1 **Title:** Genetic homogeneity of a recently introduced pathogen of chickpea, *Ascochyta rabiei*,
2 to Australia

3

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12 **Abstract**

13 The study examined the genetic structure and potential for adaption to host genotype of
14 *Ascochyta rabiei*, a major necrotrophic fungal pathogen of chickpea. For this, *A. rabiei*
15 populations derived from six major chickpea growing regions in Australia were characterized
16 using 20 polymorphic microsatellite markers. The overall gene ($H = 0.094$) and genotypic
17 ($D = 0.80$) diversities among the entire population were low, indicating the establishment of a
18 recent founder population. Since, no significant genetic differentiation was detected among
19 growing regions, subsequent anthropogenic dispersal was proposed, mainly through seed
20 movement. The highest genotypic diversity and allelic richness was detected at Kingsford,
21 South Australia, thought to be the site of industry establishment in the 1970s and hence the
22 centre of introduction. Despite assessing 206 isolates collected in 2010 from host genotypes
23 with differential disease responses, no significant co-occurrence of fungal haplotype with
24 host genotype was detected. Rather a single haplotype that accounted for 70% of the total
25 isolates assessed was detected on all host genotypes assessed and from all regions. Therefore,
26 we propose that up until 2010, host reaction was not a major influence on the Australian *A.*
27 *rabiei* population structure. Additionally, the detection of a single mating type only, MAT1-2
28 indicated asexual reproduction, further influencing low haplotype diversity and resulting in a
29 population comprising of multiple clones with relatively few haplotypes compared to
30 populations in other continents.

31

32 **Keywords** Pathogen invasion, *Ascochyta rabiei*, Microsatellite, Founder populations,
33 genetic diversity

34 Introduction

35 Global trading of agricultural products facilitates opportunities for exotic pathogens to be
36 introduced to and invade new geographic areas. An estimated 65 to 85% of exotic plant
37 pathogens are foreign in their current residing locations (Pimentel et al. 2001). An introduced
38 pathogen may result in major ecological and economical consequences (Rossman 2009). For
39 Australia, this has included *Rhynchosporium commune* on barley (Linde et al. 2009),
40 *Phytophthora cinnamomi* on woody plants (Podger et al. 1965) and *Ascochyta rabiei* (Pass.)
41 Labr. (syn *Phoma rabiei* and teleomorph *Didymella rabiei* (Kovachevski)) (Kaiser and
42 Muehlbauer 1988; Navas-Cortés et al. 1998) on chickpea. *A. rabiei* limits both yield and seed
43 quality. First observed on chickpea in South Australia in 1973, it was thought to have arrived
44 via commercially distributed seed (Galloway and MacLeod 2003). Subsequent eradication
45 attempts failed with significant losses reported across southern Australia in 1998, resulting in
46 loss of farmer confidence and near collapse of the industry (Galloway and MacLeod 2003).

47

48 *A. rabiei* is a bipolar heterothallic fungus with one mating type locus and two compatible
49 mating forms (MAT1-1 and MAT1-2) (Trapero-Casas and Kaiser 1992; Wilson and Kaiser
50 1995). The recognition and recombination of two compatible mating type alleles enables
51 sexual reproduction. However, when only one mating type allele is present, the fungus
52 reproduces asexually. The sexual form, *Didymella rabiei* produces pseudothecia early in the
53 growing season and when environmental conditions are conducive; these mature and forcibly
54 discharge ascospores, which are dispersed by wind and can travel several kilometres to infect
55 neighbouring crops (Kaiser et al. 1992). During asexual reproduction, pycnidiospores
56 (conidia) spread mainly through rain-splash over short distances (Shtienberg et al. 2000).
57 Until 2000, only one mating form was detected in Australia (Phan et al. 2002).

58

59 Migration of invasive organisms leads to potential selective emergence of adapted isolates in
60 novel geographic regions and on specific host genotypes. Despite the fact that founder
61 populations are generally characterised by low genetic diversity and allelic richness
62 (Templeton et al. 1995), examples of major epidemics subsequent to founder population
63 establishment include the introduction of *Mycosphaerella fijiensis* to banana in Latin
64 America, the Caribbean and Africa from South East Asia (Rivas et al. 2004) and the
65 introduction of *Cryphonectria parasitica* to chestnut from Asia to North America (Milgroom
66 et al. 2008). Evidence suggests that founder events are often followed by genetic expansion
67 within the population through recombination and mutation, producing novel genotypes and
68 quickly increasing the effective population size (Linde et al. 2009). Accordingly, the
69 evolutionary potential of the pathogen may be increased and subsequently adapt to overcome
70 host resistances.

71

72 There are two different adaptive patterns that can occur in invasive pathogens; general
73 adaptation to the most common and prevalent host genotype, and maladaptation to different
74 host genotypes, leading to fragmented pathogen populations as a result of genetic drift
75 (Gandon et al. 1996 and 1998). Several factors such as the magnitude of gene flow,
76 population size (Gandon and Michalakis 2000), and the type of host resistance (quantitative
77 vs qualitative) (Gandon 2002) will determine the population structure of the pathogen.

78

79 Currently, the best available host resistance to *A. rabiei* is partial and governed by multiple
80 quantitative resistance loci (Huettel et al. 2002; Iruela et al. 2006; Tar'an et al. 2007).
81 Quantitative host resistance is thought to be more durable than major gene resistance
82 (Johnson 1984). Although pathogen aggressiveness may evolve slowly (Gould et al. 1991),
83 its gradual evolution and adaptation may lead to an 'erosion' of resistance (McDonald and
84 Linde 2002), especially if a monoculture farming system is applied (Gandon 2002).
85 Resistance erosion is caused by selection, increasing frequencies of isolates with high fitness
86 and virulence (Gandon and Michalakis 2000; Andrivon et al. 2007).

87

88 | Directional selection on plant pathogens generally produces a clonal population,
89 characterised by large numbers of relatively few clonal lineages and an overall low genetic
90 variability (McDonald and Linde 2002). This was previously observed for powdery mildew
91 on barley (Brown and Wolfe 1990) and rice blast, *Magnaporthe grisea* (Babujee and
92 Gnanamanickam 2000). The potential risk from rapid selection for highly virulent clonal
93 lineages within the asexually reproducing Australian *A. rabiei* population is currently
94 unknown. Therefore, to determine the risk of a pathogen, knowledge of the current
95 population diversity and structure is required (McDonald and Linde 2002). This will inform
96 management strategies to hopefully reduce the risk for an epidemic through integrated
97 practices (McDonald et al. 1996; Poczai et al. 2012).

98

99 Despite cultivation problems with eroding host resistance, Australian farmers are still
100 encouraged to grow chickpea as it is considered a high return cash crop (FAOSTAT 2013;
101 ABARES 2012). As one of the leading global chickpea producers, Australia has directed
102 90% of its export market to various legume producing countries such as India, Pakistan,
103 Bangladesh and Sri Lanka (Pulse Australia 2012; AgVantage commodities 2012; Pulse
104 Australia 2013). In addition, chickpea fixes atmospheric nitrogen and is viewed as an
105 important component for rotational cropping with wheat and oilseed, for increased
106 sustainability, and reduced environmental and economic impacts of fertilizer application.

107

108 This study aimed to determine the potential risk to disease management from clonal lineages
109 within the Australian *A. rabiei* population through: (i) an extensive study of population
110 structure of isolates from within and across the major chickpea growing regions in Australia

up until 2010, (ii) assessing the mating type distribution and linkage disequilibrium, and (iii) assessing whether host resistance imposes positive (directional) or diversifying selection on the Australian *A. rabiei* population.

Materials and methods

Sample collections

To determine the overall population diversity of *A. rabiei* in Australia, isolates were collected from commercial chickpea crops and National Variety Trials (NVT) sites during 1999 to 2010. A total of 241 isolates from five geographic regions: Victoria (VIC) (37), New South Wales (NSW) (72), Western Australia (WA) (34), Queensland (QLD) (15) and South Australia (SA) (83) were collected. SA comprised two populations, Melton (42) and Kingsford (41) (Online Resource 1).

To investigate local adaptation and selection, 206 isolates were collected in 2010 at each major chickpea growing region in Australia (SA, VIC and NSW) from specific host genotypes with varying degrees of susceptibility to *A. rabiei* (Online Resource 1). At each site, isolates were collected from either a resistant (R) to moderately resistant (MR) host genotype, and from either a susceptible (S) to highly susceptible (HS) host genotype. The selected genotypes were: Genesis090 (R) and Howzat (S) for SA, Genesis090 (R) and Kaniva (S) for VIC, and HatTrick (R) and Flipper (MR) for NSW (DPI 2013; PBA 2009).

For both the overall population diversity and host-selection studies, samples derived from sites that were within a 50 km radius of each other were considered as one population and isolates sampled from sites that were at least 200 km apart were considered as separate according to the dispersion distance of conidiospores (Kaiser et al. 1992). Each commercial chickpea crop was approximated to be a 1 km² quadrat. From within each field, a total of five to ten random samples were taken from each of the five intensive sampling sites (one in each corner and one in the centre). At NVT sites, each row and range number corresponded to a different variety. At these sites, the sampling scheme to determine the overall population diversity comprised of a collection of isolates sought randomly from separate infected plants showing symptoms of ascochyta blight, regardless of the cultivar. For the host-selection study, three to five random samples from each row and range of the chosen varieties were collected from the same field. Each infected leaf sample was collected from a different plant across one row.

Isolation, culturing and DNA extraction

147 Isolates were cultured dependant on the visibility of pycnidia. When pycnidia were present, a
148 single pycnidium per lesion was picked with a sterile needle from an infected chickpea leaf,
149 stem or pod and inoculated into 2 mL of sterile distilled water before streaking onto V8 juice
150 growth agar. Samples with no visible pycnidia were surface sterilised and placed on V8 juice
151 growth agar. All plate cultures were incubated for 14 days at $22^{\circ}\text{C} \pm 2^{\circ}\text{C}$ with a 12/12 hour
152 light/dark photoperiod and single spored. *A. rabiei* was isolated from only one lesion per
153 infected plant to minimise the likelihood of sampling clones due to the short distance of
154 conidia dispersal through rain splash.

155

156 Single spored isolates were inoculated into 10 mL Czapek Dox broth (Difco, Australia) and
157 incubated for two weeks at $22^{\circ}\text{C} \pm 2^{\circ}\text{C}$ in the dark. Mycelia were then harvested and DNA
158 was extracted with a DNeasy Plant Mini Kit (Qiagen, USA).

159

160 Mating type determination

161 The mating type of 604 isolates (Online Resource 1) from 1999 to 2010 was determined with
162 a *MAT*- specific multiplex PCR assay developed by Barve et al. (2003). A combination of
163 three primers amplified the alpha (α) domain of the MAT1-1 and the high mobility group
164 (HMG) box of the MAT1-2 with expected band sizes of 700 and 450 bp, respectively.
165 Multiplex PCR conditions were carried out as described previously in Barve et al. (2003).
166 AR21 (ATCC 76502, MAT1-1) and AR20 (ATCC 76501, MAT1-2) isolates from W.J.
167 Kaiser were used as the mating type testers (T. Peever, Washington State University, pers.
168 comm. 2009).

169

170 Microsatellites

171 A total of 20 loci previously identified for *A. rabiei* were used to characterize the Australian
172 *A. rabiei* populations (Geistlinger et al. 2000; Leo et al. 2011) (Table 1). Genotyping was
173 performed using the Multiplex-Ready PCR technique according to the protocol developed by
174 Hayden et al. (2008). PCR products were separated using a 96-capillary ABI 3730 DNA
175 Analyzer at the Australian Genome Research Facility (AGRF, Melbourne). Alleles were
176 analysed using GeneMapper v4.0 software (Applied Biosystems).

177

178 To ensure neutrality of all the SSR loci used in this study, [W.J. Kaiser mating type testers](#)
179 [from the USA and Syria \(AR20, AR21, AR628, AR655, and WSFS 03-01\) and a population](#)
180 [of 84 ascospore progeny isolates resulting from a cross of AR20 and AR21](#) (T. Peever,
181 Washington State University, pers. comm. 2009) were included.

182

183 Data Analysis

184 Isolates with the same allele sizes at all loci were considered as clones. Clonal fraction was
185 calculated to determine the occurrence and frequencies of clones within a population. The
186 Simpson's diversity index (D) (Simpson 1949) and its corresponding evenness test (E_D) were
187 used to calculate the genotypic diversity and evenness of each population. These calculations
188 were conducted using GenoDive (Meirmans and Van Tienderen 2004).

189

190 For all allele-based analyses, unless otherwise stated, clonal genotypes were removed from
191 populations to minimise bias due to over-representation of alleles in clones. An Ewens-
192 Watterson neutrality test (Manly 1985) was performed on all 21 loci. Loci that were not
193 selectively neutral were excluded from the analyses.

194

195 To estimate the genetic diversity of the *A. rabiei* population in Australia, the number of
196 alleles, number of private alleles and Nei's gene diversity (Nei 1973) across all loci were
197 calculated in GenAlEx v6.41 (Peakall and Smouse 2006). To account for populations with
198 different sample sizes, rarefaction analyses using HP-Rare (Kalinowski 2005; Department of
199 Ecology, Montana State University, Bozeman, Montana) were performed to determine the
200 allele and private allelic richness of each population. Allelic richness of populations was
201 compared with a pair-wise probability t -test. Bootstrapping with 999 permutations was
202 conducted to test for population differences in clonal diversity using GenoDive (Meirmans
203 and Van Tienderen 2004).

204

205 Pairwise genetic distances (PhiPT) between individuals were used to construct a principal
206 coordinate analysis (PCA) in GenAlEx v6.41 (Peakall and Smouse 2006) to further define the
207 genetic clusters of individuals within the overall population. Correlations between genetic
208 distance and log (geographical distance) matrices were assessed using a Mantel test (Mantel
209 1967) in GenAlex v6.41 using 999 randomizations and PhiPT as measures of pairwise
210 genetic distance in the matrix.

211

212 To examine the relationships among isolates, minimum spanning trees were constructed with
213 Network 4.6.1.1 (Fluxus Technology Ltd., Clare, Suffolk, England) using microsatellite
214 repeat numbers of isolates representing the full data set (Australian collection = 241 isolates)
215 and isolates from specific cultivars in the selection study ($N = 206$). A median joining
216 network calculation was done with default parameters where the weight of each marker = 10,
217 $\epsilon = 0$ and Frequency >1 criterion to simplify the network with a large number of individuals
218 (Bandelt et al 1999). Higher ϵ values were also evaluated, while the haplotype groupings
219 remained the same, the hubs interconnecting different groupings were more complex making
220 the interpretation of results more complicated, hence $\epsilon = 0$ was utilised for both haplotype

networks. After calculating the median joining network, the data was further processed with MP calculation to eliminate unnecessary median vectors and links to produce the shortest (MP or Streiner) network trees (Polzin and Daneshmand 2003).

Host genotype selection

Additional to gene, genotypic diversity indexes and the median joining network as calculated above, frequencies of haplotypes within each *A. rabiei* population derived from specific host genotypes were calculated. Pair-wise and multiple comparisons to detect differences in pathogen populations sampled from different host genotypes were assessed with an analysis of molecular variance (AMOVA) using GenAlEx v6.41 (Peakall and Smouse 2006) using non-clone corrected data.

Linkage disequilibrium

Gametic disequilibrium was calculated with an Index of association (I_A) test (Maynard Smith et al. 1993) on non-clone corrected datasets. The $I_A = 1 - V_O/V_E$, where V_O and V_E were the observed and expected variance of K and K was the number of loci for which two individuals differed. Values of V_O/V_E per population were calculated by GenAlEx 6.1 (Peakall and Smouse 2006) using 999 permutations. When the ratio of $V_O/V_E = 1$ and $I_A = 0$, the population was described to undergo sexual recombination or linkage equilibrium. Probability of variance of a randomised data set (V_r) to the observed value (V_O) at 5% nominal level calculated by the same software was also used to support the linkage disequilibrium results using 999 permutations.

Results

Overall population structure of Australian *A. rabiei*

Genetic diversity

A total of 241 *A. rabiei* isolates from six different chickpea growing regions in Australia were analysed with 21 polymorphic microsatellite markers. Twenty loci were selectively neutral when screened against the six USA and Syria (Kaiser) mating type testers and the 84 progeny isolates with an Ewens-Watterson test. The probability segregation ratio of each marker locus was assessed to be approximately 1:1 in the progeny population calculated using 1000 simulated samples. However, locus ArH07D was found to be under directional selection ($P = 0.9350$) and was thus excluded from the analyses. From the 20 microsatellite loci, the number of alleles present in the total Australian population ranged from 2 to 6 per locus, with ArH05T being the most polymorphic. The overall gene diversity for each locus ranged from 0.008 to 0.299. The highest percentage of polymorphic loci was observed in both SA populations Kingsford and Melton (65%) with the least polymorphic loci in NSW (30%)

(Table 1). Gene diversity for the six populations ranged from $H = 0.028$ to 0.174 , with an overall diversity of $H = 0.094$ (Table 2). Additionally, allelic richness and private allele richness for each population was low, with an overall population value of 1.55 and 0.22 , respectively (Table 2). Allelic richness did not differ significantly among populations ($P > 0.05$), except for between populations with the lowest (NSW) and highest (SA-Kingsford) ($P = 0.04$) allelic richness.

Across all populations, a total of 95 haplotypes were identified from the 241 isolates analysed (Table 2). The highest genotypic diversity ($D = 0.98$) was in Kingsford with 32 unique haplotypes from 41 isolates. Fewer haplotypes were observed in other populations. A two sided bootstrap test of the genotypic diversity showed that SA-Kingsford was significantly different to other populations except for QLD ($P > 0.025$). The genotype evenness of SA-Kingsford was close to 1 ($E_D = 0.76$ (Table 2)), suggesting that haplotypes were distributed evenly within this population. Genotype evenness indices of all other populations were less than 0.5 , suggesting uneven frequency distribution of genotypes (Table 2).

A number of haplotypes were common among all six populations. Of the 95 haplotypes identified, 73 haplotypes were found once only, 22 were detected two to seven times in different populations. The two most common haplotypes were detected 89 and 18 times and occurred in all six populations, which accounted for 36.9% and 7.5% of the overall metapopulation, respectively. Median joining haplotype network analyses showed one large node which corresponded to the most common haplotype (89 individuals, ARH01) and another node for the second most common haplotype (18 individuals, ARH046) derived from all collection regions (Figure 1). These two common haplotypes are connected via a single haplotype (ARH074) which had a one step mutational event of a single locus (ME14-1-83) from ARH01 and a two step mutational event of a different locus (ME14-1-42) from ARH046, indicating that the two common haplotypes are closely related. The other single haplotypes found around the two common nodes indicated stepwise evolution from the common haplotypes through mutational events that have occurred at several loci. The maximum number of mutational steps in a locus (ArR12D-L1) between haplotypes is 40, whereas H haplotypes separated by long branches indicate separate introductions (Figure 1).

Comment [aeleo1]: Celeste, is this correct?

Mating types and gametic disequilibrium

All isolates ~~contained~~yielded a single amplicon of 450 bp, expected for a MAT1-2 idiomorph. Non-clone corrected data sets from six chickpea regions in Australia were tested for their linkage disequilibrium to further confirm the mating type test and the asexual status of non-identical genotypes. Using the I_A and P ($V_r > V_o$) tests, all populations except for NSW were in significant ($P < 0.05$) linkage disequilibrium (Table 2).

297 *Genetic differentiation among populations*

298 The majority of the total genetic diversity (96%) was detected within populations. Pairwise
299 PhiPT values showed low levels of differentiation among populations, ranging from 0.000 to
300 0.096. Only four of the pairwise comparisons of PhiPT were significant ($P < 0.05$; Table 3),
301 mostly involving the population from NSW. Likewise, the overall PhiPT value of the total
302 Australian population was low (PhiPT = 0.040 with $P > 0.05$).

303

304 PCA did not reveal that isolate clustering corresponded to geographic origin (Figure 2). A
305 few haplotypes, mostly from the NSW population, were outliers and may presumably have
306 contributed to the significant differentiation observed between NSW and other populations.
307 Likewise, the Mantel test also showed no genetic differentiation in relation to geographical
308 distance ($R^2 = 0.284$).

309

310 *Host genotype selection*

311 *Gene and genotypic diversity*

312 Eight of the 20 loci (ME14-1-29, ME14-1-5, ME14-1-37, ME14-1-23, ME14-1-91 ME14-1-
313 41, ME14-1-33, ME14-1-16) were monomorphic among the isolates, independent of host
314 genotype. Up to five alleles per locus were observed from the 12 polymorphic loci (Table 4).
315 The gene diversity per source host genotype was low, ranging from $H = 0.005$ (Flipper,
316 NSW) to 0.038 (HatTrick, NSW) (Table 54). Gene diversities did not differ significantly ($P >$
317 0.05) among cultivar-derived isolate populations. Across regions and source cultivars, a total
318 of 35 unique haplotypes were identified from 206 isolates ($D = 0.68$), (Table 54). The highest
319 clonality was detected in SA, with isolates from Genesis090 more clonal ($D = 0.43$, clonal
320 fraction = 0.93, $E_D = 0.25$) than isolates from Howzat ($D = 0.32$, clonal fraction = 0.88, $E_D =$
321 0.21). The least clonal reproduction was observed on the VIC cultivars, Kaniva and
322 Genesis090 (Table 54).

323

324 Within regions, sourcing from resistant or susceptible varieties contributed 0 to 3% of the
325 genetic variation observed, with the highest variation observed in SA. None of the allele
326 frequencies in any of the loci tested differed with a chi-square test (data not shown).
327 Similarly, PhiPT values of pairwise comparisons of populations originating from hosts
328 differing in their disease reaction within a region, was low and not significant (Table 54).

329

330 Differences between resistant and susceptible cultivars, or regional populations, contributed
331 only 0 to 1% to the genetic variation observed in Australia, with most of the diversity found
332 among isolates. PhiPT pairwise comparisons of populations originating from resistant hosts
333 (Genesis090 and HatTrick) derived from VIC, SA and NSW were very low and non

significant ($P > 0.05$) (Table 65). A similar scenario was found with population comparisons from susceptible hosts (Kaniva and Howzat) from VIC and SA (PhiPT = 0.007 and $P > 0.05$).

No evidence of isolate selection by host genotype was found. Howzat and Kaniva shared a common haplotype (ARH01), which accounted for 79% of isolates on susceptible populations. This isolate was also prevalent on Genesis090 and HatTrick where it accounted for 65% of the total population found on the resistant varieties. Susceptible and resistant cultivars shared 12 out of 35 haplotypes in which one of them occurred 131 times (ARH01) (Online Resource 1), accounting for 70% of the total susceptible and resistance populations in Australia. One large central node representing 148 individuals from all resistant and susceptible host genotypes was also seen in the median joining haplotype network (Figure 3).

Discussion

Overall gene and genotypic diversity of *A. rabiei* in Australia

Compared to *A. rabiei* genetic diversity detected elsewhere in the world ($H = 0.30$ to $H = 0.78$; Rhaïem et al. 2007; Nourollahi et al. 2010), Australian populations contained a low overall gene ($H = 0.094$) diversity and a high clonal fraction of up to 0.75. This was similar but slightly higher to that detected by Phan et al. (2002) ($H = 0.02$), most likely due to a larger sample size and the utilisation of more informative SSR markers compared to those developed by Geistlinger et al. (2000) (Leo et al. 2011). It is perhaps not surprising to find such low diversity in the Australian population, since the disease became noticeable only in the early 1990s. Coupled with the population's probable inability to reproduce sexually (only one mating type present) and therefore its inability to create novel genotypes through recombination, the low genetic diversity found in Australia is typical of recent founder populations (Nei 1975).

The number of founder events in Australia is unknown, but could have involved the original introduction of a small number of individuals, which have since become invasive and led to the clusters seen in the median joining haplotype network and PCA. Invasive species form a stochastic loss of genetic diversity as observed in the Australian *A. rabiei* population, with two common haplotypes dominating in all chickpea growing regions (Figure 1). Meanwhile ten unique isolates from QLD, NSW and WA that were explained mostly on principle coordinate 1 (Figure 2), could represent separate multiple introductions to Australia, perhaps from diverse donor populations (Nei et al. 1975; Barrett and Husband 1990). These outliers are also presented on the long branches on the median joining network (Figure 1), suggesting that they are not derived from the most common haplotype and are most likely from separate introductions.

The Australia chickpea industry was established with accessions imported from ICRISAT, India (Brinsmead 1994), Syria (ICARDA) and Turkey (AARI) (Siddique et al. 2004). A

genotypic comparison at the same SSR loci among Australian isolates and those from the regions where the originator accessions were sourced, will aid to determine the likely major routes of invasion. Subsequent widespread dispersal of *A. rabiei* to all the chickpea growing areas in Australia, presumably anthropogenically via seed, may have contributed to the homogenetic pathogen population structure observed.

Anthropogenic dispersal is a major factor in plant disease dispersal and acts to homogenise plant pathogen populations. A lack of isolation by distance (non-significant correlation between genetic and geographic distance), low genetic differentiation among regional populations and little contribution of geographic region to the genetic variation observed, all suggest significant anthropogenic dispersal of *A. rabiei* across Australia. Furthermore, an even distribution and the presence of dominant haplotypes across the country signified introductions of the same haplotypes to all the regions.

Identifying the initial founder population in Australia is important to help determine if genetic changes are caused by local adaptation, genetic drift or other evolutionary forces (Dlugosch and Parker 2008). The original founder population is predicted to have the highest genotypic diversity due to genetic drift further reducing diversity in subsequent founder populations (Hallatscheck and Nelson 2008). Among all the Australian *A. rabiei* populations, the SA-Kingsford population contained the highest genotypic diversity, as well as the most alleles (40 out of 70 total alleles), gene diversity ($H = 0.174$) and allelic richness ($A_R = 1.83$), indicating it as the original founder population and subsequent source of *A. rabiei* diversity in Australia. Indeed, [besides the first introduction of desi chickpea, Tyson at regional trials held in New South Wales and Queensland \(Siddique and Sykes 1997\)](#), the first [few](#) incursions in Australia through importation and sowing of infected seed in 1973 was near Kingsford (J. Davidson, South Australian Research and Development, pers. comm. 2011) and the first Ascochyta outbreak in Australia occurred in a commercial chickpea crop in SA in 1995 (Kaiser 1997 and Khan et al 1999). Kingsford is also a long running chickpea trial site with a history of a diverse range of host genotypes (J. Davidson, South Australian Research and Development, pers. comm. 2011).

Since the majority of Australian isolates are clonal and the population does not appear to contain both mating types, it is likely that more recent differences among isolates are due to gradual mutation (Montarry et al. 2006). This was perhaps demonstrated by Elliott et al. (2011), who found clonal isolates with different pathogenicities. Although this theory would require validation since the clonality proposed is based on just 20 independent and selectively neutral loci.

Although the Australian *A. rabiei* population has a low overall genetic diversity, a pair-wise regional comparison of PhiPT and bootstrap values showed that NSW was significantly different from Victoria and two of the South Australian populations ($P < 0.05$). Two plausible reasons for this differentiation are: (i) a separate recent introduction of *A. rabiei* to

NSW which may not have had the chance to spread; ii) adaptive evolution caused by local selective pressure. These may help explain the epidemic recently observed on the newly deployed moderately resistant cultivar, HatTrick in NSW (K. Moore, NSW Department of Primary Industry, pers. comm. 2010). An influx of genetic variation within an invasive population may strengthen adaptive potential (Ellstrand and Schierenbeck 2000; Bossdorf et al. 2005), also directly through the introduction of haplotypes that are well adapted to various environmental traits (Marshall and Brown 1981; Blair and Wolfe 2004). The unique haplotypes detected in northern NSW and QLD, the most recently established chickpea growing regions, may indicate that the population is rapidly evolving within these regions due to selective adaptation to new agro-geographical and host factors (Montarry et al. 2006).

Mating and linkage disequilibrium of the overall Australian isolates

All isolates were MAT1-2, indicating that the population cannot reproduce sexually. This is corroborated by low genotypic diversities and high clonal fractions, indicative of asexual reproduction. Linkage disequilibrium tests on non-clone corrected populations showed that each population except for NSW experienced linkage disequilibrium which is a characteristic of a clonally reproducing population. The exception is the NSW population which is in linkage equilibrium. Apparent linkage equilibrium ~~was may~~ most likely ~~achieved- caused by two reasons, the first being because it is NSW is~~ -a founder population of closely related individuals, as noted by the fact that NSW has the highest clonal fraction value, and the second being an artefact caused by the lack of pattern among loci and population due to an intermediate number of haplotypes in a large sample size rather than an error in the linkage disequilibrium test.

Australia appears to only have one mating type of *A. rabiei*, likely limiting the pathogen to asexual reproduction, as indicated by the detection of only MAT1-2 in all isolates assessed. However, there is a small probability that MAT1-1 does occur in Australia, albeit at very low frequencies. This is because chickpea seed sourced from overseas locations such as Canada and Syria are infected with both mating types (Armstrong et al. 2001; Atik et al. 2011). Also the teleomorph was reported from chickpea stubble in Western Australia in 2003 (Galloway and MacLeod 2003). In other parts of the world, mating types appear to be present in skewed proportions. For example, in Tunisia and Central Anatolia, more MAT1-1 type isolates were observed than MAT1-2 (Bayraktar et al. 2007; Rhaeim et al. 2007; Rhaeim et al. 2008). In India, Greece, Italy and Morocco, MAT1-2 is dominant (Navas-Cortes et al. 1998; Ali et al. 2010). Considering that many original seed imports to Australia were from India (Brinsmead 1994; Siddique and Sykes 1997), it is possible that more MAT1-2 was introduced, and perhaps MAT1-1 has reduced due to genetic drift. Indeed, mating type analysis of 37 *A. rabiei* isolates collected from seven different regions in India showed that all are MAT1-2 (Barve et al 2004), rendering the genetic diversity to be less diverse (total of 15 alleles) in comparison to other parts of the world (total of 26 alleles) using one microsatellite locus due to the tendencies of asexual reproduction of the fungus (Barve et al 2004; Santra et al 2001). Since these isolates were analysed with RAPD (Santra et al 2001) and just one microsatellite

locus (Barve et al 2004), its gene diversity of the Indian isolates could not be easily compared to the Australian *A. rabiei* gene diversity. Further research such as utilising more microsatellite loci on the Indian isolates will enable a comparison of its diversity and genetic similarity to the ones collected in Australia.

Although current Australian *A. rabiei* is reproducing asexually and generating clonal populations, this does not preclude a significant breakdown in host resistance. Whilst sexually reproducing fungi have the advantage of recombination, which may lead to higher fitness and faster adaptation in changing environments (Browne and Hoopes 1990), asexually reproducing fungi may contain combinations of well-adapted alleles that also lead to high pathogenic fitness and are preferentially inherited to result in high frequencies within a population (Browne and Hoopes 1990; Stukenbrock et al. 2006). Through positive selection, genotypes that have broad tolerance to host resistance and low fitness variance to abiotic factors such as fungicides will generally be rapidly replicated. Either as a result of founder effect and/or positive selection in our study, high frequencies of two dominant clonal haplotypes were found in all growing regions. Since these are obviously adapted over a wide range of environments and hosts, and mutations of these haplotypes may pose the greatest risk to disease management strategy including host resistance in the near future,

Host genotype selection

Progressive deployment of resistant cultivars causes pathogen populations to evolve in response to changes in the host population (McDonald et al. 1996). Environmental heterogeneity plays a significant role in shaping the pattern of species diversity (Rosenzweig 1995). Two types of adaptations are usually observed in heterogeneous environments; generalised adaptation resulting in specialised fungal haplotypes which are optimally adapted to the most prevalent host, and localised adaptation (maladaptation) of fungal haplotypes that are uniquely adapted to a specific host. Both lead to the selection of fungal haplotypes with the highest pathogenic fitness that generally increases in frequency as they have higher reproductive and survival capacities (Mettler and Gregg 1969; Leonard 1977). Due to the cultivation of a wide range of host genotypes, we expected that localised adaptation will occur and there will be a significant difference in the genetic composition among the populations sampled from different host genotypes with differing levels of susceptibility. Instead, we found low genetic diversity, high clonality and no significant difference among all host genotypes, which provided little evidence of selection or adaptation towards any of the host genotypes. Furthermore, no or very low genetic differentiation was found between populations from different host genotypes within the same geographic region, and the majority of genetic variation was distributed among individuals and was not due to differences among source host genotypes. The resistant and susceptible host genotypes within and among regions were found to share 12 out of 35 haplotypes in which one of them was shared 131 times out of 206 isolates tested, indicating it to be one of the ~~fittest~~ common

isolates in the current populations as shown on the median joining network. The most common isolate may be the first to be introduced in Australia and the high frequency of the isolate may demonstrate that it had the most extensive time to spread in the chickpea growing regions. A single large node comprising most of the haplotypes in the median joining network also indicated that the populations are derived from a single haplotype. These indicated no selection for a particular fungal clone on either the resistant or susceptible chickpea genotypes.

Selection is thought to have the least impact on population subdivision and adaptation in comparison to other evolutionary forces such as mating system, mutation and gene flow (McDonald et al. 1996). However, this does not mean that diversifying selection is not operational in the Australian *A. rabiei* pathosystem. To detect selection, the population tested needs to be in equilibrium (Kassen and Bell 1998). The current *A. rabiei* populations were in linkage disequilibrium likely due to the asexual reproduction system. Therefore, although there may be a trade-off between fungal haplotypes that are fit and aggressive, selection processes will not be detectable. Populations with clonal lineages are usually highly mutable (Lebreton et al 1998; Montarry et al 2006), and beneficial mutations can aid adaptation of specific fungal haplotypes. However, in some cases, accumulation of mutations in several fungal haplotypes may cause little functional interference in the environment and result in a more generalised pathogen adaptation (Reboud and Bell 1997). In a study by Elliott et al. (2011), pathogenicity on Australian *A. rabiei* isolates was diverse, even within clonal SSR lineages as identified in this study, further suggesting that selection does not play a major role in these populations.

However, Australia's position of population uniqueness and any advantages for disease management may be on the cusp of change with the recent wide adoption of new and different resistance genes and the ever present potential for further introductions. To identify the risk for directional selection and consequent erosion of specific resistant cultivars through introduction of potential increased pathogenicities, a more thorough spatial and temporal investigation of the population is required (Ahmed et al. 1996; Otto 2000) Therefore, it will be important to monitor for changes in allele or haplotype frequencies of the unique and clonal isolates in subsequent seasons.

Acknowledgements

The study was supported by an Australian Research Council-Linkage grant (LP0990385). We thank Dr Judith Lichtenzveig and Ms Julie Lawrence (Australian Centre for Necrotrophic Fungal Pathogens, Curtin University) for their valuable support in the genotyping work. We also thank Drs Vicki Elliott, Jenny Davidson, Kurt Lindbeck, Kevin Moore, Gail Chiplin and Malcolm Ryley for their assistance with sample collection.

537

538 **References**

- 539 ABARES (2012) The Australian Government Department of Agriculture, Fisheries and
540 Forestry (DAFF). <http://www.daff.gov.au/abares>. Accessed 10 January 2014
- 541 AgVantage Commodities (2012) AgVantage Commodities Pty Ltd: Commodity info.
542 <http://agvantagetrade.com.au/commodity-info/>. Accessed 10 January 2014
- 543 Ahmed HU, Mundt C, Hoffer ME, Coakley SM (1996) Selective influence of wheat cultivars
544 on pathogenicity of *Mycosphaerella graminicola* (anamorph *Septoria tritici*).
545 *Phytopathology* 86:454-458
- 546 Ali S, Leconte M, Walker A-S, Enjalbert J, de Vallavieille-Pope C (2010) Reduction in the
547 sex ability of worldwide clonal populations of *Puccinia striiformis* f. sp. *tritici*. *Fungal*
548 *Genet Biol* 47:828-838
- 549 Andrivon D, Pilet F, Montarry J, Hafidi M, Corbière R, Achbani EH, Pellé R, Ellissèche D
550 (2007) Adaptation of *Phytophthora infestans* to partial resistance in potato: evidence
551 from French and Moroccan populations. *Phytopathology* 97:338-343
- 552 Armstrong C, Chongo G, Gossen B, Duczek L (2001) Mating type distribution and incidence
553 of the teleomorph of *Ascochyta rabiei* (*Didymella rabiei*) in Canada. *Can J Plant Pathol*
554 23:110-113
- 555 Atik O, Baum M, El-Ahmed A, Ahmed S, Abang MM, Yabarak MM, Murad S, Kabbabeh S,
556 Hamwieh A (2011) Chickpea ascochyta blight: disease status and pathogen mating type
557 distribution in Syria. *J Phytopathol* 159:443-449
- 558 Babujee L, Gnanamanickam S (2000) Molecular tools for characterization of rice blast
559 pathogen (*Illagnaporthe grisea*) population and molecular marker-assisted breeding.
560 *Curr Sci* 78:248-257
- 561 Bandelt HJ, Forster P, Röhl A (1999) Median-joining networks for inferring intraspecific
562 phylogenies. *Mol Biol Evol* 16:37-48
- 563 Barrett SC, Husband BC (1990) Variation in Outcrossing Rates in *Eichhornia paniculata*:
564 The Role of Demographic and Reproductive Factors. *Plant Spec Biol* 5:41-55
- 565 Barve M, Arie T, Salimath S, Muehlbauer F, Peever T (2003) Cloning and characterization of
566 the mating type (MAT) locus from *Ascochyta rabiei* (teleomorph: *Didymella rabiei*)
567 and a MAT phylogeny of legume-associated *Ascochyta* spp. *Fungal Genet Biol* 39:151-
568 167
- 569 Barve MP, Santra DK, Ranjekar PK, Gupta VS (2004) Genetic diversity analysis of a world-
570 wide collection of *Ascochyta rabiei* isolates using sequence tagged microsatellite
571 markers. *World J Microbiol Biotech* 20:735-741

572 Bayraktar H, Dolar FS, Maden S (2007) Mating type groups of *Ascochyta rabiei*
573 (Teleomorph: *Didymella rabiei*), the causal agent of chickpea blight in Central
574 Anatolia. Turk J Agric For 31:41-46

575 Blair AC, Wolfe LM (2004) The evolution of an invasive plant: an experimental study with
576 *Silene latifolia*. Ecology 85:3035-3042

577 Bossdorf O, Auge H, Lafuma L, Rogers WE, Siemann E, Prati D (2005) Phenotypic and
578 genetic differentiation between native and introduced plant populations. Oecologia
579 144:1-11

580 Brinsmead B (1994) Chickpea (*Cicer arietinum*). The Australian New Crops Newsletter. I.
581 Wood and R. Fletcher, ISSN 13282026

582 Brown J, Wolfe M (1990) Structure and evolution of a population of *Erysiphe graminis* f. sp.
583 *hordei*. Plant Pathol 39:376-390

584 Browne RA, Hoopes CW (1990) Genotype diversity and selection in asexual brine shrimp
585 (*Artemia*). Evolution 44:1035-1051

586 Department of Primary Industry (DPI) (2013) DPI: Chickpea varieties.
587 [http://www.dpi.vic.gov.au/agriculture/grain-crops/crop-production/growing-](http://www.dpi.vic.gov.au/agriculture/grain-crops/crop-production/growing-chickpea/chick-pea-varieties)
588 [chickpea/chick-pea-varieties](http://www.dpi.vic.gov.au/agriculture/grain-crops/crop-production/growing-chickpea/chick-pea-varieties). Accessed 10 January 2014

589 Dlugosch K, Parker I (2008) Founding events in species invasions: genetic variation, adaptive
590 evolution, and the role of multiple introductions. Mol Ecol 17:431-449

591 Elliott VL, Taylor PW, Ford R (2011) Pathogenic variation within the 2009 Australian
592 *Ascochyta rabiei* population and implications for future disease management strategy.
593 Australas Plant Pathol 40:568-574

594 Ellstrand NC, Schierenbeck KA (2000) Hybridization as a stimulus for the evolution of
595 invasiveness in plants? Proc Natl Acad Sci 97:7043-7050

596 FAOSTAT (2013) Food and Agriculture Organization of the United Nations.
597 <http://faostat.fao.org/>. Accessed 10 January 2014.

598 Fry WE, Goodwin SB (1997) Resurgence of the Irish potato famine fungus. Biosci 47:363-
599 371

600 Galloway J, MacLeod WJ (2003) *Didymella rabiei*, the teleomorph of *Ascochyta rabiei*,
601 found on chickpea stubble in Western Australia. Australas Plant Pathol 95:1279-1286

602 Gandon S (2002) Local adaptation and the geometry of host-parasite coevolution. Ecol Lett
603 5:246-256

604 Gandon S, Capowiez Y, Dubois Y, Michalakis Y, Olivieri I (1996) Local adaptation and
605 gene-for-gene coevolution in a metapopulation model. Proc R Soc London Ser B: Biol
606 Sci 263:1003-1009

607 Gandon S, Ebert D, Olivieri I, Michalakis Y (1998) Differential adaptation in spatially
608 heterogeneous environments and host-parasite coevolution Genetic structure and local
609 adaptation in natural insect populations. Chapman & Hall, New York

610 Gandon S, Michalakis Y (2000) Evolution of parasite virulence against qualitative or
611 quantitative host resistance. Proc R Soc London Ser B: Biol Sci 267:985-990

612 Geistlinger J, Weising K, Winter P, Kahl G (2000) Locus-specific microsatellite markers for
613 the fungal chickpea pathogen *Didymella rabiei* (anamorph) *Ascochyta rabiei*. Mol Ecol
614 9:1939-1941

615 Gould F, Kennedy G, Johnson M (1991) Effects of natural enemies on the rate of herbivore
616 adaptation to resistant host plants. Entomol Exp Appl 58:1-14

617 Hallatschek O, Nelson DR (2008) Gene surfing in expanding populations. Theor Popul Biol
618 73:158-170

619 Hayden M, Nguyen T, Waterman A, Chalmers K (2008) Multiplex-ready PCR: a new
620 method for multiplexed SSR and SNP genotyping. BMC Genomics 9:80-92

621 Huettel B, Santra D, Muehlbauer F, Kahl G (2002) Resistance gene analogues of chickpea
622 (*Cicer arietinum* L.): isolation, genetic mapping and association with a Fusarium
623 resistance gene cluster. Theor Appl Genet 105:479-490

624 Iruela M, Rubio J, Barro F, Cubero J, Millán T, Gil J (2006) Detection of two quantitative
625 trait loci for resistance to ascochyta blight in an intra-specific cross of chickpea (*Cicer*
626 *arietinum* L.): development of SCAR markers associated with resistance. Theor Appl
627 Genet 112:278-287

628 Johnson R (1984) A critical analysis of durable resistance. Annu Rev Phytopathol 22:309-
629 330

630 Kaiser WJ (1997) Inter-and intranational spread of ascochyta pathogens of chickpea, faba
631 bean, and lentil. Can J Plant Pathol 19:215-224

632 Kaiser WJ, Muehlbauer FJ, Hannan RM (1992) Experience with Ascochyta blight of chickpea
633 in the United States. In: Kaiser WJM, F J (ed) Expanding the production and use of
634 cool season food legumes. Kluwer Academic Publishers, USA, pp 849-858

635 Kaiser WJ, Muehlbauer FJ (1988) An outbreak of Ascochyta blight of chickpea in the Pacific
636 Northwest, USA, in 1987. Int Chickpea Newsletter 18:16-17

637 Kalinowski ST (2005) hp-rare 1.0: a computer program for performing rarefaction on
638 measures of allelic richness. Mol Ecol Notes 5:187-189

639 Kassen R, Bell G (1998) Experimental evolution in *Chlamydomonas*. IV. Selection in
640 environments that vary through time at different scales. Heredity 80:732-741

641 Khan M, Ramsey M, Corbiere R, Infantino A, Porta-Puglia A, Bouznad Z, Scott E (1999)
642 Ascochyta blight of chickpea in Australia: identification, pathogenicity and mating
643 type. Plant Pathol 48:230-234

644 Lebreton L, Laurent C, Andrivon D (1998) Evolution of *Phytophthora infestans* populations
645 in the two most important potato production areas of France during 1992–96. Plant
646 Pathol 47:427-439

647 Leo AE, Ford R, Linde CC, Shah RM, Oliver R, J TPW, Lichtenzweig J (2011)
648 Characterization of sixteen newly developed microsatellite loci for the chickpea fungal
649 pathogen *Ascochyta rabiei*. Permanent Genetic Resources added to Molecular Ecology
650 Resources database 1 October 2010 - 30 November 2010. Mol Ecol 11:418-421.
651 doi:10.1111/j.1755-0998.2010.02970.x

652 Leonard K (1977) Selection pressures and plant pathogens. Annu NY Acad Sci 287:207-222

653 Linde CC, Zala M, McDonald BA (2009) Molecular evidence for recent founder populations
654 and human-mediated migration in the barley scald pathogen *Rhynchosporium secalis*.
655 Mol Phylogenet Evol 51:454-464

656 Manly BFJ (1985) The statistics of natural selection. Chapman & Hall, London

657 Mantel N (1967) The detection of disease clustering and a generalized regression approach.
658 Cancer Res 27:209-220

659 Marshall D, Brown A (1981) The evolution of apomixis. Heredity 47:1-15

660 Maynard Smith J, Smith NH, O'Rourke M, Spratt BG (1993) How clonal are bacteria? Proc
661 Natl Acad Sci 90:4384-4388

662 McDonald BA, Linde C (2002) The population genetics of plant pathogens and breeding
663 strategies for durable resistance. Euphytica 124:163-180

664 McDonald BA, Mundt CC, Chen R-S (1996) The role of selection on the genetic structure of
665 pathogen populations: Evidence from field experiments with *Mycosphaerella*
666 *graminicola* on wheat. Euphytica 92:73-80

667 McDonald B, Miles J, Nelson L, Pettway R (1994) Genetic variability in nuclear DNA in
668 field populations of *Stagonospora nodorum*. Phytopathology 84:250-255

669 Mettler LE, Gregg TG (1969) Population genetics and evolution. Englewood Cliffs

670 Meirmans PG, Van Tienderen PH (2004) GENOTYPE and GENODIVE: two programs for
671 the analysis of genetic diversity of asexual organisms. Mol Ecol Notes 4:792-794

672 Milgroom M, Sotirovski K, Spica D, Davis J, Brewer M, Milev M, Cortesi P (2008) Clonal
673 population structure of the chestnut blight fungus in expanding ranges in southeastern
674 Europe. Mol Ecol 17:4446-4458

675 Montarry J, Corbiere R, Lesueur S, Glais I, Andrivon D (2006) Does selection by resistant
676 hosts trigger local adaptation in plant–pathogen systems? J Evol Biol 19:522-531

677 Navas-Cortés JA, Pérez-Artés E, Jiménez-Díaz RM, Llobell A, Bainbridge BW, Heale JB
678 (1998) Mating type, pathotype, and RAPDs analysis in *Didymella rabiei*, the agent of
679 Ascochyta blight of chickpea. Phytoparasitica 26:199-212

680 Nei M (1973) Analysis of gene diversity in subdivided populations. Proc Natl Acad Sci
681 70:3321-3323

682 Nei M (1975) Molecular population genetics and evolution. Front Biol 40:I

683 Nourollahi K, Javannikkhah M, Naghavi MR, Lichtenzweig J, Okhovat SM, Oliver RP,
684 Ellwood SR (2010) Genetic diversity and population structure of *Ascochyta rabiei* from
685 the western Iranian Ilam and Kermanshah provinces using MAT and SSR markers.
686 Mycol Prog 10:1-7

687 Otto SP (2000) Detecting the form of selection from DNA sequence data. Trends Genet
688 16:526-529

689 Peakall R, Smouse PE (2006) GENALEX 6: genetic analysis in Excel. Population genetic
690 software for teaching and research. Mol Ecol Notes 6:288-295

691 Phan HTT, Ford R, Taylor PWJ (2002) Population structure of *Ascochyta rabiei* in Australia
692 based on STMS fingerprints. Fungal Divers 13:111-129

693 Pimentel D, McNair S, Janecka J, Wightman J, Simmonds C, O'Connell C, Wong E, Russel
694 L, Zern J, Aquino T, Tsomondo T (2001) Economic and environmental threats of alien
695 plant, animal, and microbe invasions. Agric Ecosyst Environ 84:1-20

696 Poczai P, Varga I, Bell NE, Hyvönen J (2012) Genomics Meets Biodiversity: Advances in
697 Molecular Marker Development and Their Applications in Plant Genetic Diversity
698 Assessment. In: Caliskan M (ed) The Molecular basis of Plant Genetic Diversity.
699 InTechopen

700 Podger FD, Doepel RF, Zentmyer GA (1965) Association of *Phytophthora cinnamomi* with a
701 disease of Eucalyptus marginata forest in Western Australia. Plant Dis Rep 49:973-947

702 Polzin T, Daneshmand, SV (2003) On Steiner trees and minimum spanning trees in
703 hypergraphs. Oper Res Lett 31;12-20

704 Pulse Australia (2012) Australian Pulse Market News: A background to global pulse market
705 drivers.
706 [http://www.pulseaus.com.au/pdf/Australian%20Pulse%20Market%20News/Australian](http://www.pulseaus.com.au/pdf/Australian%20Pulse%20Market%20News/Australian%20Pulse%20Market%20News%20Background%20Facts%202012.pdf)
707 [%20Pulse%20Market%20News%20Background%20Facts%202012.pdf](http://www.pulseaus.com.au/pdf/Australian%20Pulse%20Market%20News/Australian%20Pulse%20Market%20News%20Background%20Facts%202012.pdf). Accessed 10
708 January 2014

709 Pulse Australia (2013) Australian Pulse Market News.

710 <http://www.pulseaus.com.au/pdf/Australian%20Pulse%20Market%20News/2013/Pulse>
 711 [%20Australia%20Market%20News%20September%2020%202013.pdf](http://www.pulseaus.com.au/pdf/Australian%20Pulse%20Market%20News/2013/Pulse). Accessed 10
 712 January 2014

713 Pulse Breeding Australia (PBA) (2009) Pulse Australia: Chickpeas in South Australia and
 714 Victoria.
 715 <http://www.pulseaus.com.au/pdf/Chickpeas%20for%20SA%20%26%20Vic.pdf>.
 716 Accessed 10 January 2014

717 Reboud X, Bell G (1997) Experimental evolution in *Chlamydomonas*. III. Evolution of
 718 specialist and generalist types in environments that vary in space and time. *Heredity*
 719 78:507-514

720 Rhaïem A, Cherif M, Dyer P, Peever T (2007) Distribution of Mating Types and Genetic
 721 Diversity of *Ascochyta rabiei* Populations in Tunisia Revealed by Mating-type-specific
 722 PCR and Random Amplified Polymorphic DNA Markers. *J Phytopathol* 155:596-605

723 Rhaïem A, Cherif M, Peever T, Dyer P (2008) Population structure and mating system of
 724 *Ascochyta rabiei* in Tunisia: evidence for the recent introduction of mating type 2. *Plant*
 725 *Pathol* 57:540-551

726 Rivas G-G, Zapater M-F, Abadie C, Carlier J (2004) Founder effects and stochastic dispersal
 727 at the continental scale of the fungal pathogen of bananas *Mycosphaerella fijiensis*. *Mol*
 728 *Ecol* 13:471-482

729 Rosenzweig ML (1995) Species diversity in space and time. Cambridge University Press, UK

730 Rossman AY (2009) The impact of invasive fungi on agricultural ecosystems in the United
 731 States. *Biol Invasions* 11:97-107

732 [Santra DK, Singh G, Kaiser WJ, Gupta VS, Ranjekar PK, Muehlbauer FJ \(2001\) Molecular](#)
 733 [analysis of *Ascochyta rabiei* \(Pass.\) Labr., the pathogen of ascochyta blight in chickpea.](#)
 734 [Theor Appl Genet 102:676-682](#)

735 Shtienberg D, Vintal S, Brenner S, Retig B (2000) Rational management of *Didymella rabiei*
 736 in chickpea by integration of genotype resistance and postinfection application of
 737 fungicides. *Phytopathology* 90:834-842

738 [Siddique KHM, Sykes J \(1997\) Pulse production in Australia past, present and future. Austral](#)
 739 [J Exp Agric 37:103-111](#)

740 Siddique KHM, Regan KL, Baker MJ (2004) New ascochyta blight resistant, high quality
 741 kabuli chickpea varieties for Australia Paper presented at the New directions for a
 742 diverse planet Brisbane, Australia

743 Simpson EH (1949) Measurement of diversity. *Nat* 163:688

- 744 Stukenbrock EH, Banke S, McDonald BA (2006) Global migration patterns in the fungal
745 wheat pathogen *Phaeosphaeria nodorum*. Mol Ecol 15:2895-2904
- 746 Tar'an B, Warkentin T, Tullu A, Vandenberg A (2007) Genetic relationships among
747 Chickpea (*Cicer arietinum* L.) genotypes based on the SSRs at the quantitative trait
748 Loci for resistance to Ascochyta Blight. In: Ascochyta blights of grain legumes.
749 Springer, pp 39-51
- 750 Templeton AR, Routman E, Phillips CA (1995) Separating population structure from
751 population history: a cladistic analysis of the geographical distribution of mitochondrial
752 DNA haplotypes in the tiger salamander, *Ambystoma tigrinum*. Genetics 140:767-782
- 753 Trapero-Casas A, Kaiser W (1992) Development of *Didymella rabiei*, the teleomorph of
754 *Ascochyta rabiei*, on chickpea straw. Phytopathology 82:1261-1266
- 755 Wilson AD, Kaiser WJ (1995) Cytology and genetics of sexual incompatibility in *Didymella*
756 *rabiei*. Mycologia 87:795-804