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Worldwide population structure of the wheat rust fungus *Puccinia striiformis* in the past

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ABSTRACT

Puccinia striiformis is a basidiomycete causing yellow rust on wheat. The availability of historic samples of this pathogen from the 'Stubbs collection' enabled us to investigate past population structure and temporal dynamics on a global scale. A set of 212 single genotype urediniospore isolates, representing samples collected from five continents between 1958 and 1991, were genotyped using 19 polymorphic microsatellite markers. The population genetic analyses revealed the existence of seven genetic groups in the past worldwide *P. striiformis* population. This genetic grouping generally corresponded with geographical sample origin except for the Middle East, where six of the seven genetic groups were represented. The presence of many genetic groups in the Middle Eastern population reflected a low differentiation from the populations in East Africa (F_{ST} = 0.052) and in South Asia (F_{ST} = 0.064). A high diversity and recombinant population structure was observed in China and South Asia, while a clonal population structure was observed in NW Europe, East Africa and the Mediterranean region. The high genetic diversity in the Himalayan region supported recent studies suggesting a putative center of diversity for *P. striiformis* in this area. Four of the 89 multilocus genotypes detected were resampled in different geographical regions suggesting long-distance migration in the past. Comparison of the past populations with more recent ones, represented by 309 isolates mainly collected between 2001 and 2009, revealed temporal divergence for all populations except for Northwest Europe. Overall, we observed a clear subdivision within the worldwide population structure of *P. striiformis* and migration in the past.

1. Introduction

Research on the population genetics of microorganisms has been greatly facilitated by the development of informative molecular tools and powerful statistical software to process population genetic data (Brown, 1996; Excoffier and Heckel, 2006). This has offered new opportunities to study the population biology, ecology and evolution of important crop pathogens in much more detail, e.g., *Mycosphaerella graminicola* (*Zymoseptoria tritici*) (Goodwin et al., 2011; Linde et al., 2002), *Sclerotinia sclerotiorum* (Atallah et al., 2004), *Phytophthora infestans* (Yoshida et al., 2013) and rust fungi (Ali et al., 2014a; Berlin et al., 2012; Hubbard et al., 2015).

In recent years, the epidemiology and the population genetics of *Puccinia striiformis*, a rust fungus causing yellow (stripe) rust on wheat, have received much attention because it has spread to new areas where it was previously absent or scarce (Hovmøller et al., 2008; Milus et al., 2006; Wellings et al., 2003). Rapid evolution of new virulent races, which may infect previously resistant crop varieties, is another important feature of the yellow rust fungus (Hovmøller and Justesen, 2007; Wellings and McIntosh, 1990). Further, it was recently discovered that *Berberis* spp. can serve as an alternate host of the fungus (Jin et al., 2010), which may result in recombination and emergence of novel genotypes (Rodriguez-Algaba et al., 2014). The pathogen is present in most wheat growing areas of the world, and disease epidemics have been associated with large yield losses when management efforts using resistant wheat varieties and/or fungicides were inadequate for disease control (Wan et al., 2004; Wellings, 2011).

The urediniospores of *P. striiformis* may be carried by wind over long distances (Brown and Hovmøller, 2002; Zadoks, 1961) and cause the spread of virulent strains to new areas (Ali et al., 2014a; Hovmøller et al., 2002; Hovmøller et al., 2008; Markell and Milus, 2008; Wellings and McIntosh, 1990). There is also strong indication of spore transmission by human traveling, which lead to the initial spread of yellow rust to Australia in 1979

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(Wellings, 2007). Already in the 1960s efforts were initiated to develop international *P. striiformis* surveillance activities based on race phenotyping (Stubbs, 1988). These activities have been intensified in recent years after the worldwide spread of aggressive strains (Hovmøller et al., 2008; Milus et al., 2009) and the more severe yellow rust epidemics in wheat growing areas since the year 2000 (Chen, 2005; Hovmøller et al., 2010; Wan et al., 2004; Wellings, 2011). These events have emphasized the need for a better understanding of population diversity and dynamics of the yellow rust fungus at national and global scales (Hovmøller et al., 2010).

Several studies have documented clonal population structure in Europe, North America and Australia (Enjalbert et al., 2005; Hovmøller et al., 2002; Markell and Milus, 2008; Steele et al., 2001), whereas a recombinant population structure was found in China, Pakistan and Nepal (Ali et al., 2014a; Liang et al., 2013). Recent analyses of the contemporary worldwide populations revealed significant population subdivision, which to a large extent reflected the geography (Ali et al., 2014a). New findings further suggested that the Himalayan and near-Himalayan regions may represent a center of diversity and a putative center of origin for *P. striiformis* (Ali et al., 2014a).

Population studies of pathogenic fungi often represent a short time period and limited sampling areas unless there is access to historical collections of such pathogens. In the case of *P. striiformis*, a unique collection of liquid nitrogen-preserved urediniospore samples representing six continents was initiated by the Dutch plant pathologist J. C. Zadoks and further developed by the Dutch plant pathologist R. W. Stubbs between the late 1950s and the early 1990s (Stubbs, 1988; Thach et al., 2015; Zadoks, 1961). In the present study we explored a representative subset of isolates collected from wheat from the 'Stubbs collection' using microsatellite markers and population genetic analyses. We investigated the hypotheses whether population subdivision and signatures of recombination did exist in the past. The objectives were to (i) investigate population subdivision in the past, (ii) detect past genetic diversity and signature of recombination within geographically spaced populations, (iii) infer on migration in the past and finally (iv) infer on the temporal dynamics from the past to the present by including the data set representing the contemporary worldwide populations reported by Ali et al. (2014a).

Table 1
The geographical sampling region and the number *P. striiformis* isolates from 'Stubbs collection' entering analyses of worldwide population structure of the wheat rust pathogen in the past.

Geographical sampling region	Country	Number of isolates (*n*)	Sampling year
China	China	17	1982, 1983, 1987, 1989
South Asia	Nepal	19	1980, 1985–1991
	Pakistan	28	1973, 1975–1979, 1981–1983, 1985, 1988, 1990–1991
	Afghanistan	4	1972, 1981, 1986–1987
Middle East	Egypt	3	1975, 1977
	Iran	2	1973, 1989
	Iraq	1	1973
	Israel	9	1972–1973, 1980, 1988
	Lebanon	4	1972–1973, 1975
	Saudi Arabia	4	1975–1976
	Syria	9	1980, 1986, 1988, 1990–1991
	Turkey	1	1973
	Yemen	1	1984
East Africa	Ethiopia	6	1977, 1981, 1986–1987
	Kenya	27	1973–1974, 1976, 1978, 1980, 1982–1983, 1986–1991
	Tanzania	8	1975, 1977, 1979, 1987
Mediterranean	Algeria	5	1972, 1975, 1979, 1980
	Greece	3	1973, 1982
	Italy	11	1975, 1978–1983, 1985
	Libya	1	1990
	Morocco	1	1980
	Portugal	5	1975–1976, 1980, 1982
	Spain	7	1978, 1980, 1985, 1987
	Tunisia	4	1976, 1978, 1990
NW Europe	Belgium	3	1973, 1976, 1988
	Denmark	1	1975
	France	4	1959, 1974–1975
	Germany	6	1960, 1962, 1977, 1978
	Netherlands	12	1958, 1962, 1971–1972, 1975, 1977–1979, 1988–1989
	Sweden	1	1973
	Switzerland	1	1978
	United Kingdom	1	1965
Australia and North America	Australia	1	1985
	USA	2	1972, 1980
Total		212	

2. Materials and methods

2.1. Fungal material

A set of 212 isolates were retrieved from the historic 'Stubbs collection' from six geographical regions: NW Europe ($n = 32$), Mediterranean region ($n = 37$), East Africa ($n = 41$), Middle East ($n = 34$), South Asia ($n = 51$) and China ($n = 17$) (Table 1). Of these, 195 isolates were multiplied and urediniospores were harvested and stored at −80 °C before DNA extraction (Thach et al., 2015). These isolates were selected to represent maximum race diversity within each geographical region over time (from 1958 to 1991) (Table S1). Urediniospore samples of 17 additional Chinese isolates sampled in the 1980s were retrieved directly from liquid nitrogen (−196 °C), of which six had prior race information.

2.2. DNA extraction

DNA was extracted using a cetyltrimethyl ammonium bromide (CTAB) method (Justesen et al., 2002). Ten milligram urediniospores were ground using two steel balls (diameter: 5 mm) with an added equal volume of acid-washed sand in a Geno/Grinder® 2010 (SPEX SamplePrep, USA) at 1500 rpm for 3 × 30 s. DNA was extracted as described by Justesen et al. (2002) except for using 0.7 ml extraction buffer and chloroform. The DNA pellet was rinsed with 0.7 ml 70% ethanol, air-dried at room temperature and dissolved in 100 µl TE-buffer (10 mM Tris pH 8.0, 1 mM EDTA). The DNA was quantified with a NanoDrop 1000 Spectro-photometer (Thermo Fisher Scientific, USA), and DNA samples were diluted to 50 ng/µl before storage at −20 °C until further use.

2.3. Microsatellite genotyping

Microsatellite genotyping was performed with 19 previously developed and described SSR (Simple Sequence Repeat) primer pairs (Ali et al., 2011; Bahri et al., 2009a; Enjalbert et al., 2002). These 19 SSRs were amplified in two multiplexes as described in Rodriguez-Algaba et al. (2014). The amplification products were analyzed on Applied Biosystems 3730xl DNA Analyzer (Life Technologies Corporation, USA) by the Uppsala Genome Center, Uppsala University, Sweden, while the alleles were manually scored in GeneMarker® (Soft-Genetics, USA). Reference isolates were included in the genotyping to identify the allele sizes comparable with the previous study of Ali et al. (2014a). In cases in which allele sizes were difficult to score, multiplex PCRs were repeated or PCR for an individual SSR locus was run separately to confirm the allele

size. In cases of missing alleles, individual PCRs were run with increased primer concentration (final concentration of 0.5 μM for each primer) to confirm the results.

2.4. Analyses of population subdivision

Population subdivision was analyzed with multivariate and model-based Bayesian clustering methods, which only take into account the genotypic information and disregard information on e.g., geographical origin, which could hide information on possible admixtures (Dutech et al., 2010). Multivariate analysis using Discriminant Analysis of Principal Component (DAPC) assigned the genotypes into genetic groups (K) with the ADEGENET package in the R program (Jombart et al., 2010). Corresponding assignment graphs were generated in Excel. The optimal number of genetic groups was assessed based on the Bayesian Information Criterion (BIC) graph generated by ADEGENET and was further confirmed in a model-based Bayesian method implemented in STRUCTURE (Evanno et al., 2005; Pritchard et al., 2000). Population divergence between regions in the past was determined by F_{ST} values estimated with GENETIX v. 4.03 (Belkhir et al., 2004). Preliminary population differentiation analyses between the countries in South Asia showed that the Pakistani, Nepalese and Afghani populations could not be distinguished from each other ($F_{ST} < 0.02$). Isolates from these countries were grouped together in the further analyses and termed the 'South Asian' population.

2.5. Analyses of genetic diversity

The quality of the 19 SSR primers was tested using GenClone 2.0 (Arnaud-Haond and Belkhir, 2007) to see if the microsatellite loci were able to detect multilocus genotypes (MLGs) and if the number of SSR loci used was sufficient to detect all possible MLGs. Detection of unique MLGs under panmixia (Arnaud-Haond and Belkhir, 2007) and resampling of MLGs in order to infer migration were also carried out in GenClone 2.0. Genotypic diversity within geographically spaced populations, i.e. genetic diversity, linkage disequilibrium and the number of most resampled MLGs, was estimated with MultiLocus 1.3 (Agapow and Burt, 2001). Expected and observed heterozygosity were estimated in GENETIX v. 4.03 and p-values of these were calculated in GENEPOP 4.3 (Rousset, 2008). The software FSTAT (Goudet, 2001) calculated the gene diversity based on clone-corrected multilocus data to avoid resampling of clone mates during epidemic seasons. High genetic diversity, low linkage disequilibrium and non-significant difference between expected and observed heterozygosity were considered as signatures of recombination (Ali et al., 2014b).

2.6. Analysis of temporal dynamics

For the analysis of temporal dynamics the microsatellite data set of the past populations (1958–1991) was compared with data of a set of 309 isolates from the same regions representing the contemporary worldwide *P. striiformis* population (Ali et al., 2014a). The latter showed clear population subdivision into six genetic groups based on a larger sample set of isolates mainly sampled between 2001 and 2009 from six continents. The two data sets representing isolates from six regions (NW Europe, Mediterranean, Middle East, Pakistan, Nepal and China) were analyzed using the same microsatellite markers except RJN2. To make the comparison, divergence over time within each geographically spaced population was determined by F_{ST} values calculated from allelic data over time in GENETIX v. 4.03. Inspection of resampling of MLGs over time and space was conducted with GenClone 2.0 to infer about migration and temporal maintenance of the most frequent MLGs, as described in Ali et al. (2014b). The following six geographical regions were considered: NW Europe, Mediterranean, Middle East, Pakistan (including isolates from Afghanistan), Nepal and China. The data of South Asia was split into Pakistan/Afghanistan and Nepal for the temporal analysis to consider that those regions were divergent in the contemporary worldwide population (Ali et al., 2014a). A low number of isolates from the USA and Australia were excluded because they represented the same clonal lineage as the European population (Ali et al., 2014a; Hovmøller et al., 2008).

3. Results

3.1. Population subdivision

The 212 past *P. striiformis* isolates representing six geographical regions were initially grouped into six to eight genetic groups (equivalent to $K = 6$ to $K = 8$), estimated within the whole population based on the BIC-generated graphs (Fig. S1). When considering the stability of the clustering over runs, a division into seven genetic groups was the optimal ($K = 7$; Fig. 1), and this was also confirmed by STRUCTURE (Fig. S2). At $K = 2$ the NW European and the majority of the Mediterranean isolates clustered together in one group, while the other group contained South Asian, Middle Eastern and East African isolates. The Chinese isolates were found in both groups. Increasing the number of clusters to $K = 3$ resulted in East African, Middle Eastern and some South Asian and Mediterranean isolates to form a group. At $K = 4$ isolates from mainly East Africa and some from the Middle Eastern and Mediterranean regions clustered into a separate group. Increasing the clusters to $K = 5$ resulted in a split of the Chinese/South Asian group, in which the majority of Chinese isolates formed a unique group. At $K = 6$ a new group was formed consisting of mainly Middle Eastern isolates and few East African isolates. Finally, at $K = 7$ a large part of the Mediterranean population was assigned into a new group which also included some isolates mainly from South Asia and the Middle East. Any further increase in number of clusters did not generate additional information about population subdivision.

The genetic groupings generally corresponded with the geographical sampling origin (Fig. 1), except for the Middle East where six of the seven groups were represented. Group G4 was mainly detected in the Middle Eastern population and at low frequency in East Africa. Group G1 was specific to China while G6 was specific to NW Europe although resampled in the Mediterranean region. Group G2 and G3 were predominant in South Asia, represented by Afghanistan, Nepal and Pakistan. Another group (G5) was predominant in East Africa and present in the Middle East at low frequency. Isolates of group G7 were mainly present in the Mediterranean region.

Although an overall geographical differentiation was evident from F_{ST} values, the degree of differentiation was less for some adjacent geographical regions (Table 2). The Chinese population was differentiated from all other populations revealed by F_{ST} values between 0.166 and 0.308. The NW European population was strongly differentiated from all populations ($F_{ST} > 0.308$) except the Mediterranean population ($F_{ST} = 0.053$). The Middle Eastern population was less differentiated from the South Asian population ($F_{ST} = 0.064$) and the East African population ($F_{ST} = 0.052$).

3.2. Genetic diversity and signature of recombination

An excess of observed heterozygosity as compared to the expected under Hardy–Weinberg equilibrium indicated a strong signature of clonal reproduction in NW Europe ($p = 0.000$), East Africa ($p = 0.005$) and to a lesser extent in the Mediterranean region ($p = 0.170$) (Fig. 2). Further, high linkage disequilibrium values in these areas support this conclusion (Table 3). The Chinese

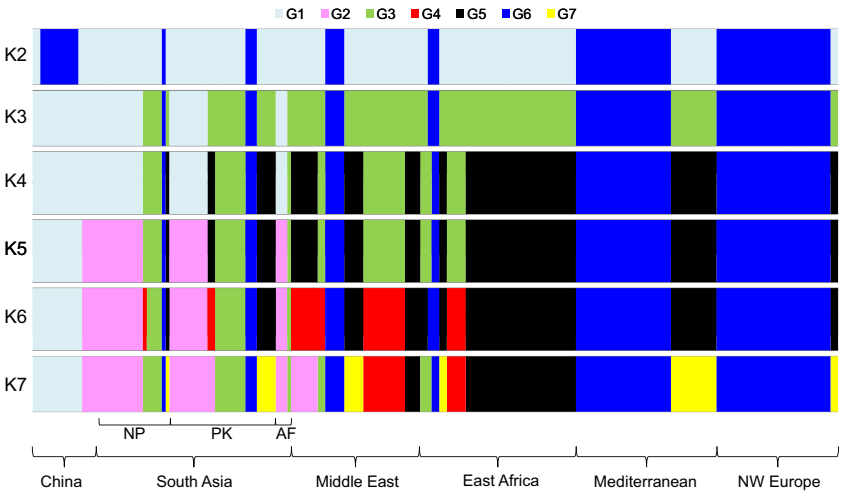

Fig. 1. Clustering of worldwide *P. striiformis* isolates from different geographical regions into genetic groups (G1–G7) based on DAPC analysis and shown by different colors.

Table 2
Differentiation between geographical spaced populations shown with F_{ST} values (all values were significant, $p < 0.001$). The clone-corrected data confirmed the F_{ST} results.

	South Asia	Middle East	East Africa	Mediterranean	NW Europe
China	0.166	0.215	0.266	0.185	0.308
South Asia	–	0.064	0.130	0.170	0.311
Middle East	–	–	0.052	0.173	0.321
East Africa	–	–	–	0.217	0.366
Mediterranean	–	–	–	–	0.053

and the South Asian populations were clearly recombinant populations with an observed level of heterozygosity not different from the expected ($p < 0.973$). Larger but non-significant differences between observed and expected heterozygosity were observed in the Middle East. The clone-corrected data, where only one representative of each genotype per geographical region was included, confirmed the trends in these results.

A total of 99 different alleles were scored across the 19 microsatellite loci (Table S2) resulting in 89 MLGs (Fig. S3). Overall, a high genotypic diversity was found in all the geographical populations (Table 3). The highest diversity was detected in the Chinese population, in which the genotypic diversity was close to one (0.985). Fifteen unique MLGs and the lowest value of linkage disequilibrium (0.121) were a clear signature of recombination, which were observed in the Chinese population. In contrast, for the clonal NW European population the lowest genotypic diversity (0.758) and number of MLGs of all the regions were observed along with high linkage disequilibrium (0.720). The values for the remaining regions were generally between these extremes.

The gene diversity varied among the geographical populations (Table 3). We observed the highest gene diversity in the South Asian populations across all calculated values, i.e., the mean number of alleles per locus (4.105), the gene diversity (0.487) and the allele richness (3.444). The overall lowest gene diversity was found in the NW European population where only 2.474 mean alleles per locus were detected and the allele richness was 2.474. The within population diversity found in the remaining geographical populations was generally in between these extreme values. The excess of observed heterozygosity but relatively high genetic diversity in the Middle Eastern population make the signature of recombination inconclusive for this region.

3.3. Migration of multilocus genotypes across geographical regions

The resampling of MLGs across geographical locations was assessed to infer on potential migration in the past. The five most resampled MLGs (MLG-17, MLG-22, MLG-35, MLG-47 and

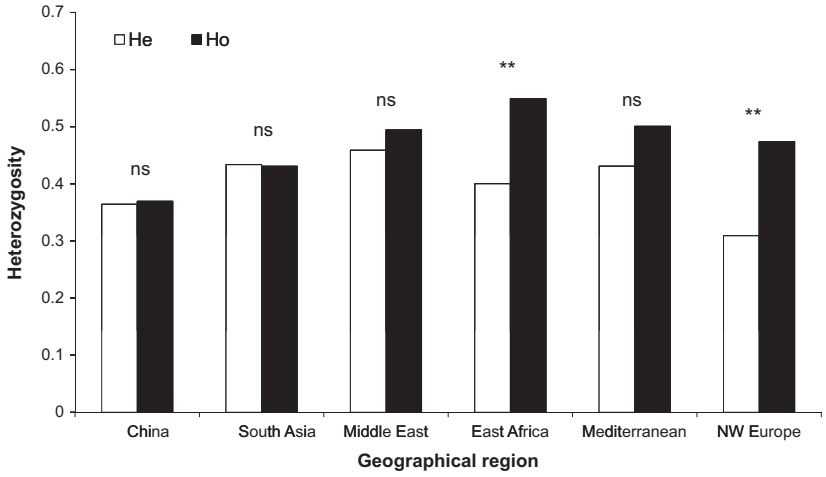

Fig. 2. Expected heterozygosity (He) and observed heterozygosity (Ho) of geographical spaced populations of *P. striiformis* shown for the whole dataset. The clone-corrected data overall supports the results although the heterozygosity levels in East Africa and NW Europe were non-significant.

Table 3
Genetic diversity of geographical spaced populations of *P. striiformis*. The gene diversity results are based on clone-corrected data.

		Genotypic diversity				Gene diversity		
Geographical population	Number of samples	MLGs	Genotypic diversity	Number of samples of most abundant MLG	Linkage disequilibrium (Rd)	Mean number of alleles per locus	Gene diversity	Allele richness
China	17	15	0.985	2	0.121	2.684	0.351	2.590
South Asia	51	22	0.840	20	0.343	4.105	0.487	3.444
Middle East	34	22	0.957	6	0.345	3.263	0.465	2.965
East Africa	41	14	0.815	13	0.469	3.158	0.485	2.975
Mediterranean	37	14	0.847	11	0.836	2.737	0.374	2.613
NW Europe	32	10	0.758	15	0.720	2.474	0.396	2.474

MLG-62) accounted for 91 of 212 isolates (Fig. 3). The most frequent genotype, MLG-22, was resampled in 30 isolates mainly from NW Europe and the Mediterranean region over several years. These 30 isolates consisted of multiple race phenotypes typical of NW Europe (Table S3). MLG-47 was resampled in 20 isolates mainly from the Mediterranean region but also from NW Europe, the Middle East, South Asia and East Africa. This MLG consisted of only four races that all shared virulence to *Yr6*, *Yr7* and *Yr8*. The third most common MLG in the study was MLG-62 resampled in 18 isolates only from South Asia. These isolates consisted of four races with similar virulence patterns, which were distinct from those of MLG-22 and MLG-47. MLG-17 (12 isolates) and MLG-35 (11 isolates) differed by only one SSR allele and were mainly present in East Africa, but resampled at low frequency in the Middle East. Eleven of the twelve isolates of MLG-17 consisted of a single race (common virulence to *Yr2*, *Yr6*, *Yr7*, *Yr8*, *Yr9*, *Yr25* and *YrAvS*), whereas MLG-35 contained three similar but less virulent races, suggesting a common origin within the area. Interestingly, virulence phenotypes found within MLG-47 and MLG-35 were identical for the majority of the isolates, although the two MLGs differed in six loci and the sampling locations were far apart.

3.4. Temporal dynamics from past to present

We investigated the temporal dynamics of *P. striiformis* by comparing isolates from the past (1958–1991) with the current populations (1992–2009) (Table 4). Significant differentiation was observed for the Nepalese (F_{ST} = 0.235), the Mediterranean (F_{ST} = 0.245) and Pakistani (F_{ST} = 0.167) populations. A lower differentiation was observed for the Chinese population (F_{ST} = 0.141) and the Middle East population (F_{ST} = 0.084). The NW European population was the most stable over the years (F_{ST} = 0.044). Considering resampling of MLGs between the historic isolates from the 'Stubbs collection' and the contemporary populations, only two MLGs were resampled. These MLGs were typical of the NW European genetic group, further endorsing the notion of long-term persistence of this population.

4. Discussion

A unique historic collection of isolates of *P. striiformis*, known as the 'Stubbs collection', made it possible to study the past worldwide population structure of this important wheat pathogen. Microsatellite markers of isolates representing the time period 1958–1991 and 34 countries in six geographical regions were genotyped. Further, the accessibility to a data set representing the contemporary worldwide population of *P. striiformis* mainly sampled between 2001 and 2009 (Ali et al., 2014a) enabled analyses of the temporal dynamics for certain regions.

4.1. Worldwide population subdivision and its divergence over time

Seven distinct genetic groups were detected in the past worldwide *P. striiformis* population, which generally corresponded with the geographical origin, except for the Middle East. This revealed the existence of population subdivision in the past *P. striiformis* population, despite its long-distance migration capacity (Brown and Hovmøller, 2002; Hovmøller et al., 2008). This overall population subdivision was in accordance with the contemporary population structure (Ali et al., 2014a), except for the Middle East where isolates representing the six genetic groups were observed in the past. Indeed, migration across geographical regions was observed in the past (Beresford, 1982; O'Brien et al., 1980; Zadoks, 1961). One plausible explanation for our observation may be that the Middle East has received migrants from many other regions due to its geographical location, resulting in its low F_{ST} values with South Asian and East African populations.

Interestingly, comparison of the past populations with the contemporary populations revealed divergence over time within the geographical regions, except for NW Europe. The NW European population has been reported to be maintained through clonal reproduction (Enjalbert et al., 2005; Justesen et al., 2002). The mutation and subsequent selection over time by host resistance genes would have resulted in the development of diverse pathotypes in a less diverse genetic background (de Vallavieille-Pope et al., 2012; Hovmøller and Justesen, 2007), albeit lacking any temporal divergence. However, since 2011 this population is being replaced to a large extent by new races of exotic origin, emphasizing that dramatic change within a region may appear rapidly (Hovmøller et al., 2015).

Among the populations with divergence over time, several explanations are possible. The Middle Eastern and Mediterranean populations in the past were divergent and more diverse than the contemporary populations (Ali et al., 2014a). The temporal divergence in the Mediterranean region may be explained by invasion and establishment of the aggressive strain PstS2 since 2003 (Ali et al., 2014a; Bahri et al., 2009b). A similar case was reported for another related strain, PstS1, which replaced the *P. striiformis* populations in the USA and Australia (Markell and Milus, 2008; Wellings, 2007). The temporal divergence in the South Asian and Chinese populations could be a result of difference in sampling strategy in highly recombinant and diverse populations (Ali et al., 2014a; Mboup et al., 2009), where further subdivision could be possible within a geographical region, as reported for Pakistan (Ali et al., 2014b).

4.2. Long-distance migration

The resampling of a number of MLGs suggested long-distance migration of *P. striiformis* in the past. The resampling of MLG-47, of which 17 of 20 isolates shared virulence to *Yr2*, *Yr6*, *Yr7* and *Yr8*, may be the most clear example suggesting long-distance migration in the past. The majority of these isolates were sampled in the Mediterranean region with some additional isolates in Pakistan, Tanzania and the Netherlands. This race has previously been termed '6E16' and frequently reported in the Mediterranean region over many years (Bahri et al., 2009b; Enjalbert et al., 2005; Stubbs, 1988). The resampling of MLG-17 and MLG-35 in East Africa and

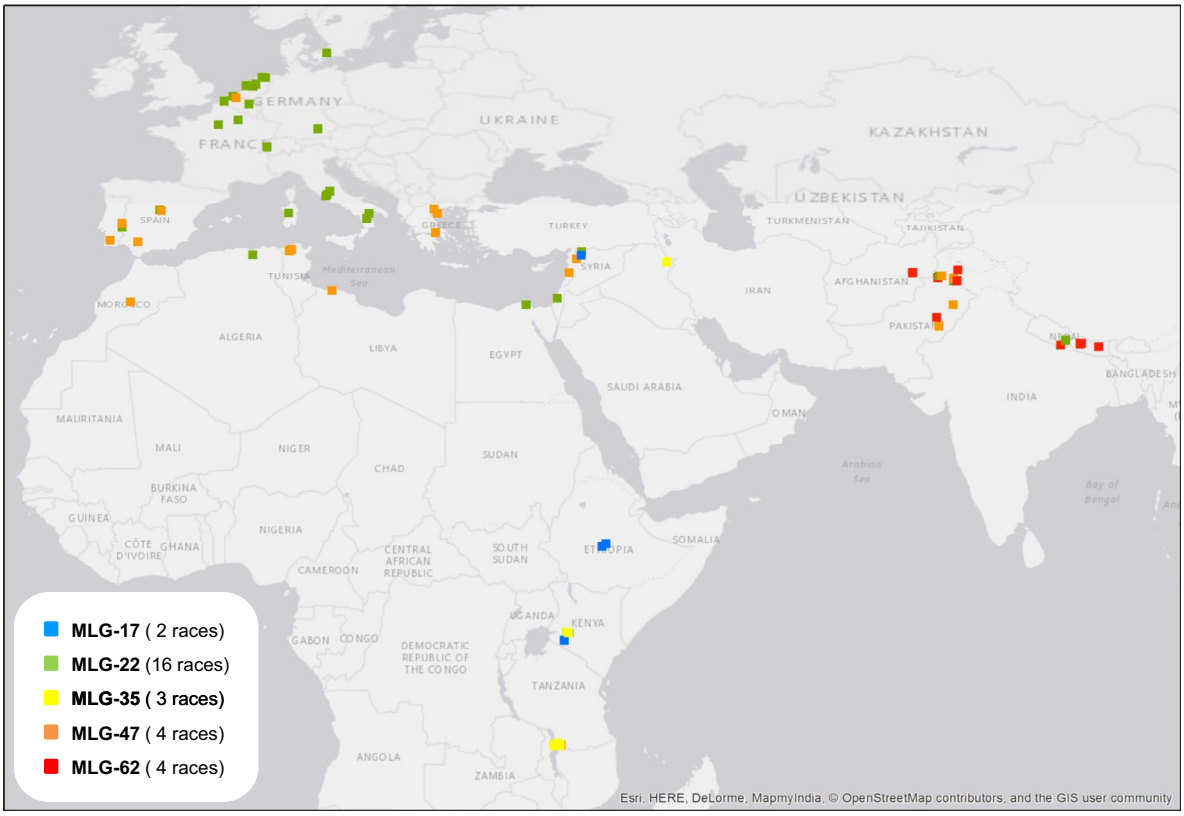

Fig. 3. Migration and approximate location of the five most resampled multilocus genotypes (MLG-17, MLG-22, MLG-35, MLG-47 and MLG-62) of historic *P. striiformis* isolates. MLG-17 was shared by 12 isolates and two races (most common virulence to *Yr2*, *Yr6*, *Yr8*, *Yr9*, *Yr25* and *YrAvS*) sampled between 1983 and 1991; MLG-22 was shared by 30 isolates and 16 races sampled between 1959 and 1989; MLG-35 was shared by 11 isolates and three races (most common virulence to *Yr2*, *Yr6*, *Yr7*, *Yr8* and *YrAvS*) sampled between 1973 and 1980; MLG-47 was shared by 20 isolates and four races (most common virulence to *Yr2*, *Yr6*, *Yr7*, *Yr8* and *YrAvS*) sampled between 1962 and 1990 and MLG-62 was shared by 18 isolates and four races (most common virulence to *Yr1*, *Yr2*, *Yr6*, *Yr7*, *Yr8*, *Yr25* and *YrAvS*) sampled between 1981 and 1991. MLG-22 was also resampled once in Australia (not shown on the map).

Table 4
Temporal divergence of worldwide geographical spaced *P. striiformis* populations of the past (1958–1991) compared to more recent populations (1992–2009[a]). Isolates sampled in the past were from the 'Stubbs collection' and the more recent isolates were those used in a previous study (Ali et al., 2014a).

Geographical population	Number of isolates		F_{ST}	*P*-value
	Past	Recent	Past and recent	Past and recent
China	17	71	0.134	0.000
Nepal	19	55	0.236	0.000
Pakistan[b]	32	75	0.178	0.000
Middle East	34	60	0.084	0.000
East Africa[c]	41	0	–	–
Mediterranean	37	21	0.236	0.000
NW Europe	29	27	0.034	0.016

[a] The majority of the isolates were sampled from 2001–2009.
[b] Isolates from Afghanistan were included in the Pakistani population.
[c] East African isolates from the recent population were only sampled from Eritrea, and they were excluded from the temporal analysis because Eritrea was not represented in the past population.

the Middle East is another example suggesting migration over long distances. Based on the results of Walter et al. (2011), MLG-17 could not be differentiated from isolates representing the high temperature adapted and aggressive strain, PstS1, which was first reported in 2000 in America and in 2002 in Australia. MLG-17 and MLG-35, which differed by only one microsatellite marker, consisted of races of similar virulence phenotypes. Ten of the eleven isolates of MLG-17 consisted of a single virulence phenotype (common virulence to *Yr2*, *Yr6*, *Yr7*, *Yr8*, *Yr9* and *Yr25*), which is identical to the phenotype of the two aggressive strains (PstS1 and PstS2) spreading worldwide in recent years (Hovmøller et al., 2008; Markell and Milus, 2008; Milus et al., 2009; Wellings, 2007). More than 50% of the isolates of MLG-35 also consisted of a single virulence phenotype (common virulence to *Yr2*, *Yr6*, *Yr7*, *Yr8* and *YrAvS*), which differed by only two–three virulences from the predominant strains PstS1/PstS2. This virulence phenotype has been observed in East Africa and Iraq since the mid-1970s, when the sampling of yellow rust and characterization of races were initiated in these areas by R. W. Stubbs (Stubbs, 1988).

4.3. Reproductive mode

The different levels of heterozygosity and reproductive modes within the past geographical populations of *P. striiformis* supported the recent results of worldwide population structure (Ali et al., 2014a; Enjalbert et al., 2005; Hovmøller et al., 2002; Mboup et al., 2009). The overall population structure in terms of recombination signature was stable over time. Clonal populations were detected in NW Europe, East Africa and in the Mediterranean region, whereas recombinant populations were present in China and South Asia. We observed that the Chinese population in the past had a recombinant population structure and a high genotypic diversity, which have also been reported for populations in China in more recent times (Ali et al., 2014a; Duan et al., 2010; Mboup et al., 2009). Further, the high genetic diversity in the South Asian region confirmed a recombinant population structure, which supported the recent conclusion that the Himalayan and near-Himalayan regions represent a center of diversity for *P. striiformis* (Ali et al., 2014a; Ali et al., 2014b).

5. Conclusion

The present study has shown the existence of seven genetic groups in a worldwide representative subset of *P. striiformis* isolates in the past sampled between 1958 and 1991. A clear population structure was detected at the worldwide scale, which included clonal populations in NW Europe, the Mediterranean region and East Africa and recombinant populations in China and South Asia. These findings supported the Himalayan and near-Himalayan regions as a center of diversity for *P. striiformis*. Clear indications of long-distance migration in the past were detected and temporal differences were observed in several regions.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.fgb.2015.12.014.

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