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Isozyme Uniformity and Virulence Variation in *Puccinia graminis* f. sp. *tritici* and *P. recondita* f. sp. *tritici* in Australia

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Abstract

During a routine survey of pathotypes of *Puccinia graminis* f. sp. *tritici* (the wheat stem rust pathogen) and *P. recondita* f. sp. *tritici* (the wheat leaf rust pathogen) present in Australia during the 1981–82 growing season, 16 pathotypes of *P. graminis tritici* and 12 pathotypes of *P. recondita tritici* were detected in total samples of 193 and 180 isolates of the two pathogens respectively. For both pathogens the distribution of isolates amongst the different pathotypes was highly uneven. The commonest pathotype of *P. graminis tritici* (pathotype 343-1,2,3,5,6) comprised 50% of isolates while the commonest pathotype of *P. recondita tritici* (pathotype 104-2,3,6) made up 69% of isolates. Many pathotypes of both species were represented by single isolates only.

Large subsamples of these field-collected isolates (58 isolates of *P. graminis tritici* representing seven pathotypes and 66 of *P. recondita tritici* representing six pathotypes) were randomly selected and used to assess isozyme variation in extant Australian populations of these two pathogens. No variation was detected within either pathogen species in the isozyme phenotypes of 11 different enzyme systems.

These data are compared and contrasted with published data on patterns of variation at avirulence/virulence and isozyme loci in these species.

Introduction

Despite the economic importance of the major cereal rust fungi, current knowledge of their population genetics is limited and is based largely on surveys of avirulence/virulence characteristics. Such surveys are routinely carried out nationally (e.g. Roelfs and Rothman 1974; Green 1979; Luig 1979; Martens 1979; Samborski 1979), to determine which of a considerable array of host resistance genes can be exploited in the breeding of resistant commercial cultivars. These surveys indicate that rust pathogen populations are often highly variable genetically at virulence loci. The degree of variation differs from year to year and from location to location and may be affected by factors such as the diversity of cultivars grown and the level of disease on the crop. For example, Luig and Watson (1970) identified from 4 to 20 different pathotypes of *Puccina graminis tritici* each year in northern New South Wales over the period 1954–55 to 1968–69. Moreover, analyses of the genetic constitution of individual pathotypes has indicated that they are often heterozygous at several loci controlling pathogenicity. For example, Samborski and Dyck (1968, 1976) have shown that a considerable amount of heterozygosity exists within North American isolates of *P. recondita tritici*. One pathotype in particular was heterozygous with respect to 11 of 12 genes investigated.

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These findings raise two important questions:

(1) Are other loci within the pathogen genome as highly heterozygous as the virulence loci or are virulence loci exceptional in this regard?

(2) Are other loci as polymorphic as virulence loci or, again, are virulence loci exceptional in this regard?

The development of procedures for the electrophoretic analysis of soluble enzymes in cereal rust pathogens (Burdon and Marshall 1981; Burdon *et al.* 1982) provides an additional set of genetic markers with which to examine these questions. As a step towards this end we report here a study involving a comparison between virulence and isozyme variation within field populations of two of the Australian cereal rust fungi, viz. *Puccinia graminis* Pers. f. sp. *tritici* Eriks. & Henn. (the wheat stem rust pathogen) and *P. recondita* Rob. ex Desm. f. sp. *tritici* Eriks. & Henn. (the wheat leaf rust pathogen).

Materials and Methods

Virulence Survey

Collections of both *P. graminis tritici* and *P. recondita tritici* were received from various co-operators in all Australian states. On receipt, each sample was used to inoculate two groups of differentials (described below) which were then kept in a glasshouse at approximately 20°C until reaction types were manifest. The reaction types were then assessed and the racial identity of the isolate determined.

All collections were classified according to a scheme where the standard race number was determined by the reaction to infection of modified, truncated, international standard sets, while a further subdivision into components was made by testing sets of supplementary local differentials. For *P. graminis tritici* the truncated international set consisted of: Einkorn (*Sr21*), Marquis (*Sr7b*), Reliance (*Sr5*), Emmer (*Sr9e*), Mindum (*Sr9d*) and Acme (*Sr9g*). The supplementary local differentials were (1) Eureka (*Sr6*); (2) Yalta (*Sr11*); (3) Gamenya (*Sr9b*); (4) Mengavi (*SrTt1*); (5) Gala (*Sr17*); (6) Mentana (*Sr8*); (7) Norka (*Sr15*); (8) Festiguay (*Sr30*); (9) *Agropyron intermedium* derivative; (10) Entrelargo de Montijo; (11) Barleta Benvenuto and (12) (COR) Coorong Triticale (*Sr27*). For *P. recondita tritici* the modified, truncated international set consisted of: Webster (*Lr2a*), Mentana (*Lr3*) and Tarsa (*Lr1*). The supplementary local differentials were (1) Thew (*Lr20*); (2) Gaza (*Lr23*); (3) Spica (*Lr14a*); (4) Kenya W1483 (*Lr15*); (5) Klein Titan; (6) Gatcher (*Lr27*) and (7) Songlen (*Lr17*).

Isozyme Survey

Production and germination of spores

Fifty-eight isolates of *P. graminis tritici* and 66 of *P. recondita tritici* were chosen at random from the isolates of both pathogens received for identification in the virulence survey during the 1981–82 growing season. After race determination, each isolate was grown separately on a susceptible cultivar of *Triticum aestivum* L. Urediniospores of each isolate were collected, dried and then stored at 4°C or in liquid nitrogen until required. Methods for germination of urediniospores have already been described (Burdon *et al.* 1982).

Electrophoretic analysis

Starch-gel electrophoresis of homogenized, germinated spores was performed as described earlier (Burdon *et al.* 1982). The following enzymes were investigated: catalase (CAT, EC 1.11.1.6), arylesterase (EST, EC 3.1.1.2), aminopeptidase (cytosol) (synonym leucine aminopeptidase; LAP, EC 3.4.11.1) and dihydrolipoamide reductase (synonym NADH diaphorase; NADHD, EC 1.6.4.3)—assayed on citrate gels; aspartate aminotransferase (synonym glutamate oxalate transaminase; GOT, EC 2.6.1.1), glucosephosphate isomerase (synonym phosphoglucoisomerase; PGI, EC 5.3.1.9), hexokinase (HEX, EC 2.7.1.1) and 6-phosphoglucose dehydrogenase (6-PGD, EC 1.1.1.43)—assayed

on borate gels; glutamate dehydrogenase (GDH, EC 1.4.1.2), malate dehydrogenase (MDH, EC 1.1.1.37) and phosphoglucomutase (PGM, EC 2.7.5.1)—assayed on histidine gels.

Results

Virulence Survey

During the routine wheat rust virulence survey conducted through the 1981–82 growing season a total of 193 isolates of *P. graminis tritici* and 180 isolates of *P. recondita tritici* were received for identification. These isolates were found to comprise a total of 16 and 12 different pathotypes of the two pathogens respectively, although for both species at least half the isolates were of one pathotype alone (*P. graminis tritici*: pathotype 343-1,2,3,5,6; *P. recondita tritici*: pathotype 104-2,3,6). This marked unevenness in the frequency of the different pathotypes is shown in Fig. 1. For both pathogens, the three commonest pathotypes comprised over 80% of all isolates. On the other hand, 56% of all pathotypes of *P. graminis tritici* were represented by a single isolate only (9 out of 16 pathotypes). 42% of all *P. recondita tritici* pathotypes were similarly represented (5 out of 12).

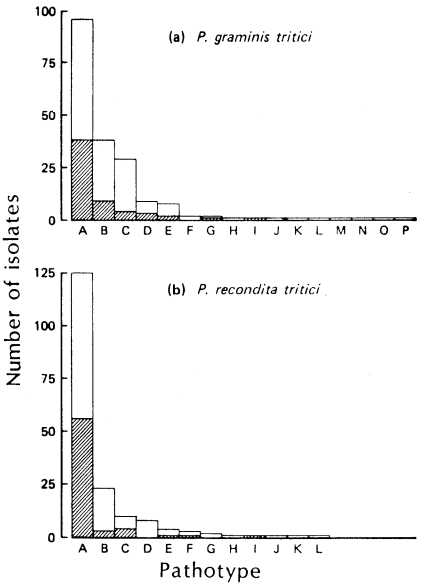

Fig. 1. Number of isolates of each pathotype of wheat stem and leaf rust examined in the 1981–82 virulence survey. (*a*) The number of isolates of *P. graminis tritici*: Pathotype A = 343-1,2,3,5,6; B = 34-2; C = 98-1,2,3,5,6; D = 222-1,2,3,5,6,7; E = 222-1,2,3,5,6; F = 21-2; G = 34-1,2,3,5,6,7; H = 21-1,2; I = 21-2,3,7; J = 21-5; K = 21-1,2,3,7,8,9; L = 11-1,2,3,5,6; M = 34-2, COR; N = 222-1,2,3,5,6,E1; O = 343-1,2,3,4,5,6; P = 343-1,2,3,5,6,*Sr9g*+. (*b*) Number of isolates of *P. recondita tritici*: Pathotype A = 104-2,3,6; B = 10-1,2,3,4; C = 76-2,3,6; D = 162-2,3,6; E = 104-2,3,6,(7); F = 104-1,2,3,6; G = 76-2,3; H = 68-1,2,3,4; I = 26-3; J = 162-1,2,3,6; K = 122-2,3; L = 104-2,3,6 + MIL. The shaded area of each histogram represents the number of isolates of each pathotype examined isozymically.

The distribution and number of pathotypes of the two species in different parts of Australia was also uneven (Fig. 2). *P. graminis tritici* tended to be collected more frequently in the south than the north, while *P. recondita tritici* showed the opposite trend. Thus 18·8% of Victorian, 45·6% of southern New South Wales, 57·8% of

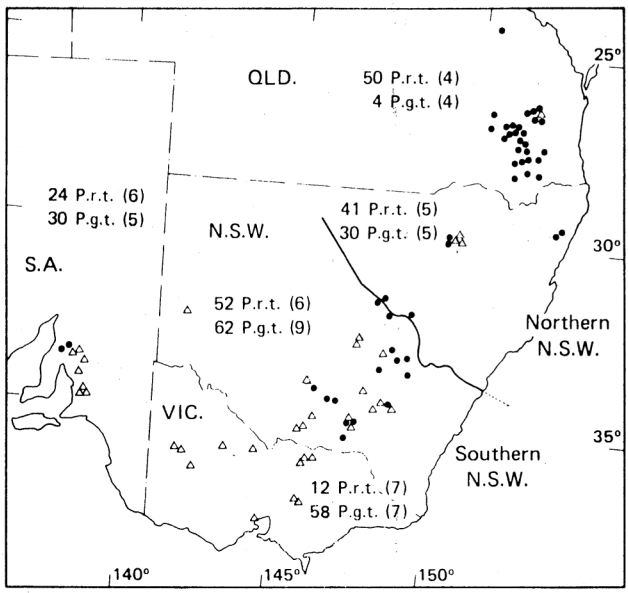

Fig. 2. Geographic distribution of the number of isolates and pathotypes (the number of pathotypes in parentheses) of *P. graminis tritici* (P.g.t.) and *P. recondita tritici* (P.r.t.) received from southern and eastern Australia in the 1981–82 growing season. △,● Distribution of most of the isolates of *P. graminis tritici* and *P. recondita tritici* used in the isozyme analysis respectively. In addition to the numbers shown, nine isolates of *P. graminis tritici* from Western Australia and one isolate of *P. recondita tritici* from Tasmania were included in the virulence survey. Three of the Western Australian and one of the Tasmanian isolates were assessed isozymically.

Table 1. Number of loci examined, their homozygous state and the R_F values of all bands for pathogens *P. graminis tritici* and *P. recondita tritici*

Isozyme system	*P. graminis tritici*			*P. recondita tritici*		
	No. of loci	Homozygous?	R_F	No. of loci	Homozygous?	R_F
CAT	1	Yes	0·19	1	Yes	0·36
EST	2	Yes	0·68 0·98	2	No	0·61 ⎫ 0·72 ⎫ [B] 0·63 ⎭ 0·74 ⎭
GDH	1	Yes	0·07	1	Yes	0·05
GOT	1	Yes	0·28	1	Yes	−0·03
HEX	1	Yes	0·48	2	Yes	0·32 0·47
LAP	1	No	0·46 0·52	1	Yes	0·54
MDH	1	Yes	0·05	1	Yes	0·07
NADHD	1	No	0·13 0·21	1	Yes	0·13
6-PGD	1	Yes	0·58	1	Yes	0·47
PGI[A]	1	No	0·22 0·28 0·37	1	Yes	0·22
PGM	1	No	0·21 0·27	1	Yes	0·20

[A] Only 30 of the 58 isolates of *P. graminis tritici* were examined for this enzyme.
[B] Bracketed values represent continuous bands.

northern New South Wales and 92·6% of Queensland isolates were of *P. recondita tritici*. The commonest pathotypes of each of the pathogens were extremely widespread and dominated the rust flora of nearly all states. Most other pathotypes were detected at too low a frequency to show any definite pattern.

Isozyme Survey

Within each pathogen species, all 11 enzyme phenotypes examined were invariant for all isolates. Table 1 shows the number of loci scored for each enzyme system, whether the enzyme phenotype was consistent with an interpretation of genetic homozygosity or heterozygosity, and the R_F values of each of the bands considered. In *P. graminis tritici*, of the 12 loci scored, only four were heterozygous (viz. LAP, NADHD, PGI and PGM), while of the 13 loci scored for *P. recondita tritici* only two (both EST loci) were heterozygous. In both pathogens, these loci were uniformly heterozygous for all individuals; no polymorphisms were detected in any of the 58 isolates of *P. graminis tritici* or the 66 isolates of *P. recondita tritici* examined.

Discussion

An analysis of the virulence structure of the two pathogen populations shows them to be diverse in both their racial composition and, to a lesser extent, in the geographic distribution of the races within the main wheat growing areas. Thus, while single pathotypes of *P. graminis tritici* (343-1,2,3,5,6) and *P. recondita tritici* (104-2,3,6) dominated the respective floras throughout most of eastern and southern Australia, the incidence of some of the more minor pathotypes fluctuated considerably. About 32% of isolates of *P. graminis tritici* from Queensland and northern New South Wales belonged to pathotype 34-2 while in southern New South Wales only 8% were of this type, in Victoria 21% and in South Australia 11%. *P. recondita tritici* also showed similar regional differences in virulence. For example, pathotype 104-2,3,6, the dominant pathotype in Queensland, New South Wales and Victoria, was supplanted in importance by pathotype 10-1,2,3,4, in South Australia.

The actual number of different pathotypes also appears to vary geographically. However, because comparisons of absolute values may be confounded by differences in sample size, Shannon diversity indices (Shannon and Weaver 1949) were calculated after first grouping the isolates from southern and eastern Australia into the two regional groupings used by Luig and Watson (1970) (region 1: Queensland and northern New South Wales; region 2: southern New South Wales, Victoria and South Australia). The Shannon diversity index incorporates a measure of both the number of phenotypes encountered and the evenness of distribution of isolates between them. Thus while more pathotypes of *P. graminis tritici* were encountered in region 2 (11 pathotypes) than in region 1 (7 pathotypes), the actual racial diversity in the two regions was very similar (Shannon values of 0·636 and 0·562 respectively). For *P. recondita tritici*, the actual number of pathotypes recorded was again greater in the south than in the north (12 *v.* 5) but, in this case, the corrected measure of racial diversity also reflected this regional difference (Shannon values of 0·661 *v.* 0·230 respectively).

Despite this pronounced variation in virulence, no variation whatsoever was encountered in the isozyme phenotypes among isolates of either pathogen population. Both virulence survey subsamples used in the isozyme study appear to reflect the

diversity of the pathogen populations. For both pathogens, the isolates analysed for isozyme variation were taken at random from those submitted for the virulence survey and are reasonably well distributed through eastern and southern Australia. This reflects the frequency with which the two species were encountered in different areas (Fig. 1). The racial identity of the isolates used in the isozyme survey also reflects the frequency distribution of different races in the virulence survey as a whole (Fig. 1). The 58 isolates of *P. graminis tritici* belonged to 7 of the 16 pathotypes detected in the virulence survey while the 66 isolates of *P. recondita tritici* belonged to 6 of the 12 pathotypes detected.

For each pathogen species, the difference between the genetic structure of the populations as reflected by the virulence survey and the isozyme study is further emphasized by a consideration of the levels of polymorphism and heterozygosity in these systems. As both pathogens are maintained in Australia through a process of repeated asexual generation with no intervening sexual phase (Watson 1981), it is possible to have heterozygosity without heterogeneity (or polymorphism) among the isolates. Thus no polymorphisms were detected within the 12 isozyme loci detected in *P. graminis tritici* or the 13 detected in *P. recondita tritici*. By way of contrast, an examination of all the pathotypes detected in the virulence survey (or only those used in the isozyme study) shows that of the 18 host genes being considered for *P. graminis tritici* (6 from the international set; 12 from the local differentials), the average proportion of polymorphic avirulence/virulence loci within the pathogen population was 0·78 (0·44 for the population subsample used in the isozyme study). For *P. recondita tritica*, where 10 host genes were considered (3 from the international set; 7 from the local differentials), the equivalent values were both 0·80.

The level of heterozygosity within individual enzyme systems for the isolates of both pathogens was either 0 (e.g. CAT, GDH, GOT) or 1 (e.g. LAP, PGI, PGM for *P. graminis tritici*, EST for *P. recondita tritici*). However, the average heterozygosity over all enzyme systems was only 0·33 for the *P. graminis tritici* subpopulation and 0·15 for that of *P. recondita tritici*. These results appear to contrast with published estimates of heterozygosity at virulence loci in cereal rust pathogens. For instance, Luig and Watson (1961) found a heterozygosity value of 0·81 in an Australian isolate of Stakman race 21 (the progenitor of many of the pathotypes extant in Australia today). Similarly high levels of heterozygosity at virulence loci have been recorded in a number of North American studies of both *P. graminis tritici* and *P. recondita tritici* [*P. graminis tritici*: 0·53 (Johnson 1954), 0·75 (Wilcoxson and Paharia 1958); *P. recondita tritici*: 0·70 (Haggag *et al.* 1973; Samborski and Dyck 1968, 1976)]. While these heterozygosity estimates are derived from entirely different populations to those examined in this study, the values are in general agreement with those determined by Luig and Watson (1961) and emphasize the possibility of a real difference in the levels of heterozygosity present at these different loci in the rust fungi. However, the difference in levels of heterozygosity recorded by these two systems of analysis may, at least partly, reflect differences in the detectability of systems. Nothing is known about the possibility of undetected biochemical variation at virulence loci; however, theoretical work has shown that starch-gel electrophoresis *may* detect only a third of the heterozygosity that exists at enzyme loci (Marshall and Brown 1975). If this is true in the present circumstances, then the difference between the present isozyme values and published virulence heterozygosity values would not be significant.

Despite this note of caution, the comparisons detailed above suggest that fundamental differences may exist in the mechanisms responsible for the production and maintenance of variation in virulence and isozyme loci in natural populations of rust pathogens. Equally, however, much of the present lack of isozyme variation may reflect a peculiarity of the Australian rust pathogen flora. In particular, the uniformity of isozyme phenotypes may largely be explained on the basis of a common origin of all existing pathotypes. Thus, the two pathotypes of *P. recondita tritici* with which Waterhouse (1952) worked in the 1920's and 1930's, and which until 1945 accounted for all the Australian isolates, were differentiated on the cultivar Thew (*Lr20*). When Gabo (*Lr23*) and Spica (*Lr14a*) were released, in 1942 and 1952, respectively, they were resistant to the existing pathotypes. This resistance was subsequently overcome by the pathogen and although neither *Lr23* nor *Lr14a* are present in current widely grown cultivars, the present Australian pathotypes of *P. recondita tritici* are still virulent for these genes. It is, therefore, conceivable that the present pathotypes are descendants from those which attacked Gabo and Spica a few years after their introduction to cultivation.

Similarly, while it is known that different isozyme phenotypes have been associated with the various overseas introductions of *P. graminis tritici* which have occurred over the last 60 years (Burdon *et al.* 1982), each subsequent introduction has generally eliminated all pre-existing forms. As a result, the *P. graminis tritici* population present in Australia today appears to be derived from, at the most, the last few introductions. All of these were isozymically identical.

If a common-origin hypothesis is accepted as the reason for the isozymic uniformity of these cereal rust pathogens, then other questions arise. For example, why has there been no detectable mutation within Australian populations of these species for novel isozyme alleles? At times, the population size of both pathogens is very large and, even if mutation rates are very low, then occasional novel types should occur. Are these all detrimental, or are they all eliminated during subsequent oversummering 'bottleneck' periods? Clearly, the data presented here point to a lack of knowledge concerning the population biology of the cereal rust fungi. The levels of isozyme polymorphism and the level of heterozygosity in the same isozyme systems were low. This contrasts with results obtained from a consideration of virulence attributes which show avirulence/virulence polymorphisms and heterozygosity at individual loci to be common.

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