Pathogenicity Associations in *Puccinia graminis* f. sp. *tritici* in the United States

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ABSTRACT

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Associations of pathogenicity in predominantly asexually reproducing populations of *Puccinia graminis* f. sp. *tritici* were analyzed for eight selected years between 1929 and 1978 on 10 standard differential wheat lines. In 1975, pathogenicity associations on 16 "single gene" lines were examined for sexual and asexual fungal populations. Associations were frequent and highly significant in all years of testing with the standard differential lines; for one-fourth of the paired combinations, the association vacillated from positive to negative over the eight years. Associations of pathogenicity were much more frequent and stronger in the asexual than in the sexual population in 1975. These data provide evidence that, in asexually reproducing populations of *P. graminis* f. sp. *tritici*, virulences and avirulences are effectively linked; thus, selectively important associations and associations occurring by chance are indistinguishable. Associations found in sexual populations are more likely to be maintained by selection. Negative associations for pathogenicity existed for host gene pairs *Sr*7b/15, 11/9a, 8/15, 8/17, 9a/10, 9a/Tmp, 9a/9e, and 36/15 in the sexual population. Experiments are advocated to find if selection for increased pathogen fitness on one host genotype leads to decreased fitness on other host genotypes. Combinations of resistance genes for which pathogens have low fitness could be introduced into cultivars for potentially more durable rust resistance.

Additional key words: stem rust of wheat, virulence analysis.

Traditionally, pathogen populations have been described by the identities, relative frequencies, or absolute numbers of races; a race being defined by similar disease reactions caused by isolates on preselected differential host lines. An alternative approach is to examine the frequency of virulence in the population to single cultivars and to particular combinations of cultivars (2,4,35). This allows examination of pathogenicity associations, that is, whether individuals in the pathogen population are more likely to have particular combinations of virulence or avirulence to a given pair of cultivars than expected by chance. Associations may be positive (in coupling) due to an excess of isolates virulent on both cultivars or avirulent on both cultivars or they may be negative (in repulsion), when there is an overabundance of isolates that are virulent on one cultivar but avirulent on the other cultivar.

Nonrandom associations of genes define, in part, the genetic structure of the population. Such associations for pathogenicity could arise in populations despite frequent genetic recombination because genes controlling pathogenicity are linked or allelic or because genes determining pathogenicity to one cultivar have pleiotropic or epistatic effects that alter the disease response on another cultivar. Another possibility is that selection favors individuals with particular combinations of pathogenicity. These explanations have intriguing possibilities for disease control, since particular combinations of host genes that are difficult for the pathogen to overcome genetically could be combined in one cultivar for a more durable resistance (2,32,33). Positive pathogenicity associations also provide clues on which cultivars share resistance genes (14,15).

Recent literature points to the difficulties in obtaining and interpreting data on nonrandom associations for pathogenicity (9,34). Associations may be spurious if sampling is not random or if individuals from different populations are lumped together in analyses. Once the existence of a real association is confirmed, the cause of the association is difficult to determine. Although plant pathologists hope to discover associations that have a genetic basis, nonrandom associations of pathogenicity may occur for other reasons. Of particular relevance in the study of many plant pathogens is the effect of low levels of genetic recombination on pathogenicity associations. Studies of *Puccinia graminis* (Pers.) f. sp. *tritici* (19) show that populations with predominantly asexual reproduction are usually composed of a smaller number of distinct genotypes than populations with frequent sexual reproduction and that certain genotypes may persist for several years. Thus, the particular combinations of virulence and avirulence found in the

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most common genotypes will determine the pathogenicity association patterns in the population. Since with asexual reproduction all genes affecting pathogenicity are effectively linked, it is difficult to know which were actually important in the success of the genotype.

The effects of asexual reproduction on pathogenicity associations are difficult to visualize in many pathogen populations because detailed data on population structure are lacking. Analysis of pathogenicity associations of populations of *P. graminis* f. sp. *tritici* offers two advantages in this regard. First, due to the economic importance of this pathogen, population surveys have been made in the United States from 1918 to the present. With these historical data, the consistency of pathogenicity associations can be examined over a period of years with varying race composition. By 1928, eradication of barberry (*Berberis vulgaris* L.), alternate host of the sexual generation of the fungus, was largely complete in the major wheat producing areas (19); thus, the predominant mode of reproduction has been asexual. A second advantage is that populations of *P. graminis* f. sp. *tritici* that yearly go through a sexual stage still exist in certain parts of the United States, such as in the Pacific Northwest. Thus, comparisons of pathogenicity associations can be made between sexual and asexual populations of the same pathogen.

Our objectives were to determine the consistency of pathogenicity associations over time and to examine the effect of the reproductive mode of the pathogen on the frequency and strength of associations.

MATERIALS AND METHODS

Pathogenicity associations historically. Data on populations of *P. graminis* f. sp. *tritici* are available from annual race surveys made in the United States from 1918 to the present. The annual survey traditionally covered the Great Plains in several trips, with stops being made at small grain fields every 16–32 km and at nurseries planted with several cultivars. Isolates were then inoculated on the standard differential series consisting of the cultivars Little Club, Marquis, Reliance, Kota, Arnautka, Mindum, Spelmar, Kubanka, Acme, Einkorn, Vernal, and Khapli. Low (avirulent) or high (virulent) disease reactions were scored. Survey and greenhouse methods for the eight selected years discussed in detail later in this paper are found in references (16, 18, 23–30).

Pathogen populations were polymorphic in virulence toward 10 of the 12 differential lines during this period; known resistance genes for these 10 differential lines for races found in North America are listed in Table 1. These differential lines have not been used as commercially grown cultivars in any more than trace amounts since 1918, with the exception that 14–19% of the acreage was planted to Marquis in the 1920s and 1930s. Some of the resistance genes in these differential lines have been used in commercially grown cultivars (eg, 5, 7b, 9d, and 9e); however, such cultivars have usually had a combination of many resistance genes (17,19). Since the effect of a resistance gene may be masked when other resistance genes are present, selection pressures for countering any one gene may be reduced. Over the years, the proportion of the pathogen population virulent on these differentials has generally increased, but a great deal of variation is evident (Table 1).

Pathogenicity associations in sexual and asexual populations. In the 1975 annual survey, an unusually large number of collections (148) were obtained from wheat and barley fields in Idaho and Washington in an area where sexual reproduction of the pathogen on the barberry alternate host occurs annually. Urediniospores in these collections were one or two asexual generations removed from the sexual cycle. Isolates obtained from these collections were separated from the collections from the asexually reproducing population east of the Rocky Mountains. There were 100 races in the 427 isolates from the sexual population and 17 races in the 2,377 isolates from the asexual population. Isolates from the sexual and asexual populations were inoculated on 16 "single gene" lines, each having one of the following known genes for resistance: *Sr*5, 6, 7b, 8, 9a, 9b, 9d, 9e, 10, 11, 13, 15, 16, 17, 36, and Tmp and disease reactions were scored. A complete description of these populations and methods has been published (19).

Statistical analysis. To test the association of pathogenicity to test cultivars a and b, a two-way contingency table was used that lists the number of isolates in each of four categories: VV—virulent on both cultivars; VA—virulent on cultivar a, but avirulent on cultivar b; AV—avirulent on cultivar a, but virulent on cultivar b; and AA—avirulent on both cultivars (see Table 2 for examples). The traditional statistical method used to compare the observed and expected numbers is the chi-square test. A log-likelihood test, called the *G*-test of independence, was designed specifically for the model of the contingency test used in pathogenicity association analysis, in which marginal totals are not fixed for either criterion (22). Although interpretations of data based on either the chi-square test or *G*-test will be similar, if discrepancies arise, the *G*-test is considered to be more reliable (1). We have used the Williams correction (22) in our tests, which reduces the possibility of making type I errors (rejection of a true null hypothesis). For the large data sets examined in this paper, it was impractical to use multi-way contingency tables, in which disease reactions on three or more differential lines are examined simultaneously. Analyses using only paired-association tables could provide misleading information if frequencies of the VV, VA, AV, and AA phenotypes are dependent on whether isolates are virulent or avirulent on other differential lines.

A positive pathogenicity association occurs if observed values significantly exceed expected values for categories VV and AA; a negative association occurs if observed values in categories VA and AV are greater than the expected.

If expected values for any cell in a table are less than five, the

TABLE 1. Population descriptions of *Puccinia graminis* f. sp. *tritici* on 10 standard differential wheat lines in eight selected annual race surveys in the United States

Year	Isolates (no.)	Races (no.)	Race number[a] (percentage) of three most common races			Percentage of population virulent on:										*G*-statistic[c] (range)
						Mq[b]	Rl	Kota	Ank	Mdm	Spl	Kb	Acme	Enk	Vn	
1929	720	23	49(26)	38(21)	36(15)	72	54	99	32	32	32	34	77	60	1	0.12–906.48
1934	737	20	56(28)	34(22)	36(20)	93	80	96	33	33	33	60	97	27	0	0.24–930.17
1949	669	17	56(45)	17(24)	38(22)	72	68	95	27	27	27	73	78	54	1	0.08–783.12
1955	755	23	15(47)	56(18)	17(14)	91	71	91	65	65	65	84	92	81	47	12.07–982.21
1958	775	25	56(29)	11(23)	15(20)	90	79	95	56	56	56	88	93	69	20	3.58–1052.13
1960	721	11	56(67)	15(17)	11(7)	97	93	97	26	26	26	93	95	33	23	11.57–819.02
1967	706	7	15(61)	11(14)	151(12)	81	99	88	88	76	76	79	91	96	61	11.34–779.41
1978	790	7	15(70)	151(18)	113(7)	74	99	82	99	73	73	74	100	99	71	447.93–922.94

[a] See cited reference 26.

[b] Full name (known resistance genes): Mq = Marquis (*Sr*7b, 18, 19, 20, X); Rl = Reliance (*Sr*5, 16, 18, 20); Kota (*Sr*7b, 18, 28, Kt'2'); Ank = Arnautka (*Sr*9d, $+_b$); Mdm = Mindum (*Sr*9d, $+_a$, $+_b$); Spl = Spelmar (*Sr*9d, $+_a$, $+_b$); Kb = Kubanka (*Sr*9g, $+_c$); Acme (*Sr*9g, $+_c$); Enk = Einkorn (*Sr*21); Vn = Vernal (*Sr*9e); see reference 20. Genes designated by a "+" are additional resistance genes that have not been fully characterized; genes with the same subscript letter are identical.

[c] See cited reference 22.

G-test is no longer suitable, and the association was not analyzed. This situation usually occurred when most of the pathogen population was virulent or avirulent on a particular differential line.

Variation in sample size affects the detection of significance with the G-statistic; ie, the same proportional difference between observed and expected frequencies will be more likely to be statistically significant with a large sample size than with a small sample size. The sample size (= number of isolates) for the surveys from 1918 to the present differ greatly; although the average is 962, they span a range from 68 to 2,415. Sample size, however, had little or no effect on the number of races detected ($r = -0.12$, $P > 0.2$). To remove the confounding problem of sample size in our pathogenicity association analyses, data for eight of the years with similar sample sizes were chosen from those within the 65-yr period (Table 1).

The sample size of the 1975 asexual population (2,377 isolates) was much larger than the size of the sexual population (427 isolates); thus associations between pathogenicity would be more likely to be detected in the asexual population simply due to its sample size. To avoid this problem, these data were analyzed in two ways: asexual population (complete) refers to analyses made by using the total data set while asexual population (adjusted) refers to analyses in which the number of individuals in each race was normalized to the number expected in a population of 427, by multiplying the number by 427 and dividing that product by 2,377.

Two-way contingency tables were set up for all possible combinations of the 10 selected standard differential lines for each of the eight years chosen between 1929 and 1978 and of the "single gene" lines by using the 1975 sexual and asexual population data. For all pairs of cultivars for which expected values of each of the cells exceeded five, the G-statistic was calculated. Significant associations were determined to be positive or negative.

RESULTS

Homogeneity of survey data. Nonrandom sampling and mixing isolates from different populations may lead to false conclusions about pathogenicity associations (34). The collection of spores for the annual race surveys is not based on a random sampling design, but care has been taken to make it as representative as possible. Two potential problems are that survey data combine spores obtained from different geographical regions and from nurseries and fields; these collections could differ in race composition. Of the eight years studied between 1929 and 1978, only data from the 1978 survey were subdivided according to these criteria. Thus, to determine how seriously bulking of data in the race survey affects interpretation of pathogenicity associations, the 1978 data were examined for homogeneity.

Geographical effects were analyzed by subdividing the 1978 data into eight ecological areas defined by their type of wheat production (18). With a 7×8 contingency table, the frequencies of the seven races (15, 151, 113, 11, 56, 17, and 29) were found to vary among the eight areas ($G = 156.98$, df = 42, $P < 0.005$). However, the three areas that represented 88% of the isolates (areas 3, 5, and 6) in the Great Plains were homogeneous in race composition ($G = 9.79$, df = 9, $0.1 < P < 0.5$). Values of the G-statistic obtained when these three homogeneous regions were pooled and those obtained from the complete data set (all eight regions) were very highly correlated (Spearman rank correlation, $r = +0.95$, $P < 0.001$). Associations that were highly significant in the homogeneous subsample were also highly significant and of the same direction (positive or negative) in the total survey data. Thus, although race composition has significant geographic variation, the regions with different frequencies of races have only a few isolates since they are not major wheat producing areas; therefore, inclusion of their data does not affect the overall conclusions.

Urediniospores collected from commercial fields and from rust nurseries could differ in race composition due to differences in host resistance. The 1978 data were separated into isolates from field and from nursery collections (18) and a 2×7 contingency test was used to examine race composition in the two groups. Frequencies of races in the field and nursery collections were not significantly different ($G = 7.167$, df = 7, $0.1 < P < 0.5$).

Obviously, surveys made over a 65-yr period have been conducted by different researchers and it is impossible to know the details of their sampling techniques. The fact that the interpretation of pathogenicity associations in 1978 does not change when isolates from different regions of the country and from both fields and nurseries are combined gives us confidence, however, that the use of survey data for *P. graminis* f. sp. *tritici* in the United States is not likely to produce spurious associations. Since *P. graminis* f. sp. *tritici* does not overwinter in the major wheat producing areas in the United States and is reestablished each year by wind-blown spores from the south, it is less likely to show geographic population differentiation than pathogens that complete their life cycles in one location.

Pathogenicity associations historically. For the eight years and 10 differential lines, a total of 360 two-way contingency tables were set up to analyze pathogenicity associations. Of these 360 tables, 96 could not be used because one or more of the four cells had an expected frequency less than five; this occurred because the pathogen population was highly skewed towards virulence or avirulence in its reaction to certain cultivars. For the 264 associations that could be analyzed, only 12 were not significant at the 0.05 level ($G < 3.841$, df = 1). Over 90% of the other 252 associations had a G-statistic that greatly exceeded even the G-statistic for the 0.001 level of significance ($G = 10.828$, df = 1); note the ranges for the G-statistic in Table 1. Thus, although analyzing all possible two-way combinations increases the probability of detecting some significant associations, the very large G-statistics obtained gives us confidence that the relationships we found are real.

Of particular interest is the analysis of associations of pathogenicity for a certain pair of differential lines over time. For the 45 two-way comparisons of the 10 lines over the eight-year period, 27 stayed positive throughout, four stayed negative throughout, 11 vacillated between negative and positive associations, and three changed from a nonsignificant association to a significant association (Table 3). Associations including either Einkorn or Reliance varied more than associations of pathogenicity for other pairs of differential lines.

The frequency of positive associations identified by using the standard differential lines can be partially explained by the fact that some differential lines possessed resistance genes in common. The clearest example is the absolute positive association of disease reactions on Mindum and Spelmar; throughout the years all isolates have either been virulent on both cultivars or avirulent on both. These cultivars share three resistance genes to the North American population of *P. graminis* f. sp. *tritici* (Table 1). For all

TABLE 2. Observed and expected numbers of isolates of *Puccinia graminis* f. sp. *tritici* in the four possible patterns of virulence and avirulence on two wheat lines

Association[x]	Wheat line	Virulence pattern			
a. Positive[y]	Acme	V	V	A	A
	Marquis	V	A	V	A
	Observed no.	502	53	14	151
	Expected no.	398	157	118	47
	$G = 411.03$, positive association				
b. Negative[z]	Reliance	V	V	A	A
	Marquis	V	A	V	A
	Observed no.	206	182	310	22
	Expected no.	278	110	238	94
	$G = 159.98$, negative association				

[x] The G-test for independence (22) was used to analyze the associations. Expected numbers for each cell are based on the null hypothesis of independence of disease reactions.

[y] Significantly positive association for pathogenicity to Marquis and Acme in 1929.

[z] Significantly negative association for pathogenicity to Marquis and Reliance in 1929.

years except 1967 and 1978, disease reactions on Arnautka were also identical to those of Mindum and Spelmar; in recent years, however, races 113 and 151 have appeared that are virulent on Arnautka but avirulent on Mindum and Spelmar. Consequently, the *G*-statistic for the positive associations of pathogenicity between Arnautka and Mindum and between Arnautka and Spelmar are now much lower, although still strongly significant.

Other examples in which long-lasting positive associations between disease reactions on differential lines were correlated with shared host genes include Marquis and Kota, Kubanka and Acme, and Reliance and Kota (Tables 1 and 3). Reliance and Marquis share two genes (*Sr*18, 20) affecting a small part of the North American population of *P. graminis* f. sp. *tritici*; however, the pathogenicity association has shifted back and forth between significantly negative and positive associations. Thus, the resistance genes not shared by the two cultivars may in certain years and for certain isolates change the association.

Compared to the six of seven consistently positive associations for combinations of differential lines that share resistance genes, only 21 of 38 associations stayed positive throughout for combinations of differential lines without resistance genes in common (Tables 1 and 3).

During the first half of the 20th century, there were several major changes in race frequency (Table 1). Since these races are defined by isolate's reactions on the differential lines used in this study, the particular races present and their relative frequencies will clearly determine the associations of pathogenicity for particular cultivars. For example, for the association between pathogenicity to Marquis and Arnautka, a positive association was found in all years (the 1978 association was not studied due to expected frequencies less than five). Similar statistical levels of association occurred in years with different race compositions: for example, in 1929 ($G = 8.25$), races 11, 21, 36, 49, and 38 were predominant while in 1960 ($G = 12.18$) a similar association developed due to the predominance of races 11, 15, and 56. In contrast, associations of pathogenicity for Reliance and Arnautka shifted between negative and positive over the years. Changing race frequencies were responsible for switches in the direction of the pathogenicity association change; for example, the large frequencies of races 15 (virulent on both) and 29 (avirulent on both) in the 1955 survey that were absent in 1949 are responsible for the switch from a negative to a positive association.

Pathogenicity associations in sexual and asexual populations. For both sexual and asexual populations, associations of certain host genes could not be used since either the population was uniformly virulent or avirulent on them or because all associations involving these genes had an expected cell frequency less than five; therefore, the following lines were not used: sexual—*Sr*6, 9b, 13; asexual (complete)—*Sr*5, 9d, 13, 16; and asexual (adjusted)—*Sr*5, 8, 9a, 9d, 13, 16. Of the remaining pathogenicity associations, 46 of the 105 sexual associations, 65 of the 66 asexual (complete) associations, and all 45 of the asexual (adjusted) associations had expected cell frequencies greater than five and could be used. Analyses of the asexual (complete) and (adjusted) data sets were in total agreement on whether or not an association was significant and on the direction (positive or negative) of significant associations. The only differences between the two data sets were: the strength of associations (value of the *G*-statistic) was greater, as expected, in the "complete" data set with a larger sample size; and associations involving *Sr*8 or 9 could be examined with the "complete" data set.

Large differences were obvious in comparisons of the data from the sexual and asexual populations. Nearly half (22/46) of the sexual associations were not significant at the 0.05 level ($G < 3.841$, df = 1) compared to 5% (3/65) of the asexual (complete) and 4% (2/45) of the asexual (adjusted) associations. Values of the *G*-statistic for significant associations were much higher for the asexual than for the sexual population (ranges for asexual, 4.30–27.75; asexual [complete], 6.94–2,502.54; asexual [adjusted], 4.27–447.59). The pathogenicity associations that were highly significant ($P < 0.001$) in the sexual population are listed in Table 4.

Since many associations had to be excluded from analysis due to expected cell frequencies less than five, it was often not possible to compare the *G*-statistic for a particular pathogenicity association in both the sexual and asexual populations. In 12 of the 18 associations in which data from both the sexual and asexual (complete) populations were available, associations were both significant and of the same direction (positive or negative). These 12 associations were: *Sr*9e/9a, 11/9a, 8/15, 8/17, 9a/10, 9a/15, 9a/17, 9a/Tmp, 7b/36, 7b/15, 36/15, and 15/17. Four of the remaining associations had a nonsignificant *G*-statistic in one population and a significant value in the other; only two associations (*Sr*7b/8, 9a/36) were both significant but of opposite directions in the two populations.

DISCUSSION

Both the eight years of data from tests on the standard differential lines and the data on sexual and asexual populations show how pathogenicity associations in *P. graminis* f. sp. *tritici* are strongly nonrandom and can be dependent on the frequencies of a few major races in the pathogen population. Where sexual reproduction was common, the number of races was much larger and more similar in frequency (19); under this situation, many fewer nonrandom pathogenicity associations were found. Not all virulences and avirulences assort randomly (even in the sexual population) suggesting that: particular combinations of genes controlling pathogenicity are under selection, that pleiotropic effects of virulence genes are important, or that a single sexual generation per year is insufficient for breaking linkage relationships.

In the asexually reproducing populations, a few races may predominate for many years; this may suggest that individuals with the combinations of genes represented by these races have particularly high fitness. However, with the effective total linkage of genes in each race in a predominantly asexually reproducing population, it is difficult to determine which particular gene(s) are

TABLE 3. Pathogenicity associations of *Puccinia graminis* f. sp. *tritici* for pairs of standard differential lines for eight selected years between 1929 and 1978

	Reliance	Kota	Arnautka	Mindum	Spelmar	Kubanka	Acme	Einkorn	Vernal
Marquis	V[a]	+[b]	+	+	+	+	+	−[c]	+
Reliance		+	V	V	V	V	V	V	+
Kota			V	NS+[d]	NS+	+	−	−	+
Arnautka				+	+	+	+	V	+
Mindum					+	+	+	V	+
Spelmar						+	+	V	+
Kubanka							+	NS−[e]	+
Acme								−	+
Einkorn									+

[a] V = Varied associations, both positive and negative in the selected years.
[b] + = Consistently positive associations for the selected years.
[c] − = Consistently negative associations for the selected years.
[d] NS+ = Pathogenicities were not significantly associated at the $P = 0.05$ level ($G < 3.841$, df = 1) in 1929 and 1934, but were positively associated in the other six years.
[e] NS− = Pathogenicities were not significantly associated at the $P = 0.05$ level ($G < 3.841$, df = 1) in 1929, but were negatively associated in the other seven years.

favored by selection. For example, when an excess of isolates sharing a certain combination of virulence or avirulence to two cultivars is found, it may be because the joint occurrence of these traits is adaptive, but it could be equally possible that the race(s) with this particular pathogenicity association have other gene combinations that are responsible for the high fitness. Similarly, the shifting back and forth from positive to negative associations for certain pathogenicity combinations over the years in the historical data probably do not reflect large-scale changes in selection for or against these associations. Instead, these associations may be selectively "neutral" and the changes in association have instead resulted from different combinations of these virulences or avirulences occurring in races that prove successful, and become prevalent, for other reasons. Thus, the interpretation of pathogenicity associations in pathogen populations with limited genetic recombination is as difficult as the determination of "unnecessary" genes for virulence: in both cases, the selective importance of a particular gene or combination of genes is confounded with the properties of the complete genotype (12).

The difficulties in separating selection on a genotype and on particular genes or gene combinations is not just a dilemma for plant pathologists. This same query has been central in the development of population genetics concepts. Associations between alleles at different loci (= linkage disequilibrium) can arise from selection favoring certain gene combinations. If genes are often in linkage disequilibrium, we cannot understand the action of selection on the individual by extrapolating from our detailed knowledge of single-locus theory. Information is therefore needed on the extent of and reasons for associations between genes in natural populations.

With fungal plant pathogens, pathogenicity associations have been found in *Puccinia recondita* f. sp. *tritici* (2,14), *Puccinia graminis* f. sp. *tritici* (15), *Bremia lactucae* (10), and *Pyricularia oryzae* (9) but have been rare in *Rhynchosporium secalis* (6), *Erysiphe graminis* (35), and *Phytophthora infestans* (21). Studies have differed in the methods used to collect isolates; thus, depending on the homogeneity of the population studied, some results may be questionable (34). Many of the examples of linkage disequilibria known for nonplant pathogens involve chromosomally linked loci (eg, *Drosophila* [13], *Cepea nemoralis* [8], *Homo sapiens* [31]), but few studies report linkage of virulence loci (3). As with the data on *P. graminis* f. sp. *tritici* presented here, the lack of frequent genetic recombination in several pathogen species is probably responsible for many of the associations.

From any purely descriptive survey of an organism, it will be impossible to be sure of the reasons for associations of traits, especially associations maintained by selection. Is it, therefore, useful to analyze pathogenicity associations in population surveys? Each case must be considered separately. The data presented here for *P. graminis* f. sp. *tritici* suggest that if reproduction is largely asexual in the pathogen species, it may be impossible to separate selectively important and selectively neutral associations, since nearly all virulences and avirulences show nonrandom associations. However, for situations in which genetic recombination is known to occur more regularly, field data provide clues on what associations might be worthy of further investigation. For example, despite the generally low levels of associations of pathogenicity in the 1975 sexual population for the host pairs listed in Table 4, combinations of virulences and avirulences were not independent of each other. Given the very large numbers and even distribution of races in this population, the consistent association of these virulences and avirulences, whether positive or negative, suggests that these combinations might be maintained by selection. Of the eight significantly negative associations, a disproportionate number involve pathogenicity to either *Sr*9a or *Sr*15 (Table 4); these host genes may be of particular interest to the wheat breeder.

Experiments are needed to test hypotheses generated by the discovery in field data of associations for pathogenicity to two cultivars. One valuable approach is the use of selection experiments: beginning with a genetically diverse population, the pathogen can be maintained for several generations on one cultivar, thus selecting for isolates with high fitness on that cultivar. The ability of the pathogen population to infect the second cultivar can be examined throughout the selection process. If field survey data indicated disease reactions on the two cultivars (a and b) were positively associated, one would predict that selection for greater fitness on cultivar a would also lead to greater fitness on cultivar b, due to a correlated response to selection (7). The converse would be expected if disease reactions are negatively associated. If fitness to the unselected cultivar is not changed by the selection process, then the virulences are truly independent. Examples of this approach are the work of Leonard (11) with *P. graminis* f. sp. *avenae* and Gould (5) with a phytophagous mite, *Tetranychus urticae*. Both found negative correlations between parasite fitness on the host plants tested, suggesting that it may be difficult to select for individuals with superior ability to infect or feed on both hosts.

Similar experiments could be done with *P. graminis* f. sp. *tritici* and other plant pathogens. For example, beginning with genetically diverse spore collection, such as that derived from the 1975 sexual population, the fungus could be maintained for several generations on a line with *Sr*15. The original and selected population could then be inoculated on differential lines such as *Sr*5, 7b, 8, 9a, 9d, 16, 17, and 36. If the pathogenicity associations found in the United States survey data (Table 4) are indicative of associations with a genetic basis, then virulence frequencies for *Sr*5, 9d, and 16 would not change, for 7b, 8, and 36 would decrease, and for *Sr*9a and 17 would increase. Particularly interesting would be experimental information on the 12 associations for which a consistent direction of association (positive or negative) was found between the sexual and asexual populations. Despite the large difference in strength of the association (as indicated by the *G*-statistic), these data suggest that genetic forces may be influencing associations of pathogenicity to some degree in the asexual population.

A better understanding of virulence distributions is needed in plant pathology. The primary means of control of wheat stem rust in the last 25 yr has been through breeding of cultivars with multiple resistance genes. The practical application of negative correlations such as those documented by Leonard (11) and Gould (5) has exciting potential: cultivars could be bred that have combinations of resistance genes for which pathogen populations are unlikely to have a high degree of fitness. However, pest populations exposed to such cultivars may eventually evolve to forms that do not have the same associations, and they may overcome the resistance. Clearly, however, choosing combinations of resistance genes based on knowledge of the pathogen's current genetic constraints could improve chances of disease control. The difficulty is identifying

TABLE 4. Pairs of "single gene" differential lines for which there were highly significant pathogenicity associations in the 1975 sexual population of *Puccinia graminis* f. sp. *tritici*. A total of 46 gene pairs were tested

Host gene pairs	*G*-statistic[a]	Direction of association
9d/5	82.99	+
9e/9a	17.87	−
9e/16	14.53	+
7b/8	19.27	+
7b/36	35.69	+
7b/15	14.41	−
11/9a	23.52	−
11/16	14.53	+
8/15	11.58	−
8/17	13.73	−
9a/10	11.58	−
9a/15	11.70	+
9a/Tmp	27.75	−
36/15	14.41	−
36/5	13.06	+
15/17	32.91	+
Tmp/16	12.61	+

[a] G (0.001) = 10.83, df = 1; see cited reference 22.

which combinations of resistance genes may offer this type of protection. This paper illustrates the problems of relying solely on survey data for organisms with limited genetic recombination; instead we propose that survey data could be used to suggest gene pairs that can be experimentally tested in selection experiments.

LITERATURE CITED

1. Bliss, C. I. 1967. Statistics in Biology. Vol. 1, Page 44. McGraw-Hill, New York.
2. Browder, L. E., and Eversmeyer, M. G. 1977. Pathogenicity associations in *Puccinia recondita tritici*. Phytopathology 67:766-771.
3. Burnett, J. H. 1975. Mycogenetics. Page 268. John Wiley & Sons, London.
4. Day, P. 1974. Genetics of Host-Parasite Interaction. Page 110. W. H. Freeman and Co., San Francisco.
5. Gould, F. 1979. Host range evolution in a population of the phytophagous mite *Tetranychus urticae* Koch. Evolution 33:791-802.
6. Hansen, L. R., and Magnus, H. A. 1973. Virulence spectrum of *Rhynchosporium secalis* in Norway and sources of resistance in barley. Phytopathol. Z. 76:303-313.
7. Hartl, D. L. 1980. Principles of Population Genetics. Pages 299-300. Sinauer Associates, Sunderland, MA.
8. Jones, J. S., Leith, B. H., and Rawlings, P. 1977. Polymorphism in *Cepaea*: A problem with too many solutions. Annu. Rev. Ecol. Syst. 8:109-143.
9. Kiyosawa, S. 1980. On the virulence analysis of pathogen race frequencies. Ann. Phytopathol. Soc. Jpn. 46:582-592.
10. Lebeda, A. 1981. Population genetics of lettuce downy mildew (*Bremia lactucae*). Phytopathol. Z. 101:228-239.
11. Leonard, K. J. 1969. Selection in heterogeneous populations of *Puccinia graminis* f. sp. *avenae*. Phytopathology 59:1851-1857.
12. Leonard, K. J., and Czochor, R. J. 1980. Theory of genetic interactions among populations of plants and their pathogens. Annu. Rev. Phytopathol. 18:237-258.
13. Prakagh, S., and Lewontin, R. C. 1968. A molecular approach to the study of genic heterozygosity in natural populations, III. Direct evidence of coadaptation in gene arrangements of *Drosophila*. Proc. Nat. Acad. Sci. USA 59:398-405.
14. Prescott, J. M. 1967. A study of variation in race populations of *Puccinia recondita* f. sp. *tritici*. M.S. thesis. Oklahoma State University, Stillwater. 49 pp.
15. Prescott, J. M. 1970. Resistance and virulence relationships in the *Triticum aestivum* : *Puccinia graminis tritici* system. Ph.D. dissertation. University of Minnesota, St. Paul. 75 pp.
16. Roelfs, A. P. 1984. Race specificity and methods of study. Pages 131-162 in: The Cereal Rusts. Vol. I. W. R. Bushnell and A. P. Roelfs, eds. Academic Press, Orlando, FL. 546 pp.
17. Roelfs, A. P. 1985. Epidemiology in North America. In: The Cereal Rusts. Vol. II. A. P. Roelfs and W. R. Bushnell, eds. Academic Press, Orlando, FL. (In press).
18. Roelfs, A. P., Casper, D. H., and Long, D. L. 1979. Races of *Puccinia graminis* f. sp. *tritici* in the U.S.A. during 1978. Plant Dis. Rep. 63:701-704.
19. Roelfs, A. P., and Groth, J. V. 1980. A comparison of virulence phenotypes in wheat stem rust populations reproducing sexually and asexually. Phytopathology 70:855-862.
20. Roelfs, A. P., and McVey, D. V. 1979. Low infection types produced by *Puccinia graminis* f. sp. *tritici* and wheat lines with designated genes for resistance. Phytopathology 69:722-730.
21. Shattock, R. C., Janssen, B. D., Whitbread, R., and Shaw, D. S. 1977. An interpretation of the frequencies of host specific phenotypes of *Phytophthora infestans* in North Wales. Ann. Appl. Biol. 86:249-260.
22. Sokal, R. R., and Rohlf, F. J. 1981. Biometry. 2nd ed. Pages 731-738. W. H. Freeman and Co., San Francisco.
23. Stakman, E. C., Levine, M. N., and Wallace, J. M. 1929. The value of physiologic-form surveys in the study of the epidemiology of stem rust. Phytopathology 19:951-959.
24. Stakman, E. C., and Loegering, W. Q. 1950. Physiologic races of *Puccinia graminis* in the United States 1945–49. U.S. Dep. Agric., Bureau of Entomology and Plant Quarantine E-267.
25. Stakman, E. C., Loegering, W. Q., Cassell, R. C., and Hines, L. 1943. Population trends of physiologic races of *Puccinia graminis tritici* in the United States for the period 1930 to 1941. Phytopathology 33:884-898.
26. Stakman, E. C., Stewart, D. M., and Loegering, W. Q. 1962. Identification of physiologic races of *Puccinia graminis* var. *tritici*. U.S. Dep. Agric., ARS E617 (Revised). 53 pp.
27. Stewart, D. M., Cotter, R. U., and Christensen, J. J. 1961. Physiologic races of *Puccinia graminis* in the United States in 1960. Plant Dis. Rep. 45:448-453.
28. Stewart, D. M., Cotter, R. U., Roberts, B. J., and Christensen, J. J. 1959. Physiologic races of *Puccinia graminis* in the United States in 1958. Plant Dis. Rep. Suppl. 258:189-196.
29. Stewart, D. M., Cotter, R. U., Roberts, B. J., and Hayden, E. B. 1956. Physiologic races of *Puccinia graminis* in the United States in 1955. Plant Dis. Rep. Suppl. 239:99-105.
30. Stewart, D. M., and Romig, R. W. 1968. Prevalence of physiologic races of *Puccinia graminis* f. sp. *avenae* and *tritici* in the USA in 1967. Plant Dis. Rep. 52:642-646.
31. Thomson, G. 1981. A review of theoretical aspects of HLA and disease associations. Theor. Popul. Biol. 20:168-208.
32. Vanderplank, J. E. 1975. Principles of Plant Infection. Pages 152-157. Academic Press, New York.
33. Wolfe, M. S., Barrett, J. A., Shattock, R. C., Shaw, D. S., and Whitbread, R. 1976. Phenotype-phenotype analysis: Field application of the gene-for-gene hypothesis in host-pathogen relations. Ann. Appl. Biol. 82:369-374.
34. Wolfe, M. S., and Knott, D. R. 1982. Populations of plant pathogens: some constraints on analysis of variation in pathogenicity. Plant Pathol. (Lond.) 31:79-90.
35. Wolfe, M. S., and Schwarzbach, E. 1975. The use of virulence analysis in cereal mildews. Phytopathol. Z. 82:297-307.