Isozyme and Virulence Variation in Asexually Reproducing Populations of *Puccinia graminis* and *P. recondita* on Wheat

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ABSTRACT

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Simultaneous isozyme and virulence surveys of North American asexual populations of *Puccinia graminis* and *P. recondita* on wheat detected marked differences between the pathogens in absolute levels of isozymic diversity and in the relative levels of isozyme and virulence diversity. In both pathogens, many virulence phenotypes were detected. In *P. recondita*, however, this diversity of virulence contrasted sharply with the very low level of isozymic diversity found (two phenotypes; one variable locus), while in *P. graminis* rust nine different isozyme phenotypes were detected. It is suggested that the two isozyme phenotypes of *P. recondita* present in the existing population represent past introductions, while most, if not all, of the nine isozyme phenotypes occurring in the population of *P. graminis* probably have their origin in the sexual population extant in the Great Plains in the 1920s.

The results of recent studies (3) in Australia have demonstrated that assessments of the population structure of cereal rust pathogens may vary dramatically depending on the particular genetic markers used. Thus, during the course of a single wheat growing season (1981–1982) a routine virulence survey detected 16 different races of *P. graminis* Pers. f. sp. *tritici* Eriks. and Henn. and 12 of *P. recondita* Robs. ex Desm. f. sp. *tritici*. By contrast, isozyme surveys of the same populations detected no variation whatsoever within either of these pathogens. This remarkable difference in the diversity of the same pathogen population may be summarized in major differences in the level of polymorphism and probable heterozygosity as determined by isozyme and virulence analysis. Inevitably, one major question arising from this study is whether this disparity reflects real differences in factors controlling various parts of the fungus genome or whether they simply reflect circumstances unique to Australia. So far, no similar comparative studies have been carried out elsewhere in the world.

In North America, as in Australia, a considerable amount of information is available concerning the population structure of cereal rust pathogens (10). On the basis of pathogenicity patterns produced on host lines carrying single resistance genes, the North American population of *P. graminis* f. sp. *tritici* occurring in the eastern and central United States can be subdivided into eight separate subpopulations. Each of these subpopulations is composed of between two and five closely related races each differing from one another by one to three virulence genes only. The subpopulations, on the other hand, differed from one another by an average of 11 virulence genes. The closely related races within each group are, presumably, derived from one another or from a common ancestor, by mutations at loci governing pathogenicity (12; A. P. Roelfs, *unpublished*). The origin of the individual groups is unclear. This situation contrasts with that in Australia where single genotypes have arrived, totally supplanted existing races, and have then, again through mutation at loci governing virulence, rapidly given rise to a pathogen population of high diversity (race 21 gave rise to over 50 mutational variants during the 1960s: 6,14).

The marked difference in the structure of populations of *P. graminis* f. sp. *tritici* in Australia and North America makes the later population a particularly interesting one to examine electrophoretically. Comparisons of data from these two regions of the world should help determine the relative contribution of different sources of diversity to variation in this pathogen. The wheat leaf rust pathogen, *P. recondita* f. sp. *tritici*, was also included in this study because although far less is known about its population structure in either Australia or North America, its total uniformity in the former continent again poses interesting questions.

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MATERIALS AND METHODS

Virulence survey. Collections of *P. graminis* f. sp. *tritici* and *P. recondita* f. sp. *tritici* were obtained from a variety of sources throughout northern Mexico, the United States east of the Rocky Mountains, and southern Canada. Upon receipt, urediniospores were collected and inoculated to a susceptible wheat cultivar (for *P. graminis* f. sp. *tritici* this was McNair 701, CI 15288; for *P. recondita* f. sp. *tritici*, Thatcher) that had previously been treated with maleic hydrazide to enhance spore production. Each collection was used to inoculate 20–30 seedlings in a single pot of the susceptible host. After 14–16 days, three leaves, each with a single uredinium, were saved, reincubated overnight to germinate loose urediniospores and replaced in isolated booths for 36–48 hr. Urediniospores were then collected separately from each uredinium with a cyclone collector. Each uredinium provided sufficient spores to inoculate, in light mineral oil, 7-day-old plants of the differential sets used to evaluate the pathogen races. Plants were then placed overnight in a dew chamber at 18 C. The next morning, the chamber was illuminated with 10,000 lux of fluorescent light (for *P. graminis* f. sp. *tritici* only) while the temperature rose to 30 C over a 4-hr period so that the dew evaporated slowly. Plants were then transferred to a greenhouse in which temperatures varied between 18 and 30 C. Infection types were recorded on a 0–4 scale 10–12 days after inoculation.

Plants were grown in 7-cm square plastic pots filled with vermiculite. Five to eight seeds of four differential lines were sown

per pot, each line being placed in separate predetermined corners. The three or four pots required for the full differential were then grouped together in a shallow plastic tray and fertilized 5 and 8 days after planting with 2.5 g of a water-soluble fertilizer (23-19-17, N-P-K).

Cultures of *P. graminis* f. sp. *tritici* were evaluated on 16 near-isogenic host lines with known genes for resistance, viz. *Sr*5, 6, 7b, 8, 9a, 9b, 9d, 9e, 10, 11, 13, 15, 16, 17, 36, and *Tmp*. These genes had been chosen over a period of years because they respond differentially to collections from the population of *P. graminis* f. sp. *tritici* extant east of the Rocky Mountains (9). Cultures of *P. recondita* f. sp. *tritici* were evaluated on 12 near-isogenic host lines with known genes for resistance, viz. *Lr*1, 2a, 2c, 3a, 3b, 9, 10, 16, 17, 18, 19, and 24.

Isozyme survey. Because previous work on both *P. graminis* f. sp. *tritici* (east of the Rocky Mountains) and *P. recondita* f. sp. *tritici* had shown these pathogens to rely exclusively on asexual reproduction for survival (9), a completely random sampling of the two populations was not considered the best procedure for detecting and estimating population variability at isozyme loci. Instead, a stratified sampling procedure based on grouping individual collections into clonal groups (based on their identical pathogenicity) and then sampling randomly within these groups was used. This procedure was modified somewhat to ensure a reasonable geographic spread of isolates so that any one region was not over-represented. In this way, a total of 54 isolates (10 races) of *P. graminis* f. sp. *tritici* (Table 1) and 44 isolates (20 races) of *P. recondita* f. sp. *tritici* (Table 2) were taken from those identified during the 1983 virulence surveys and examined isozymically.

Virulence surveys from previous years (post-1956) have detected a total of 23 races in the Great Plains population of *P. graminis* f. sp. *tritici*. Some of these races are no longer extant, having been replaced by other closely related races (differences of one or two virulence genes), while others normally occur at such a low frequency in the population (<1%) that, in any given year, they may not be detected. Where possible, multiple examples of all of these races (total of 56 isolates; Table 1) were recovered from liquid nitrogen storage and examined to determine whether isozyme patterns detected in the 1983 survey material were the same as those with identical or closely related virulence from earlier years.

A similar approach of examining isolates from past years was used with *P. recondita* f. sp. *tritici*, although here far less is known about relationships among different races (22 isolates; Table 2).

Starch-gel electrophoresis was carried out on extracts obtained from homogenized, germinated spores. Rapid and synchronous germination of urediniospores was obtained through a modification of the method detailed by Maheshwari and Sussman (8). Fifty milligrams of freshly collected urediniospores were suspended in 35 ml of a calcium phosphate-potassium phosphate buffer, pH 7.0, containing 10^{-4} M nonyl alcohol and 0.01% Tween-20 (polyoxyethylene sorbitan monolaurate). Self-inhibitors of germination were removed by vigorous agitation for 5 min on a reciprocal shaker in diffuse light at room temperature. The suspension was then centrifuged, the supernatant discarded, and the spores resuspended in another 30 ml of buffer solution. The suspension was then shaken for 90–120 min at room temperature after which virtually all spores had germinated. The suspension was again centrifuged, the supernatant was discarded, and the remaining spores were resuspended in a few drops of 1 M phosphate buffer, pH 7.0. This suspension of spores was frozen in liquid nitrogen until required.

For electrophoretic analysis, the frozen germinated spores were allowed to thaw, ground with a mortar and pestle, and the crude extract was absorbed on paper chromatography wicks (6 × 6 mm). These were inserted in a single sample slot in a horizontal starch gel, each sample being duplicated, and electrophoresis was then carried out in one continuous (histidine, pH 8.0) and two discontinuous (borate, pH 7.8, and citrate, pH 8.0) systems (1,2). In the continuous system, electrophoresis was conducted for 5 hr, and in the discontinuous systems it was allowed to proceed until the borate or citrate front had migrated 9 cm from the sample slot. Each gel was then cut horizontally into three slices and assayed for the following range of enzymes: aconitate hydratase (ACO, EC

TABLE 1. Number and source of all isolates of *Puccinia graminis* f. sp. *tritici* examined electrophoretically

Standard race	Phenotype[a]	Source of isolates: 1983 Survey	Source of isolates: Liquid N_2	Total number
11	RCR	3	2	5
	RHR	···	2	2
15	TBM	···	3	3
	TDM	···	4	4
	TLM	···	3	3
	TNM	8	1	9
	TNM[b]	30	1	31
	TDM[b]	···	1	1
17	HDL	1	4	5
	HNL	1	2	3
29-32	HJC	···	3	3
	RJC	···	3	3
56	MBC[c]	1	4	5
	MBC	1	3	4
113	RKQ	···	2	2
	RKQ[b]	···	3	3
	RPQ	···	4	4
	RTQ	3	1	4
	RTQ[b]	···	2	2
151	QCB	4	1	5
	QFB	2	1	3
151-32	QSH	···	3	3
	RSH	···	3	3
Total		54	56	110

[a] Asterisk indicates standard Cereal Rust Laboratory race designation code, fully described in (11).
[b] Also virulent on *Sr*17.
[c] Also virulent on *SrTmp*.

TABLE 2. Number and source of all isolates of *Puccinia recondita* f. sp. *tritici* examined electrophoretically and the association between isozyme phenotype and *Lr*2a and *Lr*2c loci

Virulence phenotype	Esterase isozyme phenotype 'aa': 1983 Survey	'aa': Storage	'ab': 1983 Survey	'ab': Storage
Distribution of virulence phenotypes among two esterase phenotypes				
UN-1†	1	1	···	···
UN-2	5	2	···	···
UN-3	···	1	8	4
UN-5	7	6	···	···
UN-6	1	1	6	···
UN-9	···	···	···	1
UN-13	7	4	···	···
UN-14	···	···	2	···
UN-17	7	2	···	···
Association between reaction to *Lr* 2a and *Lr* 2c loci and esterase phenotypes				
Avirulent to *Lr*2a virulent to *Lr*2c	1	1	16	4
Avirulent to *Lr*2a avirulent to *Lr*2c	13	9	···	1
Virulent to *Lr*2a virulent to *Lr*2c	14	7	···	···

† Additional differentials separated these UN groups into 1, 4, 1, 5, 4, 1, 2, 1, and 1 races, respectively.

4.2.1.3), catalase (CAT, EC 1.11.1.6), arylesterase (EST, EC 3.1.1.2), aminopeptidase (synonym leucine amino peptidase, LAP, EC 3.4.11.1), and dihydrolipoamide reductase (synonym NADH diaphorase, NADHD, EC 1.6.4.3)—assayed on citrate gels; aspartate aminotransferase (synonym glutamate oxalate transaminase, GOT, EC 2.6.1.1), and glucosephosphate isomerase (synonym phosphoglucoisomerase, PGI, EC 5.3.1.9)—assayed on borate gels; and glutamate dehydrogenase (GDH, EC 1.4.1.2), malate dehydrogenase (MDH, EC 1.1.1.37) and phosphoglucomutase (PGM, EC 2.7.5.1)—assayed on histidine gels (1,2).

RESULTS

Virulence survey. A total of 1,295 isolates (466 individual collections) of *P. graminis* f. sp. *tritici* and 512 isolates (379 individual collections) of *P. recondita* f. sp. *tritici* were identified during the 1983 cereal rust virulence survey in the United States. These isolates were found to comprise 12 and 22 races of the two pathogens, respectively, and were distributed across eight of the nine ecological areas defined for wheat rust fungi (Fig. 1, lane 11; D. L. Long, *personal communication*). Samples from the remaining area, that west of the Rocky Mountains, were drawn from a sexual population of *P. graminis* f. sp. *tritici* and were excluded from this investigation.

The distribution of the number of isolates amongst these races was very different for the two pathogens. Although 10 races of *P. graminis* f. sp. *tritici* were identified, 70% of the isolates (907) were of one race, race 15-TNM virulent on *Sr*17, 24% (311 isolates) were of race 15-TNM avirulent on *Sr*17, while the next most common race, 151-QCB, comprised 3% of the population (39 isolates). The remaining seven races each comprised a maximum of 1% of the isolates. The race designation code used for *P. graminis* f. sp. *tritici* throughout this work is that developed at the Cereal Rust Laboratory, University of Minnesota. A full description of the host responses represented by the coding is given elsewhere (9,11).

By way of contrast, there were many more races of *P. recondita* f. sp. *tritici* identified during 1983 and no one race dominated the population to the extent of 15-TNM. *P. recondita* f. sp. race UN-5 (virulent on *Lr*1, 3a, and 10) was the most common (26% or 133 isolates) while a further three races UN-2 (virulent on *Lr*3a and 10), UN-3 (virulent on *Lr*2c, 3a, 3b, and 9), and UN-17 (virulent on *Lr*2a, 2c, 3a, and 10) each made up more than 8% of the population (8, 13, and 15% or 41, 67, and 77 isolates, respectively).

Isozyme survey. The stratified sampling system utilized in the sampling of the populations of *P. graminis* f. sp. *tritici* and *P. recondita* f. sp. *tritici* resulted in a wider geographic distribution of isolates (Fig. 1) and a greater representation of rare races than would have resulted from a truly random sample. For example, the most common race of *P. graminis* f. sp. *tritici*, race 15-TNM virulent on *Sr*17, made up 70% of the isolates in the virulence survey but only 55% of those examined electrophoretically. Moreover, common races were sampled from an extremely wide geographic range. Thus, isolates of race 15-TNM were drawn from 15 states (Arkansas, California, Georgia, Illinois, Indiana, Iowa, Kansas, Louisiana, Mississippi, Minnesota, Nebraska, North Dakota, Oklahoma, South Dakota, and Texas) and Canada.

***P. graminis* f. sp. *tritici*.** Half of the enzyme phenotypes examined (GOT, LAP, NADHD, PGI, and PGM) showed variation among the 10 races obtained from the 1983 survey. No variation was found among different isolates of the same race. The remaining five enzymes (ACO, CAT, EST, GDH, and MDH) were invariant (and homozygous) for all isolates. Table 3 shows the number of loci, the number of alleles at each locus, and the R_f values of each of the alleles that were considered. Isozyme phenotypes for the *Pgm*1 locus were not recorded in all cases. While phenotypes are provided where all isolates in a particular group

Fig. 1. The geographical distribution of the isolates of *Puccinia graminis* f. sp. *tritici* that were collected during the 1983 cereal rust survey and examined electrophoretically. Of all the ecological regions from which isolates were received, only those from region 9, the Pacific Northwest, were excluded (12).

were recorded (Fig. 2), phenotypes are not provided for the virulence groupings 11-RHR or 29-32 HJC, RJC where data for all isolates were not recorded. For this reason, values for the *Pgm*1 locus have not been used to distinguish different groups.

The genetic basis of electrophoretic variation of five of these enzymes (GOT, LAP, NADHD, PGI, and PGM) has been studied in a controlled cross. For each, the ratio of progeny classes fitted that expected for Mendelian segregation of alternative alleles at a single locus (J. J. Burdon, A. P. Roelfs, and A. H. D. Brown, *unpublished*).

Electrophoretic examination of the 56 isolates of *P. graminis* f. sp. *tritici* recovered from liquid nitrogen storage (Table 1) revealed isozyme phenotypes that were entirely consistent with those obtained during the electrophoretic survey of the 1983 isolates. Variation among the 23 races was detected for the same five enzyme systems (GOT, LAP, NADHD, PGI, and PGM). Again, no variation was found among different isolates of the same race or between these isolates and those with the same virulence from the 1983 survey.

The basic hypothesis assumed from an examination of the isozyme data obtained from both the 1983 survey isolates and those recovered from liquid nitrogen storage is that isolates of the same virulence race and grouping are electrophoretically identical. The evidence available to support the contention that any particular individual virulence race is isozymically uniform is obviously weak, as only three to five isolates per race were screened. However, in all cases in which more than one isolate of a given virulence race was examined (22 of 23 cases), the isozyme phenotype of each was identical. This was even the case for the two most common races recovered in 1983 (15-TNM virulent and avirulent on *Sr*17) where 30 and 8 isolates, respectively, were examined. Additionally, in cases where direct comparisons were possible between the isozyme phenotypes of races collected in 1983 and those derived from long-term storage (Table 1), there was always a complete coincidence of patterns. Finally, support for the hypothesis is further strengthened if one views the entire data. Thus in a population containing a variant at a frequency of p, the probability of randomly selecting k isolates that fit a predetermined pattern of isozyme groupings is $(1-p)^{k-1}$. These probabilities can be multiplied over all virulence races, so that over the entire set of isolates examined, there is a probability of $(1-p)^{86}$ of selecting 103 isolates that fit a predetermined isozyme pattern ($k-1 = 109-23$). This then gives a 95% confidence limit that a variant within the population at a frequency of 0.034 would have been detected (for a 99% confidence limit the frequency is 0.052).

Taking all the data together it was clear that there were nine distinct isozyme patterns into which all 110 isolates (survey and stored isolates) of *P. graminis* f. sp. *tritici* fitted (Fig. 2). Grouping the isolates on the basis of isozyme phenotypes alone resulted in only closely related races being grouped. Of the 27 distinct resistance genes or alleles for which these races show variable response, the maximum number of virulence differences between any two races with the same isozyme phenotype was 3.0 (between 15-TBM avirulent on *Sr*17 and 15-TNM virulent on *Sr*17, and between 113-RKQ and 113-RPQ), while the average difference was 1.56. Between groups, on the other hand, the maximum number of virulence differences was 21.0 (between 56-MBC and 113-RTQ), while the average difference was 10.90. (The resistance genes involved were: *Sr*5, 6, 7a, 7b, 8, 9a, 9b, 9d, 9e, 10, 11, 12, 14, 15, 17, 21, 23, 30, 35, 36, Tt-3, *Tmp*, X, dp-2, Wst-2, U, and H.) The only

TABLE 3. Number of loci, number of alleles per locus, and the R_f values of all alleles for the pathogens *Puccinia graminis* f. sp. *tritici* and *Puccinia recondita* f. sp. *tritici*

Isozyme system	*P. graminis* f. sp. *tritici* Loci (no.)	Alleles (no.)	R_f	*P. recondita* f. sp. *tritici* Loci (no.)	Alleles (no.)	R_f
ACO	1	1	0.20 a[z]	1	1	0.11 a
CAT	1	1	0.25 b	1	1	0.28 a
EST	2			2		
	Est1	1	0.98 a	*Est1*	1	0.72 a
	Est2	1	0.74 a	*Est2*	2	0.62 a
						0.59 b
GDH	1	1	0.08 a	1	1	0.06 a
GOT	1	3	0.33 a	1	1	−0.03 a
			0.27 b			
			0.23 b			
LAP	1	3	0.49 b	1	1	0.44 a
			0.47 c			
			0.44 d			
MDH	1	1	0.05 a	1	1	0.09 a
NADHD	1	2	0.26 b	1	1	0.13 a
PGI	2		0.16 c	1	1	0.18 a
	Pgi1	1	0.56 a			
	Pgi2	2	0.27 a			
			0.23 b			
PGM	2					
	Pgm1	3	0.59 a	1	1	0.20 a
			0.56 b			
			0.53 c			
	Pgm2	2	0.28 b			
			0.21 c			

[z] Alphabetic designation of alleles with letters assigned according to a scale of decreasing electrophoretic mobility. Letter designation has been determined from these and other cultures (*unpublished*).

exception to this pattern was found in races 11-RCR and 11-RHR. In terms of their virulence, these two races are very similar (differing in virulence for *Sr*6 and *Sr*30 only). Isozymically, however, they were quite distinct (Fig. 2: see *Got, Lap*, and *Pgm2*).

***P. recondita* f. sp. *tritici*.** All but one of the 10 enzyme systems examined were invariant (and homozygous) for all isolates of *P. recondita* f. sp. *tritici*. The remaining system, EST, was polymorphic at the *Est*2 locus. Table 3 shows the number of loci, the number of alleles at each locus, and the R_f values of all of the bands observed. Isozymic examination of 22 isolates collected in previous years (and stored in liquid nitrogen) produced the same results, there being only two distinct isozyme patterns in the North American *P. recondita* f. sp. *tritici* population (Fig. 2). Although the number of isolates of each race of *P. recondita* f. sp. *tritici* examined was often small (Table 2), the total sample size was sufficient to provide a general picture of the level of electrophoretic diversity in this pathogen.

The two subpopulations of *P. recondita* f. sp. *tritici* defined by the observed difference at the *Est2* locus, i.e., races/isolates homozygous for the electrophoretically most mobile '*a*' allele and those heterozygous at this locus, '*ab*,' also divided the pathogen population on virulence grounds (Table 2). The *Est2* '*aa*' subpopulation consisted of isolates either avirulent or virulent to both *Lr*2a and *Lr*2c. The *Est2* '*ab*' population consisted of isolates avirulent to *Lr*2a but virulent to *Lr*2c. Other commonly occurring virulences appear to fall at random within these two isozymic populations. The only exceptions to this pattern were race UN-6 (two isolates), which had an *aa* isozyme phenotype and was avirulent to *Lr*2a and virulent to *Lr*2c, and race UN-9 (one isolate), which had an *ab* isozyme phenotype and was avirulent to both of these resistance alleles.

DISCUSSION

Population structure of *P. graminis*. In the virulence survey of the population of *P. graminis* f. sp. *tritici* occurring in the eastern and central United States in 1983, 12 races were recorded in a total sample of 1,295 isolates. The frequency distribution of these virulence races was extremely uneven, with the most common race comprising 70% of isolates, the three most common accounting for 97% of isolates, while the remaining nine races occurred with a combined frequency of only 3%.

Considerable variation was also detected in isozyme phenotypes in this population of *P. graminis* f. sp. *tritici*. Six isozyme phenotypes were found among the 10 races recorded in the 1983 virulence race survey (see Table 1 and Fig. 2). No isozymic differences were detected between isolates of the same virulence race, or between the eight isolates of races 15-TNM avirulent on *Sr*17 and the 30 of 15-TNM virulent on *Sr*17, between the single isolates of races 17-HDL and 17-HNL, or races 56-MBC avirulent on *SrTmp* and 56-MBC virulent on *SrTmp* or between races 151-QBC and 151-QFB (four and two isolates, respectively; see Table 1). Isozymic examination of 20 isolates of these races collected previously (1956 onward) showed that isolates with the same virulence phenotype had the same isozyme phenotype regardless of when they were originally collected.

A further extension of the isozyme survey to include multiple isolates of 13 other races (Table 1), which were not collected in the 1983 survey either because of their very low frequency in the population, or because they are no longer extant, revealed a further three isozyme phenotypes. The nine distinct isozyme phenotypes detected in the population of *P. graminis* f. sp. *tritici* of the eastern and central United States contrasts with the eight virulence pheno-

Fig. 2. Diagrammatic representation of the nine isozyme phenotypes of *Puccinia graminis* f. sp. *tritici* and the two of *Puccinia recondita* f. sp. *tritici* detected in asexual populations of these species in the United States. *Cereal Rust Laboratory race codes for these groups are, from left to right: 11-RC, 11-RH; 15-T_; 17-HD, HN; 29-32 HJ, RJ; 56-M; 113-RK, RP, RT; 151-QC, QF; and 151-32 QS, RS. **Alphabetic designation of alleles, with alleles being assigned letters according to a scale of decreasing electrophoretic mobility. 'a' Is the most mobile. Letter designation has been determined from studies of these and other cultures (J. J. Burdon and A. P. Roelfs, *unpublished*). The relative electrophoretic mobilities of isozyme bands of the two pathogens are given in Table 3. + Incomplete data set; ++ Not present in *Puccinia recondita* f. sp. *tritici*.

type groupings which had been recognized previously (12; and A. P. Roelfs, *unpublished*). Within these virulence groupings, individual races differ at only one to three virulence loci, while, with only one exception, all races within the same virulence group had the same isozyme phenotype (Table 4). The only exception was found between races 11-RCR and 11-RHR. These two races differ solely in their virulence to *Sr*6 and *Sr*30. Isozymic analysis of these two races reveals, however, that they are genetically quite distinct. Race 11-RCR is heterozygous at the *Lap, Got,* and *Pgm2* loci while Race 11-RHR is homozygous at the *Lap* and *Pgm2* loci and heterozygous for one different allele at the *Got* locus (Fig. 2).

An examination of the relationships determined among the various pathogen races in the population of *P. graminis* f. sp. *tritici* clearly indicates that virulence and isozyme analysis are two complementary, yet distinct, methods of assessing pathogen populations (Table 4). A comparison of mean virulence and isozyme differences between groups found no correlation between the two methods of analysis ($r = 0.1890$) and in fact, the same level of isozymic dissimilarity (5.00, the maximum value detected) was found between the third most closely related virulence groups (11-RCR and 29-32- _JC) and the most dissimilar ones (56-MBC and 113-R_Q). Bearing in mind these differences, it is reassuring that these separate systems of analysis should divide the pathogen population into virtually the same clonal groups. At the same time, however, isozyme analysis offers new insight, in revealing that the race 11 virulence phenotypes are drawn from two pathogen clones.

Potential sources of variation. One intriguing and important question that inevitably arises in a consideration of these subpopulations within *P. graminis* f. sp. *tritici* is that of their origin. In general, there are four ways in which variation may develop and be maintained in rust pathogen populations; mutation, somatic hybridization, introduction from elsewhere, and sexual recombination. Mutation at loci governing virulence has almost certainly been the origin of the limited amount of variation detected within the nine different isozyme-virulence phenotype groupings. It is extremely unlikely that the large virulence differences among the nine subpopulations arose by a similar process from a single ancestral form. Similarly, although somatic hybridization has frequently been induced in the laboratory (5,13) there are only two documented cases of this process leading to the occurrence of new races in the field (1,7,14). While somatic hybridization cannot be totally eliminated as a source of some of the nine *P. graminis* f. sp. *tritici* clones under consideration, its apparent rarity makes this explanation unlikely. Introduction of new races from overseas has been a major source of new variation in the Australian *P. graminis* f. sp. *tritici* flora on at least three occasions (2,14). In the present North American population, however, while some of the subpopulation groups may have arisen through accidental introduction, possible sources of such introductions are unknown as similar combinations of virulence and isozyme phenotype have not been detected elsewhere in the world (*unpublished*).

Overall, it seems that the most likely source of the different groups of clones is to be found in the process of sexual recombination. The eradication of common barberry (*Berberis vulgaris* L.) from the wheat fields of eastern and central United States was completed for all practical purposes by 1928 (9). As a result, the present groups could not have arisen recently through sexual recombination. However, at least some of them probably represent biotypes that were prevalent in the *P. graminis* f. sp. *tritici* population at the time it was deprived of its sexual cycle (12). If this is the major source of variation in this population of *P. graminis* f. sp. *tritici*, then presumably other phenotypes extant at the end of the 1920s have fallen by the wayside over the intervening years.

Comparison of *P. graminis* f. sp. *tritici* in North America and Australia. The only previously published data concerning the isozymic structure of fungal pathogen populations is that of a similar survey of *P. graminis* f. sp. *tritici* in Australia (3). While a comparison of allele frequencies in the two populations show some differences with respect to particular alleles (Table 5), the allelic diversity of the two is very similar. However, genetic diversity measures of this type are generally not appropriate for comparisons of two asexually reproducing populations. The Australian population of *P. graminis* f. sp. *tritici* is, in fact, composed of a single multilocus phenotype while the North American population is composed of nine.

Studies of *P. graminis* f. sp. *tritici* in Australia over the past 60 years have detected periodic, massive shifts in the virulence pattern of the population as new phenotypes invade and supplant preexisting ones (14). Only over very short periods of time has more than one clonal line been present in the population. In North America, on the other hand, nine different isozyme phenotypes appear to have coexisted at least since the mid-1950s (and probably

TABLE 4. A comparison of the relationships within and between the various virulence phenotype groupings with respect to both virulence and isozyme phenotype.

	11-RCR	11-RHR	15-T_M	17-H_L	29-32_JC	56-MBC	113-R_Q	151-Q_B	151-32_SH
Mean virulence differences[a]									
11-RCR	0.00								
11-RHR	2.00	0.00							
15-T_M	8.00	10.00	1.80						
17-H_L	8.50	10.50	9.10	0.50					
29-32_JC	7.50	7.50	10.30	7.00	0.50				
56-MBC	10.50	12.50	13.50	15.00	10.00	0.50			
113-R_Q	11.00	11.40	14.12	9.70	10.30	19.50	0.50		
151-Q_B	11.50	13.50	17.10	11.00	10.00	14.00	9.30	0.50	
151-32_SH	8.50	8.50	12.10	12.00	10.00	14.00	10.70	10.00	0.50
Mean isozyme dissimilarity[b]									
11-RCR	0.00								
11-RHR	3.00	0.00							
15-T_M	4.00	4.00	0.00						
17-H_L	2.00	3.00	1.00	0.00					
29-32_JC	5.00	2.00	5.00	4.00	0.00				
56-MBC	5.00	3.00	4.00	4.00	1.00	0.00			
113-R_Q	3.00	3.00	1.00	1.00	5.00	5.00	0.00		
151-Q_B	5.00	5.00	3.00	3.00	3.00	3.00	2.00	0.00	
151-32_SH	4.00	1.00	4.00	3.00	1.00	2.00	4.00	4.00	0.00

[a] Mean virulence differences within and between phenotype groupings. Comparisons were based on the 27 distinct resistance genes or alleles for which these races varied.

[b] Mean isozyme dissimilarity within and between different phenotype groupings. Comparisons were determined on all isozyme loci (Fig. 2) except *Pgm1* and expressed as the number of different alleles present at any individual locus (maximum value of two) summed over all loci (24.0 indicates total dissimilarity).

TABLE 5. Isozyme allele frequencies in the 1981 Australian and 1983 North American populations of *Puccinia graminis* f. sp. *tritici*

Enzyme locus	Allele frequencies							
	Australia[a]				North America[a]			
	a	b	c	d	a	b	c	d
Aco	-[b]	-	-	-	1.00	···	···	···
Cat	···	1.00	···	···	···	1.00	···	···
Est1	1.00	···	···	···	1.00	···	···	···
Est2	1.00	···	···	···	1.00	···	···	···
Gdh	1.00	···	···	···	1.00	···	···	···
Got	···	···	1.00	···	†[d]	···	0.99	†
Lap	0.50	···	0.50	···	···	0.47	†	0.52
Mdh	1.00	···	···	···	1.00	···	···	···
Nadhd	···	0.50	0.50	···	···	0.48	0.52	···
6-Pgd	1.00	···	···	···	···	-	-	-
Pgi1	···	-	-	-	1.00	···	···	···
Pgi2	0.50	0.50	···	···	0.52	0.48	···	···
Pgm1	···	-	-	-	0.94	0.04	0.02	···
Pgm2	···	0.50	0.50	···	···	0.99	†	···

[a] The data for the Australian population is derived from Burdon et al (3). Six different isozyme phenotypes were recorded in North America and one in Australia in the respective years. Isozyme genotypes of the different races were weighted by racial abundance.

[b] Systems were not evaluated in Australia.

[c] Not detected.

[d] Present at frequency <0.01.

since the late 1920s). Why different clonal lines coexist in North American and not in Australia is difficult to determine but may be related to the obvious requirement that a new line must have some selective advantage in at least some environments to become established in the first place, the possibility that different clones have been selected at various times by different resistant cultivars and thus that present day coexistence is a relic of past cultivar use, and differences in off-season survival. There is some evidence in North America that different clonal lines of *P. graminis* f. sp. *tritici* may overwinter in different parts of the southern United States. For example, early in the season, race 11-RCR is proportionally more common in Florida while race 15-TNM seems to survive in southern Texas and northern Mexico (A. P. Roelfs, *unpublished*). Nothing is known about this sort of possibility in Australia.

Population structure of *P. recondita* f. sp. *tritici*. In contrast to *P. graminis* f. sp. *tritici*, the population of *P. recondita* f. sp. *tritici* occurring in North America showed a much greater diversity of virulence phenotypes while being isozymically much more uniform. In a total sampling of 512 isolates, 22 races of *P. recondita* f. sp. *tritici* were detected. None of these dominated the population to the same extent as *P. graminis* f. sp. *tritici* race 15-TNM, virulent on *Sr*17 (the frequency of the most common race of *P. recondita* f. sp. *tritici*, race UN-5 was 26%). The population is composed of two isozyme phenotypes, those homozygous at *Est2* (*aa*) and those heterozygous at the same locus (*ab*). Commonly occurring virulences appear to fall at random within these two isozymic subpopulations except for those relating to *Lr*2 alleles.

As a result of extensive studies of the wheat cultivars Brevit, Carina, Loros, and Webster, Dyck and Samborski (4) concluded that three alleles for resistance of *P. recondita* f. sp. *tritici* occur at the *Lr*2 locus. They also suggested that this pathogen has two systems for virulence corresponding to these alleles. In one of these, a single recessive gene *p*2 overcomes the resistance at all alleles, while the second differentially affects the different alleles. When the North American population of *P. recondita* f. sp. *tritici* is subdivided into its two isozyme phenotypes, the *Est2 aa* subpopulation consists of isolates either avirulent or virulent to both *Lr*2a and *Lr*2c while the *Est2 ab* group consists of isolates avirulent to *Lr*2a but virulent to *Lr*2c. The only exceptions to this pattern were two isolates that were avirulent to *Lr*2a and virulent to *Lr*2c but had an *aa Est2* phenotype, and one isolate which was avirulent to both *Lr*2a and *Lr*2c but had an *ab Est2* phenotype. Despite these exceptions, a 2×2 contingency table comparing *Lr*2 virulence with the two *Est2* phenotypes found a significant association between the *Est2 ab* phenotype and the combination of avirulent to *Lr*2a and virulence to *Lr*2c, while the *Est2 aa* phenotype was significantly associated with the combination of either avirulent or virulence at both these loci (Table 2; $\chi_1^2 = 53.11$, $P < 0.005$). The origin of these two different isozyme phenotypes is open to speculation. However, as there has never been a functional sexual cycle for *P. recondita* f. sp. *tritici* in the United States, the simplest explanation is restricted to a choice between two separate introductions from elsewhere or a single introduction followed by a mutation at the *Est2* locus shortly thereafter. Available data do not allow discrimination between these two options.

A comparison of populations of *P. recondita* f. sp. *tritici* in Australia and North America shows a much greater similarity between the two populations than was found in the case of *P. graminis* f. sp. *tritici*. In Australia, the pathogen population is isozymically homozygous and uniform at all loci examined (3; subsequent work has shown the two *Est* loci considered heterozygous in that paper to be homozygous), while the same is the case in North America except for the *Est2* locus where phenotype frequencies were *aa*, 0.636; *ab*, 0.364.

The virtual lack of isozymic variation in both populations of *P. recondita* f. sp. *tritici* or in the Australian population of *P. graminis* f. sp. *tritici* contrasts markedly with the considerable variation found in the asexual North American population of *P. graminis* f. sp. *tritici*. Of the four populations, the first three have never possessed a functional sexual cycle while the fourth, although now entirely asexual, was part of a sexually reproducing population until the late 1920s. A case can be made to suggest that much of the variation occurring in the present asexual North American population of *P. graminis* f. sp. *tritici* dates back to that time—a clear indication of the impact that sexual recombination may have on the present and future structure of a population.

LITERATURE CITED

1. Burdon, J. J., Marshall, D. R., and Luig, N. H. 1981. Isozyme analysis indicates that a virulent cereal rust pathogen is a somatic hybrid. Nature 293:565-566.
2. Burdon, J. J., Marshall, D. R., Luig, N. H., and Gow, D. J. S. 1982. Isozyme studies on the origin and evolution of *Puccinia graminis* f. sp. *tritici* in Australia. Aust. J. Biol. Sci. 35:231-238.
3. Burdon, J. J., Luig, N. H., and Marshall, D. R. 1983. Isozyme uniformity and virulence variation in *Puccinia graminis* f. sp. *tritici* and *P. recondita* f. sp. *tritici* in Australia. Aust. J. Biol. Sci. 36:403-410.
4. Dyck, P. L., and Samborski, D. J. 1974. Inheritance of virulence in *Puccinia recondita* on alleles at the *Lr*2 locus for resistance in wheat. Can. J. Genet. Cytol. 16:323-332.
5. Ellingboe, A. H. 1961. Somatic recombination in *Puccinia graminis* var. *tritici*. Phytopathology 51:13-15.
6. Luig, N. H., and Watson, I. A. 1970. The effect of complex genetic resistance in wheat on the variability of *Puccinia graminis* f. sp. *tritici*. Proc. Linn. Soc. N.S.W. 95:22-45.
7. Luig, N. H., and Watson, I. A. 1972. The role of wild and cultivated grasses in the hybridization of formae speciales of *Puccinia graminis*. Aust. J. Biol. Sci. 25:335-342.
8. Maheshwari, R., and Sussman, A. S. 1970. Respiratory changes during germination of urediospores of *Puccinia graminis* f. sp. *tritici*. Phytopathology 60:1357-1364.
9. Roelfs, A. P. 1982. Effects of barberry eradication on stem rust in the United States. Plant Dis. 66:177-181.
10. Roelfs, A. P. 1985. Epidemiology in North America. In: The Cereal Rusts. Vol. II. A. P. Roelfs and W. R. Bushnell, eds. Academic Press, New York. (In press).
11. Roelfs, A. P., Casper, D. H., and Long, D. L. 1984. Races of *Puccinia graminis* in the United States and Mexico during 1983. Plant Dis. 68:902-905.
12. 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.
13. Watson, I. A. 1957. Further studies on the production of new races from mixtures of races of *Puccinia graminis* var. *tritici* on wheat seedlings. Phytopathology 47:510-512.
14. Watson, I. A. 1981. Wheat and its rust parasites in Australia. Pages 129-147 in: Wheat Science-Today and Tomorrow. L. T. Evans and W. J. Peacock, eds. Cambridge University Press, Cambridge, England.