A Comparison of Virulence Phenotypes in Wheat Stem Rust Populations Reproducing Sexually and Asexually

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ABSTRACT

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The number and distribution of 16 loci expressing virulence were compared in two populations of wheat stem rust in the USA. In 1975, samples were collected from a population on barley and wheat in Idaho and Washington that undergoes sexual reproduction annually and from a population east of the Rocky Mountains that reproduces asexually. Virulence was determined by inoculating 16 wheat lines, each differing by a single gene for stem rust resistance. The sexual population had a larger frequency of distinct phenotypes expressed as a percentage. In the sexual population, the frequency was 23.5% with 426 isolates and in the asexual population it was 0.07% with 2,377 isolates. Simpson's measure of diversity was 0.974 and 0.501 for the two populations, respectively. Mean number of loci expressing virulence per isolate was about 6 and 10 for the sexual and asexual populations, respectively. The distribution of numbers of loci expressing virulence differences between pairs of isolates was nearly random in the sexual population, while it was characterized by clusters of phenotypes in the asexual population. These clusters differed from one another by 4 to 10 genes expressing virulence; genotypes within clusters differed by 1 to 2 loci. None of the loci, examined in all of their paired combinations, deviated strongly from expected mean frequencies based on products of individual locus frequencies, providing no evidence for strong positive or negative fitness effects associated with individual genes.

Additional key words: Puccinia graminis, Triticum, selection, virulence genes.

Eradication of common barberry (*Berberis vulgaris* L.) from the wheat fields of the central Great Plains of North America was completed, for all practical purposes, by 1928. The effects of this eradication on populations of stem rust of wheat (*Puccinia graminis* Pers. f. sp. *tritici*) have been the subject of much debate and speculation. One obvious effect noted was that the source of local, often early, epidemics of stem rust was eliminated (13). The claim (11) has been made that barberry eradication increased the average useful life of resistance genes in wheat (*Triticum aestivum* L.). Comparisons of this sort are confounded, however, by differences in agronomic practices, the extent of monoculture, and the wheat cultivars used before and after eradication. Thus, all perceived differences in useful life of resistant cultivars cannot necessarily be attributed to eradication of barberry and its concomitant effect on genetic variability in stem rust.

The widespread occurrence of a sexual stage in most rust life cycles would seem to attest to the value of such a stage to the organisms. Just how, and to what extent, sexual recombination is important to biological populations is debatable. Muller (7) presented a model showing that for large populations, one important advantage of sexual recombination is the rapid incorporation of multiple rare-but-favorable mutations into a single genotype. Asexual populations, it was pointed out, can only incorporate these mutations in a series, one after another, so that only after the first mutant occurs in a large number of individuals (a number approximated in frequency by the reciprocal of the mutation rate [1]) will the second mutation be likely to become incorporated in an individual possessing the first mutation.

In 1968, Maynard Smith (5) presented an alternative model showing that in very large asexual populations, two mutations will be brought together much more rapidly than Muller's model predicted, primarily because of the recurrent, rather than unique, nature of mutational events. The model dealt with only two mutations at a time and was replaced by another, more complete, model in 1971 (6). If certain conditions were met, the latter model predicted two effects of sexual reproduction: first accelerated evolutionary adaptation; and second more variable progeny, resulting in production of some offspring of high fitness. The first effect will occur if the population size, N, is about equal to or larger than $10\,\mu^{-1}$, where μ is the mutation rate. The second effect required frequent, large environmental fluctuations. Both of these conditions are met in stem rust. Populations are generally very large, and the major component of the environment of the rust fungus—the host—changes frequently and dramatically, as compared with that of parasites in natural biota or with that of nonparasites. Additionally, the physical environment changes as the season progresses and the pathogen spreads to new areas.

Accelerated evolutionary adaptation is difficult or impossible to detect directly in plant pathogens, as indicated by the problem, stated above, of interpreting the basis for an increase in the length of useful life of a rust-resistance gene. The other effect of sexual recombination predicted by Maynard Smith (6), greater variability in the population, can be directly measured if sufficient marker genes are available. The objective of this study was to determine some effects of depriving the stem rust fungus of the sexual stage on the amount and kind of variability in the population, by comparing such a population with one in which the sexual stage is still functioning. This was done by determining the distribution of 16 loci expressing virulence among individuals of the two populations. Any difference between the populations would indicate that the sexual stage strongly influences the structure of the population and would be the first kind of evidence necessary to show differences in rate of evolution in sexual vs asexual populations.

MATERIALS AND METHODS

The data used in this paper are from the 1975 annual race survey (10). At the end of the survey it was obvious that two different pathogen populations had been sampled. For study of these populations, it was necessary to reorganize the data according to differences in the initial source of inoculum.

The rust population from east of the Rocky Mountains generally

is asexual in origin, and we will call it the asexual population. Data from all stem rust isolates obtained from aecial collections and from uredial hosts other than wheat and barley were purged. This eliminated the few isolates of sexual origin in this population. The remaining population was represented by 2,377 isolates from 759 collections.

The collections representing the asexual population were obtained from several sources. About 25% of the collections were submitted by research and extension personnel from state universities and departments of agriculture. Private companies and individuals submitted about 5% of the collections. The remaining 70% were made by the Cereal Rust Laboratory, Crop Quality Council, and field personnel of the Animal and Plant Health Inspection Service. The latter collections were generally made along selected routes through small-grain growing areas. Fields were inspected at 32-km intervals, and a collection was made whenever rust was found. Plots along the route were also visited and collections were made from susceptible cultivars within. In 1975, 39% of all collections came from commercial fields.

TABLE 1. Distribution of wheat cultivars and stem rust collections in the USA in 1975

Area[a] and cultivar[b]	Sr gene(s)[c]	Field response[d]	Percentage of acreage[e]	Percentage of collection
South Texas			0.1	2
Penjamo 62	6	R	64	
Chaparell	5,17	R	23	
Nadadores 63	11,17	R	12	
Central Texas			1.4	0.5
Sturdy	None	S	61	
Milam	6	R	12	
Knox type	None	S	12	
Southern and Ohio Valley			16.1	10
Arthur type	6,8,Tt-1	R	64	
Monon	16	S	7	
Yorkstar type	None	S	6	
Southern Great Plains			42.5	32
Scout type	17	R	31	
Triumph type	Tmp	S	16	
Centurk type	5,6,8,9a,17	R	10	
Eagle	None	R	8	
Tascosa-Sturdy-Wichita type	None	S	20	
Northeastern States			1.9	4
Arthur type	6,8,Tt-1	R	36	
Redcoat	?	R	24	
Yorkstar type	None	S	18	
Blueboy	None	S	12	
Upper and Mid-Mississippi Valley			0.8	11
Parker	None	S	29	
Gage	?	R	24	
Centurk	5,6,8,9a,17	R	17	
Minter	7b,17	R	4	
Timwin	Tt-1	R	4	
Kenosho	Tt-1	R	4	
North Central Plains			28.8	38
Waldron type	seg. 5,11	R	24	
Durum	9e	R	23	
Era type	5,6,8,17	R	14	
Fortuna	5,6,17	R	8	
Chris	5	R	5	
Northwestern Great Plains			8.4	3
Winalta	17,Tmp	S	21	
Cheyenne	5	S	19	
Lancer	17	R	15	
Warrior	None	S	4	
Trapper type	6	R	4	
Winoka	5,Tmp	S	4	

[a] Area described in Roelfs and McVey (8).
[b] Cultivar distributions estimated from Reitz and Hamlin (7).
[c] Sr genes for seedling resistance known to be present in the cultivar and evaluated in this study. Data in part from F. J. Gough, G. J. Green, N. H. Luig, J. M. S. Martinez-Gonzalez, R. A. McIntosh, D. V. McVey, I. A. Watson, and N. D. Williams (*personal communications*).
[d] Field response of cultivar in 1975. R = resistant (however, small or scattered infections may have occurred) S = susceptible.
[e] Percentage of the U.S. wheat acreage east of the Rocky Mountains in this area and percentage of the area planted to the listed cultivars.

The area east of the Rocky Mountains has been divided into eight ecological areas for wheat stem rust (10). These areas were drawn so that there was maximum uniformity in races in each area. The host cultivars differed to some extent among areas, thereby exerting different selection pressures. These areas, along with the principal (> 75%) cultivars grown therein, are shown in Table 1. The genotypes of these cultivars, in relation to the genes studied, are also given, along with their responses to the principal races identified in the area. The resistance of the cultivars was frequently due to genes other than those studied; eg, the resistance of Eagle and Waldron. In other cultivars, eg, Centurk and the Arthur type, the resistance was due primarily to the genes studied. The distribution of collections among areas and the percentage of the wheat acreage in each area are shown in Table 1. With the exception of the upper Mississippi Valley, each area was sampled more or less representatively. The excess of collections from the upper Mississippi was the result of the use of trap plots and the sampling done at the beginning and end of surveys by the Cereal Rust Laboratory.

The sexual population was obtained from Latach County, ID, and Whitman County, WA, by V. Delegans. These 426 isolates were from 148 collections made between 17 July and 9 September, of which 118 were made in August. The first aecial infections were reported in mid-June, and the first uredia were found in mid-July. Thus, most collections of uredia were only a few asexual generations from a sexual generation. These collections were made principally from the barley cultivars Steptoe and Vangard and the wheat cultivar Gaines, none of which is known to possess race-specific resistance. Thus, these 148 collections represent heavy sampling of a relatively small area.

Upon receipt of collections, uredospores were collected and inoculated to a susceptible host that had been treated previously with maleic hydrazide to enhance spore production. Isolates from the asexual population were increased on McNair 701, CI 15288. Those from the sexual population were increased on Line E (11) because some members of the sexual population were avirulent on the resistance genes *Sr*McN and *Sr*LC that are present in the cultivars McNair 701 and Little Club, respectively (11), which are commonly used as susceptible hosts. Each collection was used to inoculate 24–40 seedlings in a single pot of the susceptible host cultivar. After 14–16 days, three leaves, each with a single uredium, were saved, reincubated overnight to germinate loose uredospores, and replaced in isolation booths for 36 to 48 hr. Uredospores were then collected separately from each uredium with a cyclone collector. Each uredium provided sufficient spores to inoculate 7-day-old plants of the 16 "single gene" lines. Light-weight mineral oil was used as a spore carrier. 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 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 the temperatures varied from 18 to 30 C. Infection types were recorded 10–12 days after inoculation.

Plants were grown in vermiculite and fertilized 5 and 8 days after planting with a water-soluble fertilizer (23-19-17, N-P-K) at 2.5 g per 16 host lines.

The cultures were evaluated on 16 host lines with known genes for resistance; ie, *Sr*5, 6, 7b, 8, 9a, 9b, 9d, 9e, 10, 11, 13, 15, 16, 17, Tt-1, and Tmp. These genes had been chosen over a period of years (11) because they respond differentially to collections from the asexual wheat stem rust population.

Frequencies were obtained for each of the 15 individual

virulences (corresponding to single resistance genes in the host) that occurred in both populations; virulence on the 16th gene, *Sr*13, was absent from both. From the products of these frequencies, expected frequencies for all possible paired combinations of the 15 virulences were calculated. This calculation is based on the absence of forces, most notably selection, that might influence the genotype frequencies. Observed frequencies of paired gene combinations were recorded. After an arcsin transformation was made, deviations of observed from expected frequencies were averaged over all 14 combinations for each gene. A paired *t*-test was applied to determine whether significant deviations occurred for each of the 15 virulences.

RESULTS

Tables 2 and 3 present the observed and expected frequencies, expressed as percentages, for all paired virulence combinations in the sexual and asexual populations, respectively. For simplicity, we use the term gene interchangeably with virulence, with the understanding that in most cases, a single gene for virulence corresponding to each resistance gene is unproven. Mean deviations of observed from expected frequencies for each gene in all of its combinations and standard deviations of these means are given in Table 4 for both populations. For example, the gene for virulence to *Sr*8, in all of its combinations with the 14 other genes in the sexual population, had 14 individual deviations of observed from expected (that with *Sr*10, for instance expressed as a proportion was −0.05) with a mean of +0.01%. The standard deviation of the 14 individual deviations was 0.21% (Table 4). A paired *t*-test on the mean deviation of virulence to *Sr*8, calculated with the arcsin transformation, indicated that this small positive deviation was nonsignificant. In the sexual population, deviations were generally low; the highest mean deviation was only 0.08%. Standard deviations for these means were also low, with the result that five of the mean deviations were statistically different from zero: four negatively (indicating possible selection against the gene) and one positively (indicating possible selection in favor of the gene). The asexual population had somewhat larger mean deviations than did the sexual population. The former also had significantly larger mean variances, as measured by an F-test for homogeneity of variance. The larger variances associated with the mean deviations in the asexual population resulted in only two of the genes with deviations that were significantly different from zero; both were positive. It seems surprising that mean deviations in the asexual population were as small as they were. Unfortunately, we have no prior experience on which to base a judgment about the size of mean deviations one might expect in an asexual population. However, in the asexual population, in which genes are not randomly associated with one another because of lack of recombination and in which the number of genotypes is clearly limited, deviations can be expected to be large, since expected frequencies are calculated on the assumption of random gene association. The obverse of this observation is that the data in Table 4 demonstrate that genes in the sexual population are more nearly randomly distributed than in the asexual population.

Frequencies of resistance genes in the wheat cultivars found in the eastern and central USA in 1975 were calculated from the data shown in Table 1. These frequencies are not exact because only about 78% of the wheat is accounted for in the table. The correlation coefficient between pathogen and host gene frequencies was not significant, indicating the latter did not strongly influence the former. Some of the virulence genes were present in high frequency when they were apparently unnecessary (ie, those matching *Sr*7b, 9d, 9e, 10, 11, 16, Tt-1, and Tmp), while others were not frequent even though a relatively high percentage of the wheats contained the resistance gene that specifically correspond with them (ie, those matching *Sr*6 and 17).

A simple way of expressing variation among isolates from a population is the percentage of distinct phenotypes obtained (3, page 32). In the sexual population, 100 distinct phenotypes were identified in the 426 isolates sampled, giving 23.5%. Variation using this measure was much less in the asexual population, in which 17 phenotypes were identified in 2,377 isolates, giving 0.7%.

TABLE 2. Percentage of isolates from a sexual population of stem rust of wheat that were virulent on selected *Sr* genes (diagonal) and actual and theoretical percentages of virulence for diallel pairs of these genes

Theoretical / Actual	Percentage of isolates virulent on indicated *Sr* gene(s)														
	5	6	7b	8	9a	9b	9d	9e	10	11	15	16	17	Tt-1	Tmp
5	**69**	1	13	15	44	1	48	2	67	2	64	44	52	13	2
6	4	**1**	*[a]	*	*	*	1	*	1	*	1	*	1	*	*
7b	15	4	**19**	4	12	*	13	1	18	1	18	12	14	4	1
8	15	1	8	**21**	14	*	15	1	20	1	20	14	16	4	1
9a	47	*	7	11	**63**	1	44	2	61	2	59	40	47	12	2
9b	*	*	*	*	*	**1**	1	*	1	*	1	1	1	*	*
9d	57	*	15	17	44	*	**69**	2	66	2	64	44	51	13	2
9e	*	*	*	*	*	0	*	**4**	4	*	3	2	3	1	*
10	66	1	15	15	57	*	65	4	**97**	4	90	62	72	18	3
11	4	*	4	3	*	0	4	3	4	**4**	3	2	3	1	*
15	64	1	13	18	60	1	63	1	91	1	**93**	59	69	18	3
16	48	1	12	15	41	1	46	4	61	4	57	**64**	48	12	2
17	54	1	11	12	50	0	49	*	71	*	73	49	**75**	14	2
Tt-1	16	0	8	5	10	1	10	3	19	3	16	12	14	**19**	1
Tmp	2	0	2	2	0	0	2	2	2	2	4	2	0	2	**3**

[a] * = Less than 0.6% virulence.

TABLE 3. Percentage of isolates from an asexual population of stem rust of wheat that were virulent on selected *Sr* genes (diagonal) and actual and theoretical percentages of virulence for diallel pairs of these genes

Theoretical / Actual	Percentage of isolates virulent on indicated *Sr* gene(s)														
	5	6	7b	8	9a	9b	9d	9e	10	11	15	16	17	Tt-1	Tmp
5	**99**	11	81	85	14	12	98	76	87	81	23	98	20	80	76
6	2	**11**	9	9	2	1	11	8	10	9	2	11	2	9	8
7b	81	2	**82**	70	11	10	81	63	72	67	19	81	16	66	63
8	88	11	76	**86**	12	10	85	66	76	70	20	85	17	70	66
9a	21	2	4	5	**14**	2	14	11	12	11	3	14	3	11	11
9b	13	3	4	12	4	**12**	12	9	10	10	3	12	2	10	9
9d	98	4	81	87	14	13	**99**	76	87	81	23	98	20	86	76
9e	77	0	77	74	*[a]	*	77	**77**	68	63	18	76	15	63	59
10	87	9	80	84	2	10	87	77	**88**	72	20	87	18	72	68
11	82	10	74	79	2	11	80	72	80	**82**	19	81	16	66	63
15	23	11	6	14	14	13	14	*	11	11	**23**	23	5	19	18
16	99	11	82	87	13	13	99	77	88	82	22	**99**	20	86	76
17	19	9	2	10	10	10	19	*	11	8	20	20	**20**	16	15
Tt-1	81	4	81	77	4	4	12	77	78	74	4	81	1	**81**	62
Tmp	77	0	77	74	0	0	77	77	77	72	*	77	*	77	**77**

[a] * = Less than 0.6% virulence.

TABLE 4. Deviation of observed from expected virulence frequencies in sexual and asexual wheat stem rust populations for each of 15 host genes paired with each other gene

Sr gene	Sexual population (n = 14)		Asexual population (n = 14)	
	Mean deviation(%)	Standard deviation(%)	Mean deviation(%)	Standard deviation(%)
5	0.03	0.24	0.00	0.40
6	−0.01	0.09	−0.19	2.25
7b	0.01	0.35	−0.02	2.05
8	0.01	0.21	0.25 *[a]	0.73
9a	−0.08 *	0.22	−0.44	2.61
9b	−0.08 *	0.13	−0.02	1.97
9d	0.00	0.18	−0.40	4.95
9e	0.02	0.43	−0.22	4.10
10	−0.03 *	0.05	0.01	1.05
11	0.04	0.53	0.02	0.91
15	−0.02	0.13	−0.32	4.20
16	0.02 *	0.06	0.02 *	0.04
17	−0.06 *	0.25	−0.38	3.85
Tt-1	0.01	0.22	−0.51	6.95
Tmp	−0.02	0.49	−0.33	4.57

[a] * = Statistically different from zero at $\alpha = 0.05$, paried *t*-test. An arcsin transformation was applied to the raw data.

Historical data show a similar picture. Because of some extremely small sample sizes in early years, however, the measure of frequency of distinct phenotypes is a biased measure with which to compare variation in the rust populations sampled each year. Instead, the number, rather than frequency, of distinct phenotypes detected each year beginning in 1918, on a standard set of differential wheat cultivars was plotted in Fig. 1. Averages for each successive 10-yr period were 17.5, 10.7, 6.5, 7.7, 7.3, and 4.9 phenotypes. The drop in number of detected phenotypes corresponds with the eradication of barberry which was in an advanced stage in 1926 (13). A progressive decrease in variability of wheat, the extent of which is unknown, may have also influenced the number of detected rust phenotypes over the years. The small sample sizes, as low as 70, in the first nine years of the survey, make it likely that the number of detected phenotypes would have increased if samples had been taken of comparable size to those of later years, when they ranged from about 300 to over 2,000.

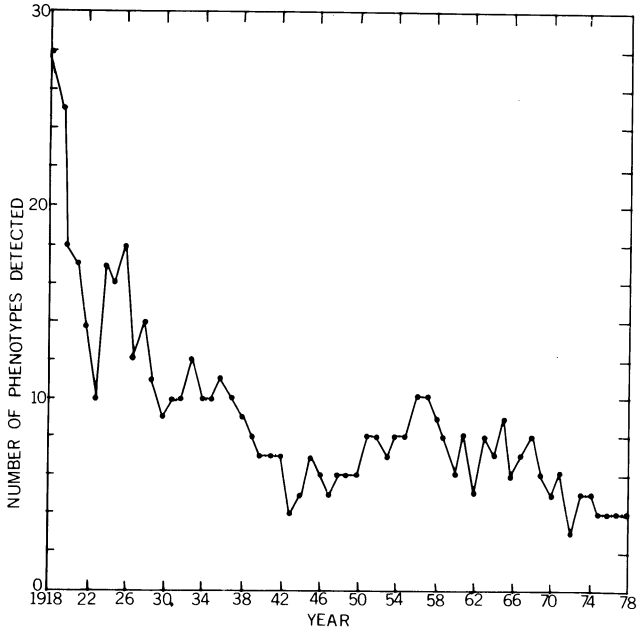

Fig. 1. Number of unique phenotypes detected on the standard differential host series in samples of the USA population of stem rust of wheat for the years 1918–1978.

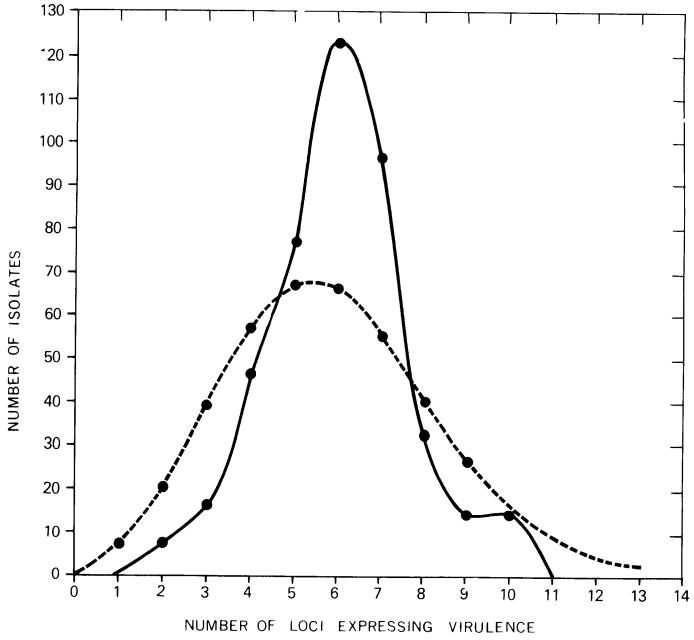

Fig. 2. Frequency distribution of numbers of loci expressing virulence in wheat stem rust isolates that originated from aecia, collected in Washington and Idaho in 1975. Sixteen virulence genes were potentially detectable. Observed —— Expected- - - -.

A second measure of variation is Simpson's Measure of Diversity (8, page 223):

$$D = 1 - \sum_{j} \frac{N_j(N_j-1)}{N(N-1)}$$

in which N_j = The number collected of the jth phenotype
N = The sample size

This statistic includes both components of diversity: number of phenotypes and evenness of distribution of phenotypes. For the sexual population $D = 0.974$, while for the asexual population $D = 0.501$. The sexual population is therefore more diverse than the asexual population.

A third measure of variation is the distribution of genes for virulence among the isolates. The observed frequency distribution for the sexual population is shown in Fig. 2, and that for the asexual population is shown in Fig. 3. The expectation if genes are randomly distributed among isolates is a Poisson distribution about the mean number of genes per isolate, as plotted in the two figures. This expectation is based on nonidentity of genes, and ignores individual genotype frequencies. An alternative expectation would involve products of frequencies of virulence and avirulence alleles in all combinations. Because of difficulties in obtaining such an expected distribution, and the lack of appropriate information to do it correctly, it was not attempted. In neither population was the Poisson expectation met by the observed results. The sexual population, however, had a nearly symmetrical distribution that departed from the expected primarily because of deficiencies in the extreme classes, those with very few or very many loci expressing virulence. The distribution in the asexual population was asymmetrical and bimodal. Most isolates had 9–11 (primarily 10) loci expressing virulence, while fewer had 5–8 (primarily 6). Other classes were not represented.

The number and identity of resistance genes in the host, which were not the same for the two populations, could influence the distribution of virulence genes among isolates and hence weaken the comparison. Thus, the distribution of genes among isolates can be expressed by the number of gene differences in each of all possible paired combinations of genotypes, weighted according to the product of the frequencies of the two genotypes being compared. Summing these over a number of different genes gives a frequency distribution of number of gene differences. Compared with the number of genes per isolate, this measure of variation logically should be less strongly influenced by the number of resistance genes in the host, provided that matching resistance

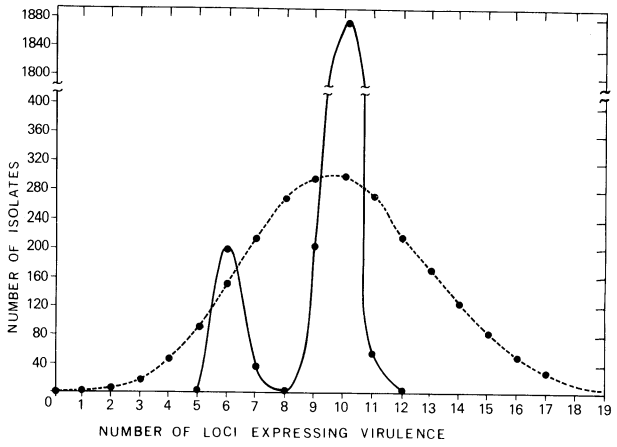

Fig. 3. Frequency distribution of numbers of loci expressing virulence in wheat stem rust isolates that originated asexually and were collected in the eastern and central USA in 1975. Observed —— Expected - - - -.

genes do not regularly occur together in the prevalent, and generally susceptible, wheat cultivars. This condition is largely met because as seen in Table 1, cultivars with several resistance genes that are prevalent are resistant to the common rust phenotypes in a region. Most or all of the rust phenotypes in the region were unable to reproduce on such cultivars and would not be the end result of selection pressure to acquire all matching virulence genes. Fig. 4 shows the distribution of virulence loci differences for the sexual population. The expected distribution, based on random gene association, is a Poisson distribution. Overall, the fit of observed to expected was close, although the two were not statistically the same. Fig. 5 shows the distribution of virulence loci differences for the asexual population. The distribution shows a "clustering" of isolates into groups separated from one another by four-to-ten virulence loci differences. The isolates within these groups differ from one another by zero, one, or (more rarely) two loci. Fig. 6 illustrates the structure of the asexual population, ignoring frequency, in three dimensions, where race groups are spheres whose diameters are proportional to the mean number of gene differences among the group members. The spheres are separated from one another by straight-line distances that are proportional to the mean number of gene differences between groups.

Finally, a frequency distribution of the frequencies of all paired combinations of loci expressing virulence for the two populations is shown in Fig. 7. Two differences are notable. First, the sexual population had a lower mean locus-pair frequency than did the asexual population. Second, the distribution of locus-pair frequencies was decidedly bimodal in the asexual population; (ie, locus-pair frequencies were either low or high, with a paucity of intermediate values). This distribution was not evident in the sexual population, in which most locus-pair frequencies were less than 20%. This difference in distribution is consistent with the principle that in the asexual population, the unit of selection is largely the genotype, whereas in the sexual population, it is largely the individual gene, since our earlier data indicate that genes are being nearly randomly reshuffled in the sexual population.

DISCUSSION

All of the evidence presented in this study supports the same general conclusion: that there are extreme differences in the structure of the Washington-Idaho and the Central USA rust populations. The variation in the sexual population is, as Maynard Smith (6) predicted, much greater than in the asexual population. Many, but not necessarily all, of the differences can be attributed to the elimination of the sexual stage from the latter population. This is supported by previous work with the cereal rusts (11,18).

Additional genes for resistance to wheat stem rust are known for which selected individuals of each population were evaluated;

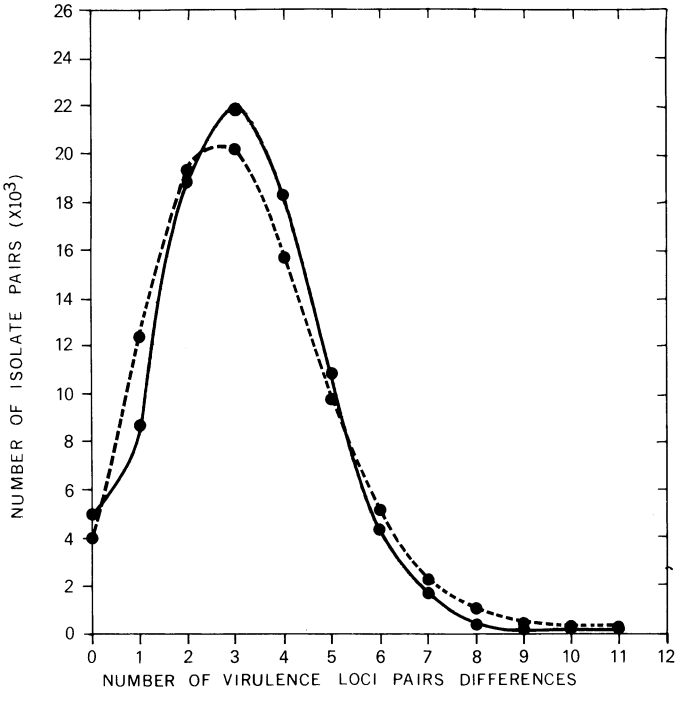

Fig. 4. Frequency distribution of numbers of virulence locus differences between pairs of wheat stem rust isolates that originated from aecia, collected in Washington and Idaho in 1975. Sixteen virulence genes were potentially detectable. Observed —— Expected - - - -.

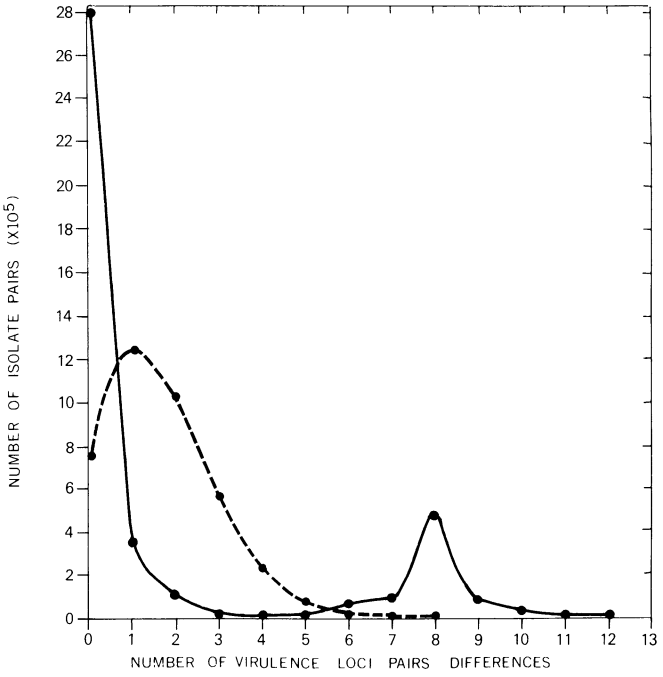

Fig. 5. Frequency distribution of number of virulence locus differences between pairs of isolates of stem rust of wheat that originated asexually, collected in the eastern and central USA in 1975. Sixteen virulence genes were potentially detectable. Observed —— Expected - - - -.

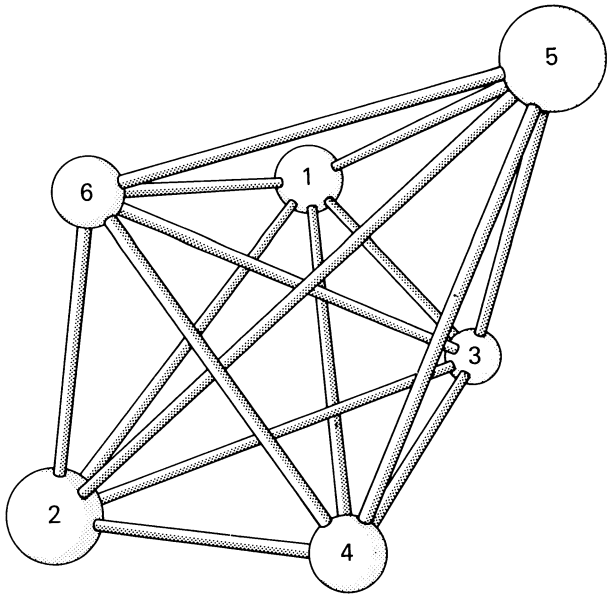

Fig. 6. Scaled illustration of the distribution of virulence differences between isolates of wheat stem rust from the eastern and central USA that originated asexually. Each sphere represents a cluster of genetically similar isolates, and its diameter is proportional to the maximum number of loci at which isolates of the cluster differ in virulence. Distances between spheres are proportional to the mean number of virulence differences between clusters. Races 151-QCB, -QFB; races 113-RKQ, -RPQ, -RTQ; race 56-MBC; races 11-RCR, -RHR; races 15-TLM, -TDM, -TNM; races 151-QSH, 32-RSH.

because not all isolates were evaluated for these additional genes, the data are not included. However, the asexual population varied for virulence to *Sr*7a, 12, 14, 21, 23, 30, X, dp2, while the sexual population varied on these genes plus *Sr*9f, 12, 18, 19, 28, Kt'2', LC, McN, and U and on a number of other genes occurring in "susceptible" lines used as backgrounds for the designated lines. Thus, the differential responses of the 16 resistance genes that were studied seem to be representative of the differences that would have been observed if more genes for resistance had been studied.

One source of probable error in the direct comparison of these two populations is that the host cultivars of one of them contain some of the resistance genes being employed as markers while those of the other do not. Because of the presence of these genes in the host of the asexual population, a certain amount of selection for phenotypes that could overcome these genes undoubtedly has occurred. This would be true even if it were known that rust was collected only from cultivars containing none of the resistance genes. In such a case the incoming inoculum that initiated the collected pustules would have been under selection pressure from the commercial wheat cultivars containing some of these resistance genes. Exactly how much this weakens or affects the comparisons presented here is uncertain. The lack of correlation between frequencies of matching host and pathogen genes indicates that such selection pressure, if it occurred, was relatively light or that it has been operating for only a short time.

The percentage of distinct phenotypes is a potentially useful expression for comparing the amount of variation in different population of the same or different pathogens. The results with crown rust obtained by Simons et al (12) agreed with ours, although they did not actually calculate this value. Collections from Texas, where the sexual host, buckthorn, is absent, showed 17% variation on 24 presumably single-gene differentials for rust resistance, whereas collections made on or near buckthorn at St. Paul had a value of 42%. Both of these values are larger than those we obtained for asexual and sexual populations, respectively, probably as a result of the use of more virulence markers. The measurement should be used more widely, for all types of pathogens for which suitable markers are available. Certain constraints should be observed when using it however. Sample sizes of populations compared should be similar. If the frequencies of different phenotypes are dissimilar, as they often are, then the number of phenotypes obtained is not linearly related to sample size. The detection of rare phenotypes requires a larger sample size than does that of the common phenotypes. Differences in distribution of phenotype frequencies between the two populations also weakens this comparison as occurred in this study. The second constraint is that the number of markers will obviously dictate the number of unique phenotypes, so the number of markers should be the same if different pathogens are to be compared. Ploidy must also be considered if marker alleles show complete dominance. Perhaps the most difficult assumption that must be met for this to be a valid measure of variation is that the sample must be representative of the population. This assumption was least met for the Washington-Idaho sample, since it was collected in only two counties. This error was not critical, inasmuch as the amount of variation present in the population should have been underestimated by such a sample, and the sample contained a great deal of variation in comparison with either the post-eradication stem rust population or the asexual population. Given the nearly random association of the 15 loci expressing virulence in the sample, it seems improbable that a larger or more representative sample of the Washington-Idaho population would have revealed either more variation or a different population structure.

Simpson's D is useful because unlike percentage of different phenotypes it is nearly independent of sample size, and it is not influenced by the occurrence of rare phenotypes in the populations that are difficult to detect in finite sampling.

The shape of the frequency distribution of phenotypes for the two populations was also markedly different. The sexual population had a rather smooth, concave histogram of frequencies, when arrayed as most-to-least frequent. The most frequent phenotype had a frequency that was only slightly greater than 0.10. At the other extreme, there were many phenotypes that were represented by three, two, or one isolates, resulting in a long right-hand tail. The asexual population was characterized by a single, very frequent phenotype (0.69 of all collections) and 16 infrequent phenotypes ranging from 0.08 to 0.0008.

Measurement of the distribution of individual genes among

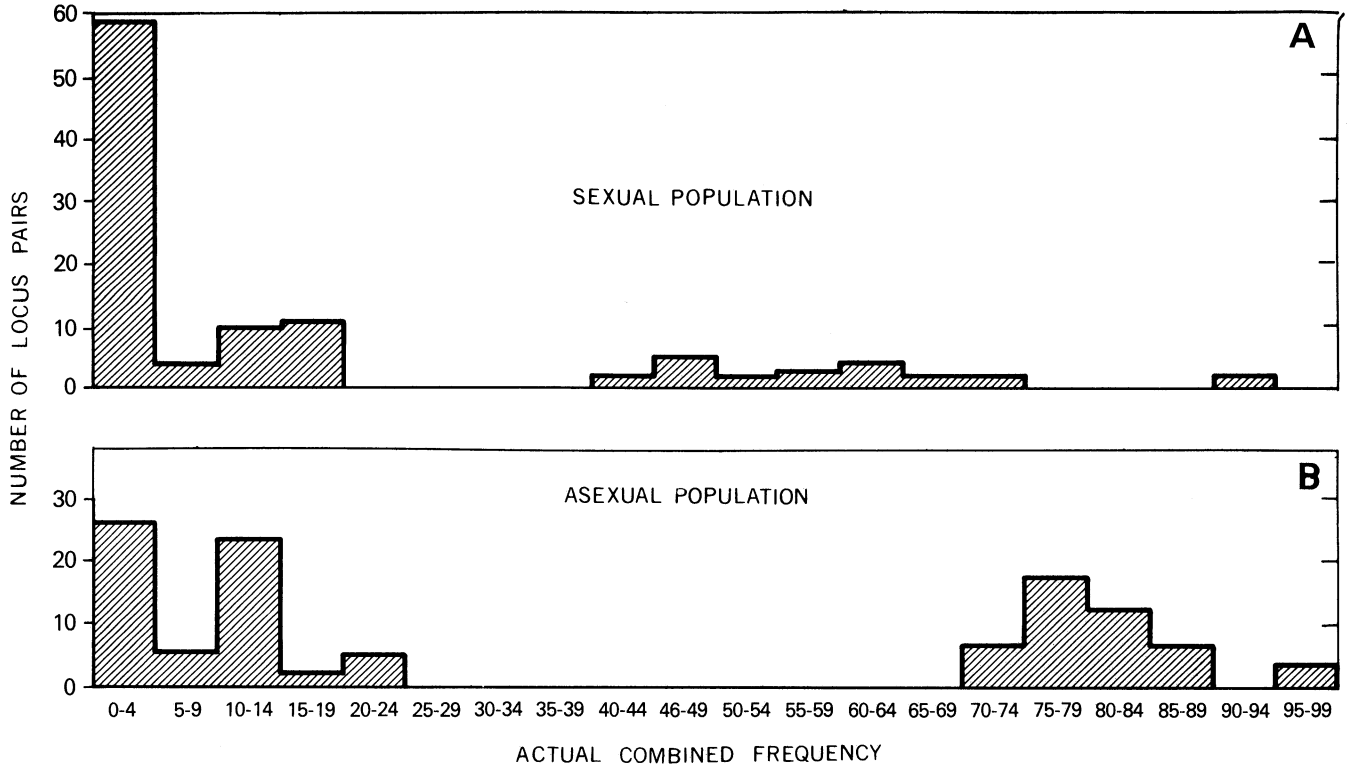

Fig. 7. Frequency distribution of the frequencies of all possible pairs of loci expressing virulence in **A**, a sexually reproducing population of stem rust of wheat and **B**, an asexually reproducing population. Sixteen loci were studied.

isolates is somewhat more useful for the sexually reproducing population than for the asexually reproducing population, because none of the 15 virulence loci in the study matches a resistance gene that is present in wheat cultivars grown in the Washington-Idaho area. In contrast, several of the matching resistance genes are present in at least a portion of the wheats grown in the Great Plains.

There was no easy way in this study to account for the influence of frequency of specific resistance genes in the host on frequency of matching virulence genes in the pathogen, as has been done in at least one other more highly controlled study (16,17). Sources of error in trying to account for this influence include: the regional localization of many resistance genes in only a portion of the epidemiological unit area of the pathogen coupled with pathogen movement between regions; the representation of only a small number of all possible combinations of resistance genes; the erratic frequency representation of the individual resistance gene in the host; the occurrence of a variety of genetic backgrounds exerting real but unknown influence on race fitness, possibly differentially; and the disproportionate representation of rust collections from certain regions. These possibilities weaken any conclusions drawn from the lack of correlation between the host and parasite gene frequencies or about disparity in frequency for specific genes in the host and parasite.

Since none of the virulence genes in the sexual population appear to be necessary for survival on the wheat host, some conclusions can reasonably be drawn about gene distribution in this population. The most striking feature of this distribution is the large number and high frequency of apparently unnecessary virulence genes. This feature might be taken as strong evidence against the concept, called stabilizing selection by Vanderplank (14), that unnecessary virulence genes impart reduced fitness of genotypes containing them, compared with those that do not. This interpretation would be premature, however. The role of such genes in enhancing or reducing fitness of the pathogen on the barberry host is not known. Since the sites of these collections were not far removed from the alternate host, we cannot be certain that these genes were unnecessary to the pathogen, throughout its life cycle. A possibility is that, on barberry, rust genotypes containing homozygous avirulence loci (with reference to wheat resistance genes) are less fit than are those that are heterozygous. This would maintain both alleles and allow, via recombination, recessive virulence to be expressed in the population each season.

The distribution of numbers of virulence genes shown in Fig. 2 is much narrower that that of the Poisson expectation. This cannot be construed to mean that there is selection against extreme classes, however, since there is a wide range of virulence frequencies in the population. Such a range can be expected to result in a narrower distribution than that of the Poisson. There is no strong evidence for selection against the extreme lower classes of zero and one gene. The expectation based on gene frequency, which can be calculated for these classes relatively easily, is that both classes are very infrequent, such that the probability of detecting them in a sample of 426 is low. This probably holds true also at the other extremes. The difficulty in obtaining a reasonable expectation for intermediate classes precluded its attempt. The mean number of expressed loci for virulence in the sexual population, which is about six, is about four genes fewer than the number found in the large majority of races of the asexual population (Fig. 3). This difference was not found with crown rust, in which the asexual and sexual population contained roughly the same mean number of expressed loci for virulence (12). In the asexual population of stem rust, the complete absence of races with five or fewer loci expressing virulence was partly determined by the presence in the host of matching resistance genes (Table 1). Such races simply could not survive on many of the winter and spring wheat cultivars grown in the Great Plains and adjoining areas. Beyond this, however, it was not possible to account for the rather unusual distribution of genes among isolates and adjoining areas. Beyond this, however, it was not possible to account for the rather unusual distribution of genes among isolates by considering only the selective force exerted by resistance genes in the host. The occurrence of a very limited number of phenotypes precluded any conclusion about the absence of races with 8 or with more than 11 loci expressing virulence.

In the sexual population (Fig. 4), the deviations from randomness of observed gene associations occurred as small deficiencies in extreme classes with a concomitant surplus of intermediate classes. This distribution might be partly a reflection of the selection, seen in Fig. 2, against extreme (high or low) gene numbers, resulting in a slight deficiency in the classes exhibiting large numbers of gene differences (on the right-hand side of the curve in Fig. 4). Such an effect should, however, also increase the frequency of lower numbers of gene differences the opposite of what was observed (Fig. 4). It is possible that there was some selection either against genotypes that differ from one another by only a few genes or for genotypes that are more dissimilar. Again, as in Fig. 2, the range of gene frequencies, which itself is influenced by selection, affects the kind of expectation that should be used to best describe random gene association.

The distribution of gene differences in the asexual population was not random. The population structure, best visualized in Fig. 6, is similar in overall pattern to that reported for stem rust populations in Canada (2) and Australia (4), where reproduction also is asexual. The genotypes occur in six groups of great genetic similarity, separated from one another by larger genetic distance. This pattern results in the bimodal distribution of gene differences (Fig. 5). Two different genotypes either differ slightly, when they are members of the same group, or differ greatly, when they are members of different groups. There is no intermediate level of difference. This pattern is what one should expect if either no, or very inefficient, mechanisms of genetic recombination are operating. As has also, and more carefully, been shown in Australia (4), most differences between members of the same groups are the result of single locus mutations, and recombination is of little importance in determining intragroup variation. At least some of the different groups have their origin in the adapted biotypes that were prevalent in the stem rust population at the time when it was deprived of the sexual mechanism of recombination. Clearly, if a substitute mechanism, such as parasexuality, has been operating in the population, the structure of the Great Plains population indicates that the mechanism provides only minimal genetic reshuffling.

Vanderplank (14) first introduced the idea that more complex races of a pathogen tend to occur more rarely than simple races. Wolfe et al (16, 17) pointed out that complex races should be rarer even in the absence of selection, since they usually represent more specifically defined genotypes than do simple races. Thus, for recessive virulence genes, the frequency of the complex genotype is expected to be the product of the frequencies of the individual virulence genes. Wolfe et al found for barley mildew populations that some genotype combinations occurred at frequencies lower or higher than expected, but that most were very close to the expected. Vanderplank (15) later revised his appraisal of the reason for the rarity of complex races by calculating expected frequencies using the logic of Wolfe et al (17). For stem rust of wheat in Canada, Vanderplank (15) postulated that the virulence genes matching *Sr*6 and *Sr*9d had an adverse, probably interactive or epistatic, effect on pathogen fitness, since isolates possessing these genes, although expected to occur, were absent from surveys of the Canadian population. In the asexual population of the present study, the observed frequency of this gene combination was 7% lower than its expected frequency, a result that, (if Vanderplank's analysis is correct) supports the conclusion that the combination lowers fitness. Many other combinations of genes, however, also show deviations of similar or greater magnitude. The genes *Sr*6 and *Sr*9d, when taken each in paired combination with all other genes, did not significantly depress observed frequencies, and they had no greater nonsignificant deviations than other genes.

In both the sexual and asexual populations, as seen in Table 4, average deviations of observed from expected frequencies of the 15 genes in all paired combinations were quite low, in most cases much less than 1%. Individual deviations varied considerably more in the asexual population than in the sexual population, so that the mean standard deviation was significantly larger in the asexual than in the sexual population. This difference is explained by the effective

"linkage" of genes in an asexually reproducing population. Some virulence genes—those present in the commonest genotypes—are linked in coupling, resulting in combined frequencies higher than expected, based on random assortment, while others—those not occurring together in common genotypes—are linked together in repulsion, resulting in combined frequencies lower than expected. The low mean deviations result when a gene's deviations include the effects of both coupling and repulsion linkages. In their analyses, Vanderplank (15) and Wolfe et al (16) assumed that the virulence genes occurred independently of one another. Fig. 5 clearly shows, however, that in an asexually reproducing population, such as the Canadian population of Vanderplank (15) and that of the present study, this assumption is not met. For such populations, there is no simple relationship between individual gene frequencies and those of combinations of these genes. In time units important in natural evolution, linkage effects are not important because given enough time all linkages are broken; however, in time units important in an agricultural context, linkages due to asexual reproduction in stem rust have remained for many years. For such populations, deviations of observed from expected combined frequencies are not a valid basis for conclusions about the fitness associated with virulence genes; expected frequencies are not simply obtained. The analysis is instructive, nevertheless, because it allows another comparison to be made between the sexual and asexual population. Fig. 4 shows that the assumption of independent gene distribution is applicable for the sexually reproducing population of Washington-Idaho. This observation leads to the conclusion that none of the 15 virulence genes was strongly inherently unfit, at least not to the extent that it caused a large negative deviation of observed from expected frequency in the majority of its associations. Furthermore, none of the genes was strongly favored. The five significant deviations may indicate negative selection against the genes matching *Sr*9a, 9b, 10, and 17, and positive selection for the gene matching *Sr*16, but the intensity of such selection was not great, as indicated by the small magnitude of the mean deviations. Alternatively, the deviations could be the result of linkage between some of the loci giving sufficient numbers or magnitudes of deviations to cause an overall significant deviation. Other workers (14,16) have indicated that selection is a likely force accounting for deviations involving specific combinations of genes. Such selection to depress or enhance the frequency specifically of combinations of genes would entail nonlinear fitness interactions or perhaps epistasis with respect to fitness. These effects would not be manifested in mean deviations of the type shown in Table 4. Furthermore, if individual virulence genes are at a selective advantage or disadvantage, and assuming such selection has been operating long enough to have a major influence on gene frequency, and assuming no other forces are operating, they would eventually be nearly fixed or absent in the population, respectively. Accordingly, either other forces are operating to offset strong fitness effects associated with virulence genes, to give the nine of 16 intermediate (.05–.95) genotype frequencies that are observed, or there are not strong selective forces associated with the genes (that have been in operation long enough to determine gene frequency). The latter case is supported by the small size of mean deviations in the sexual population in Table 4, which reflects performance of individual genes in many combinations.

As Vanderplank (15) contends, certain specific gene combinations may yet prove to be valuable because of the reduced fitness associated with them. Identification of specific combinations was not possible in this study, however, because of a lack of replication.

The locus-pair frequency distribution for the two populations indicates a serious problem in interpretation of data from asexually reproducing pathogen populations. The frequency of a virulence gene in such populations is not necessarily correlated with selective advantage of the gene. In the absence of genetic recombination, all genes in a genotype are 100% linked, so that the success of a gene in the population is a function of the success of the genotype in which it happens to occur. Presumably, then, if selection has been operating sufficiently long, those locus-pairs that occur at low frequencies (asexual population, Fig. 7) are in more poorly adapted genotypes, while those locus-pairs that occur at high frequencies are in better adapted genotypes. The adaptation of each genotype, of which there are only a few in the asexual population, is determined partly by major virulence genes that allow the genotype to attack the predominant wheat cultivars and partly by the background genes. All of these are, in the short run, fixed. The picture is complicated by the changes in selective advantage for a given genotype from one location to another. Despite this complication, overall there is a lack of intermediate locus-pair frequencies, which indicates a lack of genotypes that are selectively "neutral."

The distribution of locus-pair frequencies in the sexual population does not show a strong bimodality because both major and background genes are free to recombine, thus providing not only more genotypes, but genotypes that represent a wide, continuous range of fitnesses. The preponderance of very low locus-pair frequencies might indicate that individual virulence genes, taken singly by virtue of their being averaged over many backgrounds and in association with many other virulence genes, cannot account for most of the selective advantage or disadvantage of the genotypes in which they occur. They therefore occur in lower frequencies. In addition, the absence of matching resistance genes in the wheat host should moderate the selective advantage of the virulence genes. In this regard, the comparison between the locus-pair frequencies of the two populations (Fig. 7) is weakened.

LITERATURE CITED

1. CROW, J. F., and M. KIMURA. 1970. An Introduction to Population Genetics Theory. Harper and Row, New York, NY. 591 pp.
2. GREEN, G. J. 1975. Virulence changes in *Puccinia graminis* f. sp. *tritici* in Canada. Can. J. Bot. 53:1377-1386.
3. LEWONTIN, R. C. 1974. The Genetic Basis of Evolutionary Change. Columbia University Press, New York, NY. 346 pp.
4. LUIG, N. H. 1977. The establishment and success of exotic strains of *Puccinia graminis tritici* in Australia. Proc. Ecol. Soc. Aust. 10:89-96.
5. MAYNARD SMITH, J. 1968. Evolution in asexual and sexual populations. Am. Nat. 102:469-473.
6. MAYNARD SMITH, J. 1971. What use is sex? J. Theor. Biol. 30:319-335.
7. MULLER, H. J. 1932. Some genetic aspects of sex. Am. Nat. 8:118-138.
8. PIELOU, E. C. 1969. An introduction to Mathematical Ecology. Wiley Interscience, New York, NY. 286 pp.
9. RIETZ, L. P., and W. G. HAMLIN. 1978. Distribution of the varieties and classes of wheat in the United States in 1974. U.S. Dept. Agric. Statist. Bull. 604. 98 pp.
10. ROELFS, A. P., and D. V. McVEY. 1976. Races of *Puccinia graminis* f. sp. *tritici* in the U.S.A. during 1975. Plant Dis. Rep. 60:656-660.
11. ROELFS, A. P., and D. V. McVEY. 1979. Low infection types produced by *Puccinia graminis* f. sp. *tritici* and wheat lines with designated genes for resistance. Phytopathology 69:722-730.
12. SIMONS, M. D., P. G. ROTHMAN, and L. J. MICHEL. 1979. Pathogenicity of *Puccinia coronata* from buckthorn and from oats adjacent to and distant from buckthorn. Phytopathology 69:156-158.
13. STAKMAN, E. C., AND J. G. HARRAR. 1957. Principles of Plant Pathology. Ronald Press Co., New York, NY. 581 pp.
14. VANDERPLANK, J. E. 1968. Disease Resistance in Plants. Academic Press, Inc., New York, NY. 206 pp.
15. VANDERPLANK, J. E. 1975. Principles of Plant Infection. Academic Press Inc., New York, NY. 210 pp.
16. WOLFE, M. S., J. A. BARRETT, R. C. SHATTOCK, D. S. SHAW, and R. WHITBREAD. 1976. Phenotype-phenotype analysis: field application of the gene-for-gene hypothesis in host-pathogen relations. Ann. Appl. Biol. 82:369-374.
17. WOLFE, M. S., S. E. WRIGHT, J. A. BARRETT, and P. O'DONALD. 1973. Population genetics of cereal mildews. Abstract 1,330 in: Proc. 2nd Int. Congr. Plant Pathol., Minneapolis, MN.
18. YOUNG, H. C., JR., and J. M. PRESCOTT. 1977. A study of race populations of *Puccinia recondita* f. sp. *tritici*. Phytopathology 67:528-532.