

Mutation to Wider Virulence in *Puccinia graminis* f. sp. *tritici*: Evidence for the Existence of Loci Which Allow the Fungus To Overcome Several Host Stem Rust Resistance Genes Simultaneously†

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Mutants of *Puccinia graminis* f. sp. *tritici* were obtained which were able to overcome simultaneously several host stem rust resistance (Sr) genes effective against the wild-type culture. These results suggest that, in addition to those P_{sr} loci which relate specifically to host Sr genes in a "gene for gene" manner, one or more general loci may be present in this pathogen. The product(s) of these general genes may be necessary for the expression of various host Sr genes. The evolution of a super race capable of overcoming many Sr genes for resistance seems likely, as such a pathogen would not have to give up the many proteins predicted by the gene-for-gene relationship. Moreover, it appears that specificity in the wheat rust system is more complicated than suggested by the gene-for-gene concept.

A "gene for gene" relationship exists between host and parasite in the black stem rust disease of wheat (5, 9). The development of this disease, which results in a uredial pustule, is affected by a series of corresponding gene pairs in host and parasite. A particular pathogen race will colonize a particular host cultivar, resulting in the formation of a high-infection-type, or type 4, pustule, but only when the parasite possesses alleles for high pathogenicity at loci corresponding to host loci containing alleles for low reaction. Each host low-reaction allele must be overcome by a particular pathogen high-pathogenicity allele if a high-infection type is to occur (7).

Wheat low-reaction alleles are generally dominant to high-reaction alleles. Rust high-pathogenicity alleles are generally recessive to alleles for low pathogenicity. Low-infection types, i.e., uredial pustules ranging from type 0; to type 3, result when a low-reaction/low-pathogenicity combination occurs at at least one corresponding pair of loci. A high-infection-type (i.e., type 4) pustule arises only when one of the other three possible combinations, low reaction/high pathogenicity, high reaction/low pathogenicity, or high reaction/high pathogenicity, occurs at every corresponding pair of loci.

It is not yet understood how pairs of dominant low-reaction and low-pathogenicity alleles in host and parasite interact to accomplish a "hurdle" (2) or "stop signal" (10) in symbiotic development resulting in a low-infection type. Various hypotheses have been suggested (1, 2, 4, 6). Although to date no translational products of any low-reaction or low-pathogenicity gene have been identified, the cloning of a dominant low-pathogenicity gene in *Pseudomonas syringae* by Staskawicz et al. (15) suggests that techniques in modern molecular genetics may provide answers as to the expression of these genes.

The results from a mutation study in *Puccinia graminis* Pers. f. sp. *tritici* Eriks. & E. Henn. are reported here. The original purpose of this study was to obtain various parasite differentials via mutation rather than via the conventional method of controlled crosses, which are quite difficult to achieve in this fungus. The results obtained were unexpected and suggest that the mechanism for creating blocks in the development of this antagonistic symbiosis may be even more complex than suggested by the gene-for-gene relationship.

MATERIALS AND METHODS

Isolates. Single uredial pustule isolations were made from culture 111x36F1 of *P. graminis* f. sp. *tritici* on Little Club wheat seedlings. For a description of this culture, see Loegering and Powers (9). To determine culture purity, isolates were screened on the monogene line Cns/ISr18-Ra, which has the Sr18 allele for low reaction. As 111x36F1 has the genotype for low pathogenicity at the P_{sr}18 locus, a low-infection type, specifically, 0; results. Most cultivars of wheat now being grown possess Sr18; thus, possible contaminants would be expected to have the genotype for high pathogenicity at the P_{sr}18 locus and be able to overcome the Sr18 gene for low reaction. These would be expected to produce a type 4 pustule on this host monogene line. The absence of such pustules among the necrotic flecks was used as an indicator of purity in the various single-pustule isolates obtained from culture 111x36F1.

All single-pustule isolates were found to be free of contaminants. One such isolate was selected for further study. The inoculum was increased by injecting an aqueous suspension of urediospores from this isolate into the leaf sheath of 4-week-old Little Club plants. After 2 weeks, the newly expanded leaves became covered with pustules, the urediospores from which were collected and used immediately in the mutation studies.

X-ray studies. Freshly collected urediospores were exposed by using a Picker X-ray machine at 150 pkV and 9 mA at a distance from the source to provide an exposure of 1.6

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† This work is dedicated to the memory of my mentor, coauthor, and friend, William Q. Loegering, who passed away on 15 December 1987.

krads/min. Spores were subjected to exposures of 0, 24, 48, 96, 128, or 224 krads.

EMS studies. Freshly collected urediospores were placed in small culture tubes containing 1 ml of ethyl methanesulfonate (EMS) in 0.01% aqueous Tween 20. Concentrations of EMS used were 0, 0.005, 0.008, 0.010, 0.040, and 0.080 M. Although various surfactants and concentrations were tested, 0.01% Tween 20 was the most effective in keeping the spores in even suspension and was the least inhibitory to spore germination after washing.

The urediospores were kept in suspension in the EMS solutions for 2 h at room temperature by occasional stirring with glass rods. The spores were collected on a Millipore membrane filter (0.65- μ m pore size) and washed with at least 1 liter of distilled water to remove the EMS and Tween 20. The spores were removed from the filters in 15-ml volumes of distilled water.

Screening for mutations to high pathogenicity. After mutagenesis, the urediospores were used to inoculate 6-day-old seedlings of Cns/Sr18-Ra (Sr18) and Cns/Sr7b-Ra (Sr7b) monogene lines of wheat. Culture 111x36F1 is known to be heterozygous for low pathogenicity at both Psr18 and Psr7b loci (9) and produces a type 0; on Sr18 and a type 2 pustule on Sr7b host monogene lines. The occurrence of a higher-infection-type pustule would be most easily explained by the loss of the dominant allele (i.e., Vv to $_v$) and therefore as a mutation from low pathogenicity to high pathogenicity at either Psr locus.

Just prior to inoculation of the wheat seedlings, the cuticle was removed by rubbing the leaves gently between wetted thumb and forefinger. The plants were placed in moist chambers in the greenhouse and misted until water droplets became visible on the leaf surfaces. The X-ray-treated urediospores were mixed with talc and dusted onto the wet seedlings. The EMS-treated spores were suspended in distilled water and sprayed over the wet seedlings with an atomizer. After inoculation of the seedlings, polyethylene covers were placed over the moist chambers. The following morning, these covers were partially removed to allow the leaf surfaces to dry gradually. By late afternoon, the seedlings were moved to a greenhouse bench.

After 2 weeks, the wheat seedlings were observed for uredial pustules. Those higher than type 0; on an Sr18 host or higher than type 2 on the Sr7b host were considered presumptive mutations to wider virulence. Such presumptive mutant urediospores were collected in water and injected into leaf sheaths of 4-week-old Little Club plants. After 2 weeks, the newly expanded leaves were covered with pustules, and the spores were collected and stored in glass vials under liquid nitrogen as described by Loegering et al. (8).

Testing for temporary variants. After liquid nitrogen storage for 2 to 3 weeks, all presumptive mutant cultures were reinoculated onto Sr18 and Sr7b hosts to test for temporary variants, the occurrence of which has been reported by Rowell et al. (12).

Determining host range of mutants. Because most cultures studied routinely in the greenhouse possess the allele for high pathogenicity at the Psr18 locus and produce a higher infection type than 0; on the Sr18 host, it was necessary to determine whether the presumptive mutants which developed on the Sr18 hosts were contaminants. The presumptive mutants were inoculated onto a set of host differentials which included the following wheat cultivars: Little Club; Norka-Sr15; W2961; the monogenic substitution lines with W2961 background line C-Sr7a, SrN, SrL1, SrL2, and Sr9e;

Chinese Spring; and the monogenic substitution lines with Chinese Spring background Sr18, Sr7b, Sr8, Sr9a, Sr11, and Sr16.

The infection types resulting on these host differentials were compared with those known to result from inoculation with any other rust culture studied in the greenhouse during this period. The results from these initial host range studies suggested that a number of these presumptive mutants were neither contaminants nor simple mutants at the Psr18 or Psr7b loci. Urediospores were stored under liquid nitrogen, and the host range studies were repeated 5 years later and again 8 years later. As some variation in infection type occurs on some of these host differentials due to temperature variation, the wild-type 111x36F1 culture was included as a control in every host range experiment. Infection type was rated as high only when it was higher than that produced when the host differential was inoculated with the wild-type culture.

Infection types were based on uredial pustules as described by Stakman et al. (14) and are as follows: 0; = hypersensitive flecks; 1 = minute uredia; 2 = small uredia with green islands; 3 = medium uredia; 4 = large uredia; X = variable-sized uredia. Plus and minus indicate variation within infection type. Chlorosis or necrosis is indicated by c or n, respectively. Two infection types written together (i.e., 0;1, 0;2⁻, or 2⁺3⁻) indicate variation. A type 0;1 would be predominantly hypersensitive flecks with occasional minute pustules on the same leaf. A type 0;2⁻ would be flecks with occasional minute pustules with green islands. A type 2⁺3⁻ would be small uredia, most with green islands.

Rust cultures. The rust cultures discussed in this report are being stored at the U.S. Department of Agriculture Cereal Rust Laboratory, University of Minnesota, St. Paul.

RESULTS

Forty-three isolates were obtained which produced a higher infection type than the wild-type culture on either Sr18 or Sr7b hosts. Of these, 19 proved to be temporary variants to wider virulence and reverted back to wild type after storage in liquid nitrogen. The remaining 24 isolates are believed to be mutants, as none of these had a host range similar to any other culture being studied in the greenhouse during the period of the study. Also, no spontaneous mutants or contaminants occurred on plants inoculated with 111x36F1 urediospores which had not been subjected to either X-ray or EMS during the initial screening for mutants on Sr18 and Sr7b hosts. EMS treatment accounted for 8 of these mutants and X-ray treatment accounted for 16. The 48- and 96-krad X-ray exposures yielded the majority of these mutants (Table 1).

The infection types resulting on host differentials after 5 and 8 years of storage of the urediospores of these mutants were nearly identical to those observed after storage for only a few weeks. Thus, the mutations appear to be permanent. Mutants M16, M32, M21, and M7, which originally produced a high-infection type on either an Sr18 or an Sr7b host, lost the ability to overcome either of these host low-reaction genes after storage. However, each of these mutants retained the ability to overcome Sr7a. M7 retained its ability to overcome SrN as well (Table 2).

Three mutants (M16, M21, and M32) overcame only one host low-reaction gene (Table 2, Sr7a). Five mutants overcame two host low-reaction genes: four of these (M2, M4, M5, and M6) overcame SrL2 and Sr18, although the infection types were not identical; the other (M7) overcame Sr7a

TABLE 1. Infection types produced on host differentials by parental cultures 111-55A and 36-55A, the wild-type F₁ hybrid 111x36F1, and various mutants

Culture (mutagen)	Infection type ^a										
	7a ^b	N	L1	L2	9e	18	7b	8	9d	11	16
Parental											
111-55A	3 ^c	0;	0;	0;	0;	0;	2 ⁻	2	2 ⁼	0;	2 ⁼
36-55A	4	3 ^c	3	4	0;1	4	4	4	2 ⁻	0;	4
Wild type											
111x36F1	3 ^c	0;	0;	0;	0;	0;	2 ⁻	0;1	0;2 ⁼	0;	2 ⁼
Mutant											
X-ray (krads)											
M2 (48)	3 ^c	0;	0;	3	0;	3	2 ⁼	0;	0;	0;	0;1
M4 (48)	3 ^c	0;	0;	4	0;	3	2 ⁻	0;	0;	0;	0;1
M5 (48)	3 ^c	0;	0;	3 ^c	0;	3 ⁻	2 ⁻	0;	0;	0;	0;1
M6 (48)	3 ^c	0;	0;	3 ^c	0;	3	2 ⁻	0;	0;	0;	0;1
M7 (48)	4	3	0;	0;	0;	0;	2 ⁻	0;1	2 ⁼	0;	2 ⁼
M16 (224)	4	0;	0;	0;	0;	0;	2 ⁻	0;	0;	0;	0;1
M30 (24)	4	3 ⁻	3	0;	0;1	4	4	4	4	0;	3 ^c
M31 (48)	4	1 ⁿ	0;	4	0;	4	2 ⁻	0;1	0;	0;	2 ⁻
M32 (48)	4	1 ⁿ	0;	0;	0;	0;	2 ⁻	0;1	0;	0;	2 ⁻
M34 (48)	4	1 ⁿ	0;	4	4	4	4	2 ⁻	3 ^c	4	4
M36 (96)	3 ^{-c}	0;	0;	2 ⁺⁺⁺	4	4	3 ^c	3 ^{+c}	3 ^{+c}	3 ^{+c}	3 ^{+c}
M37 (96)	4	3	3	3	0;	4	3 ^c	2	3 ^{+c}	0;	3 ^{+c}
M38 (96)	4	2 ⁺⁺⁺	2 ⁺⁺⁺	3 ^c	0;	4	3 ^c	2	3 ^{+c}	0;	2
M39 (96)	4	2 ⁺⁺⁺	2 ⁺	3 ^c	0;	4	3 ^c	2	3 ^{+c}	0;	2
M40 (96)	4	2 ⁺⁺⁺	2 ⁺	2 ⁺	0;	4	2	2	3 ^{+c}	0;	2
M41 (96)	3 ^{-c}	0;	0;	2	3 ^{+c}	3 ^{+c}	3 ^c	3 ^{+c}	3 ^{+c}	3 ^c	3 ^{+c}
EMS (mM)											
M18 (5)	4	3	4	3	0;1	4	3 ^c	2	2 ⁼	0;	3
M19 (10)	4	0;	0;	3	0;1	4	3 ^c	3 ^c	2 ⁼	0;	3 ^c
M20 (10)	4	0;	0;	3	0;1	4	2	2 ⁻	2 ⁼	0;	3 ^{-c}
M21 (5)	4	0;	0;	0;	0;1	0;	2 ⁻	0;	2 ⁼	0;	1
M24 (8)	4	3 ⁻	X	2 ⁺ 3 ⁻	0;1	3	2 ⁻	4	4	0;	3 ^{-c}
M25 (40)	4	2	3 ^c	4	0;	4	4	4	4	0;	3 ^c
M26 (40)	4	3 ⁻	2 ⁺	0;	0;1	4	2	4	4	0;	3 ^c
M27 (40)	4	3 ⁻	2 ⁺	0;	0;1	4	2 ⁺	4	4	0;	3 ^c

^a 0; = hypersensitive flecks; 1 = minute uredia; 2 = small uredia with green islands; 3 = medium uredia; 4 = large uredia; X, = variable sized uredia. + and - indicate variation within infection type. c and n indicate chlorosis and necrosis, respectively. Two infection types written together indicate variation on the same leaf.

^b Host Sr gene (low reaction).

and SrN. One mutant (M31) overcame three host low-reaction genes (Sr7a, SrL2, and Sr18). M20 overcame five low-reaction genes (Sr7a, SrL2, Sr18, Sr8, and Sr16). M19 overcame six low-reaction genes, including those overcome by M20 as well as Sr7b. Four overcame seven host low-reaction genes. Of these, M26 and M27 were similar, differing slightly on the Sr7b host. Seven of the 24 mutants overcame eight low-reaction genes. Of these, M38 and M39 were similar, differing slightly only on the SrL1 host. M36 and M41 overcame the same eight low-reaction genes but differed in infection type produced on several hosts. M37 and M34 overcame nine host low-reaction genes but differed in host range (Tables 1 and 2).

Although all of the mutants exhibited increased virulence against one or more host low-reaction genes, several also had reduced virulence against certain host low-reaction genes. Compared with the wild-type culture, M2, M4, M5, M6, M16, and M21 produced lower infection types on the Sr16 host (Table 1). M18, M24, M25, M26, M30, M37, M38, M39, and M40 produced lower infection types (0;1) on the Sr9a host than the wild-type culture (type 4; data not shown). Interestingly, these are some of the more virulent mutants, each being able to overcome seven to nine host

low-reaction genes effective against the 111x36F1 wild-type culture.

The Sr18 and Sr7b hosts were used in the initial screening for mutations to wider virulence. Therefore, it should not be surprising that mutants capable of overcoming the Sr18 locus were the most common (20 of the 24). Nine of the mutants overcame Sr7b; three of these produced a type 4 and the rest produced a type 3c pustule. Other host loci which were overcome commonly included Sr7a (overcome by 18 mutants), SrL2 (17 mutants), Sr8 (15 mutants), Sr9d (12 mutants), Sr16 (12 mutants), SrN (11 mutants), and SrL1 (9 mutants).

Sr9e and Sr11 were the most effective host low-reaction genes as each were overcome by only 3 of the 24 mutants. The wild type 111x36F1 is probably homozygous at both of these loci as both parental strains are low-reaction types at both of these loci. However, one mutant (M34) overcame both of these host low-reaction genes (Table 2).

All mutants capable of overcoming SrL1 or SrN or both were also able to overcome Sr7a. All capable of overcoming Sr9d or Sr16 or both were able to overcome Sr8. All capable of overcoming Sr8 could overcome Sr7b. The three mutants producing a high-infection type on Sr9e hosts also produced

TABLE 2. Comparison of mutants of *P. graminis* f. sp. *tritici* culture 111x36F1 which exhibit wider virulence than the wild-type culture

Mutant	Host Sr genes overcome by mutant but not by wild type											Total ^a
	7a	N	L1	L2	9e	18	7b	8	9d	11	16	
M16	+											1
M32	+											1
M21	+											1
M7	+	+										2
M2				+		+						2
M4				+		+						2
M5				+		+						2
M6				+		+						2
M31	+			+		+						3
M20	+			+		+		+			+	5
M19	+			+		+	+	+			+	6
M40	+	+	+	+		+		+	+			7
M26	+	+	+	+		+		+	+		+	7
M27	+	+	+			+		+	+		+	7
M24	+	+		+		+		+	+		+	7
M38	+	+	+	+		+	+	+	+			8
M39	+	+	+	+		+	+	+	+			8
M18	+	+	+	+		+	+	+			+	8
M36				+	+	+	+	+	+	+	+	8
M41				+	+	+	+	+	+	+	+	8
M30	+	+	+			+	+	+	+	+	+	8
M25	+	+	+	+		+		+	+		+	8
M37	+	+	+	+		+	+	+	+		+	9
M34	+			+	+	+	+	+	+	+	+	9
Total ^b	18	11	9	17	3	20	9	15	12	3	12	

^a Number of loci overcome by mutant but not by wild type.

^b Number of mutants overcoming this locus.

a high-infection type on Sr11 hosts. All mutants overcoming SrL2, Sr9e, Sr7b, Sr8, Sr9d, Sr11, and Sr16 were able also to overcome Sr18 (Table 2), perhaps because most of these mutants were screened initially with the Sr18 host.

Surprisingly, mutants often produced an infection type lower than the expected type 4. For example, mutation at the PsrL2 locus would be expected to result in a type 4 pustule on the SrL2 host. Of the 17 mutants overcoming SrL2, 13 produced type 2 and 3 pustules instead of the expected type 4. Likewise, 6 of the 20 overcoming Sr18 and 7 of the 9 overcoming Sr7b produced infection types lower than a type 4 (Table 2).

The low-infection types observed for Sr7a, Sr7b, Sr9d, Sr9e, Sr11, Sr16, and Sr18 agreed with those described by Roelfs and McVey (11); however, they suggest the Sr8 low-infection type to be a 2. We observed a type 2⁻ with the avirulent parental 111-55A culture, but consistently obtained a 0;1 with the 111x36F1 culture. As reference was always made to this 111x36F1 culture used as the wild-type control in every host range study, infection types above 0;1 were considered high. Of the 15 isolates considered mutations to high pathogenicity at the Psr8 locus, 7 produced a type 2 on the Sr8 host (Table 1).

All cultures produced high-infection types on the variety Little Club. This cultivar lacks any of the above-mentioned low-reaction genes and was used as a control in all host range studies.

DISCUSSION

Previous studies of mutation to wider virulence in the flax rust fungus, *Melampsora lini*, have revealed generally point

mutations at a single locus in the pathogen from low to high pathogenicity (3, 13). Such mutants were observed to overcome only one host gene for low reaction not overcome by the wild-type culture. Rowell et al. (12) used X-irradiation to generate mutants in the same 111x36F1 culture of *P. graminis* f. sp. *tritici* used in this study and found only point mutations. Their initial screening was on the wheat cultivar Marquis, which is believed to possess both Sr18 and Sr7b alleles for low reaction. Of 128 mutants obtained, none produced a type 4 pustule on Marquis; therefore, none were able to overcome the Sr18 and Sr7b loci simultaneously. In the present study, 3 of 24 mutants were virulent on both ISr18-Ra and ISr7b-Ra monogenic lines. The host differentials used in the Rowell et al. (12) study are now known to possess two or more low-reaction genes. The host differentials used in this study are believed to be truly monogenic and should be more sensitive for detecting the ability of a mutant to overcome a specific Sr gene.

It is possible that the unusual results obtained in this study arose because the putative mutants were merely contaminants. We believe this is unlikely, as no mutant resembled any other culture of this fungus being studied in the greenhouse during the course of these experiments. Moreover, the patterns of virulence and avirulence for most of our mutant cultures were even different from those cultures carried in our collection preserved in liquid nitrogen. Indeed, many of the mutants would be identified as new races, using the key of Stakman et al. (14). The mutagenesis and initial isolation of presumptive mutants were conducted during the winter, before there was any rust observed in the fields in the area around Columbia, Mo., and contamination from such natural sources would have been unlikely. No contaminants occurred on uninoculated control ISr18-Ra or ISr7b-Ra plants or on those inoculated with wild-type spores, although the numbers of such control plants equalled the number of plants inoculated with spores subjected to mutagenesis. The work was initiated with spores from a single uredial pustule which were increased by using methods which have not resulted in contamination in the past. Moreover, a minimum of 14 different genotypes were isolated. We believe these isolates to be true mutants of culture 111x36F1.

As most of these mutants are able to overcome several host low-reaction genes, it is unlikely that the mutations occurred in existing low-pathogenicity alleles. As many as nine different loci would have to have been affected in some of these mutants. One possible explanation is that mutagenic treatment prevented an entire nucleus of these dikaryotic cells from being expressed. For example, the loss of the 111-55A nucleus should enable a mutant to overcome Sr7a, SrN, SrL1, SrL2, Sr18, Sr7b, Sr8, and Sr16, leaving only Sr9e, Sr9d, and Sr11 effective. None of the mutants obtained in this study could be explained by such a hypothesis, although M25 could have occurred through the loss of the 111-55A nucleus along with a simple deletion in the Psr9d locus in the 36-55A nucleus.

A gene-for-gene relationship exists between this fungus and wheat that blocks disease development. Low-infection types occur when dominant low-reaction genes are present in the host, and generally dominant low-pathogenicity genes specific to these low-reaction genes are also present in the parasite. Such low-reaction genes can be overcome when the pathogen has the allele for high pathogenicity at corresponding loci. Classical genetic studies provide strong evidence for the existence of these low-reaction-specific loci for pathogenicity in this fungus. However, data presented here suggest that these high-pathogenicity genes are not the only genes

present in the fungus which enable this parasite to overcome host low-reaction genes. Perhaps parasite gene products in addition to the low-pathogenicity gene products are needed to induce a resistant reaction or low-infection type in wheat plants containing low-reaction genes. Unlike the low-pathogenicity gene products, these might not be specific to a single host low-reaction gene. Mutation of such a more general gene to an amorphous allele would then result in the failure of several host low-reaction genes even though the specific low-pathogenicity genes were present in the parasite.

Efforts were made to determine groups of low-pathogenicity genes which might come under the control of such a general suppressor gene. For example, every mutant capable of overcoming SrL1 was also able to overcome Sr7a, SrN, Sr18, and Sr8. Perhaps a relationship between the corresponding Psr loci exists. Unfortunately, no consistent pattern could be detected.

Some insight might be gained from the observation that overcoming a host low-reaction gene often resulted in a lower infection type than the expected type 4 pustule. For example, the wild-type culture, 111x36F1, is obviously heterozygous at the PsrL2 locus, as one parent, 111-55A, produces a type 0; on the SrL2 host and the other, 36-55A, produces a type 4. Mutation of the low-pathogenicity allele at the PsrL2 locus should result in a type 4 on the SrL2 host. Of the 17 mutants overcoming SrL2, only 4 produced a type 4 pustule. The others ranged from types 2 to 3. Similarly, only three of the nine mutants overcoming Sr7b produced a type 4 pustule; the rest produced type 3^c pustules. Six of the 20 mutants overcoming Sr18 produced lower infection types than type 4, ranging from 3⁻ to 3⁺.

One explanation would be that these host differentials are not truly monogenic. For example, Cns/ISr18-Ra could have an additional low-reaction gene which, in the presence of the corresponding low-pathogenicity gene in the pathogen, results in a type 3 pustule. Mutation of the Psr18 locus to high pathogenicity would allow for the expression of this second low-reaction/low-pathogenicity pair, blocked previously by the epistatic effect of the Sr18/Psr18 pair. Similarly, Cns/ISr7b-Ra could have a second low-reaction gene which in the presence of low pathogenicity produces a type 3^c.

Alternatively, the mutation may be occurring in a pathogen locus which modifies the expression of several low-reaction/low-pathogenicity pairs, resulting in a larger pustule for any of these low-reaction/low-pathogenicity pairs. In the presence of this mutation, an Sr7b/Psr7b pair which should give a low infection type of 2⁻ produces a 3^c, and the SrL2/PsrL2 pair results in a type 2 pustule instead of a 0.

The hypothesis that pathogens have genes affecting host range in addition to low-pathogenicity genes is testable by classical genetic techniques. Consistent with this hypothesis, the various mutants and wild-type culture obtained in this study differ from each other by only one or perhaps a very few genes. Alternatively, with a gene-for-gene explanation, they should differ by as many as nine genes. Crosses could be made among such mutants or between mutants and culture 111x36F1 to determine whether F2 generations segregate in monohybrid or polyhybrid ratios with respect to their ability to overcome host low-reaction genes.

The possibility that pathogen host range is limited by a few suppressor genes is cause for some concern. The gene-for-

gene concept suggests that a super race of the parasite capable of overcoming all possible host low-reaction genes is only a remote possibility, as such a race would have to give up a large number of proteins. The possibility of the evolution of such a race is enhanced greatly when several host low-reaction genes can be overcome at once through the loss of a single or relatively low number of proteins in the parasite, as indicated in this study. This study suggests further that the genetics of specificity in the wheat rust system is more complex than suggested by the gene-for-gene concept alone.

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