

Rapid global spread of two aggressive strains of a wheat rust fungus

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Abstract

Rust fungi can overcome the effect of host resistance genes rapidly, and spores can disperse long distance by wind. Here we demonstrate a foreign incursion of similar strains of the wheat yellow rust fungus, *Puccinia striiformis* f. sp. *tritici*, in North America, Australia and Europe in less than 3 years. One strain defined by identity at 15 virulence loci and 130 amplified fragment length polymorphism (AFLP) fragments was exclusive to North America (present since 2000) and Australia (since 2002). Another strain of the same virulence phenotype, but differing in two AFLP fragments, was exclusive to Europe (present since 2000–2001) as well as Western and Central Asia and the Red Sea Area (first appearance unknown). This may be the most rapid spread of an important crop pathogen on the global scale. The limited divergence between the two strains and their derivatives, and the temporal–spatial occurrence pattern confirmed a recent spread. The data gave evidence for additional intercontinental dispersal events in the past, that is, many isolates sampled before 2000 in Europe, North America and Australia had similar AFLP fingerprints, and isolates from South Africa, which showed no divergence in AFLP, differed by only two fragments from particular isolates from Central Asia, West Asia and South Europe, respectively. Previous research has demonstrated that isolates of the two new strains produced up to two to three times more spores per day than strains found in USA and Europe before 2000, suggesting that increased aggressiveness at this level may accelerate global spread of crop pathogens.

Keywords: clonal evolution, long-distance dispersal, phylogeography, *Puccinia striiformis*, stripe rust, yellow rust

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Introduction

The pace at which microbes spread to new niches is a critical determinant of the emergence or re-emergence of infectious diseases in humans, animals and plants (Anderson *et al.* 2004; Hui 2006), affecting health (Binder *et al.* 1999) and food security (Strange & Scott 2005). Crop pathogens are spread long distance mainly by human activity resulting from trade and travel or by wind-dispersed spores, allowing the pathogens to colonize new areas or re-establish

in regions where the climate is seasonally unfavourable (Brown & Hovmøller 2002). For obligate biotrophic fungi, which are completely dependent on living host tissue for reproduction, the production of huge numbers of wind-dispersed spores is essential for spread and survival (Brown *et al.* 2002).

In this study, we have focused on the recent spread and evolution of a cereal rust fungus, *Puccinia striiformis* f. sp. *tritici*, which is a dikaryotic basidiomycete causing yellow rust (stripe rust) on wheat. Unlike other wheat rusts, yellow rust is not known to complete a sexual reproductive cycle as no alternative host has been identified (Stubbs 1985) and no recombination under natural conditions has been

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reported (Hovmøller *et al.* 2002; Enjalbert *et al.* 2005). *Puccinia striiformis* f. sp. *tritici* has highly specialized host preferences and follows the pattern of a gene-for-gene relationship (Flor 1971). Thus, the pathogen can be grouped into virulence phenotypes, defined by its compatibility with host plants possessing different resistance genes (Brown 2003). Although virulence represents the lack of recognition between pathogen and host, the term 'virulence phenotype' is used throughout this study to designate the combined phenotypic state of the virulence/avirulence loci considered.

Genetic variability at the DNA level is often low in *P. striiformis* f. sp. *tritici* at national and regional scales as compared to the diversity for virulence phenotype (Steele *et al.* 2001; Hovmøller *et al.* 2002; Enjalbert *et al.* 2005). Therefore, population studies of this pathogen are often based on techniques with a high power of discrimination, like amplified fragment length polymorphism (AFLP; Justesen *et al.* 2002), despite the dominant nature of AFLP fragments (Vos *et al.* 1995).

Recent epidemics of yellow rust have appeared in new areas, for example, warm areas in eastern USA (Chen 2005; Milus *et al.* 2006). In 1996, the disease emerged for the first time in South Africa (Boshoff *et al.* 2002) and in 2002, yellow rust first appeared in Western Australia (Wellings *et al.* 2003). Isolates sampled from eastern USA since 2000 had unusual virulence phenotypes for the region (Chen 2005; Markell & Milus 2008), similar to the virulence phenotype of most isolates from Western Australia (Wellings 2007). A similar phenotype was reported for the first time in Central and northern Europe in 2000–2001 (Flath & Barthels 2002; Hovmøller & Justesen 2007a). Nearly all isolates from eastern USA sampled since 2000 were closely related, based on AFLP fingerprints, and clearly different from representative isolates collected all over USA before 2000 (Markell & Milus 2008). Isolates representing this new population showed a significant increase in aggressiveness, that is, the ability to cause severe disease more quickly, especially at high temperature, compared to isolates collected before 2000 (Milus *et al.* 2006; Milus *et al.* 2008).

In this study, we investigated *P. striiformis* f. sp. *tritici* diversity at the global scale and tested a hypothesis of recent intercontinental spread of wheat yellow rust as opposed to local evolution of similar virulence phenotypes. Representative subsets of past and present isolates from Europe, North America and Australia were selected according to origin and virulence phenotype. These samples represented the majority of diversity in virulence phenotype in the fungus since the mid-1970s in these regions. Additional random samples were collected from areas in Central, West and South Asia, the Arabian Peninsula and northeast Africa (Red Sea Area), representing diverse ecological niches in terms of agricultural practices, host characteristics, precipitation and temperature regimes.

The combined effect of this sampling strategy and a rigorous phenotype assessment based on both virulence and AFLP markers provided a unique opportunity to test the role of long-distance dispersal of an important crop pathogen. The phylogeographical pattern of pathogen evolution provided the basis for a discussion of the sequence of events and dispersal mechanisms.

Materials and methods

Sampling

Most *Puccinia striiformis* f. sp. *tritici* isolates (Table 1) were obtained by the authors from local field trials and commercial fields as single lesions on detached wheat leaves and kept on 0.5% water agar containing 35 p.p.m. benzimidazole until multiplication on wheat cultivar 'Cartago'. Additional isolates were supplied by C. Wellings (Australia), X. Chen (Western USA), Z. Pretorius (South Africa), C. de Vallavieille-Pope, R. Bayles and L. Boyd (Europe), R. Singh (Mexico) and E. Duveiller (Nepal). Except for isolates sampled in Australia, they were multiplied under quarantine facilities at the Faculty of Agricultural Sciences, University of Aarhus, Denmark.

Determination of virulence phenotypes

Isolates were assayed for virulence phenotype using a set of 30 wheat yellow rust differential cultivars and lines according to Hovmøller & Justesen (2007a). The virulence phenotype of Australian isolates were derived from Wellings (2007) and a common scoring by C. Wellings, Plant Breeding Institute, University of Sydney and the Correspondence of this paper. The virulence phenotype of each isolate is available via Table S1, Supplementary material.

AFLP fingerprinting

A subset of 151 isolates representing all of the diversity with respect to virulence phenotype, region and sampling year was DNA fingerprinted by AFLP in Denmark. Spores (10–20 mg) of each isolate were mixed with equal amounts of acid-washed sand and ground with two steel balls (5 mm in diameter) in a Geno/Grinder 2000 at 1500 strokes/min for 3 × 30 s followed by DNA extraction (Justesen *et al.* 2002). The AFLP procedure was carried out as described previously (Hovmøller & Justesen 2007b). All isolates were analysed with nine primer combinations, which were selected in a preliminary screening to be the most informative and to give fingerprints of high quality: P11(AA)/M13(AG), P12(AC)/M26(TT), P16(CC)/M14(AT), P19(GA)/M24(TC), P19(GA)/M25(TG), P20(GC)/M11(AA), P22(GT)/M15(CA), P22(GT)/M22(GT), and P26(TT)/

Table 1 Collections and sources of *Puccinia striiformis* f. sp. *tritici* isolates

Country	Region	Sampling period	No. of isolates	Sample reference	
Denmark	North Europe (EU)	1993–2005	595	Authors	
Eritrea		Red Sea Area (RS)	2002–2005		111
Yemen	2003, 2005		15		
Kazakhstan	Central Asia (CA)		2003		7
Kyrgistan		2003	9		
Uzbekistan		2003	5		
Pakistan	South Asia (SA)	2004	16		E. Duveiller
Nepal		2005	25		
Iran	West Asia (WA)	2005	9		Authors
Azerbaijan		2005	20		
USA	North America (NA)	1981–2005	16	X. Chen	
USA		unknown	2		
Mexico		1989–2002	4		
Australia (east)	Australia (AU)	1979–1999	10	C. Wellings	
Australia (west)		2002–2004	5		
Republic of South Africa	South Africa (RA)	1996–2001	7	Z. Pretorius	
United Kingdom	North Europe (EU)	1975–1998	18	R. A. Bayles & L. Boyd	
Italy	South Europe (EU)	1998	1	R. A. Bayles	
France		1997	1	C. de Vallavieille-Pope	

M14(AT). Primer sequences have been described previously (Vos *et al.* 1995). Only polymorphic fragments of high resolution and a size range between 80 and 550 nt were considered. Isolates sharing identical AFLP patterns were defined as a unique AFLP phenotype. The term 'strain' was used to designate isolates sharing both virulence and AFLP phenotype.

Data analyses

Isolates were pooled into eight groups based on geographical origin (Table 1): Australia (AU), Central Asia (CA), Europe (EU), North America (NA), Red Sea Area (RS), Republic of South Africa (RA), South Asia (SA) and Western Asia (WA). The numbers of observed virulence and AFLP phenotypes in the eight regions were calculated. Global distributions since 2001–2002 of two particular strains, which were observed on five continents in this study, were estimated from the virulence phenotype distribution in the present data set, large-scale pathogen surveys in eastern USA (Markell & Milus 2008), northern Europe (Hovmøller & Henriksen 2008), South Africa (Boshoff *et al.* 2002) and Australia (Wellings 2007), and by AFLP phenotyping of subsets of isolates from these areas.

Analyses of phylogenetic relationships among isolates were based on phylogenetically informative markers and a single isolate was selected to represent each AFLP phenotype. The number of steps (t) required to resolve the phylogeny of the most parsimonious trees was calculated by MIX in PHYLIP (Phylogeny Inference Package version 3.5c., Felsenstein 1989). Subsequently the number of homo-

plasies, or double events (h) was calculated as the difference between the number of polymorphic fragments (v) and number of steps in the tree (t), that is, $h = t - v$. These calculations were made for the overall phylogeny as well as for partitions of the phylogeny associated with certain geographical areas.

An unrooted tree was constructed by the unweighted pair-group method using arithmetic means (UPGMA) in Neighbour in PHYLIP (Felsenstein 1989) and visualized by TreeView (Page 1996). Distances between pairs of isolates were computed using the distance $d = 1 - s$, where s is the simple matching coefficient (Sneath & Sokal 1973): $s = m/n$ where m is the number of band positions where a band is present in both isolates plus the number of positions where a band is absent in both isolates and n is the total number of band positions. The distance matrix was computed using Windist in the WinBoot program (Yap & Nelson 1996). The WinBoot program using 1000 replications calculated bootstrap values for the central branches.

Results

A total of 64 virulence phenotypes were detected among the 876 isolates (Table 2) based on the use of an extended set of differential cultivars/lines representing at least 17 yellow rust resistance genes. The subset of 151 isolates, which was assayed by 130 polymorphic AFLP fragments, revealed 41 phenotypes. The number of virulence or AFLP phenotypes in the individual sampling areas was low compared to the number of polymorphic characters considered (17 for virulence and 130 for AFLP).

Table 2 Virulence and AFLP phenotypes of worldwide *Puccinia striiformis* f. sp. *tritici* isolates

	Red Sea Area	West Asia	Central Asia	South Asia	Europe	South Africa	North America	Australia	Total
Virulence (17 polymorphic characters)									
No. of isolates	126	29	21	41	615	7	22	15	876
No. of virulence phenotypes	7	7	6	7	24	3	10	7	64
AFLP (130 polymorphic characters)									
No. of isolates*	37	17	15	15	24	7	21	15	151
No. of AFLP phenotypes	4	6	7	7	11	1	6	4	41

*Selected among above according to virulence phenotype, sampling site and year.

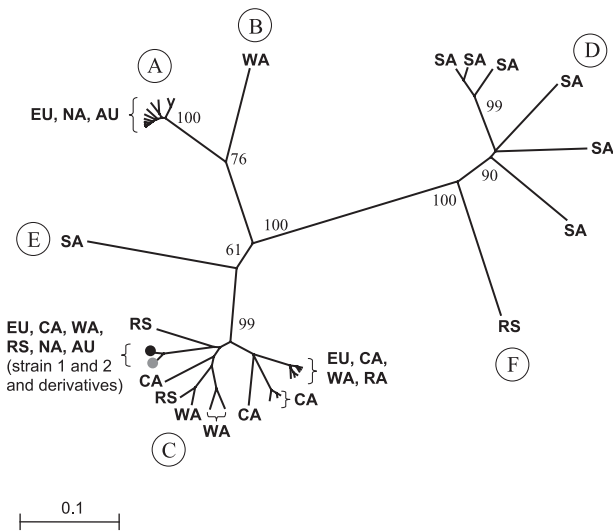


Fig. 1 Unrooted tree generated from AFLP data for *Puccinia striiformis* f. sp. *tritici* based on 151 isolates representing all of the geographical and virulence diversity found in this study. The bar indicates distance ($d = 1 - s$, where s is the simple matching coefficient). Bootstrap values based on 1000 replications are shown for central branches. The tree had three main groups A ($n = 38$), C ($n = 84$) and D ($n = 13$) each consisting of several AFLP phenotypes, n being the number of isolates in each group, and three solitary groups B ($n = 2$), E ($n = 2$) and F ($n = 12$), in total 38 AFLP phenotypes. Strain 1 and derivative (grey) was represented by 13 isolates and strain 2 and derivatives (black) by 35 isolates. Western and Central Asia (WA, CA), Australia (AU), Europe (EU), North America (NA), Republic of South Africa (RA), South Asia (SA), Red Sea Area (RS).

Of the 130 polymorphic AFLP fragments, 117 were phylogenetically informative, that is, present or absent in at least two phenotypes. Based on these, a total of 268 steps were required for resolving any of 100 equally parsimonious trees. Homoplasies, or double events, which were observed for 76 of the 117 informative fragments, accounted for 151 of the 268 steps. The level of homoplasy varied from 2 to 10 steps per homoplasious fragment (data not shown).

An unrooted tree constructed from a matrix of pairwise distances calculated based on the 117 informative AFLP fragments, resulted in a branching into six major groups (A to F), which was supported by relatively high bootstrap values except for group E (Fig. 1). The overall topologies of this tree and the most parsimonious trees were identical except for group E, which in the latter was positioned closer to group F and D (data not shown). These features suggest a generally consistent and robust data set.

The tree revealed some striking patterns according to geographical origin and sampling time. For instance, all isolates collected before 2000 in Europe, North America and eastern Australia clustered in group A, suggesting a common origin. Despite the limited divergence for AFLP, group A represented 40 virulence phenotypes (data not shown), implying a rapid evolution of virulence. Group B, which consisted of isolates of an atypical virulence phenotype sampled from tetraploid (durum) wheat in West Asia, was clearly separated from other groups, whereas C contained multiple phenotypes from Central and West Asia, the Red Sea Area, South Africa, North America, Australia and Europe since 2000. The relative difference in diversity between the European population before 2000 (A), sampled through a period of 25 years, and the populations in Central, South and West Asia (C) may be even larger taking into account that sampling in the latter areas was carried out within short periods from 2003 to 2005. The greatest diversity and divergence from European and North American isolates was observed for isolates from South Asia, that is, Pakistan (D) and Nepal (D and E). Group F showed no divergence although it represented many isolates sampled through four successive seasons in the tropical highlands of Eritrea.

The most striking feature of the data set was represented by two closely related AFLP phenotypes (Fig. 1, group C). In addition, 30 of 48 isolates of these two phenotypes were identical with respect to virulence phenotype (Table 3). Thus, the isolates of the two AFLP phenotypes were categorized as 'strain 1' and 'strain 2', respectively, or derivatives hereof.

Isolates of both strains differed by at least 14 markers from any other isolate in group C and by at least 42 markers

Table 3 AFLP and virulence phenotypes of two closely related *Puccinia striiformis* f. sp. *tritici* strains and their derivatives. AFLP phenotype: fragment present (1), absent (0). Virulence phenotype: numbers correspond to yellow rust resistance genes, Sd, Su and Sp designate resistances in Strubes Dickkopf, Suwon92-Omar and Spaldings Prolific, respectively, avirulence is shown by '—', and parenthesis indicate interactions either influenced by unrecognized resistance in test cultivars or a heterozygous state of pathogen isolates (Hovmöller & Justesen 2007a)

Strain	AFLP phenotype*		Virulence phenotype													Sd	Su	Sp	No. of isolates	Origin of sample
	P12M26 150	P20M11 333	1	2	3	4	6	7	8	9	10	15	17	32						
1	1	0	—	(2)	—	—	6	7	8	9	—	—	—	—	(Sd)	—	—	12	USA, Mexico, Australiat	
1a	1	0	1	(2)	—	—	6	7	8	9	—	—	—	(Sd)	—	—	1	Mexico		
2	0	1	—	(2)	—	—	6	7	8	9	—	—	—	(Sd)	—	—	18	Europe, C. & W. Asia, Yemen		
2a	0	1	—	(2)	—	—	6	7	8	9	10	—	—	(Sd)	—	—	9	Eritrea		
2b	0	1	—	(2)	—	—	6	7	8	9	—	—	—	(Sd)	Su	—	8	Yemen		

*AFLP phenotype is based on 130 markers of which only P12M26.150 and P20M11.333 showed polymorphism between strain 1 and 2;

†Virulence phenotype based on an interpretation of results from Wellings (2007).

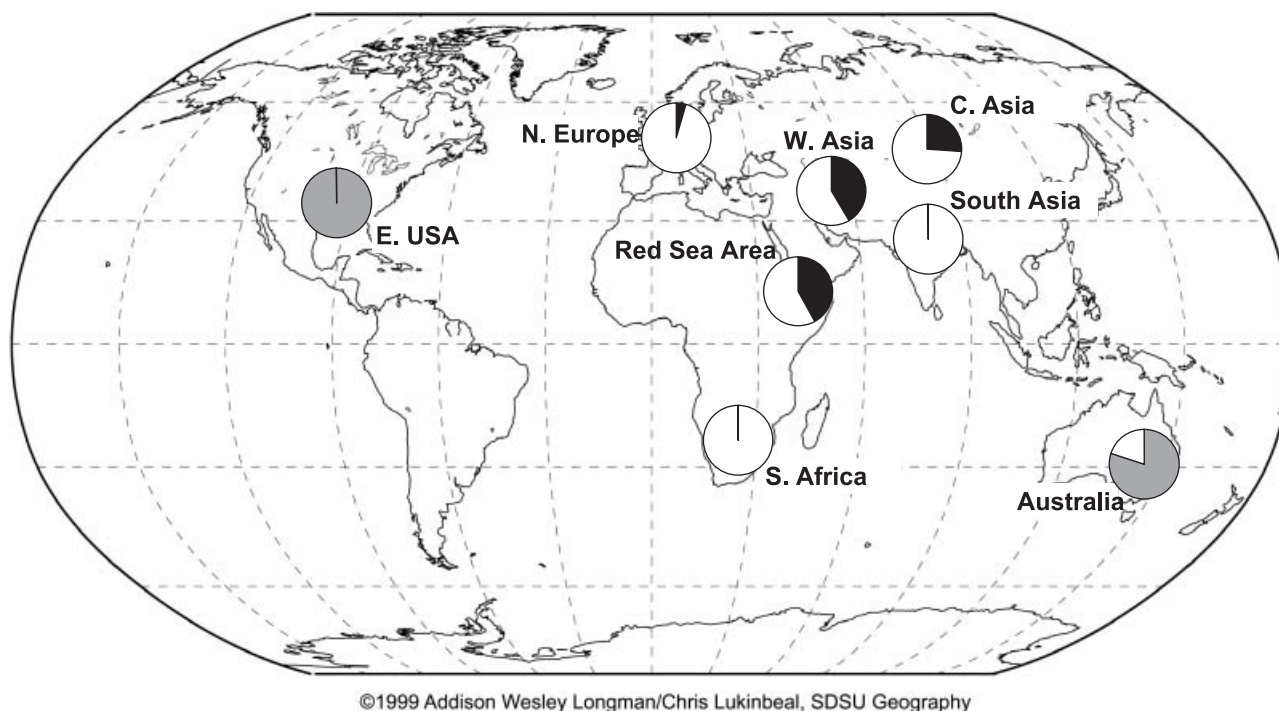


Fig. 2 Pie charts showing frequencies (2001–2005) of strain 1 (grey) and strain 2 (black) of aggressive *Puccinia striiformis* f. sp. *tritici* rust and the sum of all other strains (white). Data were based on virulence phenotype distribution in the present data set and from published results from large-scale virulence surveys in eastern USA (Markel & Milus 2008), South Africa (Boshoff *et al.* 2002) and Australia (Wellings 2007), and AFLP fingerprinting of subsets of isolates from these areas.

from any isolates collected before 2000 in Europe, USA and Australia (group A). A difference between strain 1 and 2 of only two AFLP fragments, including the most homoplasious fragment in the data set (P20M11.333), suggests a fairly recent common origin. This conclusion was supported by the limited divergence in virulence phenotype of the two strains (Table 3). Nevertheless, strain 1 was exclusive

to North America (present since 2000) and Australia (since 2002) and strain 2 to Europe (since 2000–2001), Western/Central Asia and the Red Sea Area (first appearance unknown).

Strain 1 became rapidly established in the *Puccinia striiformis* f. sp. *tritici* populations in the USA and Australia, respectively (Fig. 2). In eastern USA, almost 100% of

isolates sampled since 2001 were of this type (Markell & Milus 2008) and 80–95% of isolates tested in the Australian yellow rust survey since 2002 were of a similar virulence phenotype (Wellings 2007). This conclusion is based on the published work referenced above and the present study comprising representative isolates from these areas. In contrast, strain 1 (grey) was never observed in other parts of the world where strain 2 (black) was present. Strain 2 was first confirmed present in northern Europe in 2001 and remained at relatively low frequencies in subsequent years, most likely because it was only supported by few host cultivars in this area (data not shown). First appearance of strain 2 in Africa and Asia is not known, since it was present in moderate to high frequencies in 2002–2003 when the first sampling in this study took place in these regions.

In summary, during the period from 2000 to 2002, a foreign incursion of closely related, exotic yellow rust was observed at three continents, North America, Australia and Europe, including areas where the yellow rust disease was previously considered nonsignificant on wheat (eastern USA) or even absent (West Australia). The data gave no support for a hypothesis that the new strains had emerged through evolution in the established pathogen population before 2000 in these areas.

Discussion

The present study has shown that wheat rust fungi may become widespread across multiple continents at a faster rate than suggested by previous reports on the spread of crop pathogens including cereal rusts (Fry *et al.* 1993; Brown & Hovmöller 2002; Line 2002; Kolmer 2005; Leonard & Szabo 2005; Stukenbrock *et al.* 2006). Furthermore, two particular strains and their derivatives were found at multiple sites in relatively warm or dry wheat growing areas, where severe yellow rust epidemics have been observed in recent years, for example, the tropical highlands of East Africa (Yahyaoui *et al.* 2004), eastern USA (Chen 2005; Markel & Milus 2008) and West Australia (Wellings 2007). Based on these studies, and the present data set, strain 1 as defined in this study first appeared in North America in 2000 and in West Australia in 2002. The strain showed no divergence for any of 130 AFLP markers or 17 virulence loci assayed. The closely related strain 2, diverging by two AFLP markers from strain 1, appeared most likely in Central Europe in 2000, based on virulence phenotype data (Flath & Barthels 2002). The presence of strain 2 in northern Europe was confirmed in 2001 (Hovmöller & Justesen 2007a).

These findings raise several questions, for example, why did two particular strains of a crop pathogen spread so fast at a global scale? How could they establish in areas, which were previously considered unfavourable for the

yellow rust disease? Does the phylogeny provide additional information about the sequence of events and dispersal mechanisms?

Recent research suggests that the new yellow rust epidemics in North America may be driven by an increase in aggressiveness — the ability to cause disease more quickly and at temperatures once considered too warm for this fungus (Milus *et al.* 2006; Milus *et al.* 2008). The generation time (latent period) was approximately 2 days shorter for strain 1 and 2 and derivatives ('new' strains) compared to isolates of representative strains sampled before 2000 from multiple regions in North America and Europe ('old' strains). Isolates of the new strain produced almost twice the number of spores per lesion area per day compared to old isolates at a daily temperature regime previously considered favourable for yellow rust (Rapilly 1979). However, at high temperatures, ranging from 12 °C at night to a maximum of 28 °C at daytime, the isolates of both new strains produced up to three times more spores per lesion area per day than representative isolates of old strains. In a typical yellow rust epidemic going on for 80–100 days (Zadoks 1961), such differences would be highly significant and would cause severe disease more rapidly. The dramatic increase in spore production potential may explain why the new strain spread so rapidly on a global scale, for example, by increasing the probability of 'rare events' to occur such as long-range spore dispersal by wind (Brown & Hovmöller 2002), or accidental spread via human travel, for example due to contaminated clothing (Wellings *et al.* 1987).

The fact that strain 2 has been detected repeatedly at several locations in northern Europe, where most cultivars are resistant to the present virulence phenotype(s) of the aggressive strain, stress the potential risks for European agriculture (Hovmöller & Justesen 2007a). Two or three sequential mutations at specific avirulence loci, matching yellow rust resistance genes in European wheat cultivars, would result in loss of the current genetic control of the new aggressive strains of yellow rust in Europe.

The limited divergence of the two strains in virulence phenotype is a clear indication of very recent common origin, taking into account the rate of evolution of virulence in *Puccinia striiformis* f. sp. *tritici* (Hovmöller & Justesen 2007b). They observed an effective phenotypic mutation rate ranging from 2.2×10^{-3} to 5.6×10^{-3} per generation for virulence loci subject to selection by host resistance genes and a rate approximately 5 to 10 times lower for virulence loci not subject to selection, that is, 'unnecessary virulence' (the difference may reflect a sampling bias resulting from differences in the rate of detection of the two categories of virulence mutants). A two-fragment difference in AFLP, with an average mutation rate ranging from 1.5 to 4×10^{-6} per AFLP fragment/pathogen generation, may suggest divergence at least 10 years ago. However, this estimation

does not take into account that the rates of evolution for some fragments may deviate substantially from the average rate. This was the case for the fragment P20M11.333 (Table 3), which was homoplasious for 10 steps in the most parsimonious tree (maximum for the data set).

The recent appearance of strain 1 in North America and Western Australia is in accordance with previous virulence phenotype data (Wellings 2007; Markell & Milus 2008). Interestingly, strain 1 was exclusive to North America and Australia, while strain 2 was exclusive to other parts of the world, including West Asia where it may have been present since the early 1990s (elaborated from virulence phenotype data by Yahyaoui *et al.* 2002). However, since strain 2 lacks fragment P12M26.150, which was otherwise present in all isolates of group B-F (Fig. 2), it represents the most recent evolutionary state of the two strains and not the immediate progenitor of strain 1. Thus, these data did not allow the source area for the exotic incursion of *P. striiformis* f. sp. *tritici* into North America in 2000 to be determined. The incursion of strain 1 into Australia in 2002 may originate from the same unknown area or North America.

The sequence of detection and geographical distribution of strains 1 and 2, the expected travel time for wind borne spores across the Atlantic or the Pacific Oceans, and the sensitivity of yellow rust spores to ultraviolet radiation may suggest an introduction of strain 1 to North America and Australia resulting from human mediated travel and commerce. The alternative, a first introduction by spores carried by wind is less likely because live yellow rust spores may only survive ultraviolet radiation in upper atmosphere for a few days, for example, exposure for only 1 day at sunny conditions reduced spore germination to less than 0.1% (Maddison & Manners 1972) and the required travel time is likely longer. For instance, the spread of sugar cane rust spores by wind from West Africa (Cameroon) to North America (Dominican Republic) was estimated to take 9 days (Purdy *et al.* 1985), and spread of coffee rust from Angola to Brazil was estimated to take 5–7 days (Bowden *et al.* 1971). Watson & de Sousa (1982) estimated 2 to 6 days for wind transport of spores, depending on the height in the atmosphere, from southern Africa to Australia. The subsequent spread of the new strain of yellow rust in North America (Chen 2005; Markell & Milus 2008) and Australia (Wellings 2007) is most likely mainly caused by spores dispersed by wind, as usual for cereal rust pathogens, combined with aggressive growth on wheat crops covering large acreages.

In addition to this recent global spread of new yellow rust strains, the data gave evidence for additional inter-continental dispersal events in the past. For instance, almost all isolates sampled in northern Europe, North America and Australia before 2000, were closely related by AFLP phenotype, suggesting a common origin. Therefore,

these data also support a possible first introduction into Australia in 1979 of a single yellow rust strain from Europe, as discussed by Wellings *et al.* (1987), and a subsequent clonal evolution by mutation (Wellings & McIntosh 1990). All isolates from South Africa, where yellow rust first appeared in 1996 (Boshoff *et al.* 2002), were closely related to strains of yellow rust from Central- and West Asia and southern Europe (cf. Enjalbert *et al.* 2005). Since 2004, similar isolates have been repeatedly detected in northern Europe (Hovmøller & Justesen 2007a). First, this suggests that yellow rust spread to South Africa from either West- or Central Asia or a common unknown source, and second, that the fungus has spread frequently at long distances in recent years.

AFLP proved highly informative in this study, where the main aim was to detect recent events in terms of pathogen dispersal and evolution. The dominant nature of AFLP fragments coupled with the dikaryotic feature of the yellow rust fungus implied some restrictions for resolving genetic diversity and population structure in great detail (Kosman & Leonard 2005), but this was by far compensated for by the high power of discrimination among strains which was essential for this study. Previously, microsatellites (Enjalbert *et al.* 2005), random amplified polymorphic DNA (Steele *et al.* 2001) and isozymes (Newton *et al.* 1985) have revealed limited or no diversity in *P. striiformis* f. sp. *tritici*.

In conclusion, this study has shown that the combination of rigorous DNA fingerprinting and virulence phenotyping made it possible to detect global dispersal events and to evaluate the timing and sequence of events for a biotrophic and asexually reproduced fungus. The results reinforce the recent alarm by the Food and Agriculture Organization that a new strain of the wheat stem rust fungus that originated in East Africa is at risk of spreading globally (Stokstad 2007). This knowledge is considered of utmost importance for assessing the risks of new wheat rust epidemics in different parts of the world and may form a basis for designing new control strategies to combat important crop pathogens posing a threat to agricultural crops on the global scale.

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Supporting Information

The following supporting information is available for this article:

Table S1 Origin and virulence phenotype of 151 global samples of *Puccinia striiformis* f. sp. *tritici*.

This information may be found in the online version of the article.

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