Lack of polymorphism suggests a recent bottleneck of *Aloidendron pillansii*

Exploration into the population of *Aloidendron pillansii* from phylogeographical analysis of molecular data

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October 2014

Dissertation submitted to the University of Cape Town, in partial fulfilment of the requirements for the award of an Honours Degree in Biological Sciences

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Abstract

The Karoo-Namib is a species rich region in which many iconic and keystone species are found, such as *Aloe pillansii.* The recent population history of *A.pillansii* is poorly understood. However the suggested climatic shifts that occurred throughout the Holocene era may have affected its distribution, demographics and gene flow. The glacial/interglacial refugia hypothesis predicts that the southernmost population served as a refuge population and that the subsequent expansion of the population was to the north in concert with the northward expansion of the winter rainfall regime. I evaluated this hypothesis by linking the molecular data (cpDNA and nDNA) of 84 individuals from three main populations with phylogeographical techniques. Based on the combination of percentage of mutations percentage per million years range and the chloroplast sequences, it has been estimated that the time of divergences was between 3.45 to 16.67 million years ago. The molecular analysis identified a significant lack of genetic diversity within and among the three dominant populations of *A.pillansii*. This suggests that the species experienced a severe bottleneck event prior to its recent expansion that has been suggested to have occurred within the time frame of 100 to 1000 years ago. This pattern is compared with its sister taxa *Aloe dichtotma,* which possesses variation within and among its populations. The lack of genetic variation evident within *A. pillansii* leaves it vulnerable to future climate shifts as low genetic variation within a species lowers the ability of that species to adapt to both environmental and climatic changes. This thesis has provided a brief insight into the population history of *A.pillansii,*but further research is needed.

Keywords: *Aloe pillansii*, bottleneck and variation

1. Introduction

Species are often limited in their distributions by a range of biotic and abiotic factors (Potts 2011; Chase and Meadows 2007). The regions where species occur and do not occur is often based on their relative rate evolution and speciation through time and space (Elith and Leatherwick 2009, Dynesius and Jansson 2000). Individuals rarely produce a solitary population with an equal connectivity amongst each individual within the species. Individuals may be isolated from each other by distance or by complex environmental scenarios that could have an influence on their dispersal (Potts 2011; Elith and Leatherwick 2009). Traditionally, the ability to understand the distributional shifts of a species through time relied on mapping the movement of the species and dating fossils and pollen (Holmes et al 2003). However, the historical information gathered about the species is often fragmented and the fossil or pollen remains may not exist for particular taxa (Elith and Leatherwick 2009). Additionally, in many regions around the world and in southern Africa particularly, climatic conditions or landscapes necessary for the formation of preserved archives have not occurred (Potts 2011).

Recently, plant phylogeography has allowed for an increased understanding of the historical factors that have influenced the directional migration of species in response to Holocene glacial and inter-glacial cycles through different regions of the world (Beheregaray 2008). Phylogeography encompasses the processes and principles which govern the genetic lineages and the geographic distributions within and among populations (Avise 1998) and provides the potential to gain a greater understanding of the history of plant communities. This is especially useful in places like Southern Africa which is home to three biodiversity hotspots (Chase and Meadows 2007).

The plant diversity of Southern Africa's arid landscapes is astonishing, particularly within the winter rainfall zone of the karoo, with its large array of succulents (Bolus *et al.* 2004). The Karoo-Namib region is home to over 6300 species of which at least 38% are endemic (Hilton-Taylor 1996). The Karoo-Namib region is largely comprised of widespread plateaus (Holmes *et al.* 2013). The Great Escarpment separates the eastern coastal regions from the plateaus. The arid interior area is the dominant spatial and geological aspect of the region. Holmes *et al.* (2003) showed that based on depositional evidence; there has been a shift in climatic conditions throughout the Holocene. The results were interpreted to suggest that the Karoo-Namib region has experienced an array of palaeoenvironments since the last glacial/interglacial periods.

Southern Africa is located at the boundary of the temperate, tropical and subtropical climate systems (Chase and Meadows 2007). The subcontinent is influenced by several circulation systems, including those of atmospheric and oceanic origins (Weldeab *et al.* 2013, Chase and Meadows 2007). This results in different season rainfall regimes occurring over specific regions within the subcontinent.

Across Southern Africa there are two prevailing rainfall regimes (Chase and Meadows 2007). The northern and eastern regions receive summer rainfall which comprises more than 66% of the annual rainfall. This is caused by the interaction between pressure cells and easterly flows. In contrast the winter rainfall regime extends from south western Namibia to Cape Agulhas along the South Atlantic coastline (Chase and Meadows 2007). Arid and semi –arid landscapes such as the southern Namib Desert and the South African Namaqualand region receive more than 65% of their annual rainfall in the winter months (Chase and meadows 2007,Cowling *et al.* 1999). Between the dominant rainfall regimes lies a narrow zone which receives both winter and summer rainfall which is often referred to as the all-year rainfall zone.

The current winter rainfall regime of southern Africa has been hypothesised (eg Stuut *et al*. 2004; van Zinderen Bakker , 1976) to have been caused by an increase in the glaciation of the Antarctic sea (Zachos *et al*. 2001; Potts 2011) with the resultant equatorward displacement of the south Atlantic high-pressure cell (Potts 2011). These shifts would influence the climates of southern Africa resulting in an increase in humidity and winter rainfall throughout the glacial periods that persisted in southwestern Africa (Chase and Meadows 2007). However, the body of evidence for environmental change in southern Africa has been meagre (Weldeab *et al*. 2013). This has been largely because the environment of the subcontinent experiences conditions that are not favourable for preserving paleaoecological data (Chase and Meadows 2007). These environments include a range of arid conditions and seasonal rainfall which have fluctuated over time.

Van Zinderen Bakker (1976) proposed the hypothesis of the shift in the latitudinal circulatory systems over Southern Africa throughout the glacial-interglacial periods (Chase and Meadows 2007). The idea was largely based upon the expansion of the pressure systems and the displacement of the intertropical convergence zone (ITCZ) (the ITCZ is a zone in which north-easterly and south-easterly trade winds meet) (Phillips 2013). Thereafter he developed models for the expansion of the winter rainfall regime across southern Africa during glacial periods (Chase and Meadows 2007). The conceptual model developed by Van Zinderen Bakker (1976), which implies an expansion of the winter rainfall regime throughout southwestern Africa within the last glacial period, has been supported by recent evidence (Chase and Meadows 2007).

The importance of the proposed research on the phylogeography of *Aloe pillansii* is multifaceted. One of the foremost reasons is that the species is in decline. Midgley et al (1997) and Bolus *et al*. (2004) have provided evidence of the decline in the northern population of this species. Data produced by (Williamson 1998) has shown that there is a decline in the number of juveniles and seedlings in the northern populations which would influence subsequent recruitment in these populations. This trend will result in the senescence of the population in future. Furthermore, populations have a relatively small distribution and range which has the potential for the populations to be isolated. This is another aspect of the species that needs attention due to the fact that conservation efforts are threatened by population isolation. *A.pillansii* is listed as an endangered species on the IUCN Red List which increases the need for the conservation of the species.

Aloe pillansii has previously fallen within the Asphodelaceae subfamily: Alooideae which encompassed seven alooid genera (Grace *et al.* 2013). However recently there has been a reconsideration of the classification which a contracted generic group for Aloe species. This gave rise to the novel genera of Aloidendron (Grace *et al.* 2013). Within the novel genus is *A.pllansii, A.dichotoma, A.ramosissimum, A. barberae and A. eminems* (Adams *et al.* 2002).The taxa within the genus Aloidendron has been grouped based upon descriptive and chemical similarities(Dagne et al. 2000). This indicates how closely related *A.pllansii is* to *A.dichotoma* and A.ramosissimum*,*

This study sought to investigate the population history of Aloe pillansii in South Africa and Namibia. My approaches include a phylogenetic analysis to determine the position of *A.pillansii* within the Aloe clade. To assess the time of the divergence of *A.pillansii* from its closest relatives, molecular detaining approach will be used. Based on the study by (Stager *et al*. 2012) which suggests a warming period that occurred 900 to 1400 years before present. It can be hypothesised that *A.pilansii* may have experienced a recent bottleneck event. Using the approached mentioned it is possible to infer the occurrence of the bottleneck and if so, how severe was it? Should the results of this thesis supports the proposed hypotheses, it will provide context for the population history of not just *A.pillansii* but of other tree species within the Karoo-Namib region as well. The results would also provide knowledge of the role that climate change has had on past species distributions. This helps in the construction of future species distribution models which are crucial for the conservation of those species most likely affected by future changes in climate.

2. Methods and Materials

2.1 Species Description

Aloe pillansii is a keystone species of the Succulent Karoo biome (Duncan *et al.* 2005) and is rare and endemic to the region (Bolus *et al.* 2004). Trees grow to a height of 10 m or greater with a corresponding stem diameter that is between 1 – 2 m wide (Bolus 2004). A characteristic feature of these trees is the dichotomous branching which often occurs at the midpoint of the stem's length (www.plantzafrica.com) (Fig 1). Older individuals often possess trunks that are swollen and bottle shaped which develop to support the branches (www.plantzafrica.com). *A.pillansii* is distinguishable from *A.dichotoma* and *A. ramosissima by its rosulate leaves* and by its inflorescences which develop horizontally upon the lowest leaf (Bolus 2004) (Fig 1.)In *A.dichotoma* and *A. ramisissima* their inflorescences are held erect above the leaf rosettes (www.plantzafrica.com).Distinctively, the younger leaves appear glaucous in comparison to species that are closely related (www.plantzafrica.com). The flowering season occurs from September to December wherein the spherical capsules ripen and split open to release wind dispersed seeds (Bolus 2004).

Figure 1. ***Aloe pillansii*** **showing (A) dichotomous branching and (B) the leaves and inflorescences** (www.arkive.org)

2.2 Species Distribution

Aloe pillansii occurs in regions that experience an arid climate with rain falling largely in the winter months. The specific habitat occupied by *A.pillansii* includes mountainous regions, locations adjacent to river beds as well as low lying gravel slopes. The global population spatial range is located within Namibia and South Africa. The most northern cluster of populations occurs in southwestern Namibia and is separated from the central cluster of populations in South Africa by the Orange River (Fig 2). There is a third, disjunct population in the southern part of its distribution in South Africa.

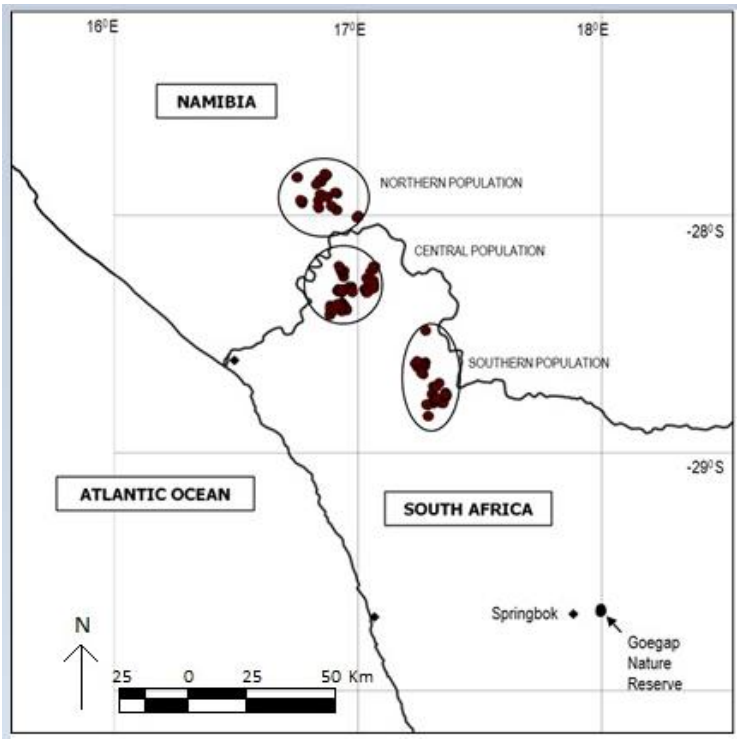

Fig 2: The entire spatial range and pattern of the populations of *Aloe pillansii* (map adapted from Elsabe Swart)

2.3 Sampling and DNA extraction

Populations of *Aloe pillansii* were identified at 22 localities within South Africa and Namibia respectively. The locations were found across the spatial range of the northern, central and southern cluster of populations of *Aloe pillansii* (Fig 2). Leaf material samples of the individuals found at the identified locations were collected by Elsabe Swart. DNA was extracted using the CTAB buffer DNA protocol described by Doyle and Doyle (1987).

Table 1: *Aloe pillansii* populations analysed (84 individuals) in this study (* represents the populations where the GPS coordinates were not available).

			GPS Coordinates	
Country	**Location**	**Sample Code**	**Latitude**	**Longitude**
South Africa	Rooiberg	R	33°24'36.23''	19°45'42.95''
South Africa	Keerdam	K	30°02'36''	17°35'10''
South Africa	Creshe Mountain*	C		
South Africa	Swartberge	S	33.3667°	22.3542°
South Africa	Hartmens Zebra Mountain	HMZ	48.550°	11.3315°
South Africa	Rosyntjieberg	RB	28°18'01.12''	17°12'53.65''
South Africa	Mountains west of Namas	MWN	29°58'36.51''	19°05'18.22''
South Africa	Beacon Mountain*	BM		
South Africa	Orange River	OR	28°44'55.33''	19°21'17.09''
South Africa	Goegap	GNR	29°06'25.86''	19°21'47.91''
South Africa	Zebrasfontein*	ZFN		
South Africa	Five Sisters	FS	28°13'16.11''	16°55'41.47''
South Africa	Cornell's Kop	CK	28°25'05.36''	16°53'05.36''
Namibia	Mi Millans*	MM		
South Africa	Kodasmyn	KDN	28.0539°	17.0347°
South Africa	Anniskop	AP	28°23'08.82''	16°52'34.65''
Naminia	McMillan*	MCM		
Namibia	Rosh Pinah	RP	27°57'53.78''	16°45'36.50''
Namibia	Namaskluft	NT	27.94983°	16.753063°
Namibia	Namaskluft RD Farm (Inselberg 1)	NI1	27.95640°	16.68541°
Namibia	Namas Inselberg 2	NI2	27.45698°	16.54238°
Nambia	Valley Opposite Namas*	VON		

2.4 Nuclear and chloroplast sequencing

The nuclear region sampled was the internal transcribed spacer (ITS). Five chloroplast regions were sampled:, *trnS-trnG, trnT-trnD,rpl32-trnL* and *trnT-trnL* and *psbA-trnH*.Primers used in the PCR reaction are given in Table 1. The PCR volume for each genome region evaluated per individual consisted of 20-25 mg of plant DNA material, 17.6 µl of PCR water, 3.0 µl reaction buffer and $MgCl_2$, 1.2 µl dNTPS, 1.0 µl of tailed forward and reverse primer s (Table 2) and 0.2 µl of Taq DNA polymerase. The PCR amplification protocol used for both the ITS and chloroplast region was carried out using a Veriti® 96-Well Fast Thermal Cycler using the protocol labelled "BogStandard". The protocol consisted of a denaturing step at 94°C for 3 minutes, 35 cycles, with each including 94°C for 45 seconds, 52°C for 45 seconds, 72°C for 1 minute and 30 seconds,and a final step of 72°C for 7 minutes. The PCR products were sequenced using the forward primers at the University of Stellenbosch at their Central DNA sequencing Facility

Table 2. The primers used for the PCR amplification for the selected Nuclear and Chloroplast regions of *Aloe pillansii* (Potts 2011)

Genome	Region	Primer Name	Primer Sequence	Primer Sequence Reference
Chloroplast	trnS-trnG	C1 (Forward)	AGATAGGGATTCGAACCCTCGGT	(Figlar and Nooteboom 2004)
		C4 (Reverse)	TTTTACCACTAAACTATACCCGC	(Figlar and Nooteboom 2005)
	trnT-trnD	F1 (Forward)	ACC AAT TGA ACT ACA ATC CC	(Clark 2007)
		F4 (Reverse)	CCC TTT TAA CTC AGT GGT A	(Clark 2007)
	rpl32-trnL	V1 (Forward)	CAGTTCCAAAAAAACGTACTTC	(Shaw *et al* 2007)
		V2 (Reverse)	CTGCTTCCTAAGAGCAGCGT	(Shaw *et al* 2007)
	trnT-trnL	H5 (Forward)	CAT TAC AAA TGC GAT GCT CT	(Clark 2007)
		H6 (Reverse)	TCT ACC GAT TTC GCC ATA TC	(Clark 2007)
	psbA	A7 (Forward)	GTTATGCATGAACGTAATGCTC	(Sang *et al.*1995)
		A8 (Reverse)	AACCTTGGTATGGAAGTTATG	(Sang *et al.*1995)
Nuclear	ITS	ITS4 (Forward)	TCC TCC GCT TAT TGA TAT GC	(White *et al.* 1990);(Pots 2011)
		ITS5 (Reverse)	GGA AGG AGA AGT CGT AAC AAG G	(Sang *et al.*1995)

2.5 Sequence assembly and alignment

The nuclear and chloroplast DNA sequences produced were assembled using BioEdit sequence Alignment Editor Version 7.2.5 (http://www.mbio.ncsu.edu/bioedit/bioedit.html) and then aligned using a ClustalW Multiple alignment (Thompson et al 1997).

2.6 Phylogenetic analysis

We used the psbA-trnH region to reconstruct phylogenetic relationships among the three western tree sites (Daru *et al.* 2012).Based on the previous study, where these comprised a well-supported monophyletic group, sister to *A.barberae*. We used the latter as an outgroup. Molecular Evolutionary Genetics Analysis (MEGA) version 6.0 (Tamura *et al.* 2013). Nodal support was estimated by bootstrapping. With 1000 bootstrap replicates.

2.7 Molecular dating

The absence of a fossil record which could be used to calibrate the phylogeny and population-level data, we employed a simple molecular clock approach (e.g. Sarich and Wilson, 1973). Since chloroplast mutation rates vary widely across angiosperms, and across DNA regions, we used a very broad range (1.54 x 10-9 - 3.6 x 10-8 per site per year) of plausible mutation rates (Zhang & Huett 2003). For the population study, these values were used to estimate expected nucleotide diversity across our set of sequences for various time intervals (Daru *et al.* 2012)

The time of divergence was obtained by firstly determining the number of differences within the psbA-trnH sequences between *A.pillansii* and *A.dichotoma*. The differences were then divided by the sequence length. The resultant value was then divided by two as at the point of divergence between *A.pillansii* and *A.dichiotoma*, the tree splits into two branches. This was then divided by the mutation percentage per million years range which produced the range of the time of divergence for *A.pillansii*.

3. Results

3.1 Aloe pillansii sequence assembly and alignment

The nuclear DNA (ITS) alignment contained multiple traces that could not be reliably phased. This region was thus discarded from subsequent analyses. The genetic data (cpDNA) analysed showed no variation within the selected gene regions within and between the sampled populations of the species. This was made clear by the presence of a single chloroplast haplotype that was identified across the entire spatial range for each chloroplast region (Table 3). The nuclear DNA (ITS) alignment

contained multiple traces which made it unclear as to the accuracy of the sequences and therefore could not be used in the analysis.

Based on the broad range of mutation rates used here, the expected diversity of the total 700 positions sampled is considerably higher.Even at the lowest plausible rates, we would have expected to see at least 1.68 to 2.52 mutations over 1000 years, and 16.8 over 100, 000. At the higher rate, our expectation for 700 sites is a range 2.52 for 1000 and 25.2 over 100 000 years.

Table 3. Haplotype Diversity for the *A.pillansii populations*

			cpDNA Haplotypes				
Country	Location	Sample Code	*TrnS-TrnsG*	*TrnT-TrnD*	*rpl32-TrnL*	*TrnT-TrnL*	*psbA*
South Africa	Rooiberg	R	1	1	1	1	1
South Africa	Keerdam	K	1	1	1	1	1
South Africa	Creshe Mountain	C	1	1	1	1	1
South Africa	Swartberge	S	1	1	1	1	1
South Africa	HartmensZebra Mountain	HMZ	1	1	1	1	1
South Africa	Rosyntjieberg	RB	1	1	1	1	1
South Africa	Mountains west of Namas	MWN	1	1	1	1	1
South Africa	Beacon Mountain	BM	1	1	1	1	1
South Africa	Orange River	OR	1	1	1	1	1
South Africa	Goegap	GNR	1	1	1	1	1
South Africa	Zebrasfontein	ZFN	1	1	1	1	1
South Africa	Five Sisters	FS	1	1	1	1	1
South Africa	Cornell's Kop	CK	1	1	1	1	1
South Africa	Mi Millans	MM	1	1	1	1	1
South Africa	Kodasmyn	KDN	1	1	1	1	1
South Africa	Anniskop	AP	1	1	1	1	1
Namibia	McMillan	MCM	1	1	1	1	1
Namibia	Rosh Pinah	RP	1	1	1	1	1
Namibia	Namaskluft	NT	1	1	1	1	1
Namibia	Namaskluft RD Farm (Inselberg 1)	NI1	1	1	1	1	1
Namibia	Namas Inselberg 2	NI2	1	1	1	1	1
Nambia	Valley Opposite Namas	VON	1	1	1	1	1

3.2 Phylogenetic analysis and Molecular Dating

The phylogenetic tree recovered under ML resolves *A. pillansii* as sister to the remaining two taxa (Fig 3). The relationship between *A. dichotoma* and *A. ramossisima* is only moderately well supported. Based on the reasonable mutation rates for chloroplast DNA of 0.024% per million years to 0.116% per million years (Potts *et al*.2013), *A.pillansii* diverged from its close relatives 16.67 TO 3.45 million years ago (Fig 4).

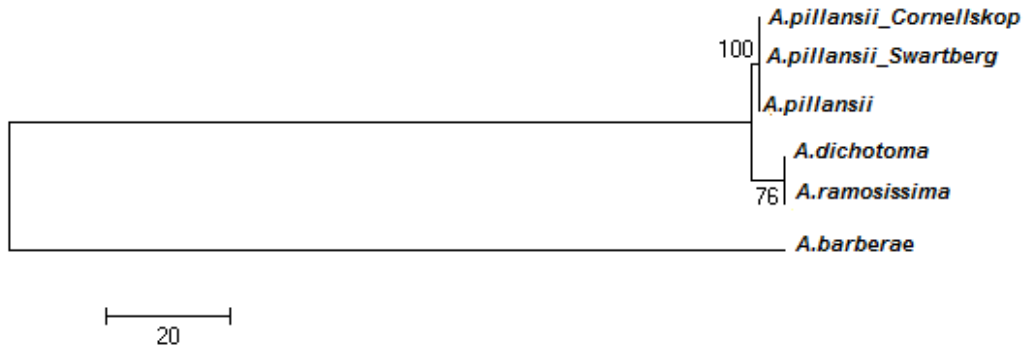

Figure 3. A phylogram of Aloidendron species for the psbA-trnH sequences using Mega and 1000 bootstrap repetitions

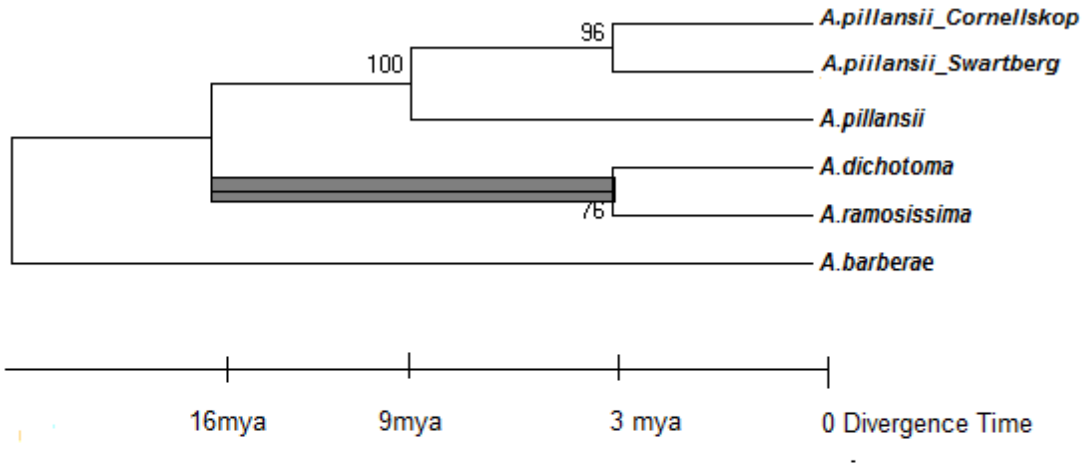

Figure: A phylogenetic estimate of the divergence time for Aloidendron species

4. Discussion

Two key findings emerge from the present study. First, *A.pillansii* is a relatively young species that is estimated to have diverged from its nearest relatives only 16.67 3.45 million years ago. The time of divergence coincides with the recent radiation of Rushioideae that occurred between 3.8 and 8.7 million years ago (Klak *et al.*2004).This was proposed to be as a result of the expansion of the winter rainfall niche which occurred 5 million years ago as well as the suggested increase in aridification of the region (Klak *et al. (2004).*

The second key observation is the complete lack of chloroplast polymorphism across a large sample of individuals covering the entirety of its range. A possible explanation for this pattern could be selection, which would reduce levels of substitution. However, this is unlikely as all the regions sampled are spacers, and thus likely to be under few if any selective constraints. Indeed all the regions are typically used in population analyses (Sunnucks 2000). In addition, the *psbA-trnH* region shows sufficient variation between *A. pillansii* and *A. dichotoma* to resolve a convincing phylogeny, again arguing against a pervasive slowdown in rates as a potential explanation**.**

An alternative is that the species has experienced a recent, drastic bottleneck. Based on our simple calculations of expected diversity, it is estimated that the severe bottleneck event experienced by *A.pillansii* occurred within the time frame of 1000 to 100 years before present. A broad range of mutation rates were used given that the origin of land plants cannot be pushed back that far. Given more time, the probability of attaining zero mutations could have been calculated using a conservative coalescent approach. The bottleneck event may have been as the result of the Medieval Climate Anomaly that occurred between 900 and 1400 years before present (Stager *et al*. 2012). Tyson *et al*. (2000) suggests that the overall mean temperature over the winter rainfall zone of Southern Africa experienced a 3 to 4 degree Celsius (°C) increase in temperature. Within arid and semi-arid regions, organisms are at their tolerance limits, and may not have the ability to continue to exist under warmer conditions (Noble and Gitay 1996). This may have been the scenario that caused *A.pillansii* to experience a severe bottleneck.

The low cpDnA diversity within *A.pillansii* suggests that the species experienced a decrease of its effective size which was followed by a population growth (Fedorov and Stenseth 2001) which may have been caused by a severe bottleneck. The lack of variation implies that the species survived in a single refugium from which it recently expanded and this idea is supported by the presence of a single haplotype across the chloroplast regions (Stevens and Hogg 2003). A similar study by

Tollefsrud *et al.* (2008) used palaeoecological and genetic data to infer that the northern range of *Picea abies* survived the glacial period from a solitary refugium.

With the bottleneck event suggested to have occurred between 100 to 1000 years ago, it would therefore be logical for the recent expansion of the species to have occurred within the same period. One possible explanation for the expansion is the Little Ice Age that occurred 700 years before present (Tyson *et al.* 2000).Stager *et al.* (2012) reports remarkably wetter conditions during the majority of the last 7 centuries. Isotope evidence of the Little Ice Age presented by Tyson *et al.* (2000) is found within Namibia, the Namib Desert, the Northern Western regions of South Africa and the Western Cape Province. The regions mention encompasses the entire spatial range of *A.pillansii* is located which provides additional support for the expansion of the species being as a result of the Little Ice Age.

However, because no significant variation was identified, it was not possible to evaluate the gene flow between the populations and the significance of the recent expansion of the species. The results of the molecular analysis showed that the individuals of the different populations have significantly low levels of genetic differences amongst each other and when compared to the individuals of the other populations. One possible reason for the lack of the genetic diversity within *A.pillansii* was as a result of the species experiencing a bottleneck event prior to its most recent expansion. It has been demonstrated by Leimu *et al*. (2006) that species with small population sizes which are isolated from one another often have a lower genetic diversity when compared to those with larger population sizes. This is suggested to be as a result of the species being affected by a bottleneck and/or genetic drift (Leimu *et al.* 2006). The analysis carried out by Leimu *et al.* (2006) suggests that population size could be as a crucial variable in understanding the differences in genetic diversity between populations. When a population has undergone a bottleneck event and as a result has remained small for several generations, the resultant loss of rare alleles is a primary factor in the reduced genetic variation within that population (Barrett and Kohn 1991). According to Jansson and Dynesius (2002), climate change has negatively influenced the genetic divergence of species within southern Africa. In comparison with its sister species, *A.pillansii* has a lower genetic diversity than *A.dichotoma*. This has been observed within the species *Corylus avellana.* In comparison with its sister species, many chloroplast DNA haplotypes are not observed as a result of the species experiencing bottleneck events during the glacial periods (Palmé & Vendramin 2002). In their study, Palmé & Vendramin (2002) identified that geographical distributions influenced the variation of the chloroplast haplotypes within *Corylus avellana* and its sister taxa.

4.1 Implications for the species

From the analysis it suggests that *A.pillansii* had gone through a bottleneck prior to its recent expansion. This has resulted in the species losing its genetic diversity which is apparent in the lack of genetic variation within and among its dominant populations. This loss of genetic diversity is suggested to be influenced through genetic drift and inbreeding (Charlesworth and Charlesworth 1999). Inbreeding within populations has the potential to influence the accumulation of deleterious recessive genes. These genes may have an effect on the ability of the species to withstand environmental stress (Lacy 1997). Inbreeding has the potential to increase the vulnerability to harmful diseases, decrease the ability to compete for resources, decrease fecundity and increase mortality (Keller and Waller 2002). With inbreeding, the unfavorable traits are expressed in the offspring due to the similarities of the parental genomes (Keller and Waller 2002). The increasing similar the parental genomes are the more consistent the unfavorable traits are expressed in the offspring. This will produce offspring that are less fit in terms of adaptations and survival to environmental and climatic changes.

The loss of allelic diversity is influenced by the levels of genetic variation present in the population prior to the bottleneck event (Kramer and Sarnelle 2008). The Allee effect is described as the reduction of the growth rate of a population as the density of that population declines (Courchamp et al. 1999). The Allee effect has the ability to limit the genetic loss by limiting the minimum effective size of the population (Maruyama and fuerst 1985). The minimum effective size of the population is maintained throughout the bottleneck which in turn determines the genetic composition of the population after the bottleneck. This influences the genetic variation of the resultant population (Wade and McCauley 1988; Nei et al. 1975)

Climate change has the potential to influence the ability of species to be adapted to a set of conditions (both climatic and environmental) within a specific region (Bellard *et al*. 2012).It has been suggested that within southern Africa there would be an increased frequency of harsh climatic events which include droughts (Hoffman *et al.* 2009) particularly in the winter rainfall zone of southern Africa where *A.pillansii* is found.

It is suggested that the survival of a species throughout severe instances of drought is largely influenced by genetic differences present within the species (Gutshick and BassirRad 2003, McDowell *et al*. 2008). During periods of drought there are dramatic changes to the environment in terms of the relative niches a species could occupy as well as the availability of resources.

Haensler *et al.* (2011) had projected using Regional Climate (RCM) a climate change of a 30 to 50% drying which would take effect at the end of the twenty-first century. In addition, (Stager *et al.* 2012) reports from model simulations which suggest an increase in the aridity within the winter rain fall zone of South Africa. The suggested increase in aridity is said to decrease the total runoff by a maximum of 30% by the year 2050 (Stager *et al.* 2012).This could be detrimental to the numerous plant species that are endemic to the Succulent Karoo (Meadows 2006; Stager *et al.* 2012). A successful drought strategy that increases a species resistance to a drought includes the avoidance of tissue dehydration and the maintenance of their tissue water potential (Chaves *et al*. 2009). These drought survival strategies are associated with adaptive traits that arise from the buildup of beneficial genes and the removal of deleterious genes through the process of selection. This usually arises within a population that has a high level of genetic diversity.

In the case of a species like *A.pillansii* which has a low genetic diversity, the chances of surviving an extreme climatic event such as a drought are dangerously low. The populations of *A.pillansii* are relatively isolated from one another which make them inherently more susceptible to environmental and climatic fluctuations (Keller and Waller 2002). The nature of the populations being isolated from one another may result in related individuals producing offspring (Eldrige *et al*., 1999; Lynch, 1996; Slate *et al*., 2000; Keller and Waller 2002) that could possess an accumulation of deleterious genes which would not collectively produce a successful response for survival to periods of droughts. The overall ability of a species to adapt to a climatic change event such as a drought is the presence of a percentage of genetic variation within the population (Booy *et al.* 2000)

The future for *A.pillansii* does not look bright unless conservation efforts are greatly increased. At present there has been a steady decline in the number of individuals in a population which amongst other factors has been attributed to drought stress as a result of climate change (Bolus *et al.* 2010). This impact is likely to continue without the proper intervention by the relative authorities. For the continued survival of the species, conservation efforts need to be focused on the ability of the species to be able to adapt to a changing environment. Such efforts should be concerned with increasing the low genetic variation within the species (Hendrik and Kalinowski 2000). Genetic restorations of populations with low genetic diversity may benefit from the introduction of individuals that are from a related subspecies (Hendrik and Kalinowski 2000). This process will aid in avoiding extinction of the species as well as the removal of deleterious genes and allow the species with sub-populations to attain a normal level of genetic variation. At present there is a lack of information which provides an example of whether this genetic restoration initiative has occurred with *A.pillansii* and a related species.

5. The way forward

Using the standard genetic markers to identify variation with the nuclear and chloroplast regions has not been successful. To identify greater variation for the nuclear and chloroplast gene sequences, microsatellites could be used. Microsatellites are sequences that are composed of a single sequence of no more 6 bases long (Litt and Luty 1989). Microsatellites are progressively replacing or being used in conjunction with other DNA markers (Bowcock *et al*., 1994; Goletti *et al*., 1994; Taylor *et al.*1994; Estoup *et al*., 1996). They have been identified within the genomes of each organism that has thus far been analyzed (Weber and May 1989). Designing a set of species-specific microsatellites will potentially increase the chance of gathering information on the expansion of the population of *Aloe pillansii* which would allow us to infer on the time of the expansion and the direction of that expansion. Molecular and phylogeographical data that explores the expansion of the species would provide insights into whether the expansion of the species was coupled with the expansion of the winter rainfall regime as well as whether the expansion of the species was in fact northward which would suggest that the southernmost population served as the ancestral population.

Distribution models provide evolutionary and ecological insights into a species' history as well as to predict the species distributions through time and space (Potts 2011). These models incorporate numerical data that make use of both the species occurrence or the abundance of a species in relation to its environmental estimates (Elith and Leathwick 2009). This concept of analysis may provide insights into the historical and future geographic distributions of *A.pillansii.* The future geographic distribution of a species is associated with soil, climate and an array of additional physical factors (Merow *et al.* 2013). This would suggest that the distribution has the potential to change in response to a shift in the prevailing climate. Distribution models can therefore be developed to allow for predictions of the response of the species distribution to climate change (Merow *et al.* 2013).

6. Conclusions

In this study it was found that there was a lack of chloroplast haplotypes within and between the individuals of the populations of *A.pillansii* which suggested a lack of variation. In comparison with a sister species *A.dichotoma,* the lack of variation was suggested to be as a result of *A.pillansii* experiencing a bottleneck event prior to its recent expansion.The bottleneck event has been suggested to have occurred within the time frame 0f 100 to 1000 years before present. Based on the lack of the variation observed within the species, it is suggested that *A.pillansii* experienced a severe bottleneck event. It remains unclear as to whether the expansion of the species coincided with the expansion of the winter rainfall regime that occurred throughout the Holocene era. For species that lack variation, inbreeding and gene drift are serious issues to contend with. Inbreeding results in a buildup of unfavorable alleles that affect the ability of the species to adapt to environmental and climatic conditions. To completely understand the population history of *A.pillansii* further research is required

Acknowledgments

I would like to thank my supervisors Prof. Terry Hedderson and Prof.Timm Hoffman for making themselves available to provide support, advice and a sympathetic ear. I special thank you goes out to Tracy Nowell who guided me through every step of my laboratory analysis. Lastly I would like to thank my family and friends for their support through the year.

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