leucine aminopeptidase [LAP; cytosol aminopeptidase; α -aminoacyl-peptide hydrolase (cytosol), EC 3.4.11.1], and β -glucosidase (BGLU; β -D-glucoside glucosylhydrolase, EC 3.2.1.21). Each enzyme system was dimorphic for fast vs. slow variants. Both the color (COL) and the isozyme variants were transmitted stably through repeated transfers to fresh PDA medium. The black vs. cream-colored and the fast vs. slow isozyme variants were designated 1 and 2, respectively. These numbers were combined in the order PGI, PGM, LAP, BGLU, and COL in designating multilocus phenotypes; thus, for example, phenotype 21211 is slow for PGI, fast for PGM, slow for LAP, fast for BGLU, and black colored. These five putative loci, each with two alternative allelic states, potentially identify $2^5 = 32$ phenotypes (haploid genotypes), among which 14 were observed. Such multilocus phenotypes (genotypes) will hereafter be referred to as haplotypes. Because *R. secalis* reproduces asexually in nature (12), the array of haplotypes can be considered to be equivalent to an allelic series within which mutation (including somatic recombination) is expected to produce new haplotypes over time. Color and isozyme data were recorded for all 1331 isolates. Virulence vs. avirulence was determined for 79 clonal isolates on a set of 14 barley differentials (4), 40 from RSII and 39 from RSV; these isolates were selected to include 2 or more representatives of each of the 9 most frequent among the 14 different haplotypes that were observed.

RESULTS AND DISCUSSION

The design of the experiment permits comparisons of the genetic structure of populations RSII and RSV for the following features.

Variant Frequencies. Overall frequencies of variant 1 for each of the five loci (PGI, PGM, LAP, BGLU, COL) in the total sample (RSII and RSV combined) were 0.680, 0.789, 0.296, 0.793, and 0.787, respectively. The fast variants of PGI, PGM, BGLU, the black color variant, and the slow variant of LAP were much more frequent than their alternative variants in both RSII and RSV. Variant frequencies were, however, not the same in the two populations and, even though interpopulational differences were small, they were statistically significant for all variants except PGM (Table 1). Thus, the population genotypes of CCII and CCV had differing effects on the ability of four of the five alternative variants to infect the two host populations.

Haplotype Frequencies. Fourteen among the 32 possible haplotypes were observed, 13 in RSII and 12 in RSV. The six most frequent haplotypes were 21211 (0.30) and 11211 (0.28), followed by 11212 (0.10), 12122 (0.09), 12121 (0.04), and 12111 (0.04); the eight remaining haplotypes (summed frequency = 0.15) were individually infrequent or rare. Haplotype frequencies differed significantly [overall $\chi^2_{(10)} = 299.7$; $P < 0.0001$] in the two populations. The frequencies of eight of the haplotypes were found to differ significantly when single degrees of freedom were isolated (Table 2). One

Table 1. Numbers (*n*) and frequencies (*F*) of the fast isozyme and black variants in populations RSII and RSV and probabilities (*P*) that frequency differences between the two populations are different

Locus	RSII		RSV		<i>P</i>
	<i>n</i>	<i>F</i>	<i>n</i>	<i>F</i>	
PGI	427	0.63	475	0.73	<0.001
PGM	520	0.77	530	0.81	0.239*
LAP	239	0.35	129	0.20	<0.001
BGLU	493	0.73	557	0.85	<0.001
COL	609	0.90	469	0.72	<0.001

*Not significant.

haplotype, 11121, was in high frequency in RSII but in low frequency in RSV, whereas the opposite was the case for haplotype 11212. Two haplotypes observed in low frequency in RSII (12212, 12112) and one observed in low frequency in RSV (22211) were private; nonappearance of these rare haplotypes in our sample from the opposite population may have been due to (i) local founder effects during the period of initial infection from seed-borne inoculum, (ii) subsequent sampling accidents (genetic drift), or (iii) selection against some haplotypes.

Multilocus Associations. Multilocus associations among the five markers were assessed using the discrete log-linear procedure of Fienberg (13). Likelihood ratio tests were used in a series to identify and eliminate nonsignificant interaction terms after which log-linear models were constructed to fit the remaining terms. Models were fit in a hierarchical manner such that a higher-order term was included only when lower-order terms failed to fit the data; when a higher-order term was included, all of its lower-order relatives were also included. A stepwise procedure was used in model selection (14). The best fitting model for RSII was characterized by the two highest-order interaction terms (PGI-PGM-BGLU) (PGM-LAP-BGLU-COL), whereas the best fitting model for RSV was characterized by the two highest-order interaction terms (PGI-PGM-LAP) (PGI-LAP-COL). The different form of the model in the two populations indicates that the multilocus structure of RSII was different from that of RSV—i.e., that the allelic combinations that were most frequent in RSII were not the same as the allelic combinations that were most frequent in RSV.

Table 2. Numbers (*n*) and frequencies (*F*) of haplotypes in populations RSII and RSV and probabilities (*P*) that frequency differences between the populations are different

Haplotype	RSII		RSV		<i>P</i>
	<i>n</i>	<i>F</i>	<i>n</i>	<i>F</i>	
11111	5	0.01	17	0.03	<0.01
11121	104	0.15	5	0.01	<0.0001
11211	159	0.26	217	0.33	<0.001
11212	2	<0.01	127	0.19	<0.0001
12111	43	0.06	9	0.01	<0.001
12112*	6	0.01	0	0	NS
12121	23	0.03	36	0.06	NS
12122	57	0.08	57	0.09	NS
12211	26	0.04	7	0.01	<0.01
12212*	2	<0.01	0	0	NS
21111*	1	<0.01	5	0.01	NS
21211	248	0.37	157	0.24	<0.001
21212*	1	<0.01	2	<0.01	NS
22211	0	0	16	0.02	<0.001
Total	677		655		

NS, not significant.

*The four haplotypes for which expected numbers were <5 were combined into a single class in tests of significance.

Spatial Distribution of Haplotypes. Because random lots of seed from generation 55 of CCII and generation 43 of CCV were used to sow the 10×10 m plots of CCII and CCV, respectively, the distribution of haplotypes is expected to be uniform within and among the 1.25×5.0 m subplots of the main plots, assuming no disturbing forces intervened after sowing and prior to sampling. Observed distributions of haplotypes within the CCII plot and the CCV plot are given in Fig. 1, in which subplots are shown as they were arranged in the field and data are presented as frequencies of haplotypes within each 1.25×5.0 m subplot. There were five to seven haplotypes in most subplots (range, 3–10; mean, 6.03); most subplots included all six among the most common haplotypes. However, one or two haplotypes predominated in most subplots and the predominant haplotype often differed from subplot to subplot; furthermore, the predominant haplotype was often the same in adjacent subplots. This pattern of variation suggests that specific haplotypes became established on single barley plants during the period of initial infection from seed-borne inoculum and that these founder events led to foci of infection from which haplotypes spread by rain splash both within subplots and into neighboring subplots. Thus, by season's end, haplotype variability came to be distributed in a nonrandom fashion in a fine-scaled mosaic pattern within and among subplots.

Population structure was analyzed by F statistics (15–17); for these analyses, data from adjacent subplots were pooled forming eight subplots measuring 2.5×5.0 m (mean sample size per subplot was 83 isolates). Partitions of the total genetic diversity (H_T) into components due to average diversity within subplots (H_S), average diversity between all subplots within populations (D_{SP}), and average diversity between the two populations (D_{PT}), gave H_T (0.781) = H_S (0.669) + D_{SP} (0.095) + D_{PT} (0.017). Thus, most of the genetic variability is attributable to diversity within subplots (H_S/H_T

= 0.86) and most of the rest of the diversity is attributable to differences among subplots within populations ($D_{SP}/H_T = F_{SP(T)} = 0.12$; $\chi^2 = 2NF_{SP(T)} = 319$; $P < 0.001$). We postulate that the observed differences in genetic variability within subplots resulted from founder events followed by rain-splash spread by secondary infections from the resulting foci of infection and that the observed differences among subplots resulted from subsequent spreading into neighboring subplots. The proportion of between-population diversity relative to total diversity, D_{PT}/H_T , was 0.022 = F_{PT} , which is equivalent to Wright's F_{ST} under random mating. Although genetic differentiation between the two populations accounted for only 2% of the total diversity, the difference between the two populations is statistically significant ($\chi^2 = 2NF_{ST} = 58$; $P < 0.001$). The results of the genetic diversity analyses thus provide numerical support for the conclusions reached from visual inspection of Fig. 1—namely, that genetic variability is much more extensive within than between RSII and RSV. This result parallels the pattern of geographical distribution of resistance to *R. secalis* observed in a random sample of 350 accessions of barley from a worldwide collection (18); diversity for resistance within the 350 accessions was larger (53% of total diversity) than diversity among the accessions. This result suggests that the optimum evolutionary strategy may be development, within local populations, of complementary patterns of genetic variation for resistance in the host and virulence in the pathogen. Robinson (2) reached a similar conclusion from theoretical arguments.

Virulence of Haplotypes. The virulence of the nine most frequent haplotypes defined by the enzyme and color variants was determined by testing the ability of each haplotype to infect a set of 14 barley differentials (4) and a number of barley lines known to carry specific alleles for resistance (11). The nine haplotypes fell into seven classes on the basis of the

FIG. 1. Frequency of haplotypes collected in subplots of the plots of CCII (Left) and CCV (Right). Plots and subplots are shown as they were arranged in the field. Data are presented as the frequency of each haplotype within each subplot.

Table 3. Numbers (*n*) of each haplotype tested against the set of 14 barley differentials and the mean number of differentials infected by each haplotype

Haplotype	<i>n</i>	Mean
11212	9	3.22 (a)
12211	5	5.40 (ab)
12121	5	6.00 (abc)
11111	5	7.00 (bd)
11211	13	7.23 (bd)
21211	20	8.10 (bd)
11121	6	10.00 (de)
12122	14	10.93 (e)
12111	2	11.00 (cde)

Means followed by the same letter are not significantly different (Fisher's protected least significant difference method; ref. 19).

average number of differentials they were able to infect (Table 3); the members of the least virulent class infected 3.22 differentials and the members of the most virulent class infected 11.00 differentials, on the average. This result establishes that the haplotypes differed widely in virulence and also that statistically significant associations exist between haplotypes and virulence. The virulence data also show that the isolates from CCII, the more resistant population (7–10), infected a significantly larger number of differentials on the average (mean, 8.95) than the isolates from CCV (mean, 6.44). This relationship also held when the mean virulence of each isolate was weighted by its frequency (mean, 8.60 for CCII and 6.10 for CCV). The least virulent haplotype (11212), which made up 19% of the isolates in RSV, accounted for only 0.3% of the isolates in RSII. Several earlier experiments (7–10) have shown that the frequency of individuals resistant to various races of *R. secalis* had become much higher in the later generations of CCII than in the later generations of CCV. Furthermore, lines resistant to multiple races were much more frequent in CCII than in CCV (8, 20)—e.g., 36% and 3%, respectively, of lines from CCII and CCV were triply resistant to races 40, 61, and 74. In the present study, >10% of isolates collected from CCII were able to infect multiply resistant tester lines, whereas none of the isolates from RSV was virulent on such lines.

The above results indicate that CCII and CCV exerted very different selective forces on the variable natural inoculum to which they have been exposed over a period of 56 and 44 host generations, respectively. Associations within haplotypes between virulence and the other genetic variants are especially important because they show that selection operating on virulence affects all other variants and, hence, all aspects of the genome. This is especially the case when, as in *R.*

secalis, asexual reproduction (12) drastically limits opportunity for recombination to break up existing associations between virulence genes and other genes. The other side of the coin is that selection due to variants with unknown functions and unknown roles in adaptation must also have had correlated effects on the frequency of virulence genes in RSII and RSV, which, in turn, must affect allelic frequencies of genes governing resistance vs. susceptibility in CCII and CCV. The picture that emerges is that the genetic systems of *H. vulgare* and *R. secalis* are complementary: interactions among loci affecting many different traits, including interactions among host resistance and pathogen virulence genes, have correlated effects on the genetic structure of the host as well as that of the pathogen population.

This research was supported in part by grants from the U.S. Public Health Service (National Institutes of Health Grant GM-32429), the National Science Foundation (BSR83110869), and the Science and Education Administration, U.S. Department of Agriculture (79-59-2063-1-204-1).

1. Flor, H. H. (1955) *Phytopathology* **45**, 680–685.
2. Robinson, R. A. (1976) *Plant Pathosystems* (Springer, New York).
3. Shipton, W. A., Boyd, W. J. R. & Ali, S. M. (1974) *Rev. Plant Pathol.* **53**, 839–861.
4. Jackson, L. F. & Webster, R. K. (1976) *Phytopathology* **66**, 719–725.
5. Allard, R. W. & Kahler, A. L. (1972) in *Proceedings of the Sixth Berkeley Symposium on Mathematical Statistics and Probability*, eds. LeCam, L. M., Neyman, J. & Scott, E. L. (Univ. of California Press, Berkeley), Vol. 5, pp. 237–254.
6. Allard, R. W. (1988) *J. Hered.* **79**, 225–238.
7. Jackson, L. F., Kahler, A. L., Webster, R. K. & Allard, R. W. (1978) *Phytopathology* **68**, 645–650.
8. Muona, O., Allard, R. W. & Webster, R. K. (1982) *Theor. Appl. Genet.* **1**, 209–214.
9. Jackson, L. F., Kahler, A. L., Webster, R. K. & Allard, R. W. (1978) *Phytopathology* **68**, 645–650.
10. Saghai-Maroo, M. A., Webster, R. K. & Allard, R. W. (1983) *Theor. Appl. Genet.* **66**, 279–283.
11. McDonald, B. A., Allard, R. W. & Webster, R. K. (1988) *Crop Sci.* **28**, 447–452.
12. McDermott, J. M., McDonald, B. A., Allard, R. W. & Webster, R. K. (1989) *Genetics* **121**, in press.
13. Fienberg, S. E. (1977) *The Analysis of Cross-Classified Categorical Data* (MIT Press, Cambridge, MA).
14. Goodman, L. A. (1971) *Technometrics* **13**, 33–61.
15. Wright, S. (1965) *Evolution* **19**, 395–420.
16. Nei, M. (1973) *Proc. Natl. Acad. Sci. USA* **70**, 3321–3323.
17. Chakraborty, R. (1960) *Genetics* **96**, 721–723.
18. Zhang, Q., Webster, R. K. & Allard, R. W. (1987) *Phytopathology* **77**, 352–357.
19. Chew, V. (1976) *HortScience* **11**, 348–357.
20. Webster, R. K., Saghai-Maroo, M. A. & Allard, R. W. (1986) *Phytopathology* **76**, 661–668.