Genetic structure of *Mycosphaerella graminicola* populations from Iran, Argentina and Australia

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Abstract

Restriction fragment length polymorphism (RFLP) markers were used to assess the genetic structure of populations of *Mycosphaerella graminicola* collected from wheat fields. A total of 585 isolates representing 10 field populations were sampled from Iran, Argentina and Australia. The genetic structure of *M. graminicola* populations from Iran and Argentina is described for the first time. Results were compared to previously investigated populations from Israel, Uruguay and Australia. Populations from Iran exhibited high clonality and low gene diversity, suggesting an inoculation event. Populations from uninoculated fields in Argentina had gene and genotype diversities similar to previously described European and North American populations. Genotype diversity was high for populations from Australia and tests for multilocus associations were consistent with sexual recombination in these populations. Gene diversity was low and fixed alleles were found for several loci. These findings are consistent with a relatively small founding population for Australia. These 10 new populations were integrated into a genetic distance comparison with 13 global populations that were characterized earlier.

Introduction

Knowledge of the genetic structure of pathogen populations can be used to infer processes affecting population genetics that can be applied in an agricultural context. For example, knowledge of the pathogen centre of origin can focus the search for sources of genetic resistance. A high degree of similarity between geographically separated populations may indicate gene flow between populations, with possible implications for the effectiveness of control strategies (McDonald and Linde, 2002).

Neutral RFLP genetic markers have been used to characterize the genetic structure of *Mycosphaerella graminicola* populations for more than 15 years (e.g. McDonald and Martinez, 1990, 1991a, b; Boeger et al., 1993; Chen and McDonald, 1996; Schneider et al., 2001; Linde et al., 2002; Zhan et al., 2003, 2004). *Mycosphaerella graminicola* is a heterothallic ascomycete that causes Septoria tritici leaf blotch on wheat. This pathogenic fungus is distributed worldwide and can cause yield losses of up to 40% (Eyal, 1999). Population genetic analysis of *M. graminicola* revealed that the majority of genetic variation is distributed on small spatial scales (Linde et al., 2002), and that gene flow has occurred on a global scale (Zhan et al., 2003).

Zhan et al. (2003) analyzed 15 *M. graminicola* populations from five continents and showed that global gene diversity was highest in populations from Israel. Thus the Fertile Crescent was

hypothesized to be the most likely centre of origin for the pathogen. However, Israel was the only population sampled from the Fertile Crescent, so it is desirable to sample other populations from the Fertile Crescent to further test the hypothesis. Similarly, only one population from South America was included in the original analysis, and additional collections are needed to determine if the single collection was representative of such a large region.

Zhan et al. (2003) found that the genetic diversity within populations was high for all but three of the 15 populations analyzed. Two of the populations with low genetic diversity were collected in Australia. The low number of isolates considered in both populations coupled with the non-hierarchical sampling procedures used for one of the populations led to doubts concerning the validity of the hypothesis that the observed genetic structure was due to genetic drift resulting from a founder effect.

The objectives of the present study were: (1) to test if the genetic structure of *M. graminicola* populations from Iran resembles the genetic structure of a previously characterized population from Israel and to determine if regional gene flow is occurring among the Middle Eastern populations; (2) to investigate the genetic structure of fungal populations from Argentina and to place these findings into a regional and more global setting; (3) to further test the hypothesis that the genetic structure of *M. graminicola* populations from Australia resulted from a founder event and to infer the most likely source for these populations.

Materials and methods

Fungal populations

Mycosphaerella graminicola populations considered in this analysis were collected from 14 wheat fields in five countries from three continents (Table 1). The sampled regions included the Fertile Crescent, South America and Australia. The population from Israel was described previously (Linde et al., 2002). Populations from Iran were sampled in 2001 from fields at two different experiment stations. Population one (IRA1) originated from Mehran, in the Province Ilam in western Iran near the border of Iraq. Population two (IRA2)

originated from Dezfool, in the Province Khozestan in southwestern Iran. The two collection sites were approximately 240 km apart and separated by a mountain range. The population from Uruguay (URU) was the only population from South America described previously (Zhan et al., 2003). Collections from Argentina were made in one of the main wheat growing areas near Buenos Aires. In 1998, population 1 was collected at Los Hornos (ARG1). Population 2 was collected at Balcarce (ARG2) at three different time points during the same year. In 2000, three additional populations were sampled from three fields at two different experimental field stations: population 3 (ARG3) and 4 (ARG4) were collected at Barrow whereas population 5 (ARG5) was again collected at Los Hornos. ARG4 was collected in the same area as ARG3 from two different durum wheat cultivars. ARG5 was collected in the same area as ARG1 with a difference of two years between the samplings. Barrow and Los Hornos are approximately 450 km apart. Five populations from Australia representing the western (AUS1) and eastern (AUS2, AUS3, AUS4 and AUS5) part of the continent were included in this study. AUS1 consists of isolates collected from 1978 to 1991 in Western Australia. AUS2 was collected near Wagga Wagga in 1992 from a single field using hierarchical sampling. Previous interpretations of Australian genetic structure based on these collections (Zhan et al., 2003) were limited due to the small sample sizes and non-hierarchical sampling strategies, so additional collections were needed. In 2001, three additional field populations were collected from farmers fields in Eastern Australia near Wagga Wagga. One population (AUS3) was collected 2 km from the collection site of AUS4; AUS5 was collected at a distance of approximately 40 km from the collection sites for AUS3 and AUS4.

Fungal isolation, DNA extraction, and detection of restriction fragment length polymorphisms

The methods described by Chen et al. (1994) were used for the isolation and culturing of the fungal collections. DNA was extracted with the Qiagen DNA Plant mini extraction kit according to the specifications of the manufacturer (Qiagen GmbH, Hilden, Germany). Restriction digestion with *Pst*I, Southern blotting, and RFLP analysis were as

Table 1. Description of *Mycosphaerella graminicola* populations used in this study

Region/Countries	Locations	Population	Year(s) collected	Host variety	Sampling strategy	Source (previous publication)
Fertile Crescent						
Israel	Nahal Oz	ISR	1992	4 bread wheat cvs	Transect	O. Yarden (Linde et al., 2002; Zhan et al., 2003)
Iran	Mehran	IRA1	2001	Unknown bread wheat cvs	Random, different spots in fields	M. Javan-Nikkah
	Dezfool	IRA2	2001			
South America						
Uruguay	Colonia	URU	1993	2 bread wheat cvs	Hierarchical	M. Diaz de Ackermann (Zhan et al., 2003)
Argentina	Los Hornos	ARG1	1998	8 bread wheat cvs	Hierarchical	C. Cordo
	Balcarce	ARG2		Unknown bread wheat cvs	Random, different spots in fields	
	Barrow	ARG3	2000	8 bread wheat cvs	Hierarchical	
	Barrow	ARG4		2 durum wheat cvs		
	Los Hornos	ARG5		8 bread wheat cvs		
Australia						
West Australia	West Australia	AUS1	1978–1991	Unknown bread wheat cvs	Random, different spots in fields	R. Loughman (Zhan et al., 2003)
East Australia	Wagga Wagga	AUS2	1992	Unknown bread wheat cvs	Hierarchical	B. Ballantyne (Zhan et al., 2003)
	Tank	AUS3	1999	Unknown bread wheat cvs	Hierarchical	B.A. McDonald/A. Milgate
	Mascot	AUS4				
	PAD-17	AUS5				

described previously for *M. graminicola* (Linde et al., 2002). The seven probes used to obtain allele frequencies were *pSTS*192, *pSTS*14, *pSTL*10, *pSTL*53, *pSTS*43, *pSTL*31, and *pSTS*2. It was demonstrated previously that probe *pSTS*192 hybridizes simultaneously to two loci on different chromosomes (McDonald and Martinez, 1991a, b), providing us with eight RFLP loci in total for each isolate. Data from the RFLP fingerprinting probes *pSTL*70 and *pSTL*40 were combined to identify clones.

Mating type analysis

The mating types of Australian and Iranian isolates were determined with a multiplex polymerase chain reaction using primers MAT1-1F, MAT1-1R, MAT1-2F and MAT1-2R (Waalwijk et al., 2002). MAT1-1 isolates produced a 340 bp fragment and MAT1-2 isolates produced a 600 bp fragment. Mating type data from the Argentinean populations could not be collected because insufficient DNA remained after the preparation of the Southern blots.

Data analysis

Each probe defined a different RFLP locus. DNA fragments or combinations of fragments were treated as different alleles. Isolates with the same RFLP fingerprints and multilocus haplotype were considered clones of the same genotype.

To quantify genotypic variation within populations, the genotype diversity measure of Stoddart and Taylor (1988) was calculated for all populations. To compare $\hat{G}$ in collections with different sample sizes, $\hat{G}$ was divided by N to calculate the percentage of maximum possible diversity that was obtained. In addition, the clonal fraction was measured as $1 - [(\text{number of different genotypes})/(\text{total number of isolates})]$ (Zhan et al., 2003). All further analyses were conducted on clone-corrected datasets to avoid overrepresentation of alleles in frequently occurring clones unless stated differently. The gene diversity measure of Nei (1973) and the average observed number of alleles was estimated using PopGene (Version 1.32; 2000, Yeh et al., Molecular Biology and Biotechnology Centre, University of Alberta, Canada, URL http://www.ualberta.ca/~fyeh/index.htm).

Analysis of multilocus associations (Brown et al., 1980) was used to measure the extent of gametic disequilibrium for each of the populations used in this study. These estimates were based on a dataset that included only isolates that had complete data for all loci and were calculated using PopGene. The index of association I_A was calculated based on 100 artificially recombined datasets using Multilocus (Version 1.2; 2000, Agapow and Burt; Department of Biology, Imperial College, UK, URL http://www.bio.ic.ac.uk/evolve/software/multilocus/). For the latter calculations, missing data were fixed during randomizations. Significant departures from an expected 1:1 ratio in mating type frequencies were tested with a χ^2-test.

Differences in allele frequencies among populations were calculated using a contingency χ^2-test (Workman and Niswander, 1970). The Bonferroni correction (Weir, 1997) was applied to all χ^2-tests to reduce Type 1 error. Measures of genotypic diversity ($\hat{G}$) were compared using a *t*-test (Chen et al., 1994). Gene flow (Nm) within and among regions was estimated based on the average degree of population subdivision (G_{ST}) across all eight loci (Newton et al., 1997). Statistical significance of differences in number of alleles among populations was tested by bootstrap analyses using Resampling Stats for Excel (Version 3.0, Resampling Stats). For each replication, the total number of alleles was recorded from a random sample of 15 strains (the actual size of the second smallest population after clone-correction). This procedure was repeated 100 times. The mean and variance of total allele number was calculated and used for a *t*-test. Genetic distances (Nei, 1978) between and among 13 previously described populations (Linde et al., 2002; Zhan et al., 2003) as well as the 10 new populations described here were calculated using PopGene. The pair-wise genetic distances were used to draw a phenogram using the NTSYS software package (Version 2.1, 2000) based on a cluster analysis using UPGMA.

Results

Genotypic and gene diversity for Iranian populations

A total of 144 isolates from two different field sites in Iran were analyzed (Table 1). Forty-three genetically distinct haplotypes were found; thus about 30% of all isolates were unique haplotypes. For both Iranian populations the average number

of alleles, the genotype measure of Stoddart and Taylor and gene diversity were low (Table 2). The genotypic diversity measure was significantly lower ($P < 0.01$) in the population IRA1 compared to the population IRA2. Fixed alleles were found for three loci in each of the Iranian populations. In IRA1 alleles were fixed for the loci *pSTS*192A (allele 1), *pSTS*2 (allele 1) and *pSTS*43 (allele 1). In IRA2 alleles were fixed for *pSTS*192A (allele 1), *pSTL*31 (allele 2) and *pSTS*43 (allele 1).

IRA1 departed from gametic equilibrium as indicated by Brown's multilocus analysis and mating type frequencies differed significantly from the expected 1:1 ratio ($P < 0.05$) (Table 3). For IRA2, a balanced mating type ratio was found for the clone-corrected data set, but both the multilocus analyses and the index of association indicated a deviation from equilibrium. In population IRA1, one haplotype occurred 46 times (64%). The probability that this haplotype would occur by chance in a random mating population was 23% based on the allele frequencies of the clone-corrected dataset of population IRA1.

Genetic variation among populations from the Fertile Crescent

The population from Israel was described earlier (Zhan et al., 2003; Linde et al., 2002) and displayed high gene diversity and a maximum value for genotypic diversity. Genotypic diversity estimates were significantly lower for the Iranian populations ($P < 0.01$) in comparison to the population from Israel. A bootstrap analysis revealed that the population from Israel had the highest total number of alleles (mean = 24.4, SD = 0.7) and that the total number of alleles was significantly ($P < 0.01$) lower for the populations from Iran (IRA1: mean = 13.4, SD = 0.5; IRA2: mean = 13, SD = 0.3).

A contingency χ^2-test revealed that allele frequencies differed significantly for three loci (*pSTS*14, *pSTS*43 and *pSTL*31) between all populations from the Fertile Crescent. A pair-wise comparison between IRA1 and IRA2 revealed significant differences in the allele frequencies of loci *pSTS*14, *pSTL*10 and *pSTL*31. Results were unchanged after Bonferroni correction. G_{ST} was higher between the two Iranian populations than between the population from Israel and the two populations from Iran (Table 4).

Among the Iranian populations, one haplotype was shared, occurring 16 times in IRA1 and three times in IRA2. The expected probability for this haplotype to occur under the assumption of random mating is 3.5% based on the allele frequencies of a clone-corrected dataset combining the two Iranian populations.

Table 2. Gene and genotype diversity data for *M. graminicola* field collections from three regions

Region/Countries	Population	No. of strains	No. of haplotypes	No. of shared RFLP loci	Clonal fraction	Average observed number of alleles	Loci with fixed alleles	$\hat{G}/N$ (%)[a]	Gene diversity[b]
Fertile Crescent									
Israel	ISR	158	158	8	0	6.8	0	100	0.50
Iran	IRA1	72	18	8	0.75	1.8	3	3	0.23
	IRA2	72	25	8	0.65	1.6	3	14	0.26
South America									
Uruguay	URU	41	41	8	0	3.6	1	100	0.44
Argentina	ARG1	62	46	8	0.36	2.8	2	53	0.30
	ARG2	58	42	8	0.28	3.0	1	49	0.27
	ARG3	73	66	8	0.10	3.3	0	80	0.38
	ARG4	44	15	8	0.66	1.4	6	10	0.10
	ARG5	39	9	8	0.77	2.0	2	13	0.36
Australia									
West Australia	AUS1	32	27	7	0.16	1.3	5	73	0.11
East Australia	AUS2	26	19	8	0.27	2.4	4	54	0.20
	AUS3	50	48	8	0.04	2.3	2	93	0.16
	AUS4	46	45	8	0.03	1.6	4	88	0.12
	AUS5	71	64	8	0.90	2.0	2	84	0.15

[a]Stoddart and Taylor (1988).
[b]Nei (1973).

Table 3. Tests for multilocus associations and mating type ratios for *M. graminicola* field collections

Region/Countries	Population	Multilocus associations					Frequencies		
		N^a	S_k^{2b}	L^c	$I_A{}^d$	P-value[e]	MAT1-1	MAT1-2	χ^2-value[f]
Fertile Crescent									
Israel	ISR	158 (145)	1.765	1.961	0.370	0.38	0.51	0.49	0.06
Iran	IRA1	18 (10)	1.810	1.648	0.457	0.06	0.29	0.71	5.18*
	IRA2	25 (18)	2.093	1.907	0.582	0.01	0.52	0.48	0.08
South America									
Uruguay	URU	41 (40)	1.76	2.104	0.004	0.47	0.52	0.48	0.08
Argentina	ARG1	46 (36)	1.894	1.770	0.712	0.01			
	ARG2	42 (31)	1.894	1.599	0.406	0.02			
	ARG3	66 (15)	2.172	2.608	0.299	0.97			
	ARG4	15 (4)	0.250	0.250	0.007	0.50			
	ARG5	9 (5)	1.606	1.507	2.305	0.01			
Australia									
West Australia	AUS1	27 (13)	0.5047	0.7498	0.052	0.22	0.23	0.77	40.63*
East Australia	AUS2	19 (12)	0.7174	1.3637	−0.107	0.73	0.54	0.46	0.17
	AUS3	48 (30)	0.9253	1.4282	−0.017	0.53	0.51	0.49	0.17
	AUS4	45 (37)	0.5526	0.9453	−0.195	1	0.49	0.51	0.04
	AUS5	64 (25)	0.8381	1.5330	−0.107	0.94	0.55	0.45	1.24

[a]Population size used for the analysis of the index of association; population size used for the analysis of multilocus associations is indicated in parenthesis (Brown et al., 1980).
[b]Observed variance of the number of heterozygous comparisons.
[c]Upper confidence limit.
[d]Index of association as calculated in Multilocus.
[e]P-value to indicate significant deviations from populations in disequilibrium based on estimates of the index of association.
[f]χ^2-values based on a 1:1 ratio of mating type.
* indicates mating type frequencies are significantly different at $P < 0.05$.

Genotypic and gene diversity within the Argentinean populations

Among 276 *M. graminicola* isolates from five field sites in Argentina, 178 distinct genotypes were found (Table 1); thus about 65% of all isolates were unique haplotypes. A high level of clonality was found in the populations ARG4 and ARG5 as indicated by low genotype diversity values (Table 2). Genotypic diversity for ARG3 was significantly higher ($P < 0.01$), than for ARG1 and ARG2 and these were significantly higher than for populations ARG4 and ARG5 ($P < 0.01$). The observed number of alleles was highest for ARG3,

Table 4. Population differentiation (G_{ST}) (below diagonal), and gene flow (Nm) (above diagonal) among *M. graminicola* populations from Israel and Iran (IRA)

	Israel	IRA1	IRA2
Israel	–	3.7	2.4
IRA1	0.12	–	1.7
IRA2	0.17	0.23	–

as was gene diversity. A bootstrap analysis revealed that the total number of alleles was comparable for ARG1 (mean = 17.3, SD = 0.5), ARG3 (mean = 17.2, SD = 0.7), and ARG2 (mean = 17.1, SD = 0.4) and significantly lower ($P < 0.01$) for ARG5 (mean = 15.7, SD = 0.7) and ARG4 (mean = 10.9, SD = 1). The populations from Uruguay and ARG3 did not deviate from gametic equilibrium as indicated by both Brown's multilocus analysis and the index of association (Table 3).

Genetic variation among populations from South America

The population from Uruguay was described previously (Zhan et al., 2003) and displayed a level of gene diversity and an average number of alleles similar to previously described populations from North America and Europe. The null hypothesis of equal allele frequencies across all loci was rejected for all populations from South America by testing for homogeneity in gene frequencies

using a contingency χ^2-test. Applying the test for pair-wise comparisons between all populations from South America including Uruguay, a varying number of significant differences was detected. It ranged from one locus (pSTL31) in the case of comparing ARG1 and ARG2 to five loci (pSTS192A, pSTS192B, pSTS14, pSTS2 and pSTL31) in the case of comparing ARG4 and ARG5 to ARG3. Population subdivision between ARG4 and all other populations was highest with an average G_{ST} of 0.53 (Table 5). Low G_{ST} values with corresponding high Nm values were found between Uruguay, ARG1 and ARG3 and between ARG1 and ARG2.

One haplotype occurred 18 times at the ARG4 site and another haplotype occurred 11 times at the ARG5 site. The ARG4 and the ARG5 populations also shared these two haplotypes. The haplotype observed 18 times in ARG4 ($N = 44$) was detected four times in ARG5 ($N = 39$). The probability that this haplotype would occur by chance under the assumption of random-mating is 1.4% based upon the clone-corrected allele frequencies of the combined dataset. The haplotype that was observed 11 times in ARG5 was found eight times in ARG4. The probability that this haplotype would occur in random-mating populations was 1.2% based upon the clone-corrected allele frequencies of the combined dataset.

Genotypic and gene diversity within Australian populations

The populations AUS1 and AUS2 were described previously (Zhan et al., 2003) and displayed low gene diversity and average number of alleles. A total of 167 not previously described isolates from three different field sites (AUS3, AUS4 and AUS5) revealed 156 distinct genotypes (Table 1). The normalized genotypic diversity measures of Stoddart and Taylor ranged from 83% to 93% (Table 2). No significant differences in genotypic diversity were detected for AUS3, AUS4 and AUS5 and these populations did not depart from gametic equilibrium or from a 1:1 ratio in mating type frequencies (Table 3). Gene diversity was low as was the average observed number of alleles for all three populations. A bootstrap analysis revealed similar total number of alleles among AUS3 (mean = 10.5, SD = 0.6), AUS4 (mean = 10.2, SD = 0.6) and AUS5 (mean = 10.9, SD = 0.3). Allele numbers for the populations AUS1, AUS3, AUS4 and AUS5 were significantly lower ($P < 0.01$) than for the populations from Israel (mean = 24.4, SD = 0.7), Uruguay (mean = 21.0, SD = 0.5), and Argentina, ARG1 (mean = 17.3, SD = 0.5), ARG2 (mean = 17.1, SD = 0.4) and ARG3 (mean = 17.2, SD = 0.7).

Genetic variation among populations from Australia

Allele frequencies differed significantly across all Australian populations for four loci. The only significant difference ($P < 0.05$) between the allele frequencies of AUS3, AUS4 and AUS5 was found at locus pSTS43. Population subdivision was high between AUS1 and AUS2 and the more recently collected populations AUS3, AUS4 and AUS5 with an average G_{ST} of 0.43. Population subdivision was low among AUS3, AUS4 and AUS5 with an average G_{ST} of 0.02 and the corresponding Nm values were high (Table 6). A deletion at the pSTS192A locus that had not been described previously occurred at high frequencies in all Australian populations (19% in AUS1, 12% in AUS2, 94% in AUS3, 71% in AUS4 and 94% in AUS5). The deletion could also be found at low frequencies in populations from Denmark (1%), Germany (4%) and Switzerland (6%) but not in populations from Israel or North America.

Table 5. Population differentiation (G_{ST}) (below diagonal), and gene flow (Nm) (above diagonal) among *M. graminicola* populations from Uruguay and Argentina (ARG)

	Uruguay	ARG1	ARG2	ARG3	ARG4	ARG5
Uruguay	–	4.6	3.3	8.6	0.7	1.6
ARG1	0.10	–	24.1	0.5	2.1	1.4
ARG2	0.13	0.02	–	4.1	0.4	1.3
ARG3	0.05	0.51	0.11	–	0.8	2.1
ARG4	0.43	0.20	0.53	0.38	–	2.9
ARG5	0.24	0.26	0.27	0.20	0.15	–

Table 6. Population differentiation (G_{ST}) (below diagonal), and gene flow (*Nm*) (above diagonal) among *M. graminicola* populations from Australia (AUS)

	AUS1	AUS2	AUS3	AUS4	AUS5
AUS1	–	1.0	0.7	0.7	0.7
AUS2	0.33	–	0.7	0.6	0.7
AUS3	0.41	0.42	–	20.5	23.0
AUS4	0.43	0.45	0.02	–	19.4
AUS5	0.43	0.43	0.02	0.02	–

Global phenogram

A comparison of genetic distance between and among the newly described populations and populations described previously (Zhan et al., 2003) revealed that ARG3 is located in the same clade where most of the European and North American populations were located and grouped with Uruguay. ARG1 and ARG2 formed a group in the same clade. ARG4 and ARG5 formed a separate clade distinct from all other populations. The new Australian populations (AUS3, AUS4 and AUS5) formed a distinct clade with the previously described AUS2 (Figure 1).

Discussion

Is Iran within the centre of origin?

A high level of diversity in the nuclear genome is typical for field populations of *M. graminicola* (Zhan et al., 2003). A previous global comparison of populations identified the Fertile Crescent as the most likely centre of origin for the pathogen based on the high degree of mitochondrial and nuclear diversity found within the region. The two populations from Iran deviated strongly from these previous findings even though gene flow on a regional scale was expected. Genotype diversity, average observed numbers of alleles, as well as gene diversity estimates were surprisingly low for both populations from Iran. The high level of clonality within the populations was as unexpected as the relatively high number of fixed alleles. Also unexpected were the differences between the two Iranian populations regarding genotypic diversity measures as well as mating type frequencies. The natural occurrence of one genotype at the high frequency found in IRA1 has not been reported before, neither have we found previous examples of identical haplotypes spread over large spatial scales in naturally infected *M. graminicola* fields. The low probability that random mating would generate this shared haplotype in two separate populations lends support to the hypothesis that the source of these isolates was artificial inoculation instead of natural infection. It was confirmed that fields at the research station where IRA2 was collected had been inoculated previously (M. Turabi, Seed & Plant Improvement Institute, Iran, pers. communication). We were not able to confirm inoculation for IRA1. The only previous observation of several clones being distributed over spatial scales larger than 1–2 m was found in a Mexican population collected at the CIMMYT disease nursery in Patzcuaro (Zhan et al., 2003). For the Patzcuaro field site, it was confirmed that the field had been inoculated (Eyal, 1999). The characteristics of this Mexican population that displayed low genetic diversity with both mating types occurring at roughly the same frequency are similar to IRA2. The frequent occurrence of one haplotype in IRA1 suggests a recent inoculation event with that haplotype.

If both Iranian populations were derived from inoculation events, we cannot draw meaningful conclusions from a comparison between the two populations or the Israeli population, and we cannot use these Iranian populations to further test our hypothesis that the Fertile Crescent is the centre of origin for *M. graminicola*. But we consider it noteworthy that both fingerprinting probes hybridized to significantly fewer bands in the Iranian populations, a result consistent with our findings in the Israeli collection of isolates. For *pSTL*70 the number of hybridizing bands between 9.4 and 2.0 kb across all isolates ranged from a mean value of 1.1 (SD = 0.29) for the Iranian populations and a mean value of 2.3 (SD = 2.04) for the population from Israel to a mean value of 5.2 (SD = 2.9) in the case of the Argentinean
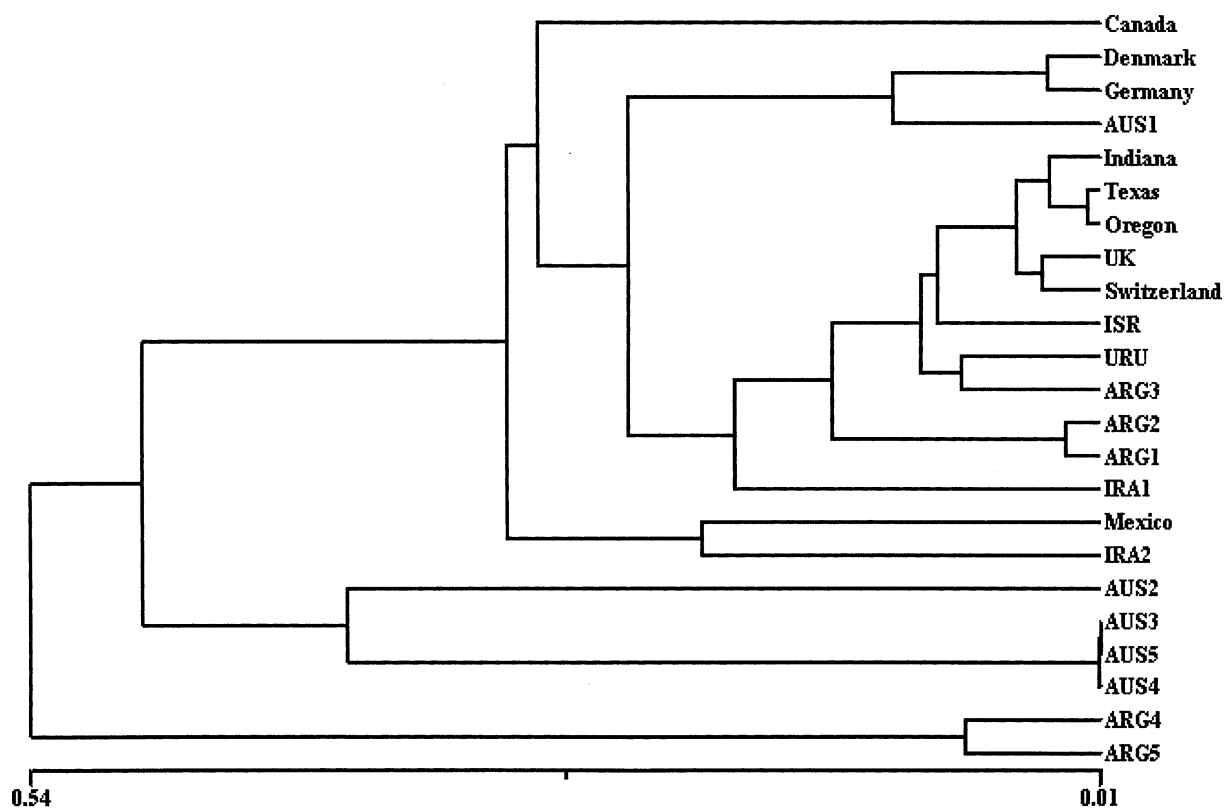
Figure 1. UPGMA phenogram based on Nei's genetic distance (1978) among *M. graminicola* populations from five continents. Data from field populations not described in this paper are from Zhan et al. (2003) and Linde et al. (2002).

populations. The differences between the isolates from South America and the Fertile Crescent were significant ($P < 0.01$). Thus we believe that the isolates used in these Iranian trials probably originated from the Fertile Crescent, and they may be useful in future studies of phylogeography based on DNA sequence loci (Banke and McDonald, 2005).

Are Argentinean populations representative of South America?

The genetic structure of two populations from Argentina (ARG4 and ARG5) was similar to the genetic structure of the populations from Iran in that ARG4 and ARG5 exhibited a high level of clonality within populations and they shared two haplotypes. In addition, ARG4 had only two variable RFLP loci among 44 isolates. These populations originated from different host species: ARG4 was isolated from durum wheat, whereas ARG5 was isolated from bread wheat. Kema et al.

(1996) proposed designating two host-specialized forms within *M. graminicola* based on virulence tests that indicated host-specialization. But on the molecular level, it was shown that frequencies of alleles at nuclear RFLP loci differed very little between host-adapted populations (Zhan et al., 2004). Based on genetic distance, ARG4 and ARG5 are more related to each other than to any other South American population. The most likely explanation for these observations is that the same source of inoculum was introduced into these field sites. These two populations were therefore excluded from further analysis.

Populations ARG1, ARG2 and ARG3 were collected from different places within the main wheat growing area of Argentina. The low level of population differentiation between ARG1 and ARG2 suggests that regional gene flow had a significant impact on the genetic structure of these populations. The degree of gene flow was similar to what was described earlier for *M. graminicola* populations in Switzerland (Linde et al., 2002).

The most recently collected population from Argentina, ARG3, had the greatest similarity to the population from Uruguay. ARG1, ARG2 and ARG3 differed significantly in genotype diversity. Brown's analysis of multilocus association as well as the index of association indicated a deviation from gametic equilibrium in the case of ARG1 and ARG2 but not for ARG3.

One possible explanation for these differences could be that the climate in Argentina during the 1998 growing season did not favour sexual reproduction in ARG1 and ARG2 whereas during the 2000 growing season, the situation might have favoured the sexual cycle, leading to gametic equilibrium and high genotypic diversity in the case of ARG3. ARG2 consisted of isolates collected at three time points during the growing season from different cultivars. Thus the genetic structure of this population may have changed through time due to selection on the different hosts and this could have generated disequilibrium. ARG1 was isolated from a total of 33 leaves representing eight different wheat varieties. Though a hierarchical sampling strategy was chosen, it remains possible that too little leaf material was collected to allow meaningful comparisons. Considering these limitations, we believe that ARG3 is the most representative Argentinean population in the scope of this study.

Is the genetic structure of Australian populations consistent with a founder effect?

It was hypothesized earlier that the low degree of gene diversity in Australian populations was due to a combination of founder events, recurring bottlenecks, geographical isolation, and effective quarantine measures (Zhan et al., 2003). However, the poor sampling strategy and the low number of isolates originating from Australia put severe limitations on the interpretation of previous findings. Our analysis of the AUS3, AUS4 and AUS5 populations lend strong support to the original hypothesis that the genetic structure of Australian *M. graminicola* populations reflects significant random genetic drift. This argument is based on three lines of evidence: (1) the relatively high number of fixed alleles; (2) the relatively low values for gene diversity and average number of alleles, and (3) the unusually high frequency of the deletion at locus pSTS192A.

Possible applications

For microsatellite loci, it has been suggested (Banke and McDonald, 2005) that rare alleles can be useful for tracing recent migration patterns. Rare RFLP alleles can be used in the same way. The deletion at locus pSTS192A was found at a high frequency in Australia and at low frequencies in populations from Switzerland, Germany and Denmark. In an earlier survey of 1673 *M. graminicola* isolates (Zhan et al., 2003) seven different mitochondrial haplotypes could be distinguished. For AUS2 the occurrence of mitochondrial type 5 was reported at a low frequency. Apart from Australia and the Middle East, this mitochondrial type was found only in the fungal population from the UK. Both of these observations give support to the hypothesis that fungal populations in Australia represent a founder event that might date back to the first European colonists.

We believe that both populations in Iran and two of the populations in Argentina originated from inoculated fields because they had unusual genetic structures. These examples illustrate well the importance of making collections from naturally infected farmers fields in order to draw meaningful conclusions regarding the genetic structure of pathogen populations that exist naturally in agroecosystems.

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