Selection in a Heterogeneous Population of *Puccinia recondita* f. sp. *tritici*

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ABSTRACT

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A heterogeneous population of *Puccinia recondita* f. sp. *tritici* developed from randomly mated pycnial infections on *Thalictrum speciosissimum* was selected for 12 generations on four wheat lines with different resistance genes to determine if unnecessary genes for virulence were deleterious to general levels of fitness in the wheat leaf rust fungus. Diversity of virulence phenotypes, as measured with the Shannon index, declined least in the population selected on the susceptible line Thatcher and most in the population selected on the resistant cultivar Roblin (*Lr1, Lr10, Lr13,* and *Lr34*). Phenotypes with virulence to six differential lines predominated in the populations selected on Thatcher, the Thatcher isogenic line containing *Lr3ka* (Tc*Lr3ka*) and Tc*Lr11*. The frequencies of virulence to *Lr2a, Lr11,* and *Lr17* significantly increased in the population selected on Thatcher, and frequencies of virulence to *Lr3ka* and *Lr30* significantly decreased. In the Roblin population, frequency of virulence to *Lr2a* significantly decreased, and frequency of virulence to *Lr24* significantly increased. Frequency of virulence to *Lr2c* and *Lr17* significantly increased in the population selected on Tc*Lr3ka*. Genes influencing fitness may be linked to the virulences that showed consistent and significant change in frequency over generations. Differences in the effective population size in the initial generation, caused by resistance genes in the host lines, may account for some of the differences in frequency of virulence phenotypes between the four selection populations.

Additional keywords: specific resistance, specific virulence, *Triticum aestivum*, virulence polymorphism.

Selective forces that maintain genetic polymorphism for virulence in plant pathogen populations are critical in influencing the durability of the corresponding resistance genes in host cultivars. As is well-known, the widespread release of cultivars with specific resistance genes often causes directional increase in frequencies of specific genes for virulence in pathogen populations (8,10). What is less well understood are other selective forces that affect frequencies of the genes for virulence in the absence of the corresponding host resistance. Vanderplank (24) originally described the idea that pathogen genotypes with unnecessary genes for virulence are less fit than are genotypes that have only the minimum number of virulence genes required by the pathogen to successfully parasitize the host. Leonard and Czochor (17) have argued that all virulence polymorphisms would be transient if there were no selection against unneeded virulence genes in pathogen populations. Also, if virulences were selectively neutral in the absence of host resistance genes, it should be difficult to find genes that condition useful levels of resistance (24), because the pathogen population would already be sufficiently polymorphic for the corresponding virulence to render the resistance ineffective.

Strategies designed to prolong effectiveness of host resistance, such as multilines (2), cultivar mixtures (25), and gene recycling (3), implicitly depend on pathogen genotypes with unnecessary virulences being less fit. Different types of experiments have been conducted to test how pathogen populations are selected on susceptible hosts. Leonard (15,16) and Bronson and Ellingboe (1) conducted studies designed to test the fitness effects of specific virulence alleles in populations of fungal pathogens derived from sexual crosses. Martens (20), Katsuya and Green (9), and Loegering (18) conducted competition studies with mixtures of *Puccinia graminis* races on oats and wheat. Grant and Archer (6) calculated selection coefficients for unnecessary virulence genes from race survey data of *P. g. tritici* in Australia.

In a previous study with *Puccinia recondita* Roberge ex Desmaz. f. sp. *tritici* (Eriks. & E. Henn.), the wheat leaf rust fungus, Kolmer (11) determined that in a mixture of field races (virulence phenotypes) commonly found in North America the relative frequency of the virulence phenotypes did not change significantly when maintained on a susceptible host for eight uredinial generations. In the present study, a highly diverse population of *P. r. tritici* derived from a random-mating population (13) was used to minimize linkage disequilibria between virulence and fitness genes (14,17) and to increase the diversity of the population for selection to act on. The objective was to determine if phenotypes with fewer unnecessary virulence genes were generally selected on susceptible and resistant hosts after 12 uredinial generations.

MATERIALS AND METHODS

Initial population and host-selection lines. A random-mating population of *P. r. tritici* derived from 47 single-pustule isolates was developed by bulking 5 mg of spores from each individual isolate and inoculating the bulked population onto adult plants of the Thatcher wheat (*Triticum aestivum* L.) line containing resistance gene *Lr16* (Tc*Lr16*, RL 6005) to produce teliospores. Flag leaves with teliospores were conditioned for teliospore germination (13) and placed over young leaves of *Thalictrum speciosissimum* incubated in humidity chambers. Over 1,000 pycnial infections were obtained. Pycnial infections on the same leaves were randomly intermated. Three to five single-pustule isolates were obtained from each aecium. Two hundred fifty-seven single-uredinial isolates were derived from the intermating population. Details of teliospore germination, mating of pycnial infections, and virulence characteristics of the aeciospore-derived isolates have been described previously (13,14). Spores (3–5 mg) from each isolate were bulked together to form the initial generation (G_0).

All isolates from the random-mating population were virulent to cultivar Thatcher (RL 6101), which was used as the susceptible host in the selection experiment. Cultivar Roblin (RL 4483), which contains seedling resistance genes *Lr1* and *Lr10* and adult plant genes *Lr13* and *Lr34*, was used as a host with complex resistance. Over 85% of the isolates in G_0 were virulent to both *Lr1* and *Lr10*. Isolates virulent to *Lr1* and *Lr10* in the progenitor asexual population of G_0 produced intermediate avirulent infection types on adult plants of Roblin. Isogenic Thatcher lines with seedling genes *Lr3ka* (Tc*Lr3ka*, RL 6007) and *Lr11* (Tc*Lr11*, RL 6053) were used as hosts with simple resistances. These two lines were chosen because virulence frequencies to these two genes were at intermediate levels in the initial population. Frequency of virulence to *Lr3ka* and *Lr11* in the 257 individual aeciospore-derived isolates was 62 and 25%, respectively (14).

General procedures. The study was based on a sequence of culturing populations of uredinia from G_0 on each of the selective host lines for 12 uredinial generations. Spores (25 mg) from G_0 were inoculated onto adult plants of each of the selection lines. Urediniospores were collected from each line in each generation and inoculated onto plants of the same selection line for the next generation. For each generation, the selection lines were grown in a greenhouse at 15–20 C with 8 h of supplemental fluorescent light per day in 10 15-cm Fiberpots (Kord Products Ltd., Bramatea, Ontario, Canada) filled with a mixture of soil and peat moss, five plants per pot. The plants were treated regularly with 20-20-20 N-P-K water-soluble fertilizer. The selection lines were inoculated with 25 mg of urediniospores suspended in light industrial oil when the plants were at the heading stage, Feekes scale 10.5 (5), and were incubated overnight at 18 C in an unlit dew chamber. After incubation, each selection line was maintained in a separate greenhouse to prevent cross contamination among the selected rust populations. After 14 days, rust was collected from each selection line every 2–3 days by tapping the infected plants over sheets of waxed paper. Collected urediniospores were stored at −70 C until used to inoculate the next generation.

Evaluation of virulence phenotypes. Isolates from G_0 and selection generations 3 (G_3), 6 (G_6), 9 (G_9), and 12 (G_{12}) from each selection line were evaluated for virulence phenotypes with the 12 Thatcher isogenic lines in the *Prt* differential set (19). Fifteen 10.16-cm pots with 7-day-old plants of the susceptible cultivar Little Club (C.I. 4066) were inoculated with 5 mg of uredinia from each population evaluated for virulence phenotypes. The Little Club seedlings were treated at emergence with maleic hydrazide to prevent emergence of secondary leaves and to increase pustule size. One week after inoculation the plants were trimmed so only one uredinium remained on each plant. Urediniospores from single uredinia were collected in a 00 gelatin capsule when secondary rings had formed. Oil (333 μl) was added to each capsule, and the spore suspensions were atomized onto 7-day-old *Prt* differential sets. The differentials were incubated overnight at 18 C in an unlit dew chamber and were moved to a greenhouse bench at 15–20 C with 8 h of supplemental fluorescent light per 24-h period. The differential sets were evaluated 12 days after inoculation; infection types 0–2 were considered avirulent; infection types 3 and 4 were recorded as virulent (19). Each single-uredinial isolate was assigned a three-letter designation based on high- or low-infection types to the 12 differentials. Generally, 50–60 single-uredinial isolates from each selection population in the four evaluated generations were tested for virulence phenotypes. As a control to estimate sampling variance, 60 single-uredinial isolates from G_0 were sampled and evaluated each time a selection generation was evaluated.

Analysis of selection populations. The phenotypic diversity of the four G_0 populations and the evaluated generations in the selection populations were determined by the Shannon index (7), which indicates the number of distinct phenotypes and the evenness of the frequency distribution. Frequencies of the predominant virulence phenotypes were plotted over generations in each selection population. Phenotypes also were grouped according to the number of differential lines to which each had virulence (zero to seven), and the frequency of each class was plotted over generations. Five by two chi-square contingency tests (23) were used to determine if frequencies of virulence to individual resistance genes in the *Prt* differentials varied significantly from G_0 to G_{12} in the four selection populations. Four by two chi-square contingency tests were used to determine if virulence frequencies to the individual resistance genes varied significantly in the four samplings of G_0. Logits of the individual virulence frequencies in each selection population were regressed on generation number to determine if virulence frequencies changed significantly over generations. A one-tailed t test at the 0.05 confidence level was used to determine if the slopes of the regression lines were significantly different from zero (23). Logistic regression to determine significant change in frequency for individual virulences was initially used by Leonard (15).

RESULTS

G_0 control populations. TBB at an average of 13.6% was the most common virulence phenotype sampled in the four G_0 control populations (Table 1). All other phenotypes were found at an average of less than 10%. A total of 45 virulence phenotypes were identified in the G_0 populations. The average Shannon index of diversity for the G_0 populations was 3.09 (Fig. 1), with a 95% confidence interval of 0.18. Using chi-square, frequencies of virulence to specific resistance genes did not significantly differ between G_0 samplings except for virulence to *Lr17* and *Lr30*, to which frequencies varied from 11 to 27 and from 22 to 46%, respectively (Table 2). This variance may have been due to the evaluation of control populations over inconstant temperatures in the greenhouse because the typical intermediate (;12–22+) avirulent infection types conditioned by genes *Lr17* and *Lr30* can be temperature sensitive (4).

Selection populations. Diversity, as measured by the Shannon index, declined least in the Thatcher population to 2.07 in G_{12} (Fig. 1). Diversity in the Roblin population declined most to 1.25 in G_{12}. Diversity in the Tc*Lr3ka* population declined to 1.64 in G_{12}. In the Tc*Lr11* population, diversity was measured at less than 1.00 in G_9 but increased to 1.49 in G_{12} (Fig. 1). All selection populations in every generation had lower Shannon indexes than had the four G_0 samplings.

In the Thatcher population, TBJ appeared to be the most fit phenotype because it increased steadily from less than an average of 2% in G_0 to over 40% in G_{12} (Fig. 2A). Phenotypes TBD and KBG increased from less than an average of 2% in G_0 to 12 and 9%, respectively, in G_{12}. Phenotypes virulent to six differential lines increased steadily to over 50% in G_{12} (Fig. 3A). The frequencies of isolates expressing virulence to plants with *Lr2a, Lr11,* and *Lr17* increased significantly over generations, as indicated by the significant chi-square values and significant positive slopes of the virulence frequency logits regressed on generation number (Table 3). Phenotype TBJ was virulent to all three of these genes, whereas TBD and KBG were each virulent

to two of the three genes (Table 1). Virulence frequencies to *Lr2c* varied significantly over generations, as indicated by the significant chi-square values. However, the nonsignificant slope of the regression indicated that virulence to this gene did not change in a consistent manner. Virulence frequencies to *Lr3ka* and *Lr30* decreased significantly over the selection generations (Table 3). The parallel trend in virulence frequency to these two genes was due to the tight linkage between virulences to *Lr3ka* and *Lr30* (14,22). Virulence frequencies to *Lr1, Lr24,* and *Lr26* in the Thatcher population did not change significantly over generations, as seen in the nonsignificant chi-square values and slopes from regression of virulence frequency logits on generation number (Table 3).

In the Roblin population, phenotype MDB increased steadily from less than an average of 5% in G_0 to 46% in G_{12} (Fig. 2B). Phenotype PBR also increased from less than 5% in G_0 to 40% in G_{12}. Phenotype MBB was not detected in G_3 and G_6 but increased to 12% in G_{12}. TBD increased to 15% in G_6 but declined to 0% in G_{12}. Phenotypes virulent to three differential lines increased to over 45% in G_{12} (Fig. 3B). In this selection population, virulence to *Lr24* significantly increased, and virulence to *Lr2a* significantly decreased over generations (Table 3). Virulence frequencies to *Lr2c, Lr3ka, Lr11, Lr17,* and *Lr30* changed significantly over generations according to the chi-square values; however, the slopes of the logistic regressions were not significantly different from zero. Virulence to *Lr1* was fixed at 100% after G_3 because Roblin had *Lr1*.

In the Tc*Lr3ka* population, phenotype PBR increased erratically to 40% in G_{12} (Fig. 2C). Phenotype TBN increased steadily from 1.9% in G_0 to 30% in G_{12}. PBL increased to 33% in G_9 but declined to 12% in G_{12}. Phenotypes virulent to six differential lines increased to 75% in G_{12} (Fig. 3C). The increase in frequencies of isolates virulent to plants with *Lr2c* and *Lr17* over generations were significant by the chi-square values and the positive slopes of the virulence frequency logits regressed on generation number (Table 3). Frequencies of virulence to *Lr11* and *Lr30* also varied significantly over generations according to the chi-square values; however, slopes of the regression lines were not significantly different from zero. Virulence frequencies to *Lr1, Lr2a,* and *Lr24* did not change significantly over generations. Virulence to *Lr26* was fixed near 0% over all generations, and virulence to *Lr3ka*

TABLE 1. Mean frequencies (%) of *Puccinia recondita* f. sp. *tritici* virulence phenotypes in four samplings of population G_0 developed from randomly mated pycnial infections on *Thalictrum speciosissimum*

Virulence phenotype	Virulences	Mean frequency (%)
BBB[y]	none	3.8 ± 2.1[z]
BBG	11	1.0 ± 1.7
CBB	3	1.0 ± 1.7
CBG	3,11	0.5 ± 1.5
CBJ	3,11,17	1.4 ± 2.7
FBB	2c,3	1.5 ± 1.6
KDL	2a,2c,3,3ka	0.5 ± 1.5
KDM	2a,2c,3,3ka,30	0.6 ± 1.9
MBB	1,3	5.2 ± 4.8
MBD	1,3,17	1.3 ± 2.6
MBG	1,3,11	2.2 ± 3.2
MBJ	1,3,11,17	0.4 ± 1.4
MBL	1,3,3ka	4.2 ± 6.3
MBM	1,3,3ka,30	4.5 ± 3.5
MBN	1,3,3ka,17	0.9 ± 1.7
MBP	1,3,3ka,17,30	0.9 ± 1.7
MBR	1,3,3ka,11,30	2.1 ± 3.2
MBS	1,3,3ka,11,17	0.4 ± 1.4
MCB	1,3,26	2.4 ± 1.1
MDB	1,3,24	3.9 ± 1.0
MDL	1,3,3ka	1.4 ± 4.5
MDM	1,3,24,3ka,30	2.5 ± 3.7
MDR	1,3,24,3ka,11,30	0.4 ± 1.4
PBB	1,2c,3	2.7 ± 2.9
PBD	1,2c,3,17	0.5 ± 1.5
PBG	1,2c,3,11	1.0 ± 2.0
PBL	1,2c,3,3ka	3.9 ± 6.3
PBM	1,2c,3,3ka,30	5.9 ± 5.9
PBN	1,2c,3,3ka,17	0.5 ± 1.5
PBP	1,2c,3,3ka,17,30	1.3 ± 2.6
PBQ	1,2c,3,3ka,11	1.5 ± 2.9
PBR	1,2c,3,3ka,11,30	5.0 ± 4.4
PDL	1,2c,3,24,3ka	1.5 ± 1.7
TBB	1,2a,2c,3	13.6 ± 9.0
TBD	1,2a,2c,3,17	1.6 ± 1.7
TBJ	1,2a,2c,3,11,17	1.4 ± 2.9
TBL	1,2a,2c,3,3ka	2.6 ± 3.3
TBM	1,2a,2c,3,3ka,30	4.1 ± 6.6
TBN	1,2a,2c,3,3ka,17	1.9 ± 3.9
TBP	1,2a,2c,3,3ka,17,30	2.3 ± 4.2
TBQ	1,2a,2c,3,3ka,11	0.9 ± 2.9
TBR	1,2a,2c,3,3ka,11,30	2.5 ± 1.5
TDL	1,2a,2c,3,24,3ka	0.6 ± 1.9
TDM	1,2a,2c,3,24,3ka,30	1.3 ± 2.6
TDQ	1,2a,2c,3,24,3ka,11	0.9 ± 2.7

[y] Three-letter code describes virulence phenotypes in *Prt* nomenclature (19).
[z] 95% confidence interval.

TABLE 2. Mean frequencies and chi-quare values for homogeneity of virulence frequencies to leaf rust resistance genes in four samplings of a *Puccinia recondita* f. sp. *tritici* population (G_0) developed from randomly mated pycnial infections on *Thalictrum speciosissimum*

Gene	Mean frequency (%)	χ^2	*P*-value
Lr1	90.0 ± 6.0[y]	2.45	0.483
Lr2a	34.5 ± 5.5	0.82	0.845
Lr2c	59.9 ± 5.6	0.83	0.842
Lr3ka	54.9 ± 17.1	7.15	0.067
Lr11	21.5 ± 2.2	0.43	0.934
Lr17	14.7 ± 15.3	10.99[z]	0.012
Lr24	13.5 ± 7.2	2.79	0.424
Lr26	2.4 ± 0.4	1.12	0.941
Lr30	33.3 ± 17.6	9.05[z]	0.029

[y] 95% confidence interval of the mean.
[z] Significant at $P = 0.05$.

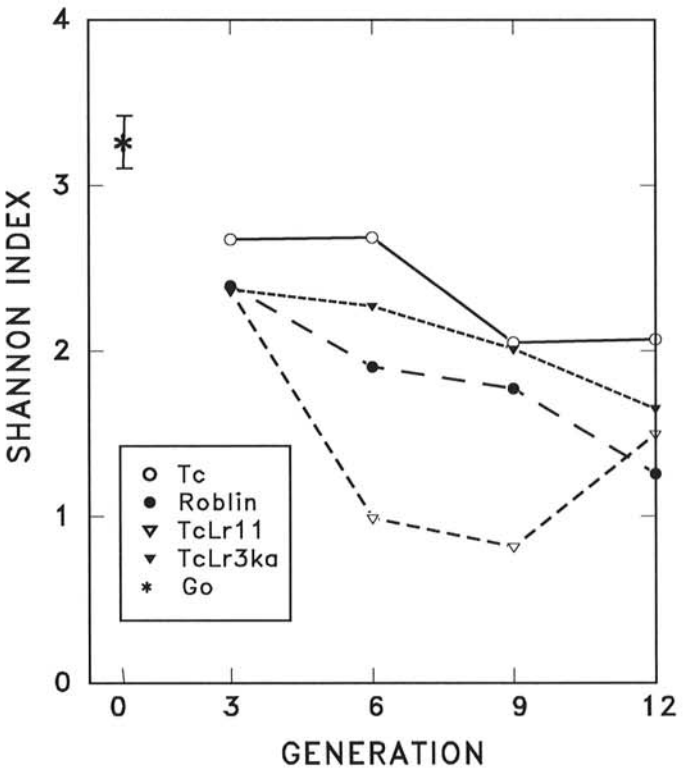

Fig. 1. Mean and 95% confidence interval of Shannon index of phenotypic diversity of G_0 and Shannon index values for the Thatcher (Tc), Roblin, Tc*Lr11*, and Tc*Lr3ka* selection populations in G_3, G_6, G_9, and G_{12} of a heterogeneous *Puccinia recondita* f. sp. *tritici* population.

was fixed at 100%.

In the Tc*Lr11* population, phenotype PBR increased to 75 and 76% in G_6 and G_9, respectively, and declined to 45% in G_{12} (Fig. 2D). Phenotype TBJ increased after G_6 to 30% in G_{12}. Phenotypes virulent to six differentials increased to 80% by G_9 and declined to 70% in G_{12} (Fig. 2D). Virulence frequency to *Lr11* was fixed at 100% in the selection generations, whereas virulences to *Lr24* and *Lr26* were near zero in all generations (Table 3). Virulences to *Lr1* and *Lr17* did not vary significantly in frequency. Virulence frequencies to *Lr2a, Lr2c, Lr3ka,* and *Lr30* varied significantly over generations according to the chi-square values; however, the slopes of the logistic regression lines were not significantly different from zero (Table 3).

In the G_0 populations, the weighted average number of resistance genes in the *Prt* (19) differential set to which each isolate was virulent was 4.26. In G_{12} of the Thatcher population, the weighted average number of virulences per isolate was 5.09. Among the phenotypes in G_0 virulent to *Lr1* and *Lr10*, the weighted average number of virulences per isolate was 4.55. In G_{12} of the Roblin population, the average number of virulences per isolate was 4.11. Among phenotypes in G_0 virulent to *Lr3ka*, the weighted average number of virulences was 5.16, and in the Tc*Lr3ka* population at G_{12}, the average number of virulences per isolate was 5.96. The phenotypes virulent to *Lr11* in G_0 had a weighted average of 4.96, and at G_{12} in the Tc*Lr11* population, the average number of virulences per isolate was 5.10.

DISCUSSION

In the *P. r. tritici* population selected on Thatcher, phenotypes virulent to six leaf rust differential lines increased steadily from G_0 to G_{12}. Because Thatcher was susceptible to all the isolates in G_0, any virulences to the Thatcher isogenic lines containing *Lr* genes may be considered unnecessary. Isolates with fewer virulences did not increase in later generations, as would be expected if unnecessary virulences in general had deleterious fitness effects. The most common phenotypes at G_{12} in the Thatcher population, TBJ, TBD, and KBG, had six, five, and four unnecessary virulences. As such, there was little evidence of stabilizing selection such as Vanderplank (24) described.

Similarly, isolates with the fewest virulences besides the required virulence to *Lr3ka* or *Lr11* were not the prevalent isolates in

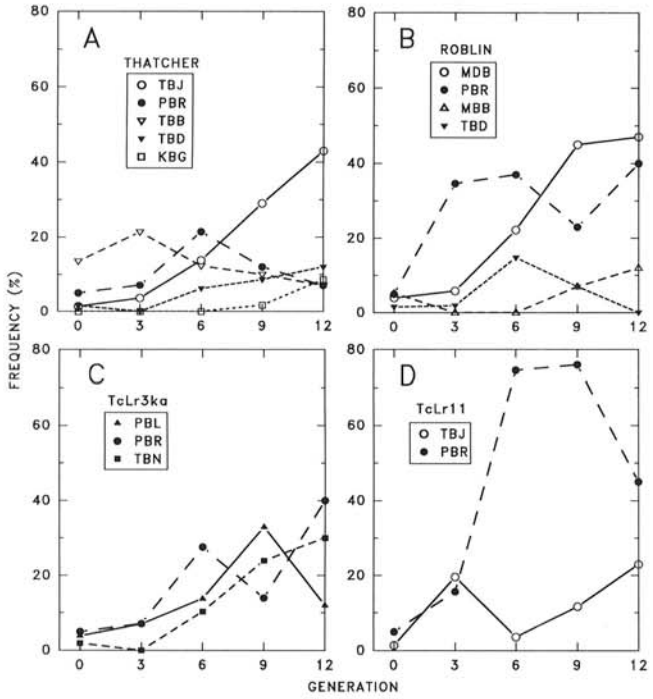

Fig. 2. Frequency (percent) of the predominant *Puccinia recondita* f. sp. *tritici* virulence phenotypes selected over 12 generations on wheat lines Thatcher, Roblin, Tc*Lr3ka*, and Tc*Lr11*.

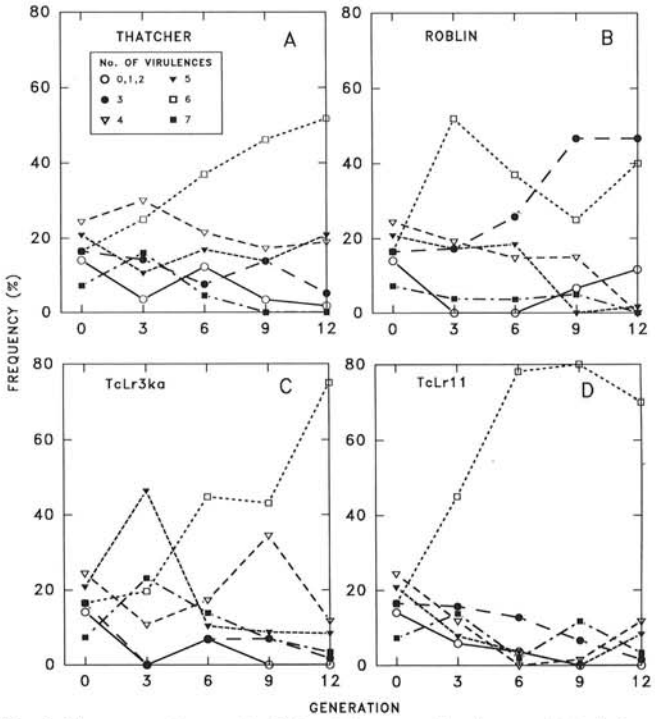

Fig. 3. Frequency (percent) of *Puccinia recondita* f. sp. *tritici* virulence phenotypes virulent on zero to seven isogenic wheat leaf rust differential lines selected over 12 generations on wheat lines Thatcher, Roblin, Tc*Lr3ka*, and Tc*Lr11*.

TABLE 3. Chi-square values for homogeneity of virulence frequencies to leaf rust resistance genes and slopes from regression of logits of virulence frequencies on generation number in populations of *Puccinia recondita* f. sp. *tritici* cultured for 12 generations on four wheat hosts with different resistance genes

Gene	Thatcher population			Roblin population			Tc*Lr3ka* population			Tc*Lr11* population		
	χ^2	Slope[x]	*t*-value	χ^2	Slope	*t*-value	χ^2	Slope	*t*-value	χ^2	Slope	*t*-value
Lr1	2.45	−0.0178	1.054	...[y]			3.95	0.0351	1.780	2.18	0.0027	0.192
Lr2a	26.37 a[z]	0.0616	4.245 a[z]	27.43 a	−0.1409	2.539 a	0.94	0.0096	1.896	27.20 a	−0.0375	0.835
Lr2c	21.77 a	0.0430	1.511	35.02 a	−0.0561	1.500	41.06 a	0.0741	2.690 a	18.74 a	0.0435	1.652
Lr3ka	25.65 a	−0.0677	6.481 a	19.39 a	−0.0391	1.580	...			35.04 a	0.0144	0.329
Lr11	31.04 a	0.0693	5.345 a	15.58 a	0.0122	0.415 a	22.18 a	0.0317	0.898	...		
Lr17	28.14 a	0.0709	13.094 a	10.06 a	−0.7270	1.821	9.82 a	0.0411	2.820 a	5.93	0.0224	1.323
Lr24	2.65	−0.0040	0.216	44.44 a	0.0833	3.351 a	0.56	−0.0034	0.3523	...		
Lr26	4.21	−0.0027	0.007	1.96	−0.0371	1.174	...			...		
Lr30	20.94 a	−0.0599	3.009 a	17.52 a	−0.1500	0.400	46.51 a	0.0243	0.443	48.34 a	0.0414	0.900

[x] Slope of log $(q_n/1 - q_n)$ regressed on generation, q_n = virulence frequency.
[y] Virulence frequencies were near 0.0 or 1.0 in all generations.
[z] Numbers followed by a are significant at $P = 0.05$.

the Tc*Lr3ka* and Tc*Lr11* selection populations, respectively. In these two populations, phenotypes with six virulences increased over generations and were the most predominant phenotypes at G_{12}. The common phenotypes at G_{12} in the Tc*Lr3ka* population, PBR, TBN, and PBL, had five, five, and three unnecessary virulences, respectively. Likewise, at G_{12} in the Tc*Lr11* population, the most common phenotypes, PBR and TBJ, had five unnecessary virulences each.

In this study, a population of *P. r. tritici* phenotypes was intermated to more evenly distribute virulences with respect to genes that condition fitness and to increase the diversity of virulence phenotypes for selection to act on. In previous studies (13,14), one generation of random mating greatly reduced the overall level of virulence associations in the G_0 population compared to the progenitor asexual population from which it was derived. However, some significant associations between virulence pairs remained in the sexual population, indicating that genes that condition fitness may not have been randomly distributed among the different virulence phenotypes. Genes affecting fitness may still be associated with specific virulence loci. In the *P. r. tritici* population developed for this study, virulences to *Lr2a, Lr11,* and *Lr17* may be associated with genes that confer higher fitness on Thatcher (Table 3). Furthermore, the actual fitness effects of the virulence genes themselves may be too small to determine in this type of experiment if other genes with larger effects on fitness also are segregating in the population (1).

Similarities in the predominant phenotypes in the Thatcher, Tc*Lr3ka*, and Tc*Lr11* selection populations indicated that selection for the same fitness genes may have occurred in these populations. Also, phenotypes with virulence to six differentials predominated in all three populations. Fitness appeared to be associated with virulence to *Lr2a, Lr11,* and *Lr17* in the Thatcher population. Virulence to *Lr17* also increased in the Tc*Lr3ka* population. In this population, phenotype TBN, which is virulent to *Lr2a* and *Lr17* had the most consistent increase over generations. Phenotype TBJ, which was the most fit isolate in the Thatcher population, was avirulent to *Lr3ka*, which would preclude this phenotype from reproducing on lines with this gene. In the Tc*Lr11* population, TBJ increased steadily after G_6 and may have replaced PBR as the most common phenotype in further generations.

The differing sizes of the effective populations at G_0 in the four selection populations almost certainly had an effect on the relative increase and decline of virulence phenotypes over generations. In the Thatcher population, all phenotypes in G_0 were considered to be in the effective population because the isolates were virulent to Thatcher. In this selection population, phenotypes TBB and PBR were the most common phenotypes at G_3 and G_6, respectively, and declined to less than 10% by G_{12}. The increase in early generations was most likely due to the relatively high frequency of both phenotypes in G_0. TBB and PBR declined in later generations as they were replaced by phenotypes that were at lower initial frequencies in G_0 but that had higher fitness levels.

Founder effects caused by the elimination of phenotypes avirulent to *Lr3ka* and *Lr11* at G_1 in the Tc*Lr3ka* and Tc*Lr11* populations, respectively, almost certainly affected the subsequent selection of phenotypes in these populations. The effects of random drift or sampling error from generation to generation would be greater in populations with smaller initial effective population sizes. Phenotype PBR was the most common phenotype with virulence to *Lr3ka* and *Lr11*, respectively, in the Tc*Lr3ka* and Tc*Lr11* populations at G_0. This, along with sampling error, may explain the prevalence of PBR in the Tc*Lr3ka* and Tc*Lr11* populations. PBR also was a common phenotype at G_{12} in the Thatcher and Roblin populations and may have a high overall average fitness level. However, PBR steadily declined in the Thatcher population after G_6, which suggests the high incidence of PBR in the other three populations was due to high initial frequency in the effective populations at G_0 and to sampling error.

In the Roblin population, a different phenotype, MDB, increased steadily and was the most common isolate at G_{12}. This phenotype was not commonly found in any other selection population. The increase of MDB was probably associated with increased spore production or virulence to *Lr13* and/or *Lr34*. It is possible that increased fitness on *Lr13* and/or *Lr34* is associated with virulence to *Lr24* and avirulence to *Lr2a* in the Roblin selection population. MDB was the most common of the phenotypes virulent to *Lr24* and avirulent to *Lr2a* in G_0. Other similar phenotypes, such as MDL and MDM, may not have increased because of lower initial frequency at G_0. Phenotype PBR may have increased only because of its relatively high initial frequency at G_0 and/or because it also had a higher general level of fitness on lines with *Lr13* and *Lr34*. It is unlikely that *Lr1* and *Lr10* in Roblin exerted much selection pressure because over 85% of the G_0 population was virulent to both genes. The effective population size for these two genes would be similar to the entire G_0 population.

At G_{12}, Roblin still showed a resistant response to the rust population even though specific phenotypes had been selected. Selection may not have been for complete virulence to *Lr13* or *Lr34* but, rather, for increased spore production among isolates that produce intermediate avirulent infection types to these genes.

Uredinial field populations of *P. r. tritici* previously have been found to be highly heterozygous at virulence loci (14). It was hypothesized that heterozygosity for virulence may impart selective advantage in *P. r. tritici* populations. Because avirulence is usually dominant, and if heterozygotes have a selective advantage, selection on a susceptible host, such as Thatcher, should have resulted in a final population with a lower average number of specific virulences per isolate. However, the average number of virulences per isolate actually increased in the Thatcher population. The selection that occurred in a small population over relatively few generations in this study may be quite different from the selective process that has occurred for a much longer period of time in the uredinial field populations of *P. r. tritici* in North America. The relationships between specific virulences and fitness that were observed in this experiment are probably unique to this particular small population of *P. r. tritici* phenotypes.

In a previous selection study with the progenitor asexual uredinial field population, the relative frequencies of the virulence phenotypes changed very little after being selected for eight generations on Thatcher (11). The uredinial field population must have been heterozygous for fitness genes, because the phenotypes in the sexual population effectively segregated for fitness when selected on Thatcher. Uredinial field isolates might differ relatively little for fitness on Thatcher because these isolates are already well adapted to hexaploid bread wheat. Sexual recombination would have created new genotypes with much greater fitness differences than those between genotypes in a well-adapted asexual population.

Leonard (15) determined in a population of *P. graminis* f. sp. *avenae* that races avirulent to specific resistance genes had fitness values 14–46% higher than had virulent races. In contrast, Bronson and Ellingboe (1) determined that reduced fitness segregated independently of virulence genes in F_1 populations of *Erysiphe graminis* f. sp. *tritici*. In the present study, certain virulences were associated with higher or lower fitness, whereas other virulences were independent.

To determine the effects of virulence genes on fitness, populations should ideally be designed so individual genes are the units of selection. This would eliminate disequilibria between fitness genes and virulence loci and yield selection coefficients that can be solely attributed to alleles at virulence loci. In the case of cereal rust fungi, this necessarily requires that each selection generation alternate with a generation of random mating. However, because *P. r. tritici* exists in asexual populations in North America, selection for fitness in the uredinial stage may be confounded by selection for ability to complete the sexual cycle. In the present study, although G_0 was derived from a random-mating population, all subsequent selection occurred in a population of genotypes that did not undergo any further sexual recombination. Østergård (21) recommended in this type of population that a selection coefficient for each phenotype be calculated

and the phenotypes sorted according to virulence to each specific resistance gene. Selection coefficients for alternate alleles at virulence loci are then determined from the weighted average selection coefficients of the individual phenotypes. In the present study, the large number of phenotypes relative to the sample sizes precluded Østergård's (21) suggested analysis. In the Thatcher population, only five phenotypes were detected in G_0 and all the sampled generations. A much larger sample size would have been needed to detect the phenotypes present at very low frequencies.

In summary, this study determined there was no general relationship between the average number of unnecessary virulences and apparent fitness in populations of *P. r. tritici*. Rather, fitness appeared to be associated or dissociated with certain individual virulences. Analysis of the annual virulence surveys of *P. r. tritici* in Canada (10,12) also have shown that prevalence and selection of phenotypes has no general relationship with the number of unnecessary virulences. However, *P. r. tritici* populations in North America have retained high levels of virulence diversity, which indicates selection for virulence to host resistance genes is effectively balanced by other selective forces in this pathogen population.

LITERATURE CITED

1. Bronson, C. R., and Ellingboe, A. H. 1986. The influence of four unnecessary genes for virulence on the fitness of *Erysiphe graminis* f. sp. *tritici*. Phytopathology 76:154-158.
2. Browning, J. A., and Frey, K. J. 1969. Multiline cultivars as a means of disease control. Annu. Rev. Phytopathol. 7:355-382.
3. Crill, P. 1977. An assessment of stabilizing selection in crop variety development. Annu. Rev. Phytopathol. 15:185-202.
4. Dyck, P. L., and Johnson, R. 1983. Temperature sensitivity of genes for resistance in wheat to *Puccinia recondita*. Can. J. Plant Pathol. 5:229-234.
5. Feekes, W. 1941. De tarwe en haar milieu. Versl. Tech. Tarwe Commun. Hoitsema, Groningen 12:523-888.
6. Grant, M. W., and Archer, S. A. 1983. Calculation of selection coefficients against unnecessary genes for virulence from field data. Phytopathology 73:547-551.
7. Groth, J. V., and Roelfs, A. P. 1987. Analysis of virulence diversity in populations of plant pathogens. Pages 63-67 in: Populations of Plant Pathogens. M. S. Wolfe and C. E. Caten, eds. Blackwell Scientific Publications, Palo Alto, CA.
8. Johnson, T. 1961. Man-guided evolution in plant rusts. Science 133:357-362.
9. Katsuya, K., and Green, G. J. 1967. Reproductive potentials of races 15B and 56 of wheat stem rust. Can. J. Bot. 45:1077-1091.
10. Kolmer, J. A. 1989. Virulence and race dynamics of *Puccinia recondita* f. sp. *tritici* in Canada during 1956–1987. Phytopathology 79:349-356.
11. Kolmer, J. A. 1990. Selection of virulence phenotypes in a heterogeneous, asexual population of *Puccinia recondita* f. sp. *tritici*. Phytopathology 80:1377-1381.
12. Kolmer, J. A. 1991. Evolution of distinct populations of *Puccinia recondita* f. sp. *tritici* in Canada. Phytopathology 81:316-322.
13. Kolmer, J. A. 1992. Effect of sexual recombination in two populations of the wheat leaf rust fungus *Puccinia recondita*. Can. J. Bot. 70:359-363.
14. Kolmer, J. A. 1992. Virulence heterozygosity and gametic phase disequilibria in two populations of *Puccinia recondita* (wheat leaf rust fungus). Heredity 68:505-513.
15. Leonard, K. J. 1969. Selection in heterogeneous populations of *Puccinia graminis* f. sp. *avenae*. Phytopathology 59:1851-1857.
16. Leonard, K. J. 1977. Virulence, temperature optima, and competitive abilities of isolines of races T and O of *Bipolaris maydis*. Phytopathology 67:1273-1279.
17. Leonard, K. J., and Czochor, R. J. 1980. Theory of genetic interactions among populations of plants and their pathogens. Annu. Rev. Phytopathol. 18:237-258.
18. Loegering, W. Q. 1951. Survival of races of wheat stem rust in mixtures. Phytopathology 41:55-65.
19. Long, D. L., and Kolmer, J. A. 1989. A North American system of nomenclature for *Puccinia recondita* f. sp. *tritici*. Phytopathology 79:525-529.
20. Martens, J. W. 1973. Competitive ability of oat stem rust races in mixtures. Can. J. Bot. 51:2233-2236.
21. Østergård, H. 1987. Estimating relative fitness in asexually reproducing plant pathogen populations. Theor. Appl. Genet. 74:87-94.
22. Samborski, D. J., and Dyck, P. L. 1976. Inheritance of virulence in *Puccina recondita* on six backcross lines with single genes for resistance to leaf rust. Can. J. Bot. 54:1666-1671.
23. Steel, R. G., and Torrie, J. H. 1980. Principles and Procedures of Statistics. McGraw-Hill, New York. 633 pp.
24. Vanderplank, J. E. 1968. Disease resistance in plants. Academic Press, New York. 206 pp.
25. Wolfe, M. S., and Barrett, J. A. 1980. Can we lead the pathogen astray? Plant Dis. 64:148-155.