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Support for a stepwise mutation model for pathogen evolution in Australasian *Puccinia striiformis* f.sp. *tritici* by use of molecular markers

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Since its initial detection in Australia in 1979, wheat yellow (stripe) rust (*Puccinia striiformis* f.sp. *tritici*) has evolved in Australia and New Zealand into more than 20 pathotypes with assorted virulence characteristics. This evolution is believed to have occurred in a stepwise fashion from an original single pathotype, with no subsequent new introductions. A combination of random amplified polymorphic DNA (RAPDs) and amplified fragment length polymorphisms (AFLPs) was used to examine the level of molecular variation in Australian and New Zealand isolates, and to compare this with variation amongst other isolates of *P. striiformis*. Using 60 RAPD primers on seven Australian isolates representing seven different pathotypes collected between 1979 and 1991, more than 300 potentially polymorphic loci were analysed and no polymorphisms were detected. Using the same primers on two UK isolates, 3% of loci showed a polymorphism. A similar level of polymorphism was found between UK isolates using AFLP primers, and between 5 and 15% of fragments were polymorphic between an isolate from the UK, an isolate from Denmark, and one from Colombia. However, no AFLP polymorphisms were found amongst 14 Australian and New Zealand isolates tested, at over 100 potentially polymorphic loci. The lack of molecular variation in the Australian and New Zealand collection is consistent with the stepwise mutation theory of pathotype evolution from a single introduction.

Keywords: AFLP, pathotype, RAPD, variation, wheat stripe rust, wheat yellow rust

Introduction

Yellow (stripe) rust (*Puccinia striiformis* f.sp. *tritici*, PST) has for centuries been a common disease of European wheat, but was detected on wheat in Australia only in October 1979 (O'Brien *et al*., 1980), where it rapidly became established as an endemic disease. A single pathotype was introduced initially, and more than 20 new pathotypes have subsequently been detected in Australia and New Zealand, including some of economic importance to commercial wheat cultivars and others with no obvious selective advantage (Wellings & McIntosh, 1990).

Genetic variation in rust fungi, which gives rise to new pathotypes, is believed to be controlled by the combined effects of mutations and immigration or influxes of new genotypes with subsequent sexual reproduction or somatic hybridization between or within the immigrant and local populations (Watson, 1981). PST is hemicyclic (urediniospores and teliospores only), and no alternate host or sexual cycle has yet been identified for the fungus. In Europe the airborne spread of urediniospores between countries growing a wide array of different wheat cultivars contributes to pathotype diversity. The geographic isolation of Australia from other cereal-growing regions of the world means that it is an ideal location for studying the contribution of mutation and somatic recombination to the production of new rust pathotypes. Somatic recombination of whole nuclei during germ-tube fusion can result in new pathotypes, as demonstrated by Wright & Lennard (1980), who produced a novel pathotype of yellow rust by mixing urediniospores of two pathotypes and inoculating them on the same plant. However, there is no evidence that this occurs in the field, particularly as many commercially grown wheat cultivars are susceptible to only a limited number of

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pathotypes. If somatic recombination is common, the population should be made up of pathotypes having combinations of virulences from coexisting pathotypes. In Australia and New Zealand, constant monitoring of pathotypes shows that newly detected ones appear to differ from pre-existing ones only at single virulence loci, suggesting a sequential pattern of single gene mutations for virulence (Wellings & McIntosh, 1990). This stepwise mutation model implies that spontaneous mutation from avirulence to virulence is the most important mechanism for generating variation in virulence characteristics.

In this study, molecular variation in Australian and New Zealand isolates was examined and compared with the amount of variation found amongst other isolates. Molecular variation has previously been detected in rust genomes with RAPDs (random amplified polymorphic DNA), which have been used to study *Uromyces appendiculatus* (MacLean *et al.*, 1995), *P. triticina* (Kolmer *et al.*, 1995) and North American isolates of *P. striiformis* (Chen *et al.*, 1993; Chen *et al.*, 1995). A detectable level of polymorphism was found both between and within rust species. In particular, variation was found between and within North American PST pathotypes (Chen *et al.*, 1993). There was no evidence of molecular markers correlating with pathotypes, and the results were taken as an indication of a wide degree of genetic variability within the North American yellow rust population. RFLPs (restriction fragment length polymorphisms) were detected in Chinese isolates of PST when a moderately repetitive DNA sequence from PST was used as a probe (Shan *et al.*, 1996, Shan *et al.*, 1998).

In this study a combination of RAPD primers and AFLPs (amplified fragment length polymorphisms) was used on Australian, New Zealand, European and a Colombian isolate of PST, representing a range of isolates of varying pathotypes, in order to examine the level of molecular variation and to provide evidence to support the stepwise mutation model for PST evolution.

Materials and methods

Yellow rust propagation and isolation

Sixteen Australian, two New Zealand, one Danish, one Colombian and 18 UK isolates of PST were analysed; their origins and virulence characteristics are listed in Table 1. A single isolate of wheat brown rust (*P. triticina*) was included for comparison in RAPD variation tests. Isolates from NIAB (Cambridge, UK) and Cobbitty (Australia) were from spore collections, and were not isolated from single spores in this study. Single-spore isolation of three European pathotypes (237E141, 40E8 and 32E160) was carried out on the universally susceptible wheat (*Triticum aestivum*) cultivars Sappo or Vuka. For pathotype confirmation, 16 wheat cultivars and *Triticum spelta album* were used (the World and European series of differentials) (McIntosh *et al.*, 1995). Propagation of non-UK isolates is not permitted in the UK, so pathotype identification of Australian isolates was performed in Cobbitty, Australia.

Plants were maintained in growth rooms at 20°C day/12°C night with a minimum 16 h photoperiod supplemented where necessary by high-pressure sodium lighting, and grown to a height of about 5 cm (1–2 weeks), at which stage the soil was drenched with 10 mL 0·04% (w/v) maleic acid hydrazide to prevent further plant cell division. Seedlings were sprayed with a fine mist of water and inoculated with 20–50 mg yellow rust urediniospores mixed with 10× volume of talc dusted over the leaves prior to incubation overnight at 100% relative humidity and 12°C to induce spore germination. Each isolate was cultured in a separate growth room and spores were collected daily on aluminium foil. Pure isolates were obtained by embedding the cut end of a leaf carrying a single rust pustule into medium containing 5% (w/v) glucose and 1% (w/v) agar in a 9 cm diameter Petri dish. Inverted plates were incubated at 20°C in constant light for 2–4 days and urediniospores were collected daily. Viable spore samples were stored in screw-cap sealed tubes, for the short term at 4°C in the dark over silica gel, and for the long term in liquid nitrogen.

DNA isolation

The method of M. Hovmøller (Danish Institute of Plant and Soil Science, personal communication) was used to extract DNA from urediniospores. Urediniospores (50 mg) mixed with an equal volume of acid-washed sand were ground in liquid nitrogen. The powder was transferred to a chilled 1·5 mL microfuge tube to which 500 μL CTAB buffer [2·5% (w/v) sorbitol; 1% (w/v) sarkosyl; 0·8% (w/v) CTAB; 4·7% (w/v) NaCl; 1% (w/v) EDTA; 1% (w/v) PVP] and 50 μL 10 mg mL^{-1} proteinase K were added. The mixture was vortexed and incubated at 65°C for 1·5 h, then extracted with 0·5 mL chloroform. The top aqueous phase (≈400 μL) was transferred to a clean tube and 5 μL DNase-free RNase (15 U mL^{-1}) was added. After 1 h incubation at 37°C, a further 0·5 mL chloroform was added and the supernatant separated and mixed with 800 μL ethanol for precipitation at −20°C for 2 h. Centrifugation for 20 min at 11600 ***g*** pelleted the DNA, which was rinsed with 70% (v/v) ethanol, vacuum-dried, and resuspended in 20 μL TE (pH 8·0). DNA concentrations were quantified by comparison with standards in gel electrophoresis and the volume used in PCR mixes was adjusted accordingly.

RAPD analysis

Operon arbitrary 10-mer primer kits B, C, D, E, F, G and H (VH Bio Limited, Newcastle-upon-Tyne, UK) were used for RAPD analysis following the method of Williams *et al.* (1990). Each 25 μL RAPD PCR mixture

Table 1 Origin and pathotype of *Puccinia striiformis* f.sp. *tritici* isolates screened with RAPDs and AFLPs

Identification/ Accession number[a]	Collection	Country of isolation	Virulent on cultivars with Yr resistances	Year collected
104E137A- (791533)[AR]	PBI Cobbitty	Australia	2,3,4	1979
104E137A- (albino)[A]	PBI Cobbitty	Australia	2,3,4	Unknown
104E137A + (821552)[A]	PBI Cobbitty	Australia	2,3,4,Avocet	1982
104E137A + (911506)[A]	PBI Cobbitty	Australia	2,3,4,Avocet	1991
104E137A + (941540)[A]	PBI Cobbitty	Australia	2,3,4,Avocet	1994
108E141A- (841744)[A]	PBI Cobbitty	Australia	2,3,4,6	1984
108E141A- (881563)[A]	PBI Cobbitty	Australia	2,3,4,6	1988
108E141A- (941537)[A]	PBI Cobbitty	Australia	2,3,4,6	1994
108E141A + (831917)[AR]	PBI Cobbitty	Australia	2,3,4,6,Avocet	1983
108E141A + (832100)[A]	PBI Cobbitty	Australia	2,3,4,6,Avocet	1983
108E141A + (861513)[A]	PBI Cobbitty	Australia	2,3,4,6,Avocet	1986
108E141A + (891512)[A]	PBI Cobbitty	Australia	2,3,4,6,Avocet	1989
360E137A + (841924)[R]	PBI Cobbitty	Australia	2,3,4,5,Avocet	1984
104E153A + (851595)[R]	PBI Cobbitty	Australia	2,3,4,8,Avocet	1985
108E205A + (841943)[R]	PBI Cobbitty	Australia	2,3,4,6,Avocet,	1984
110E143A + (861725)[A]	PBI Cobbitty	Australia	2,3,4,6,7,Avocet	1986
106E139A- (821589)[AR]	PBI Cobbitty	New Zealand	2,3,4,7	1982
106E139A- + Sk (911582)[AR]	PBI Cobbitty	New Zealand	2,3,4,7,Selkirk	1991
32E160[AR]	PBI Cambridge	UK	2,Carsten's V	1955
40E8[R]	PBI Cambridge	UK	3	1952
237E141[AR]	PBI Cambridge	UK	1,2,3,4,6,9	1988
232E137[R]	PBI Cambridge	UK	2,3,4,9	1975
76/71[R]	NIAB	UK	1,2,3	1976
75/27[R]	NIAB	UK	2,3,4	1975
93/21[R]	NIAB	UK	3,4	1993
95/92[R]	NIAB	UK	3,4,6	1995
83/62[R]	NIAB	UK	1,2,3,6	1983
84/1[R]	NIAB	UK	1,2,3,4,6	1984
87/21[R]	NIAB	UK	2,3,4,6	1984
88/28[R]	NIAB	UK	1,2,3	1988
88/31[R]	NIAB	UK	2,3,4,6,9	1988
89/148[R]	NIAB	UK	1,2,3,4,6,9	1989
90/505[R]	NIAB	UK	1,2,3,4,7	1990
88/127[R]	NIAB	UK	1,2,3,4,7	1988
88/77[R]	NIAB	UK	2,3,4,6,7	1988
94/519[A]	NIAB	UK	1,2,3,9,17,Carsten's V	1994
DK68/94[A]	John Innes Centre	Denmark	Unknown	Unknown
66E0[A]	John Innes Centre	Colombia	7	Unknown

[R]Isolates that were used for RAPD analysis.

[A]Isolates that were used for AFLP analysis.

[a]Australian, New Zealand, Columbian and first four UK pathotypes are designated by binary notation converted to decanary (Johnson *et al.*, 1972).

consisted of 0·83 Units *Taq* DNA polymerase, 10 μM primer, 0·2 mM each dNTP, 1 mM $MgCl_2$ and approximately 0·2 ng sample DNA. Amplification was carried out using a RoboCycler thermal cycler (Stratagene, La Jolla, CA, USA) for 40 cycles of: 94°C for 30 s, 34°C for 2·5 min and 72°C for 2 min, followed by a final incubation of 72°C for 10 min. PCR products were separated by gel electrophoresis on 1% (w/v) agarose gels in 1 × TBE. Gels were stained with ethidium bromide, visualized under UV using a UVP IMAGESTORE transilluminator and photographed with a video camera using UVP IMAGESTORE Software.

AFLP analysis

AFLP primers and adapters for *Mse*I- and *Pst*I-digested DNA were used according to the method of Thomas *et al.* (1995), except that the streptavidin selection step was omitted. Instead, after addition of adapter sequences, digested DNA from each isolate was preamplified with unlabelled nonselective primers: *Mse*I universal (GAC GAT GAG TCC TGA GTA A) and *Pst*I universal (CTC GTA GAC TGC GTA CAT GCA G). Preamplification reactions contained 0·6 μL primer (*Pst*I universal 50 ng mL^{-1}), 0·6 μL primer (*Mse*I universal 50 ng mL^{-1}), 0·11 μL *Taq* DNA polymerase (5 U μL^{-1}), 0·8 μL 5 mM dNTP, 2 μL 10 × PCR buffer, 5 μL template DNA (from 50 μL restriction–ligation reaction), and water up to 20 μL total volume. The AFLP preamplification PCR programme, performed in a Robocycler, was 29 cycles of: 94°C for 30 s, 56°C for 1 min, and 72°C for 1 min.

Preamplification products were diluted 1 : 50 in TE and stored at −20°C.

For selective AFLP amplification, the *Pst*I-selective primer was end-labelled with $\gamma[^{33}P]$-ATP. Selective AFLP reaction mixes contained 0·6 μL unlabelled *Mse*I-selective primer (50 ng mL^{-1}), 1·6 μL 2·5 mM dNTP, 0·11 μL Taq DNA polymerase (5 U μL^{-1}), 2 μL 10 × PCR buffer, 0·5 μL labelled *Pst*I-selective primer and 5 μL DNA from the diluted preamplification. AFLP PCR was performed in a Robocycler programmed for 1 min at 94°C followed by 10 cycles of: 94°C for 40 s, 65°C for 1 min, 72°C for 1 min; then 25 cycles of: 94°C for 40 s, 56°C for 1 min, 72°C for 1 min. Products were analysed by autoradiography following electrophoresis through 4·5% (w/v) denaturing polyacrylamide gels as described by Thomas *et al.* (1995).

Alternatively, *Pst*I-selective primers were labelled with one of the fluorescent primers HEX or TET (MWG Biotech Ltd, Milton Keynes, UK) and analysed on an ABI Prism 310 DNA sequencer. For calculating fragment sizes, the internal Genescan-500 ROX length standard (PE Applied Biosystems, Warrington, UK) labelled with 6-carboxy-X-rhodamine (ROX) was used. Semi-automated AFLP fragment analysis was performed with GENESCAN analysis software version 3·1 and data transferred to ABI GENOTYPER analysis software version 1·1.1r6 (PE Applied Biosystems) for automatic sizing. The *Pst*I-selective primers used in this study were: P16, CTC GTA GAC TGC GTA CAT GCA GCC; P18, CTC GTA GAC TGC GTA CAT GCA GCT; P19, CTC GTA GAC TGC GTA CAT GCA GGA; and P26, CTC GTA GAC TGC GTA CAT GCA GTT. The *Mse*I-selective primers used were: M12, GAC GAT GAG TCC TGA GTA AAC; M15, GAC GAT GAG TCC TGA GTA ACA; M17, GAC GAT GAG TCC TGA GTA ACG; and M88, GAC GAT GAG TCC TGA GTA ATG C.

Results

RAPD variation in PST

Sixty RAPD primers were used to compare PST (UK isolates 40E8 and 237E141) with one *P. triticina* isolate. More than 300 RAPD bands were amplified, and approximately 60% were polymorphic between the two rust species. Within the species approximately 3% of RAPD bands were polymorphic between the two PST isolates, which differed at least at seven avirulence loci. Two RAPD primers that showed polymorphisms between 40E8 and 237E141 are illustrated in Fig. 1, and to confirm the reproducibility of this polymorphism, DNA was tested from two separate single-spore isolates of 237E141.

The same 60 primers were used on seven Australian and New Zealand isolates of PST, representing seven different pathotypes and collected between 1979 and 1991 (Table 1). No reproducible polymorphisms were

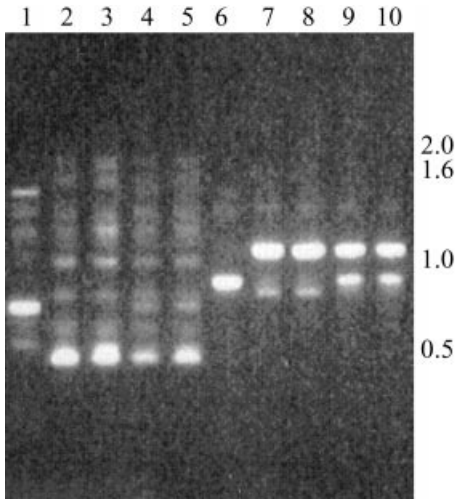

Fig. 1 RAPD gel analysis for *Puccinia triticina* (wheat brown rust, lanes 1 and 6) and UK isolates of *P. striiformis* f.sp. *tritici* (wheat yellow rust: isolate 40E8, lanes 2, 3, 7 and 8; isolate 237E141, lanes 4, 5, 9 and 10). Primers used were F12 (lanes 1–5) and F13 (lanes 6–10).

detected amongst these isolates with any of the primers used (Fig. 2, lanes 19–25). To determine whether this lack of polymorphism in the Australian and New Zealand isolates was a result of the relatively short evolutionary period between 1979 and 1991, compared with the two UK isolates 40E8 (1952) and 237E141 (1988), a range of UK isolates was obtained from NIAB that had been collected between 1975 and 1995. These isolates were screened with 20 primers that had shown polymorphisms between 40E8 and 237E141 (B3, B8, C8, D5, D6, D11, D12, E2, E4, F7, F13, G3, G7, G16, H7, H8, H14, H17, H18, H20). Figure 2 shows a representative gel. Polymorphisms were readily detected between UK isolates, although no RAPD polymorphism was detected between any two isolates of the same pathotype (e.g. lanes 16 and 17). Furthermore, there was no obvious correlation between virulence factors in isolates and RAPD polymorphisms, as determined by scoring presence/absence of polymorphic bands against each of the known virulence factors 1, 2, 3, 4, 6, 7, 9 and 17 (results not shown).

AFLP variation in PST

Five isolates (three from the UK, 237E141, 32E160, 94/519; one from Denmark, DK68/94; and one that originated in Colombia, 66E0) were tested with AFLPs and polymorphic bands were detected between them. On average, 6·5 polymorphic bands were revealed with each primer pair, and banding profiles were different between the isolates compared. Figure 3 shows a section from an autoradiograph showing the results for three of the isolates screened using two different AFLP primer combinations. Total variation with six primer pairs was greater between 32E160 and 66E0 (38 polymorphic bands) than between 32E160

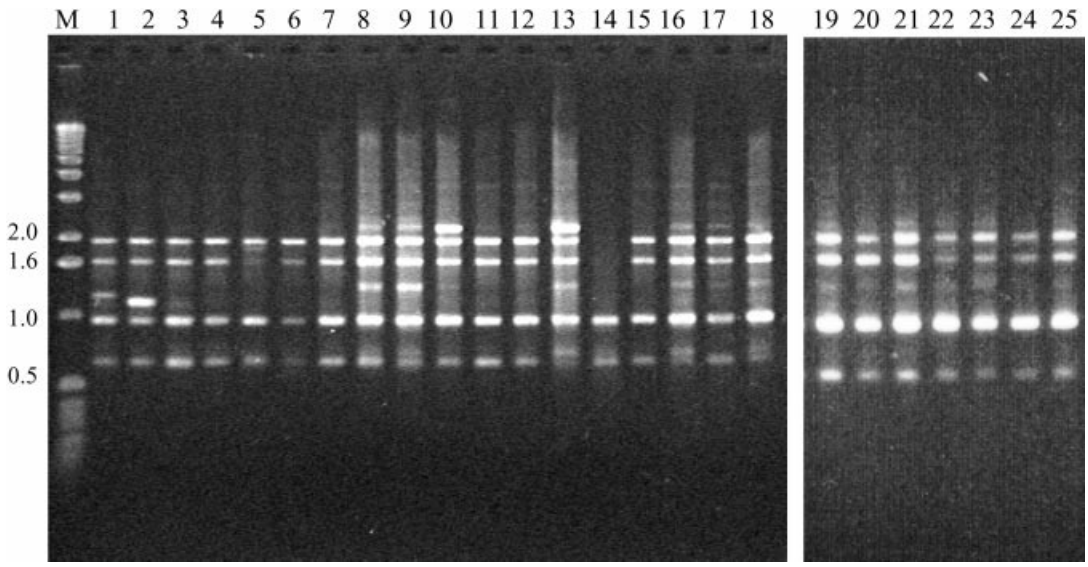

Fig. 2 RAPD gel analysis for UK (lanes 1–4 and 6–18), Colombian (lane 5), Australian and New Zealand (lanes 19–25) isolates of *P. striiformis* f.sp. *tritici*. Primer used was C8. The DNA used as template was: 1, 237E141; 2, 32E160; 3, 40E8; 4, 232E137; 5, 66E0; 6, 76/71; 7, 75/27; 8, 93/21; 9, 95/92; 10, 83/62; 11, 84/1; 12, 87/21; 13, 88/28; 14, 88/31; 15, 89/148; 16, 90/505; 17, 88/127; 18, 88/77; 19, 104E137A − (791533); 20, 106E139A − (821589); 21, 108E141A + (831917); 22, 360E137A + (841924); 23, 108E205A + (841943); 24, 104E153A + (851595); 25, 106E139A- + Sk (911582). Molecular weight markers (in kb) are indicated on the right side of the photograph (M = 1 kb ladder; Promega, Southampton, UK).

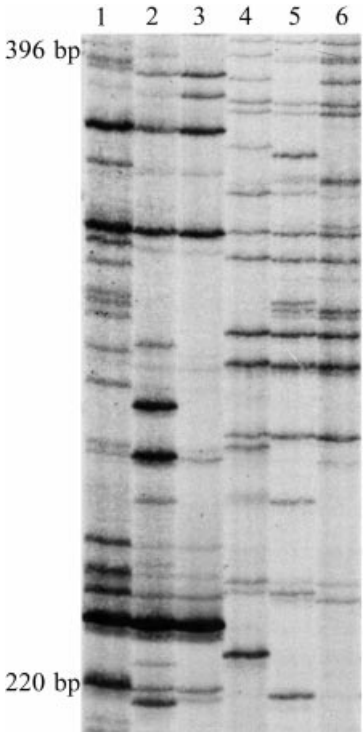

Fig. 3 AFLP gel analysis of a Colombian isolate (66E0, lanes 1 and 4), a UK isolate (32E160, lanes 2 and 5) and a Danish isolate (DK68/94, lanes 3 and 6) of PST. Primers used were P16 and M88 (lanes 1–3), and P16 and M12 (lanes 4–6).

and DK68/94 (13 polymorphic bands). With primers P16/M15, four bands were polymorphic between 66E0 and the other isolates; however, 32E160, DK68/94 and 237E141 all shared the same banding profiles (not shown). All five isolates tested could be uniquely identified by at least one band when compared with six primer combinations, and the polymorphisms revealed by AFLPs were more repeatable than those detected with RAPDs.

AFLPs in the Australian collection of PST

Two isolates, 32E160 and 66E0, along with 14 Australian and New Zealand isolates (marked with [A] Table 1), were analysed by AFLPs using fluorescent primers. Figure 4 shows the results for one set of primers on nine of these isolates. For the 33 peaks marked on this figure, seven were polymorphic between 66E0 and 32E160, which supports the results obtained using radioactive primers (Fig. 3). However, no polymorphisms were found between any of the Australian and New Zealand isolates for any of these peaks. Similar results were obtained using other primer pairs on the same isolates, and no detectable polymorphisms have yet been found amongst the Australian and New Zealand isolates at over 100 potentially polymorphic loci. The Australian and New Zealand isolates showed a greater similarity to the UK isolate 32E160 than to Colombian isolate 66E0 (Fig. 4). There were three polymorphisms detected between 32E160 and the isolates from Australia and New Zealand at the 100

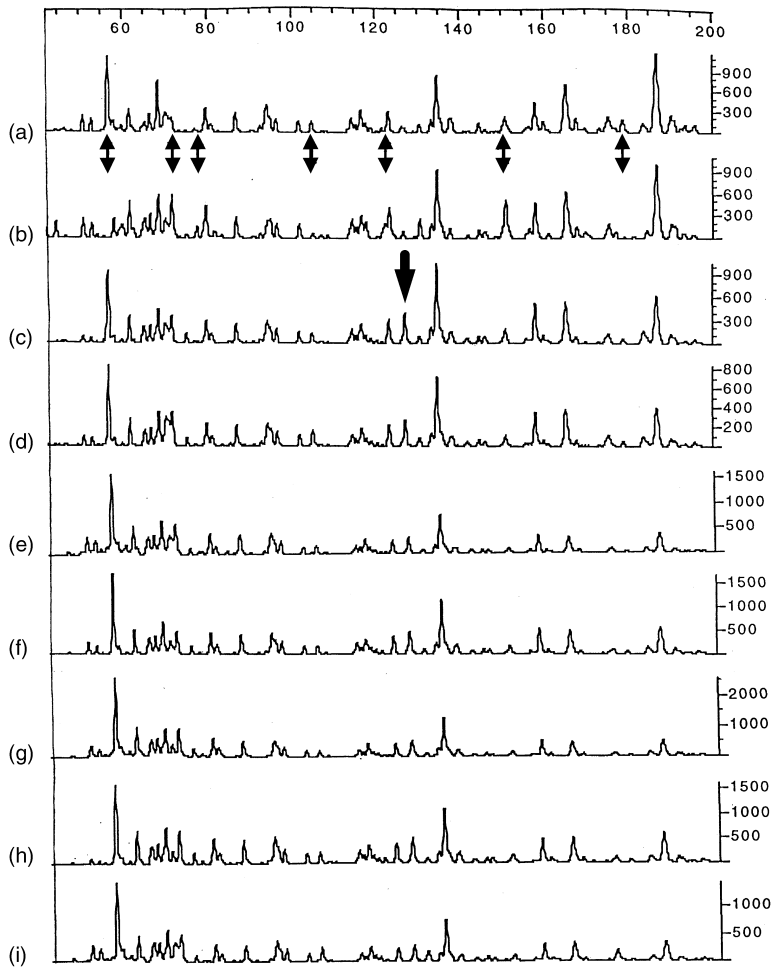

Fig. 4 AFLP polymorphisms between isolates using the fluorescent *Pst*I primer P16 labelled with HEX and the unlabelled *Mse*I primer M 12. Isolates were (a) 32E160; (b) 66E0; (c) 108E141A + (832100); (d) 108E141A + (861513); (e) 108E141A + (831917); (f) 108E141A- (841744); (g) 104E137A + (821552); (h) 104E137A + (911506); and (i) 110E143A + (861725). Horizontal scale represents size of fragments in base pairs, and their vertical scale is a measure of fluorescence signal intensity. Peaks that are polymorphic between UK isolate 32E160 and Colombian isolate 66E0 are marked with a double arrow, and the peak that is exclusively in the Australian and New Zealand isolates is marked with a single arrow.

potentially polymorphic loci tested, compared with over 20 between 66E0 and these isolates.

Discussion

RAPDs and AFLPs are techniques for monitoring polymorphisms in random sequences amongst isolates of organisms. Because no sexual cycle is known for the wheat yellow rust fungus, it is not possible to monitor the segregation of markers in progeny from defined crosses. The approach of using RAPDs and AFLPs on isolates from collections in the UK and Australia was therefore adopted to monitor the level of variation relative to the virulence characteristics. Eighteen Australian and New Zealand isolates collected over 15 years and representing 10 different pathotypes were analysed, and no repeatable molecular variation was detected with either AFLPs or RAPDs. Amongst UK isolates, the molecular variation detected was up to 3% polymorphic bands between isolates with both RAPDs and AFLPs. However, AFLPs were compared on a smaller sample of UK isolates that had already shown polymorphisms with RAPDs. The level of polymorphism between UK isolate 32E160 and Danish isolate DK68/94 was approximately 5%, and approximately 15% between 32E160 and Colombian isolate 66E0. Generally it was found that AFLPs were superior to RAPDs for the comparison of PST isolates, as they were more reproducible and revealed more polymorphic bands with fewer primers.

This study has shown that within the species there is limited molecular variation, a finding that is consistent with previous studies on PST. Newton *et al.* (1985) failed to detect isozyme or dsRNA variation between 29 British isolates, although some variation in dsRNAs was found amongst Australian and New Zealand isolates (Dickinson *et al.*, 1990). Chen *et al.* (1993) tested 115 North American isolates of PST with 11 RAPD primers and found that 47% of bands were polymorphic. Different populations have different levels of molecular polymorphism: more RAPD diversity has been found in the North American population than in the European and Australian collections. These differences could relate to the ages of the populations and to the relative number of migration events occurring between populations. The isolates collected in Australia and New Zealand are likely to have been derived from one isolate (pathotype 104E137A-) introduced in 1979, and there is no evidence that other exotic isolates have since been introduced to Australia or New Zealand. Interestingly,

the Australian population showed closer similarity to UK isolate 32E160 than Colombian isolate 66E0 on AFLP analysis, which strongly supports the belief of an original accidental introduction into Australia from Europe. The pattern of single stepwise changes for virulence that was observed by Wellings & McIntosh, 1990) is more consistent with mutation as the main contribution to pathotype variation than with somatic recombination. Newton *et al*. (1986) observed spontaneous mutation of white-spored mutants of barley yellow rust (*P. striiformis* f.sp. *hordei*), and isozyme analysis confirmed that mutation at a single locus was more likely to be the origin of the colour variant than somatic recombination between a yellow isolate of barley yellow rust and an albino wheat yellow rust when they were grown together on a universally susceptible plant. The monomorphic nature of Australian isolates with respect to molecular markers is evidence that they are very closely related to each other, and also suggests that stepwise mutation of single virulence genes is the most probable means by which new pathotypes have originated.

The lack of variation in these random molecular markers, compared with the remarkable changes that have occurred in pathogenicity of the Australian and New Zealand isolates over the same period, is surprising. Altogether, over 300 potentially polymorphic loci were sampled, although not between all isolates. The rapid generation of new pathotypes of *P. striiformis* in Australia might be indicative of a high mutation rate in the fungus combined with selection for new or altered virulences. In other fungi, changes from avirulence to virulence have been shown to result from both point mutations within avirulence genes, such as for *Avr4* in *Cladosporium fulvum* and *nip1* in some races of *Rhynchosporium secalis*, and from deletions such as for *Avr9* in *C. fulvum* (Knogge, 1996). However, in the present study there was no evidence for a high mutation rate or chromosomal deletions in PST, and further analysis is required to determine whether the evolution of new pathotypes is caused by random mutation/deletion events or by more specific mechanisms at avirulence gene loci.

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