The Genetic Structure of Populations of *Puccinia graminis* f. sp. *tritici*

D. R. Knott

Professor, Department of Crop Science and Plant Ecology, University of Saskatchewan, Saskatoon, Canada S7N 0W0.
Accepted for publication 25 July 1986.

Vanderplank (9,10) noted that in populations of *Puccinia graminis* f. sp. *tritici* in North America combinations of pairs of genes for virulence on particular genes for resistance in the host (*Sr* genes) did not occur in the frequencies expected from their individual frequencies. From his analysis, Vanderplank proposed that genes for virulence, and by reflection genes for resistance, fell into two groups. The ABC group was comprised of *Sr*6, *Sr*9a, *Sr*9b, *Sr*15, and *Sr*17, and the XYZ group was comprised of *Sr*7b, *Sr*9e, *Sr*10, *Sr*11, *Sr*Ttl, and Tmp. Genes within a group were associated, whereas genes in opposite groups were dissociated.

The assumption made in this type of analysis is that the phenotypes observed are a representative sample from a randomly mating population. Knott (3) and Roelfs and Martens (7) pointed out that the North American population of *P. g.* f. sp. *tritici* is essentially asexual and only a limited number of races are observed in any year. One race, 15-TNM, has predominated in recent years, making up 68% of the population in 1975, the year analyzed most completely by Vanderplank (10). Knott (3) noted that 15-TNM is virulent on all of the genes in the XYZ group and avirulent on all of those in the ABC group. Recently, Burdon and Roelfs (2) did an isozyme analysis of North American isolates and identified only nine basic types. Thus, using two distinct types of markers, the fungus population has been shown to consist of a very limited number of phenotypes out of the very large number possible.

The survey data are clear in showing that certain combinations of pairs of virulence genes occur either more frequently or less frequently than expected. The question is, what underlying mechanisms produce the observed frequencies? To account for the observed association or dissociation of certain genes for virulence, Vanderplank (9,10) proposed a speculative hypothesis involving interactions between genes at different loci. For example, in discussing the genes for virulence on *Sr*6 and *Sr*9e he states (10) that, "the effect of the gene for virulence for *Sr*6 is evidently changed by the presence or absence of the gene for virulence for *Sr*9e. This is epistasis in the pathogen." Presumably he does not mean that the virulences controlled by the two genes are affected, but rather that the gene combinations affect the fitness of cultures carrying them.

Vanderplank (9–11) placed considerable emphasis on the dissociation of virulence on *Sr*6 and *Sr*9e in North American and Australian populations of *P. g.* f. sp. *tritici*. The actual and theoretical frequencies for the four identifiable phenotypes involving the *P*6 and *P*9e loci in the United States in 1975 and in Australia from 1961–1962 to 1968–1969 are given in Table 1. It is assumed that recessive genes *p*6 and *p*9e condition virulence and *P*6 and *P*9e condition avirulence on *Sr*6 and *Sr*9e, respectively. The heterozygous and homozygous dominant genotypes are indistinguishable. The theoretical frequencies are calculated on the assumption that the isolates are a representative sample from a randomly mating population. Actually, in 1975 in the United States, only 10 races had frequencies of 1% or more, and they fell into just five of the nine isozyme types established by Burdon and Roelfs (2). Race 15-TNM, which is avirulent on *Sr*6 and virulent on *Sr*9e, made up 68% of the isolates. Only two other races, 15-TDM (5%) and 15-TLM (3%), were virulent on *Sr*9e and both were also avirulent on *Sr*6. All three are similar in virulence and belong to one isozyme type. Thus, it is probable that *p*9e became established only once in the North American population of *P. g.* f. sp. *tritici* and in a genotype carrying *P*6. Since then, the combination has been maintained by asexual reproduction. The small differences in virulence among the three races virulent on *Sr*9e almost certainly evolved by mutation.

Vanderplank (11) in his reply to Knott (3) and Roelfs and Martens (7) argued that the dissociation between *p*6 and *p*9e holds independently of race 15-TNM. He is correct, but it is easy to see why. Excluding race 15-TNM, two other closely related races, 15-TDM (5%) and 15-TLM (3%), are avirulent on *Sr*6 and virulent on *Sr*9e. The second most frequent race, 151-QSH (8%) has the reciprocal phenotype, virulent on *Sr*6 and avirulent on *Sr*9e. Thus, the apparent dissociation after excluding 15-TNM depends on just three races, which make up half of the remaining 32% of the isolates.

Vanderplank (11) is critical of the fact that I discussed (3) only North American data but, of course, his analysis (9,10) was largely based on North American surveys. However, Vanderplank's conclusions based on the Australian surveys are even more questionable. It is well established (4) that the Australian population of *P. g.* f. sp. *tritici* has undergone several sudden changes as a result of the influx of one or two new races that rapidly replaced the existing races. These races reproduced asexually and evolved by mutation, often in response to the release of cultivars carrying particular *Sr* genes.

In 1954, race 21 (*P*6*P*9e) was first discovered in eastern Australia. In the following years, race 21 and mutants derived from it dominated the Australian population of *P. g.* f. sp. *tritici* (4). Vanderplank (9, p. 20) analyzed the data of Luig and Watson (5) for the period 1961–1962 to 1968–1969 (a modification of his analysis is given in Table 1). Of the 5,861 isolates, 82.3% were *P*6*P*9e, the same as the original race 21, 16.7% were *p*6*P*9, and only 1.1% were *P*6*p*9e. None were *p*6*p*9e although on the basis of random combination, 10.5 of the 5,861 isolates should have been. Vanderplank (9) concluded that *p*6 and *p*9e were dissociated. However, the data of Luig and Watson (5) also show that *p*6

TABLE 1. The actual and theoretical frequencies of the four identifiable phenotypic combinations at the *P*6 and *P*9e loci for isolates of *Puccinia graminis* f. sp. *tritici* from Australia for 1961–1962 to 1968–1969 and the United States for 1975[a]

	Australia				U.S.A.			
	Actual		Theoretical		Actual		Theoretical	
Phenotypes	(no.)	(%)	(no.)	(%)	(no.)[b]	(%)	(no.)	(%)
*P*6*P*9e	4,822	82.3	4,832.5	82.5	241	10.4	432	18.6
*P*6*p*9e	63	1.1	52.5	0.9	1,838	79.2	1,647	71.0
*p*6*P*9e	976	16.7	965.5	16.5	241	10.4	51	2.2
*p*6*p*9e	0	0	10.5	0.2	0	0	190	8.2
Totals	5,851	100.1	5,861.0	100.1	2,320	100.0	2,320	100.0

[a] Australian data from Vanderplank (9) and U.S. data from Roelfs and McVey (8).

[b] Calculated from the percentage figures for races given by Roelfs and McVey (8) and based on 96% of the isolates. The remaining 4% of the isolates were reported only as less than 0.6%.

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.

occurred most frequently in isolates from Western Australia, particularly in the latter part of the period. By contrast, with one exception, isolates carrying *p*9e occurred only in eastern Australia. Thus, it is possible that the combination of *p*6 + *p*9e seldom if ever occurred and never became established. There would then be no opportunity for natural selection to operate against the genotype, assuming that it is less fit as a result of Vanderplank's postulated epistatic interactions. Wolfe and Knott (12) warned of the danger of obtaining misleading results by combining heterogenous data derived from collections in different areas.

The frequencies of the four phenotypes involving the *P*6 and *P*9e loci are very different in the Australian and U.S. populations (Table 1). If, in fact, the same selective forces had been at work in both populations, more similar results would have been expected. On the other hand, the results are easily explained by the establishment and asexual reproduction of a few genotypes of *P. g.* f. sp. *tritici* in each area.

Vanderplank's statement (11) that the Mexican data support the ABC-XYZ grouping is surprising. The data are limited, partly because there was no variation for pathogenicity on some resistance genes in 1975 (8). For this reason the data on virulence on *Sr*9e (0%), *Sr*15 (100%), and *Sr*Tmp (0%) have to be eliminated in the analysis. In addition Roelfs and McVey (8) do not show the pathogenicity of the isolates on *Sr*17. When virulence on these genes is eliminated, the results largely contradict the ABC-XYZ groupings (Table 2). Of the 12 pairs of virulence genes with one gene from each of the ABC and XYZ groups, only two (*p*9a*p*10 and *p*9a*p*11) show the predicted negative deviations of the actual from the theoretical percentages, and some of the positive deviations are quite large, exceeding 10% (*p*7b*p*9a, *p*9a*p*Ttl, and *p*9b*p*Ttl). Of the nine pairings between genes in the same group, only five show the predicted positive deviations and two of the four negative deviations are large, (*p*7b*p*10 and *p*10*p*Ttl). The reason for the difference between the Mexican and U.S. results is evident from an examination of the races identified. In Mexico in 1975, 99% of the isolates fell into six races, which represented only three isozyme types (the remaining isolates were not completely identified). All six races were also present in the United States, but their frequencies were very different. In Mexico three closely related races, 113-RKQ, 113-RPQ, and 113-RTQ, all of one isozyme type, made up 75% of the isolates compared with 3% in the United States. Because all three races are virulent on *Sr*9a and *Sr*9b from the ABC group, and *Sr*7b and *Sr*Ttl from the XYZ group, the virulences on all four genes are associated, rather than *p*9a and *p*9b being dissociated from *p*7b and *p*Ttl as Vanderplank would predict. Thus, shifts in races from area to area affect pathogenicity associations. Alexander et al (1) found that pathogenicity associations also change over time as race frequencies change.

The Mexican data provide another example of the effects of a single race. Only one race, 151-QSH, was virulent on *Sr*10. The apparent association of *p*10 with or dissociation from another gene for virulence depended on whether 151-QSH carried the second gene or not. If it did not, then the gene was strongly dissociated from *p*10. This was the case for *p*9a (ABC group) and *p*7b (XYZ group).

Vanderplank (11) is correct that the apparent dissociations and associations do not reflect only the predominant race. Other key races can have an important effect as well, as shown above. It happens that in the U.S. race 15-TNM has the major effect. Race 15-TNM has the virulence formula (effective/ineffective resistance genes) *Sr*6, 9a, 9b, 13/5, 7b, 8, 9d, 9e, 10,11, Ttl(*Sr*36), and Tmp. The genes *Sr*6, 9a, and 9b are in the ABC group, and 7b, 9e, 10, 11, Ttl, and Tmp in the XYZ group. The genes *Sr*5, 8, 9d, and 13 are not included in either group. The percentage of isolates from the United States that were virulent on *Sr*5 and *Sr*9d was 99% and on *Sr*13, 0%. With essentially no variability, no test for association of virulence on these three genes with virulence on other genes is possible. Vanderplank (9) does not include *Sr*8 in either of his groups of genes. Based on the virulence formula of race 15-TNM, *Sr*8 should be in the XYZ group. In fact the frequencies of the combinations of *p*8 with other virulence genes fit the predicted with two exceptions (6). The exceptions are the combinations of *p*8 + *p*6 and *p*8 + *p*9b, for which the deviations of the actual frequencies from the expected are +2% in each case, when they should be negative. These deviations occur because *p*6 and *p*8 are combined in the second most frequent race and none of the three genes is present in the third most frequent. Thus, there is really no major exception to the conclusion that apparent associations are artifacts caused mainly by the predominance of 15-TNM.

An obvious feature of Table 4.1 in Vanderplank (10), which gives the analysis of the data for 1975 in the United States, is that all of the genes for virulence in the ABC group show low frequencies (11–23%), whereas those in the XYZ group show high frequencies (77–88%). It would be difficult to set up a system of fitness values such that pairs of genes for virulence within either the ABC group or the XYZ group are favored, but the ABC genes all end up with low frequencies and XYZ genes with high frequencies. Surely some of the pairs of ABC genes would be sufficiently favored to reach a higher frequency and some of the pairs of XYZ genes would be less favored and have a lower frequency. The basic pattern clearly depends on which genes are present in and which are absent from race 15-TNM.

Contrary to all of the evidence that asexual reproduction of limited numbers of races is the cause of the apparent association and dissociation of virulence genes, Vanderplank (11) states that, "sexuality (i.e., the occurrence of the sexual cycle) would be an issue only if ABC-XYZ groupings in sexual populations disagreed with those in asexual populations because of sexuality." He uses the data of Alexander et al (1) to argue that the association and dissociation of genes for virulence is the same in sexual and asexual populations of *P. g.* f. sp. *tritici* in North America. It is well known in population genetics that one cycle of random mating does not result in genetic equilibrium. However, it should move the population towards equilibrium, which would result in a reduction in the number of associations. This is exactly what Alexander et al (1) found. Nearly half (22/46) of the pathogenicity associations in the sexual population were not significant compared with 5% (3/65) in the asexual population. Furthermore, the values of the *G*-statistic for significant associations were much higher for the asexual than for the sexual populations. Similarly, Roelfs and Groth (6) analyzed the same data and showed that "genes in the sexual population are more nearly randomly distributed than in the asexual population." A comparison of the actual and theoretical frequencies for pairs of virulence genes, one from each of the ABC and XYZ groups, shows that in the asexual population the differences are mostly large and negative (Table 3). In the sexual population the differences are much smaller and occasionally positive. Thus, the data clearly show that the sexual cycle quickly reduces the apparent associations and dissociations.

All of the evidence indicates that the apparent association and dissociation of pairs of genes for virulence is most simply explained by the fact that a limited number of races become established in the fungus population and then reproduce asexually. Which genotypes become established may depend on which genes for resistance are present in the wheat cultivars being grown, which genotypes happen to survive the low point of the annual rust cycle

TABLE 2. The actual and theoretical frequencies of pairs of genes for virulence in the Mexican population of *Puccinia graminis* f. sp. *tritici* in 1975[a]

	Actual (theoretical) percentage of isolates virulent on						
	ABC group			XYZ group			
Sr gene	6	9a	9b	7b	10	11	Ttl
6	84	67(70)	84(77)	67(63)	17(14)	72(67)	67(63)
9a		83	75(76)	75(62)	0(14)	63(66)	75(62)
9b			92	75(69)	17(16)	80(74)	80(69)
7b				75	0(13)	63(60)	75(56)
10					17	17(14)	0(13)
11						80	63(60)
Ttl							75

[a] Data from Roelfs and McVey (8).

TABLE 3. The actual and theoretical frequencies of pairs of genes for virulence, one of each pair from the ABC group and one from the XYZ group, in a sexual and an asexual population of *Puccinia graminis* f. sp. *tritici* in the United States in 1975[a]

		Actual (theoretical) percentage of virulent isolates					
		XYZ group					
	Sr gene	7b	9e	10	11	Ttl	Tmp
Sexual population							
	6	4(*)[b]	*(*)	1(1)	*(*)	0(*)	0(*)
ABC	9a	7(12)	*(2)	57(61)	*(2)	10(12)	0(*)
group	9b	*(*)	0(*)	*(1)	0(*)	1(*)	0(*)
	15	13(18)	1(3)	91(90)	1(3)	16(18)	4(3)
	17	11(14)	*(3)	71(72)	*(3)	14(14)	0(2)
Asexual population							
	6	2(9)	0(8)	9(10)	10(9)	4(9)	0(8)
ABC	9a	4(11)	*(11)	2(12)	2(11)	4(11)	0(11)
group	9b	4(10)	*(9)	10(10)	11(10)	4(10)	0(9)
	15	6(19)	*(18)	11(20)	11(19)	4(19)	*(18)
	17	2(16)	*(15)	11(18)	8(11)	1(16)	*(15)

[a]From Roelfs and Groth (6).
[b]* = Frequencies less than 0.6%.

(overwintering in North America or oversummering in Australia), or which genotypes happen to have the best overall fitness. Most rust genotypes will carry some pairs of genes for virulence that are in the same group of Vanderplank's two groupings and some that are in different groups. Under Vanderplank's hypothesis the fitness of any one genotype would depend on the balance between opposing forces for association and dissociation and would be unpredictable.

Vanderplank has published several interesting and stimulating books. One of their major values has been that they stimulate thinking, discussion, and research. However, many of his hypotheses are speculative and must be examined critically and subjected to tests.

LITERATURE CITED

1. Alexander, H. M., Roelfs, A. P., and Groth, J. V. 1984. Pathogenicity associations in *Puccinia graminis* f. sp. *tritici* in the United States. Phytopathology 74:1161-1166.
2. Burdon, J. J., and Roelfs, A. P. 1985. Isozyme and virulence variation in asexually reproducing populations of *Puccinia graminis* and *P. recondita* on wheat. Phytopathology 75:907-913.
3. Knott, D. R. 1984. The association and dissociation of genes for virulence in wheat stem rust. Phytopathology 74:1023.
4. Luig, N. H. 1983. A survey of virulence genes in wheat stem rust, *Puccinia graminis* f. sp. *tritici*. Verlag Paul Parey, Berlin and Hamburg. 199 pp.
5. 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.
6. 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.
7. Roelfs, A. P., and Martens, J. W. 1984. The virulence associations in *Puccinia graminis* f. sp. *tritici* in North America. Phytopathology 74:1022.
8. Roelfs, A. P., and McVey, D. V. 1976. Races of *Puccinia graminis* f. sp. *tritici* in the U.S.A. during 1975. Plant Dis. Rep. 60:656-660.
9. Vanderplank, J. E. 1982. Host Pathogen Interactions in Plant Disease. Academic Press, New York. 207 pp.
10. Vanderplank, J. E. 1984. Disease Resistance in Plants. Second edition. Academic Press, Orlando, FL. 194 pp.
11. Vanderplank, J. E. 1985. Virulence structure in *Puccinia graminis* f. sp. *tritici*: A reply. Phytopathology 75:1079.
12. Wolfe, M. S., and Knott, D. R. 1982. Populations of plant pathogens: Some constraints on analysis of variation in pathogenicity. Plant Pathol. 31:79-90.