The Effect of Sexual and Asexual Reproduction on the Isozyme Structure of Populations of *Puccinia graminis*

J. J. Burdon and A. P. Roelfs

Senior research scientist, Division of Plant Industry, CSIRO, P.O. Box 1600, Canberra City, A.C.T. 2601 Australia; and research plant pathologist, Cereal Rust Laboratory, Agricultural Research, U.S. Department of Agriculture, University of Minnesota, St. Paul 55108.
Cooperative investigation, U.S. Department of Agriculture and the University of Minnesota. J. J. B. acknowledges with gratitude, support provided by the Australian-American Education Foundation, the E. C. Stakman Visiting Professorship Endowment, and the Australian Wheat Industry Research Council. This research was supported in part by USDA Competitive Grant 59-2271-1-1-687-0. Scientific Journal Series Paper 13,968, Minnesota Agricultural Experiment Station.
Accepted for publication 11 April 1985.

ABSTRACT

Burdon, J. J., and Roelfs, A. P. 1985. The effect of sexual and asexual reproduction on the isozyme structure of populations of *Puccinia graminis*. Phytopathology 75:1068-1073.

Isozyme markers were used to compare the genetic structure of a sexually and an asexually reproducing population of the wheat stem rust pathogen occurring in the United States in 1972, 1975, and 1976. The structure of the sexual population was determined directly by electrophoretic analysis of individual isolates collected in the appropriate year and stored since in liquid nitrogen. The structure of the asexual population was estimated from a known association of particular isozyme and virulence phenotypes. In all 3 yr, the sexual population was always more diverse than the asexual one whether this was measured by allelic richness, Nei's gene diversity index, or by the frequency distribution of the number of heterozygous loci per individual. Moreover, the genetic structure of the sexual population approximated to that expected under panmixis (random mating) whereas that of the asexual population showed marked deviations. These deviations were associated with the clonal nature of the asexual population and the apparently complete association of isozyme and virulence phenotype. By contrast, no association was detected between individual isozyme alleles or between individual isozyme alleles and virulence genes in the sexual population.

Additional key words: *Puccinia graminis* f. sp. *tritici*, pathogenicity.

The development and maintenance of genetic variation in wheat stem rust pathogen populations is controlled by the combined effects of mutation, somatic hybridization and recombination, sexual recombination, and introduction from other populations (5). The relative contribution of these different mechanisms to the existing genetic structure of a population is of considerable importance in determining the rapidity and effectiveness with which individual pathogen populations may adapt to changing environmental circumstances. In *Puccinia graminis* f. sp. *tritici*, populations exist in which the sexual stage has never occurred, in which the sexual stage ceased to be functional about 50 yr ago, and in which the sexual stage is a vital part of winter survival every year. As a result, comparisons among such populations provide a unique opportunity to investigate the role of sexual recombination in maintaining variability within populations.

In Australia, where the sexual stage of the life cycle of *P.g.* f. sp. *tritici* has never occurred (16), the virulence and isozyme structure of the population periodically undergoes a massive change as a new pathogen race invades and supplants the pre-existing types. In this new population a high level of diversity in virulence is achieved and maintained through mutation at loci governing virulence (3). In contrast, the North American asexual population occurs in an area where the sexual cycle was an important part of the life cycle until the eradication of common barberry in the late 1930s (11). In this population, diversity in both isozyme and virulence characters occur. The limited diversity of isozyme phenotypes most probably represents clonal lines that have descended unchanged from biotypes prevalent in the pathogen population when its sexual cycle was interrupted (6,11). Part of the diversity in virulence is also derived from these clonal lines and part from a process of mutation within these lines at loci governing virulence.

The combined results of electrophoretic and virulence studies of these two populations of *P.g.* f. sp. *tritici* (3,6,11,16) provide some circumstantial evidence concerning the importance of sexual processes in maintaining variation. However, although a comparison of the virulence diversity of two North American populations, one reproducing sexually and the other asexually, has already shown greater diversity for virulence in the sexual population (11), no direct information is available concerning the effectiveness of sexual reproduction in maintaining variation in characters like isozyme alleles.

The purpose of the study reported here was to address this problem by examining the effect of the two different modes of reproduction (asexual and sexual) on the level and diversity of

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.

isozyme variation found within the respective North American populations of *P.g.* f. sp. *tritici*.

MATERIALS AND METHODS

Virulence survey. The data used in this paper are from the 1972, 1975, and 1976 annual wheat stem rust surveys (12–14) with particular emphasis being placed on that from 1975, the year in which the sexual population was best represented. The collections representing the asexual population were obtained from a variety of sources throughout northern Mexico, the United States east of the Rocky Mountains, and southern Canada. Those representing the sexual population, on the other hand, were obtained from a very limited area of the Pacific Northwest (11). Most of the latter collections were made within one or two asexual generations of a sexual generation.

Upon receipt, isolates were evaluated on 16 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*. Details of the procedure adopted during virulence evaluation are described elsewhere (6).

Isozyme survey. *Asexual population.* A previous study of isozyme and virulence variation in the North American asexual population of *P.g.* f. sp. *tritici* over the past 25 yr (6) revealed a complete association between isozyme phenotypes and nine major virulence groupings occurring within the population. Within each major virulence grouping, all individual isolates had exactly the same isozyme phenotype. On the other hand, each virulence grouping differed from every other by a minimum of one isozyme allele. These findings were based on an electrophoretic analysis of 110 separate isolates of *P.g.* f. sp. *tritici* representing 23 different virulence races within the nine major groups (6). While this inevitably meant that on average only three to five isolates per race were screened, no differences were detected among isolates of any given race derived from long-term storage, from a survey of the extant population, or from among isolates of the two most common races present in the asexual population in 1983 (30 and 8 isolates of 15-TNM virulent and avirulent on *Sr*17, respectively). Furthermore, investigation of the likelihood of all isolates examined fitting a specific set of isozyme patterns showed a 95% chance of detecting any electrophoretic variant occurring within the asexual population with a frequency of 0.034 or greater (for a 99% chance of detection the frequency is 0.052) (6).

In the present study, this association of virulence and isozyme phenotype patterns has been used to predict, from their virulence formulae, the isozyme phenotypes of all isolates of *P.g.* f. sp. *tritici* collected from the asexual population during the 1972, 1975, and 1976 cereal rust surveys. Even if the assumption of complete association between virulence and isozyme phenotypes is invalid, the frequency of other isozyme variants is likely to be too low to materially affect the genetic measures used here (Nei's gene diversity index and Wright's fixation index) to compare the asexual and sexual populations of *P.g.* f. sp. *tritici*.

Sexual population. The approach used to determine the isozyme phenotypes of individual members of the asexual population could not be utilized in the sexual population because of the high probability that random recombination of virulence and isozyme alleles would produce individuals with the same virulence phenotype but dissimilar isozyme phenotypes (or vice versa).

To study isozyme variation in the sexual population, therefore, 21, 92, and 44 separate isolates of the sexual population occurring in the Pacific Northwest in 1972, 1975, and 1976, respectively, were recovered from liquid nitrogen storage and their purity and racial identity were checked on the differential set of 16 host lines. Starch gel electrophoresis was then performed on homogenized, germinated spores of these isolates, by using a procedure described elsewhere (6). The following enzymes were investigated: aconitate hydratase (ACO, EC 4.2.1.3), catalase (CAT, EC 1.11.1.6), arylesterase (EST, EC 3.1.1.2), aminopeptidase (synonym leucine aminopeptidase; 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.

Although the isolates examined electrophoretically represented only a portion of the total number received and identified in any particular year, comparison of the number and percentage of isolates virulent on individual stem rust resistance genes from the original 1975 survey sample with those recovered from liquid nitrogen storage showed little difference (Table 1). For the 15 *Sr* genes listed in Table 1, the two samples differed significantly ($P<0.05$) only in the frequency of individuals virulent for *Sr*16.

RESULTS

A total of twelve isozyme loci in ten different enzyme systems were investigated in this study. Of these, the *Aco*, *Cat*, *Est1*, *Gdh*, and *Mdh* loci were monomorphic and invariant in both the asexual and sexual populations of *P.g.* f. sp. *tritici*. The genotypic frequencies of the seven remaining enzyme loci which were variable in at least one of the two populations are given in Table 2. A comparison of the frequency of alleles at these loci shows marked differences in both the magnitude and consistency of changes in the asexual and sexual populations.

In the asexual population, the assumption of electrophoretic invariance within races has allowed the estimation of isozyme allele frequencies in the original survey population. While this will not allow for any electrophoretically variant types, the argument developed earlier indicates the frequency of these would be too low to materially affect changes in the frequency of the commonest alleles. The resultant large sample sizes do, however, mean that virtually all changes in the frequency of alleles in the asexual population are statistically highly significant (Table 3). In Table 3 the degree of heterogeneity among the different years (viewed as subpopulations) has been measured by determining diallelic estimates of the coefficient of subpopulation structure F_{ST} (17), based on the most common allele, for each of the variable loci. In

TABLE 1. Number and percentage of isolates from the 1975 sexual population of *Puccinia graminis* f. sp. *tritici* virulent for selected *Sr* genes

		Number and percentage of isolates virulent on indicated *Sr* gene:														
		5	6	7b	8	9a	9b	9d	9e	10	11	15	16	17	36	*Tmp*
Virulence survey population[a]	No.	293	4	81	89	267	4	293	17	411	17	394	271	318	81	13
	%	69	1	19	21	63	1	69	4	97	4	93	64	75	19	3
Isozyme Subsample[b]	No.	59	0	19	23	53	0	56	2	89	0	85	48	71	10	0
	%	64	0	21	25	58	0	61	2	97	0	92	52	77	11	0
χ_1^2[c]		0.86	0.87	0.12	0.72	0.92	0.87	2.34	0.72	0.01	3.81	0.03	4.42*[d]	0.19	3.53	2.89

[a] Population sample examined during routine virulence survey.
[b] Those individuals examined in the isozyme survey.
[c] Results of Chi-square tests for significance of differences between the two samples.
[d] Asterisk indicates statistical significance, $P<0.05$.

the asexual population these values are all statistically significant, indicating considerable heterogeneity among the 3 yr in the frequency of the various isozyme alleles.

In the sexual population, allele frequencies were more even over the 3 yr of the study with only those at the *Est2* showing significant heterogeneity (Table 3) despite consistent, if small changes, in the frequency of other alleles (e.g., *Lap* c, *Pgi2* a, and *Pgm1* b).

The main feature of interest in this study is the effect that different modes of reproduction have on the level of diversity within populations of *P.g.* f. sp. *tritici*. To determine this, various measures of genetic diversity were assessed in the asexual and sexual populations. The first of these, Nei's gene diversity index (h), is given by the equation: $h = 1 - \Sigma x_i^2$, in which x_i is the frequency of the i-th allele (10). This provides a composite measure of both the number of alleles present (allelic "richness") and the evenness of distribution of individuals among the various alleles (2). For the majority of loci, h values were greater in the sexual than the asexual population although the reverse was true for the *Nadhd* and *Pgi2* loci (Table 4). This reflects both the general tendency for a greater number of alleles per locus in the sexual population (3.0 versus 2.3 in the asexual population) and a more even allelic distribution. At the two loci where h values were greater in the asexual population (*Nadhd* and *Pgi2*), the number of alleles present was the same in both populations, but in the asexual population the high frequency of race 15-TN (heterozygous at both loci) resulted in a more even distribution of allele frequencies.

At the level of the individual locus, h values were of similar magnitude in all 3 yr and were consistently and statistically different between the two populations (Table 4). At the level of the population as a whole, however, mean h values for all seven variable loci were consistently but not statistically greater in the sexual population. Even if mean h values are determined over all 13 loci examined, differences between the two populations still remain nonsignificant, although standard errors are necessarily smaller [h (1975) = 0.155 ± 0.229 and 0.197 ± 0.251 for the asexual and sexual populations, respectively].

Another important feature of a population is the number of heterozygotes present at each sampling time. In assessing heterozygosity on a gene-by-gene basis, quite marked differences were detected between the asexual and sexual populations (Table 5). The average of the observed heterozygosities over all the variable loci assayed showed a steady, but statistically nonsignificant, increase in the asexual and decrease in the sexual population. In many cases, however, the behavior of the frequency of individuals which were heterozygous for any particular locus showed significant heterogeneity between years. This was particularly the case in the asexual population where the large sample size which resulted from the isozyme genotype prediction procedure resulted in statistically significant fluctuations ($P = 0.01$) being detected in the frequency of individuals heterozygous at any locus over the study period (Table 5). In the sexual population, on the other hand, only the frequency of individuals heterozygous at the *Est2* locus varied significantly (Tables 3 and 5).

Heterozygosity within a population may also be assessed in terms of the genotype as a whole—that is, the number of loci for which any individual is heterozygous. A comparison of the frequency distribution of the number of heterozygous loci per individual in the two populations of *P.g.* f. sp. *tritici* in 1975 shows a marked difference (Fig. 1). In the asexual population, an overwhelming proportion of individuals (78.5%) were heterozygous at three loci (mean number, 2.73). No individuals were totally homozygous nor were any heterozygous for more than five loci. In the sexual population, on the other hand, the frequency of individuals in the different heterozygous loci categories approximated a normal distribution (mean number, 2.08), with highly heterozygous (six loci) and totally homozygous individuals present.

So that differences in the genotypic distribution could be detected separately from differences caused by a change in gene frequency, composite values of Wright's Fixation index (F) were estimated (Table 6). These F values were computed by dividing the observed frequency of heterozygotes by the expected frequency and

TABLE 2. Allele frequencies for the seven variable enzyme loci studied in the asexual[a] and sexual populations of *Puccinia graminis* f. sp. *tritici* occurring in the United States in 1972, 1975, and 1976

Enzyme locus	Date	Allele frequencies in asexual population[b]					Allele frequencies in sexual population[b]				
		a	b	c	d	n[c]	a	b	c	d	n[c]
Est2	1972	1.00				1,925	0.71	0.29			21
	1975	1.00				2,356	0.96	0.04			89
	1976	1.00				1,667	0.83	0.17			41
Got	1972	0.04		0.96	—[d]	1,925	0.43		0.57		14
	1975	0.05		0.95	—[d]	2,356	0.32		0.67	0.01	76
	1976	0.02		0.98		1,667	0.37		0.63		37
Lap	1972		0.28	0.07	0.65	1,925		0.43	0.45	0.12	21
	1975		0.39	0.10	0.51	2,356	—[d]	0.36	0.30	0.34	92
	1976		0.44	0.04	0.52	1,667		0.48	0.28	0.24	44
Nadhd	1972		0.39	0.61		1,925		0.03	0.97		20
	1975		0.46	0.54		2,356		0.04	0.96		90
	1976		0.47	0.53		1,667		0.01	0.99		44
Pgi2	1972	0.64	0.36			1,925	0.86	0.14			21
	1975	0.59	0.41			2,356	0.84	0.16			92
	1976	0.55	0.45			1,667	0.82	0.18			44
Pgm1	1972	0.55	0.34	0.11		1,925	0.22	0.50	0.28		16
	1975	0.79	0.16	0.05		2,356	0.23	0.63	0.12	0.02	70
	1976	0.88	0.09	0.03		1,667	0.19	0.66	0.12	0.03	16
Pgm2	1972		1.00	—[d]		1,925		0.69	0.31		21
	1975		0.99	0.01		2,356	—[d]	0.59	0.40	—[d]	92
	1976		1.00			1,667	0.01	0.65	0.33	0.01	44

[a] Data for the asexual population were calculated from a knowledge of the isozyme phenotypes of the clones comprising the population (5).
[b] Alleles are labeled (a, b, c, d) in order of decreasing electrophoretic mobility and account for alleles known to occur, but not present in these populations.
[c] Number (n) of isolates studied.
[d] Present at frequencies less than 0.01.

subtracting this quotient from unity. The value of F expected under panmixia (random mating and no selection) is zero, an increasing deficiency in the number of heterozygotes gives F values that are negative.

In the sexual population, no significant changes occurred in the F values of individual loci over the period of the study. In addition, none of these values deviated significantly from that expected under a random mating, no selection model (zero). In the asexual population, on the other hand, there was a significant change in F values for the *Lap* (−0.121 to −0.393 to −0.634), *Nadhd* (−0.650 to −0.840 to −0.876) and *Pgi2* (−0.565 to −0.704 to −0.807) loci. Moreover, the F values obtained for these and the *Pgm1* locus all differed significantly from zero (Table 6).

TABLE 3. The degree of heterogeneity in allele frequencies among years in the asexual and sexual populations of *Puccinia graminis* f. sp. *tritici* as measured by diallelic estimates of the coefficient of subpopulation structure (Wright's F_{ST})

Enzyme locus	Asexual population		Sexual population	
	F_{ST}[a]	Common allele	F_{ST}[a]	Common allele
Est2	...	...	0.085**	a
Got	0.003**	c	0.004	c
Lap	0.017**	d	0.011	b
Nadhd	0.004**	c	0.005	c
Pgi2	0.005**	a	0.001	a
Pgm1	0.090**	a	0.010	b
Pgm2	0.002**	b	0.006	b

[a] Asterisks indicate statistical significance, $P<0.01$; chi-square values were calculated according to the formula: $\chi_2^2 = 2\ N_T F_{ST}$ in which N_T is the total population size summed over all 3 yr (see 17).

TABLE 4. Nei's gene diversity index values (h) and standard error (SE(h)) of these for all seven variable loci studied in the asexual and sexual populations of *Puccinia graminis* f. sp. *tritici* occurring in the United States in 1972, 1975, and 1976

Year and enzyme locus	*P. g.* f. sp. *tritici* population			
	Asexual		Sexual	
	h	[a]*SE(h)	h	[a]*SE(h)
1972				
Est2	0.000	...	0.408	0.060
Got	0.073	0.006	0.490	0.027
Lap	0.495	0.007	0.598	0.032
Nadhd	0.478	0.003	0.049	0.047
Pgi2	0.461	0.004	0.245	0.077
Pgm1	0.566	0.005	0.623	0.044
Pgm2	0.002	0.001	0.428	0.054
Mean	0.296	0.257	0.406	0.202
1975				
Est2	0.000	...	0.075	0.027
Got	0.097	0.006	0.446	0.028
Lap	0.575	0.004	0.668	0.005
Nadhd	0.496	0.001	0.075	0.027
Pgi2	0.485	0.003	0.266	0.037
Pgm1	0.348	0.008	0.537	0.037
Pgm2	0.010	0.002	0.491	0.016
Mean	0.287	0.247	0.365	0.232
1976				
Est2	0.000	...	0.284	0.055
Got	0.045	0.005	0.464	0.030
Lap	0.537	0.003	0.635	0.023
Nadhd	0.498	0.001	0.022	0.022
Pgi2	0.494	0.002	0.298	0.052
Pgm1	0.220	0.009	0.518	0.085
Pgm2	0.000	...	0.471	0.036
Mean	0.256	0.249	0.385	0.201

[a] *SE(h) for individual loci calculated using the approach of Simpson (15). The SE(h) mean is an empirically derived value for the h values for the seven variable loci.

DISCUSSION

The data presented here support the general conclusion that considerable differences exist between the genetic structure of the asexual and sexual populations of *P.g.* f. sp. *tritici* occurring in the United States. The diversity of the sexual population was consistently greater than that of the asexual population whether this was measured by unweighted (e.g., mean number of alleles per

TABLE 5. The frequency of heterozygous individuals of *Puccinia graminis* f. sp. *tritici* in the asexual and sexual populations of this pathogen in the United States in the years 1972, 1975, and 1976

Enzyme locus	Proportion of individuals heterozygous in:			
	1972	1975	1976	χ_2^2[a]
Asexual population[b]				
Est2	0.0	0.0	0.0	0.0
Got	0.076	0.102	0.046	42.32**
Lap	0.554	0.801	0.878	561.47**
Nadhd	0.789	0.913	0.934	218.99**
Pgi2	0.722	0.826	0.893	176.41**
Pgm1	0.208	0.082	0.061	232.19**
Pgm2	0.002	0.010	0.0	25.82**
Mean	0.336	0.391	0.402	...
Sexual population				
Est2	0.380	0.080	0.293	15.55**
Got	0.571	0.440	0.351	2.08
Lap	0.550	0.554	0.477	5.96
Nadhd	0.053	0.034	0.023	0.33
Pgi2	0.300	0.207	0.227	0.63
Pgm1	0.357	0.427	0.500	0.52
Pgm2	0.250	0.517	0.386	6.44
Mean	0.352	0.326	0.322	...

[a] Contingency chi-square test of heterogeneity in the number of isolates heterozygous at particular loci over the three years. Values marked with asterisks are significant, $P = 0.01$.

[b] Data for the asexual population were calculated from a knowledge of the isozyme phenotypes of the clones comprising the population (6).

TABLE 6. Wright's Fixation values (F) and standard error SE(F) of these for the seven variable enzyme loci studied in the asexual and sexual populations of *Puccinia graminis* f. sp. *tritici* in 1972, 1975, and 1976

Enzyme locus	Fixation values (F) for *P.g.* f. sp. *tritici*			
	Asexual population		Sexual population	
	F	SE(F)	F	SE(F)
1972				
Est2	...	...	+0.067	0.224
Lap	−0.121	0.013	+0.123	0.218
Got	−0.008	0.023	−0.167	0.261
Nadhd	−0.650	0.013	−0.026	0.026
Pgi2	−0.565	0.013	−0.167	0.067
Pgm1	+0.632	0.006	+0.398	0.242
Pgm2	−0.001	0.001	+0.443	0.211
1975				
Est2	...	...	−0.040	0.106
Lap	−0.393	0.012	+0.170	0.103
Got	−0.052	0.003	−0.003	0.115
Nadhd	−0.840	0.010	+0.554	0.230
Pgi2	−0.704	0.011	+0.222	0.127
Pgm1	+0.765	0.003	+0.175	0.118
Pgm2	−0.005	0.001	+0.039	0.105
1976				
Est2	...	...	−0.034	0.147
Lap	−0.634	0.013	+0.248	0.141
Got	−0.023	0.003	+0.242	0.164
Nadhd	−0.876	0.011	−0.012	0.012
Pgi2	−0.807	0.012	+0.236	0.177
Pgm1	+0.722	0.003	+0.034	0.229
Pgm2	...	...	+0.131	0.153

locus; 1.75 and 1.33, respectively) or weighted methods (Nei's diversity index; Table 4); at the level of the individual locus or at that of the whole population (Table 4; Fig. 1).

In the sexual population none of the Wright's fixation values (F) for variable loci differed significantly from zero (Table 6). This implies that the once-yearly cycle of sexual reproduction in this population is sufficient to produce a subsequent uredinial population which has a genetic structure equivalent to that expected under panmixis (random mating). Such a random mating outcome is to be expected from the inbuilt system of double randomization of gametes that occurs, firstly, during the spread of basidiospores from the asexual host to the sexual one and, secondly, during the later transfer of pycniospores from one pycnium to another by foraging insects.

A not surprising consequence of this process is the occurrence of an enormous array of different multi-locus virulence and isozyme phenotypes. Among the 92 isolates examined from the 1975 sexual population, 61 different virulence and 80 different isozyme multi-locus phenotypes were recorded. Thirty-nine of the different virulence multi-locus phenotypes occurred only once, 15 occurred twice, five occurred three times while the remaining two occurred four times. This great diversity of virulence and isozyme multilocus phenotypes ensured that no association between particular multi-locus phenotypes could be detected. Individual isolates with the same virulence phenotype were generally quite distinct electrophoretically (e.g., two isolates virulent on *Sr*5, 7b, 9a, 9d, 10, 15, and 17 differed by three isozyme alleles as did three isolates virulent on *Sr*5, 9a, 9d, 10, 15, and 17). In fact, of all pair-wise combinations of isolates possible, only four pairs had exactly the same virulence and isozyme multi-locus phenotypes.

In contrast, the genetic structure of the asexual population clearly diverged from that expected under a random mating regime (Table 6). Of the six loci which varied in this population, the F values of five differed significantly from zero. Four F values indicated an excess of individuals heterozygous at the loci concerned; one a deficiency. This divergence from random mating expectation is not unexpected as the asexual population in the United States has been deprived of its sexual cycle since the late 1930s. The present population is composed of nine separate isozymic clones (6) which are believed to be direct descendants of some of the genotypes present at the time of barberry eradication (6,11). Alternatively it may be argued that the divergent F values for the asexual population result from a mixing of samples from different populations with different gene frequencies. However, there is little evidence to support this view. Although statistically significant variations in the frequency of occurrence of different racial groups have been detected among the different geographic regions covered by this population (1), the actual effect of this is likely to be small. The three areas from which more than 80% of isolates were collected in 1975 have been shown to be homogenous in their racial composition (1).

The general departure of the genetic structure of the asexual population from that expected under random mating results in large part from the complete association that has been assumed to occur between the isozyme and virulence phenotypes of individuals within each of the clones that make up the asexual population (6). Thus, the consistent trends which occurred in F values over the years 1972, 1975, and 1976 (e.g., *Lap* changed from −0.121 to −0.634; Table 5) reflect the increasing frequency of one particular clonal line, the race 15-TN virulence phenotype group. This virulence group increased in frequency from 55% of isolates in 1972, 79% in 1975 to 87% in 1976. This clonal line is heterozygous at the *Nadhd*, *Lap*, and *Pgi2* loci (all with F values indicative of an excess of heterozygotes) and is the main cause of the departure of the population from Hardy-Weinberg equilibrium. Moreover, the increasing dominance of the race 15-TN group is also largely responsible for the relatively low gene diversity values recorded (Table 4), the lower allelic richness, the tendency for the proportion of individuals heterozygous at individual loci to increase markedly over time (Table 5) and the markedly asymmetric frequency distribution of the number of heterozygous isozyme loci occurring per individual (Fig. 1).

The results reported here are in agreement with a previous study which investigated the virulence structure of these two populations (11). In that study the sexual population was also found to be more diverse than the asexual one. Comparative studies of different populations of pathogenic fungi using isozyme markers are, however, in their infancy (4) and no study similar to the present one is available for comparison. However, similar effects of the mode of reproduction on the genetic structure of populations have been observed in cladoceran populations. For example, in populations of the crustacean *Daphnia magna* annually reconstituted from sexually produced eggs, isozyme genotypes are distributed according to Hardy-Weinberg equilibrium proportions (8). On the other hand, in populations where reproduction is largely parthenogenetic, genotypic arrays may show considerable deviation from expected proportions and the number of clonal lines comprising the population may be limited (7). Similar tendencies for asexually reproducing populations to be composed of a relatively few distinct clones have been noted before for bacteria (*Escherichia coli* [14]) and plants (*Taraxacum officinale* [9]).

The results presented here demonstrate differences between the genetic structure of the sexually and asexually reproducing populations of *P.g.* f. sp. *tritici* in the United States. In the latter population, the inability of individual clonal lines to recombine with one another restricts the range of isozyme genotypic arrays that are present. In the former population, on the other hand, the process of random mating during every sexual cycle ensures the occurrence of a considerable diversity of genotypes.

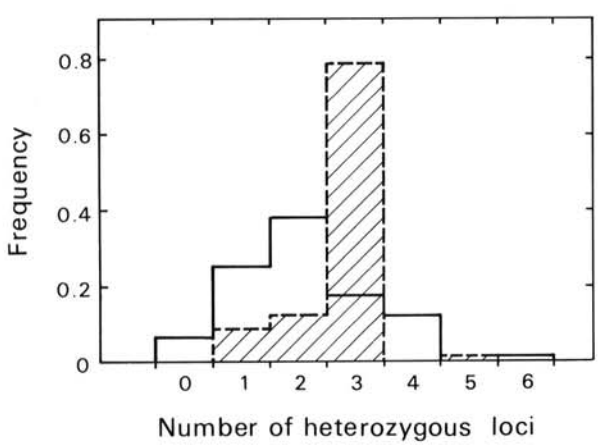

Fig. 1. Frequency distribution of the number of heterozygous loci per individual of *Puccinia graminis* f. sp. *tritici* from the asexual and sexual populations occurring in the United States in 1975. Values for the asexual population were calculated from a knowledge of the isozyme phenotypes of the clones comprising the population (6). Asexual population, dotted line; sexual population, solid line. (Contingency table test of heterogeneity between the two samples showed $\chi^2_{12} = 602.9$, $P < 0.01$).

LITERATURE CITED

1. Alexander, H. M., Roelfs, A. P., and Groth, J. V. 1985. Pathogenicity associations in *Puccinia graminis* f. sp. *tritici* in the United States. Phytopathology 74:1161-1166.
2. Brown, A. H. D., and Weir, B. S. 1983. Measuring genetic variability in plant populations. Pages 219-239 in: Isozymes in Plant Genetics and Breeding, Part A. S. D. Tanksley and T. J. Orton, eds. Elsevier Science Publishers, Amsterdam.
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. Burdon, J. J., and Marshall, D. R. 1983. The use of isozymes in plant disease research. Pages 401-412 in: Isozymes in Plant Genetics and Breeding, Part A. S. D. Tanksley and T. J. Orton, eds. Elsevier Science Publishers, Amsterdam.
5. 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.

6. Burdon, J. J., and Roelfs, A. P. 1985. Isozyme and virulence variation in asexually reproducing populations of wheat leaf and stem rust. Phytopathology 75:907-913.
7. Hebert, P. D. N. 1974. Enzyme variability in natural populations of *Daphnia magna*. II. Genotypic frequencies in permanent populations. Genetics 77:323-334.
8. Hebert, P. D. N. 1974. Enzyme variability in natural populations of *Daphnia magna*. III. Genotypic frequencies in intermittent populations. Genetics 77:335-344.
9. Lyman, J. C., and Ellstrand, N. C. 1984. Clonal diversity in *Taraxacum officinale* (Compositae), an apomict. Heredity 53:1-10.
10. Nei, M. 1975. Molecular Population Genetics and Evolution. North-Holland Publishing Co., Amsterdam.
11. 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.
12. Roelfs, A. P., Long, D. L., Casper, D. H., and McVey, D. V. 1977. Races of *Puccinia graminis* f. sp. *tritici* in the U.S.A. during 1976. Plant Dis. Rep. 61:987-991.
13. Roelfs, A. P., and McVey, D. V. 1973. Races of *Puccinia graminis* f. sp. *tritici* in the U.S.A. during 1972. Plant Dis. Rep. 57:880-884.
14. Selander, R. K., and Levin, B. R. 1980. Genetic diversity and structure in *Escherichia coli* populations. Science 210:545-547.
15. Simpson, E. H. 1949. Measurement of diversity. Nature 163:688.
16. 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.
17. Workman, P. L., and Niswander, J. D. 1970. Population studies on Southwestern Indian tribes. II. Local genetic differentiation in the Papago. Am. J. Human Genet. 22:24-49.