Population structure of *Ascochyta rabiei* in Australia based on STMS fingerprints

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Nineteen sequence-tagged microsatellite site (STMS) primer pairs were used to determine the genetic structure within an Australian population of *Ascochyta rabiei* collected from Victoria, South Australia, Western Australia and New South Wales. None of the alleles found in Australia showed significant differences in allele frequencies or were at a gamete disequilibrium. A very low level of gene diversity (Ht = 0.02) was found within the Australian population with the majority of the diversity (92%) distributed within subpopulations. In contrast, high diversity was detected among the international isolates (Ht = 0.45) with 22% attributed to differentiation between countries. Of the 20 loci assessed, 16 were homozygous in the Australian population, and gene flow from four heterozygous loci was high. Of the seven genotypes identified within the Australian population, one was found in all Australian subpopulations and accounted for 82.9% of the total isolates tested. The Canadian and USA populations were more similar to each other than to the Australian or Syrian populations. Results from this study will be useful in breeding for chickpea resistant cultivars and developing necessary quarantine regulations.

Key words: *Ascochyta rabiei*, gene flow, genetic diversity, population structure, STMS markers.

Introduction

Ascochyta blight, caused by *Ascochyta rabiei* (Pass.) Labrousse, teleomorph *Didymella rabiei* (Kovachevski), is considered to be the most serious disease of chickpea worldwide (Nene and Reddy, 1987) as well as in Australia (Collard *et al*., 2001). The pathogen can infect all above-ground plant parts at any stage of crop development, leading to rapidly spreading necrotic lesions and tissue collapse. During the growing season, the pathogen reproduces asexually on the host and is spread short distances within a field by rainsplash dispersal of conidia. Although the perfect stage of *D. rabiei* has been identified on over-wintering chickpea debris in several countries (Kaiser,

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1995) and more recently in Australia (Galloway and MacLeod, 2003), the nature of the primary inoculum in a chickpea field is still unclear. Previously, conidia derived from infected seeds or chickpea debris were considered as the only source of primary inoculum (Nene and Reddy, 1987). However, recent evidence suggests that the teleomorph is more widespread than previously thought and that ascospores may also serve as primary inoculum (Trapero-Casas and Kaiser, 1992; Trapero-Casas *et al*., 1996)

The disease can cause total yield loss in years of severe epidemic (Chaube and Mishra, 1992). The potential for the disease can be decreased through the use of resistant cultivars, fungicide applications and cultural practices like rotation, removal of trash and sowing clean seeds.

Since the durability of disease resistance may be affected by the adaptive potential of the pathogen population, pathogens with a high adaptive potential are more likely to overcome control methods faster than pathogens with a low adaptive potential. The adaptive potential of a pathogen population can be predicted based on the population structure (McDonald and Linde, 2002). For durable host resistance and to ensure an effective fungicide regime against *A. rabiei*, information on the amount and distribution of genetic variation within and between the pathogen's populations (population structure) in Australia is important. Also, there is a need to determine if there are differences between the Australian *A. rabiei* population and international populations, since more diverse international sources pose a threat of increased adaptive potential to the Australian population.

The genetic variation within and between plant pathogen populations has traditionally been assessed using phenotypic traits. The variability in pathogenicity of *A. rabiei* has been studied within and between populations collected in different countries (Reddy and Kabbabeh, 1985; Jamil *et al*., 1993; Porta-Puglia *et al*., 1996; Navas-Cortés *et al*., 1998). However, assessing variation among populations, based on pathogenicity alone, is difficult due to inconsistency in sets of host cultivars used to assess pathotypes and varying experimental and environmental conditions. There are also difficulties in standardising assay procedures and disease rating scales between studies, which may potentially lead to an over- or under-estimation of pathogen diversity (Udupa *et al*., 1998).

In recent years, DNA markers have been widely deployed to compliment traditional markers in the analysis of genetic diversity of *A. rabiei* (Morjane *et al*., 1994; Fischer *et al*., 1995; Udupa *et al*., 1998; Santra *et al*., 2001) and diagnosis of the pathogen (Phan *et al*., 2002). Molecular markers commonly used for assessing genetic variation of *A. rabiei* have been based on Restriction Fragment Length Polymorphism (RFLP) patterns with multilocus probes

(Morjane *et al*., 1994; Udupa *et al*., 1998); and Polymerase Chain Reaction (PCR) fingerprinting with arbitrary primers, such as Random Amplified Polymorphic DNA (RAPD) analysis that amplify random regions of the genome (Fischer *et al*., 1995; Udupa *et al*., 1998).

Another marker system, Sequence-Tagged Microsatellite Site (STMS), has several advantages over RFLP and RAPD markers. STMS markers are designed from the flanking regions of simple sequence repeats (SSR). These markers are based on arrays of randomly repeated microsatellite motifs (one to six nucleotides per motif) and may be highly polymorphic due to a variable internal repeat number. Insertions and deletions of repeat units are the result of DNA polymerase slippage that may occur during replication or unequal recombination (Levinson and Gutman, 1997; Sia *et al*., 1997). They are PCR-based, may provide single locus detection like RFLP, and can be detected without using radioisotopes. Moreover, STMS markers can be applied to elucidate the identity of unknown or contaminated DNA samples because of their species-specificity and high sensitivity (Tenzer *et al*., 1999).

STMS markers may be codominant and are therefore, widely used in fungal population genetics (Groppe *et al*., 1995; Powell *et al*., 1995; Tenzer *et al*., 1999). For *A*. *rabiei*, 20 specific STMS primer pairs were recently developed by Geistlinger *et al*. (2000). These primer pairs may be useful in studying genetic variability of *A*. *rabiei* populations.

This study reports the use of STMS markers to assess population genetic structure of *A*. *rabiei* in Australia; to compare that to a collection of *A*. *rabiei* isolates from the USA, Canada and Syria; and to identify the level of gene flow of *A*. *rabiei* between different chickpea growing regions in Australia. For the scope of this paper, "population genetic structure" refers to the amount of genetic variation among individuals in a population, the way in which this variation partitioned and behaved.

Materials and methods

Fungal isolates

The isolates of *A*. *rabiei* used in this study were collected from infected chickpea plants in eight to ten fields within each of the four major chickpea growing regions of Australia (Table 1) and supplied by the Victorian Institute for Dryland Agriculture, Department of Primary Industries; The South Australian Research and Development Institute (SARDI); the Centre for Cropping Systems, Western Australia; and the New South Wales Agriculture Tamworth Centre for Crop Improvement. All isolates were received as mycelium cultures on either sterile water or Potato Dextrose Agar (PDA)

Table 1. List of *Ascochyta rabiei* isolates used in this study.

Isolate number	Isolate name	Passport data (country, state, year, collection site)
1	AR94	Australia, Victoria, 1998, Rupanyup
2	AR103	Australia, Victoria, 1998, Kalkee
3	AR108	Australia, Victoria, 1998, Donald, Tyson
4	AR116	Australia, Victoria, 1998, Donald, Amethyst
5	AR123	Australia, Victoria, 1998, Jeffcott
6	AR133	Australia, Victoria, 1998, Netherby
7	AR124	Australia, Victoria, 1998, Dimboola
8	AR126	Australia, Victoria, 1998, Glenlee
9	AR129	Australia, Victoria, 1998, Rainbow
10	AR135	Australia, Victoria, 1998, Sheep Hills
11	AR61	Australia, South Australia, 1996, Blyth
12	AR140	Australia, South Australia, 1998, Salter Springs
13	AR141	Australia, South Australia, 1998, Bethel (1)[a]
14	AR142	Australia, South Australia, 1998, Tanaunda
15	AR144	Australia, South Australia, 1998, Roseworthy (1)
16	AR152	Australia, South Australia, 1998, Bethel (2)
17	AR153	Australia, South Australia, 1998, Bethel (3)
18	AR178	Australia, South Australia, 1998, Roseworthy (2)
19	AR78	Australia, Western Australia, 1998,
20	AR286	Australia, Western Australia, 1999, Bruce Rock
21	AR293	Australia, Western Australia, 2000, Dowerin
22	AR294	Australia, Western Australia, 2000, Mingenew (1)
23	AR296	Australia, Western Australia, 2000, Geraldton (1)
24	AR298	Australia, Western Australia, 2000, Geraldton (2)
25	AR300	Australia, Western Australia, 2000, Mingenew (2)
26	AR307	Australia, Western Australia, 2000, Morawa-Ming
27	AR311	Australia, Western Australia, 2000, Quindanning (1)
28	AR314	Australia, Western Australia, 2000, Quindanning (2)
29	AR69	Australia, New South Wales, 1998, Wagga(1)
30	AR316	Australia, New South Wales, 1999, Wagga (2)
31	AR317	Australia, New South Wales, 1999, Wagga (3)
32	AR319	Australia, New South Wales, 1998, Croppa Creek
33	AR320	Australia, New South Wales, 1998, Boggabri
34	AR321	Australia, New South Wales, 1998, Edgeroi
35	AR322	Australia, New South Wales, 1998, Walgett
36	AR68	Australia, New South Wales, 1998, Goondiwindi
37	AR48	USA, collection from Pullman, WA
38	AR49	USA, collection from Pullman, WA
39	Oct_9814	USA, collection from Pullman, WA
40	Oct_9815	USA, collection from Pullman, WA
41	Oct_9811	USA, collection from Pullman, WA
42	Oct_9816	USA, collection from Pullman, WA
43	Apr_9601	Syria, collection from Saskatchewan (Race 1)

Table 1. (continued).

Isolate number	Isolate name	Passport data (country, state, year, collection site)
44	Apr_9603	Syria, collection from Saskatchewan (Race 3)
45	Apr_9604	Syria, collection from Saskatchewan (Race 4)
46	Apr_9605	Syria, collection from Saskatchewan (Race 5)
47	Apr_9606	Syria, collection from Saskatchewan (Race 6)
48	Jul_9803	Canada, collection from Saskatchewan
49	Jul_9807	Canada, collection from Saskatchewan
50	Jul_9817	Canada, collection from Saskatchewan
51	Jul_9818	Canada, collection from Saskatchewan
52	Sep_9806	Canada, collection from Saskatchewan

[a] = several fields from the same region were sampled

plates. A further two isolates from New South Wales (AR316, AR317) were collected by the authors.

Two USA isolates (AR48 and AR49) were supplied from Washington University, USA. The rest of the USA isolates and all Canadian and Syrian isolates were supplied by Agriculture and Agri-food Canada, Saskatoon, Saskatchewan, Canada. The five isolates from Syria represented five races of the pathogen according to Reddy and Kabbabeh (1985).

Single spore cultures of all 51 isolates were maintained on either Coon's agar (Johnston and Booth, 1983) or Potato Dextrose Agar (PDA), grown at 20ºC with 12 hours of fluorescent and UV lights and 12 hours of darkness.

DNA extraction

Mycelium from each isolate was grown in 50 ml volumes of Czapek-Dox liquid medium (Johnston and Booth, 1983) in 250 ml flasks incubated on a rotary shaker (115 rpm) at 20 ± 2ºC for three days in darkness. Mycelial mats were obtained after centrifuging the isolate cultures at 5000 rpm for 10 minutes. Total genomic DNA was extracted from mycelium using the DNAeasy Plant Mini Kit (Qiagen Inc., USA).

STMS primers and PCR amplification

Twenty STMS primer pairs, specific to *A. rabiei* (Geistlinger *et al*. 2000), were first screened on DNA of five isolates (AR94, AR 61, AR78, AR48 and AR49, data not shown). PCR with the *A. rabiei*-STMS primer pairs was performed as described by Geistlinger *et al*. (2000), with some modifications (annealing temperatures of PCR of different primer pairs ranged from 57ºC to 60ºC) to identify which primers produced clear and reproducible

markers. The PCR was carried out using a PTC-200 thermocycler (MJ Research, Inc., USA) in 15 µL reaction volumes. The annealing temperature for each primer combination was optimized. PCR products were electrophoresed on 5% denaturing polyacrylamide gels in Tris-borate EDTA (TBE) buffer (Sambrook *et al*., 1989). The resolved PCR products were then detected by silver staining (Promega Corporation, USA). Only markers that were reproducible and clearly resolvable were scored and used for analysis.

Molecular data analysis

Each STMS primer pair was presumed to amplify a different microsatellite locus or loci. DNA fragments with different sizes were treated as alleles at each microsatellite locus. Isolates from any given location (VIC, SA, NSW, WA, USA, Syria and Can) was considered to be a separate subpopulation. Four subpopulations collected in Australia and three from overseas were considered to be two populations.

Allele frequencies and heterozygosity for each locus, number of genotypes in each subpopulation and significant differences in allele frequencies between pairs of subpopulation (Raymond and Rousset, 1995) were calculated using the Tools For Population Genetic Analysis (TFPGA) program (version 1.3; Miller, 1997). Gene diversity in the total world wide population (Ht), gene diversities within subpopulations (Hc), between subpopulations in Australia or overseas (Hs) as well as the proportion of gene diversity attributable to differentiation among populations (Gst) and the proportion of gene diversity attributable to differentiation among subpopulations (Gcs) were calculated using POPGENE program version 1.31 (http://www.ualberta.ca/~fyeh/fyeh). Unbiased measures of genetic identities and genetic distances between any two subpopulations were obtained following the distance algorithm of Nei's (1978) using the TFPGA program (version 1.3; Miller, 1997). Gametic disequilibria between pairs of loci and χ^2 tests for significance for each population (Weir, 1979) were also estimated using the POPGENE program.

The measures of differences in allele frequencies and gamete disequilibrium were not carried out in the overseas population since the numbers of isolates from each country were too small to draw any valid conclusions.

Gene flow was estimated from Gst or Gcs estimates as Nm = 0.5(1 - Gst)/Gst or Nm(Gcs) = 0.5(1 - Gcs)/Gcs, where Nm is the average number of migrants among populations or subpopulations (Slatkin and Barton, 1989). Two was used in the denominator instead of four because *A*. *rabiei* is haploid.

Results

Of the 20 *A. rabiei*-specific STMS primer pairs developed by Geistlinger *et al*. (2000), 18 amplified a single locus, one amplified two loci and one amplified a complicated profile that may have included multiple loci. The data from the multiple band profile was excluded from analysis due to difficulty in allele assignment. The remaining 19 STMS primer pairs amplified 20 loci with 76 alleles. Three of the unilocus 18 primer pairs were monomorphic across all 52 isolates (Table 2). The rest generated unilocus polymorphic markers clearly detectable on PAGE as shown in Figure 1.

Allele frequency and heterozygocity data for all loci are showed in Table 2. Of the total 76 alleles, fifty-one (68%) were found only overseas. Of the 25 alleles identified in the Australian population, 16 were monomorphic across all isolates (Table 2). All alleles found in Australia showed no significant differences in allele frequencies between the Australian subpopulations ($P > 0.05$, data not showed).

The total gene diversity (Ht) worldwide was estimated to be 0.29 with 48% attributable to variation within subpopulations, 22% was due to differentiation among subpopulations within regions (inside or outside Australia), and 30% was due to differentiation within subpopulations (Table 3).

When each region was analyzed separately, the mean total and within subpopulation gene diversities, Ht and Hs, respectively, were much greater in the overseas population (0.45 and 0.35) than in the Australian population (0.0198 and 0.0182, Table 4). An extremely low level of gene diversity (Ht = 0.02) was detected among all Australian isolates using Nei's (1978) measure of genetic distance, of which the majority (92%) was distributed within subpopulations, only 8% of the gene diversity was distributed among Australian subpopulations (Table 4). In Australia, WA was the most divergent subpopulation (H = 0.03; Table 6). Locus-by-locus comparisons were made between pairs of Australian subpopulations and a significant departure ($P < 0.05$) from equilibrium was not found in any comparison (data not showed).

In contrast to the Australian population, a high level of diversity was detected among the overseas population (Ht = 0.45, Table 4). The maximum gene diversity was detected in Canada (H = 0.38), followed by USA (H = 0.36) and Syria (H = 0.32, Table 5). Some STMS loci showed very high levels of diversity among the international isolates. The heterozygosity indices of loci amplified from primer pairs ArR08T, ArR10T, ArH02T and ArA02D were more than 0.80 (Table 2). The majority (78%) of the differentiation among these overseas isolates was found within each country and 22% was distributed

Table 2. Allele frequencies and heterozygosity at 20 loci generated from 19 STMS primer pairs in Australian and overseas populations of *Ascochyta rabiei*.

Primer/ Locus	Repeat of cloned allele[1]	Allele	Australian population		Overseas population	
			Allele frequency[2]	Het[3]	Allele frequency[2]	Het[3]
*Ar*A11T	$(GAA)_8$	1	1.0	0.0	0.88	0.23
		2	0.0		0.13	
*Ar*R08T	$(GAA)_{13}$	1	1.0	0.0	1.0	0.0
*Ar*R10T	$(CAGCAA)_2(CAA)_8$	1	1.0	0.0	0.19	0.67
		2	0.0		0.50	
		3	0.0		0.25	
		4	0.0		0.06	
*Ar*H02T	$(GAA)_{58}(GTA)_6$	1	0.94	0.11	0.25	0.86
		2	0.03		0.13	
		3	0.03		0.0	
		4	0.0		0.19	
		5	0.0		0.13	
		6	0.0		0.06	
		7	0.0		0.13	
		8	0.0		0.13	
*Ar*H06T	$(CAA)_9(CAG)_7(CAA)_{21}$	1	1.0	0.0	0.13	0.90
		2	0.0		0.13	
		3	0.0		0.13	
		4	0.0		0.06	
		5	0.0		0.19	
		6	0.0		0.06	
		7	0.0		0.13	
		8	0.0		0.13	
		9	0.0		0.06	
*Ar*H04T	$(CTT)_{10}$	1	1.0	0.0	0.81	0.31
		2	0.0		0.19	

Table 2. (continued).

Primer/ Locus	Repeat of cloned allele[1]	Allele	Australian population		Overseas population	
			Allele frequency[2]	Het[3]	Allele frequency[2]	Het[3]
*Ar*S03T	$(GTT)_5(GCT)_3(GTT)_5$	1	1.0	0.0	0.69	0.44
		2	0.0		0.31	
*Ar*R12D	$(CA)_{15}$	1	0.94	0.11	0.63	0.59
		2	0.03		0.06	
		3	0.03		0.0	
		4	0.0		0.13	
		5	0.0		0.13	
		6	0.0		0.06	
*Ar*R04D	$(GTGTGTAT)_2(N)_8(GT)_{10}$	1	1.0	0.0	0.63	0.48
		2	0.0		0.38	
*Ar*R01D	$(GTGTGTGG)_6$	1	1.0	0.0	0.56	0.56
		2	0.0		0.06	
		3	0.0		0.38	
*Ar*A02D	$(CACACAA)_5$	1	1.0	0.0	1.0	0.0
*Ar*H02D	$(CA)_4N(CA)_5(N)_2(CA)_4$	1	1.0	0.0	1.0	0.0
*Ar*H07D	$(GT)_{23}$	1	1.0	0.0	0.19	0.85
		2	0.0		0.25	
		3	0.0		0.06	
		4	0.0		0.13	
		5	0.0		0.13	
		6	0.0		0.19	
		7	0.0		0.06	
*Ar*H08D	$(CA)_3(CT)_8(CA)_8(CT)_{10}$	1	1.0	0.0	0.75	0.42
		2	0.0		0.13	
		3	0.0		0.13	
*Ar*H11D	$(GACA)_6$	1	1.0	0.0	0.81	0.31
		2	0.0		0.19	

Table 2. (continued).

Primer/ Locus	Repeat of cloned allele[1]	Allele	Australian population		Overseas population	
			Allele frequency[2]	Het[3]	Allele frequency[2]	Het[3]
*Ar*A02T	$GAA(N)_9(GAA)_8$	1	1.0	0.0	0.31	0.69
		2	0.0		0.38	
		3	0.0		0.31	
*Ar*A06T	$(CAACAC)_7(N)_9(CAC)_3$	1	1.0	0.0	0.88	0.23
		2	0.0		0.06	
		3	0.0		0.06	
*Ar*A03T-1	$(GAA)_{31}$	1	1.0	0.0	0.06	0.70
		2	0.0		0.31	
		3	0.0		0.44	
		4	0.0		0.13	
		5	0.0		0.06	
*Ar*A03T-2	$(GAA)_{31}$	1	1.0	0.0	0.94	0.12
		2	0.0		0.06	
*Ar*H05T	$(CTT)_{18}$	1	0.94	0.11	0.13	0.91
		2	0.03		0.06	
		3	0.03		0.0	
		4	0.0		0.19	
		5	0.0		0.06	
		6	0.0		0.13	
		7	0.0		0.13	
		8	0.0		0.13	
		9	0.0		0.06	
		10	0.0		0.06	
		11	0.0		0.06	
Average		3.8	0.016	0.02	0.46	0.46

[1]Sequences obtained from Geistlinger *et al*. (2000).
[2]allele frequency was calculated using TFPGA program (version 1.3; Miller, 1997).
[3]Nei's unbiased heterozygosity was calculated using TFPGA program (version 1.3; Miller, 1997).

among countries (Table 4). The average of Gst (0.22) indicated a substantial amount of genetic differentiation existed among the overseas subpopulations .

Based on STMS data, a total of seven distinct genotypes were identified in the Australian population. One genotype, found in all Australian subpopulations, accounted for 83% of the Australian isolates tested. The other six genotypes identified in the Australian populations comprised only one isolate each. Among the six genotypes, one each was found in Victoria and Western Australia, two each were found in the South Australian and New South Wales subpopulations.

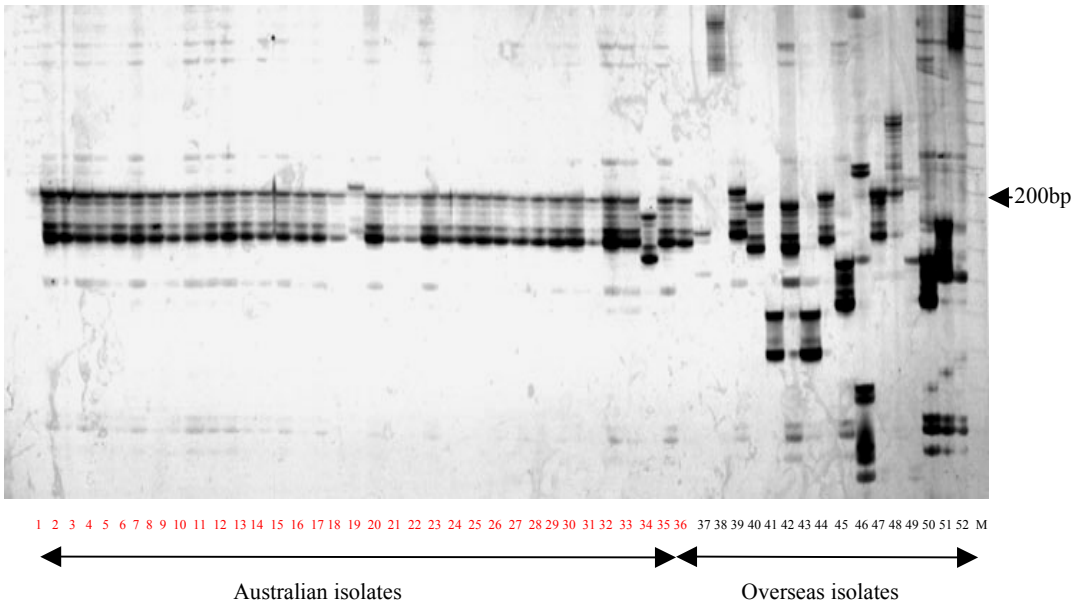

Fig. 1: Profile of alleles amplified with STMS primer pair *Ar*H05T from the 36 Australian isolates and 16 overseas isolates. Isolate numbers are the same as in Table 1. M: 30–330 bp DNA ladder (Invitrogen, Australia).

Gene flow (Nm) varied considerably within populations (Table 4), from 1.74 among overseas subpopulations to 5.73 among Australian subpopulations (Table 4). This level of gene flow is sufficient to prevent differentiation among all the subpopulations tested (Nm ¡Ý 1 means no differentiation; Slatkin and Barton, 1989). The average amount of gene flow world wide was estimated to

Table 3: Nei's analysis (1973) of gene diversities, population differentiation and gene flow of the total population.

Locus	Sample size	Ht[1]	Hc[2]	Hs[3]	Gst[4]	Gcs[4]	Nm(Gst)[5]	Nm(Gcs)[5]
*Ar*A06T	52	0.11	0.07	0.10	0.08	0.31	5.69	1.13
*Ar*A08T	52	0.00	0.00	0.00	-	-	-	-
*Ar*A11T	52	0.53	0.17	0.27	0.48	0.39	0.55	0.78
*Ar*R08T	52	0.56	0.35	0.42	0.25	0.15	1.51	2.74
*Ar*H02T	52	0.59	0.31	0.38	0.36	0.18	0.89	2.32
*Ar*H06T	52	0.16	0.11	0.14	0.13	0.17	3.50	2.50
*Ar*H04T	52	0.23	0.18	0.18	0.21	0.03	1.94	15.00
*Ar*R01D	52	0.32	0.21	0.29	0.09	0.30	5.25	1.19
*Ar*S03T	52	0.28	0.13	0.20	0.27	0.36	1.38	0.90
*Ar*H05T	52	0.32	0.22	0.23	0.27	0.04	1.38	11.48
*Ar*R04D	52	0.00	0.00	0.00	-	-	-	-
*Ar*R12D	52	0.00	0.00	0.00	-	-	-	-
*Ar*A02D	52	0.55	0.25	0.36	0.35	0.29	0.93	1.23
*Ar*H02D	52	0.19	0.13	0.17	0.11	0.23	3.88	1.70
*Ar*H07D	52	0.15	0.11	0.13	0.12	0.18	3.76	2.35
*Ar*H08D	52	0.47	0.28	0.32	0.33	0.13	1.04	3.45
*Ar*H11D	52	0.11	0.08	0.10	0.06	0.22	7.88	1.75
*Ar*A02T-1	52	0.59	0.21	0.29	0.50	0.29	0.50	1.25
*Ar*A02T-2	52	0.06	0.05	0.05	0.04	0.14	12.25	3.00
*Ar*R10T	52	0.63	0.35	0.45	0.29	0.21	1.23	1.85
Mean	52	0.29	0.16	0.20	0.30	0.22	1.18	1.82

[1]Gene diversities in the total world wide population.
[2]Gene diversities within subpopulations.
[3]Gene diversities between subpopulations.
[4]Gene diversity attributable to differentiation among populations (Gst) and among subpopulations (Gcs).
[5]Nm = estimate of gene flow from Gst or Gcs.
All of these parameters were calculated using POPGENE program version 1.31.

be 1.18 (Table 3) which is close to a level that would prevent populations from diverging by genetic drift. Inside Australia, the level of gene flow was more than 3 times that detected outside Australia (5.73), this value was calculated based on 4 STMS heterozygous loci identified among Australian subpopulations only (Table 4). The other 16 loci, homozygous across all Australian isolates tested, could not be included in calculating Nm.

When Australia was treated as a "country" subpopulation, the gene distance detected between pairs of subpopulations was largest between Australian and Syrian subpopulations (34%), followed by that detected between the USA and Australian subpopulations (25.5%). The most similar subpopulations were those of the USA and Canada (15%). Among Australian

Table 4: Nei's analysis (1973) of gene diversities, population differentiation and gene flow of Australian and overseas populations.

	Australian populations					Overseas populations				
Locus	**Sample size**	**Ht[1]**	**Hs[2]**	**Gst[3]**	**Nm[4]**	**Sample size**	**Ht[1]**	**Hs[2]**	**Gst[3]**	**Nm[4]**
*Ar*A06T	36	0.00	0.00	-	-	16	0.23	0.16	0.31	1.13
*Ar*A08T	36	0.00	0.00	-	-	16	0.00	0.00	-	-
*Ar*A11T	36	0.00	0.00	-	-	16	0.64	0.39	0.39	0.78
*Ar*R08T	36	0.11	0.10	0.07	6.19	16	0.83	0.69	0.17	2.47
*Ar*H02T	36	0.00	0.00	-	-	16	0.88	0.72	0.18	2.32
*Ar*H06T	36	0.00	0.00	-	-	16	0.32	0.27	0.17	2.50
*Ar*H04T	36	0.00	0.00	-	-	16	0.43	0.41	0.03	15.00
*Ar*R01D	36	0.11	0.10	0.07	6.19	16	0.54	0.35	0.35	0.91
*Ar*S03T	36	0.00	0.00	-	-	16	0.48	0.31	0.36	0.90
*Ar*H05T	36	0.00	0.00	-	-	16	0.54	0.52	0.04	11.48
*Ar*R04D	36	0.00	0.00	-	-	16	0.00	0.00	-	-
*Ar*R12D	36	0.00	0.00	-	-	16	0.00	0.00	-	-
*Ar*A02D	36	0.00	0.00	-	-	16	0.83	0.59	0.29	1.23
*Ar*H02D	36	0.00	0.00	-	-	16	0.40	0.31	0.23	1.70
*Ar*H07D	36	0.00	0.00	-	-	16	0.31	0.25	0.18	2.35
*Ar*H08D	36	0.06	0.05	0.10	4.67	16	0.66	0.58	0.13	3.34
*Ar*H11D	36	0.00	0.00	-	-	16	0.24	0.19	0.22	1.75
*Ar*A02T-1	36	0.00	0.00	-	-	16	0.68	0.49	0.29	1.25
*Ar*A02T-2	36	0.00	0.00	-	-	16	0.12	0.11	0.14	3.00
*Ar*R10T	36	0.12	0.11	0.08	5.6	16	0.88	0.68	0.24	1.62
Mean	36	0.0198	0.0182	0.08	5.73	16	0.45	0.35	0.22	1.74

[1]Gene diversities in the Australian or overseas populations.
[2]Gene diversities between subpopulations in Australia or overseas.
[3]Gene diversity attributable to differentiation among subpopulations (Gst).
[4]Nm = estimate of gene flow from Gst, Nm = 0.5(1 - Gst)/Gst.
All of these parameters were calculated using POPGENE program version 1.31.

subpopulations, the most divergent were WA and NSW (0.15%), and the closest were NSW and VIC or NSW and SA (0.05%, Table 6).

Discussion

Population structure of Australian A. rabiei

Each subpopulation consisted of only eight to ten isolates, collected from each of the four major chickpea growing areas in Australia. A primary screening using seven RAPD primers on 20 to 30 isolates from each chickpea growing region in Australia revealed a very high level of similarity among Australian *A. rabiei* isolates (Phan, unpublished data), concluding that a small number of isolates was sufficient to study genetic diversity.

Table 5. Nei's (1973) gene diversities of each locus across seven subpopulations. (VIC = Victoria, SA = South Australia, NSW = New South Wales, WA = Western Australia, USA = United States of America, Syr = Syria, Can = Canada).

Locus	VIC	SA	NSW	WA	USA	Syria	Can
*Ar*A06T	0.00	0.00	0.00	0.00	0.00	0.48	0.00
*Ar*A08T	0.00	0.00	0.00	0.00	0.00	0.00	0.00
*Ar*A11T	0.00	0.00	0.00	0.00	0.61	0.00	0.56
*Ar*R08T	0.00	0.22	0.18	0.00	0.72	0.64	0.72
*Ar*H02T	0.00	0.00	0.00	0.00	0.72	0.72	0.72
*Ar*H06T	0.00	0.00	0.00	0.00	0.00	0.48	0.32
*Ar*H04T	0.00	0.00	0.00	0.00	0.44	0.48	0.32
*Ar*R01D	0.18	0.00	0.00	0.22	0.72	0.32	0.00
*Ar*S03T	0.00	0.00	0.00	0.00	0.28	0.32	0.32
*Ar*H05T	0.00	0.00	0.00	0.00	0.44	0.64	0.48
*Ar*R04D	0.00	0.00	0.00	0.00	0.00	0.00	0.00
*Ar*R12D	0.00	0.00	0.00	0.00	0.00	0.00	0.00
*Ar*A02D	0.00	0.00	0.00	0.00	0.50	0.64	0.64
*Ar*H02D	0.00	0.00	0.00	0.00	0.44	0.00	0.48
*Ar*H07D	0.00	0.00	0.00	0.00	0.28	0.00	0.48
*Ar*H08D	0.00	0.00	0.00	0.22	0.61	0.48	0.64
*Ar*H11D	0.00	0.00	0.00	0.00	0.00	0.00	0.56
*Ar*A02T-1	0.00	0.00	0.00	0.00	0.67	0.48	0.32
*Ar*A02T-2	0.00	0.00	0.00	0.00	0.00	0.00	0.32
*Ar*R10T	0.00	0.22	0.00	0.22	0.67	0.64	0.72
Mean	0.009	0.02	0.009	0.03	0.36	0.32	0.38

A low level of gene diversity was detected among all *A. rabiei* Australian subpopulations even though they were separated by large geographical distances (> 650 km). On average, 92% of the gene diversity was distributed on a local level (subpopulation). The low Gst value (0.08) indicated little evidence for geographical subdivision inside Australia. This was further suggested by the fact that allele frequencies were the same in all subpopulations tested and all alleles were at gamete equilibrium.

On the other hand, a high level of gene diversity of the pathogen was known to exist outside Australia. This may suggest that the Australian population originated from a recent introduction, involving a relatively small number of individuals representing a limited sample of genotypes from the source population, a process known as the "founder effect" (Hartl and Clark, 1997).

The geographic distances among the subpopulations ranged from 650 km to 2720 km with only minor differences in the distribution of gene diversity among regional populations within Australia. Conidia dispersed by rainsplash are not expected to travel long distance (Brennan *et al*., 1985), so it is unlikely

Table 6. Nei's unbiased (1978) measures of genetic identities and genetic distances between pairs of subpopulations.

Subpopu.[1]	VIC[2]	SA	NSW	WA	USA	Syr	Can
VIC	-	0.9989[3]	0.9995	0.9992	0.7695	0.7072	0.7865
SA	0.0011	-	0.9995	0.9987	0.7746	0.7160	0.7954
NSW	0.0005	0.0005	-	0.9985	0.7695	0.7085	0.7918
WA	0.0008	0.0013	0.0015	-	0.7807	0.7156	0.7901
USA	0.2620	0.2553	0.2620	0.2476	-	0.7986	0.8604
Syr	0.3464	0.3341	0.3446	0.3347	0.2248	-	0.7839
Can	0.2402	0.2289	0.2335	0.2355	0.1504	0.2434	-

[1]Subpopu. = Subpopulation, including: VIC = Victoria, SA = South Australia, NSW = New South Wales, WA = Western Australia, USA = United States of America, Syr = Syria, Can = Canada).

[2]Measures of genetic identities are shown above the diagonal and genetic distances are shown below the diagonal.

[3]Maximum value of 1.0 occurs when the same alleles occur at the same frequencies for each pair of subpopulations.

that these subpopulations were linked by movement of conidia. It is more likely that gene flow among these subpopulations occurred through infected seeds, fungal ascospores, or infected plant materials which were disseminated over long distances.

Evidence for gene flow among Australian subpopulations

Gene flow is one of the evolutionary forces that causes significant impact on the genetic structure of a population. In the absence of gene flow, genetic drift will cause isolated populations to develop different allele frequencies at neutral loci, leading to population differentiation. Gene flow among the Australian subpopulations of *A. rabiei* was evident from the distribution of the genotypes in the subpopulations. One of the seven genotypes was found in all Australian subpopulations and accounted for 83% of the Australian isolates tested. As well, of the 20 STMS loci used in this study, 16 were homogenous across all isolates.

In addition, the shared alleles at neutral loci could be used to make an indirect measure of gene flow. The Gst of the four heterozygous STMS loci were used to estimate the average number of migrants (Nm) that would need to be exchanged among populations of each generation to account for the low value of Gst = 0.08. Nm was 5.73, suggesting that a level of gene flow was nearly six times greater than needed to prevent the subpopulations from diverging by genetic drift.

Comparison to a collection of overseas isolates

In contrast to the Australian population, a much higher level of diversity was detected within and between populations of overseas isolates. The high level of diversity that exists in *A. rabiei* populations overseas has been reported in other studies. Santra *et al*. (2001) found a similar index of only 0.55 among USA *A. rabiei* isolates; in the same study, Indian isolates showed a similar index of 0.49 to Pakistani isolates and from 0.33 to 0.52 to Syrian isolates. Another study conducted by Morjane *et al*. (1994) detected considerable genetic variation (0.22) on a microgeographic scale, distinguishing a total of 12 different *A. rabiei* fingerprint genotypes out of 50 isolates sampled from one single chickpea field in Tunisia. A high level of diversity was also detected among 53 *A. rabiei* isolates in Syria (H = 0.70) by Udupa *et al*. (1998). The similarity detected between isolates from within the Canadian and USA populations was probably due to geographical proximity as well as the frequent commercial exchange of chickpea seed between these regions.

The low genetic diversity of *A. rabiei* detected in Australia compared to the diversity detected in the overseas population revealed a potential risk to Australia's chickpea industry. Introduced genetic diversity from the overseas populations could increase the genetic diversity of the pathogen in Australia and thus poses a potential threat to the successful use of resistance genes in local disease management programs. An increase in variation may lead to increased adaptation potential particularly in the presence of multiple mating systems. New epidemics could be caused by a few introduced isolates that are already adapted to overcome resistance. This was previously demonstrated to be the case with the introduction of the wheat rust pathogen to Australia (Burdon *et al*., 1982).

STMS markers are well-suited for studying fungal genetic diversity and population structure; and findings were consistent with results obtained using RAPD markers to analyze the same set of *A. rabiei* isolates (Phan, unpublished data). SSR markers have also been used with restriction enzymes to correlate DNA fingerprintings with aggressiveness for six different pathotypes (Weising *et al*., 1991).

The low level of gene diversity with the same pattern of allele frequencies over large geographical scale (four chickpea growing states in Australia) with a few genotypes suggest that the traditional method of introducing major resistant genes into good agronomic cultivars may provide long term control of ascochyta blight disease. However, strict quarantine regulations will need to be enforced to prevent the introduction of more diverse isolates into Australia. As well, knowledge on the movement ability (gene

flow) of the pathogen is valuable to help Australian chickpea breeders to develop durable resistant cultivars in Australia. Potential resistant cultivars can be screened on the most popular and diverse population of *A. rabiei* isolates already present in the country.

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